AGE-RELATED MACULAR DEGENERATION

Clinical and Molecular Studies

Eveline Kersten

Age-related macular degeneration

Clinical and molecular studies

Eveline Kersten

The work presented in this thesis was carried out within the Radboud Institute for Health Sciences.

Research described in this thesis financially supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 634479, the European Research Council under the European Union's Seventh Framework Programme (FP/2007–2013)/ERC grant agreement no. 310644 (MACULA), Foundation Fighting Blindness USA (Grant C-GE-0811-0548-RAD04), Salentein fellowship, MD Fonds, Oogfonds, Algemene Nederlandse Vereniging ter Voorkoming van Blindheid, Stichting Nederlands Oogheelkundig Onderzoek, and Gelderse Blindenstichting.

Financial support for the publication of this thesis was kindly provided by Radboud University Nijmegen, Radboud Institute for Health Sciences, Bayer B.V., Théa Pharma B.V., and Synga Medical B.V..

All funding organizations had no role in the design or conduct of this research. They provided unrestricted grants.

ISBN	978-94-6323-671-3
Design cover	Jack Roskam
Design inside	Gildeprint
Photograph of the author	DUTZU photography
Printed by	Gildeprint

Age-related macular degeneration

Clinical and molecular studies

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op vrijdag 5 juli 2019
om 12.30 uur precies

door

Eveline Kersten

geboren op 12 december 1989
te Nijmegen

Promotoren
Prof. dr. C. B. Hoyng
Prof. dr. A.I. den Hollander

Copromotor
Dr. E.K. de Jong

Manuscriptcommissie
Prof. dr. G.J. van der Wilt
Prof. dr. R. Silva (University of Coimbra, Portugal)
Dr. F.D. Verbraak (Amsterdam UMC)

Table of contents

Chapter 1

General Introduction

BACKGROUND

Age-related macular degeneration (AMD) is a complex, multifactorial disease affecting the central retina. It is characterized by progressive changes to the macula eventually leading to central vision loss. In western countries, AMD is the leading cause of visual impairment among elderly people.[1] AMD and its accompanying vision loss are associated with difficulties performing daily activities, increased emotional distress and depression. It negatively influences the quality of life, not only of the affected individual but often also that of close relatives.[2] Since 2006, the number of people with visual impairment secondary to AMD has decreased in Europe. This is most probably due to the introduction of anti-vascular endothelial growth factor treatment for a subset of AMD patients along with healthier lifestyles.[3] However, as a consequence of the ageing population, the number of patients worldwide is expected to increase substantially from 196 million in 2020 to 288 million in 2040. Therefore, AMD remains a significant public health problem.[4]

STAGES OF AMD

Early and intermediate AMD

The major hallmark of the early and intermediate AMD stages are yellowish deposits between the retinal pigment epithelium (RPE) and Bruch's membrane, known as drusen.[5] These drusen consist of various lipids and proteins, and are considered to be cellular residues and debris from RPE cell degeneration constituting chronic inflammation.[6-8] The appearance of drusen varies greatly and several subtypes can be distinguished.[9] Drusen can be classified based on their diameter: small (<63 µm), intermediate (63-124 µm), and large drusen (≥125 µm). Additionally, drusen can be categorized by their appearance: small hard drusen have sharp edges and uniform colour density, whereas soft drusen are less sharp demarcated, often larger in size, and can be distinct (uniform colour density) or indistinct (graded colour density).[9-12] Larger soft drusen are associated with higher risk of developing advanced AMD,[13-16] whereas small hard drusen are considered to be normal changes related to aging as they are very common and confer minimal risk of development of advanced AMD.[10]

A specific subtype of small drusen, known as cuticular drusen, can be distinguished (Figure 1A). These are numerous (≥50) discrete, small, round drusen scattered throughout the macula, often extending to the peripheral retina. Cuticular drusen are best visible on fluorescein angiography and have a typical "stars in the sky" appearance.[9,17] This subphenotype has been associated with an earlier age of onset and is frequently clustered in families, suggesting a stronger genetic component in the development of cuticular drusen.[18,19] Additionally, all drusen can undergo calcification. Calcified drusen, also known as refractile drusen, have a

characteristic glistening or crystalline appearance on color fundus imaging (Figure 1B), and have been associated with the development of geographic atrophy.[20,21]

Next to drusen, reticular pseudodrusen can be observed in AMD patients. These reticular pseudodrusen differ both phenotypically and anatomically from drusen. Reticular pseudodrusen are subretinal deposits, in contrast to genuine drusen that are located beneath the RPE. Phenotypically, reticular pseudodrusen can be recognized as grey-white spots arranged in a reticular network (Figure 1C). Also, they are more commonly located at the superotemporal quadrant of the macula.[22] Reticular pseudodrusen are associated with development of advanced AMD, especially geographic atrophy.[22-24]

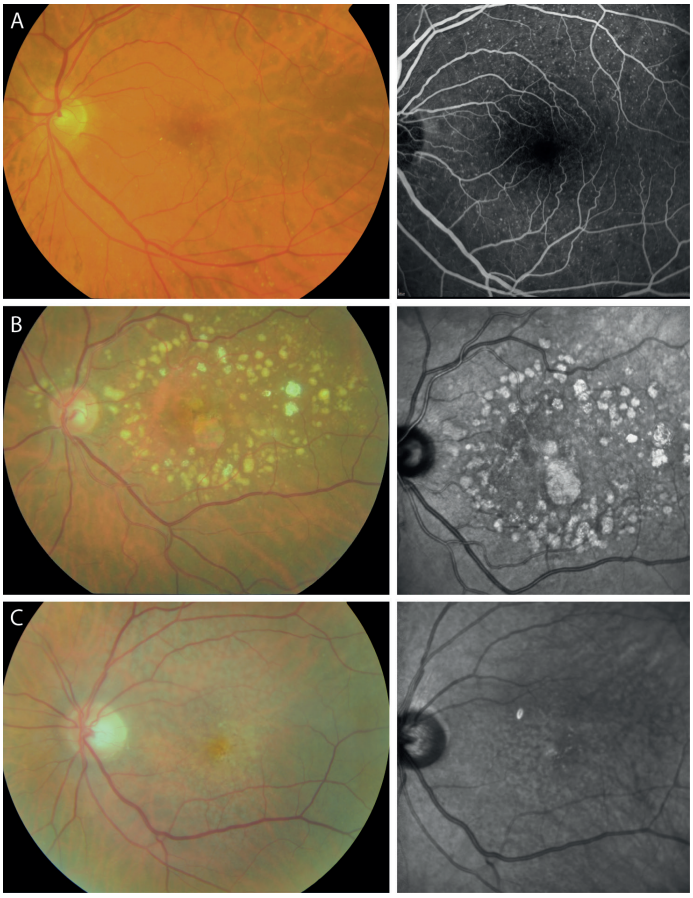


Figure 1. Examples of different types of drusen

A. Cuticular drusen on color fundus photograph (left) and fluorescein angiography (right). B. Crystalline drusen on color fundus photograph (left) and infrared imaging (right). C. Reticular pseudodrusen on color fundus photograph (left) and infrared imaging (right).

Other frequently observed features of early and intermediate AMD are hypo- and hyperpigmentations of the RPE.[5] The presence of these pigmentary abnormalities together with intermediate drusen are associated with 4-fold increased risk of advanced AMD compared to intermediate drusen alone.[14]

Advanced AMD

In time, early and intermediate AMD can progress to advanced AMD. Two subtypes of advanced AMD can be distinguished: geographic atrophy and neovascular AMD. Geographic atrophy (GA) is characterized by the presence of a sharp demarcated hypopigmented area, corresponding with RPE cell atrophy and photoreceptor degeneration, in which choroidal vessels become increasingly visible.[5] These atrophic areas enlarge gradually and cause slowly progressive vision loss.[25] The distinctive characteristic of neovascular AMD is choroidal neovascularization (CNV), which is the formation of new fragile blood vessels originating from the choroid. These new vessels can cause fluid leakage and/or hemorrhage resulting in a serous RPE detachment accompanied by a rapid loss of vision, and eventually fibrovascular scarring occurs.[26]

Table 1. Classification of AMD according to the CIRCL protocol based on color fundus photographs (CFP), spectral domain-optical coherence tomography (SD-OCT) and fluorescein angiography (FA)

Stage	Description
No AMD	No drusen and pigmentary changes Small drusen (diameter <63 µm) or pigmentary changes only Less than 10 small drusen and pigmentary changes
Early AMD	10 or more small drusen and pigmentary changes 1-14 intermediate drusen (diameter 63-124 µm)
Intermediate AMD	15 or more intermediate drusen 1 or more large drusen (diameter ≥125 µm) RPE atrophy (diameter ≥175 µm) outside the central circle of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid
Advanced AMD	Any or all of the following: central geographic atrophy, or evidence of neovascular AMD
Geographic atrophy	Sharply demarcated round or oval area of depigmentation of the RPE (diameter ≥175 µm) with increased visibility of choroidal vessels within the central circle of the ETDRS grid secondary to AMD
Neovascular AMD	Choroidal neovascular lesion within the ETDRS grid secondary to AMD with evidence of fluid, blood, or fibrovascular tissue on FP, signs of active or previous CNV on FA, and/or retinal or subretinal fluid and/or tissue secondary to AMD on SD-OCT

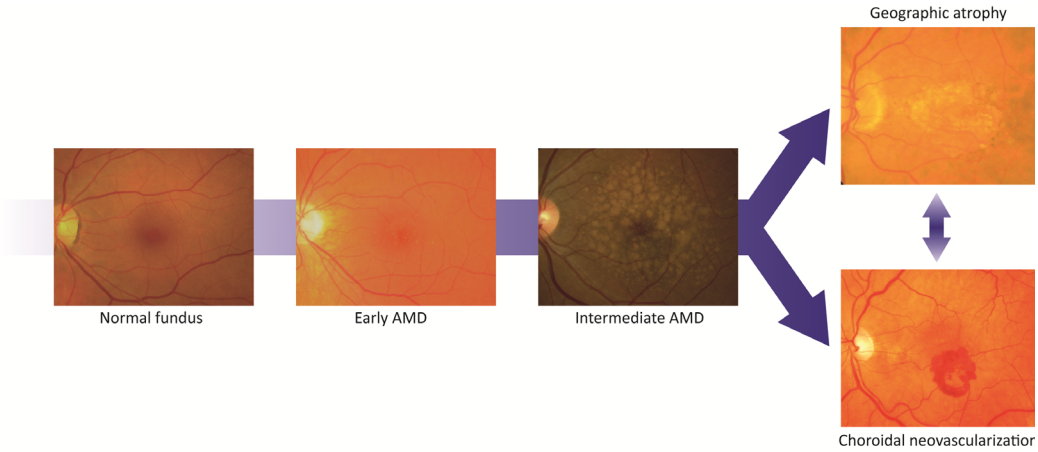


Figure 2. Visualization of disease progression and examples of different AMD stages according to the CIRCL protocol

AMD Classification

The various severity stages and large diversity of phenotypes in AMD ask for standardization. In clinical practice standardization is necessary for establishing an accurate diagnosis, and determining prognosis and management strategy. In research, standardization is essential for comparison of study results. A detailed grading system for AMD has already been published in 1991: "The Wisconsin Age-Related Maculopathy Grading System".[12] A few years later, the International Age-Related Maculopathy Epidemiological Study Group proposed a classification protocol and grading system for AMD.[27] This classification system distinguishes 'early' and 'late' AMD. Early AMD is characterized by presence of drusen and RPE pigmentary abnormalities, and late AMD is characterized by the presence of GA of the RPE or neovascular AMD defined as RPE detachment, hemorrhages, and/or scars. Multiple classification protocols have been developed and adapted for epidemiological studies and clinical trials since then.[10,11,15,28-31] These classification systems are based on (detailed) evaluation of color fundus photographs, and include number and size of drusen, presence of pigmentary abnormalities and signs of GA or CNV. Grading of AMD in this thesis is based on the standard grading protocol of the Cologne Image and Reading Center and Laboratory (CIRCL; https://augenklinik.uk-koeln.de/forschung/arbeitsgruppen-labore/circl/), and also includes evaluation of spectral domain-optical coherence tomography and fluorescein angiography. A detailed description of the different AMD stages and corresponding examples of color fundus images are displayed in Table 1 and Figure 2, respectively.

RISK FACTORS FOR DEVELOPMENT AND PROGRESSION OF AMD

Nongenetic risk factors

AMD is a multifactorial disease, which means that a combination of multiple environmental and genetic factors act together and trigger the development of AMD. Around one third of AMD risk is explained by nongenetic risk factors.[32] As the name already suggests, age is the most important risk factor for AMD development. Population-based studies demonstrated that the prevalence of AMD increases significantly with age.[3,33] A recent meta-analysis, combining data of 14 population-based studies in Europe, showed an increase in prevalence of early and intermediate AMD from 3.5% in people aged 55 to 59 years up to 17.6% in people 85 years and older. For advanced AMD, these prevalences increase from 0.1% to 9.8%, respectively.[3]

Smoking has also been consistently associated with development of AMD. Current smokers have a two- to three-fold increased risk of AMD.[34-36] Although past smokers still have a higher risk compared to never smokers, cessation decreases the risk of AMD development even in elderly.[35,36] Smoking cessation should therefore be encouraged by ophthalmologists to modify a patient's risk of AMD. Other commonly reported nongenetic risk factors include low dietary intake of antioxidants and omega-3 fatty acids, obesity and reduced physical activity.[34,37]

Approximately 1 in 7 people will develop any kind of non-advanced AMD over the age of 70,[3] however not all of them progress to an advanced disease stage. Rates of progression to advanced AMD vary greatly in literature due to differences in follow-up time and study design.[38-40] However, this conversion to advanced AMD is clinically very relevant as this is accompanied by vision loss and can have consequences for the management and monitoring of AMD patients. Nongenetic risk factors associated with progression to advanced AMD include age, smoking, and BMI.[41,42] Additionally, phenotype is also considered a major predictor for progression to advanced AMD, with the more severe phenotype conferring the highest risk for progression.[38,39,41,42]

Family history

Family and twin studies have played an important role in understanding the genetic contribution of AMD. Already in the '90s it was reported that AMD aggregates in families, and also high concordance in twin studies led to the conclusion that there must be a familial component in AMD.[43-48] This familial component could either be explained by shared genetic susceptibility, common exposure to environmental risk factors or a combination of both.

First-degree relatives of an AMD patient have an increased risk of AMD development (odds ratio [OR] range, 2.4 to 19.3).[43-45,49-52] Additionally, it has been suggested that siblings are more likely to develop the same advanced phenotype as their proband,[53] although most studies report large phenotypic heterogeneity within families.[54-56]

Although at present a large proportion of the heritability of AMD has been explained, not all AMD families can be fully explained by known risk factors and family history seems to remain a (substitute) risk factor for development of AMD.[57,58]

Genetic risk factors

The heritability of AMD is estimated to be 46-71%,[32] and significant progress has been made in the identification of genetic variants associated with AMD risk. Since the identification of a risk-conferring common variant in the complement factor H (*CFH*) gene in 1995,[59-61] genome-wide association studies have identified many (mainly common) genetic variants that are associated with AMD. The largest genome-wide association study to date included 16,144 advanced AMD cases and 17,832 control individuals, and reported 52 genetic variants distributed across 34 loci to be independently associated with AMD.[62] Until now, both common variants with small to modest effect sizes and several low- to rare frequency variants have been identified to contribute to disease susceptibility (Figure 3). Together these variants explain more than half of the heritability.[62] The remaining unexplained heritability may be (partly) attributed to additional rare genetic variants.[63-65] Next-generation sequencing technology has provided an opportunity to discover these variants using whole-exome or whole-genome

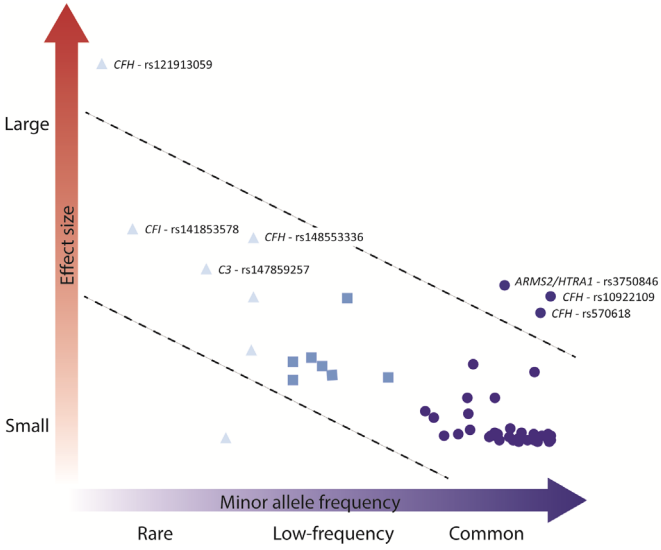


Figure 3. Genetic architecture of age-related macular degeneration

Light blue triangles indicate rare variants, blue squares indicate low-frequency variants, and dark blue circles indicate common variants with population frequencies of <1%, 1-5%, ≥5% respectively. The area above dashed lines indicate the area with the highest feasibility of identifying genetic variants because of their high effect size. Common genetic variants with stronger effects can be detected in relatively small case-control studies, however to identify variants with modest to small effect sizes larger cohorts and high-resolution genotyping are needed (area between the dashed lines). To identify rare variants specific sequencing strategies using next-generation sequencing are necessary. Genetic variants associated with AMD depicted in this figure are derived from Fritsche et al.[62]

sequencing methods. Sequencing of AMD families seems to be an ideal approach to further identify rare, more penetrant variants, as these families cannot completely be explained by clustering of known environmental and genetic risk factors. In recent years, already a number of rare genetic variants have been identified in AMD families.[66-73] Rare variants with smaller risk effects will be hard to identify, and larger consortium-based studies are needed to identify these.

PATHWAYS INVOLVED IN AMD PATHOGENESIS

The complement system

The complement system is part of our innate immune system. It protects the host from invading pathogens by inducing inflammatory responses, opsonization of pathogens and lysis of foreign cells, but also contributes to host homeostasis by clearance of apoptotic cells and cellular debris.[74] Activation of the complement cascade can be initiated through three distinct pathways (the classical, lectin, or alternative pathway), which eventually all merge into one terminal cytolytic pathway.[74] In the eye there is a continuous low-level activation of the alternative pathway.[75] This complement activity is initiated by spontaneous conversion of complement component 3, and leads to a cascade of conversions of inactive proteins to their active forms. Consequently, convertases (C3- and C5-convertase) and anaphylatoxins (C3a and C5a) are produced, and this cascade finally results in the formation of the membrane attack complex (Figure 4).[74,76] To avoid tissue damage, complement activation is closely regulated by several complement inhibitory proteins. Factor H is an important inhibitor circulating in plasma and acts through several mechanisms: by acting as cofactor for factor I mediated proteolysis of C3b, accelerating decay of C3-convertase, and competing with factor B in binding to C3b and thereby preventing formation of C3-convertase.[74,76]

There is cumulative evidence that dysregulation of the alternative pathway of the complement system plays a pivotal role in AMD pathogenesis. First, histopathological studies demonstrated the presence of several complement proteins, complement activators, and complement regulatory proteins in drusen.[6,7,77] Subsequently, genetic studies showed strong associations between AMD and complement genes, in particular the *CFH* gene.[59-62] Moreover, the variants in complement-associated genes together account for nearly 60% of the AMD genetic risk.[78] Finally, increased systemic levels of complement activation products have been consistently reported,[79-82] suggesting that complement activation in AMD is not restricted to the eye.

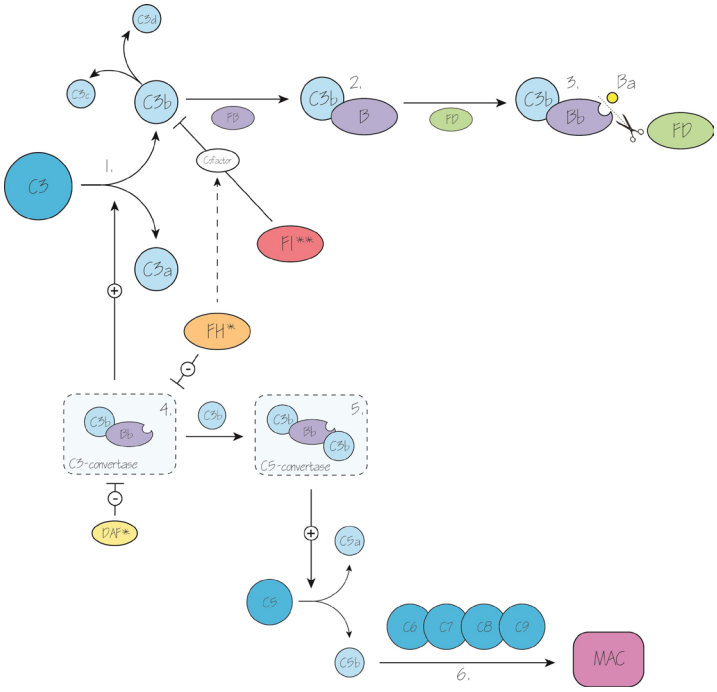


Figure 4. Overview of the alternative pathway of the complement system
(1) Complement component 3 (C3) splits into C3a and C3b by spontaneous hydrolyzation or by the C3-convertase (C4bC2) resulting from activation of the classical or lectin pathway. (2) Factor B (FB) can bind C3b to form C3bB. (3) The bound factor B is then cleaved by factor D (FD) which results in the formation of the C3-convertase: C3bBb (4). This C3-convertase can cleave C3, which leads to more C3b and in turn increased formation of the C3-convertase (known as the C3 amplification loop). The C3-convertase can also bind another C3b molecule to form C3bBb3b, which is a C5-convertase (5). This C5-convertase can convert C5 into C5a and C5b. (6) C5b then sequentially binds C6, C7, C8, and multiple C9 molecules to form the terminal complement complex (SC5b-9), also known as the membrane attack complex.
* The C3-convertase is inhibited by several complement regulators, among which decay accelerating factor (DAF) and factor H (FH).
** Factor I (FI) can breakdown C3b via several digestion steps to C3c and finally C3d. This protease activity, however, requires a cofactor, such as FH.

Oxidative stress

Multiple sources of oxidative stress make the macula susceptible to oxidative damage. Reactive oxygen species (responsible for oxidative stress) are abundant in macular tissue due to its high metabolic activity, continuous photoreceptor shedding, and exposure to bright light.[83,84] Increased oxidative stress is considered to contribute to AMD pathogenesis. Support for this theory mainly comes from epidemiological studies linking several environmental risk factors for AMD to oxidative stress. Smoking, one of the most important environmental risk factors, enhances production of reactive oxygen species thereby adding to oxidative stress.[83,85]

Also, high dietary intake of antioxidants is associated with a reduced AMD risk,[37,86] implicating imbalance of the oxidative stress system in AMD. Moreover, increased levels of oxidation products and decreased levels of antioxidants have been reported in AMD patients.[87,88]
Genetic studies have suggested a role for oxidative stress in AMD as well. Mitochondria are an important source of reactive oxygen species formation and are highly susceptible for oxidative damage.[84] A number of studies have associated variations in mitochondrial DNA with AMD and are suggestive of a pathogenic role of oxidative stress in AMD.[89-91] Studies investigating variations in genes encoding antioxidant proteins have been inconclusive.[92-95]

Other pathways

Several other pathways have been implicated in AMD pathogenesis. These include lipid metabolism, extracellular matrix remodeling, and the angiogenesis signaling pathway. Lipids are abundantly present in drusen and altered systemic lipid measurements have been reported in AMD patients.[96,97] Especially elevated high-density lipoprotein levels seem to be related with increased AMD risk.[97-99] Involvement of lipid metabolism in AMD is further strengthened by genetic associations of lipid-related genes (e.g. *APOE*, *CETP*, *LIPC, ABCA1*).[62,78] Genetic studies also points towards a role for the extracellular matrix pathway in AMD.[62,78] An important extracellular matrix structure in the retina is Bruch's membrane, located between the RPE and the choroid. Many histopathological studies have pinpointed alterations at Bruch's membrane as the first changes in AMD.[100]
Another important pathway in AMD pathogenesis is the angiogenesis signaling pathway. Genetic associations have been reported for several genes encoding angiogenic growth factors involved in the initiation of neovascularization (e.g. *VEGFA* and *TGFBR1*).[62]

TREATMENT

Prevention

Lifestyle interventions can positively influence the natural course of AMD and slow down progression to an advanced disease stage. Smoking cessation is highly recommended and has been proven beneficial in reducing risk, even in elderly.[35] Ensuring a healthy body weight, regular physical activity, and a diet rich in antioxidants (fruit and vegetables) may also confer protection against AMD progression.[86,101-103] Additionally, nutritional supplementation can be beneficial for a subgroup of patients. The Age-related Eye Disease Study (AREDS) and its successor, the AREDS2, are the largest investigations into vitamin supplementation in AMD. In the AREDS, daily supplementation with vitamin C, vitamin E, β-carotene and zinc oxide reduced the risk for development of advanced AMD in 5 years with approximately 25% in patients with a high risk for progression.[104] The AREDS2 was initiated to evaluate the effects of lutein, zeaxanthin and omega-3 fatty acids supplementation. Although no further risk

reduction was established when adding these nutrients to the original AREDS formulation, β-carotene was replaced by lutein and zeaxanthin because of the increased risk of lung cancer in smokers receiving β-carotene.[105] The improved AREDS2 formula, containing 500 mg vitamin C, 400 IU vitamin E, 10 mg lutein, 2 mg zeaxanthin, 80 mg zinc oxide, and 2 mg copper is recommended for patients with intermediate or unilateral advanced AMD.

Neovascular AMD

Although neovascular AMD affects the minority of AMD patients, it accounts for the majority of visually impaired AMD patients.[106] Since the introduction of intravitreal injections with anti-vascular endothelial growth factor (VEGF) agents in 2006, the number of AMD patients with severe visual impairment has decreased significantly.[107] Unfortunately, anti-VEGF treatment does not lead to improvement or stabilization of vision in every patient, and results in a declined visual acuity after one year in approximately 10% of the patients.[108] Currently available anti-VEGF drugs include bevacizumab, ranibizumab, and aflibercept. Efficacy and safety of these drugs are comparable, however, aflibercept has the advantage that it can be injected every two months instead of monthly.[108,109] Although bevacizumab use is not officially approved for treatment of neovascular AMD, it is often used off-label as first choice treatment by ophthalmologists due to its favorable cost-effective profile.

Dry AMD

No treatment is yet available for the early, intermediate and advanced atrophic stages of AMD, together also referred to as 'dry AMD'. Various clinical trials targeting different pathways are ongoing (Table 3). Promising results have been reported for complement inhibiting drugs, particularly for lampalizumab. Intravitreal administration of lampalizumab was associated with 20% reduction in atrophy growth in the MAHALO phase II clinical trial.[110] The efficacy of lampalizumab was further evaluated in two large multicenter phase III trials. However, both the SPECTRI study (NCT02247531) and the CHROMA study (NCT02247479) did not meet their primary endpoint in reducing atrophy growth.[111]

Table 3. Overview of clinical trials for dry AMD. Derived from https://clinicaltrials.gov/

Agent	Mechanism of action	Administration	Current stage	(Preliminary) results
Anti-inflammatory agents and complement inhibitors				
Lampalizumab	Inhibits complement system by interaction with complement factor D	Intravitreal injection	Phase II – completed (NCT02288559/NCT01602120) Phase III – completed (NCT02247479) Phase III – terminated (NCT02247531)	20% reduction in GA area progression, subgroup analysis of CFI risk-allele carriers showed 44% reduction in area progression [110] Did not reduce mean change in GA lesion area compared to sham treatment at 48 weeks[111]
Eculizumab	Inhibits complement system by inhibition of complement factor C5	Intravenous	Phase II – completed (NCT00935883)	No significant decrease in growth rate of GA compared to placebo[112]
LFG316	Inhibits complement system by interaction with complement factor C5	Intravitreal injection	Phase II – completed (NCT01527500)	No results reported
CLG561 with and without LFG316	Inhibits complement system by inhibition of properdin	Intravitreal injection	Phase II – completed (NCT02515942)	No results reported
ARC1905 (Zimura)	Inhibits complement system by interaction with complement factor C5	Intravitreal injection	Phase II/III – recruiting (NCT02686658)	No results reported
AL-78898A (POT-4)	Inhibits complement system by inhibiting cleavage of complement factor C3	Intravitreal injection	Phase II – terminated (NCT01603043)	Terminated due to high likelihood that continued enrollment would reach predefined study stopping criteria based upon the number of patients with drug deposit formation (not published)
APL-2	Inhibits complement system by inhibiting cleavage of complement factor C3	Intravitreal injection	Phase II – completed (NCT02503332) Phase III – not yet recruiting (NCT03525600/NCT03525613)	Preliminary results demonstrated 29% reduction in GA growth (press results by Apellis Pharmaceuticals)
Fluocinolone acetonide (Iluvien)	Inhibits inflammation (corticosteroid)	Intravitreal injection	Phase II – terminated (NCT00695318)	No difference in change of GA size compared to sham (not published)

Sirolimus	Immunosuppressant by inhibition of mTOR	Subconjunctival injection Intravitreal injection Intravitreal injection	Phase I/II – completed (NCT00766649) Phase I/II – completed (NCT01445548) Phase II – terminated (NCT01675947)	No positive anatomic or functional effects, might have a negative effect on visual acuity[113] No evidence of anatomical or functional benefit, might be associated with increased retinal/RPE atrophy[114] Study injections discontinued due to safety concerns[115]
Glatiramer acetate (copaxone)	Immunomodulation	Subcutaneous injection	Phase II/III – unknown (NCT00466076)	Preliminary results demonstrated a reduction in drusen area[116,117]
Doxycycline (ORACEA)	Prevents photoreceptor loss (MMP inhibitor)	Oral	Phase II/III – recruiting (NCT01782989)	No results reported
Visual cycle modulators				
Fenretinide	Inhibits retinol binding to RBP leading to decreased formation of A2E	Oral	Phase II – completed (NCT00429936)	Trend for reduced annual growth rate of GA and trend for reduced incidence of CNV[118]
ACU-4429 (emixustat)	Inhibits retinol isomerisation decreasing accumulation of toxic retinal by products (A2E)	Oral	Phase II – completed (NCT01002950) Phase II/III – completed (NCT01802866)	Dose-dependent reversible effect on rod function, findings support further evaluation[119] No reduction in GA growth rate[120]
Antioxidants and neuroprotectants				
OT-551	Reduces oxidative stress (SOD mimetic and NRF2 activator)	Topical drops	Phase II – completed (NCT00306488)	Lower mean decrease in VA, however all secondary outcomes not significant, therefore limited or no benefit as treatment for GA[121]
Brimonidine tartrate	Neuroprotection (α2-adrenergic receptor agonist)	Intravitreal implant	Phase II – completed (NCT00658619) Phase II – ongoing (NCT02087085)	No significant difference in change of GA size compared to sham (not published) No results reported

Agent	Mechanism of action	Administration	Current stage	(Preliminary) results
Ciliary Neurotrophic Factor (NT-501)	Retards loss of photoreceptor cells during degeneration	Intraocular implant	Phase II – completed (NCT00447954)	Appears to slow progression of vision loss, especially in eyes with VA of 20/63 or better at baseline. No significant difference in progression of GA lesion size[122]
Tandospirone (AL-8309B)	Protects retina from light damage (selective serotonin 1A agonist)	Topical drops	Phase III – terminated (NCT00890097)	Did not affect lesion growth and treatment is regarded ineffective[123]
Reducers of toxic byproducts of amyloid ß				
RN6G	Prevents accumulation of amyloid β-40/β-42 byproducts	Intravenous	Phase II – terminated (NCT01577381)	Not enough subjects or data to perform meaningful analyses (not published)
GSK933776	Inhibits accumulation of amyloid β byproducts	Intravenous	Phase II – completed (NCT01342926)	No difference in change of GA size compared to placebo (not published)
Vascular enhancers				
MC-1101 (Macuclear)	Increases choroidal blood flow	Topical drops	Phase II/III – unknown & terminated (NCT02127463 & NCT01601483)	No results reported
Isopropyl unoprostone (UF-021)	Increases retinal and choroidal bloodflow	Topical drops	Phase II – completed (NCT01379560)	No results reported
Alprostadil (prostaglandin E1)	Increases choroidal blood flow	Intravenous	Phase III – terminated (NCT00619229)	Interim analysis indicated that the number of originally planned patients were insufficient to reach statistical significance and was therefore terminated[124]

Stem cell therapy				
MA09-hRPE	Replacement of dysfunctional RPE by human embryonic stem cells	Subretinal stem cell transplant	Phase I/II – unknown (NCT01674829) Phase I/II – completed (NCT01344993)	No results reported First evidence for medium- to long-term safety and tolerability, improvement in VA of the treated eye suggests biological activity of the transplanted cells[125]
HuCNS-SC	Replacement of dysfunctional RPE by human central nervous system stem cells	Subretinal stem cell transplant	Phase I/II – completed NCT01632527) Phase II – terminated (NCT02467634)	No results reported Business decision – unrelated to any safety concerns
Gene therapy				
AAVCAGsCD59	Inhibits formation of MAC	Intravitreal injection	Phase I – active, not recruiting (NCT03144999)	No results reported

Abbreviations: CNV, choroidal neovascularization; CFI, complement factor I; GA, geographic atrophy; MAC, membrane attack complex; mTOR, mechanistic target of rapamycin; NRF2, Nuclear factor erythroid 2-related factor 2; RBP, retinol-binding protein; RPE, retinal pigment epithelium; SOD, superoxide dismutase; VA, visual acuity.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to increase our understanding of the clinical and molecular characteristics of AMD development and progression. Specifically, differences between AMD families and nonfamilial (sporadic) AMD were studied, and a prediction algorithm for conversion from early to advanced AMD was established to facilitate risk assessment and genetic counseling. Additionally, insights from molecular studies also facilitate genetic counseling and can contribute to identification of molecular drivers or biological pathways relevant for AMD. In this thesis, c*hapter 2* focuses on clinical studies, and *chapter 3* on molecular studies.

Clinical studies

Familial aggregation of AMD has been described and might imply a stronger genetic component and smaller contribution of environmental factors in familial AMD. C*hapter 2.1* evaluates potential differences between familial and sporadic AMD patients with respect to environmental risk factors for AMD. Additionally, phenotypical characteristics of familial and sporadic AMD are discussed. In clinical practice, particularly members of AMD families are interested in their risk of AMD. However, most prediction models have been established for AMD based on demographic, environmental and common genetic risk factors. Even though these factors can cluster in families, it is expected that densely affected families cannot be fully explained by known risk factors and may harbor rare genetic variants. *Chapter 2.2* assesses the proportion of AMD families that cannot be explained by common genetic and environmental risk factors and discusses risk prediction in AMD families.

Additionally, identification of predictive factors for progression to advanced AMD may contribute to a more efficient and personalized approach in monitoring and support of patients already diagnosed with early or intermediate AMD. *Chapter 2.3* presents a prediction model for conversion to advanced AMD including genetic, environmental, demographic risk factors and detailed phenotypic characteristics.

Although no treatment exists yet for dry AMD, therapies are in development. It is important to select those patients that will most likely benefit from these therapies. Patients carrying a rare variant in complement-associated genes seem an ideal patient group for complement-inhibiting therapies. *Chapter 2.4* describes phenotypical characteristics of AMD patients carrying a rare variant in the *CFH* gene, which could help ophthalmologists to select patients for additional genetic testing and upcoming complement-inhibiting therapies.

Molecular studies

Molecular biomarkers can help unravel mechanisms of disease and identify new targets for therapy. *Chapter 3.1* provides an overview of systemic compounds investigated in relation

to AMD and discusses their usefulness as AMD biomarker. The use of hypothesis-free techniques in biomarker detection holds great promise. *Chapter 3.2* evaluates the metabolic profile of AMD patients using a targeted metabolomics approach, aiming to contribute to the identification of biomarkers and metabolic pathways for AMD.

With the rise of next-generation sequencing techniques, significant progress has been made in the discovery of low-frequency and rare genetic variants contributing to AMD risk. Using genetic data of approximately 40.000 individuals, the International AMD Genomics Consortium identified seven independent rare variants to be associated with AMD. *Chapter 3.3* describes the geographic distribution of these rare variants, as population-specific variants have implications for genetic counseling, carrier screening and personalized treatment. Finally, *Chapter 3.4* evaluates the occurrence of genetic variants in genes underlying AMD-mimicking dystrophies in a dry AMD cohort. The clinical utility of genetic screening for macular dystrophies in AMD patients is discussed, since it can be clinically difficult to distinguish dry AMD from AMD-mimicking dystrophies.

General discussion and summary

Chapter 4 further discusses the studies described in this thesis and places them in a broader, future, perspective. Finally, c*hapter 5* provides a summary of the findings described in this thesis.

REFERENCES

1. Bourne RR, Jonas JB, Flaxman SR, et al. Prevalence and causes of vision loss in high-income countries and in Eastern and Central Europe: 1990-2010. *Br J Ophthalmol.* 2014;98(5):629-638.
2. Mitchell J, Bradley C. Quality of life in age-related macular degeneration: a review of the literature. *Health Qual Life Outcomes.* 2006;4:97.
3. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology.* 2017;124(12):1753-1763.
4. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health.* 2014;2(2):e106-116.
5. de Jong PT. Age-related macular degeneration. *N Engl J Med.* 2006;355(14):1474-1485.
6. Hageman GS, Mullins RF. Molecular composition of drusen as related to substructural phenotype. *Mol Vis.* 1999;5:28.
7. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol.* 2002;134(3):411-431.
8. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2002;99(23):14682-14687.
9. Khan KN, Mahroo OA, Khan RS, et al. Differentiating drusen: Drusen and drusen-like appearances associated with ageing, age-related macular degeneration, inherited eye disease and other pathological processes. *Prog Retin Eye Res.* 2016;53:70-106.
10. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology.* 2013;120(4):844-851.
11. Age-Related Eye Disease Study Research G. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study Report Number 6. *Am J Ophthalmol.* 2001;132(5):668-681.
12. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology.* 1991;98(7):1128-1134.
13. Klein R, Klein BE, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology.* 2007;114(2):253-262.
14. Joachim ND, Mitchell P, Kifley A, Wang JJ. Incidence, Progression, and Associated Risk Factors of Medium Drusen in Age-Related Macular Degeneration: Findings From the 15-Year Follow-up of an Australian Cohort. *JAMA Ophthalmol.* 2015;133(6):698-705.
15. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci.* 2001;42(10):2237-2241.
16. Tikellis G, Robman LD, Dimitrov P, Nicolas C, McCarty CA, Guymer RH. Characteristics of progression of early age-related macular degeneration: the cardiovascular health and age-related maculopathy study. *Eye (Lond).* 2007;21(2):169-176.
17. Boon CJ, van de Ven JP, Hoyng CB, den Hollander AI, Klevering BJ. Cuticular drusen: stars in the sky. *Prog Retin Eye Res.* 2013;37:90-113.
18. Boon CJ, Klevering BJ, Hoyng CB, et al. Basal laminar drusen caused by compound heterozygous variants in the CFH gene. *Am J Hum Genet.* 2008;82(2):516-523.
19. van de Ven JP, Boon CJ, Fauser S, et al. Clinical evaluation of 3 families with basal laminar drusen caused by novel mutations in the complement factor H gene. *Arch Ophthalmol.* 2012;130(8):1038-1047.
20. Oishi A, Thiele S, Nadal J, et al. Prevalence, Natural Course, and Prognostic Role of Refractile Drusen in Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2017;58(4):2198-2206.

21. Suzuki M, Curcio CA, Mullins RF, Spaide RF. REFRACTILE DRUSEN: Clinical Imaging and Candidate Histology. *Retina.* 2015;35(5):859-865.
22. Zweifel SA, Imamura Y, Spaide TC, Fujiwara T, Spaide RF. Prevalence and significance of subretinal drusenoid deposits (reticular pseudodrusen) in age-related macular degeneration. *Ophthalmology.* 2010;117(9):1775-1781.
23. Marsiglia M, Boddu S, Bearelly S, et al. Association between geographic atrophy progression and reticular pseudodrusen in eyes with dry age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2013;54(12):7362-7369.
24. Schmitz-Valckenberg S, Alten F, Steinberg JS, et al. Reticular drusen associated with geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2011;52(9):5009-5015.
25. Sunness JS, Gonzalez-Baron J, Applegate CA, et al. Enlargement of atrophy and visual acuity loss in the geographic atrophy form of age-related macular degeneration. *Ophthalmology.* 1999;106(9):1768-1779.
26. Campochiaro PA, Soloway P, Ryan SJ, Miller JW. The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration. *Mol Vis.* 1999;5:34.
27. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol.* 1995;39(5):367-374.
28. Davis MD, Gangnon RE, Lee LY, et al. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch Ophthalmol.* 2005;123(11):1484-1498.
29. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch Ophthalmol.* 2005;123(11):1570-1574.
30. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology.* 1995;102(10):1450-1460.
31. Seddon JM, Sharma S, Adelman RA. Evaluation of the clinical age-related maculopathy staging system. *Ophthalmology.* 2006;113(2):260-266.
32. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch Ophthalmol.* 2005;123(3):321-327.
33. Smith W, Assink J, Klein R, et al. Risk factors for age-related macular degeneration: Pooled findings from three continents. *Ophthalmology.* 2001;108(4):697-704.
34. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol.* 2010;10:31.
35. Neuner B, Komm A, Wellmann J, et al. Smoking history and the incidence of age-related macular degeneration--results from the Muenster Aging and Retina Study (MARS) cohort and systematic review and meta-analysis of observational longitudinal studies. *Addict Behav.* 2009;34(11):938-947.
36. Velilla S, Garcia-Medina JJ, Garcia-Layana A, et al. Smoking and age-related macular degeneration: review and update. *J Ophthalmol.* 2013;2013:895147.
37. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. *Lancet.* 2012;379(9827):1728-1738.
38. Chew EY, Clemons TE, Agron E, et al. Ten-year follow-up of age-related macular degeneration in the age-related eye disease study: AREDS report no. 36. *JAMA Ophthalmol.* 2014;132(3):272-277.
39. Joachim N, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. *Ophthalmology.* 2015;122(12):2482-2489.

40. Jonasson F, Fisher DE, Eiriksdottir G, et al. Five-year incidence, progression, and risk factors for age-related macular degeneration: the age, gene/environment susceptibility study. *Ophthalmology.* 2014;121(9):1766-1772.
41. Seddon JM, Silver RE, Kwong M, Rosner B. Risk Prediction for Progression of Macular Degeneration: 10 Common and Rare Genetic Variants, Demographic, Environmental, and Macular Covariates. *Invest Ophthalmol Vis Sci.* 2015;56(4):2192-2202.
42. Perlee LT, Bansal AT, Gehrs K, et al. Inclusion of genotype with fundus phenotype improves accuracy of predicting choroidal neovascularization and geographic atrophy. *Ophthalmology.* 2013;120(9):1880-1892.
43. Klaver CC, Wolfs RC, Assink JJ, van Duijn CM, Hofman A, de Jong PT. Genetic risk of age-related maculopathy. Population-based familial aggregation study. *Arch Ophthalmol.* 1998;116(12):1646-1651.
44. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol.* 1997;123(2):199-206.
45. Silvestri G, Johnston PB, Hughes AE. Is genetic predisposition an important risk factor in age-related macular degeneration? *Eye (Lond).* 1994;8 (Pt 5):564-568.
46. Heiba IM, Elston RC, Klein BE, Klein R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. *Genet Epidemiol.* 1994;11(1):51-67.
47. Klein ML, Mauldin WM, Stoumbos VD. Heredity and age-related macular degeneration. Observations in monozygotic twins. *Arch Ophthalmol.* 1994;112(7):932-937.
48. Meyers SM, Greene T, Gutman FA. A twin study of age-related macular degeneration. *Am J Ophthalmol.* 1995;120(6):757-766.
49. Klein BE, Klein R, Lee KE, Moore EL, Danforth L. Risk of incident age-related eye diseases in people with an affected sibling : The Beaver Dam Eye Study. *Am J Epidemiol.* 2001;154(3):207-211.
50. Luo L, Harmon J, Yang X, et al. Familial aggregation of age-related macular degeneration in the Utah population. *Vision Res.* 2008;48(3):494-500.
51. Shahid H, Khan JC, Cipriani V, et al. Age-related macular degeneration: the importance of family history as a risk factor. *Br J Ophthalmol.* 2012;96(3):427-431.
52. Smith W, Mitchell P. Family history and age-related maculopathy: the Blue Mountains Eye Study. *Aust N Z J Ophthalmol.* 1998;26(3):203-206.
53. Sobrin L, Ripke S, Yu Y, et al. Heritability and genome-wide association study to assess genetic differences between advanced age-related macular degeneration subtypes. *Ophthalmology.* 2012;119(9):1874-1885.
54. Assink JJ, Klaver CC, Houwing-Duistermaat JJ, et al. Heterogeneity of the genetic risk in age-related macular disease: a population-based familial risk study. *Ophthalmology.* 2005;112(3):482-487.
55. De la Paz MA, Pericak-Vance MA, Haines JL, Seddon JM. Phenotypic heterogeneity in families with age-related macular degeneration. *Am J Ophthalmol.* 1997;124(3):331-343.
56. Postel EA, Agarwal A, Schmidt S, et al. Comparing age-related macular degeneration phenotype in probands from singleton and multiplex families. *Am J Ophthalmol.* 2005;139(5):820-825.
57. Klein ML, Francis PJ, Ferris FL, 3rd, Hamon SC, Clemons TE. Risk assessment model for development of advanced age-related macular degeneration. *Arch Ophthalmol.* 2011;129(12):1543-1550.
58. Sobrin L, Maller JB, Neale BM, et al. Genetic profile for five common variants associated with age-related macular degeneration in densely affected families: a novel analytic approach. *Eur J Hum Genet.* 2010;18(4):496-501.
59. Edwards AO, Ritter R, 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005;308(5720):421-424.
60. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* 2005;308(5720):419-421.

61. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308(5720):385-389.
62. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
63. den Hollander AI. Omics in Ophthalmology: Advances in Genomics and Precision Medicine for Leber Congenital Amaurosis and Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2016;57(3):1378-1387.
64. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature.* 2009;461(7265):747-753.
65. McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet.* 2008;9(5):356-369.
66. Duvvari MR, van de Ven JP, Geerlings MJ, et al. Whole Exome Sequencing in Patients with the Cuticular Drusen Subtype of Age-Related Macular Degeneration. *PLoS One.* 2016;11(3):e0152047.
67. Geerlings MJ, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2017;135(1):39-46.
68. Hoffman JD, Cooke Bailey JN, D'Aoust L, et al. Rare complement factor H variant associated with age-related macular degeneration in the Amish. *Invest Ophthalmol Vis Sci.* 2014;55(7):4455-4460.
69. Pras E, Kristal D, Shoshany N, et al. Rare genetic variants in Tunisian Jewish patients suffering from age-related macular degeneration. *J Med Genet.* 2015;52(7):484-492.
70. Ratnapriya R, Zhan X, Fariss RN, et al. Rare and common variants in extracellular matrix gene Fibrillin 2 (FBN2) are associated with macular degeneration. *Hum Mol Genet.* 2014;23(21):5827-5837.
71. Saksens NT, Geerlings MJ, Bakker B, et al. Rare Genetic Variants Associated With Development of Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2016;134(3):287-293.
72. Wagner EK, Raychaudhuri S, Villalonga MB, et al. Mapping rare, deleterious mutations in Factor H: Association with early onset, drusen burden, and lower antigenic levels in familial AMD. *Sci Rep.* 2016;6:31531.
73. Yu Y, Triebwasser MP, Wong EK, et al. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. *Hum Mol Genet.* 2014;23(19):5283-5293.
74. Liszewski MK, Java A, Schramm EC, Atkinson JP. Complement Dysregulation and Disease: Insights from Contemporary Genetics. *Annu Rev Pathol.* 2017;12:25-52.
75. Sohn JH, Kaplan HJ, Suk HJ, Bora PS, Bora NS. Chronic low level complement activation within the eye is controlled by intraocular complement regulatory proteins. *Invest Ophthalmol Vis Sci.* 2000;41(11):3492-3502.
76. Toomey CB, Johnson LV, Rickman CB. Complement factor H in AMD: Bridging genetic associations and pathobiology. *Prog Retin Eye Res.* 2017.
77. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J.* 2000;14(7):835-846.
78. Fritsche LG, Fariss RN, Stambolian D, Abecasis GR, Curcio CA, Swaroop A. Age-related macular degeneration: genetics and biology coming together. *Annu Rev Genomics Hum Genet.* 2014;15:151-171.
79. Scholl HP, Charbel Issa P, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS One.* 2008;3(7):e2593.

80. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. *Invest Ophthalmol Vis Sci.* 2009;50(12):5818-5827.

81. Hecker LA, Edwards AO, Ryu E, et al. Genetic control of the alternative pathway of complement in humans and age-related macular degeneration. *Hum Mol Genet.* 2010;19(1):209-215.

82. Smailhodzic D, Klaver CC, Klevering BJ, et al. Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. *Ophthalmology.* 2012;119(2):339-346.

83. Datta S, Cano M, Ebrahimi K, Wang L, Handa JT. The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD. *Prog Retin Eye Res.* 2017;60:201-218.

84. Khandhadia S, Lotery A. Oxidation and age-related macular degeneration: insights from molecular biology. *Expert Rev Mol Med.* 2010;12:e34.

85. Bertram KM, Baglole CJ, Phipps RP, Libby RT. Molecular regulation of cigarette smoke induced-oxidative stress in human retinal pigment epithelial cells: implications for age-related macular degeneration. *Am J Physiol Cell Physiol.* 2009;297(5):C1200-1210.

86. van Leeuwen R, Boekhoorn S, Vingerling JR, et al. Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA.* 2005;294(24):3101-3107.

87. Gu J, Pauer GJ, Yue X, et al. Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers. *Mol Cell Proteomics.* 2009;8(6):1338-1349.

88. Delcourt C, Carriere I, Delage M, Barberger-Gateau P, Schalch W, Group PS. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. *Invest Ophthalmol Vis Sci.* 2006;47(6):2329-2335.

89. Canter JA, Olson LM, Spencer K, et al. Mitochondrial DNA polymorphism A4917G is independently associated with age-related macular degeneration. *PLoS One.* 2008;3(5):e2091.

90. SanGiovanni JP, Arking DE, Iyengar SK, et al. Mitochondrial DNA variants of respiratory complex I that uniquely characterize haplogroup T2 are associated with increased risk of age-related macular degeneration. *PLoS One.* 2009;4(5):e5508.

91. Jones MM, Manwaring N, Wang JJ, Rochtchina E, Mitchell P, Sue CM. Mitochondrial DNA haplogroups and age-related maculopathy. *Arch Ophthalmol.* 2007;125(9):1235-1240.

92. Esfandiary H, Chakravarthy U, Patterson C, Young I, Hughes AE. Association study of detoxification genes in age related macular degeneration. *Br J Ophthalmol.* 2005;89(4):470-474.

93. Gotoh N, Yamada R, Matsuda F, Yoshimura N, Iida T. Manganese superoxide dismutase gene (SOD2) polymorphism and exudative age-related macular degeneration in the Japanese population. *Am J Ophthalmol.* 2008;146(1):146; author reply 146-147.

94. Ikeda T, Obayashi H, Hasegawa G, et al. Paraoxonase gene polymorphisms and plasma oxidized low-density lipoprotein level as possible risk factors for exudative age-related macular degeneration. *Am J Ophthalmol.* 2001;132(2):191-195.

95. Kimura K, Isashiki Y, Sonoda S, Kakiuchi-Matsumoto T, Ohba N. Genetic association of manganese superoxide dismutase with exudative age-related macular degeneration. *Am J Ophthalmol.* 2000;130(6):769-773.

96. Wang L, Clark ME, Crossman DK, et al. Abundant lipid and protein components of drusen. *PLoS One.* 2010;5(4):e10329.

97. Wang Y, Wang M, Zhang X, et al. The Association between the Lipids Levels in Blood and Risk of Age-Related Macular Degeneration. *Nutrients.* 2016;8(10).

98. Cougnard-Gregoire A, Delyfer MN, Korobelnik JF, et al. Elevated high-density lipoprotein cholesterol and age-related macular degeneration: the Alienor study. *PLoS One.* 2014;9(3):e90973.

99. Fan Q, Maranville JC, Fritsche L, et al. HDL-cholesterol levels and risk of age-related macular degeneration: a multiethnic genetic study using Mendelian randomization. *Int J Epidemiol.* 2017.

100. Johnson LV, Anderson DH. Age-related macular degeneration and the extracellular matrix. *N Engl J Med.* 2004;351(4):320-322.

101. Zhang QY, Tie LJ, Wu SS, et al. Overweight, Obesity, and Risk of Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2016;57(3):1276-1283.

102. McGuinness MB, Le J, Mitchell P, et al. Physical Activity and Age-related Macular Degeneration: A Systematic Literature Review and Meta-analysis. *Am J Ophthalmol.* 2017;180:29-38.

103. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology.* 2008;115(2):334-341.

104. Age-Related Eye Disease Study Research G. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol.* 2001;119(10):1417-1436.

105. Age-Related Eye Disease Study 2 Research G. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA.* 2013;309(19):2005-2015.

106. Ferris FL, 3rd, Fine SL, Hyman L. Age-related macular degeneration and blindness due to neovascular maculopathy. *Arch Ophthalmol.* 1984;102(11):1640-1642.

107. Campbell JP, Bressler SB, Bressler NM. Impact of availability of anti-vascular endothelial growth factor therapy on visual impairment and blindness due to neovascular age-related macular degeneration. *Arch Ophthalmol.* 2012;130(6):794-795.

108. Group CR, Martin DF, Maguire MG, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med.* 2011;364(20):1897-1908.

109. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology.* 2012;119(12):2537-2548.

110. Yaspan BL, Williams DF, Holz FG, et al. Targeting factor D of the alternative complement pathway reduces geographic atrophy progression secondary to age-related macular degeneration. *Sci Transl Med.* 2017;9(395).

111. Holz FG, Sadda SR, Busbee B, et al. Efficacy and Safety of Lampalizumab for Geographic Atrophy Due to Age-Related Macular Degeneration: Chroma and Spectri Phase 3 Randomized Clinical Trials. *JAMA Ophthalmol.* 2018;136(6):666-677.

112. Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, et al. Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. *Ophthalmology.* 2014;121(3):693-701.

113. Wong WT, Dresner S, Forooghian F, et al. Treatment of geographic atrophy with subconjunctival sirolimus: results of a phase I/II clinical trial. *Invest Ophthalmol Vis Sci.* 2013;54(4):2941-2950.

114. Petrou PA, Cunningham D, Shimel K, et al. Intravitreal sirolimus for the treatment of geographic atrophy: results of a phase I/II clinical trial. *Invest Ophthalmol Vis Sci.* 2014;56(1):330-338.

115. Gensler G, Clemons TE, Domalpally A, et al. Treatment of Geographic Atrophy with Intravitreal Sirolimus: The Age-Related Eye Disease Study 2 Ancillary Study. *Ophthalmol Retina.* 2018;2(5):441-450.

116. Landa G, Butovsky O, Shoshani J, Schwartz M, Pollack A. Weekly vaccination with Copaxone (glatiramer acetate) as a potential therapy for dry age-related macular degeneration. *Curr Eye Res.* 2008;33(11):1011-1013.

117. Landa G, Rosen RB, Patel A, et al. Qualitative spectral OCT/SLO analysis of drusen change in dry age-related macular degeneration patients treated with Copaxone. *J Ocul Pharmacol Ther.* 2011;27(1):77-82.

118. Mata NL, Lichter JB, Vogel R, Han Y, Bui TV, Singerman LJ. Investigation of oral fenretinide for treatment of geographic atrophy in age-related macular degeneration. *Retina.* 2013;33(3):498-507.

119. Dugel PU, Novack RL, Csaky KG, Richmond PP, Birch DG, Kubota R. Phase ii, randomized, placebo-controlled, 90-day study of emixustat hydrochloride in geographic atrophy associated with dry age-related macular degeneration. *Retina.* 2015;35(6):1173-1183.

120. Rosenfeld PJ, Dugel PU, Holz FG, et al. Emixustat Hydrochloride for Geographic Atrophy Secondary to Age-Related Macular Degeneration: A Randomized Clinical Trial. *Ophthalmology.* 2018.

121. Wong WT, Kam W, Cunningham D, et al. Treatment of geographic atrophy by the topical administration of OT-551: results of a phase II clinical trial. *Invest Ophthalmol Vis Sci.* 2010;51(12):6131-6139.

122. Zhang K, Hopkins JJ, Heier JS, et al. Ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for treatment of geographic atrophy in age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2011;108(15):6241-6245.

123. Jaffe GJ, Schmitz-Valckenberg S, Boyer D, et al. Randomized Trial to Evaluate Tandospirone in Geographic Atrophy Secondary to Age-Related Macular Degeneration: The GATE Study. *Am J Ophthalmol.* 2015;160(6):1226-1234.

124. Augustin AJ, Diehm C, Grieger F, Bentz J. Alprostadil infusion in patients with dry age related macular degeneration: a randomized controlled clinical trial. *Expert Opin Investig Drugs.* 2013;22(7):803-812.

125. Schwartz SD, Regillo CD, Lam BL, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet.* 2015;385(9967):509-516.

Chapter 2

Clinical studies

Chapter 2.1
Clinical Characteristics of Familial and Sporadic Age-Related Macular Degeneration: Differences and Similarities

Nicole T.M. Saksens
Eveline Kersten
Joannes M.M. Groenewoud
Mark J.J.P. van Grinsven
Johannes P.H. van de Ven
Clara I. Sánchez
Tina Schick
Sascha Fauser
Anneke I. den Hollander
Carel B. Hoyng
Camiel J.F. Boon

Published in: Investigative Ophthalmology & Visual Science. 2014 Oct 9;55(11):7085-92

ABSTRACT

Purpose: We describe the differences and similarities in clinical characteristics and phenotype of familial and sporadic patients with age-related macular degeneration (AMD).

Methods: We evaluated data of 1828 AMD patients and 1715 controls enrolled in the European Genetic Database. All subjects underwent ophthalmologic examination, including visual acuity testing and fundus photography. Images were graded and fundus photographs were used for automatic drusen quantification by a machine learning algorithm. Data on disease characteristics, family history, medical history, and lifestyle habits were obtained by a questionnaire.

Results: The age at first symptoms was significantly lower in AMD patients with a positive family history (68.5 years) than in those with no family history (71.6 years; $P = 1.9 \times 10^{-5}$). Risk factors identified in sporadic and familial subjects were increasing age (odds ratio [OR], 1.08 per year; $P = 3.0 \times 10^{-51}$ and OR, 1.15; $P = 5.3 \times 10^{-36}$, respectively) and smoking (OR, 1.01 per pack year; $P = 1.1 \times 10^{-6}$ and OR, 1.02; $P = 0.005$). Physical activity and daily red meat consumption were significantly associated with AMD in sporadic subjects only (OR, 0.49; $P = 3.7 \times 10^{-10}$ and OR 1.81; $P = 0.001$). With regard to the phenotype, geographic atrophy and cuticular drusen were significantly more prevalent in familial AMD (17.5% and 21.7%, respectively) compared to sporadic AMD (9.8% and 12.1%).

Conclusion: Familial AMD patients become symptomatic at a younger age. The higher prevalence of geographic atrophy and cuticular drusen in the familial AMD cases may be explained by the contribution of additional genetic factors segregating within families.

INTRODUCTION

Age-related macular degeneration (AMD) is a multifactorial retinal disease leading to severe vision loss among the elderly. Advanced age, female sex, smoking, and obesity (body mass index [BMI] >30) are most commonly reported as important demographic and environmental risk factors for the development of AMD.[1-6] In addition, several important genetic variants have been found to be associated with AMD, either as a risk factor or as a protective factor. The strongest associations have been reported for the single-nucleotide polymorphisms (SNPs) in the complement factor H gene *(CFH* Y402H*;* rs1061170*)* and in the age-related maculopathy susceptibility 2 gene *(ARMS2* A69S*;* rs10490924), which strongly increase the risk of developing AMD.[7-11]

Previous studies have demonstrated aggregation of AMD in families.[12,13] A family history of AMD has been reported as a significant risk factor for AMD.[14] Individuals are at a higher risk of developing AMD when a first-degree relative is affected. Moreover, having an affected parent is associated with a higher risk than having an affected sibling.[13,14] Shahid et al. showed an odds ratio (OR) for AMD of 27.8 in people with an affected parent and an OR of 12.0 for people with an affected sibling. Likewise, Luo et al. reported a relative risk for the development of AMD of 5.66 for people with an affected parent, and a relative risk of 2.95 for people with an affected sibling.

A lower age at onset has been reported in familial AMD patients and heritability of AMD subtypes has been suggested.[14,15] Even though environmental and genetic risk factors can cluster in families, the number of affected family members in large densely affected families cannot be fully explained by clustering of known risk factors.[12] Several recent studies have shown that rare, highly penetrant genetic variants can strongly increase the risk of developing AMD in families with AMD, as well as in the AMD population in general.[16-20]

Little is known about clinical differences and similarities between patients with and without a family history for AMD. The purpose of this study is to gain more insight into the clinical and phenotypic characteristics of familial and sporadic AMD patients, and to analyze if there are distinct clinical differences between these subgroups.

PATIENTS AND METHODS

Study Population

The European Genetic Database (EUGENDA, available in the public domain at www.eugenda.org) is a multicenter database for clinical and molecular analysis of AMD founded by the Radboud university medical center (Nijmegen, the Netherlands) and the Department of Ophthalmology of the University Hospital of Cologne (Cologne, Germany). This database

contains data of AMD patients and control individuals, including family history, environmental risk factors and ophthalmologic examination. For this retrospective study we evaluated data of 1828 Caucasian patients with AMD and 1715 Caucasian controls enrolled in EUGENDA of whom family history of AMD, smoking status, BMI, age, and sex data were available.
This study was performed in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethical committees at the Radboud university medical center and the University of Cologne. Written informed consent was obtained from all participants before enrolling in EUGENDA.

Questionnaire, Clinical Evaluation and Grading

Before enrollment in the EUGENDA database, all subjects were interviewed with a detailed questionnaire about disease characteristics (e.g., age at first symptoms), family history, medical history and lifestyle habits, such as smoking status, diet and physical activity. For each subject, BMI was calculated using body height and body weight as reported in the questionnaire. Based on years of smoking and number of cigarettes smoked per day we calculated the number of pack years. Each subject underwent an ophthalmologic examination, including Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity testing, dilated fundus examination and color fundus photography. The best corrected visual acuity (BCVA) was converted to Logarithm of the Minimal Angle of Resolution (logMAR) visual acuity for the purpose of statistical analysis. Two independent certified reading center graders evaluated color fundus photographs of both eyes of all subjects according to the standard protocol of the Cologne Image Reading Center and Laboratory (CIRCL).[21] Digital nonstereoscopic 30° color fundus photographs centered on the fovea were performed with a Topcon TRC 50IX camera (Topcon Corporation, Tokyo, Japan). The diagnosis and grading of AMD was based on a classification and grading scheme as described previously.[22] For all analyses in this study we used the grading of the worst affected eye, and subjects with only one gradable color fundus photograph were excluded. Additionally, in 1184 AMD subjects spectral-domain optical coherence tomography (SD-OCT, Spectralis; Heidelberg Engineering, Heidelberg, Germany) was available and evaluated for the presence of reticular pseudodrusen. In 677 subjects the presence of cuticular drusen was evaluated based on available fluorescein angiography, performed using the Spectralis HRA system (Heidelberg Engineering, Heidelberg, Germany).[23] The SD-OCT volume scans consisting of 19 or 37 parallel OCT B-scans were used for analysis, covering 6x4mm of the macula. For each OCT B-scan, 20 images were averaged using the automated real-time function.[21] Evaluation of the presence of reticular pseudodrusen on SD-OCT and cuticular drusen on fluorescein angiography was done by one senior grader.
Based on diagnosis and family history, the participants in this study were divided into four groups: sporadic AMD, sporadic control, familial AMD and familial control. We classified subjects as familial in case of confirmed or possible AMD in at least one close relative, defined

as a parent, sibling, or child. Sporadic subjects were defined as individuals who did not have a close relative with AMD.

Automatic Drusen Quantification

In addition to the human grading of AMD based on photographs, a machine learning algorithm for computer-aided diagnosis of AMD, was used for detection and quantification of drusen number and area (measured in pixels). This was described previously as accurate in detecting and quantifying drusen number and area on color fundus photographs of patients with nonadvanced AMD and control subjects,[24]. Patients with advanced AMD in the worst affected eye have been excluded for this specific analysis, because the automatic system was not designed to deal with images containing signs of advanced stage AMD. A quality score ranging from 0 to 1 was calculated, with 0 being the worst quality and 1 being the best quality. Only color fundus photographs were selected with a quality score of 0.3 or more, which corresponds to sufficient quality for human grading.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software, version 20.0 (Released 2011; IBM Corp., Armonk, NY, USA). Each potential risk factor for the development of AMD obtained from the questionnaire was included separately in a logistic regression analysis adjusted for age, sex, smoking, and BMI. The ORs were calculated for familial subjects (familial AMD versus familial controls) and sporadic subjects (sporadic AMD versus sporadic controls). Significant differences between ORs for sporadic and familial subjects were identified by interaction analysis using binary logistic regressions. All continuous variables were analyzed using an independent sample t-test or one-way ANOVA. An univariate general linear model was used when continuous variables were analyzed with correction for other variables. Categorical variables were analyzed using a χ^2 test. Differences with a *P* value less than 0.05 were considered statistically significant. Because multiple possible risk factors were analyzed and many tests of significance were performed in our study, Bonferroni correction was performed for the risk and interaction analysis of environmental factors.

RESULTS

Demographic characteristics of the cohort are shown in Table 1. All four groups were comparable for sex, smoking, and BMI. The mean age of the familial subjects was slightly lower than in sporadic subjects (69.6 and 73.0 years, respectively; $P = 4.7 \times 10^{-16}$), mainly due to younger familial control individuals. In 309 subjects who reported in the questionnaire to have a close relative with (possible) AMD, the ophthalmologically examined AMD status

of close relatives was available. To determine the degree of misclassification of subjects into familial or sporadic based on the questionnaire, we compared the family history with these examined data. In 3 of 309 cases where subjects reported in the questionnaire to have at least one close relative with AMD, no family members seemed to be affected upon ophthalmological examination. Therefore, these cases were incorrectly classified as familial. No ophthalmological information was available for relatives of sporadic subjects included in this study.

Familial and Sporadic AMD: Clinical Differences and Similarities

Information about the age at first symptoms was available in 703 AMD patients (469 sporadic subjects, 234 familial subjects, Table 2). The age at first symptoms was significantly lower ($P = 1.9x10^{-5}$) in familial AMD patients (mean, 68.5 years; SD, 9.8) than in sporadic AMD patients (mean, 71.6 years; SD, 8.7) with a mean difference of 3.1 years (95% confidence interval [CI], 1.7-4.5). When subdividing AMD in the presence or absence of the reticular pseudodrusen subtype, the age at first symptoms was also lower in familial AMD patients. In addition, AMD patients with reticular pseudodrusen have a significantly higher age at first symptoms than patients without reticular pseudodrusen, in both sporadic and familial patients ($P = 4.4x10^{-10}$

Table 1. Demographic Characteristics

	Sporadic AMD	Control	Total	Familial AMD	Control	Total
Cases, n	1330	1405	2735	498	310	808
Sex, n(%)						
Male	536 (40.3)	587 (41.8)	1123 (41.1)	181 (36.3)	132 (42.6)	313 (38.7)
Female	794 (59.7)	818 (58.2)	1612 (58.9)	317 (63.7)	178 (57.4)	495 (61.3)
Age, mean±SD*	75.6±9.1	70.5±7.3	73.0±8.6	74.0±8.4	62.6±10.2	69.6±10.7
Smoking, mean±SD†	14.6±20.1	11.6±16.6	13.1±18.4	14.5±17.9	12.2±15.9	13.6±17.2
BMI, n(%)						
<25	600 (45.1)	638 (45.4)	1238 (45.2)	233 (46.8)	139 (44.8)	372 (46.0)
25-30	558 (42.0)	599 (42.6)	1157 (42.3)	208 (41.8)	124 (40.0)	332 (41.1)
>30	172 (12.9)	168 (12.0)	340 (12.4)	57 (11.4)	47 (15.2)	104 (12.9)
Examined family history, n (%)				165(100)	144(100)	309 (100)
Familial				164(99.4)	142(98.6)	306 (99.0)
Sporadic				1(0.6)	2(1.4)	3 (1.0)

The study included 498 familial AMD patients deriving from 393 families (393 probands and 105 family members) and 310 controls deriving from 216 families (216 probands and 94 family members). In 309 familial cases information about ophthalmologic examination in their close relatives (parents, sibling, and/or children) was available and was compared to family history based on the questionnaire to determine the degree of misclassification. Familial, positive family history for AMD (confirmed or possible AMD in at least one close relative [parent, sibling or child]); sporadic, no positive family history.

* Age of participation in years

† Smoking in pack years

Table 2. Clinical Features and Staging of Sporadic and Familial AMD patients

	Sporadic AMD mean±SD	**Familial AMD** mean±SD	*P*
Age at first symptoms, y			
*AMD, total**	71.6±8.7	68.5±9.8	$1.9x10^{-5}$
AMD, reticular pseudodrusen†	76.2±7.2	72.5±7.3	0.008
AMD, cuticular drusen‡	65.8±9.2	64.5±17.7	0.740
Visual acuity per age category§			
<70	0.12±0.27	0.09±0.25	0.293
70-80	0.19±0.29	0.26±0.36	0.107
>80	0.40±0.40	0.38±0.46	0.814
Visual acuity per stage§			
Nonadvanced	0.05±0.14	0.03±0.13	0.367
Advanced	0.33±0.37	0.37±0.42	0.314
Grading, n(%)			
Early	301 (22.6)	94 (18.9)	
Intermediate	250 (18.8)	90 (18.1)	0.158
Advanced	779 (58.6)	314 (63.1)	
GA	76 (9.8)	55 (17.5)	
CNV	660 (84.7)	234 (74.5)	$3.0x10^{-4}$
Mixed	43 (5.5)	25 (8.0)	

* Data on age of first symptoms were available in 469 sporadic and 234 familial AMD patients
† Data on age of first symptoms were available in 75 sporadic and 45 familial AMD patients with reticular pseudodrusen
‡ Data on age of first symptoms were available in 32 sporadic and 19 familial AMD patients with cuticular drusen
§ Visual acuity defined as best-corrected logMAR visual acuity

and $P = 0.002$, respectively). In contrast, in AMD patients with the cuticular drusen subtype, the age at first symptoms was lower than in patients without cuticular drusen, in sporadic and familial patients ($P = 1.26x10^{-6}$ and $P = 0.074$, respectively). However, no significant difference in age at first symptoms was observed between familial and sporadic patients with the cuticular drusen subtype ($P = 0.740$).

Despite a younger age at first symptoms, BCVA of both eyes did not differ significantly between familial and sporadic AMD patients when subdivided in three age categories (data shown in Table 2). Also, if young patients (<60 years) were analyzed separately, no difference in BCVA was observed between familial and sporadic patients (data not shown). After distinguishing between advanced and nonadvanced AMD subjects, BCVA also was comparable between sporadic and familial patients.

To identify risk factors in our cohort, we analyzed several demographic and environmental factors (Table 3). Risk factors identified in sporadic and familial AMD patients were increasing

age ($P = 3.0x10^{-51}$ and $P = 5.3x10^{-36}$) and smoking ($P = 1.1x10^{-6}$ and $P = 0.005$). Interaction analysis showed a significant difference between sporadic and familial subjects for increasing age ($P = 9.4x10^{-7}$).

In terms of comorbidity (Table 3), allergy was significantly associated with a decreased risk of AMD for sporadic and familial subjects ($P = 0.002$ and $P = 0.024$, respectively). Diabetes mellitus was a risk factor for the development of AMD in sporadic patients (OR, 1.34; 95% CI, 1.02-1.75; $P = 0.035$), but not in familial cases ($P = 0.704$). No significant interaction between family history and allergy or diabetes was present. Cardiovascular disease, renal disease, and auto-immune disease were no significant risk factors for AMD in our cohort.

With regard to dietary factors, we observed that eating red meat a few times per week or daily is a significant risk factor in sporadic AMD patients (OR, 1.24; 95% CI, 1.05-1.48; $P = 0.013$ and OR, 1.81; 95% CI, 1.30-2.54; $P = 0.001$, respectively), but not in familial subjects. A protective factor for AMD in sporadic patients was eating fruit a few times per week (OR, 0.60; 95% CI, 0.40-0.90; $P = 0.013$). Intake of fruit every day did not seem to further decrease the risk of AMD (OR, 0.74; 95% CI, 0.52-1.04; $P = 0.085$). However, consumption of fruit was not significantly associated with a decreased risk of AMD in familial subjects.

Regular physical activity, one or two times a week, was significantly associated with a decreased risk for AMD in the sporadic AMD subgroup (OR, 0.67; 95% CI, 0.56-0.81; $P = 2.6x10^{-6}$) and the familial AMD subgroup (OR, 0.62; 95% CI, 0.42-0.94; $P = 0.022$).

After Bonferroni correction of the demographic and environmental risk factors for AMD, the association of increasing age with AMD in familial and sporadic subjects remained significant, as well as the association of female sex, smoking, allergy, daily red meat consumption and physical activity in sporadic patients only.

Familial and Sporadic AMD: Phenotypic Differences and Dimilarities

The prevalence of early, intermediate, and advanced stage AMD was similar in the familial and sporadic AMD patient group (Table 2). After differentiation of advanced AMD into geographic atrophy (GA), choroidal neovascularization (CNV) and mixed (GA and CNV in one patient), we found that GA was more prevalent in familial AMD patients (17.5%) than in sporadic AMD patients (9.8%; $P = 3.0x10^{-4}$), despite a comparable SNP load of *CFH* Y402H and *ARMS2* A69S between familial and sporadic patients with GA (data not shown). In 829 sporadic subjects and 355 familial subjects data on reticular pseudodrusen were available, and data on cuticular drusen were available in 520 sporadic subjects and 157 familial subjects. The prevalence of reticular pseudodrusen was comparable between familial (18.0%) and sporadic subjects (18.8%; $P = 0.749$), whereas the prevalence of cuticular drusen was significantly higher in familial AMD (21.7%) compared to sporadic AMD (12.1%; $P = 0.003$).

Table 3. Risk and Interaction Analysis for Demographic and Environmental Factors in Sporadic and Familial Subjects

	Sporadic, n(%)		Sporadic AMD vs. control		Familial, n(%)		Familial AMD vs. control		Familial AMD vs. sporadic AMD
	AMD	**Control**	OR (95% CI)*	*P*	**AMD**	**Control**	OR (95% CI)*	*P*	*P*
Age †	1330 (100)	1405 (100)	1.08 (1.07-1.09)	$3.0x10^{-51}$#	498 (100)	310 (100)	1.15 (1.13-1.18)	$5.3x10^{-36}$#	$9.4x10^{-7}$#
Sex									
Male	536 (40.3)	587 (41.8)	reference		181 (36.3)	132 (42.6)	reference		
Female	794 (59.7)	818 (58.2)	1.27 (1.07-1.51)	0.007	317 (63.7)	178 (57.4)	1.33 (0.93-1.91)	0.120	0.811
Smoking ‡	1330 (100)	1405 (100)	1.01 (1.01-1.02)	$1.1x10^{-6}$#	498 (100)	310 (100)	1.02 (1.01-1.03)	0.005	0.914
BMI									
<25	600 (45.1)	638 (45.4)	reference		233 (46.8)	139 (44.8)	reference		
25-30	558 (42.0)	599 (42.6)	1.04 (0.88-1.24)	0.635	208 (41.8)	124 (40.0)	0.80 (0.56-1.16)	0.244	0.314
>30	172 (12.9)	168 (12.0)	1.21 (0.94-1.56)	0.149	57 (11.4)	47 (15.2)	0.70 (0.41-1.17)	0.172	0.051
Comorbidity									
Cardiovascular disease§	519 (43.0)	545 (42.2)	0.84 (0.71-1.00)	0.053	200 (41.7)	83 (27.9)	1.31 (0.90-1.90)	0.163	0.019
Diabetes	157 (11.8)	113 (8.0)	1.34 (1.02-1.75)	0.035	41 (8.3)	17 (5.5)	0.88 (0.46-1.70)	0.704	0.365
Renal disease	72 (5.4)	60 (4.3)	1.15 (0.79-1.66)	0.480	28 (5.7)	11 (3.5)	0.98 (0.43-2.24)	0.959	0.954
Autoimmune disease II	101 (7.6)	93 (6.6)	1.11 (0.81-1.51)	0.520	54 (10.9)	16 (5.2)	1.82 (0.91-3.64)	0.091	0.150
Allergy	239 (18.0)	371 (26.4)	0.74 (0.61-0.89)	0.002#	88 (17.8)	92 (29.7)	0.63 (0.43-0.94)	0.024	0.294
Diet									
Use of butter/oil									
Butter/margarine	101 (15.8)	105 (14.3)	reference		53 (17.6)	30 (13.5)	reference		
Low-fat margarine	58 (9.1)	45 (6.1)	1.40 (0.84-2.33)	0.195	49 (16.3)	50 (22.5)	0.92 (0.44-1.95)	0.836	0.225
Vegetable oil	255 (40.0)	411 (56.1)	0.77 (0.55-1.08)	0.127	78 (25.9)	84 (37.8)	0.74 (0.38-1.44)	0.373	0.795
Other	226 (35.3)	172 (23.5)	1.34 (0.94-1.93)	0.110	121 (40.2)	58 (26.1)	1.35 (0.69-2.66)	0.384	0.999
Fish									
Once a week or less	871 (73.9)	1015 (74.2)	reference		357 (77.4)	244 (78.7)	reference		
Few times a week	300 (25.4)	346 (25.3)	0.99 (0.82-1.20)	0.941	103 (22.3)	65 (21.0)	0.84 (0.56-1.27)	0.412	0.765
Every day	8 (0.7)	7 (0.5)	0.80 (0.27-2.38)	0.684	1 (0.2)	1 (0.3)	0.26 (0.01-10.37)	0.470	0.677

	Sporadic, n(%)		Sporadic AMD vs. control		Familial, n(%)		Familial AMD vs. control		Familial AMD vs. sporadic AMD
	AMD	**Control**	OR (95% CI)*	*P*	**AMD**	**Control**	OR (95% CI)*	*P*	*P*
Red meat									
Once a week or less	434 (36.7)	602 (44.3)	reference		118 (25.7)	75 (24.3)	reference		
Few times a week	638 (53.9)	680 (50.1)	1.24 (1.05-1.48)	0.013	273 (59.3)	188 (60.8)	0.92 (0.62-1.39)	0.702	0.142
Every day	112 (9.5)	76 (5.6)	1.81 (1.30-2.54)	0.001#	69 (15.0)	46 (14.9)	1.16 (0.66-2.05)	0.605	0.074
Fruit									
Once a week or less	84 (7.1)	75 (5.5)	reference		36 (7.8)	26 (8.4)	reference		
Few times a week	138 (11.7)	207 (15.1)	0.60 (0.40-0.90)	0.013	73 (15.8)	70 (22.6)	0.85 (0.40-1.79)	0.661	0.370
Every day	962 (81.3)	1093 (79.5)	0.74 (0.52-1.04)	0.085	352 (76.4)	214 (69.0)	0.73 (0.37-1.44)	0.361	0.542
Vegetables									
Not every day	189 (16.0)	244 (17.7)	reference		61 (13.3)	54 (17.4)	reference		
Every day	995 (84.0)	1131 (82.3)	1.11 (0.89-1.39)	0.348	399 (86.7)	256 (82.6)	0.92 (0.56-1.52)	0.741	0.922
Physical activity									
(Almost) never	566 (44.1)	377 (27.2)	reference		177 (37.0)	84 (27.2)	reference		
1-2 times a week	509 (32.1)	629 (45.4)	0.67 (0.56-0.81)	$2.6x10^{-6}$#	227 (47.5)	174 (56.3)	0.62 (0.42-0.94)	0.022	0.942
3 times a week or more	209 (16.3)	378 (27.3)	0.49 (0.39-0.61)	$3.7x10^{-10}$#	74 (15.5)	51 (16.5)	0.75 (0.44-1.27)	0.281	0.087

* The ORs were adjusted for age, sex, smoking, and BMI.

† Age of participation in years.

‡ Smoking in pack years.

§Cardiovascular disease defined as presence or history of hypertension, angina pectoris, myocardial infarction, congestive heart failure and/or stroke/transient ischemic attack.

II Autoimmune disease defined as presence or history of rheumatoid arthritis or systemic lupus erythematosus.

Significant after Bonferroni correction

Data on the number of drusen and area of drusen within the macular area were available for 689 sporadic subjects and 203 familial subjects (Table 4). Familial subjects showed a trend toward a higher number of drusen and a larger area of drusen in the macula as compared to sporadic patients, although this was only significant for the area of drusen in subjects with intermediate AMD (P = 0.043). After correction for age, sex, BMI, and smoking, the mean area of drusen in sporadic subjects with intermediate AMD was 1114.49 and 1415.63 in familial subjects, which were no longer significantly different (P = 0.160).

Table 4. Number and Area of Drusen in Sporadic and Familial Control Individuals and Nonadvanced AMD Patients

		Sporadic		Familial		
		n	Mean±SD	n	Mean±SD	*P*
Control	Drusen, n	159	2.25±6.0	24	2.85±3.4	0.633
	Area of drusen*	159	81.65±149.6	24	128.86±129.5	0.145
Early	Drusen, n	291	8.69±20.0	93	11.69±27.37	0.254
	Area of drusen*	291	219.17±353.4	93	249.67±416.6	0.489
Intermediate	Drusen, n	239	40.25±62.4	86	46.37±48.9	0.411
	Area of drusen*	239	1167.90±1735.4	86	1598.70±1555.4	0.043

* Area of drusen in pixels

DISCUSSION

Familial and Sporadic AMD: Clinical Differences and Similarities

Familial AMD patients have a lower age at first symptoms compared to sporadic AMD patients. The phenomenon of a lower age at onset in patients with familial occurrence has been shown in other complex diseases with a significant genetic component, such as schizophrenia and Alzheimer's disease.[25,26] A lower age at onset in familial AMD patients has previously been reported by Shahid et al.[14] (70.4 years in familial patients and 73.2 years in sporadic patients), and is in accordance with the mean difference of 3.1 years in our study. A significant difference in age of onset between familial and sporadic subjects also was observed in AMD patients with reticular pseudodrusen, but not in patients with cuticular drusen. However, as a result of a positive family history, familial subjects may have an increased awareness of visual symptoms which can lead to an earlier visit at a physician for evaluation. Therefore, it should be noted that the lower age at first symptoms in familial AMD patients may be partially attributed to an ascertainment bias.

In our study, BCVA per age category did not differ between familial and sporadic AMD patients, suggesting that visual acuity does not decrease earlier or at a faster rate in familial patients, despite the lower age at first symptoms in familial AMD patients. Heightened awareness in familial patients may explain why no actual difference in BCVA was observed.

Similar to other studies,[1-3,6] smoking and advanced age were associated with the development of AMD in the current study, in sporadic and familial subjects. Furthermore, age was a more important risk factor for AMD in familial subjects as age shows a significant interaction with family history, resulting in a younger age at onset in familial subjects.

Ristau et al.[22] have reported recently that allergy is associated with a reduced risk of AMD. We did not find a significant difference for the association of allergy with AMD between familial and sporadic subjects, so the protective effect of allergy does not seem to be influenced by family history.

The pathogenesis of AMD as well as cardiovascular disease and diabetes have been linked to oxidative stress, inflammation and a vascular origin. Moreover, diabetes, cardiovascular disease, and its risk factors have been associated with the development of AMD, although this was not consistent among studies.[1,27-30] In the current study we observed that sporadic subjects with diabetes have an increased risk for AMD. However, diabetes was no risk factor for familial AMD, which may be explained by the larger genetic component in the pathophysiology of familial AMD, while sporadic AMD may be associated with a larger contribution of environmental or lifestyle factors such as diabetes (and associated factors).

Several studies reported an increased risk of AMD for patients with chronic renal disease.[31,32] It has been shown that AMD and renal diseases, such as membranoproliferative glomerulonephritis type 2 and atypical hemolytic uremic syndrome, are related to the same genetic variants of the complement pathway, including the complement factor H gene.[33-35] Therefore, we evaluated whether renal disease might be correlated with AMD in familial subjects. However, we did not find a clear association between familial AMD and renal disease in our study population, possibly due to the low number of patients with renal disease.

We also compared dietary factors between familial and sporadic subjects. It is interesting to investigate these dietary factors, since these are modifiable. The consumption of red meat at least a few times a week increased the risk of AMD in our sporadic patient cohort. This is supported by findings of Chong et al.,[36] demonstrating that higher red meat intake was associated with the development of AMD. In our study consumption of fruit a few times a week was associated with a decreased risk for the development of AMD, but we did not observe such an association with frequent consumption of vegetables. In agreement with this finding, Cho et al.[37] and Zerbib et al.[38] described a protective effect of frequent consumption of fruits for exudative AMD, but no association with the consumption of vegetables. A study by Seddon et al.[39] showed that intake of foods rich in carotenoids, in particular green leafy vegetables, decreased the risk of exudative AMD. Our study might be limited because we did

not discriminate between different kinds of vegetables and no risk calculation was performed for the progression to advanced AMD. This may explain why we did not observe a protective effect for the consumption of vegetables. Also, it must be considered that fruit consumption can be related to a more healthy lifestyle in general, and therefore, these results may be confounded by other factors, other than smoking and BMI, related to a healthy lifestyle.
In cuticular drusen, a clinical subtype of AMD that tends to cluster in families, differences in environmental and genetic risk factors have been reported compared to the AMD group as a whole.[23,40] We previously reported that the association with smoking was significantly lower in patients with cuticular drusen compared to AMD patients without cuticular drusen.[23] In this study, we observed no significant difference in environmental risk factors, such as smoking, between familial and sporadic AMD. However, several factors such as the consumption of red meat and frequent physical activity, tended to have a less important role in familial AMD than in sporadic AMD, supporting a stronger genetic component in the pathophysiology of AMD in families.

Familial and sporadic AMD: phenotypic differences and similarities

In our study population, GA was more prevalent in familial AMD patients than in sporadic patients. This cannot be explained by selection bias of familial patients, because if only the probands of the familial group (n = 262) were included in the analysis, GA was still significantly more prevalent in the familial AMD group than in the sporadic group (17.6% and 9.8%, respectively, $P = 0.001$). This finding may be explained by the stronger influence of genetic factors in the pathogenesis of familial AMD in certain phenotypic subtypes, such as GA. Previously Shahid et al.[14] suggested heritability of AMD subtypes, but these investigators were not able to confirm this hypothesis due to low numbers of subjects. Sobrin et al.[15] showed that siblings are more likely to develop the same advanced subtype as their proband. This may suggest the contribution of genetic variants in these familial patients,[16,35,41] which may increase the risk for developing GA rather than CNV. Affected members of densely affected families have been reported to bear a lower SNP load than expected based on five common known AMD risk variants; *CFH* (rs1061170 and rs1410996)*, ARMS2* (rs10490924), *C2-CFB* (rs641153 and rs9332739).[10] This supports the hypothesis that rare genetic variants in these families may explain the high prevalence of AMD.
In this study, we reported a higher prevalence of cuticular drusen in familial AMD compared to sporadic AMD, which is in agreement with previous reports.[40,42,43] However, in our cohort the prevalence of cuticular drusen was higher and 32% of the patients with cuticular drusen had a positive family history of AMD compared to 44% of the patients in a study of Grassi et al.[40] Previously, our group demonstrated that heterozygous loss-of-function mutations in the *CFH* gene are found among family members with cuticular drusen.[35,41] In addition, rare highly penetrant variants in the *CFI* gene have been identified in patients with cuticular

drusen.[16,44] Therefore, the higher prevalence of cuticular drusen in families may be explained by segregation of rare, highly penetrant variants within these families.

In addition to a possible ascertainment bias, caused by an increased awareness of disease-associated visual symptoms in familial subjects, another limitation of this study is the classification of subjects into familial or sporadic, based on the family history in the questionnaire, which may lead to misclassification. However, in a subset of the familial cases ophthalmologic examination data of their close relatives were available and compared to our classification based on the family history of the questionnaire. The degree of misclassification of the family history was very low in the familial subjects, as in only three cases (1.0%) no close relative with AMD was found by ophthalmologic examination. Unfortunately, no clinical data of family members of sporadic cases were available. The rate of misclassification may be some higher in sporadic individuals, as these subjects may not have been informed of the eye disease of close relatives or the relatives were asymptomatic and therefore, not yet diagnosed with AMD.

In conclusion, this study demonstrated that familial AMD patients have a lower age at first symptoms compared to sporadic patients. Our findings also indicate that familial AMD patients differ from sporadic patients in terms of risk factors and clinical features. The higher prevalence of GA and cuticular drusen in familial AMD patients may be explained by the contribution of additional genetic factors segregating within these families.

REFERENCES

1. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. BMC Ophthalmol. 2010;10:31.
2. Age-Related Eye Disease Study Research G. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. Ophthalmology. 2000;107(12):2224-2232.
3. Evans JR. Risk factors for age-related macular degeneration. Prog Retin Eye Res. 2001;20(2):227-253.
4. Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL, 3rd, Age-Related Eye Disease Study Research G. Risk factors for the incidence of Advanced Age-Related Macular Degeneration in the Age-Related Eye Disease Study (AREDS) AREDS report no. 19. Ophthalmology. 2005;112(4):533-539.
5. Seddon JM, Cote J, Davis N, Rosner B. Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio. Arch Ophthalmol. 2003;121(6):785-792.
6. Erke MG, Bertelsen G, Peto T, Sjolie AK, Lindekleiv H, Njolstad I. Cardiovascular risk factors associated with age-related macular degeneration: the Tromso Study. Acta Ophthalmol. 2014.
7. Edwards AO, Ritter R, 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005;308(5720):421-424.
8. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005;308(5720):385-389.
9. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet. 2005;14(21):3227-3236.
10. Sobrin L, Maller JB, Neale BM, et al. Genetic profile for five common variants associated with age-related macular degeneration in densely affected families: a novel analytic approach. Eur J Hum Genet. 2010;18(4):496-501.
11. Shastry BS. Assessment of the contribution of the LOC387715 gene polymorphism in a family with exudative age-related macular degeneration and heterozygous CFH variant (Y402H). J Hum Genet. 2007;52(4):384-387.
12. Klaver CC, Wolfs RC, Assink JJ, van Duijn CM, Hofman A, de Jong PT. Genetic risk of age-related maculopathy. Population-based familial aggregation study. Arch Ophthalmol. 1998;116(12):1646-1651.
13. Luo L, Harmon J, Yang X, et al. Familial aggregation of age-related macular degeneration in the Utah population. Vision Res. 2008;48(3):494-500.
14. Shahid H, Khan JC, Cipriani V, et al. Age-related macular degeneration: the importance of family history as a risk factor. Br J Ophthalmol. 2012;96(3):427-431.
15. Sobrin L, Ripke S, Yu Y, et al. Heritability and genome-wide association study to assess genetic differences between advanced age-related macular degeneration subtypes. Ophthalmology. 2012;119(9):1874-1885.
16. van de Ven JP, Nilsson SC, Tan PL, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. Nat Genet. 2013;45(7):813-817.
17. Helgason H, Sulem P, Duvvari MR, et al. A rare nonsynonymous sequence variant in C3 is associated with high risk of age-related macular degeneration. Nature Genetics. 2013;45(11):1371-1374.
18. Raychaudhuri S, Iartchouk O, Chin K, et al. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. Nature Genetics. 2011;43(12):1232-1236.

19. Seddon JM, Yu Y, Miller EC, et al. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. Nature Genetics. 2013;45(11):1366-1370.
20. Zhan X, Larson DE, Wang C, et al. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. Nature Genetics. 2013;45(11):1375-1379.
21. Mokwa NF, Ristau T, Keane PA, Kirchhof B, Sadda SR, Liakopoulos S. Grading of Age-Related Macular Degeneration: Comparison between Color Fundus Photography, Fluorescein Angiography, and Spectral Domain Optical Coherence Tomography. Journal of Ophthalmology. 2013;2013:385915.
22. Ristau T, Ersoy L, Lechanteur Y, et al. Allergy is a protective factor against age-related macular degeneration. Invest Ophthalmol Vis Sci. 2014;55(1):210-214.
23. van de Ven JP, Smailhodzic D, Boon CJ, et al. Association analysis of genetic and environmental risk factors in the cuticular drusen subtype of age-related macular degeneration. Mol Vis. 2012;18:2271-2278.
24. van Grinsven MJ, Lechanteur YT, van de Ven JP, et al. Automatic drusen quantification and risk assessment of age-related macular degeneration on color fundus images. Invest Ophthalmol Vis Sci. 2013;54(4):3019-3027.
25. Suvisaari JM, Haukka J, Tanskanen A, Lonnqvist JK. Age at onset and outcome in schizophrenia are related to the degree of familial loading. Br J Psychiatry. 1998;173:494-500.
26. Duara R, Lopez-Alberola RF, Barker WW, et al. A comparison of familial and sporadic Alzheimer's disease. Neurology. 1993;43(7):1377-1384.
27. Tan JS, Mitchell P, Smith W, Wang JJ. Cardiovascular risk factors and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. Ophthalmology. 2007;114(6):1143-1150.
28. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam eye study. Ophthalmology. 2003;110(4):636-643.
29. Mitchell P, Wang JJ. Diabetes, fasting blood glucose and age-related maculopathy: The Blue Mountains Eye Study. Aust N Z J Ophthalmol. 1999;27(3-4):197-199.
30. Klein R, Klein BE, Moss SE. Diabetes, hyperglycemia, and age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology. 1992;99(10):1527-1534.
31. Liew G, Mitchell P, Wong TY, Iyengar SK, Wang JJ. CKD increases the risk of age-related macular degeneration. J Am Soc Nephrol. 2008;19(4):806-811.
32. Klein R, Knudtson MD, Lee KE, Klein BE. Serum cystatin C level, kidney disease markers, and incidence of age-related macular degeneration: the Beaver Dam Eye Study. Arch Ophthalmol. 2009;127(2):193-199.
33. Nitsch D, Douglas I, Smeeth L, Fletcher A. Age-related macular degeneration and complement activation-related diseases: a population-based case-control study. Ophthalmology. 2008;115(11):1904-1910.
34. Boon CJ, van de Kar NC, Klevering BJ, et al. The spectrum of phenotypes caused by variants in the CFH gene. Mol Immunol. 2009;46(8-9):1573-1594.
35. van de Ven JP, Boon CJ, Fauser S, et al. Clinical evaluation of 3 families with basal laminar drusen caused by novel mutations in the complement factor H gene. Arch Ophthalmol. 2012;130(8):1038-1047.
36. Chong EW, Simpson JA, Robman LD, et al. Red meat and chicken consumption and its association with age-related macular degeneration. Am J Epidemiol. 2009;169(7):867-876.
37. Cho E, Seddon JM, Rosner B, Willett WC, Hankinson SE. Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. Arch Ophthalmol. 2004;122(6):883-892.

38. Zerbib J, Delcourt C, Puche N, et al. Risk factors for exudative age-related macular degeneration in a large French case-control study. Graefes Arch Clin Exp Ophthalmol. 2013.

39. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA. 1994;272(18):1413-1420.

40. Grassi MA, Folk JC, Scheetz TE, Taylor CM, Sheffield VC, Stone EM. Complement factor H polymorphism p.Tyr402His and cuticular Drusen. Archives of Ophthalmology. 2007;125(1):93-97.

41. Boon CJ, Klevering BJ, Hoyng CB, et al. Basal laminar drusen caused by compound heterozygous variants in the CFH gene. Am J Hum Genet. 2008;82(2):516-523.

42. Lerche W. [Pigment epithelium changes in drusen of the macular region]. Bericht Uber Die Zusammenkunft. Deutsche Ophthalmologische Gesellschaft. 1975(73):439-446.

43. Barbazetto IA, Yannuzzi NA, Klais CM, et al. Pseudo-vitelliform macular detachment and cuticular drusen: exclusion of 6 candidate genes. Ophthalmic Genetics. 2007;28(4):192-197.

44. Boon CJ, van de Ven JP, Hoyng CB, den Hollander AI, Klevering BJ. Cuticular drusen: stars in the sky. Progress in Retinal and Eye Research. 2013;37:90-113.

Chapter 2.2
Burden of common genetic and environmental risk factors in families affected with age-related macular degeneration

Eveline Kersten*
Yara T.E. Lechanteur*
Nicole T.M. Saksens
Maartje J. Geerlings
Tina Schick
Sascha Fauser
Eiko K. de Jong
Anneke I. den Hollander
Caroline C.W. Klaver
Carel B. Hoyng

*These authors contributed equally to this study

Manuscript submitted

ABSTRACT

Importance: Many prediction models for age-related macular degeneration (AMD) have been established in non-familial AMD patients. However, individuals most interested in their risk of AMD are usually from densely affected AMD families. The question arises if these common risk factors can explain the aggregation of AMD in these families.

Objective: To evaluate which proportion of AMD families cannot be explained by known environmental and common genetic risk factors.

Design: Retrospective case-control and family study.

Setting: Case-control and family datasets were extracted from the European Genetic Database (EUGENDA). Patient recruitment took place at the Ophthalmology department of the Radboud university medical center, Nijmegen, and University Hospital of Cologne from March 29, 2006, to April 26, 2013, and data collection from April 20, 2012, to July 27, 2017.

Participants: The case-control dataset (1188 cases, 1468 controls) was used for creation of a prediction model based on known common risk factors. Then, the model was applied to 38 affected families. Based on the predicted probabilities within each family, the observed number of affected family members was compared with the expected number of affected family members. Thereafter, phenotypic differences were evaluated between families explained by common risk factors and families not explained by these common risk factors.

Main outcome measures: Proportion of families not explained by common risk factors.

Results: In most families (68.4%), the number of affected individuals was similar to expected based on the predicted probabilities within each family. Contrarily, we identified two families with significantly more affected family members than expected. A higher prevalence of hyperpigmentation was observed in families explained by common risk factors compared to families not explained by common risk factors (61.3% vs. 38.9%).

Conclusions and relevance: Despite clustering of common risk factors in two-thirds of AMD families, a relatively large proportion of families is not readily explained by these risk factors and may harbor rare genetic variants. These families cannot be identified solely by phenotype. Individual risk prediction in AMD families remains therefore challenging and evaluation of multiple family members is recommended.

INTRODUCTION

Age-related macular degeneration (AMD) is a multifactorial, progressive retinal disorder resulting in severe visual impairment. Advanced age is one of the most important risk factors for AMD,[1,2] resulting in a rapidly growing burden of disease due to the aging population.[3-5] Numerous demographic, environmental and genetic risk factors have already been identified and many prediction models have been developed based on these common risk factors.[6-12] Prediction models can aid in the identification of individuals with a high risk for disease, enabling personalized healthcare and early detection of disease. In clinical practice, particularly individuals with affected family members are interested in their individual risk of AMD, especially when more than one family member is affected. However, existing prediction models were mainly constructed in studies representing the general population and are based on environmental and common genetic risk factors in non-familial AMD patients. Aggregation of AMD in families has been reported,[13-15] and not all of these AMD families can be fully explained by these common genetic risk factors.[16,17] Moreover, rare genetic variants with a high risk for disease have been identified in such AMD families.[18-20] Additionally, it has been reported that a common variant in the *ARMS2* gene (p.Ala69Ser, rs10490924) associated with AMD in non-familial patients is not associated with AMD in densely affected families.[21] These findings raise the question if the current models, based on environmental and common genetic risk factors, can be applied to individuals deriving from families affected with AMD. Therefore, the aim of this study was to evaluate the accuracy of prediction model based on environmental and common genetic risk factors in an AMD family cohort and to determine the proportion of families that cannot be explained by these known risk factors.

METHODS

Participants

For this study, an independent case-control dataset and a family dataset were extracted from the European Genetic Database (EUGENDA, www.eugenda.org), a large multicenter database for clinical and molecular analysis of AMD. All individuals provided written informed consent for data entry in EUGENDA. This research was approved by the local ethical committees at the Radboud university medical center and the University Hospital of Cologne and the study was performed in accordance with the tenets of the Declaration of Helsinki.

The case-control dataset was used to create a model and included 1188 cases and 1468 control individuals recruited at the department of Ophthalmology at the Radboud university medical center, Nijmegen and the Ophthalmology department of the University Hospital of Cologne. All individuals in this case-control dataset were unrelated. Additionally, a family

dataset was extracted from EUGENDA including 43 AMD families (125 cases, 39 control individuals). These AMD families had at least two first-degree relatives with AMD confirmed by color fundus photography. Final analyses included 38 AMD families; five families were excluded because data of one or two family members was incomplete.

Cases were defined as having at least 15 soft medium drusen (diameter ≥ 63µm), or advanced AMD (central geographic atrophy [GA] or choroidal neovascularization [CNV] secondary to AMD) in at least one eye. Control individuals were aged 65 years and above and did not exhibit any signs of AMD, or did not meet the criteria above (small hard drusen only, or less than 15 soft medium drusen). The diagnosis and grading of AMD was based on color fundus photographs of the more severely affected eye and was performed by independent certified reading center graders as described previously.[22] To obtain more detailed phenotypic characteristics of the AMD families, additional phenotyping was performed by an independent certified grader according the Rotterdam grading protocol based on the Wisconsin Age-Related Maculopathy Grading System[23] and the modified International Classification System.[24]

Information on lifestyle and other environmental factors was obtained through detailed interviewer-assisted questionnaires. Variables included in this study were: age at participation, sex (male/female), smoking status (never/past/current), body mass index (BMI) calculated as weight in kilograms divided by height in meters squared (normal [<25kg/m^2]/overweight [25-30 kg/m^2]/obese [>30 kg/m^2]), physical activity (no or almost never/1-2 times per week/3 or more times per week), education level (high school or less/more than high school) and family history of AMD (yes/no).

Single nucleotide polymorphism genotyping

Genomic DNA was isolated from venous blood leukocytes and genotyping was performed for susceptibility single nucleotide polymorphisms (SNPs) in the following AMD-associated genes: *ADAMTS9* (rs6795735),[25] *APOE* (rs2075650,[26] rs4420638[25]), *ARMS2* (rs10490924),[27] *B3GALTL* (rs9542236),[25] *C3* (rs433594,[28] rs2230199[29,30]), *CCDC109B* (rs4698775),[25] *CETP* (rs3764261),[31,32] *CFB* (rs4151667,[33] rs641153[33]), *CFH* (rs800292,[34] rs1061170,[35-37] rs12144939[38]), *CFI* (rs10033900),[39] *COL8A1* (rs13081855),[25] *COL10A1* (rs3812111),[25] *CYP24A1* (rs1570669),[40] *FADS1* (rs174547),[32] *GLI2* (rs6721654),[26] *GLI3* (rs2049622),[26] IER3DDR (rs3130783),[25] *IGFR1* (rs2872060),[41] *LIPC* (rs10468017),[31,32] *LPL* (rs12678919),[31,32] *MYRIP* (rs2679798),[42] *PON1* (rs705381),[43] *RAD51B* (rs8017304),[25] *SKIV2L* (rs429608),[42] *SLC16A8* (rs8135665),[25] *TGFBR1* (rs334353),[25] *TIMP3* (rs9621532),[31] *TNFRSF10A* (rs13278062),[25] *TYR* (rs621313),[26] and *VEGFA* (rs943080).[44] SNPs were genotyped using competitive allele-specific PCR assays (KASP SNP Genotyping System, LGC).

Statistical analyses

Data for all variables included in a prediction model are necessary in order to properly apply the model in a specific cohort. Due to one or more missing variables in our family dataset for existing prediction models for AMD, [6-8,11,12] these could not be applied. For the purpose of this study, first a prediction model similar to existing models was created based on the available case-control data in EUGENDA.

This prediction model for the development of AMD was constructed using binary logistic regression analyses. Univariable logistic regression analyses were performed for five non-genetic and 35 genetic variables to study associations between each single risk factor and AMD. Subsequently, all variables with a *P* value ≤ 0.10 were selected for inclusion in a multivariable model adjusted for age and gender. Backward multivariable regression analysis was performed and the final prediction model included only variables with a *P* value less than 0.10 (Supplementary Table 1). The accuracy of the prediction model to discriminate between cases and controls was evaluated using a receiver operating characteristic (ROC) curve and calculation of the corresponding area under the curve (AUC). Risk scores (calculated as the sum of regression coefficients) and the predicted probability of AMD were then computed for all individuals in the family dataset. These statistical analyses were performed using IBM SPSS Statistics software, version 25.0 (IBM Corp., Armonk, NY, USA).

Next, to determine the proportion of AMD families that cannot be explained by these known risk factors, we calculated the expected number of affected family members within a family using the RAND('BERNOULLI',*p*) function in a simulation analyses. A probability distribution for the number of expected affected individuals was provided after running 1000 simulations with the predicted probabilities within each family. Based on these probabilities we calculated the expected number of affected individuals in each family and compared these with the observed numbers of affected family members. These analyses were performed using software package SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Finally, phenotypic differences were evaluated between families explained by common risk factors and families not explained by these common risk factors using a chi-squared test.

RESULTS

Basic demographic characteristics of the case-control and family dataset are shown in Table 1. From the univariable analysis 21 variables were selected for inclusion in the multivariable model adjusted for age and gender (data available upon request). Factors included in the final model are age, smoking status, physical activity, education, family history for AMD and eleven common genetic variants located in *APOE* (rs2075650), *ARMS2* (rs10490924), *C3* (rs2230199), *CETP* (rs3764261), *CFH* (rs800292, rs1061170, rs12144939), *FADS1* (rs174547),

SKIV2L (rs429608), *TIMP3* (rs9621532), *TNFRSF10A* (rs13278062), and *VEGFA* (rs943080). The associations of these variables with AMD are shown in Supplementary Table 1. Based on these results, we calculated the predicted probabilities for each individual and analyzed the accuracy of this model. The model showed an area under the curve of 0.833 with a 95% confidence interval between 0.813-0.853. The model was then applied to the AMD family dataset to calculate the predicted probabilities for each individual in the family dataset.

Table 1. Basic demographic characteristics of the case-control and family dataset

	Case-control set		**Family set***	
	Case (n=1188)	Control (n=1468)	Case (n=104)	Control (n=36)
Age (age in years±SD)	76.4±8.4	72.4±6.64	73.8±8.4	70.3±4.5
Gender (n[%])				
Male	481 (40.5%)	624 (42.5%)	37 (35.6%)	19 (52.8%)
Female	707 (59.5%)	844 (57.5%)	67 (64.4%)	17 (47.2%)

*Final analyses included 38 of the 43 AMD families. Five families were excluded from the simulation analyses because data of one or two family members was incomplete.
Abbreviations: SD, standard deviation.

Observed vs. expected number of affected family members

Further analyses included 38 of the 43 affected AMD families. In 26 out of 38 families (68.4%) the observed number of affected family members equaled the expected number of affected family members (Table 2). In 12 families the number of affected family members outnumbered the expected number of affected family members, and in two of these families (5.3%) this was significantly higher than expected based on the simulation ($P<0.05$). The predicted probabilities in each of the family members of these two families are shown in Figure 1. Therefore it is unlikely that these families can be explained by the common risk factors included in this model.

Phenotypic differences between AMD families

Next, phenotypic differences were evaluated between cases from the 26 families explained by common risk factors and cases from the 12 families not explained by these common risk factors (Table 3). AMD patients from families not explained by risk factors tended to have larger drusen, but did not reach statistical significance. A higher prevalence of hyperpigmentation

Table 2. Comparison of observed number of affected family members with the expected number of affected family members based on the predicted probabilities within each individual family

Family	Observed number of affected individuals/Total number of family members	Expected number of affected individuals/Total number of family members	Probability that observed number of affected family members is true (0-1)
#5	3/4	4/4	0.996
#38	2/3	3/3	0.987
#7	5/9	7/9	0.975
#36	2/2	2/2	0.972
#12	3/9	5/9	0.970
#16	2/4	3/4	0.966
#14	2/5	3/5	0.951
#21	3/5	4/5	0.949
#11	2/3	2/3	0.919
#17	3/3	3/3	0.915
#29	3/3	3/3	0.900
#13	3/4	3/4	0.890
#2	2/2	2/2	0.885
#33	2/2	2/2	0.881
#15	2/2	2/2	0.859
#28	2/2	2/2	0.846
#4	3/4	3/4	0.817
#19	4/5	4/5	0.788
#25	2/2	2/2	0.781
#37	2/2	2/2	0.776
#18	2/2	2/2	0.748
#31	2/3	2/3	0.734
#32	3/4	3/4	0.724
#34	3/3	3/3	0.662
#20	3/5	3/5	0.649
#26	2/2	2/2	0.597
#8	2/2	1/2	0.421
#6	2/3	1/3	0.397
#22	4/6	3/6	0.285
#3	3/3	2/3	0.252
#30	5/5	4/5	0.239
#23	4/6	3/6	0.221
#10	3/5	2/5	0.218
#27	3/3	2/3	0.177
#1	3/4	1/4	0.092
#9	3/4	1/4	0.076
#24	3/3	1/3	0.020
#35	2/2	0/2	0.010

In 26 families the observed number of affected family members was equal to or less than the expected number of affected family members based on the common risk factors in our model (light grey). In twelve families (white) the number of affected family members outnumbered the expected number of affected family members, in two of these families (dark grey) this was significantly higher than expected based on the simulation ($P<0.05$).

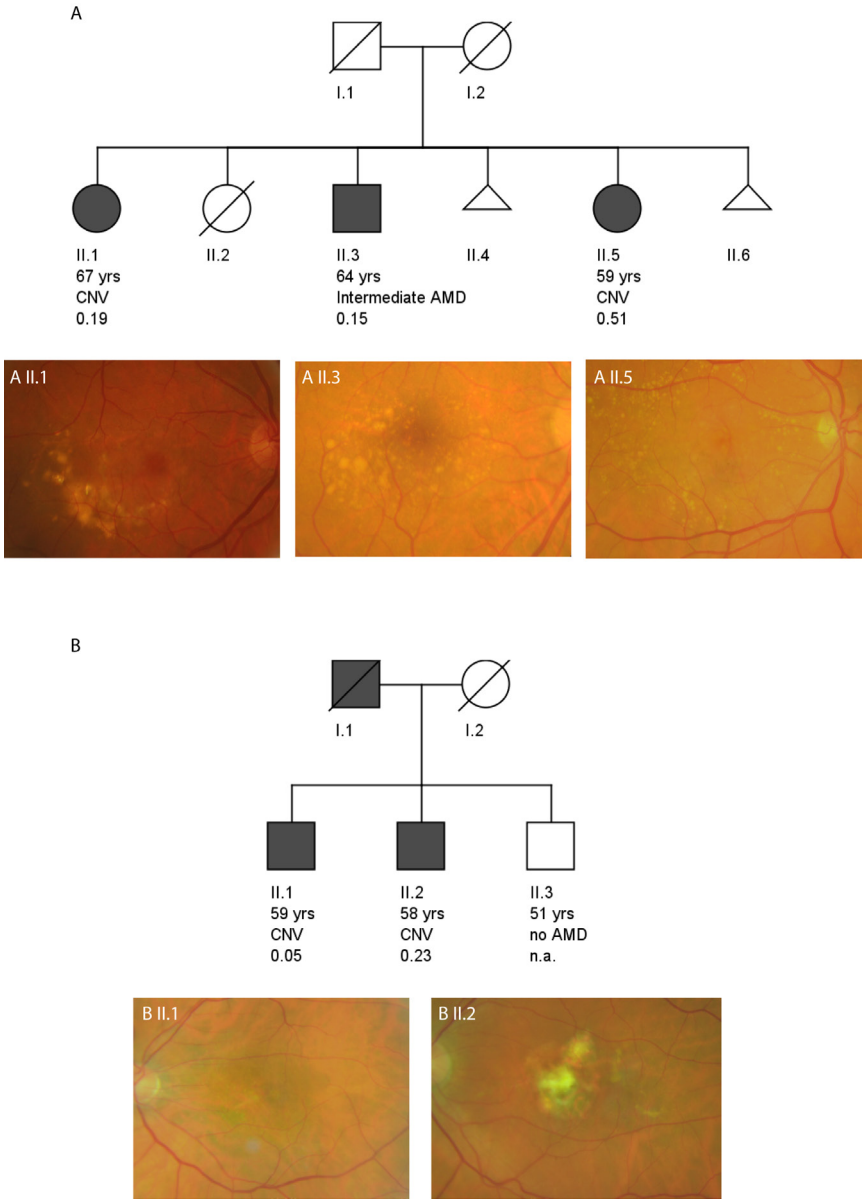


Figure 1. Pedigrees of families #24 (A) and #35 (B), in which the observed number of affected family members is much higher than expected and therefore not explained by common risk factors
Squares indicate men; Circles, women; Slashes, deceased family members; Black symbols, AMD patients; Triangle, miscarriage. The predicted probabilities based on demographic, environmental and common genetic risk factors are displayed on the bottom line beneath the symbols. No data were available for the deceased family members.

Table 3. Phenotypic characteristics in families explained by common risk factors versus families not explained by these risk factors.

Phenotypic characteristics		Cases from families explained by risk factors (n=67)*	Cases from families not explained by risk factors (n=37)**	P-value
Any drusen outside grid	Present	50 (79.4%)	28 (77.8%)	0.853
	Absent	13 (20.6%)	8 (22.2%)	
Predominant drusen type inside grid	Hard	2 (4.3%)	0 (0.0%)	
	Soft ≤125µm	9 (19.1%)	3 (10.3%)	
	Soft distinct, >125µm	12 (25.5%)	6 (20.7%)	0.100
	Soft indistinct, >125µm	17 (36.2%)	19 (65.5%)	
	Reticular	7 (14.9%)	1 (3.4%)	
Largest drusen inside grid	< 63µm	2 (4.3%)	0 (0.0%)	
	< 125µm	10 (21.3%)	1 (3.4%)	
	< 250µm	12 (25.5%)	10 (34.5%)	0.066
	≥ 250µm	14 (29.8%)	15 (51.7%)	
	Reticular	9 (19.1%)	3 (10.3%)	
Retinal area covered by drusen central circle	< 10%	10 (45.5%)	8 (38.1%)	
	< 25%	4 (18.2%)	5 (23.8%)	0.458
	< 50%	5 (22.7%)	2 (9.5%)	
	≥ 50%	3 (13.6%)	6 (28.6%)	
Retinal area covered by drusen inner circle	< 10%	21 (56.8%)	16 (57.1%)	
	< 25%	8 (21.6%)	6 (21.4%)	0.829
	< 50%	7 (18.9%)	4 (14.3%)	
	≥ 50%	1 (2.7%)	2 (7.1%)	
Retinal area covered by drusen outer circle	< 10%	27 (58.7%)	16 (55.2%)	
	< 25%	12 (26.1%)	10 (34.5%)	0.360
	< 50%	6 (13.0%)	1 (3.4%)	
	≥ 50%	1 (2.2%)	2 (6.9%)	
Hyperpigmentation	Present	38 (61.3%)	14 (38.9%)	0.032
	Absent	24 (38.7%)	22 (61.1%)	
Geographic atrophy	Present	18 (27.7%)	11 (30.6%)	0.761
	Absent	47 (72.3%)	25 (69.4%)	
Neovascular AMD	Present	28 (43.1%)	10 (27.8%)	0.128
	Absent	37 (56.9%)	26 (72.2%)	

*Cases from families #2, #4, #5, #7, # 11-#21, #25, #26, #28, #29, #31-34, #36-38
** Cases from families #1, #3, #6, #8-10, #22-24, #27, #30, #35

was detected in cases from families explained by common risk factors (38/67 cases; 61.3%) compared to cases from families not explained by common risk factors (14/37 cases; 38.9%, P = 0.032). However, other than this finding there were no statistically significant phenotypic differences between these families.

DISCUSSION

In this study, we demonstrated that a prediction model based on demographic, common genetic and environmental risk factors can be applied to most AMD families. Like other prediction models, common risk factors were included in the current study and the AUC was within range of the existing prediction models (AUC 0.68-0.94).[6] Further evaluation of the individual families showed that the number of affected family members is similar to what is expected based on the known risk factors in the majority (68.4%) of families. This is in agreement with findings from Sobrin et al., who previously reported that in most AMD families the genotypic load for five common SNPs did not significantly differ from the expected load.[17]

Previous studies reported that associations of risk factors in familial AMD may differ from non-familial AMD.[16,17,21] A weaker association for a common genetic variant in *ARMS2* (rs10490924) was observed in familial AMD patients compared to non-familial AMD patients, moreover, in densely affected families this association was not significant anymore. In addition, certain lifestyle factors seem to play a less important role in familial AMD, such as red meat consumption and increased physical exercise.[16] Furthermore, rare genetic variants with a high risk for disease have been identified in AMD families.[18-20,45-49] Our results indicate that in the majority of families clustering of common genetic and environmental risk factors explains the aggregation of AMD in these families. By contrast, we also identified two AMD families in which the number of affected individuals was much higher than expected. These families may harbor rare, penetrant genetic variants responsible for the risk of disease, thus are of particular interest for additional genetic testing, such as whole exome sequencing.[18,19] However, it must be noted that these families might also carry other common genetic variants that were not included in this study which could explain their AMD risk.

Since rare genetic variants could play an important role in the etiology of familial AMD, addition of such variants might improve risk prediction. Due to their low frequencies however, the additive predictive value of rare genetic variants in the general population is low.[50] Hence, accurate risk prediction in AMD families remains difficult and it is recommended to evaluate risk factors not only individually, but throughout the family to accurately predict disease risk in AMD families.

Limitations

Our study was limited in its cross-sectional design. Preferably, longitudinal data is used as it would allow more accurate risk prediction for progression of AMD. Also, due to lack of one or more missing variables in our dataset for existing prediction models for AMD, these could not be applied to our dataset. Therefore, first a prediction model was created based on the

available case-control data. A limitation of this study is that the model was not validated in an independent cohort. However, this model contains previously described risk factors, and were comparable to previously described models.[6-8,11,12]

CONCLUSION

In conclusion, we have shown that approximately two-thirds of AMD families in this study can be explained by clustering of common genetic and environmental risk factors. However, in a subset of families the risk of advanced AMD is not explained by these common risk factors. This suggests that other factors, such as rare genetic variants conferring a high disease risk, play a key role in the development of advanced AMD in these families. These families cannot be identified solely by phenotype. Individual risk prediction based on existing prediction models in AMD families is therefore challenging and evaluation of multiple family members is recommended.

REFERENCES

1. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol.* 2010;10:31.
2. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet.* 2018;392(10153):1147-1159.
3. Boyers LN, Karimkhani C, Hilton J, Richheimer W, Dellavalle RP. Global burden of eye and vision disease as reflected in the Cochrane Database of Systematic Reviews. *JAMA Ophthalmol.* 2015;133(1):25-31.
4. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health.* 2014;2(2):e106-116.
5. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology.* 2017;124(12):1753-1763.
6. Buitendijk GH, Rochtchina E, Myers C, et al. Prediction of age-related macular degeneration in the general population: the Three Continent AMD Consortium. *Ophthalmology.* 2013;120(12):2644-2655.
7. Chen Y, Zeng J, Zhao C, et al. Assessing susceptibility to age-related macular degeneration with genetic markers and environmental factors. *Arch Ophthalmol.* 2011;129(3):344-351.
8. Klein ML, Francis PJ, Ferris FL, 3rd, Hamon SC, Clemons TE. Risk assessment model for development of advanced age-related macular degeneration. *Arch Ophthalmol.* 2011;129(12):1543-1550.
9. Ristau T, Ersoy L, Hahn M, et al. Nongenetic risk factors for neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(8):5228-5232.
10. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci.* 2009;50(5):2044-2053.
11. Seddon JM, Reynolds R, Yu Y, Daly MJ, Rosner B. Risk models for progression to advanced age-related macular degeneration using demographic, environmental, genetic, and ocular factors. *Ophthalmology.* 2011;118(11):2203-2211.
12. Spencer KL, Olson LM, Schnetz-Boutaud N, et al. Using genetic variation and environmental risk factor data to identify individuals at high risk for age-related macular degeneration. *PLoS One.* 2011;6(3):e17784.
13. Klaver CC, Wolfs RC, Assink JJ, van Duijn CM, Hofman A, de Jong PT. Genetic risk of age-related maculopathy. Population-based familial aggregation study. *Arch Ophthalmol.* 1998;116(12):1646-1651.
14. Luo L, Harmon J, Yang X, et al. Familial aggregation of age-related macular degeneration in the Utah population. *Vision Res.* 2008;48(3):494-500.
15. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol.* 1997;123(2):199-206.
16. Saksens NT, Kersten E, Groenewoud JM, et al. Clinical characteristics of familial and sporadic age-related macular degeneration: differences and similarities. *Invest Ophthalmol Vis Sci.* 2014;55(11):7085-7092.
17. Sobrin L, Maller JB, Neale BM, et al. Genetic profile for five common variants associated with age-related macular degeneration in densely affected families: a novel analytic approach. *Eur J Hum Genet.* 2010;18(4):496-501.
18. Wagner EK, Raychaudhuri S, Villalonga MB, et al. Mapping rare, deleterious mutations in Factor H: Association with early onset, drusen burden, and lower antigenic levels in familial AMD. *Sci Rep.* 2016;6:31531.

19. Yu Y, Triebwasser MP, Wong EK, et al. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. *Hum Mol Genet.* 2014;23(19):5283-5293.
20. Saksens NT, Geerlings MJ, Bakker B, et al. Rare Genetic Variants Associated With Development of Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2016;134(3):287-293.
21. Saksens NT, Lechanteur YT, Verbakel SK, et al. Analysis of Risk Alleles and Complement Activation Levels in Familial and Non-Familial Age-Related Macular Degeneration. *PLoS One.* 2016;11(6):e0144367.
22. Ristau T, Ersoy L, Lechanteur Y, et al. Allergy is a protective factor against age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(1):210-214.
23. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology.* 1991;98(7):1128-1134.
24. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol.* 1995;39(5):367-374.
25. Fritsche LG, Chen W, Schu M, et al. Seven new loci associated with age-related macular degeneration. *Nat Genet.* 2013;45(4):433-439, 439e431-432.
26. Holliday EG, Smith AV, Cornes BK, et al. Insights into the genetic architecture of early stage age-related macular degeneration: a genome-wide association study meta-analysis. *PLoS One.* 2013;8(1):e53830.
27. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet.* 2005;14(21):3227-3236.
28. Ryu E, Fridley BL, Tosakulwong N, Bailey KR, Edwards AO. Genome-wide association analyses of genetic, phenotypic, and environmental risks in the age-related eye disease study. *Mol Vis.* 2010;16:2811-2821.
29. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat Genet.* 2007;39(10):1200-1201.
30. Yates JR, Sepp T, Matharu BK, et al. Complement C3 variant and the risk of age-related macular degeneration. *N Engl J Med.* 2007;357(6):553-561.
31. Chen W, Stambolian D, Edwards AO, et al. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2010;107(16):7401-7406.
32. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). *Proc Natl Acad Sci U S A.* 2010;107(16):7395-7400.
33. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet.* 2006;38(4):458-462.
34. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2005;102(20):7227-7232.
35. Edwards AO, Ritter R, 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005;308(5720):421-424.
36. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* 2005;308(5720):419-421.
37. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308(5720):385-389.

38. Ristau T, Paun C, Ersoy L, et al. Impact of the common genetic associations of age-related macular degeneration upon systemic complement component C3d levels. *PLoS One.* 2014;9(3):e93459.

39. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. *Eur J Hum Genet.* 2009;17(1):100-104.

40. Morrison MA, Silveira AC, Huynh N, et al. Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. *Hum Genomics.* 2011;5(6):538-568.

41. Chiu CJ, Conley YP, Gorin MB, et al. Associations between genetic polymorphisms of insulin-like growth factor axis genes and risk for age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2011;52(12):9099-9107.

42. Kopplin LJ, Igo RP, Jr., Wang Y, et al. Genome-wide association identifies SKIV2L and MYRIP as protective factors for age-related macular degeneration. *Genes Immun.* 2010;11(8):609-621.

43. Oczos J, Grimm C, Barthelmes D, et al. Regulatory regions of the paraoxonase 1 (PON1) gene are associated with neovascular age-related macular degeneration (AMD). *Age (Dordr).* 2013;35(5):1651-1662.

44. Yu Y, Bhangale TR, Fagerness J, et al. Common variants near FRK/COL10A1 and VEGFA are associated with advanced age-related macular degeneration. *Hum Mol Genet.* 2011;20(18):3699-3709.

45. Pras E, Kristal D, Shoshany N, et al. Rare genetic variants in Tunisian Jewish patients suffering from age-related macular degeneration. *J Med Genet.* 2015;52(7):484-492.

46. Ratnapriya R, Zhan X, Fariss RN, et al. Rare and common variants in extracellular matrix gene Fibrillin 2 (FBN2) are associated with macular degeneration. *Hum Mol Genet.* 2014;23(21):5827-5837.

47. Raychaudhuri S, Iartchouk O, Chin K, et al. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat Genet.* 2011;43(12):1232-1236.

48. van de Ven JP, Nilsson SC, Tan PL, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. *Nat Genet.* 2013;45(7):813-817.

49. Zhan X, Larson DE, Wang C, et al. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. *Nat Genet.* 2013;45(11):1375-1379.

50. Mihaescu R, Pencina MJ, Alonso A, et al. Incremental value of rare genetic variants for the prediction of multifactorial diseases. *Genome Med.* 2013;5(8):76.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Final multivariable model used for the prediction of advanced AMD

		Regression coefficient	OR (95% CI)	*P* value
Age of participation (y)		0.10	1.10 (1.081-1.117)	<0.001
Smoking status	Never	Reference	Reference	
	Past	0.15	1.158 (0.903-1.485)	<0.001
	Current	1.00	2.721 (1.704-4.345)	
BMI (kg/m^2)	Normal (<25)	Reference	Reference	
	Overweight (25-30)	0.13	1.139 (0.883-1.469)	0.029
	Obese (>30)	0.53	1.701 (1.149-2.518)	
Physical activity	Less than once a week	Reference	Reference	
	1 or 2 times a week	-0.37	0.688 (0.522-0.906)	<0.001
	3 or more times a week	-0.72	0.485 (0.346-0.680)	
Education	Less than high school	Reference	Reference	
	High school or more	-0.45	0.639 (0.502-0.812)	<0.001
Family history of AMD	No	Reference	Reference	
	Yes	0.82	2.265 (1.703-3.011)	<0.001
APOE, rs2075650	A:A	Reference	Reference	
	A:G	-0.36	0.695 (0.521-0.923	0.047
	G:G	0.02	1.1015 (0.338-3.070)	
ARMS2, rs10490924	G:G	Reference	Reference	
	G:T	0.98	2.673 (2.082-3.434)	<0.001
	T:T	2.36	10.579 (6.927-16.158)	
C3, rs2230199	C:C	Reference	Reference	
	C:G	0.12	1.124 (0.875-1.443)	0.014
	G:G	0.77	2.156 (1.281-3.630)	
CETP, rs3764261	C:C	Reference	Reference	
	C:A	0.45	1.564 (1.216-2.011)	0.001
	A:A	0.47	1.596 (1.082-2.354)	
CFH, rs800292	G:G	Reference	Reference	
	G:A	-0.51	0.601 (0.445-0.812)	0.003
	A:A	-0.55	0.577 (0.297-1.123)	
CFH, rs1061170	T:T	Reference	Reference	
	T:C	0.05	1.045 (0.759-1.441)	<0.001
	C:C	0.80	2.216 (1.422-3.455)	
CFH, rs12144939	G:G	Reference	Reference	
	G:T	-0.65	0.523 (0.377-0.723)	<0.001
	T:T	-0.73	0.480 (0.227-1.013)	
SKIV2L, rs429608	G:G	Reference	Reference	
	G:A	-0.70	0.496 (0.365-0.675)	<0.001
	A:A	-0.51	0.598 (0.218-1.646)	
TIMP3, rs9621532	A:A	Reference	Reference	0.017
	A:C or C:C	-0.54	0.586 (0.378-0.907)	
TNFRSF10A, rs13278062	G:G	Reference	Reference	
	G:T	0.06	1.065 (0.789-1.438)	0.073
	T:T	0.34	1.411 (1.011-1.969)	
VEGFA, rs943080	T:T	Reference	Reference	
	T:C	-0.22	0.800 (0.604-1.058)	0.074
	C:C	-0.39	0.678 (0.483-0.952)	

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio; SD, standard deviation.

Chapter 2.3
Risk factors for progression to late age-related macular degeneration: 5-year follow-up of patients with early and intermediate AMD in the EUGENDA cohort

Eveline Kersten*
Vasilena Sitnilska*
Lebriz Altay
Tina Schick
Philip Enders
Eiko K. de Jong
Thomas Langmann
Carel B. Hoyng
Anneke I. den Hollander
Sascha Fauser

* These authors contributed equally to this study

Manuscript submitted

ABSTRACT

Purpose: To evaluate the combined role of genetic, non-genetic and phenotypic risk factors for conversion from early and intermediate age-related macular degeneration (AMD) to late AMD.

Methods: This study included genetic, non-genetic and phenotypic data of 232 patients with early/intermediate AMD from the European genetic database (EUGENDA) with follow-up of at least five years. Baseline phenotypic characteristics were evaluated based on color fundus photography, spectral-domain optical coherence tomography and infrared images. Genotyping for 36 single nucleotide polymorphisms in AMD-associated risk genes and systemic lipid and complement measurements were performed. Multivariable backward logistic regression resulted in a final prediction model.

Results: During a mean follow-up of 5.9 years, 22.4% of patients progressed to late AMD. The multivariable prediction model included age, *CFH* variants (rs1061170, rs800292), pigment abnormalities, drusenoid pigment epithelial detachment (dPED), reticular pseudodrusen (RPD) and hyperreflective foci (HF). The model showed an area under the curve of 0.978 (95% Confidence Interval: 0.961-0.996) and adequate calibration (Hosmer-Lemeshow P= 0.712).

Conclusions and Relevance: In addition to advanced age and CFH variants, pigment abnormalities, dPED, RPD and HF are relevant imaging biomarkers for conversion to late AMD. In clinical routine, an intensified monitoring of patients with high risk phenotypic profile may be useful for early detection of progression to late AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is a complex progressive retinal disease and one of the leading causes of severe vision loss in the elderly population of the Western world.[1] The early stages of AMD are characterized by the presence of small, intermediate and/or large drusen and retinal pigmentary abnormalities. Early AMD can progress to late AMD, which is characterized by choroidal neovascularization (CNV) or geographic atrophy (GA). Identification of predictive factors for progression to late AMD may contribute to a more efficient and personalized approach in monitoring and support of patients with early AMD stages. Additionally, understanding disease progression is crucial for a better insight in the disease mechanisms and could potentially contribute to the development of new preventive strategies to delay the onset of late AMD stages.

Genomic heritability of AMD is estimated to be 46-71%.[2] In recent years, 52 independently associated common and rare genetic variants at 34 loci have been associated with AMD, which together explain approximately half of the heritability. The AMD-associated genes are primarily involved in the modulation of the complement system, extracellular matrix and lipid metabolism.[3] Nevertheless, the impact of these genetic variants on AMD progression has not been studied extensively. Several reports on AMD progression evaluated only a limited number of known AMD-associated variants.[4-7] A recent report developed a genetic risk score for AMD progression including the top variants of all known 34 AMD risk loci.[8] Besides genetic influence, also non-genetic risk factors (age,[4,9] BMI,[4,9,10] smoking[4,8-11]) and phenotypic characteristics such as presence of large drusen,[12,13] central drusen location,[11,14] pigmentary abnormalities,[11,14] hyperreflective foci (HF)[15,16] and reticular pseudodrusen (RPD)[17,18] have been identified as individual risk factors for the progression to late AMD. However, there is a lack of studies on AMD progression investigating the combined effect of comprehensive genetic, environmental, demographic risk factors and detailed phenotypic characteristics based on multimodal imaging.

Hence, this study aims to evaluate comprehensively the role of genetic, non-genetic and phenotypic characteristics based on multimodal imaging on conversion from early to late AMD over a period of at least five years. Furthermore, we aimed to create a multivariable prediction algorithm based on the factors and characteristics that were significantly associated with AMD progression.

METHODS

In this prospective observational study, we examined patients with early or intermediate AMD over a follow-up time of at least five years after baseline examination. The participants

were recruited from the European Genetic Database (EUGENDA, www.eugenda.org), a multicenter database of AMD patients from University Hospital in Cologne, Germany and Radboud university medical center in Nijmegen, the Netherlands. The study was approved by local ethics committees and was performed in accordance with the tenets of the Declaration of Helsinki. Before enrollment in EUGENDA, written informed consent was obtained from all participants.

At baseline, a total of 605 patients who presented signs of early or intermediate AMD and no signs of late AMD (CNV or GA) were examined at the Department of Ophthalmology of the University Hospital of Cologne, Germany, and Radboud university medical center in Nijmegen, the Netherlands, between June 2008 and January 2012. After five years, 232 of the 605 participants underwent a follow-up examination at the same departments between April 2014 and November 2016. The remainder of 605 patients did not participate in the follow-up examination due to several reasons; 185 of the 605 patients did not respond to different attempts of contact, 115 declined participation or were unable to perform the visit due to other severe medical conditions, 23 had changed their residence, 47 had died and three were excluded due to bad image quality of the follow-up examination. None of the included subjects suffered from other severe retinal pathologies such as diabetic retinopathy/maculopathy, high myopia (≥-6 diopters) or severe macular pucker.

Spectral Domain Optical coherence tomography (SD-OCT), infrared images (IRs, Spectralis HRA system; Heidelberg Engineering, Heidelberg, Germany), digital color fundus photographs (FP; Cologne: Canon UVI fundus camera; Canon, Tokyo, Japan, and Nijmegen: Topcon TRC 50IX fundus camera; Topcon, Tokyo, Japan) and fluorescein angiography (FA) were performed at baseline. At the follow-up visit, patients underwent at least SD-OCT, IRs and FP examinations. Further medical data, such as arterial hypertension (yes/no), cardiovascular diseases (yes/no), allergy (yes/no) and BMI (normal <25/overweight ≥25), were obtained by standardized interviewer-assisted questionnaires for each patient. Sociodemographic characteristics (age, sex [male/female]) and smoking status (never/ever) were also included in this questionnaire.

Grading Procedure

Severity staging was performed by grading of retinal images, including FP, SD-OCTs and IRs, according to the Beckman classification system[19] by certified graders at baseline (LA, TS) and follow-up visit (VS, LA). Discrepancies between graders were solved by open adjudication.

Presence of the following phenotypic characteristics were evaluated at baseline based on FP, SD-OCT and IRs: drusenoid pigment epithelial detachment (dPED; drusen size ≥360µm[20]), pigment abnormalities (hyper-or hypopigmentation areas in form of pigmentary clusters or sharply demarcated sections of the RPE[14]), reticular pseudodrusen (RPD; drusenoid deposit, located in the subretinal space above the RPE[21]), and HF (n<10/≥10 in all SD-OCT scans; small, highly-backscattering lesions within the neurosensory retina with greater reflectivity than the RPE[22]).

Serum Measurements and Genotyping

Venous blood samples were collected at baseline visit for systemic lipid and complement measurements and genetic analyses. Serum lipid measurements included the following: total cholesterol, high-density lipoprotein-cholesterol (HDL), triglycerides, apolipoprotein B, apolipoprotein A1, Lipoprotein a and C-reactive protein (CRP). Complement activation levels were evaluated using the ratio between activation fragment C3d and complement component C3 levels (C3d/C3 ratio). Lipid levels and complement components were measured in serum samples as described before.[23] Genotyping was performed for 36 single nucleotide polymorphisms (SNPs) in AMD-associated risk genes using the KASPar SNP Genotyping System (LGC Genomics, Berlin, Germany; Supplementary Table 1).

Statistical Analysis

All demographic, environmental, phenotypic, non-genetic and genetic variables collected at baseline were assessed to determine their association with progression to late AMD using univariable logistic regression models adjusted for age and sex. Thereafter, variables with a P-value < 0.10 were selected for inclusion in multivariable logistic regression models. First, we developed multivariable models for phenotypic and genotypic factors separately (adjusted for age and sex). Age, sex and all variables with a P value < 0.10 in univariable analyses (both genetic and phenotypic factors) were selected for inclusion in a backward logistic regression resulting in a final prediction model. The discriminative accuracy of each model was evaluated using receiver operating characteristic (ROC) curves and calculation of their corresponding area under the curve (AUC). The Hosmer-Lemeshow goodness of fit test was performed to check the calibration of the models. Mann-Whitney U Test and Pearson's chi-squared test were used to assess the differences in baseline characteristics between the included patients and the ones lost-to-follow-up.

All statistical analyses were performed using IBM SPSS Statistics software, version 22.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Baseline characteristics

Mean follow-up time of the 232 included patients with early or intermediate AMD at baseline examination was 5.9 years (range 4.5-10.4 years). Among these patients, 132 (56.9%) were female (mean age 69.2±6.2 years) and 100 (43.1%) were male (mean age 71.3±5.9 years). At baseline, 92 follow-up participants (39.7%) were categorized as early AMD and 140 (60.3%) as intermediate AMD. Further baseline characteristics of all participants in this study are shown in Table 1. Patients lost to follow-up were significantly older than included patients, reported less history of smoking, but suffered more frequently from cardiovascular disease.

Table 1. Baseline Characteristics of all Participants

	Included Patients	Patients lost to follow-up	P-Value
Number of patients, n	232	373	
Age, mean (SD), years	70.13 (6.17)	74.96 (8.67)	0.001[a]
Female sex, n (%)	132 (56.9)	236 (63.3)	0.119[b]
Follow up period, mean (SD), years	5.94 (0.91)		
Ever smoked, n (%)	142 (61.2)	172 (46.1)	0.004[b]
Hypertension, n (%)	84 (36.2)	137 (36.7)	0.690[b]
Cardiovascular diseases, n (%)	44 (19.0)	102 (27.3)	0.011[b]
Allergy, n (%)	66 (28.4)	79 (21.2)	0.065[b]
BMI ≥25, n (%)	117 (50.4)	175 (46.9)	0.584[b]
C3d/C3 Ratio, mean (SD)	1.47 (0.36)	1.52 (0.41)	0.478[a]
Total cholesterol, mean (SD), mmol/l	5.78 (1.13)	5.69 (1.17)	0.369[a]
HDL cholesterol, mean (SD), mmol/l	1.54 (0.38)	1.53 (0.40)	0.822[a]
Triglyceride, mean (SD), mmol/l	1.63 (0.74)	1.67 (0.85)	0.736[a]
ApoB, mean (SD), mg/l	958.10 (227.76)	934.35 (215.65)	0.280[a]
ApoA1, mean (SD), mg/l	1730.42 (307.98)	1723.98 (324.62)	0.389[a]
Lp(a), mean (SD), U/l	312.85 (375.56)	339.39 (432.91)	0.485[a]
CRP, mean (SD), mg/l	5.40 (5.26)	5.67 (5.45)	0.376[a]

SD: Standard Deviation, BMI: Body Mass Index, C3d/C3 ratio: Complement Activation Level as the ratio of C3d and C3 fragments, HDL: High-Density Lipoprotein, ApoB: Apolipoprotein B, ApoA1: Apolipoprotein A1, Lp(a): Lipoprotein a, CRP: C-reactive protein

[a]Pearson's chi-squared test

[b]Mann-Whitney U test

Progression Rates to Late AMD

During the mean follow-up time of 5.9 years, 52 of 232 patients (22.4%) developed a late stage of AMD, hereafter referred to as "progressors". The 180 patients who did not develop late AMD during follow-up are referred to as "non-progressors".

At follow-up, 52 of the 140 patients (37.1%) with intermediate AMD at baseline converted to late AMD at the end of the study. None of the 92 patients with early AMD at baseline converted to late AMD. Of the progressors, 29 patients (55.8%) developed unilateral late AMD (25.0% CNV, 30.8% GA), while 23 (44.2%) presented a late stage in both eyes (5.8% CNV, 21.2% GA, 17.3% mixed type).

Predictive factors for the progression to late AMD

Demographic, environmental, non-genetic, genetic, and phenotypic features at baseline were individually compared between progressors and non-progressors, adjusted for age and sex (Table 2).

Non-genetic risk factors

There was no significant difference in follow-up time (P = 0.121) and sex distribution (P = 0.446) between progressors and non-progressors, whereas the non-progressors tended to be younger (P = 0.056). History of smoking, hypertension, cardiovascular disease, allergy and increased BMI were not associated with progression to late AMD after five years of follow-up. Also, the C3d/C3 ratio and systemic serum lipid levels showed no association with progression to late AMD.

Genetic risk factors

Among the 36 tested SNPs only three tended to be associated with progression to late AMD in univariable logistic regression analyses: *CFH* rs1061170 (Odds Ratio [OR] 2.41, P = 0.25×10^{-3}), *CFH* rs800292 (OR 0.37, P = 0.005), and *CETP* rs3764261 (OR 1.94, P = 0.008). In contrast, the *ARMS2* rs10490924 risk allele showed no significant association with conversion to late AMD (OR 1.22, P = 0.431). Analysis of the ROC curve of the multivariable model that included the four SNPs with a P-value < 0.10 in the *CFH*, *CETP* and *C3* genes revealed a fairly good ability to discriminate between progressors and non-progressors with an AUC of 0.763 (95% confidence interval [CI] 0.677-0.849).

Phenotypic risk factors

Presence of HF on SD-OCT were associated with a disproportionally high risk of progression to late AMD (OR 166.78, P = 0.05×10^{-4}). Furthermore, presence of dPED (OR 35.43, P = 1.93×10^{-15}), pigment abnormalities (OR 12.27, P = 2.30×10^{-10}), and presence of RPD (OR 4.56, P = 0.004) at baseline increased the odds of progression to late stages of AMD.

A multivariable logistic regression model with all statistically significant phenotypic features (presence of HF, RPD, dPED, and pigment abnormalities) reached an AUC of 0.955 (95% CI 0.926-0.984).

Final multivariable prediction model

In a next step, all parameters with a P-value < 0.10 from the univariable analyses were combined in a backward multivariable logistic regression procedure. This resulted in a final prediction model including age, *CFH* rs1061170, *CFH* rs800292, and presence of the following phenotypic risk factors: pigment abnormalities, dPED, RPD, and HF. Within the final model, the AUC increased to 0.978 (95% CI 0.961-0.996) and showed good calibration (Hosmer-Lemeshow P = 0.712) (Table 3).

Table 2. Univariable Logistic Regression Analysis for Predictors for Conversion of Early/Intermediate to Late AMD after Five Years Follow-up (adjusted for age and sex)

Baseline	Progressors (n=52)	Non-Progressors (n=180)	Odds Ratio	95% CI	P-Value
Age, mean (SD), years	71.50 (8.34)	69.73 (5.35)	1.051	0.999-1.107	0.056
Female sex, n (%)	31 (59.6)	101 (56.1)	1.283	0.676-2.437	0.446
Follow up period, mean (SD), years	6.10 (0.93)	5.90 (0.91)	1.301	0.933-1.815	0.121
Environmental Factors					
Ever smoked, n (%)	33 (68.8)	109 (60.9)	1.620	0.783-3.355	0.194
Hypertension, n (%)	16 (33.3)	68 (37.8)	0.780	0.396-1.539	0.474
Cardiovascular diseases, n (%)	10 (20.8)	34 (18.9)	1.067	0.472-2.413	0.877
Allergy, n (%)	9 (18.8)	57 (31.7)	0.503	0.227-1.115	0.091
BMI ≥25, n (%)	26 (54.2)	91 (50.8)	1.236	0.644-2.372	0.524
Lab measurements					
C3d/C3 Ratio, mean (SD)	1.51 (0.36)	1.46 (0.36)	1.568	0.544-4.522	0.406
Total cholesterol, mean (SD),mmol/l	5.61 (1.10)	5.83 (1.13)	0.855	0.625-1.169	0.327
HDL cholesterol, mean (SD), mmol/l	1.55 (0.37)	1.54 (0.38)	1.286	0.491-3.366	0.608
Triglyceride, mean (SD), mmol/l	1.61 (0.68)	1.63 (0.76)	0.953	0.606-1.498	0.834
ApoB, mean (SD), mg/l	945.6 (267.8)	961.4 (216.9)	1.000	0.998-1.001	0.746
ApoA1, mean (SD), mg/l	1723.0 (299.8)	1732.3 (310.9)	1.000	0.999-1.001	0.984
Lp(a), mean (SD), U/l	287.4 (381.4)	319.5 (374.8)	1.000	0.999-1.001	0.588
CRP, mean (SD), mg/l	5.91 (4.95)	5.27 (5.35)	1.023	0.969-1.081	0.405
Genetics*					
ARMS2, rs10490924, T	MAF 0.34	MAF 0.30	1.220	0.744-1.999	0.431
CFH, rs1061170, C	MAF 0.64	MAF 0.41	2.405	1.503-3.847	**0.25×10^{-3}**
CFH, rs800292, A	MAF 0.11	MAF 0.25	0.372	0.188-0.737	**0.005**
CETP, rs3764261, A	MAF 0.46	MAF 0.31	1.942	1.191-3.165	**0.008**
C3, rs433594, T	MAF 0.49	MAF 0.40	1.604	0.949-2.710	**0.077**
Imaging features					
Pigment abnormality, n (%)	41 (80.4)	46 (25.6)	12.268	5.651-26.630	**2.30×10^{-10}**
DPED, n (%)	42 (80.8)	23 (12.8)	35.434	14.697-85.430	**1.93×10^{-15}**
RPD, n (%)	10 (19.2)	8 (4.4)	4.562	1.622-12.827	**0.004**
HF≥10, n (%)	17 (37.8)	1 (0.6)	166.779	18.566-1498.175	**0.05×10^{-4}**

* Not all variables are shown here; variables not shown had P-values > 0.10 (Supplementary Table 1).
CI: Confidence Interval, SD: Standard Deviation, BMI: Body Mass Index, C3d/C3 ratio: Complement Activation Level as the ratio of C3d and C3 fragments, HDL: High-Density Lipoprotein, ApoB: Apolipoprotein B, ApoA1: Apolipoprotein A1, Lp(a): Lipoprotein a, CRP: C-reactive protein, DPED: Drusenoid Pigment Epithelial Detachment, RPD: Reticular Pseudodrusen, HF: Hyperreflective Foci

Table 3. Final model resulting from backward multivariable logistic regression analysis with risk factors for development of late AMD within follow-up time of at least five years

Baseline	Progressors (n=52)	Non-Progressors (n=180)	Odds Ratio	95% CI	P-Value
Age, mean (SD), years	71.5 (8.3)	69.7 (5.4)	1.25	1.08-1.45	0.003
CFH, rs1061170, C	MAF 0.64	MAF 0.41	7.02	2.08-23.65	0.002
CFH, rs800292, A	MAF 0.11	MAF 0.25	0.07	0.01-0.64	0.019
Pigment abnormality, n (%)	41 (80.4)	46 (25.6)	11.61	2.35-57.39	0.003
DPED, n (%)	42 (80.8)	23 (12.8)	103.31	14.04-760.20	$0.5x10^{-5}$
RPD, n (%)	10 (19.2)	8 (4.4)	14.68	1.06-202.92	0.045
HF ≥10, n (%)	17 (37.8)	1 (0.6)	85.1	4.002-1810.64	0.004

CI: Confidence Interval, SD: Standard Deviation, MAF: Minor Allele Frequency, DPED: Drusenoid Pigment Epithelial Detachment, RPD: Reticular Pseudodrusen, HF: Hyperreflective Foci

Subanalysis of patients with intermediate AMD at baseline

Since all progressors had intermediate AMD at baseline, we performed a subanalysis including only patients with intermediate AMD at baseline (age OR 1.26, P = 0.004; *CFH* rs1061170 OR 6.12, P = 0.003; *CFH* rs800292 OR 0.06, P = 0.018; pigment abnormality OR 13.34, P = 0.004; dPED OR 56.05, P = $0.2x10^{-3}$; RPD OR 6.50, P = 0.171; presence of HF OR 61.25, P = 0.009; AUC=0.964 (95% CI 0.933-0.995)). Despite reduced statistical power, all features, except RPD, remained significantly associated with progression to advanced AMD thereby underlining these features have additive risk effects.

DISCUSSION

This multicenter study comprehensively analyzed the effects of genetic, non-genetic and multiple phenotypic risk factors on the conversion of early to late AMD.

In literature, progression rates of early to late AMD range from 0.5% to 76.5% (Supplementary Table 2).[4,10,11,24-26] This vast spread can be explained by varying follow-up periods (2-15 years), differences in cohorts and study designs as well as definitions of progression. The most straightforward definition of progression is conversion to late AMD in one or both eyes: in our study 22.4% of patients with early or intermediate AMD at baseline progressed to CNV and/or GA after five years, and 37.1% with intermediate AMD at baseline converted to late AMD, which is similar to previously reported progression rates.[10,27]

To date, several studies have analyzed the combined effect of genetic, environmental, demographic and phenotypic factors. However, the grading of phenotypic features was solely based on FP.[4,7-11] A multimodal approach allows for a better differentiation of specific

phenotypic features like HF[22] and it increases the sensitivity for detection of other phenotypic features like RPD and atrophy.[28,29] Recent studies included data from multimodal imaging or automatic grading systems based on SD-OCT, but in these cohorts no genetic information was available.[12,13,15,16,24,30] To our knowledge, this is the first study to present a comprehensive prediction model which considers the distinctive phenotypic features based on multimodal imaging together with genetic, demographic and environmental risk factors on the conversion of early to late AMD stages. Here, a combination of both genetic and phenotypic risk factors showed superior performance, with an AUC of 0.978. Compared to other progression studies (Supplementary Table 2), this model has one of the highest AUCs. A similar AUC was provided by Perlee et al[9] who also combined both genetic and phenotypic risk factors. The model based on only genetic factors in our cohort is inferior to the model including only phenotypic risk factors (AUC of 0.763 vs. 0.955). The high predictive value of phenotypic characteristics for AMD progression is in concordance with previous studies[7,9] and could be very valuable in clinical routine.

Our final model included age, *CFH* rs1061170, *CFH* rs800292 and phenotypic risk factors (RPE abnormalities, dPED, RPD and HF), which are all involved in local inflammatory processes. Aging is a process that is associated with continuous subclinical inflammation,[31] leading to gradual loss of RPE cells and photoreceptors.[32] Age is considered as the major risk factor for onset and progression of AMD. Besides aging, genetic polymorphisms in genes encoding components of the complement system play an important role in the pathogenesis of AMD.[3,33] In concordance with previous studies, we found a strong association of *CFH* variants with disease progression.[4,7,9,10] However, in contrast to previous studies,[4,7,9,10] *ARMS2* did not reach statistical significance in our study, which could be due to limited sample size or different study design. Likewise, systemic complement activation was not predictive for AMD progression. Previous work has shown that increased complement activation occurs in a subset of patients carrying genetic risk variants in complement-associated genes.[34,35] However, due to our limited cohort size we were not able to perform such a subgroup analysis. Additionally, measuring complement activation in aqueous humor might be a more sensitive parameter.[36] Chronic retinal inflammation is considered to play a major role in the formation of focal deposits known as drusen.[37,38] Enlargement or confluence of drusen can be clinically identified as dPED,[39] which is a known risk factor for AMD progression and presumably reflects the high degree of chronic retinal inflammation.[16,39,40] Moreover, HF and RPD, which are highly associated with development of late AMD,[15,16,18] are discussed as *in vivo* inflammation biomarkers of the disease.[41-44] Although both RPD and HF are not pathognomonic for AMD[45-48] they are related to AMD-associated genetic variants.[18,49] Given their dynamic nature, these features could serve as clinical marker for local inflammation.[15,42,50]

Increasing AMD severity is known to be associated with higher risk of progression.[4,9,11] Nevertheless, the risk factors remained associated with conversion to late AMD when

applying the model performed only on patients with intermediate AMD at baseline, which underlines the additive risk effects of features such as HF and dPED.

It is known that several systemic markers are associated with both chronic inflammation and disease activity.[51] However, in this study, no major influence of non-genetic risk factors on disease progression could be detected. Additional investigation of metabolites, proteins and epigenetics in future studies would be helpful to identify non-genetic risk factors for AMD progression.[51,52]

A high number of patients in this study was lost to follow-up due to nonresponse towards attempts to contact, other severe medical conditions or death. This is likely explained by the more advanced age of this group in comparison to included patients. As a consequence of the limited sample size of our study, we were unable to perform subanalyses for progression to either CNV or GA separately. Also, split-sample validation for our prediction model was not possible, and validation in another study is therefore a warranted next step.

Strengths of our study include usage of multimodal imaging including FP, high-resolution SD-OCT and IR, providing a more detailed assessment of retinal pathologies that might be essential for prediction of AMD conversion. Furthermore, multimodal image analysis was based on a generally accepted clinical classification system and was performed by certified graders. Our findings have practical clinical value, as patients at high risk of progression could be monitored more frequently for optimal support and early detection of advanced disease leading to better treatment outcomes.[53]

In summary, we report a 5.9-year prospective follow-up study of patients with early forms of AMD and present a prediction model for conversion to late AMD based on multimodal imaging and genetics. All features in this model are considered to be involved in local inflammation processes, which might be the main trigger for progression to late AMD. In our model, patients of advanced age, carrying *CFH*-risk alleles, and presenting with RPE abnormalities, dPED, HF and RPD are highly likely to progress to late AMD. In clinical routine, these phenotypic features can easily be detected with non-invasive high-resolution retinal imaging. In cases at high risk of progression, an intensified monitoring may aid the early detection of conversion to late AMD.

REFERENCES

1. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology.* 2017;124(12):1753-1763.
2. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch Ophthalmol.* 2005;123(3):321-327.
3. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
4. Seddon JM, Silver RE, Kwong M, Rosner B. Risk Prediction for Progression of Macular Degeneration: 10 Common and Rare Genetic Variants, Demographic, Environmental, and Macular Covariates. *Invest Ophthalmol Vis Sci.* 2015;56(4):2192-2202.
5. Sardell RJ, Persad PJ, Pan SS, et al. Progression Rate From Intermediate to Advanced Age-Related Macular Degeneration Is Correlated With the Number of Risk Alleles at the CFH Locus. *Invest Ophthalmol Vis Sci.* 2016;57(14):6107-6115.
6. Farwick A, Wellmann J, Stoll M, Pauleikhoff D, Hense HW. Susceptibility genes and progression in age-related maculopathy: a study of single eyes. *Invest Ophthalmol Vis Sci.* 2010;51(2):731-736.
7. Klein ML, Francis PJ, Ferris FL, 3rd, Hamon SC, Clemons TE. Risk assessment model for development of advanced age-related macular degeneration. *Arch Ophthalmol.* 2011;129(12):1543-1550.
8. Ding Y, Liu Y, Yan Q, et al. Bivariate Analysis of Age-Related Macular Degeneration Progression Using Genetic Risk Scores. *Genetics.* 2017;206(1):119-133.
9. Perlee LT, Bansal AT, Gehrs K, et al. Inclusion of genotype with fundus phenotype improves accuracy of predicting choroidal neovascularization and geographic atrophy. *Ophthalmology.* 2013;120(9):1880-1892.
10. Yu Y, Reynolds R, Rosner B, Daly MJ, Seddon JM. Prospective assessment of genetic effects on progression to different stages of age-related macular degeneration using multistate Markov models. *Invest Ophthalmol Vis Sci.* 2012;53(3):1548-1556.
11. Joachim N, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. *Ophthalmology.* 2015;122(12):2482-2489.
12. Abdelfattah NS, Zhang H, Boyer DS, et al. Drusen Volume as a Predictor of Disease Progression in Patients With Late Age-Related Macular Degeneration in the Fellow Eye. *Invest Ophthalmol Vis Sci.* 2016;57(4):1839-1846.
13. de Sisternes L, Simon N, Tibshirani R, Leng T, Rubin DL. Quantitative SD-OCT imaging biomarkers as indicators of age-related macular degeneration progression. *Invest Ophthalmol Vis Sci.* 2014;55(11):7093-7103.
14. Joachim ND, Mitchell P, Kifley A, Wang JJ. Incidence, Progression, and Associated Risk Factors of Medium Drusen in Age-Related Macular Degeneration: Findings From the 15-Year Follow-up of an Australian Cohort. *JAMA Ophthalmol.* 2015;133(6):698-705.
15. Christenbury JG, Folgar FA, O'Connell RV, et al. Progression of intermediate age-related macular degeneration with proliferation and inner retinal migration of hyperreflective foci. *Ophthalmology.* 2013;120(5):1038-1045.
16. Fragiotta S, Rossi T, Cutini A, Grenga PL, Vingolo EM. Predictive Factors for Development of Neovascular Age-related Macular Degeneration: A Spectral-Domain Optical Coherence Tomography Study. *Retina.* 2018;38(2):245-252.
17. Marsiglia M, Boddu S, Bearelly S, et al. Association between geographic atrophy progression and reticular pseudodrusen in eyes with dry age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2013;54(12):7362-7369.

18. Finger RP, Chong E, McGuinness MB, et al. Reticular Pseudodrusen and Their Association with Age-Related Macular Degeneration: The Melbourne Collaborative Cohort Study. *Ophthalmology.* 2016;123(3):599-608.
19. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology.* 2013;120(4):844-851.
20. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology.* 1991;98(7):1128-1134.
21. Zweifel SA, Imamura Y, Spaide TC, Fujiwara T, Spaide RF. Prevalence and significance of subretinal drusenoid deposits (reticular pseudodrusen) in age-related macular degeneration. *Ophthalmology.* 2010;117(9):1775-1781.
22. Raychaudhuri S, Iartchouk O, Chin K, et al. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat Genet.* 2011;43(12):1232-1236.
23. Paun CC, Ersoy L, Schick T, et al. Genetic Variants and Systemic Complement Activation Levels Are Associated With Serum Lipoprotein Levels in Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(13):7766-7773.
24. Folgar FA, Yuan EL, Sevilla MB, et al. Drusen Volume and Retinal Pigment Epithelium Abnormal Thinning Volume Predict 2-Year Progression of Age-Related Macular Degeneration. *Ophthalmology.* 2016;123(1):39-50 e31.
25. Vitale S, Clemons TE, Agron E, et al. Evaluating the Validity of the Age-Related Eye Disease Study Grading Scale for Age-Related Macular Degeneration: AREDS2 Report 10. *JAMA Ophthalmol.* 2016;134(9):1041-1047.
26. Lei J, Balasubramanian S, Abdelfattah NS, Nittala MG, Sadda SR. Proposal of a simple optical coherence tomography-based scoring system for progression of age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2017;255(8):1551-1558.
27. Jonasson F, Fisher DE, Eiriksdottir G, et al. Five-year incidence, progression, and risk factors for age-related macular degeneration: the age, gene/environment susceptibility study. *Ophthalmology.* 2014;121(9):1766-1772.
28. Chan H, Cougnard-Gregoire A, Delyfer MN, et al. Multimodal Imaging of Reticular Pseudodrusen in a Population-Based Setting: The Alienor Study. *Invest Ophthalmol Vis Sci.* 2016;57(7):3058-3065.
29. Holz FG, Sadda SR, Staurenghi G, et al. Imaging Protocols in Clinical Studies in Advanced Age-Related Macular Degeneration: Recommendations from Classification of Atrophy Consensus Meetings. *Ophthalmology.* 2017;124(4):464-478.
30. Veerappan M, El-Hage-Sleiman AM, Tai V, et al. Optical Coherence Tomography Reflective Drusen Substructures Predict Progression to Geographic Atrophy in Age-related Macular Degeneration. *Ophthalmology.* 2016;123(12):2554-2570.
31. Franceschi C, Bonafe M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* 2000;908:244-254.
32. Dorey CK, Wu G, Ebenstein D, Garsd A, Weiter JJ. Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci.* 1989;30(8):1691-1699.
33. Geerlings MJ, de Jong EK, den Hollander AI. The complement system in age-related macular degeneration: A review of rare genetic variants and implications for personalized treatment. *Mol Immunol.* 2017;84:65-76.
34. Paun CC, Lechanteur YT, Groenewoud JM, et al. A Novel Complotype Combination Associates with Age-Related Macular Degeneration and High Complement Activation Levels in vivo. *Sci Rep.* 2016;6:26568.

35. Geerlings MJ, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2017;135(1):39-46.

36. Schick T, Steinhauer M, Aslanidis A, et al. Local complement activation in aqueous humor in patients with age-related macular degeneration. *Eye (Lond).* 2017.

37. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol.* 2002;134(3):411-431.

38. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res.* 2001;20(6):705-732.

39. Roquet W, Roudot-Thoraval F, Coscas G, Soubrane G. Clinical features of drusenoid pigment epithelial detachment in age related macular degeneration. *Br J Ophthalmol.* 2004;88(5):638-642.

40. Ferrara D, Silver RE, Louzada RN, Novais EA, Collins GK, Seddon JM. Optical Coherence Tomography Features Preceding the Onset of Advanced Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2017;58(9):3519-3529.

41. Greferath U, Guymer RH, Vessey KA, Brassington K, Fletcher EL. Correlation of Histologic Features with In Vivo Imaging of Reticular Pseudodrusen. *Ophthalmology.* 2016;123(6):1320-1331.

42. Framme C, Wolf S, Wolf-Schnurrbusch U. Small dense particles in the retina observable by spectral-domain optical coherence tomography in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2010;51(11):5965-5969.

43. Coscas G, De Benedetto U, Coscas F, et al. Hyperreflective dots: a new spectral-domain optical coherence tomography entity for follow-up and prognosis in exudative age-related macular degeneration. *Ophthalmologica.* 2013;229(1):32-37.

44. Pang CE, Messinger JD, Zanzottera EC, Freund KB, Curcio CA. The Onion Sign in Neovascular Age-Related Macular Degeneration Represents Cholesterol Crystals. *Ophthalmology.* 2015;122(11):2316-2326.

45. Wilde C, Lakshmanan A, Patel M, Morales MU, Dhar-Munshi S, Amoaku WM. Prevalence of reticular pseudodrusen in newly presenting adult onset foveomacular vitelliform dystrophy. *Eye (Lond).* 2016;30(6):817-824.

46. Gliem M, Muller PL, Mangold E, et al. Reticular Pseudodrusen in Sorsby Fundus Dystrophy. *Ophthalmology.* 2015;122(8):1555-1562.

47. Lee H, Lee J, Chung H, Kim HC. Baseline Spectral Domain Optical Coherence Tomographic Hyperreflective Foci as a Predictor of Visual Outcome and Recurrence for Central Serous Chorioretinopathy. *Retina.* 2016;36(7):1372-1380.

48. Kang JW, Chung H, Chan Kim H. Correlation of Optical Coherence Tomographic Hyperreflective Foci with Visual Outcomes in Different Patterns of Diabetic Macular Edema. *Retina.* 2016;36(9):1630-1639.

49. Altay L, Scholz P, Schick T, et al. Association of Hyperreflective Foci Present in Early Forms of Age-Related Macular Degeneration With Known Age-Related Macular Degeneration Risk Polymorphisms. *Invest Ophthalmol Vis Sci.* 2016;57(10):4315-4320.

50. Kaszubski PA, Ben Ami T, Saade C, et al. Changes in reticular pseudodrusen area in eyes that progressed from early to late age-related macular degeneration. *Int Ophthalmol.* 2018;38(2):503-511.

51. Kersten E, Paun CC, Schellevis RL, et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv Ophthalmol.* 2018;63(1):9-39.

52. Lauwen S, de Jong EK, Lefeber DJ, den Hollander A. Omics Biomarkers in Ophthalmology. *Invest Ophthalmol Vis Sci.* 2017;58(6):BIO88-BIO98.

53. Muether PS, Hoerster R, Hermann MM, Kirchhof B, Fauser S. Long-term effects of ranibizumab treatment delay in neovascular age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2013;251(2):453-458.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Minor allele frequencies of 36 single nucleotide polymorphisms in AMD-associated risk genes in progressors and non-progressors and their association with progression to late AMD

			Progressors		Non-progressors				
Gene	**rs number**	**Minor allele**	**No. of minor alleles/ total no. of alleles**	**MAF**	**No. of minor alleles/ total no. of alleles**	**MAF**	**Odds Ratio**	**95% CI**	**P-value**
ADAMTS9AS2	rs6795735	T	36/90	0.400	138/338	0.408	0.969	0.596-1.575	0.899
APOE/TOMM40	rs2075650	G	14/90	0.156	45/338	0.133	1.289	0.646-2.572	0.472
APOE/APOC1	rs4420638	G	15/90	0.167	38/338	0.112	1.765	0.868-3.586	0.117
ARMS2	rs10490924	T	31/90	0.344	103/338	0.305	1.220	0.744-1.999	0.431
B3GALTL	rs9542236	C	38/90	0.422	158/338	0.467	0.904	0.555-1.472	0.684
C3	rs433594	T	44/90	0.489	134/334	0.401	1.604	0.949-2.710	0.077
C3	rs2230199	G	24/88	0.273	79/332	0.238	1.239	0.726-2.115	0.432
C3	rs1047286	A	23/90	0.256	77/330	0.233	1.152	0.666-1.990	0.613
CCDC109B	rs4698775	G	29/90	0.322	111/334	0.332	1.012	0.613-1.672	0.962
CETP	rs3764261	A	41/90	0.456	105/336	0.313	1.942	1.191-3.165	0.008
CFB	rs4151667	A	1/90	0.011	16/338	0.047	0.241	0.031-1.892	0.176
CFB	rs641153	A	4/90	0.044	26/338	0.077	0.520	0.170-1.590	0.251
CFH	rs800292	A	11/96	0.115	85/340	0.250	0.372	0.188-0.737	0.005
CFH	rs1061170	C	58/90	0.644	138/336	0.411	2.405	1.503-3.847	< 0.001
CFH	rs12144939	T	10/90	0.111	61/338	0.180	0.596	0.299-1.187	0.141
CFI	rs10033900	T	42/88	0.477	161/334	0.482	0.981	0.607-1.585	0.937
COL8A1	rs13081855	T	7/90	0.078	36/338	0.107	0.779	0.323-1.879	0.578
COL10A1	rs3812111	A	31/90	0.344	132/336	0.393	0.811	0.492-1.335	0.410
CYP24A1	rs1570669	G	34/90	0.378	104/336	0.310	1.334	0.836-2.129	0.227
FADS1	rs174547	C	35/90	0.389	111/338	0.328	1.326	0.794-2.214	0.281
GLI2	rs6721654	T	7/90	0.078	31/338	0.092	0.851	0.345-2.099	0.726
GLI3	rs2049622	A	43/90	0.478	175/334	0.524	0.832	0.531-1.303	0.421
IER3DDR	rs3130783	G	17/90	0.189	65/338	0.192	0.978	0.534-1.793	0.943

IGFR1	rs2872060	T	41/90	0.456	141/336	0.420	1.243	0.755-2.044	0.392
LIPC	rs10468017	T	25/90	0.278	92/336	0.274	1.015	0.618-1.667	0.954
LPL	rs12678919	G	13/90	0.144	35/332	0.105	1.526	0.735-3.171	0.257
MYRIP	rs2679798	G	46/90	0.511	155/338	0.459	1.227	0.776-1.940	0.381
PON1	rs705381	T	24/90	0.267	84/336	0.250	1.150	0.680-1.945	0.602
RAD51B	rs8017304	G	33/90	0.367	126/338	0.373	0.958	0.594-1.546	0.862
SKIV2L	rs429608	A	7/90	0.078	45/336	0.134	0.544	0.236-1.257	0.154
SLC16A8	rs8135665	T	19/90	0.211	77/336	0.229	0.940	0.521-1.695	0.836
TGFBR1	rs334353	G	18/90	0.200	81/338	0.240	0.772	0.426-1.396	0.391
TIMP3	rs9621532	C	5/90	0.056	16/336	0.048	1.121	0.382-3.284	0.835
TNFRSF10A	rs13278062	T	47/86	0.547	184/336	0.548	0.973	0.598-1.585	0.913
TYR	rs621313	G	49/88	0.557	167/336	0.497	1.283	0.802-2.052	0.298
VEGFA	rs943080	C	40/90	0.444	165/338	0.488	0.828	0.499-1.374	0.465

CI: Confidence Interval, MAF: Minor Allele Frequency

Supplementary Table 2. Overview of progression studies

Study	Cohort (patient number)	Mean FU in years (range)	Inclusion criteria	End point	Progression rate	Imaging	Demographic/ Environmental factors	Phenotypic factors	Genetic factors	Final model/Main findings	AUC
Roquet et al.[39] 2004	Retrospective study (32)	4.6y (1–17y)	-DPED	Progression to late AMD	13%-49%	FP, FA, ICG, OCT only at last visit in 30%	None	Size of dPED	None	Kaplan Meyer survival analyses: Patients with dPED have 50% chance of GA development in 7y, if dPED > 2DD or associated with metamorphopsia progression to late AMD occurs after 2y	-
Farwick et al.[6] 2010	MARS (722)	Not provided; median FU time 2.6y	-No AMD -Early AMD forms -Unilateral late AMD	Progression to early and late AMD	2% to 31.8%	FP	Age, sex, smoking	Baseline AMD status in the study and fellow eye	*ARMS2* (rs10490924) *CFH* (rs1061170) *C3* (rs2230199)	Logistic regression analyses: CFH rs1061170 was related to development of early AMD; ARMS2 rs10490924 was associated with progression to late AMD	-
Klein et al.[7] 2011	AREDS (2846)/ CAPT (297)	9.3y (Range not provided)	-No AMD -Early AMD forms -Unilateral late AMD	Progression to late AMD	24% to 82%	FP	Age, sex, education, BMI, smoking, diet, sunlight exposure, skin cancer, arthritis, hypertension, other cardiovascular diseases, diabetes, current and past medications and dietary supplements	AREDS simplified severity scale 0-4, presence of very large drusen (≥250µm), late AMD in 1 eye	*APOE* (rs7412, rs429358) *ARMS2* (rs10490924) *C2* (rs9332739) *C3* (rs2230199) *CFH* (rs1061170) *CFI* (rs13117504, rs10033900, rs2285714)	Cox proportional hazard model: Age, smoking, family history of AMD, modified AREDS simple scale score, presence of very large drusen, late AMD in 1 eye, *CFH* (rs1061170), *ARMS2* (rs10490924)	0.87
Yu et al.[10] 2012	AREDS (2560)	10.3y (2-13y)	-No AMD -Early AMD forms	Drusen size progression and progression to late AMD	19.2% to 22.5%	Not provided	Age, sex, education, BMI, smoking, antioxidant treatment	Baseline AMD status of fellow eye	*ABCA1* (rs1883025) *APOE* (rs7412/ rs429358) *ARMS2* (rs10490924) *C2* (rs9332739) *C3* (rs2230199) *CETP* (rs3764261) *CFB* (rs641153) *CFH* (rs1061170) *CFI* (rs10033900) *COL8A1* (rs13095226) *LIPC* (rs10468017) *TIMP3* (rs9621532)	Multistate Markov model: All 12 genetic variants, age, sex, BMI, smoking, education, antioxidant treatment, fellow eye status	0.90

Christenbury et al.[15] 2013	AREDS2 Ancillary SD-OCT Study (299)	2y (Range not provided)	-Intermediate AMD with large drusen (≥125µm) in at least one eye -Unilateral late AMD	Progression of HF number and distribution, progression to noncentral GA or any late AMD	46.1%	FP, SD-OCT	None	Presence, number and axial distribution of HF, axial distribution score, RPE elevation/ drusen, RPE atrophy, epiretinal membrane, cystoid macular edema, subretinal fluid	None	Logistic regression analyses: Presence of baseline HF, number of HF and axial distribution score are correlated with GA development at 2y	-
Perlee et al.[9] 2013	AREDS (2415)	Not provided	-No AMD -Early AMD forms -Unilateral late AMD	Progression to late AMD (CNV or GA)	CNV: 25% GA: 16%	FP	Age, sex, education, BMI, smoking, AREDS treatment category	AREDS Simplified Severity Scale	*ARMS2* (rs10490924) *C2* (rs9332739) *C3* (rs2230199) *CFB* (rs641153) *CFH* (rs1061170, rs2274700, rs403846, rs12144939) *CFHR4* (rs1409153) *CFHR5* (rs1750311, rs10922153) *F13B* (rs698859, rs2990510) *HTRA1* (rs11200638)	Cox proportional hazard model: For CNV: Age, smoking, all genetic variants (except *CFH* rs403846 and HTRA1 rs11200638), AREDS Simplified Severity Scale For GA: rs2274700, rs10490924, rs2230199, rs9332739, GA in Non-study Eye at Baseline, AREDS Simplified Severity Scale	0.96 0.94
Jonasson et al.[27] 2014	AGES (2864)	5y (Range not provided)	-No AMD -Early AMD forms -Late AMD	Progression from early to late AMD	22.7%	FP	Age, sex ,BMI, smoking, cod liver oil use, hypertension, pulse pressure, diabetes, total cholesterol, HDL cholesterol, CRP	None	None	Multivariate logistic regression analyses: For nAMD: Age and female sex were associated with progression For GA: Age and plasma HDL cholesterol were associated with progression	-
de Sisternes et al.[13] 2014	Retrospective study (244)	Not provided; (0.5-5y)	-Early AMD forms -Unilateral late AMD	Progression to nAMD	12.7%	SD-OCT	Age, sex, time since diagnosis	Automatic quantification of drusen number, extent, area, volume, shape, density, and reflectivity characteristics and evolution of each of these characteristics over time, fellow eye status	None	L1 penalized Poison model: All quantitative drusen features, age, sex, nAMD in fellow eye, time since diagnosis	0.70-0.92
Seddon et al.[4] 2015	AREDS (2951)	8.8y (0.5-13y)	-No AMD -Early AMD forms -Unilateral late AMD	Progression to late AMD	28.3%	FP	Age, sex, education, BMI, smoking, AREDS treatment category	Baseline AMD status Largest drusen size	*ARMS2* (rs10490924) *C2* (rs9332739) *C3* (rs2230199, rs147859257) *C9* (rs34882957) *CFB* (rs641153) *CFH* (rs1061170, rs1410996, rs121913059) *COL8A1* (rs13095226) *RAD51B* (rs8017304)	Cox proportional hazard model: all genetic variants (except *C9* variant), BMI and smoking, adjusted for age, sex, education, baseline AMD status and AREDS treatment groups	0.91

Study	Cohort (patient number)	Mean FU in years (range)	Inclusion criteria	End point	Progression rate	Imaging	Demographic/ Environmental factors	Phenotypic factors	Genetic factors	Final model/Main findings	AUC
Joachim et al.[14] 2015	BMES I-IV (3281)	Not provided; FU up to 15y	-No AMD -Early AMD forms with drusen <125µm	Progression of medium drusen to worse AMD stages and to late AMD	5%- 50%	FP	Age, sex ,smoking, blood pressure, serum lipid levels (total cholesterol, HDL cholesterol, triglycerides), white blood cell count, fish consumption, alcohol consumption, antioxidant and zinc supplementation intake, dietary lutein and zeaxanthin intake	Medium drusen area diameter, central drusen location, pigmentary abnormalities	*ARMS2* (rs10490924) *CFH* (rs1061170)	Generalized linear model (GEE): Medium drusen area and central location of medium drusen adjusted for age, sex, smoking, fish consumption, *CFH* and *ARMS2* risk alleles	-
Joachim et al.[11] 2015	BMES I-IV (2474)	5.1 to 15.6y (3.4-17.7y)	-No AMD -Early AMD forms -Unilateral late AMD	Progression from early to late AMD	1.2% to 76.5%	FP	Age, sex, smoking, fish consumption	AREDS Simplified Severity Scale, eye-specific characteristics: maximum drusen size, drusen type, drusen location, drusen area, retinal pigment abnormality	*ARMS2* (rs10490924) *CFH* (rs1061170)	Multivariable logistic regression model (GEE): All eye-specific characteristics adjusted for age, sex, smoking, fish consumption, *CFH* and *ARMS2*	-
Folgar et al.[24] 2016	AREDS2 Ancillary SD-OCT study (325)	2y (Range not provided)	-Intermediate AMD with large drusen (≥125µm) in at least one eye -Unilateral late AMD	Progression to noncentral GA or any late AMD	20.1%-21.3%	FP, SD-OCT	None	Semiautomated segmentation of RPE-drusen complex (RPEDC) volume, drusen volume and RPEDC abnormal thinning volumes	None	Bivariate logistic regression analyses: <u>For CNV:</u> RPEDC volume and drusen volume associated with progression <u>For (noncentral) GA:</u> RPEDC abnormal thinning associated with progression	-
Abdelfattah et al.[12] 2016	Retrospective cohort (89)	2y (Range not provided)	-No or early AMD forms in study eye and late nAMD in fellow eye	Progression to late AMD	38.2%	HD-OCT	Age, sex, smoking, hypertension, diabetes, cardiovascular diseases	Automatic quantification of drusen volume	None	Univariate logistic regression analyses: Drusen volume was associated with progression	-
Sardell et al.[5] 2016	VEI and BPEI (372)	2.7y (1 month-13y)	-Intermediate AMD in study eye	Progression to late AMD	36.8%	FP	Age, sex	None	*ARMS2* (rs10490924) *C2* (rs429608, rs9332739) *C3* (rs2230199) *CFB* (rs641153) *CFH* (rs10737680[rs6677604], rs1061170)	Cox proportional hazard model: Age, sex, *CFH* (rs10737680)	0.67

Veerappan et al.[30] 2016	AREDS2 Ancillary SD-OCT study (307)	3y (Range not provided)	-Intermediate AMD with large drusen (≥125µm) in at least one eye -Unilateral late AMD	Progression to noncentral GA or any late AMD	-	FP, SD-OCT	Age, smoking, statin use	OCT-reflective drusen substructures, drusen volume, GA, preatrophic features, intra- and subretinal fluid, CNV, HF, subretinal lesions, neurosensory retina volume	None	OCT-reflective drusen substructures are predictive of accelerated progression to late atrophic AMD	-
Fragiotta et al.[16] 2017	Retrospective study (73)	3.3-3.5y (Range not provided)	-Early AMD forms -Unilateral late AMD	Progression to late nAMD	-	SD-OCT	Age, sex	Type of drusen, integrity of external limiting membrane/ inner ellipsoid band/RPE, HF, DPED (incl max height and width), choroidal thickness, CNV in fellow eye	None	Binary logistic regression analyses: Presence of HF and DPED width were associated with progression to late nAMD	-
Ding et al.[8] 2017	AREDS (2721)/ AREDS2 (1700)	10.3y (1.8-12.6y)/ 4.8y (2.1-5.9)	-No AMD -Early AMD forms -Unilateral late AMD NB. Both eyes of the same participant were analyzed separately accounting for between-eye correlation	Progression to noncentral GA or any late AMD	3% to 81%/ 9%-50%	Not provided	Age, sex, education, smoking, AREDS treatment category	Baseline AREDS AMD severity score in the study and fellow eye	Genetic risk score (GRS) based on 34 top AMD risk variants from Fritsche et al.[5]	Cox proportional hazard model: Age, smoking, education, baseline severity score for study and fellow eye, GRS	0.91 (AREDS) / 0.74 (AREDS2)
Ferrara et al.[40] 2017	Case-control study (80)	5.2-5.6y (Range not provided)	-Early AMD forms -Unilateral late AMD	Progression to late AMD	-	FP, FAF, FA, SD-OCT	Age, sex	Retinal thickness measurements, photoreceptor layer integrity, RPE abnormalities, nascent GA, choroidal abnormalities	None	Multivariate stepwise logistic regression model: Total retinal thickness, RPE pigmentary hyperreflective material, features of nascent GA, choroidal vessels irregularaties	-
Lei et al.[26] 2017	Retrospective study (138)	2.4y (1–5.3y)	-Early AMD forms -Unilateral late AMD	Progression to late AMD	39.9%	SD-OCT	None	Drusen volume ≥ 0.03 mm3 within a 3-mm circle, intraretinal HF, hyporeflective foci within a drusenoid lesion and subretinal drusenoid deposits for study and fellow eye	None	Correlation and logistic regression analyses: All four OCT-features were correlated with progression to late AMD. Based on these SD-OCT features a progression scoring system was developed	-

AMD: Age-Related Macular Degeneration, AUC: Area Under The Curve, BMI: Body Mass Index, CNV: Choroidale Neovascularisation, CRP: C-reactive Protein, DD: Disc Diameter, DPED: Drusenoid Pigment Epithelial Detachment, FA: Fluorescein Angiography, FAF: Fundus Autofluorescence, FU: Follow-Up, GA: Geographic Atrophy, GEE: Generalized Estimated Equations, HDL: High-Density Lipoprotein, HF: Hyperreflective Foci, ICG: Indocyanine Green Angiography , NB: Nota Bene, nAMD: Neovascular AMD, OCT: Optic Coherence Tomography, RPD: Reticular Pseudodrusen, RPE: Retinal Pigment Epithelium, Y: Years

Chapter 2.4
Phenotype Characteristics of Patients With Age-Related Macular Degeneration Carrying a Rare Variant in the *Complement Factor H* Gene

Eveline Kersten
Maartje J. Geerlings
Anneke I. den Hollander
Eiko K. de Jong
Sascha Fauser
Tunde Peto
Carel B. Hoyng

Published in: JAMA Ophthalmology. 2017 Oct 1;135(10):1037-1044.

ABSTRACT

Importance: Rare variants in the complement factor H (*CFH*) gene and their association with age-related macular degeneration (AMD) have been described. However, there is limited literature on the phenotypes accompanying these rare variants. Phenotypical characteristics could help ophthalmologists select patients for additional genetic testing.

Objective: To describe the phenotypical characteristics of patients with AMD carrying a rare variant in the *CFH* gene.

Design, setting and participants: In this cross-sectional study, we searched the genetic database of the department of ophthalmology at the Radboudumc (tertiary ophthalmologic referral center) and the European Genetic Database for patients with AMD with a rare genetic variant in the *CFH* gene. Patient recruitment took place from March 30, 2006, to February 18, 2013, and data were analyzed from November 30, 2015, to May 8, 2017. Phenotypical features on fundus photographs of both eyes of patients were graded by two independent reading center graders masked for carrier status.

Main Outcomes and Measures: Differences in phenotypical characteristics between rare variant carriers and noncarriers were analyzed using univariable generalized estimated equations logistic regression models accounting for intereye correlation.

Results: Analyses included 100 eyes of 51 patients with AMD carrying a *CFH* variant (mean [SD] age, 66.7 [12.1] years; 64.7% female) and 204 eyes of 102 age-matched noncarriers (mean [SD] age, 67.1 [11.8] years; 54.9% female). Carrying a rare pathogenic *CFH* variant was associated with larger drusen area (odds ratio range, 6.98 [95% CI, 2.04-23.89] to 18.50 [95% CI, 2.19-155.99]; P = 0.002), presence of drusen with crystalline appearance (odds ratio, 3.24; 95% CI, 1.24-8.50; P = 0.02), and drusen nasal to the optic disc (odds ratio range, 4.03 [95% CI, 1.70-9.56] to 7.42 [95% CI, 0.65-84.84]; P = 0.003).

Conclusions and Relevance: Identification of rare *CFH* variant carriers may be important for upcoming complement-inhibiting therapies. Patients with an extensive drusen area, drusen with crystalline appearance, and drusen nasal to the optic disc are more likely to have a rare variant in the *CFH* gene. However, it is not likely that carriers can be discriminated from noncarriers based solely on phenotypical characteristics from color fundus images. Therefore, ophthalmologists should consider genetic testing in patients with these phenotypic characteristics in combination with other patient characteristics, such as early onset, cuticular drusen on fluorescein angiography, and family history of AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is a common multifactorial eye disease in Western countries,[1] however the exact pathophysiology of the disease is not yet completely understood. Environmental factors, such as age and smoking,[2,3] and both common and rare genetic variants have been identified as risk factors for AMD.[4] A large number of these genetic variants are located in genes encoding components of the complement system. Additionally, higher local and systemic complement activity has been reported in patients with AMD compared with control individuals.[5-7] Together, these findings implicate a pivotal role of the complement system in AMD.

Rare genetic variants located in the complement factor H (*CFH*) gene are among the variants that confer the highest risk for AMD.[4,8,9] The *CFH* gene encodes factor H (FH), a regulator of the alternative pathway of the complement system. Factor H inhibits the C3-convertase (C3bBb) and also acts as cofactor for factor I-mediated inactivation of C3b,[10] leading to decreased activity and thereby preventing overactivation of the complement system. Several studies showed lower systemic FH levels in patients carrying a rare *CFH* variant.[10,11] Furthermore, functional studies have reported an altered function of FH in patients carrying a rare variant in *CFH* resulting in increased complement activation despite normal systemic FH levels.[9,12,13]

While antivascular endothelial growth factor treatment is available for neovascular AMD, there is currently no effective treatment available for the early and atrophic stages of AMD. Because the complement system plays an important role in AMD pathogenesis, therapies targeting different components of the complement system are being developed. Currently, a number of phase 2/3 clinical trials are in progress, and so far two phase 2 trials have been completed with mixed results.[9,14,15] The Complement Inhibition With Eculizumab for the Treatment of Non-Exudative Age-Related Macular Degeneration (COMPLETE) study did not show decreased atrophy progression after administration of eculizumab,[16] while the MAHALO study showed beneficial effect of lampalizumab treatment on reducing atrophy progression.[17] With upcoming therapies targeting the complement system, it may be important to identify the patients who will most likely benefit from these therapies. Patients carrying a rare variant in the *CFH* gene seem to be a very suitable patient group for complement inhibiting therapies because of the associated functional consequences on complement activation.[12] However, it is expensive to genetically screen every patient with AMD in a diagnostic setting; therefore, it is desirable to preselect cases for genotyping based on phenotype. Unfortunately, there is limited literature on the phenotypes accompanying these *CFH* variants. Previously, a higher burden of extramacular drusen was reported in families carrying rare *CFH* variants compared with unrelated AMD cases; however, other distinct phenotypical characteristics were not described.[11] Another study described phenotypical characteristics in a more detailed manner,

but only included individuals carrying the rare p.Arg1210Cys variant in *CFH*.[18] We hypothesize that all pathogenic *CFH* variants share phenotypical characteristics owing to their functional influences on FH. Detailed characterization of phenotypes caused by a broad spectrum of rare *CFH* variants is lacking. Therefore, we aim to describe the phenotypical characteristics of patients with AMD carrying a rare variant in the *CFH* gene. A distinct phenotype description of these *CFH* carriers will enable ophthalmologists to select patients for additional genetic testing and complement-inhibiting therapies more efficiently.

METHODS

Study Population

In this retrospective cross-sectional study, we searched the genetic database of the department of ophthalmology at the Radboud university medical center, Nijmegen, the Netherlands (Radboudumc) and the European Genetic Database (EUGENDA), a multicenter database for clinical and molecular analysis of AMD, for individuals with a rare genetic variant in the *CFH* gene. Patient recruitment took place from March 30, 2006, to February 18, 2013. We selected AMD cases carrying protein-altering variants with a population frequency of less than 1%. We defined AMD as the presence of at least 10 small drusen (<63 µm) and pigmentary changes, intermediate or large drusen (≥63 µm), or late AMD, including subfoveal geographic atrophy (GA) and/or choroidal neovascularization (CNV) in at least one eye on color fundus images. Details of this classification are described elsewhere.[19] In total, 51 patients, with 33 different *CFH* variants, were identified and included in this study, hereafter referred to as carriers. Additionally, for each carrier, we selected from the European Genetic Database two similarly aged AMD cases (±2 years) without a rare genetic variant associated with AMD; these cases were defined as noncarriers (n = 102). For two carriers color fundus images of only one eye were available; therefore, final analyses included 100 eyes of 51 carriers and 204 eyes of 102 noncarriers. All participants indicated to be of European descent. Written informed consent was provided by all participants. The study was approved by the local ethics committee on research involving human participants, Commissie Mensgebonden Onderzoek Regio Arnhem–Nijmegen, and the local committee of University Hospital Cologne and was performed in accordance with the tenets of the Declaration of Helsinki.

Genotyping

Whole-exome sequencing (WES) and/or Sanger sequencing was previously performed. For both approaches, DNA was extracted from venous blood using standard procedures.

Most *CFH* carriers (n = 42) were identified through WES. Preparation and sequencing of the DNA samples were done as previously described.[12] In short, exome capture Nimblegen

SeqCap EZ V2 kit (Roche) paired-end sequencing was performed on an Illumina HiSeq2000 sequencer using TruSeq V3 chemistry (Illumina) followed by downstream quality control and genotyping of the samples. For this study, WES data were filtered specifically for the *CFH* gene (HUGO Gene Nomenclature Committee ID: 4883; NM_000186). Additional filtering steps on the data were implemented to select genetic variants that result in a splice-site or protein change (non-synonymous) as these variants are more likely to be pathogenic. We focused on rare genetic variants only (minor allele frequency ≤ 1%) as based on the Exome Aggregation Consortium (ExAC) database, specifically the non-Finnish European population.[20] Individual variants were confirmed with Sanger sequencing using primers designed by Primer3 software.[21] The remainder of *CFH* carriers (n = 9) was identified through conventional Sanger sequencing of the entire *CFH* gene as described in detail previously.[22] We excluded rare *CFH* variants with a described protective effect in case-control analyses (c.2850G>T p.Gln950His) or a likely benign effect in functional studies (c.2669G>T p.Ser890Ile, c.2867C>T p.Thr956Met, c.3019G>T p.Val1007Leu).[9] All *CFH* variants included in this study are described in Supplementary Table 1.

For all noncarriers, WES data were available and screened for rare genetic variants in the *CFH*, *CFI*, *C3* and *C9* genes associated with AMD. Only individuals without any rare variant in the *CFH* gene or a described pathogenic rare variant in the other AMD-associated genes[9] were included in this study as noncarriers.

Image Assessment

Digital 35° or 40° field of view color fundus photographs centered on the fovea were performed with a Topcon TRC 50IX camera (Topcon Corporation) or Canon 60UVi fundus camera (Canon), respectively. Color fundus photographs were analyzed for this study by two senior graders from an independent reading center (Moorfields Eye Hospital, London, England, UK) according to a standardized grading protocol. The following fundus features were assessed: predominant type of drusen, largest type of drusen in the central field, percentage of the area of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid covered with drusen, presence of extramacular drusen (defined as drusen outside the ETDRS grid), drusen nasal to the optic disc, reticular drusen, drusen with crystalline appearance, serogranular/serous drusen pigment epithelium detachment, pigmentary abnormalities, geographic atrophy, or signs of neovascularization.

Statistical analysis

Data were analyzed from November 30, 2015 to May 8, 2017. Demographic characteristics of the two study groups were compared using one-way analysis of variance or the χ^2 test. Phenotypical characteristics were individually assessed using binary logistic regression models. Generalized estimating equations procedures were used to correct for the fellow

eye. To compare the frequencies of late AMD subtypes between carriers and noncarriers, we performed a χ^2 test based on the more severely affected eye of each patient. In case both geographic atrophy and choroidal neovascularization were present in an individual, it was classified as mixed late AMD. A phenotypic risk score for each eye was calculated as the sum of regression coefficients of all individual phenotypical characteristics resulting from univariable generalized estimating equations logistic regression analyses. A receiver operating characteristic curve was obtained and the area under the curve was measured for this risk score. Finally, symmetry between eyes was calculated as follows: number of equal phenotypical characteristics between right and left eye divided by the number of phenotypical characteristics graded times 100%.
All statistical analyses were performed using SPSS statistics software (released 2013; IBM SPSS Statistics for Windows, Version 22.0; IBM Corp).

RESULTS

In total, 100 eyes of 51 carriers and 204 eyes of 102 noncarriers were included for analyses. Demographic and environmental characteristics were comparable between carriers and noncarriers (Table 1). The frequency of common genetic variants in *CFH*, *ARMS2,* and *C3* seems to be slightly higher in noncarriers compared with carriers. However, the minor allele frequencies of these common variants in noncarriers are comparable with the general AMD population.[23] This may imply that carriers of rare *CFH* variants are less burdened by common AMD risk variants and that their AMD risk is rather attributable to the rare variants.

When comparing the fundus features by carrier status, the odds of carrying any rare *CFH* variant increases with increasing drusen area within the ETDRS grid (odds ratio [OR] up to 6.85 when more than 50% of the ETDRS grid is covered with drusen, P = 0.004), and with the presence of serogranular/serous drusen pigment epithelium detachment (OR, 4.74; 95% CI, 1.30-17.31; P = 0.02). Additionally, drusen deposition in rare variant carriers is often not limited to the central retina; these carriers tend to have extramacular drusen (80.8%) and drusen nasal to the optic disc (43.8%) more frequently than noncarriers (73.4% and 35.1%, respectively), although these differences were not significant. The association of all assessed fundus features of carriers and noncarriers are shown in Table 2.

Because the carrier group contains both rare variants known to be associated with AMD and rare variants of unknown clinical significance, we repeated the analyses with stricter inclusion criteria comparing only cases carrying a known pathogenic variant (n = 25) with noncarriers (Table 3). Known pathogenic variants included rare *CFH* variants associated with AMD in case-

Table 1. General Characteristics of the Study Groups

Characteristic	Noncarriers (No. [%]) (n = 102; 204 eyes)	Carriers (No. [%]) (n = 51; 100 eyes)
Age at participation, mean (SD), y	67.1 (11.8)	66.7 (12.1)
Sex		
Male	46 (45.1)	18 (35.3)
Female	56 (54.9)	33 (64.7)
Smoking status		
Never	21 (22.3)	16 (39.0)
Past	53 (56.4)	17 (41.5)
Current	20 (21.3)	8 (19.5)
BMI, mean (SD)	26.1 (4.1)	26.5 (4.2)
Family history for AMD		
Yes	52 (58.4)	27 (67.5)
No	37 (41.6)	13 (32.5)
Common genetic variants, No. of minor allels/total No. of alleles (MAF %)		
ARMS2, rs10490923, T	75/176 (42.6)	17/74 (23.0)
CFH, rs1061170, C	99/176 (56.3)	29/76 (38.2)
C3, rs2230199, G	49/174 (28.2)	10/76 (13.2)

Abbreviations: *ARMS2*, age-related maculopathy susceptibility 2; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); *C3*, complement component 3; *CFH*, complement factor H; MAF, minor allele frequency.

control or segregation analyses or with a described functional effect. This subanalysis showed an even higher association between drusen area within the ETDRS grid and rare pathogenic *CFH* variant carriers (OR range, 6.98 [95% CI, 2.04-23.89] to 18.50 [95% CI, 2.19-155.99]; P = 0.002). Additionally, intermediate and large drusen located nasal to the optic disc (OR range, 4.03 [95% CI, 1.70-9.56] to 7.42 [95% CI, 0.65-84.84]; P = 0.003) and the presence drusen with crystalline appearance (OR, 3.24; 95% CI, 1.24-8.50; P = 0.02) were significantly associated with carrying a rare pathogenic *CFH* variant. Subanalysis of late AMD cases only (n = 71) showed a higher frequency of late atrophic AMD in rare pathogenic variant carriers (57.1%) compared with noncarriers (28.1%), although this was not significantly different (P = 0.12). Notably, the association between serogranular/serous drusen pigment epithelium detachment and carrier status did not remain significant but still tended to increase the odds of carrying a rare *CFH* variant. Examples of color fundus photographs of carriers of rare *CFH* variants with the associated fundus features are displayed in Figure 1.

Table 2. Phenotypical Characteristics of Carriers and Noncarriers of Rare CFH Variants

	No. of Eyes (%)			
Phenotypic characteristic	**Noncarrier (n = 204)**	**Carrier (n = 100)**	**Odds ratio (95% CI)[a]**	***P-value***
Predominant drusen type within ETDRS grid				
None or small drusen (<63 µm)	32 (15.7)	12 (12.0)	Reference	
Intermediate drusen (63-125 µm)	107 (52.5)	45 (45.0)	1.12 (0.47-2.68)	0.31
Large drusen (>125 µm)	65 (31.9)	43 (43.0)	1.76 (0.71-4.38)	
Largest drusen type within central field				
None or small drusen (<63 µm)	106 (52.5)	44 (44.9)	Reference	
Intermediate drusen (63-125 µm)	77 (38.1)	44 (44.9)	1.38 (0.75-2.52)	0.58
Large drusen (>125 µm)	19 (9.4)	10 (10.2)	1.27 (0.46-3.48)	
Proportion of grid area covered by drusen, %				
0-10	111 (54.4)	27 (27.3)	Reference	
10-25	61 (29.9)	41 (41.4)	2.76 (1.36-5.63)	0.004[b]
25-50	29 (14.2)	26 (26.3)	3.69 (1.58-8.58)	
>50	3 (1.5)	5 (5.1)	6.85 (1.37-34.37)	
Extramacular drusen				
Absent	54 (26.6)	19 (19.2)	Reference	0.27
Present	149 (73.4)	80 (80.8)	1.53 (0.72-3.24)	
Drusen nasal to the optic disc				
None or small drusen (<63 µm)	89 (65.0)	45 (56.3)	Reference	
Intermediate drusen (63-125 µm)	46 (33.6)	32 (40.0)	1.38 (0.69-2.74)	0.47
Large drusen (>125 µm)	2 (1.5)	3 (3.8)	2.97 (0.30-29.51)	
Reticular drusen				
Absent	163 (86.7)	83 (93.3)	Reference	0.14
Present	25 (13.3)	6 (6.7)	0.47 (0.18-1.27)	
Drusen with crystalline appearance				
Absent	178 (89.4)	76 (81.7)	Reference	0.15
Present	21 (10.6)	17 (18.3)	1.90 (0.80-4.48)	
SPED				
Absent	199 (97.5)	84 (89.4)	Reference	0.02
Present	5 (2.5)	10 (10.6)	4.74 (1.30-17.31)	
RPE pigmentation				
Absent	84 (45.4)	31 (32.3)	Reference	0.08
Present	101 (54.6)	65 (67.7)	1.74 (0.93-3.27)	
Geographic atrophy				
Absent	154 (76.2)	65 (70.7)	Reference	0.42
Present	48 (23.8)	27 (29.3)	1.33 (0.66-2.69)	
Neovascular AMD				
Absent	144 (72.4)	79 (82.3)	Reference	0.12
Present	55 (27.6)	17 (17.7)	0.56 (0.28-1.15)	

Abbreviations: AMD, age-related macular degeneration; *CFH*, complement factor H; ETDRS, Early Treatment Diabetic Retinopathy Study; RPE, retinal pigment epithelium; SPED, serogranular/serous drusen pigment epithelium detachment.

[a] The presented odds ratios result from univariable generalized estimating equations logistic regression analyses.

[b] *P*-value remained significant after Bonferroni correction for multiple testing.

Table 3. Associations of phenotypical characteristics with confirmed pathogenic rare *CFH* variants

	No. of Eyes (%)			
Phenotypic characteristic	**Noncarrier (n = 204)**	**Carrier (n = 48)**	**Odds ratio (95% CI)[a]**	***P-value***
Predominant drusen type within ETDRS grid				
None or small drusen (<63 µm)	32 (15.7)	4 (8.3)	Reference	
Intermediate drusen (63-125 µm)	107 (52.5)	18 (37.5)	1.35 (0.34-5.38)	0.06
Large drusen (>125 µm)	65 (31.9)	26 (54.2)	3.20 (0.80-12.75)	
Largest drusen type within central field				
None or small drusen (<63 µm)	106 (52.5)	24 (50.0)	Reference	
Intermediate drusen (63-125 µm)	77 (38.1)	19 (39.6)	1.16 (0.29-4.66)	0.97
Large drusen (>125 µm)	19 (9.4)	5 (10.4)	1.09 (0.50-2.39)	
Proportion of grid area covered by drusen, %				
0-10	111 (54.4)	6 (12.5)	Reference	
10-25	61 (29.9)	23 (47.9)	6.98 (2.04-23.89)	0.002[b]
25-50	29 (14.2)	16 (33.3)	10.21 (2.85-36.59)	
>50	3 (1.5)	3 (6.3)	18.50 (2.19-155.99)	
Extramacular drusen				
Absent	54 (26.6)	6 (12.5)	Reference	0.11
Present	149 (73.4)	42 (87.5)	2.54 (0.80-8.04)	
Drusen nasal to the optic disc				
None or small drusen (<63 µm)	89 (65.0)	12 (30.8)	Reference	
Intermediate drusen (63-125 µm)	46 (33.6)	25 (64.1)	4.03 (1.70-9.56)	0.003[b]
Large drusen (>125 µm)	2 (1.5)	2 (5.1)	7.42 (0.65-84.84)	
Reticular drusen				
Absent	163 (86.7)	43 (93.5)	Reference	0.23
Present	25 (13.3)	3 (6.5)	0.46 (0.13-1.64)	
Drusen with crystalline appearance				
Absent	178 (89.4)	34 (72.3)	Reference	0.02
Present	21 (10.6)	13 (27.7)	3.24 (1.24-8.50)	
SPED				
Absent	199 (97.5)	43 (91.5)	Reference	0.11
Present	5 (2.5)	4 (8.5)	3.70 (0.74-18.63)	
RPE pigmentation				
Absent	84 (45.4)	13 (28.3)	Reference	0.10
Present	101 (54.6)	33 (71.7)	2.11 (0.87-5.14)	
Geographic atrophy				
Absent	154 (76.2)	28 (65.1)	Reference	0.23
Present	48 (23.8)	15 (34.9)	1.72 (0.71-4.14)	
Neovascular AMD				
Absent	144 (72.4)	38 (82.6)	Reference	0.23
Present	55 (27.6)	8 (17.4)	0.55 (0.21-1.47)	

Abbreviations: AMD, age-related macular degeneration; *CFH*, complement factor H; ETDRS, Early Treatment Diabetic Retinopathy Study; RPE, retinal pigment epithelium; SPED, serogranular/serous drusen pigment epithelium detachment.

[a] The presented odds ratios result from univariable generalized estimating equations logistic regression analyses.

[b] *P*-value remained significant after Bonferroni correction for multiple testing.

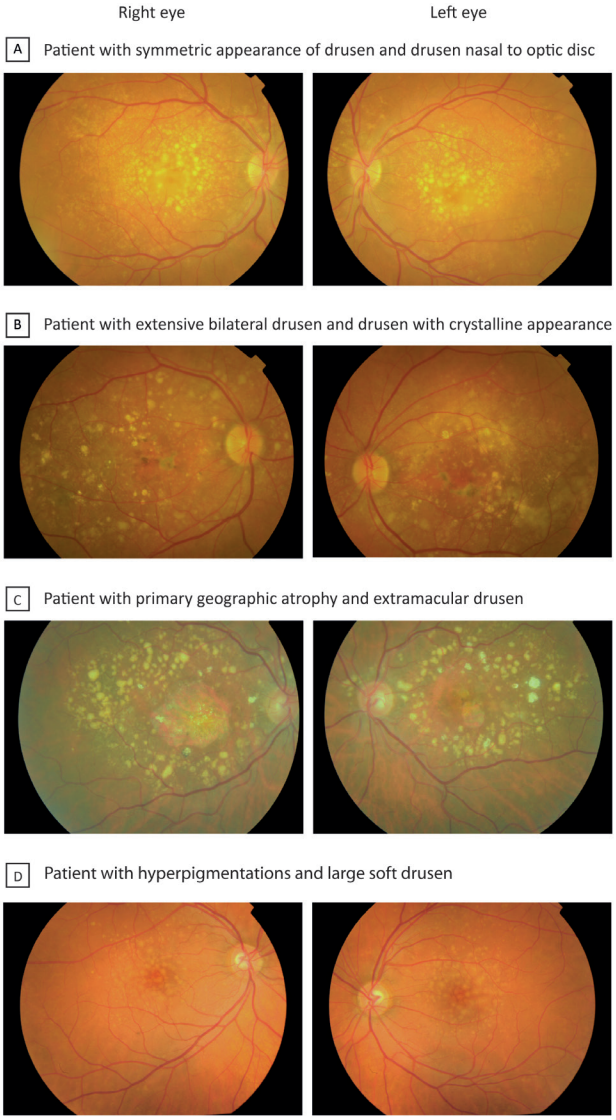


Figure 1. Color fundus photographs of carriers of rare variants in the complement factor H (*CFH*) gene
A. A woman in her 50s (*CFH* c.2537A>G, p.Gln846Arg) with a symmetric appearance of extensive drusen deposition within the Early Treatment Diabetic Retinopathy Study grid extending beyond the inferior and superior retinal arcades and nasal to the optic disc in both eyes. B. A man in his 60s (*CFH* c.550delA, p.Ile184Leufs*33) with extensive bilateral drusen deposition inside and outside the Early Treatment Diabetic Retinopathy Study grid and nasal to the optic disc, presence of drusen with crystalline appearance, and hypopigmentations and hyperpigmentations. C. Woman in her 70s (*CFH* c.524G>A, p.Arg175Gln) with primary geographic atrophy surrounded by predominantly large drusen, some with crystalline appearance, beyond the retinal arcades and the optic disc. D. A man in his 40s (*CFH* c.1198C>A, p.Gln400Lys) with hyperpigmentations and mainly centrally located large soft drusen but also extending to the peripheral retina.

Overall, rare *CFH* variant carriers tend to have more and larger drusen, and drusen are more often located outside the ETDRS grid. However, not all of these analyzed phenotypical characteristics individually reach statistical significance. Based on the findings in Table 3, we calculated a phenotypic risk score for each eye including all assessed phenotypical characteristics (Supplementary Figure 1). The mean (SD) phenotypic risk score in carriers (4.35 [2.0]) was significantly higher compared with noncarriers (2.32 [2.5]), although the ability to accurately discriminate between eyes of carriers of pathogenic *CFH* variants and noncarriers based on the phenotypic risk score was limited (area under the curve, 0.75; 95% CI, 0.65-0.85; Supplementary Figure 2). Similar results were obtained when including only the highest phenotypic risk score for each patient (area under the curve, 0.75; 95% CI, 0.61-0.88). Finally, for every patient, the grade of symmetry between eyes was determined based on the number of equal characteristics. Each study group showed a high grade of symmetry between the eyes (79.9% in noncarriers vs 79.1% in carriers of pathogenic variants) and the groups were not significantly different (P = 0.85).

DISCUSSION

In this study, we aimed to describe the phenotypical characteristics of patients with AMD carrying a rare variant in the *CFH* gene. Overall, rare *CFH* variant carriers have a more severe phenotype with more and larger drusen, often extending to the peripheral retina. Larger drusen area within the ETDRS grid and drusen located nasal to the optic disc were significantly associated with patients with AMD carrying a rare pathogenic *CFH* variant. These findings are in line with previous studies reporting extensive macular drusen accumulation and presence of extramacular drusen in patients with AMD carrying the *CFH* p.Arg1210Cys variant[18] and other rare *CFH* variants.[11]

In addition, we report an association between the presence of drusen with crystalline appearance and carrying a rare variant in *CFH*. Drusen with crystalline appearance, also known as refractile or calcified drusen, have a characteristic glistening appearance on color fundus imaging and have been associated with the development of geographic atrophy.[24,25] Thus, these patients might be at higher risk for developing geographic atrophy compared with noncarriers. In the current study, rare *CFH* variant carriers seem to develop geographic atrophy more often than choroidal neovascularization, as was already observed in rare variant carriers of other complement genes (*CFI*, *C3*, and *C9*).[26] However, probably owing to the small number of patients with late AMD, this difference was not significant.

From literature, it is known that *CFH* carriers usually have an earlier age at onset.[8,11,13,22,26,27] Owing to our study design a lower age at onset in rare variant carriers could not be analyzed. Our study was merely designed to analyze phenotypical differences between rare *CFH* carriers

and noncarriers; therefore, age-matched noncarriers were selected. As a consequence, no difference in age at onset could be observed. However, assessing the age at onset remains an important clue for ophthalmologists when considering (rare) genetic variants in a patient. Familial burden is also known to be associated with rare *CFH* variant carriers.[11,13,18,26,28] Although the number of carriers with a family history of AMD (64.7%) was not significantly different from noncarriers (53.9%), it must be emphasized that family history was obtained through interviewer-assisted questionnaires. From previous studies, it is known that *CFH* carriers often have asymptomatic family members[29,30] and, therefore, it is plausible that the percentage of carriers with a family history of AMD is underestimated.

Assuming that rare protein-altering variants located in the *CFH* gene lead to similar phenotype, this study was not restricted to one or more specific *CFH* variants but included a wide variety of rare protein-altering *CFH* variants identified by WES or Sanger sequencing in our cohort. Therefore, our analyses also included some variants that were not described before in the literature. However, when limiting the analyses to confirmed pathogenic variants only, the associations between rare variant carriers and phenotypical characteristics become more pronounced. More information on pathogenicity of variants is therefore desirable. As prediction tools do not always correctly predict a genetic variant to be functionally impaired,[31,32] other large sequencing or functional studies are needed to confirm the clinical significance of these variants.

Limitations

Because of an overlap in phenotypical characteristics between carriers and noncarriers, even when including only confirmed pathogenic variants, the sample size of our study might be insufficient to detect small to moderate associations or associations with relatively infrequent features, such as serogranular/serous drusen pigment epithelium detachment and the presence of geographic atrophy. Additionally, when correcting for multiple comparisons, only drusen area remained significantly associated with rare variant carriers, which is most likely the result of our small sample size.

Our study was also restricted by its retrospective design, therefore, for the analyses, we were limited to the images that were captured in the past. Peripheral fundus images and other image modalities were often lacking and therefore not taken into account in the current study. To assess to what extent drusen are located outside the central retina, imaging should preferably be extended to the peripheral retina. Additionally, certain phenotypical characteristics are better visualized with other imaging techniques (eg, cuticular drusen). Previously, *CFH* variants were identified in patients with the cuticular drusen subtype of AMD, and fluorescein angiography is considered the best modality to diagnose these type of drusen.[22,29,30] Furthermore, optical coherence tomography enables detailed visualization of the different retinal layer structures that are not visible on color fundus images, and has

the advantage of three-dimensional image assessment. Future prospective studies could therefore benefit from assessing multiple image modalities and imaging of the peripheral retina.

CONCLUSION

Because patients with AMD carrying a rare *CFH* variant seem a very suitable group for upcoming complement-inhibiting therapies, identification of this subpopulation may be very important to direct choice of treatment. Our results indicate that patients with an extensive drusen area, drusen with crystalline appearance, and drusen nasal to the optic disc are more likely to have a rare genetic variant in the *CFH* gene. These phenotypical characteristics could aid ophthalmologists to select patients for genetic screening. However, it is unlikely that carriers can be discriminated from noncarriers based solely on phenotypical characteristics. Therefore, ophthalmologists should consider genetic testing in patients with extensive drusen deposition, drusen with crystalline appearance and/or drusen nasal to the optic disc in combination with other patient characteristics, such as an early age at onset, cuticular drusen on fluorescein angiography, and a positive family history for AMD.

REFERENCES

1. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2(2):e106-e116.
2. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. BMC Ophthalmol. 2010;10:31.
3. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. N Engl J Med. 2008;358(24):2606-2617.
4. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016;48(2):134-143.
5. Ristau T, Paun C, Ersoy L, et al. Impact of the common genetic associations of age-related macular degeneration upon systemic complement component C3d levels. PLoS One. 2014;9(3):e93459.
6. Smailhodzic D, Klaver CC, Klevering BJ, et al. Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. Ophthalmology. 2012;119(2):339-346.
7. Schick T, Steinhauer M, Aslanidis A, et al. Local complement activation in aqueous humor in patients with age-related macular degeneration. Eye (Lond). 2017;31(5):810-813.
8. Raychaudhuri S, Iartchouk O, Chin K, et al. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. Nat Genet. 2011;43(12):1232-1236.
9. Geerlings MJ, de Jong EK, den Hollander AI. The complement system in age-related macular degeneration: a review of rare genetic variants and implications for personalized treatment. Mol Immunol. 2017;84:65-76.
10. Triebwasser MP, Roberson ED, Yu Y, et al. Rare variants in the functional domains of complement factor H are associated with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2015;56(11):6873-6878.
11. Wagner EK, Raychaudhuri S, Villalonga MB, et al. Mapping rare, deleterious mutations in factor H: sssociation with early onset, drusen burden, and lower antigenic levels in familial AMD. Sci Rep. 2016;6:31531.
12. Geerlings MJ, Kremlitzka M, Bakker B, et al. The functional effect of rare variants in complement genes on C3b degradation in patients with age-related macular degeneration. JAMA Ophthalmol. 2017;135(1):39-46.
13. Yu Y, Triebwasser MP, Wong EK, et al. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. Hum Mol Genet. 2014;23(19):5283-5293.
14. Boyer DS, Schmidt-Erfurth U, van Lookeren Campagne M, Henry EC, Brittain C. The pathophysiology of geographic atrophy secondary to age-related macular degeneration and the complement pathway as a therapeutic target. Retina. 2017;37(5):819-835.
15. Volz C, Pauly D. Antibody therapies and their challenges in the treatment of age-related macular degeneration. Eur J Pharm Biopharm. 2015;95(pt B):158-172.
16. Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, et al. Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: the COMPLETE study. Ophthalmology. 2014;121(3):693-701.
17. Yaspan BL, Williams DF, Holz FG, et al; MAHALO Study Investigators. Targeting factor D of the alternative complement pathway reduces geographic atrophy progression secondary to age-related macular degeneration. Sci Transl Med. 2017;9(395):eaaf1443.
18. Ferrara D, Seddon JM. Phenotypic characterization of complement factor H R1210C rare genetic variant in age-related macular degeneration. JAMA Ophthalmol. 2015;133(7):785-791.

19. Ristau T, Ersoy L, Lechanteur Y, et al. Allergy is a protective factor against age-related macular degeneration. Invest Ophthalmol Vis Sci. 2014;55(1):210-214.
20. Lek M, Karczewski KJ, Minikel EV, et al; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-291.
21. Untergasser A, Cutcutache I, Koressaar T, et al. Primer3; new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115.
22. Duvvari MR, Saksens NT, van de Ven JP, et al. Analysis of rare variants in the CFH gene in patients with the cuticular drusen subtype of age-related macular degeneration. Mol Vis. 2015;21:285-292.
23. Saksens NT, Lechanteur YT, Verbakel SK, et al. analysis of risk alleles and complement activation levels in familial and non-familial age-related macular degeneration. PLoS One. 2016;11(6):e0144367.
24. Oishi A, Thiele S, Nadal J, et al. Prevalence, natural course, and prognostic role of refractile drusen in age-related macular degeneration. Invest Ophthalmol Vis Sci. 2017;58(4):2198-2206.
25. Suzuki M, Curcio CA, Mullins RF, Spaide RF. Refractile drusen: clinical imaging and candidate histology. Retina. 2015;35(5):859-865.
26. Saksens NT, Geerlings MJ, Bakker B, et al. Rare genetic variants associated with development of age-related macular degeneration. JAMA Ophthalmol. 2016;134(3):287-293.
27. Hughes AE, Meng W, Bridgett S, Bradley DT. Rare CFH mutations and early-onset age-related macular degeneration. Acta Ophthalmol. 2016;94(3):e247-e248.
28. Hoffman JD, Cooke Bailey JN, D'Aoust L, et al. Rare complement factor H variant associated with age-related macular degeneration in the Amish. Invest Ophthalmol Vis Sci. 2014;55(7):4455-4460.
29. Boon CJ, Klevering BJ, Hoyng CB, et al. Basal laminar drusen caused by compound heterozygous variants in the CFH gene. Am J Hum Genet. 2008;82(2):516-523.
30. van de Ven JP, Boon CJ, Fauser S, et al. Clinical evaluation of 3 families with basal laminar drusen caused by novel mutations in the complement factor H gene. Arch Ophthalmol. 2012;130(8):1038-1047.
31. Jaffe A, Wojcik G, Chu A, et al. Identification of functional genetic variation in exome sequence analysis. BMC Proc. 2011;5(suppl 9):S13.
32. Flanagan SE, Patch AM, Ellard S. Using SIFT and PolyPhen to predict loss-of-function and gain-of-function mutations. Genet Test Mol Biomarkers. 2010;14(4):533-537

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Rare Genetic Variants Identified in the *CFH* Gene

No. of cases	Genomic startposition	Nucleotide change	Protein change	SNP ID	ExAC[a]	Prediction algorithms SIFT[b]	Polyphen2 HDIV[c]	CADD PHRED[d]	Clinical significance
Previously reported rare *CFH* variants									
1	196621254	c.7C>G	p.Leu3Val	rs139254423	0.03	T (0.26)	D (0.965)	6.682	Unknown[1,2]
1	196642221	c.172T>G	p.Ser58Ala	rs141336681	0.02	D (0.05)	B (0.151)	9.864	Risk (case-control analysis)[1,3,4]
1	196643098	c.350+6T>G	splice-donor site	rs387906550	NA	NA	NA	NA	Risk (segregation analysis)[5]
1	196646659	c.481G>T	p.Ala161Ser	rs777300338	0.002999	T (0.94)	P (0.923)	10.30	Unknown[2,3]
1	196646674	c.496C>T	p.Arg166Trp	.	NA	D (0.01)	D (1.0)	11.81	Unknown[3]
1	196646696	c.518C>G	p.Ala173Gly	.	NA	T (0.13)	B (0.209)	6.230	Unknown[2,6]
7	196646702	c.524G>A	p.Arg175Gln	.	NA	T (0.65)	B (0.005)	0.014	Risk (functional analysis)[2,7]
1	196646728	c.550delA	p.Ile184Leufs*33	.	NA	NA	NA	NA	Risk (segregation analysis)[8]
7	196646756	c.578C>T	p.Ser193Leu	.	NA	T (0.67)	D (1.0)	15.11	Risk (functional analysis)[2,7]
1	196648780	c.647T>C	p.Ile216Thr	rs183474263	NA	T (0.60)	B (0.005)	5.591	Unknown[2]
7	196654311	c.908G>A	p.Arg303Gln	rs766408580	NA	T (0.61)	D (0.976)	11.03	Unknown[1]
2	196659231	c.1198C>A	p.Gln400Lys	rs201671665	0.01	T (0.91)	B (0.04)	0.012	Risk (functional analysis)[1-4]
2	196659255	c.1222C>T	p.Gln408*	rs121913061	NA	T (0.22)	NA	15.49	Risk (segregation analysis)[5]
1	196683035	c.1507C>G	p.Pro503Ala	rs570523689	NA	T (0.23)	D (0.965)	12.43	Risk (case-control analysis)[3,4,9]
1	196684855	c.1652T>C	p.Ile551Thr	rs35453854	0.008995	T (0.3)	D (0.999)	13.01	Unknown[4]
1	196694234_196694243	c.1697-17_-8	splice-acceptor site	.	NA	NA	NA	NA	Unknown[8]
1	196695675	c.1949G>T	p.Gly650Val	rs143237092	0.03	T (0.28)	B (0.095)	13.53	Unknown[1,3,4,10]
1	196706001	c.2461C>T	p.His821Tyr	rs367687415	0.0002	T (1)	B (0.044)	0.106	Unknown[1,4]
1	196711077	c.3029C>T	p.Ala1010Val	.	NA	T (0.52)	B (0.002)	9.318	Unknown[3]
1	196712682	c.3234G>T	p.Arg1078Ser	rs121913062	0.007492	T (0.76)	B (0.035)	15.09	Risk (segregation analysis)[5]
2	196716375	c.3628C>T	p.Arg1210Cys	rs121913059	0.03	D (0.03)	B (0.024)	15.48	Risk (case-control analysis)[1-4,10-15]
Novel rare *CFH* variants									
1	196642194	c.145A>G	p.Ile49Val	rs747546121	0.001501	T (0.65)	B (0.002)	0.230	Unknown
1	196642260	c.211T>A	p.Trp71Arg	.	NA	D (0.02)	D (1.0)	16.92	Unknown

1	196646750	c.572A>G	p.His191Arg	.	NA	T (0.35)	D (0.999)	12.52	Unknown
1	196648897	c.764G>A	p.Gly255Glu	rs771112278	0.0001	T (0.07)	D (1)	15.59	Unknown
3	196654303	c.900TG>T	p.Ala300Glnfs*22	.	NA	NA	NA	NA	Unknown
1	196659226	c.1193A>G	p.Tyr398Cys	rs765210362	NA	T (0.09)	D (0.999)	10.83	Unknown
1	196659281	c.1248C>G	p.Cys416Trp	.	NA	D (0)	D (1.0)	12.77	Unknown
1	196695729	c.2003C>T	p.Pro668Leu	rs764187411	0.003003	T (0.31)	P (0.893)	13.81	Unknown
1	196697568	c.2329A>G	p.Ile777Val	rs761904009	0.001528	T (1)	B (0.001)	4.132	Unknown
1	196706077	c.2537A>G	p.Gln846Arg	.	NA	T (0.61)	B (0.004)	3.988	Unknown
3	196706112	c.2572T>A	p.Trp858Arg	.	NA	D (0)	D (1.0)	14.20	Unknown
1	196712608	c.3160G>A	p.Val1054Ile	rs757426928	0.001499	T (0.96)	P (0.955)	12.83	Unknown

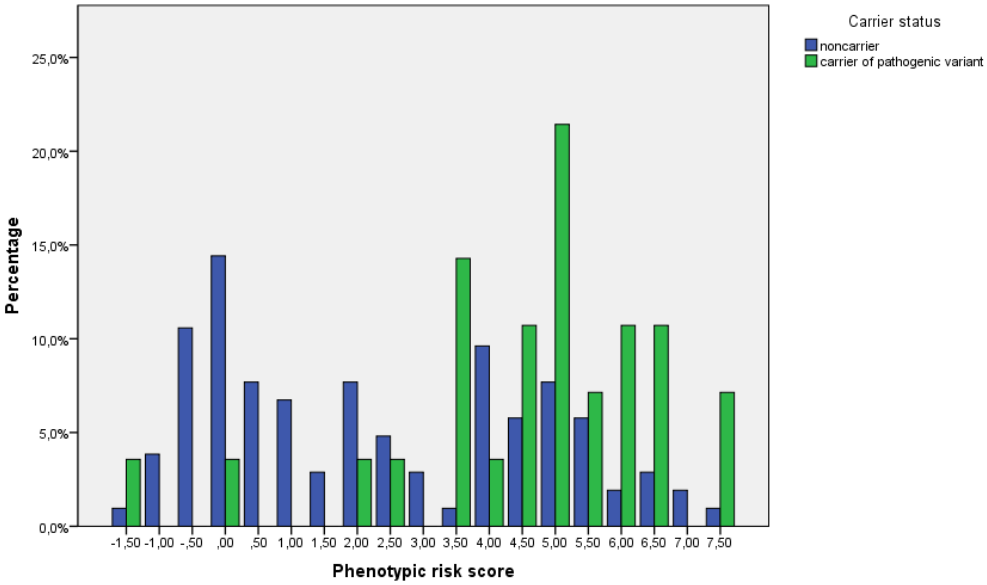
[a] ExAC: Exome Aggregation Consortium. Frequencies presented here are based on a non-Finnish European population which is best comparable to our cohort.

[b]SIFT: Sorting Intolerant from Tolerant. D: Deleterious (sift score ≤0.05); T: tolerated (sift score >0.05)

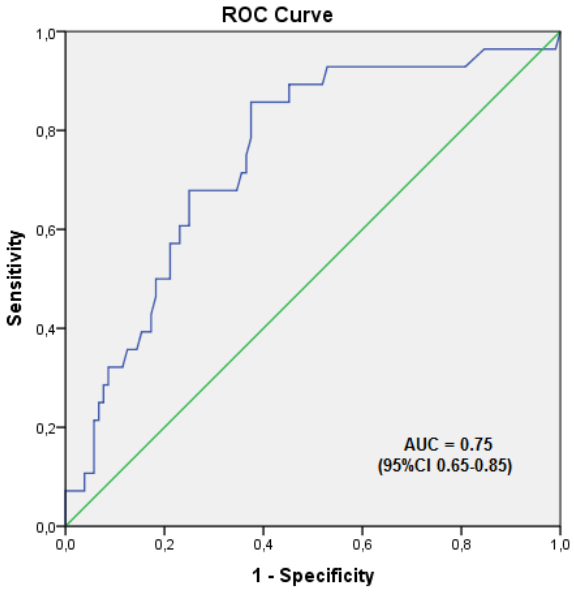
[c]PolyPhen2 HDIV: Polymorphism Phenotyping version 2. D: Probably damaging (score ≥0.957), P: possibly damaging (0.453≤ score ≤0.956); B: benign (score ≤ 0.452)

[d]CADD: Combined Annotation Dependent Depletion (phred = scaled CADD-score; CADD-PHRED score of 10 means 10% most deleterious variants, 20 = 1% most deleterious, 30 = 0.1% most deleterious, etc.)

1. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
2. Duvvari MR, Saksens NT, van de Ven JP, et al. Analysis of rare variants in the CFH gene in patients with the cuticular drusen subtype of age-related macular degeneration. *Mol Vis.* 2015;21:285-292.
3. Triebwasser MP, Roberson ED, Yu Y, et al. Rare Variants in the Functional Domains of Complement Factor H Are Associated With Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(11):6873-6878.
4. Zhan X, Larson DE, Wang C, et al. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. *Nat Genet.* 2013;45(11):1375-1379.
5. Boon CJ, Klevering BJ, Hoyng CB, et al. Basal laminar drusen caused by compound heterozygous variants in the CFH gene. *Am J Hum Genet.* 2008;82(2):516-523.
6. Duvvari MR, van de Ven JP, Geerlings MJ, et al. Whole Exome Sequencing in Patients with the Cuticular Drusen Subtype of Age-Related Macular Degeneration. *PLoS One.* 2016;11(3):e0152047.
7. Geerlings MJ, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2016.
8. van de Ven JP, Boon CJ, Fauser S, et al. Clinical evaluation of 3 families with basal laminar drusen caused by novel mutations in the complement factor H gene. *Arch Ophthalmol.* 2012;130(8):1038-1047.
9. Hoffman JD, Cooke Bailey JN, D'Aoust L, et al. Rare complement factor H variant associated with age-related macular degeneration in the Amish. *Invest Ophthalmol Vis Sci.* 2014;55(7):4455-4460.
10. Seddon JM, Yu Y, Miller EC, et al. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. *Nat Genet.* 2013;45(11):1366-1370.
11. Raychaudhuri S, Iartchouk O, Chin K, et al. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat Genet.* 2011;43(12):1232-1236.
12. Saksens NT, Geerlings MJ, Bakker B, et al. Rare Genetic Variants Associated With Development of Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2016;134(3):287-293.
13. Miyake M, Saito M, Yamashiro K, Sekiryu T, Yoshimura N. Complement factor H R1210C among Japanese patients with age-related macular degeneration. *Jpn J Ophthalmol.* 2015;59(5):273-278.
14. van de Ven JP, Nilsson SC, Tan PL, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. *Nat Genet.* 2013;45(7):813-817.
15. Recalde S, Tortajada A, Subias M, et al. Molecular Basis of Factor H R1210C Association with Ocular and Renal Diseases. *J Am Soc Nephrol.* 2016;27(5):1305-1311.



Supplementary Figure 1. Distribution of Phenotypic Risk Scores in Eyes of Rare Pathogenic *CFH* Variant Carriers (green) and Noncarriers (blue)
The x-axis represents the phenotypic risk score and the y-axis frequency as percentages within each study group.



Supplementary Figure 2. Receiver Operating Characteristic Curve of the Phenotypic Risk Score
The optimal cut-off for this phenotypic risk score is 3 with a sensitivity of 0.86 and specificity of 0.63.

Chapter 3

Molecular studies

Chapter 3.1
Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration

Eveline Kersten*
Constantin C. Paun*
Rosa L. Schellevis
Carel. B. Hoyng
Cécile Delcourt
Imre Lengyel
Tunde Peto
Marius Ueffing
Caroline C.W. Klaver
Sascha Dammeier
Anneke I. den Hollander
Eiko K. de Jong

*These authors contributed equally to this study

Published in: Survey of Ophthalmology. 2018 Jan - Feb;63(1):9-39.

ABSTRACT

Biomarkers can help unravel mechanisms of disease and identify new targets for therapy. They can also be useful in clinical practice for monitoring disease progression, evaluation of treatment efficacy and risk assessment in multifactorial diseases, such as age-related macular degeneration (AMD). AMD is a highly prevalent progressive retinal disorder for which multiple genetic and environmental risk factors have been described, but the exact etiology is not yet fully understood. Many compounds have been evaluated for their association with AMD. We performed an extensive literature review of all compounds measured in serum, plasma, vitreous, aqueous humor and urine of AMD patients. Over 3600 articles were screened resulting in more than 100 different compounds analyzed in AMD studies, involved in neovascularization, immunity, lipid metabolism, extracellular matrix, oxidative stress, diet, hormones, and comorbidities (such as kidney disease). For each compound we provide a short description of its function and discuss the results of the studies in relation to its usefulness as AMD biomarker. Additionally, biomarkers identified by hypothesis-free techniques, including metabolomics, proteomics and epigenomics, are covered. In summary, compounds belonging to the oxidative stress pathway, the complement system, and lipid metabolism are the most promising biomarker candidates for AMD. We hope that this comprehensive survey of the literature on systemic and ocular fluid compounds as potential biomarkers in AMD will provide a stepping stone for future research and possible implementation in clinical practice.

CONTENTS

1. INTRODUCTION

The term biomarker refers to an objective, measurable characteristic that is indicative of a biological process (normal, pathogenic, or in response to treatment).[1] Biomarkers can help unravel mechanisms of disease and identify (new) targets for treatment. The potential benefit of biomarkers in drug development is to allow earlier, more robust drug safety and efficacy measurements.[2] Additionally, biomarkers can be useful in clinical practice for detecting disease, monitoring disease progression, evaluation of treatment efficacy, and risk assessment. Biomarker testing is an important step towards personalized medicine in many diseases, such as cancer,[3] but also in age-related macular degeneration (AMD).

AMD is the leading cause of irreversible loss of vision among the elderly in the Western world, and the prevalence of AMD is expected to increase with population ageing.[4]

The early stage of AMD is characterized by subretinal yellowish deposits, known as drusen, and changes in macular pigmentation.[5,6] At this stage patients usually express little or no complaints. As AMD progresses, central vision becomes increasingly blurred, resulting in irreversible vision loss in the advanced stages of the disease. Two subtypes of advanced AMD can be distinguished: geographic atrophy (GA) and neovascular AMD (nAMD).[5,6] The atrophic form of AMD is characterized by cell death of the retinal pigment epithelium (RPE) and photoreceptors causing gradual vision loss.[7] Neovascular AMD, also referred to as "wet" or "exudative" AMD, is characterized by abnormal vessel growth into the retina from the choroid (choroidal neovascularization [CNV]). Leakage from these fragile neovascularizations can cause rapid loss of vision.[8] In this review, we will use the following terms for the different AMD subgroups described in literature: any AMD, early AMD, advanced AMD (geographic atrophy/neovascular/any advanced), and dry AMD (For definitions of these terms, see Table 1).

AMD is a multifactorial disease, and many risk factors for the development of AMD have been described. The most commonly reported environmental risk factors include aging, smoking, family history, low dietary intake of antioxidants and omega-3 fatty acids, and reduced physical activity.[9-11] Also, multiple genetic risk factors have been identified, consisting of genetic variants that are either common or rare in the population. A large risk effect has been reported for genetic variants located at the *CFH* and *ARMS2/HTRA1* loci.[12] Most genes associated with AMD can be clustered into five main pathways: the complement pathway, lipid transport, extracellular matrix remodeling, angiogenesis and cell survival.[13] Despite considerable progress in understanding the genetic architecture of AMD, the exact disease etiology is not yet fully understood.

In attempts to unravel the etiology of AMD, to improve patient stratification, to monitor disease progression and to discover new drug targets, many biomarker studies have been performed. In general, new analytical strategies have emerged, moving from single markers

towards complex biomarker signatures, increasing the chance for greater specificity and a higher diagnostic or predictive value.
There has been no comprehensive overview of all potential biomarkers and their applicability in AMD. Here, we present a detailed summary of the current literature on molecular compounds reported as analysed in serum, plasma, aqueous humor, vitreous and urine of AMD patients. The scope of this review is limited to non-genetic chemical compounds. For all compounds, a short description of their function is provided and the results of the studies are summarized and discussed in relation to AMD. Currently, most of these markers are not yet established as routine clinical diagnostic tools and are discussed here in order to direct future research and eventually clinical implementation. A complete overview of the studies and references is provided in Supplementary Table 1.

Table 1. Explanation of terms used in this review to describe different types of AMD

Type of AMD	Criteria
Any AMD	No specific definition of AMD reported, or Analyses were performed on all AMD stages together
Early AMD	Analyses were performed on AMD cases in absence of advanced stage disease (GA or CNV) and can include early and/or intermediate AMD
Advanced: GA	Geographic atrophy of the RPE secondary to AMD
Advanced: neovascular	Choroidal neovascular lesion (active or occult) secondary to AMD, including serous and/or hemorrhagic RPE detachment, subretinal fibrovascular tissue and scarring
Any advanced AMD	No specific definition of advanced AMD reported, or Analyses were performed on both advanced AMD stages (GA and CNV) together
Dry AMD	No specific definition of dry AMD reported, or Analyses were performed on AMD cases in absence of advanced neovascular AMD (can therefore include early AMD and/or advanced: GA)

2. NEOVASCULARIZATION AND HEMOSTASIS

Since choroidal neovascularization is one of the subtypes of advanced AMD, it is not surprising that factors involved in neovascularization and hemostasis have been extensively studied. The results of the studies describing these factors are described in section 2.1 and 2.2, respectively.

2.1 Neovascularization

2.1.1 Vascular endothelial growth factor & Soluble VEGF receptor 1

Vascular endothelial growth factor (VEGF) is currently the most important target in the treatment of nAMD, and the expression profile of VEGF has been extensively investigated

in AMD patients. VEGF acts as a hypoxia-driven local signal to induce the formation of new blood vessels. Treatments inhibiting its function can partially restore and/or maintain vision in nAMD patients.

Contrary to expectation, VEGF is not consistently upregulated in AMD patients across studies. One study showed that VEGF levels in the aqueous humor of 12 nAMD patients were highly elevated (668.9 pg/ml) compared to 10 controls (cataract patients; 108.3 pg/ml).[14] In a second study involving aqueous humor, however, significant higher VEGF levels could only be demonstrated in the most aggressive form of nAMD (type 3 neovascularization) compared to controls.[15] A third study did not report a difference in VEGF levels in aqueous humor between nAMD and controls at all.[16] Of note, a considerable range in VEGF levels in aqueous humor exists among these studies. In the study by Tong *et al*,[14] the levels of VEGF in control individuals were around 100 pg/ml, whereas the VEGF levels in the two other studies were much lower in controls (39.5 pg/ml and 63.9 pg/ml respectively).[15,16] These differences may be explained by the use of three different analytical systems, emphasizing the need for standardized assay systems for key marker compounds in eye fluids. Additionally, studies analyzing VEGF levels in vitreous samples did not detect differences between VEGF levels of nAMD cases and controls.[17,18]

Even though the measurement of VEGF levels in vitreous or aqueous humor is expected to best reflect VEGF levels in the macula, the procedure is invasive and therefore not desirable in individuals with early or intermediate AMD. Thus, for purposes of a clinical tool for diagnosis and progression, measurement of VEGF levels in more accessible body fluids such as serum or plasma is preferable. Several studies did investigate VEGF levels in AMD patients and controls in serum or plasma, with mixed results. Four studies detected significantly upregulated levels of VEGF in serum or plasma,[19-22] but these findings are contrasted with 10 other studies that reported no association.[23-32]

VEGF signaling is mediated through a complex of receptors and co-receptors, of which the soluble form of VEGF receptor 1 (sVEGFR1) has been investigated in a number of studies. As in the case of VEGF, these studies do not offer a clear direction of effect. One study investigated the levels of sVEGFR1 in vitreous and found that levels were higher in nAMD patients.[18] In contrast, two studies performed on serum could not corroborate these findings. One of the studies did not find any association,[33] the other even reported lower levels of sVEGFR1 in nAMD.[34]

2.1.2 Pigment epithelium derived factor

Pigment epithelium derived factor (PEDF) is produced by RPE cells and has anti-angiogenic properties, opposing the effects of VEGF. It has been proposed as a target to inhibit choroidal neovascularization and its expression signature in model systems suggests that it is downregulated under hypoxic conditions.[35] Two studies on vitreous support this by

demonstrating a marked reduction in PEDF levels in AMD patients versus controls.[17,18] One study analyzing aqueous humor showed the opposite result, an increase of PEDF levels in AMD patients.[14] These conflicting results are not readily explained. It is possible that in different fluids or in different parts of the eye (anterior/posterior) PEDF is regulated differently, but additional experiments are needed to determine the direction of the effect with certainty.

2.1.3 Transforming growth factor beta

Transforming growth factor beta (TGF-β) has been described to increase the expression of VEGF and is therefore also implicated in neovascularization.[36] In vitreous samples of nAMD patients, TGF-β was significantly elevated when compared to controls (patients with idiopathic macular holes).[36] An earlier study had already demonstrated that urinary TGF-β levels were increased in cases compared to controls, but only in early AMD the difference was significant.[37]

2.2 Hemostatic system

2.2.1 Fibrinogen

Fibrinogen is a hemorheological factor involved in endothelial functioning.[38] Abnormalities in this factor are linked to thrombogenesis and vascular disorders,[39] hence fibrinogen has been examined for its potential involvement in AMD. Studies have yielded mixed results. A number showed that increased fibrinogen level is a significant risk factor,[21,40-43] while others did not find evidence for an association.[44-52]

2.2.2 Plasminogen activator inhibitor 1 (PAI-1)

Plasminogen activator inhibitor 1 (PAI-1) is another main component of the fibrinolytic system.[53] Four studies have investigated whether a relation between PAI-1 and AMD exists, with some support for a positive association,[52] while other studies did not find any association.[48,51,54]

2.2.3 Platelet count

Several studies have measured platelet count. Most did not find any association between platelet counts and AMD.[21,45,55-57] Two larger studies did find lower platelet counts in AMD, but this minimally protective effect for platelets on the development of AMD was only significant in univariate analyses.[58,59]

2.2.4 Von Willebrand factor

Von Willebrand factor (VWF) is a blood glycoprotein that is essential for normal hemostasis.[60] Since vascular pathology is hypothesized to be involved in the pathogenesis of AMD, VWF was

investigated as a possible risk factor. One study showed higher levels compared to controls (but in multivariate analysis no significant correlation was found),[21] and three more studies found no association at all.[48,51,52]

In summary, many inconsistent results for factors involved in neovascularization have been reported and further work is required to determine whether these could be used as AMD biomarkers. Factors involved in hemostasis described in section 2.2 are unlikely to serve as biomarkers for AMD.

3. OXIDATIVE STRESS

The human body is dependent on an aerobic environment for survival. This constant exposure to oxygen can lead to detrimental oxidative modifications of cell components and tissues. Usually, cells are equipped with sufficient antioxidative mechanisms to maintain oxidant homeostasis, but if this balance is disrupted, oxidative stress occurs.[61] Oxidative stress in cells and tissues is characterized by an excess in molecules containing free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).
Polyunsaturated fatty acid (PUFA) molecules are present in lipids on the membranes of cells and are prone to oxidation due to the presence of susceptible double carbon bonds.[61,62] During the process of lipid peroxidation by ROS, the double carbon bond is oxidized, leading to the formation of unstable reactive carbonyl compounds (e.g. malondialdehyde).[63-66] ROS can also oxidize proteins, resulting in 2-(ω-carboxyethyl) pyrrole (CEP) protein adducts[67] and induce formation of advanced glycosylation end products (e.g. N^{ε}-carboxymethyllysine).[68,69]
Increased oxidative stress is thought to be one of the underlying factors in the occurrence of AMD.[61-63,70-72] The eye, and especially the macula, is susceptible to oxidative stress because of its high metabolic activity and the high PUFA content in the membranes of the photoreceptors.[61] High oxygen pressure from the blood in the choroid and exposure to bright light also cause increased ROS levels in the retina.[64,65,71] In addition, photoreceptors are subjected to constant shedding, and subsequent phagocytosis of the shed fragments leads to ROS generation.[61,72] Environmental factors such as smoking and alcohol consumption can also increase ROS production.[73] Therefore, factors related to oxidative stress could potentially be valuable biomarkers for the incidence and/or progression of AMD and are discussed in more detail in the sections 3.1 to 3.4. A schematic overview of these oxidative stress related factors is provided in figure 1 and a complete overview of the studies and references is provided in Supplementary Table 2.

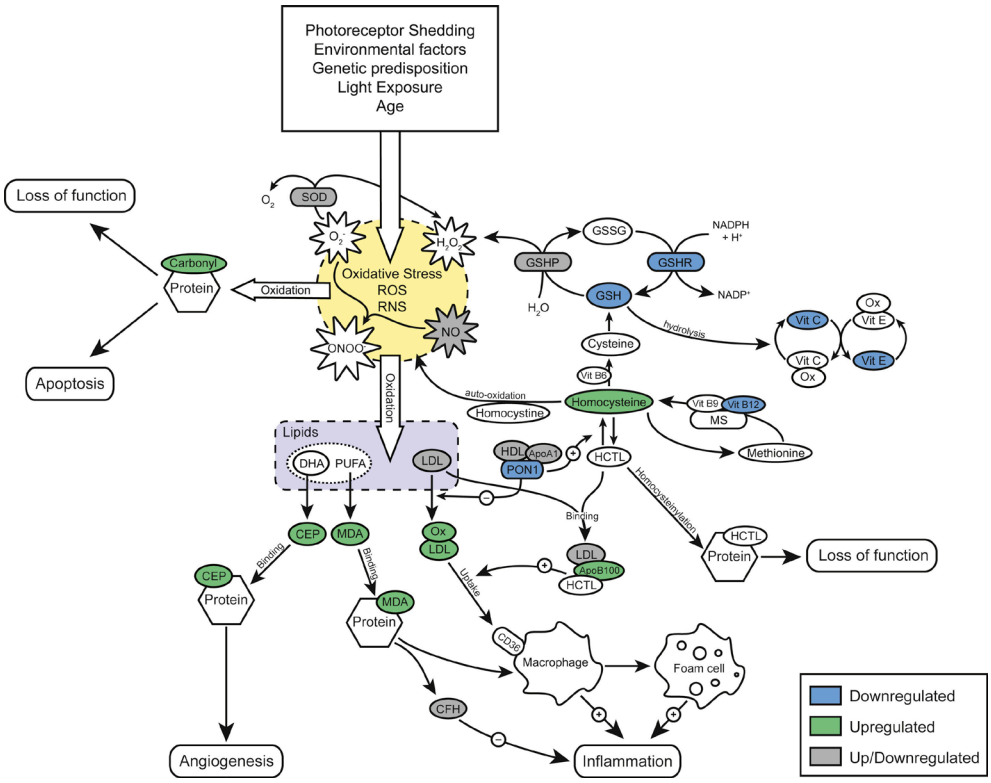


Figure 1. Networks of oxidative stress in age relatedmacular degeneration (AMD)
Spheres are colored to indicate levels in AMD patients compared to controls based on literature: upregulated (green), downregulated (blue), or inconsistent levels (gray). In this figure, studies reporting no association were not taken into account for the sake of readability. Abbreviations: Apo, apolipoprotein; CEP, 2-(u-carboxyethyl) carboxyethylpyrrole; DHA, docosahexaenoic acid; GSH, glutathione; GSHP, glutathione peroxidase; GSHR, glutathione reductase; HCTL, homocysteine thiolactone; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde; MS, methionine synthase; Ox, oxidized; PON1, paraoxonase 1; PUFA, polyunsaturated fatty acid; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; Vit, vitamin.

3.1 Oxidation products

3.1.1 Malondialdehyde (MDA)

Malondialdehyde (MDA) is one of the reactive carbonyl compounds originating from PUFA oxidation, and its presence is often used to measure lipid peroxidation levels in blood or serum samples.[63,64,74] Increased systemic levels of MDA have been consistently observed in both wet and dry AMD.[63-65,72-79] Additionally, an allele-dependent increase of MDA levels was measured in subjects carrying the A69S variant (rs10490924) in the *ARMS2* gene that is associated with AMD. Patients heterozygous or homozygous for the risk allele showed higher MDA levels.[65]

MDA is a highly reactive molecule that forms covalent bonds with the amino acids of endogenous proteins. This MDA modification can be recognized by factors of the innate immune system such as complement factor H (FH), immunoglobulin M (IgM) and macrophages.[66,80,81] Binding of MDA by IgM or macrophages leads to a pro-inflammatory response by increasing the expression of the inflammation factor IL-8,[81,82] whereas binding to FH attenuates inflammation.[81]

3.1.2 2-(ω-Carboxyethyl) pyrrole (CEP) adducts and N(6)-carboxymethyllysine (CML)

Docosahexaenoic acid (DHA) accounts for about 80% of all PUFAs in the photoreceptor outer segments, and is most prone to oxidation in human tissues.[83] Upon oxidative stress DHA is oxidized forming specific CEP-adducts.[67] Plasma CEP levels in AMD patients are elevated compared to controls.[84-86] Moreover, elevated CEP levels combined with AMD risk alleles in *ARMS2, HTRA1, CFH,* or *C3* increased the risk of AMD two- to threefold compared to genotype alone.[84]

Furthermore, plasma of AMD patients contained more and a higher diversity of CEP autoantibodies compared to controls in two studies from the same group.[67,84] Another independent study found no association between CEP autoantibodies and AMD.[85]

N(6)-carboxymethyllysine (CML) is an advanced glycation end product that originates from a protein lysine modification, is a major immunological epitope recognized by the immune system.[68] Plasma CML levels were upregulated in AMD in one study,[85] but no significant difference was found in another.[69]

Both CEP adducts and CML are present on proteins. They are recognized by the immune system[68,87] and can stimulate angiogenesis *in vivo*.[88,89] Receptor-mediated binding of CEP adducts results in an angiogenic response of endothelial cells independent of VEGF signaling.[87] Upregulation of CML and CEP levels in AMD might be implicated in the progression towards nAMD by promoting angiogenesis, but further studies are necessary to support this hypothesis.

3.1.3 Protein Carbonyl Groups (PCG) and total oxidation status

Levels of protein carbonyl groups (PCG) are often used to assess the total protein oxidation status in subjects as they are easy to measure.[90] Protein carbonyl groups consist of an oxygen molecule bound to a carbon atom with a double bond (-RC=O) resulting from protein oxidation and are therefore indicative of oxidative stress. Elevated levels of both PCG[78,91] and total oxidation status[78,92] were found in nAMD patients.

3.1.4. Oxidized low density lipoprotein (Ox-LDL)

Low-density lipoprotein (LDL) is abundantly present in and around cells and is an easy target for oxidation by ROS. LDL-cholesterol has been studied extensively in the context of AMD,

described in section 5.2; however, studies on its oxidized form (Ox-LDL) are more limited. Higher Ox-LDL levels were found systemically in AMD patients compared to controls,[93-95] but a lack of association has also been reported.[96]

Increased Ox-LDL levels are known to activate various factors of the complement system such as C3b, C5b-9 and complement factor B.[97] These factors are described in more detail in section 4.1. High Ox-LDL levels as observed in AMD might initiate apoptosis of RPE cells through disruption of the mitochondrial pro-(Bax) and anti-apoptotic (Bcl2) balance,[98] leading to GA. Additionally, uptake of Ox-LDL molecules by macrophages contributes to the formation of foam cells, implicated in the development of atherosclerotic plaques.[99]

3.2 Nitric oxide

Nitric oxide (NO) is one of the most abundant free radicals in the human body and is able to react with other ROS resulting in cell dysfunction and apoptosis.[75] It is synthesized by endothelial cells and is an important vasoactive agent affecting blood flow and other vascular functions.[100] Involvement of NO in AMD is less clear. One study observed increased levels of NO in AMD patients,[75] another study described downregulation of NO in nAMD,[74] and a third study reported no association.[22]

3.3 Homocysteine

Homocysteine is an intermediate molecule in the conversion of the amino acid methionine to cysteine and glutathione, a process mediated by multiple enzymes.[101,102] Homocysteine can auto-oxidize in plasma, leading to the formation of various reactive products such as homocysteine thiolactone (HCTL), which is also accompanied by ROS generation (Figure 1).[103] Dysregulation of the homocysteine balance has been associated with various diseases such as vascular dysfunction, autoimmune diseases and neurodegenerative disorders.[102] Increased systemic levels of homocysteine were observed in both neovascular and dry forms of AMD compared to controls,[63,94,95,103-111] and there were also higher levels in the vitreous of nAMD patients.[108] Moreover, some studies found higher homocysteine levels in nAMD compared to dry AMD.[104,105] However, other studies did not find an association between homocysteine levels and AMD.[47,51,52,112-114]

3.4 Antioxidants

Antioxidants enhance ROS clearance and prevent ROS formation thereby averting damage in the aging eye and other tissues.[71] Enzymes such as catalase, superoxide dismutase and paraoxonase prevent the accumulation of oxidized lipids by converting ROS before they can react, or by removing the oxidized products from the endogenous proteins.[71] Several vitamins and trace elements act as co-factors for these enzymes, or react with ROS to prevent accumulation.[66,72]

Multiple studies hypothesized that the antioxidant capacity in AMD patients might be impaired, and some showed a decreased overall antioxidant capacity in serum of patients.[44,77,78,91,115,116] In the next sections, we discuss levels of thiols (section 3.4.1), carotenoids (section and 3.4.2) and enzymes with antioxidant activity (section 3.4.3) in AMD patients.

3.4.1 Thiols and glutathione

Thiols mediate an important part of the balance between proper oxidation versus antioxidants in tissues. Their main characteristic is a carbon-bonded sulfhydryl group (C-SH), which can form a disulfide bridge with other thiols via redox reactions (C-S-S-C). Thiols can neutralize ROS by providing an electron during the formation of the disulfide bridge.[117] Although their normal function is to prevent oxidative stress, thiols can also promote oxidative stress in the presence of metal ions such as iron.[94]

Thiol content is either measured by focusing on the individual thiols or by evaluating total thiol (tSH) content of the blood. Glutathione (GSH) is one of the most important thiols in the body. GSH can be transformed into glutathione disulfide (GSSG) by the enzyme glutathione peroxidase (GSHP), thereby breaking down hydrogen peroxide ($2\ GSH + H_2O_2 \rightarrow GS\text{-}SG + 2\ H_2O$).[117] Glutathione reductase (GSHR) is able to transform the formed glutathione disulfide to its monomeric form ($GS\text{-}SG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$), making it available for conversion by GSHP again.[117] This circular process (Figure 1) is of vital importance for proper ROS maintenance.

Lower levels of GSH and tSH are thought to result in more ROS formation owing to absence of hydrogen peroxide clearance, resulting in subsequent oxidative damage.[71,103] Lower levels of total thiol content[92,94,103] and plasma GSH[94,103] were found in patients with AMD compared to control subjects, and both were negatively correlated with homocysteine levels;[103] however, multiple studies have found no association between systemic GSH levels and AMD.[79,118-121]

Plasma and serum GSHR levels were lowered in patients with AMD,[44,91,122] although one study did not find this association in erythrocytes.[123] Systemic GSHP levels were lowered in some studies[73,116,124,125] and higher in one study,[126] but in most studies no association was found.[44,79,91,122,123]

3.4.2 Carotenoids

Carotenoids are a group of natural red and yellow hued pigments (carotenes and xanthophylls) synthesized in most plants. The antioxidant capacity of carotenoids is based on their ability to absorb and process free electrons from ROS such as singlet oxygen ($^1O_2$) and peroxyl radicals ($ROO^\bullet$). After the uptake of an electron the carotenoid releases its energy in the form of heat, and can be used again. Humans are unable to synthesize carotenoids, and rely on dietary intake of vegetables.[127,128] In AMD, total serum carotenoid levels were decreased in two studies by the same group,[41,115] while two other studies described a lack of association.[129,130]

Two main xanthophylls are located in the macula: lutein is concentrated in the peripheral macula and zeaxanthin in the fovea. Here they are able to attenuate blue-light wavelengths, preventing the light from reaching and damaging the underlying photoreceptors.[131] In blood, lutein and zeaxanthin are transported by lipoproteins such as HDL and LDL. Zeaxanthin and lutein exert their antioxidant abilities by reacting with free radicals both in the macula and in blood.[131] Levels of lutein and zeaxanthin were found to be decreased in AMD patients in several studies.[115,132,133] One study described decreased levels of zeaxanthin but not lutein in AMD patients.[134] Others found no association for either lutein or zeaxanthin.[129,130,135-137]
β-cryptoxanthin is a carotenoid most commonly found in citrus fruits. Besides its role as an antioxidant, *in vitro* experiments have shown that β-cryptoxanthin also stimulates DNA repair mechanisms.[138] Levels of β-cryptoxanthin were decreased in patients with advanced AMD in some studies,[115,130,133,136] while others did not find a significant association with AMD.[129,132,135,137]
A decrease of α-carotene was found in patients with nAMD,[115,133] whereas higher levels of α-carotene were present in early AMD.[133] Also β-carotene levels were decreased in advanced AMD in some studies;[115,133,136] however, most studies did not find a significant association between AMD and α-carotene or β-carotene levels.[129,130,132,135-137,139,140] Importantly, supplementation of β-carotene has been associated with an increased risk of lung cancer in smokers and former smokers, and therefore long-term use to inhibit AMD progression is not recommended.[141,142]
Finally, one of the most potent antioxidants present in blood is lycopene. The main dietary sources of this red pigment carotenoid are red fruits or vegetables, such as tomatoes.[143] Levels of lycopene were either decreased in AMD patients[129,130,133] or not associated with AMD.[115,132,135-137]
In summary, when studies reported a significant association between carotenoids and AMD, the vast majority described decreased carotenoid levels in patients. This probably reflects a difference in dietary intake of these carotenoids between AMD patients and controls. Several reported that a higher intake of carotenoids is associated with a reduced risk of AMD.[144-146] Additionally, a beneficial effect was shown for supplementation with lutein and zeaxantin on progression to advanced AMD.[147-149]

3.4.3 Enzymes

Superoxide dismutase (SOD)
Superoxide dismutase (SOD) is an important antioxidant that catalyzes the conversion of superoxide ($O_2^{\bullet-}$) into oxygen and hydrogen peroxide (H_2O_2).[71] Two families of SOD exist based on their metal ion cofactor: SOD1 (CuZnSOD), which is localized to the cytoplasm and SOD2 (MnSOD), found in mitochondria.[71] Absence of SOD1 or SOD2 has been associated with early retinal cell degeneration in mice,[150,151] suggesting an important role for SOD in the eye.

With regard to AMD, several reports show elevated systemic SOD activity in AMD patients compared to controls,[76,77,152,153] others found lowered SOD activity levels,[73,75,79,91,125] and still others measured no significant association.[44,116,122,123,126] One study showed a significant difference in SOD activity between late and early AMD, with a lower SOD activity in late AMD patients.[75]
The association of both low and high SOD serum activity levels with AMD might be explained by the damaging effects of both high and low levels of SOD. High levels of SOD lead to higher H_2O_2 production, whereas low SOD activity leads to the continuing presence of $O_2^{\bullet-}$ molecules. The detrimental effects of both low SOD and high SOD activity on ROS production suggest that imbalance of the enzyme activity leads to pathological conditions and that proper SOD balance is important to maintain homeostasis.

Paraoxonase 1 (PON1)
Paraoxonase 1 (PON1) is bound to high-density lipoprotein (HDL). PON1 hydrolyzes organophosphates and lipid peroxides, and inhibits the oxidation of LDL.[63,95] Additionally, PON1 is able to detoxify HCTL, one of the highly reactive metabolites of homocysteine.[95] Active PON1 interacts with oxidized proteins or lipids, leading to its own inactivation.[63] The low serum PON1 activity levels observed in AMD patients[63,64,92] could be due to inactivation of PON1 after reacting with oxidized proteins.
Catalase
Catalases are important in ROS clearance by converting hydrogen peroxide (H_2O_2) to oxygen and water.[154] In AMD, three studies reported downregulated systemic catalase activity levels,[72,73,125] while three others reported no difference in catalase activity levels between AMD patients and controls.[75,116,123]

Taken together, dysregulation of the oxidative stress pathway and the manner in which oxidative stress is managed by the body seems to play an important role in AMD. A large number of investigators have reported decreased levels of antioxidants and elevated oxidized protein or lipid levels (Figure 1). The most promising biomarker candidates in the oxidative stress pathway are MDA and homocysteine, which were consistently reported to be increased in AMD patients. For other factors, however, the reported associations were less clear and with mixed results. This could indicate that an imbalance of the entire oxidative stress system may play a role, rather than levels of individual factors of this system specifically.

4. IMMUNITY

The involvement of the immune system in the pathology of AMD is widely accepted, and some suggest reframing AMD as an auto-immune disease.[155] The activity of the immune system in AMD, both innate and adaptive, has been implicated at several levels. Immune cell infiltrates have been shown in the retinas of AMD patients examined post-mortem,[156] with evidence of cytokine/chemokine expression at the affected site, as described in more detail in section 4.2.
Strong evidence for the involvement of the immune system in AMD also comes several GWAS studies (described in section 1).[12,157] In particular, the role of the complement system is apparent. In the next sections, we discuss immunity-related compounds, including systemic markers of the complement system (section 4.1), and elements of adaptive and innate immunity (sections 4.2-4.4). A complete overview of the studies and references is provided in Supplementary Table 3.

4.1 The complement system

The complement system is an integral part of innate immunity with essential roles in protection against foreign intruders via tissue inflammation, cell opsonization, and cytolysis. It is also involved in monitoring and maintaining host tissues by clearing cellular debris, maintaining cellular integrity, tissue homeostasis, and modifying the adaptive immune responses.[158]
Ever since histopathological studies demonstrated the presence of complement components in drusen,[159,160] the involvement of the complement system in AMD has been studied extensively and genetic evidence showing strong links between components of the alternative pathway of the complement system and AMD followed.[12,161] Although the complement system acts locally, its components can also be detected systemically in serum or plasma. A number of studies have investigated the expression levels of complement regulators, complement components and activation products in AMD patients versus controls. An overview of the alternative pathway of the complement system is provided in Figure 2.

The central molecule of the complement system is complement component 3 (C3). Enzymatic cleavage of C3 results in the generation of its active fragments C3a (a potent proinflammatory molecule) and C3b that, via several digestion steps, leads to C3d.[162] A number of studies measured systemic C3 levels but did not find an association with AMD,[163-166] whereas higher systemic levels of its active fragments, C3a and C3d, were detected in AMD patients.[163,164,166,167] These findings suggest that the processing of C3, i.e. its activation, may be associated with AMD and a number of studies have investigated this. Complement activation was measured as the ratio of C3 and its degradation product C3d (C3d/C3),[166,168,169] or as a cleaved form of C3a (C3a-desArg) in blood[170] and urine.[37] Out of the five studies that investigated complement

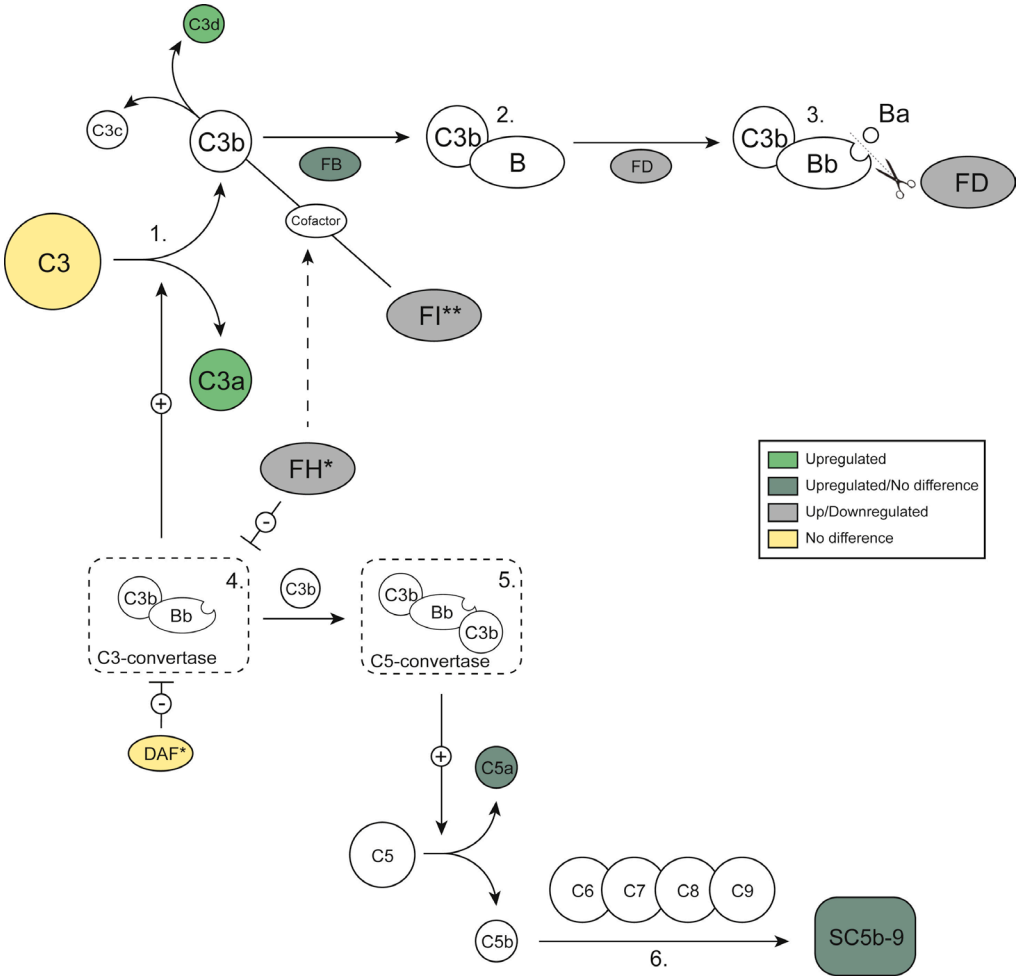


Figure 2. Overview of the alternative pathway of the complement system

Spheres are colored to indicate levels in AMD patients compared with controls based on literature: upregulated (green), upregulated/no difference (dark green), upregulated/downregulated (gray), and no difference (yellow). (1) Complement component 3 (C3) splits into C3a and C3b by spontaneous hydrolyzation or by the C3-convertase (C4bC2) resulting from activation of the classical or lection pathway. (2) Factor B (FB) can bind C3b to form C3bB. (3) The bound factor B is then cleaved by factor D (FD) which results in the formation of the C3-convertase: C3bBb (4). This C3-convertase can cleave C3 which leads to more C3b and in turn increased formation of the C3-convertase (known as the C3 amplification loop). The C3-convertase can also bind another C3b molecule to form C3bBb3b, which is a C5-convertase (5). This C5-convertase can convert C5 into C5a and C5b. (6) C5b then sequentially binds C6, C7, C8, and multiple C9 molecules to form the terminal complement complex (SC5b-9), also known as membrane attack complex. * The C3-convertase is inhibited by several complement regulators, among which decay accelerating factor (DAF) and factor H (FH). ** Factor I (FI) can breakdown C3b via several digestion steps to C3c and finally C3d, this protease activity, however, requires a cofactor, such as FH.

activation in AMD, four found higher complement activation levels in AMD patients.[166,168-170] An association of C3a-desArg in urine with AMD was not established.[37] A recent study suggests that complement activation levels may decrease at more advanced stages of the disease, but this finding needs to be confirmed in prospective AMD cohorts.[171]

Besides C3, complement component 5 (C5) is also essential in the activation cascade because it serves as the entry point for the formation of the terminal complement complex (SC5b-9).[162] The activation product of C5, C5a, is a potent anaphylatoxin. Increased levels of C5a were detected in most, but not all,[167] studies examining the role of C5a in AMD.[163,164,166] These same studies also tested whether SC5b-9 is associated with AMD. Higher SC5b-9 levels were detected in AMD in one study,[164] but the other two studies found no evidence for an association.[163,166]

The activity of the complement system is tightly controlled by regulatory factors that ensure appropriate, but not excessive, generation of terminal complexes. Among others, they include complement factor H (FH, encoded by the *CFH* gene), factor I (FI, encoded by *CFI*), factor B (FB, encoded by *CFB*), factor D (FD, encoded by *CFD*), and decay accelerating factor (DAF/CD55, encoded by *CD55*).[162]

Genetic association studies showed strong evidence of an association between the *CFH* gene and AMD.[12] Systemic levels of FH have been investigated with mixed results, however. Four studies report lower FH levels in AMD,[153,163,172,173] one study detected higher levels of FH in AMD,[174] and another four studies did not find an association with AMD.[164-166,175]

Similar to FH, FI also inhibits the activity of the complement system through inactivation of C3b. Genetic evidence for factor I involvement in AMD has been shown previously, but no conclusive evidence links FI levels to AMD in general. One study reports increased FI levels in AMD,[165] another reports decreased levels but only in patients carrying a rare genetic variant in *CFI,*[176] and two did not find any association.[163,166]

The findings for FB and FD levels in AMD are also inconsistent. Three studies reported higher FB levels in AMD patients,[164,166,167] while two others did not detect an association with AMD.[163,165] Similar results were described for FD, where three studies reported higher FD levels,[164,167,177] one study reported lower levels in AMD,[165] and another found no association with AMD.[163] Finally, two studies that examined the role of CD55 did not find evidence for an association with AMD.[178,179]

In summary, not only genetic studies but also studies measuring complement components provide evidence that link complement activation to AMD (Table 2). Some factors, however, should be taken into account when considering the use of systemic complement activation levels as a biomarker for AMD in individual patients. Often antibody based tests do not discriminate between the total amount of a specific complement factor and its processed activated part, as cleavage of the pro-form to the active mature form cannot be distinguished by

Table 2. Overview of studies measuring complement components in AMD patients compared to controls

Component	Upregulation	No difference	Downregulation
C3		Scholl et al. 2008[164] Reynolds et al. 2009[163] Silva et al. 2012[165] Smailhodzic et al. 2012[166]	
C3a	Scholl et al. 2008[164] Reynolds et al. 2009[163]		
C3d	Scholl et al. 2008[164] Hecker et al. 2010[167] Smailhodzic et al. 2012[166]		
C3a des Arg	Sivaprasad et al. 2007[170]	Guymer et al. 2011[37]	
C3d/C3	Smailhodzic et al. 2012[166] Ristau et al. 2014[168] Ristau et al. 2014[169]		
C5a	Scholl et al. 2008[164] Reynolds et al. 2009[163] Smailhodzic et al. 2012[166]	Hecker et al. 2010[167]	
SC5b-9	Scholl et al. 2008[164]	Reynolds et al. 2009[163] Smailhodzic et al. 2012[166]	
FH	Hakobyan et al. 2008[174]	Scholl et al. 2008[164] Silva et al. 2012[165] Smailhodzic et al. 2012[166] Guymer et al. 2015[175]	Reynolds et al. 2009[163] Ansari et al. 2013[172] Sharma et al. 2013[173] Sharma et al 2013[153]
FI	Silva et al. 2012[165]	Reynolds et al. 2009[163] Smailhodzic et al. 2012[166] Van de Ven et al. 2013*[176]	
FB	Scholl et al. 2008[164] Hecker et al. 2010[167] Smailhodzic et al. 2012[166]	Reynolds et al. 2009[163] Silva et al. 2012[165]	
FD	Scholl et al. 2008[164] Hecker et al. 2010[167] Stanton et al. 2011[177]	Reynolds et al. 2009[163]	Silva et al. 2012[165]
DAF/CD55		Haas et al. 2011[178] Singh et al. 2012[179]	

*Significant downregulation of FI was described in a subgroup of patients with a rare variant in the *CFI* gene.

the reagent. Moreover, complement activation levels are subject to high variability, and other causes of increased complement activity should be excluded since increased complement activation may reflect immune system activity that is not necessarily connected to disease progression. Linking exacerbated complement activation in an individual patient to his or her genetic blueprint is potentially more useful. For example, haplotypes and combinations of genotypes in several complement genes have been associated with increased complement activation levels.[167,180] In addition, several investigations have now demonstrated that FI

levels are lower in AMD patients carrying rare genetic variants in the *CFI* gene.[176,181,182] For FH levels, there were similar associations with genotype. Some but not all rare variants in the *CFH* gene were associated with reduced FH levels.[183-185] Thus, patients carrying rare variants in complement genes tend to have higher complement activation levels than AMD patients in general.[186] These insights may benefit ongoing clinical trials on the effectiveness of complement inhibitors and could prioritize patients who carry rare variants in these genes.

4.2 Cytokines

4.2.1 Interleukins

Cytokines are a large family of small proteins that play a pivotal role in cell signaling. An important group of cytokines are interleukins. Interleukins play a key signaling role in the inflammatory response. Interleukin 6 (IL-6) is a cytokine with many described functions,[187,188] and its relationship to AMD has been investigated. A number of studies reported increased levels of IL-6 in AMD patients,[19,189,190] but the majority found no association with AMD in general.[16,29,44,47,51,52,191-193] Notably, a number of these studies did find an association in subgroup analyses. For instance, an association with AMD was reported only in patients with high IL-6 levels[44] or the association with IL-6 was established only for GA patients.[47] Also, only the highest tertile of IL-6 levels was associated with progression of AMD in a prospective cohort study.[194]

Other interleukins have also been studied in relation to AMD, though to a lesser extent. In most studies these interleukins were measured in a multiplex analysis of inflammatory markers. Two studies measured multiple interleukins in serum.[29,193] In one study, there were higher serum levels of IL-1β, IL-4, IL-5, IL-10 and IL-13 in patients with nAMD,[193] but these factors were not associated with early, atrophic, or neovascular AMD in another study.[29] Higher serum levels of IL-1α and IL-17 in nAMD patients were only reported in the first study. Additionally no association was found for IL-2, IL-12 and IL-15.[193] Other studies also detected no association between IL-2,[47] IL-15[195] and AMD. For IL-8, although no association was present in two studies,[196,197] a third larger study described higher IL-8 levels in AMD patients, in particular in dry AMD.[19] Higher IL-18 levels were reported in dry, but not nAMD, in one study.[198] A second study did not find different levels between different types of AMD and controls.[195]

Although most studies focused on systemic levels of interleukins, a small number performed measurements in aqueous humor[16] and vitreous.[199] Higher IL-1β levels were found in the vitreous of nAMD patients.[199] In aqueous humor, IL-1α and IL-15 were upregulated and IL-13 was downregulated, while for IL-2, IL-4, IL-8, IL-10, IL-12 and IL-17 no differences were detected.[16]

4.2.2 Chemokines and chemokine receptors

Chemokines (chemotactic cytokines) have the ability to direct movement of cells through receptor-mediated chemotaxis. Evidence from post-mortem material as well as animal models have implicated infiltrating immune cells in pathological eye tissues, suggestive of the involvement of chemokines in these environments.[156,200-202]

Chemokine Ligand 2 (CCL2; or monocyte chemoattractant protein 1 [MCP-1]) attracts C-C Chemokine Receptor type 2 (CCR2)-expressing monocytes into tissues and is one of the most studied chemokines in AMD. Five relatively small, case-control studies did not find an association between levels of CCL2 and AMD,[16,29,175,203,204] but several larger studies did see an association with increased levels of CCL2.[153,205,206] This effect was also reported in a cross-sectional study linking higher levels of urinary CCL2 to early AMD.[37] Overall, these findings support the notion that CCL2 is involved in AMD. Interestingly, CCR2-expressing cells can also be detected systemically, and both decreased and increased levels have been associated with AMD.[20,205] Two other studies did not find any association.[207,208]

Another receptor involved in the recruitment of monocytes, CX3C Receptor 1 (CX3CR1), was measured in two AMD studies.[203,204] Only the more recent study reported CX3CR1 to be upregulated in both early and neovascular AMD.[203]

Eotaxin (eosinophil chemotactic protein/CCL11) and closely related Eotaxin-2 (CCL24) attract eosinophils. These are interesting molecules for AMD pathogenesis since CCL11 and CCL24 and their receptor CCR3 are implicated in choroidal neovascularization.[209,210] CCR3 is expressed on choroidal neovascular endothelial cells and signaling through this receptor leads to endothelial proliferation, even without the involvement of eosinophils or other immune cells. Blocking CCR3 signaling in animals led to a potent inhibition of neovascularization, even stronger than blocking VEGFA signaling.[202] Levels of CCL11 were investigated in two studies, one reporting increased levels in AMD,[29] and the other finding no differences.[209] Supportive of the findings above, two studies of the same group reported CCL24 to be upregulated in AMD.[153,210] Despite these overall promising results, systemic elevations of CCR3 on immune cells have not yet been reported. The only study investigating CCR3 on granulocytes reported no association, although there was a trend towards higher expression of CCR3 in nAMD.[209] Taken together, the CCL11/CCL24-CCR3 axis is potentially involved in human AMD pathology, but it is not yet clear whether this is mostly a local signaling, mediated through CCR3 expression on endothelial cells, or whether systemic CCR3-expressing cells could also be involved.

The chemokine ligand CXCL10, also known as interferon gamma-induced protein 10 (IP-10), attracts a range of cell types and is an inhibitor of angiogenesis.[211] Two studies showed no association with CXCL10 in serum or plasma and AMD,[204,212] and only one study showed elevated serum CXCL10 levels in AMD patients.[29] Of interest is a recent publication, showing upregulation of CXCL10 in aqueous humor of AMD patients compared to controls undergoing cataract surgery,[16] suggesting that the effect of this chemokine might be local.

The receptor for CXCL10 is CXCR3 which is expressed on a variety of cell types. Only one study investigated numbers of CXCR3-expressing cells peripherally and detected reduced presence of CD8+ T-cells expressing CXCR3 in AMD,[212] but additional research is warranted before concluding whether the CXCL10-CXCR3 axis can be reliably used as a biomarker for AMD.
It has been suggested that stem cell progenitor cells are involved in the disease etiology of AMD. Chemokine ligand CXCL12, also known as stromal cell-derived factor 1 (SDF-1), plays a role in the movement of these stem cell progenitor cells throughout the body. Four small case-control studies have investigated the plasma levels of SDF-1 in AMD patients with mixed results. Two, by the same group, report significantly lower levels of SDF-1 in patients with nAMD,[28,213] whereas another study showed the inverse effect[30] and the fourth did not report any differences between nAMD and control individuals.[20]

4.2.3 Other cytokines

Tumor necrosis factor alpha (TNF-α)
Tumor necrosis factor alpha (TNF-α), an important marker for systemic inflammation, has been investigated as such in several studies; however, no significant associations between AMD cases and controls were reported in serum or plasma.[29,32,47,175,189,192,193] Increased levels of soluble TNF-α receptor 2 were reported in a case-control study in early and neovascular AMD,[195] which in a large population-based study did not reach statistical significance but there was a trend towards upregulation in early AMD patients.[190]

Interferon gamma (IFN-γ)
Interferon gamma (IFN- γ) is an important cytokine in both innate and adaptive immunity as it induces cellular response to infections.[214] Three studies measured IFN-γ in AMD cases and controls, but none found an association with AMD.[29,193,195]

4.3 Other immune factors

4.3.1 C-reactive protein
C-reactive protein (CRP) is a marker of inflammation and a so-called acute phase protein because its levels change quickly upon disturbances of homeostasis. Evidence regarding the possible relation of this protein with AMD is inconclusive, with a roughly equal number of studies reporting higher CRP levels in AMD patients[16,19,69,111,208,215-224] or no clear evidence for an association.[40,44,46,59,165,175,225-231] Those that employed a more precise measurement of CRP (high-sensitivity CRP [hsCRP]) were also not able to provide conclusive results: five studies detected higher levels of hsCRP in AMD patients,[49,189,190,232,233] compared to five that did not show an association with AMD.[47,51,52,191,192]

4.3.2 (Soluble) Intercellular adhesion molecule and vascular cell adhesion molecule

Intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) are immunoglobulins that are usually upregulated on cell surfaces after immune signaling has taken place.[234] They form a sticky surface to which immune cells that express integrins can adhere. These molecules and their soluble counterparts are rarely investigated alone, but usually as part of a panel that measures inflammatory activity. For ICAM, one study reported higher levels to be associated with the incidence of AMD in women,[49] whereas six others did not find any association.[51,52,175,190,192,227] In the case of VCAM, one study measured higher levels in AMD patients,[190] while two studies did not find any association with AMD.[175,227] Additionally, no association with AMD progression and either ICAM or VCAM was reported.[194]

4.3.3 White blood cell count

As mentioned before in section 4, a clear link with inflammation and inflammatory processes and AMD has been established, and several immune-competent cells have been implicated in the disease etiology. As a result of local stress or inflammation, the body may respond by cellular proliferation of immune cells and recruitment of these cells to the affected site. From this perspective, white blood cell count (WBC) is an interesting parameter to measure in AMD. A relatively large number of studies have investigated WBC in AMD and some did detect increased white blood cell numbers.[56,190,224,235-237] This contrasts with the majority of studies that did not find any association.[21,45,46,51,52,55,56,58,59,106,192,230,238,239] Nevertheless, WBC may still be considered as a potential biomarker for AMD if the analysis is performed in the context of a different theoretical framework. It is conceivable that it is not the total number of cells that change, but rather the ratio between different cell types. Supporting this notion, a higher neutrophil/lymphocyte ratio has been associated to AMD and AMD subtypes.[240] A more in-depth analysis of the different cellular subtypes, such as the relative expression of cytokine/chemokine receptors, would offer more insights.

4.3.4 Pentraxin-3 (PTX3)

Pentraxin-3 (PTX3), like CRP, belongs to the pentraxin superfamily. Upon inflammation, PTX3 is produced locally by the RPE,[241] and can interact with complement component C1q and enhances activation of the classical and lectin pathways of the complement system. Additionally, PTX3 attracts complement FH, thereby inhibiting the amplification loop and preventing excessive activation of the alternative pathway.[241,242] Although one case-control study reported higher plasma PTX3 levels in nAMD,[219] a more recent study (including also early AMD and GA patients) could not replicate these findings.[241] The latter study did however describe an increased expression of the *PTX3* gene with age and inflammation-induced apical PTX3 secretion of the RPE.[241] Taken together, this suggests a more local expression of PTX3 in AMD; however, measurements of PTX3 locally in vitreous samples have not yet been performed and would therefore be a target of further research.

4.4 Antibodies

4.4.1 Anti-retinal autoantibodies

The formation of antibodies against foreign epitopes is a key element of immunity. When endogenous epitopes become the trigger for mounting an immune response, auto-immunity ensues.[243] Antibodies against epitopes found in retinal material of AMD patients have been investigated in various studies. Several studies demonstrated upregulation of circulating anti-retinal autoantibodies (ARAs) in the serum of AMD patients.[244-247] Although one study showed similar levels of ARAs in cases and controls, it did show a difference in types of antibodies specific for each disease stage.[248] Additionally, higher concentrations of circulating ARAs were detected in treatment-naïve nAMD patients compared to controls.[249,250] These levels also correlated to lesion size.[250] After the loading phase of anti-VEGF treatment, autoantibody levels decreased.[249,250] Moreover, correlations were reported between ARA levels and improvement of visual acuity, fluid reduction on Optical Coherence Tomography, and decreased leakage on fluorescein angiography after three months.[250]

Furthermore, other studies attempted to identify specific circulating ARAs associated with AMD.[251-253] Surprisingly, one study showed not only upregulation of antibodies, but also downregulation of a specific ARA in AMD. Lower antibody concentrations were reported for α-crystallin, while α-enolase and glial fibrillary acidic protein (GFAP) antibodies were both significantly higher in serum of AMD patients.[252] The latter finding is supported by results from a previous study which showed different staining patterns in serum of AMD patients, with the most frequent pattern observed being almost identical to that using anti-GFAP antibodies.[247]

In addition, using an untargeted approach, one study identified four novel retinal antigens in serum of AMD patients: retinol binding protein 3 (Rbp3), aldolase C (ALDOC), pyruvate kinase isoform M2 (PKM2), and retinaldehyde binding protein 1 (RLBP1).[253] Because Rbp3 and RLBP1 were previously reported in other ocular diseases, this study focused on ALDOC and PKM2. A significant higher reactivity to ALDOC in nAMD, but not in early AMD, was reported. Because reactivity to PKM2 was higher in both AMD groups compared to controls, this could potentially be a biomarker for the development of AMD.[253] A more recent study with a similar approach also identified ARAs with higher reactivity in AMD; heat shock 70 kDa protein 8 and 9, α-crystallin A chain, annexin A5, and protein S100-A9.[251]

4.4.2 Other autoantibodies

Serum autoantibodies have been extensively investigated by Morohoshi and colleagues using a antigen microarray analysis containing 85 autoantigens. Serum of AMD patients and controls showed a different IgG and IgM autoantibody profile, and multiple autoantibodies were significantly higher in AMD. Additionally, they calculated IgG/IgM ratios for the antibodies and evaluated whether this ratio correlated to disease severity. Anti-phosphatidylserine (PS)

IgG/IgM was significantly elevated in AMD and correlated best with AMD stage. Moreover, reactivity to PS was highly increased in retina of AMD patients compared to controls.[254]
Other investigators focused specifically on antiphospholipid antibodies, which are reported to be found in aging people and diseases associated with aging.[255] In this study, anti-cardiolipin IgG levels were associated with AMD, supported by the findings of Morohoshi *et al.* which showed higher expression of anti-cardiolipin antibodies in nAMD compared to controls.[254,255]
As described in section 3.1, anti-CEP antibodies have also been investigated in association with AMD.[67,84,85]

4.4.3 Antibodies against pathogens

Infection by pathogens leads to increased antibody titers of the foreign pathogen. Several infectious agents have been implicated in AMD and we detail the antibodies against these pathogens in this section.
Chlamydia pneumoniae is an intracellular bacterial species that has been linked to atherosclerosis.[256] Since AMD involves inflammatory processes similar to atherosclerosis, the association of *Chlamydia pneumoniae* with AMD was explored. One small case-control study found support for this with increased antibody levels in AMD patients,[257] while four larger studies did not find evidence for a relation between anti-*Chlamydia pneumoniae* antibodies and AMD.[47,192,258,259]
The cytomegalovirus (CMV) is another infectious agent that has been hypothesized to be associated with the pathogenesis of AMD, based on the relation between inflammatory processes induced by infection and the resulting vasculopathy.[258] Only two studies investigated this association. One found no evidence for an association,[260] while the other described higher levels of antibodies against CMV in nAMD compared to controls and dry AMD.[258]
Another infectious agent possibly involved in the pathogenesis of AMD is *Helicobacter pylori*. Two studies have tested an association between antibodies against *Helicobacter pylori* and AMD, but found no evidence for this, even when distinguishing between dry and neovascular AMD.[47,258]

To summarize the most important findings regarding immune related factors, involvement of the complement system in AMD is evident and complement activation products seem to be good biomarker candidates. Increased levels of inflammatory factors, such as CCL2 or CRP, have been frequently reported and support the notion that inflammatory processes underlie AMD. Yet, these are not specifically related to AMD and may therefore not be the best biomarker for clinical implementation. The use of multiplex assays for the simultaneous detection of multiple inflammatory markers (cytokines, chemokines) holds great promise, but additional data are required to determine their usefulness as AMD biomarkers. Additionally, ARAs are also associated with AMD, but at present it is unclear whether these autoantibodies

play a direct role in the etiology of the disease or rather are the result of retinal damage. Further research is therefore necessary to determine if (specific) ARAs could be used as a biomarker for AMD.

5. LIPID METABOLISM/HOMEOSTASIS

Lipid metabolism is one of the major pathways involved in the pathogenesis of AMD as evidenced by genetic associations of lipid-linked genes *CETP*, *LIPC*, *ABCA1* and *APOE*.[12,157] Moreover, drusen, the major hallmark of AMD, consist of at least 40% lipids.[261,262] Also, as mentioned in section 4.4, there are similarities in the pathogenesis of atherosclerosis and AMD.[263] Since lipids are important risk factors for atherosclerosis and CVD,[264] these might also be associated with AMD. Numerous studies have measured lipid levels in serum or plasma, and the results of these studies are summarized in sections 5.1 to 5.4. We focus on studies that reported associations with AMD and results from large population-based studies. A complete overview of all studies and references is provided in Supplementary table 4.

5.1 Lipids

Cholesterol has multiple functions. It is required for building and maintaining cell membranes, it is involved in cell signaling processes, and it is a precursor molecule for synthesis of steroid hormones, bile acids, and vitamin D.[265]

The population-based Cardiovascular Health Study reported lower levels of total cholesterol in AMD patients, of which the majority had early AMD.[46,229] Also in the Beaver Dam Eye Study lower cholesterol levels were associated with development of early AMD in women,[236] and there was a trend for lower levels of cholesterol in nAMD;[57] a more recent analysis of the Beaver Dam Eye Study data, however, did not show an association between AMD and cholesterol levels.[192] Additionally, two case-control studies described lower levels of cholesterol in AMD patients.[266,267] In contrast, higher cholesterol was associated with AMD in ten studies, though these were all case-control studies, and only half studied nAMD.[41,94,216,223,227,268-272] The vast majority of studies (Supplementary Table 4), however, did not demonstrate a difference in cholesterol levels between AMD patients and controls, including a meta-analysis of three large population-based studies,[273] and several large population-based studies.[43,50,52,58,69,96,198,225,226,228,238,274-286]

Triglycerides are molecules that have a glycerol backbone, connected to three fatty acids of variable length. Most studies did not report differences in triglyceride levels between AMD cases and controls (Supplementary Table 4). Lower triglyceride levels were reported in early AMD,[185, 371] in nAMD[219] and in any AMD.[59,69,225,280,287] In contrast, three studies reported a higher level of triglycerides to be associated with AMD,[197,269,272] of these, one study included

only women,[62] and one study found the association in women only.[197]
Phospholipids are another class of lipids, and are an important component of cell membranes. In three studies, no association was found between phospholipids and AMD.[129,137,288]

5.2 Lipoproteins

Because of the insoluble nature of lipid molecules, lipoproteins are needed for transportation of lipids through the circulation. Five different lipoproteins exist, differing in their density and size: chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).[289] Both HDL and LDL carry cholesterol between the liver and periphery.[265,287,290] The association between these two lipoproteins and AMD has been extensively studied.
For AMD, higher levels of LDL-cholesterol (LDL-C) were found in several studies. Half of these studies found this association when comparing controls to nAMD,[94,223,271,291,292] others found an association in early AMD,[120] any AMD,[216,269] and in women with dry AMD.[272] Almost all other studies, including multiple large population-based studies,[50,58,69,96,225,274,276,285,286,293] did not report an association between AMD and LDL-C (Supplementary table 4). Only the Cardiovascular Health Study associated lower LDL-C levels with early AMD patients[46] and reported a trend towards lower levels in patients with any AMD.[229] Differences in results regarding LDL-C levels can be partly due to different measurement methods across studies, as it can either be measured directly, but often it is estimated using the Friedewald equation.[294]
Since HDL-cholesterol (HDL-C) is inversely associated with CVD, one may have expected to also find this inverse association with AMD. Surprisingly, lower HDL-C levels were only described in a few studies in varying AMD stages; in late AMD,[50,292] in women with dry AMD,[272] and in early AMD.[55] Increased HDL-C levels in AMD patients were present in multiple studies.[57,58,69,96,191,208,224,226,228,236,266,276,277,284,287,295] It must be noted that most of these studies only found a weak association in a subgroup of AMD patients. The majority of the studies did not describe significant differences in HDL-C levels (Supplementary Table 4).
Three studies evaluated non-HDL-C, which is calculated by subtracting HDL-C from total cholesterol. Two studies, including a large meta-analysis of three population-based studies, reported no association with AMD,[273,287] while the third study found higher non-HDL-C to be associated with any AMD.[216]
Lipoprotein (a), Lp(a), is an LDL-like particle, which consists of apolipoprotein-B100 and apoliprotein-A. Its precise function is unclear, but higher levels of Lp(a) have been repeatedly associated with CVD.[296,297] Contrarily, no association of Lp(a) levels with AMD or progression of AMD has been described so far.[46,194,207,216,272,288,298]

5.3 Apolipoproteins

Apolipoproteins bind lipids to form lipoproteins that are responsible for lipid transport. They also function as enzyme cofactors and receptor ligands.[288] There are several classes of apolipoproteins. The overview presented in this section is restricted to apolipoprotein A1 (ApoA1), the major component of HDL-C, apolipoprotein B (ApoB), mostly found in LDL-C, and apolipoprotein E (ApoE), found in IDL-C and chylomicrons. Several investigations found an association between apolipoproteins and AMD or features of AMD.[207,272,277,287,288] The Pathologies Oculaires Liées à l'Age (POLA) study described ApoA1 to be associated with an increased risk of soft drusen,[277] and also in the European Genetic Database (EUGENDA) cohort higher levels of ApoA1 were associated with AMD, even after adjustment for genetic variants that influence lipid levels.[287] In contrast, one study reported a lower ApoA1 concentration in women with dry AMD.[272] This study also described a higher concentration of ApoB in dry AMD cases, which is in concordance with another study.[207] Higher ApoE levels were reported in advanced AMD compared to early AMD and control individuals; this difference could be due to a higher allelic burden of the *APOE* gene in these patients.[288] Other studies did not describe an association between ApoA1, ApoB or ApoE and AMD.[46,216,298,299]

5.4 Fatty acids

There are different types of fatty acids. Polyunsaturated fatty acids (PUFAs) usually derive from phospholipids or triglycerides.[62,300] The most commonly studied PUFAs in AMD are the omega-3 fatty acids DHA and eicosapentaenoic acid (EPA). Fish and other seafood are the main source of these omega-3 PUFAs.[301,302] Animal and epidemiological studies have shown a lower risk for AMD in subjects with high dietary intake of omega-3 fatty acids.[303,304] Also two interventional studies with omega-3 fatty acid supplementation have been performed; the Age-related Eye Disease Study (AREDS)2 showed no beneficial effect for omega-3 fatty acid supplementation,[147] while the Nutritional AMD Treatment 2 (NAT2) study showed a protective effect for DHA supplementation only in patient homozygous for the major allele (T) of the Y402H variant in the *CFH* gene.[305]

Considering omega-3 fatty acids as potential biomarkers, a number of studies investigated plasma or serum levels of these factors. In the Antioxydants, Lipides Essentiels, Nutrition et maladies Oculaires (ALIENOR), a population-based study, advanced AMD cases had lower plasma levels of α-linoleic acid (ALA) and DHA compared to no or early AMD. In addition, lower plasma levels of EPA were associated with GA.[302] This is in line with baseline measurements performed in the NAT2 study, that showed that nAMD cases had lower EPA and DHA levels in red blood cell membranes and lower serum EPA.[301] On the contrary, smaller case-controls studies reported no effect or opposite effects for DHA, EPA and ALA.[137,300,306,307] For plasma or serum levels of docosapentaenoic acid (DPA), another omega-3 fatty acid, no significant associations were described.[137,302,306]

Omega-6 fatty acids arachidonic acid (AA) and linoleic acid (LA), and omega-9 fatty acid oleic acid (OA) have also been measured. A small case-control study found lower levels of LA and OA, and higher levels of AA in the membranes of erythrocytes of AMD patients.[300] In line with these findings, a recent study reported higher serum AA in nAMD.[307] Two larger case-control studies, however, did not show different levels of these omega-6 and omega-9 fatty acids.[137,306]
Regarding saturated fatty acids (which are single-bonded), lower levels of palmitic acid in erythrocytes of AMD patients were reported in a small, case-control study,[300] though systemic levels were not different between cases and controls.[300,306] Also for stearic acid, no association with AMD was detected.[300,306]

Evidence for the involvement of lipids in AMD comes from epidemiologic, molecular, and genetic studies, but the exact role of systemic lipid levels is not yet clear. These studies are complicated by high variability of lipid and fatty acid levels in general and are potentially further confounded by the use of medication and/ or dietary intake, including supplements. Although a combination of factors could constitute a risk profile that may be linked to the development and progression of AMD, it is unlikely that these factors individually could act as proper biomarkers for the disease.

6. EXTRACELLULAR MATRIX

Remodeling of the extracellular matrix (ECM) plays a role in the pathogenesis of AMD.[308,309] Drusen development, as well as alterations of Bruch membrane[310,311] and infiltration of immune cells, relate to a balance between structural tightness or looseness of the extracellular environment. The constant remodeling of the ECM is carefully regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs).[312] Dysregulation of MMPs and/or TIMPs could lead to ECM changes seen in AMD and therefore, these are potentially useful biomarkers for AMD.
Genetic variations in several ECM-related genes are associated with AMD;[157,313] however, only few studies have measured plasma or serum levels of MMPs and TIMPs.[47,175,314-316] An overview of the studies and references is provided in Supplementary Table 5. Upregulation of MMP9 in plasma was associated with AMD in one study;[314] however, two other studies could not replicate these findings.[175,316] No association was found for serum MMP1 levels[175,316] or MMP2 in serum or plasma.[175,314,316]
All three studies were limited because of small samples sizes and the measurement techniques used. Moreover, in these studies both the proenzyme and active forms were measured together. Increased immunoactivity of MMPs does not necessarily mean an

increase in enzymatic activity. Other measurement techniques are required to measure MMP activity more reliably, and larger future studies are needed to elucidate the potency of MMPs as biomarkers for AMD.

One of the main constituent of the ECM in Bruch membrane is elastin.[309] Elastin, in combination with other proteins of the ECM,[317] provides strong and long lasting elasticity to the Bruch's membrane. The elastin layer degrades with age, however, and elastin metabolism may contribute to AMD where there is frequently thinning and fragmentation of the elastic layer,[310] especially in relationship to choroidal neovascularization.[235,318] There is also evidence for abnormal systemic elastin metabolism in AMD. Patients with nAMD had significantly increased susceptibility to elastolysis in the skin.[235] Patients with nAMD had significantly higher levels of serum elastin-derived peptide levels,[319] probably due to the above mentioned elevated levels of MMPs in serum.[314] Apart from elevated elastin peptide fragment levels, sera from patients with AMD contain specific autoantibodies against elastin and it has been suggested that the IgG/IgM ratio for elastin, and other, autoantibodies might allow monitoring the progression of AMD.[254] Therefore, analyzing elastin degradation product or autoantibody levels or ratios might be a useful tools as biomarkers, at least for, nAMD.

7. DIETARY FACTORS

Known risk factors for AMD include dietary factors, such as low intake of antioxidants. Some vitamins are antioxidants, whereas others act as co-factors for enzymes involved in ROS clearance,[71] as detailed in section 7.1. Trace elements have also been hypothesized to be involved in the pathogenesis of AMD and are described in section 7.2. Another marker influenced by diet is serum albumin, this is considered to be an indicator of nutritional status and inflammation and is discussed in section 7.3. Additionally, diet is also an important source for fatty acids and carotenoids both related to AMD. These are described in section 5.4 and section 3.4.2, respectively. A complete overview of the studies and references is provided in Supplementary Table 6.

7.1 Vitamins

Vitamin C can act as a ROS scavenger and it mediates reactivation of vitamin E.[71] When vitamin C hydrolyzes and reactivates vitamin E the molecule itself is inactivated, and hydrolysis by GSH can reactivate vitamin C (Fig. 1).[320] Lowered levels of vitamin C result in less vitamin E conversion to its active form. Additionally, vitamin C itself cannot fulfill its antioxidant function, and as a consequence ROS production will rise.[320] Vitamin C levels were found to be lower in AMD patients compared to controls,[77] and lower in advanced versus early AMD,[130] however most studies do not report an association between vitamin C and AMD.[41,79,115,119,140,235]

Vitamin E is anchored in the plasma membrane and prevents lipid peroxidation.[71] Lower levels of serum vitamin E in AMD patients were reported.[130,135,140,321] However, associations with vitamin E were not conclusive, since no difference in vitamin E levels have been found in several studies.[41,77,115,119,129,136,137,139,235,322]

One study reported lower levels of vitamin A in patients with nAMD.[133] However, the majority of studies did not find a significant association between vitamin A levels and AMD.[41,119,130,136,137,140,235]

B vitamins are essential molecules in homocysteine metabolism and synthesis of methionine. Both vitamin B9 (folate) and B12 (cobalamin) act as cofactors to convert homocysteine into methionine.[102] In AMD patients, lower serum levels of vitamin B12 were detected compared to controls.[106,107,109] These results were not consistently replicated, as equal levels of serum vitamin B12 in patients and controls have also been described.[113,114] Folate levels were similar between controls and AMD patients in all studies.[106,107,109,113,114,192]

Vitamin D can be produced in the dermis upon sunlight exposure or can be obtained through diet. For its activity the molecule has to be converted into its active form in the liver and kidney before it can regulate uptake of nutrients such as iron, calcium, magnesium and zinc.[323] There are inconsistent results for vitamin D levels in AMD patients. They have been described to be higher,[280] lower,[324,325] or not associated with the disease.[58,282,326-330]

7.2 Trace Elements

Trace elements are required by the human body in very low concentrations for proper physiological functioning; however, deficiency or excess amounts may be harmful.[331]

Iron is essential for retinal functioning, as phototransduction is dependent on iron-containing enzymes. Accumulation of iron, however, can be harmful. Iron can convert hydrogen peroxide (H_2O_2) into highly reactive ROS and thereby enhance oxidative stress.[332] Cadmium can also increase ROS formation,[333] and mercury can decrease oxidant defense mechanisms,[334] both leading to increased oxidative stress. In contrast, manganese, copper and zinc contribute to antioxidant activity as they are co-factors for the antioxidant enzyme SOD.[71,335] GSHP is dependent on the presence of the essential heavy metal selenium.[336] Additionally, copper and zinc are able to stabilize proteins, reducing their vulnerability to oxidation,[335] but can also lead to pathological aggregation or even precipitation of proteins.[337-339] Both zinc and manganese can reduce uptake or accumulation of toxic cadmium.[340]

Several studies reported elevated cadmium levels in blood,[58,279,341,342] aqueous humor[343] and urine of AMD patients.[342] Measurement of cadmium levels in blood might represent only recent cadmium exposure, while urinary cadmium reflects long-term exposure to cadmium and might therefore be a more accurate biomarker. A study comparing both blood and urinary cadmium levels did not show an association with AMD in the total study group; however, when stratified for smoking status, increased urinary cadmium levels were associated with AMD in

smoking individuals, suggesting a smoke-related association of cadmium with AMD.[344] Lead levels were elevated in serum and urine of both early and advanced AMD,[58,341,342] and one study reported an association between lead and AMD only for women.[198] Levels of mercury were only elevated in patients with advanced AMD.[58,341]
Selenium was in general not associated with AMD.[41,115,343] One study found a borderline significant association with AMD,[322] and another measured significantly lower levels of selenium in nAMD patients.[345] Conflicting results are reported for levels of iron,[235,343,346] copper,[129,343] manganese,[341,343] and zinc.[41,130,321,341,343]

7.3 Albumin

Albumin is essential for maintenance of plasma colloid oncotic pressure, acts as a plasma binding protein, and also has antioxidant activity.[347] Additionally, albumin is one of the most common proteins found in drusen.[348] A few studies measured serum albumin in AMD patients and controls. Two case-control studies did not show a significant association between serum albumin and AMD.[41,235] The population-based Cardiovascular Health Study and Beaver Dam Eye Study did report significantly lower serum albumin levels in early and neovascular AMD, respectively.[46,239] A more recent nested case-control study within the Beaver Dam population further analyzing these data could not confirm decreased albumin levels in AMD.[192]

Taken together, because of the highly variable diet between subjects, and varying levels of dietary factors within subjects based on fasting state, assessment of the role of these dietary factors as biomarkers in AMD remains difficult. Dietary intake and/or supplementation of antioxidants and vitamins, however, have therapeutic benefit. The AREDS trial, one of the largest investigations into vitamin supplementation in AMD, focused on daily supplementation with vitamin E, vitamin C, β-carotene and zinc, and demonstrated a lower chance of advanced AMD development in subjects taking these supplements.[149] In the AREDS2 study, an improved formula was evaluated and β-carotene was replaced by lutein/zeaxanthin because of the increased risk of lung cancer in smokers.[147,148]
Regarding trace elements, toxic heavy metals (such as lead, mercury and cadmium) are mainly associated with an increased risk of AMD, while essential heavy metals (e.g. zinc, manganese) seem to protect against the development of AMD. For most trace elements there are only a limited number of studies available in the public domain to date, and further research is required to assess their potential role as a biomarker or as protective supplement.

8. HORMONES

In this section we discuss the few hormones that have been investigated in relation to AMD: leptin, melatonin and dehydroepiandrosterone sulfate (DHEAS). A complete overview of the studies and references is provided in Supplementary Table 7.

8.1 Leptin

Since AMD is a multifactorial disease in which dietary factors and body-mass-index also play a role in the disease mechanism, it has been suggested that the principal hormone involved in food intake behavior, leptin, may be associated with AMD. Two studies support this theory; both showed a reduction in serum leptin levels in AMD patients compared to controls.[124,283] After controlling for potential confounders, including smoking, body mass index, blood pressure, and HDL-C, the association remained significant, which suggests that mechanisms other than body fat underlie the relationship between leptin levels and AMD.[283] A third study did not observe a difference in leptin levels in patients versus control individuals.[189]

8.2 Melatonin

Melatonin has strong antioxidative capacities, is expressed in the retina, and expression levels decrease during aging.[349-351] Two studies investigated levels of melatonin in AMD. One showed elevated blood levels of daytime melatonin in pseudophakic AMD patients.[352] The second study analyzed the major metabolite of melatonin in urine, 6-sulfatoxymelatonin, and described lower levels in AMD.[353] Comparing the two studies is difficult because of the differences in methodology and fluid matrix analyzed, so additional experiments linking melatonin and AMD are necessary.

8.3 Dehydroepiandrosterone sulfate (DHEAS)

DHEAS is a sulfate ester of dehydroepiandrosterone (DHEA), which is an endogenous steroid hormone synthesized from cholesterol in the adrenal glands and serves as precursor molecule for sex steroids androgen and estrogen.[354] It has been suggested that DHEAS has antioxidant effects.[223,354,355] Also, the DHEAS level in blood decreases with age.[354-356] Since both oxidative stress and aging are important risk factors for AMD,[311] the question arises whether DHEAS and AMD could be correlated. Three studies investigated the association between AMD and DHEAS, all with different outcomes; higher levels of DHEAS were reported in women with early AMD,[357] another study described low DHEAS in both dry and neovascular AMD cases,[355] and a third study did not find an association between nAMD and controls.[223]

In summary, only a limited amount of studies assessing hormones in AMD have been performed with inconclusive results and do not seem to be reliable biomarkers for AMD at this point in time.

9. FACTORS RELATED TO COMORBIDITIES

AMD has been suggested to share risk factors or coexist with other diseases, such as kidney disease, diabetes mellitus and Alzheimer disease. Factors related to these comorbidities are discussed in section 9.1 to 9.3, respectively. Although AMD has not been associated with liver disease before, some studies investigated factors related to liver function and these are described in section 9.4. A complete overview of the studies and references is provided in Supplementary Table 8.

9.1 Kidney disease

Several studies have suggested overlapping risk factors between AMD and kidney diseases.[224,358-360] A number of large, often population-based, studies have investigated kidney function, like glomerular filtration rate, but also markers that can be measured in serum/plasma like creatinine and cystatin-C. In the Beaver Dam Eye Study, serum cystatin-C was associated to the incidence of early AMD and nAMD.[359] In the Multi-Ethnic Study of Atherosclerosis (MESA) this association was only found when the highest deciles of cystatin-C were compared to other deciles with prevalence of early AMD.[361] In the Hatoyama study no association between cystatin-C and AMD was found.[191]

Several large studies investigated creatinine in patients, but no clear association between serum creatinine and AMD was found. Two reports from the Korean National Health and Nutrition Examination Survey (KNHANES) describe a significant difference between AMD patients and controls, but after adjustment for other variables no significant association was found.[58,282] The remainder of the studies, including large population-based studies such as the MESA and the Singapore Malay Eye Study, did not find any association between serum creatinine and AMD.[94,95,114,225,235,274,324]

Another indicator of renal health is blood nitrogen urea (BUN), but also for this factor no link was established with AMD.[58,235,282,359]

9.2 Diabetes Mellitus

While some cardiovascular risk factors, such as smoking, have been consistently related to AMD, there are conflicting results for an association between diabetes mellitus and AMD.[9] Several studies, mostly population-based, measured glycated hemoglobin (HbA1c) and glucose as indicators for the presence of diabetes mellitus. Only one study found lower levels of glucose in advanced AMD,[281] but none of the other studies described an association of either markers with AMD.[41,43,94,95,198,225,235,274,277-279,286,293] Several studies, all reports from the KNHANES, reported lower HbA1c levels in AMD,[58,198,279-281] however, studies from other cohorts detected no difference.[32,223,225,274,278]

9.3 Alzheimer disease

Similar to AMD, the prevalence of Alzheimer disease increases with age. This neurological disorder is characterized by amyloid plaques in the brain, with the main component being amyloid beta (Aβ).[362] In AMD, two studies identified Aβ as a component of drusen.[363,364] Also, Aβ might trigger activation of the complement cascade in AMD.[365] Several isoforms of Aβ with different amino acid lengths exist; in this section we discuss the most common isoforms: Aβ1-40 and Aβ1-42.

A small, case-control study did not show different levels of Aβ1-42 between controls and either dry or neovascular AMD;[114] however, two more recent case-control studies showed significantly higher Aβ1-42 peptide levels in AMD patients.[175,189] Also after correction for age, Aβ1-42 was significantly associated with AMD, and there was a trend towards increasing levels of Aβ with increasing disease severity.[175] An association of AMD with Aβ1-40 in these studies was less clear. A significant upregulation was described in one study in nAMD only,[175] while the other study did not report a difference between nAMD patients and controls.[189]

9.4 Liver function

So far, to our knowledge, no study has focused specifically on liver function and AMD. In a few studies indicators of liver function have been reported as part of a routine blood examination with no associations between lactate dehydrogenase, aspartate transaminase, or alanine transaminase and AMD.[58,59,235]

For hepatitis B surface antigen (HBsAg) on the other hand, an association was described in several Korean studies, a country where hepatitis B is still endemic.[58,59,282] In these studies HBsAg carrier status was positively associated with AMD. HBsAg has been detected in subretinal fluid, and it is hypothesized these individuals are therefore at increased risk for uveoretinal pathology, such as AMD.[59,282]

In conclusion, despite coexistence and overlapping risk factors with AMD, biomarkers for kidney disease, diabetes mellitus and liver disease discussed here do not seem good biomarker candidates for AMD. As an exception, Aβ could potentially be a marker of disease progression, however, larger prospective studies are required to confirm these findings. Additionally, also in terms of a potential new drug target, further evaluation of this biomarker in AMD seems worthwhile, as promising anti-Aβ therapies are being developed for Alzheimer's disease.[362]

10. HYPOTHESIS-FREE TECHNIQUES

In the past decade many advanced high-throughput *omic* technologies have been developed. These technologies enable us to analyze large numbers of markers at the same

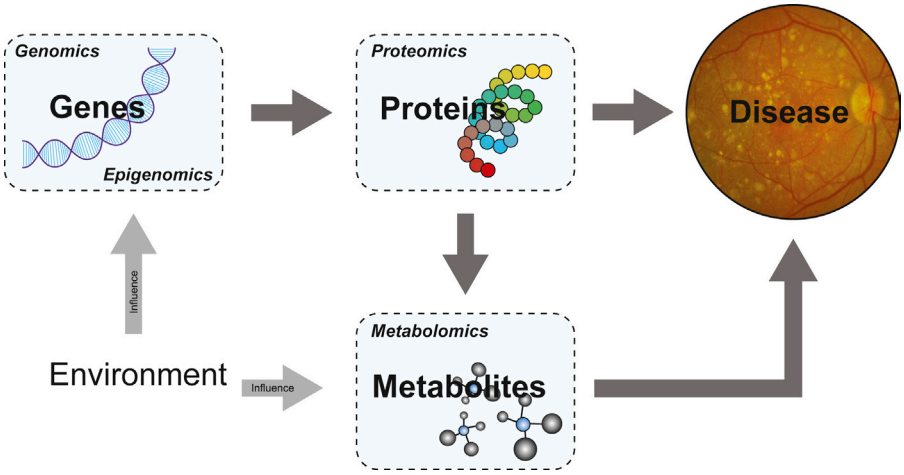


Figure 3. Omics in age-related macular degeneration

time in an untargeted and unbiased manner. Here, we discuss several *omic* technologies in association with AMD (Figure 3): proteomics (section 10.1), metabolomics (section 10.2), and epigenomics (section 10.3). Expression of circulating microRNAs can also be measured using high-throughput techniques, these are described in section 10.4.

10.1 Proteomics

The field of proteomic research uses mass spectrometry, or variations to this technique, to determine the nature of peptides or proteins in various tissues or other biological samples. The advantage of proteomic research is that it delivers results that are unbiased by preconceived notions or hypotheses. Within the field of AMD, proteomics has been employed in a number of investigations, and several have been successful in showing particular proteomic signatures in plasma, vitreous, and aqueous humor from AMD patients when compared to controls.

A small study by Kim *et al*, identified 154 proteins in aqueous humor of 9 nAMD patients and 8 cataract controls.[366] In this study seven potential biomarker candidates were selected for further analysis: ceruloplasmin, PEDF, plasma protease C1 inhibitor, TGF-β1, clusterin, cathepsin D and cystatin D. The relative abundances of TGF-β1, plasma protease C1 inhibitor, ceruloplasmin and PEDF were shown to be significantly higher in AMD samples compared to controls. Another small study, collecting and profiling aqueous humor of 6 nAMD patients and 6 cataract controls, found 68 proteins to be differentially expressed.[367] Only 9 proteins were identified in both studies, among which were some that were related to AMD previously (CCL24 and complement FI), lipocalin-1 and several members of the crystallin family. These crystallins, known for their chaperone function, may also be involved

in protein-protein interaction, prevention of apoptosis, and inhibition of inflammation among others.[368] Lipocalin-1 concentrations were quantified using enzyme-linked immunosorbent assay (ELISA) and levels were significantly elevated in the aqueous humor of nAMD patients.
A third small study performed a focused proteomic analysis on protein members of the ubiquitin pathway.[369] Difference in expression of six proteins in aqueous humor of two AMD patients compared to two controls was reported. This included the 26S proteasome non-ATPase regulatory subunit 1 (Rpn2), a protein that is also present in plasma. Rpn2 was therefore selected as potential AMD biomarker and liquid chromatography-multiple reaction monitoring (LC-MRM) mass spectrometry of another 15 aqueous humor samples showed a relative increase of Rpn2 in nAMD patients.

Kang *et al* analyzed aqueous humor samples of 26 treatment-naive patients with nAMD and 18 controls.[370] By comparing expression profiles in exosomes of aqueous humor and cultured RPE cells, six candidate proteins were selected for verification in an independent sample set by LC-MRM mass spectrometry: actin, myosin-9, heat shock protein 70, cathepsin D, cytokeratin 8, cytokeratin 14. Of these, cytokeratin 8 showed the highest area under the curve value (0.929), suggesting that it is a strong predictor for AMD. Although cytokeratins were not previously reported in other proteomic analyses in AMD, and might be valuable markers to further investigate, it is disputable whether they could qualify as manageable biomarkers. Cytokeratins are abundant contaminants in laboratories,[371] so careful replication of these findings in other laboratories is warranted.
One other study investigated in a targeted manner the involvement of Wnt modulators in aqueous humor and found that WNT inhibitory factor 1 (WIF-1) and Dickkopf-related protein 3 (DKK-3) were upregulated in nAMD.[372]
In a study of 73 nAMD patients and 15 controls, a large set of proteins were detected in vitreous humor, of which 19 were upregulated in nAMD patients.[373] Bioinformatic analyses suggested enrichment of the complement and coagulation cascades, as well as markers involved in arachidonic acid metabolism. Of the 19 proteins, five were randomly selected for Western blot validation; alpha-1-antitrypsin reached statistical significance, while apolipoprotein A1 and transthyretin showed a non-significant increase in AMD. These findings need validation in a larger sample set.
Nobl *et al* investigated vitreous samples of 108 nAMD patients and 24 controls, distributed over a discovery and validation set, and discovered 101 different proteins.[374] Using a closed testing procedure, they focused on four differentially expressed proteins as candidate AMD biomarkers: clusterin, opticin, PEDF and PH2D, which were increased in nAMD compared to controls, except for opticin, which was reduced. Upregulation of PEDF and PH2D in nAMD was described previously.[366,373] Clusterin and PEDF remained significantly increased in nAMD after validation and correction for multiple testing in an independent sample set using ELISA.
There have been limited plasma proteomic studies. Xu *et al* found 28 clinically relevant proteins

to be altered in AMD patients (n=24) compared to healthy volunteers (n=6),[375] but further investigation of these plasma proteins is necessary to validate these findings. Additionally, two studies using proteomic profiling of the same dataset identified three potential AMD biomarkers: vinculin, phospolipid transfer protein and mannan-binding lectin protease-1.[376,377] In general, proteomics of plasma or serum is a great analytical challenge due to the dominant fraction of highly abundant proteins, which have effectively prevented the discovery of novel proteomic biomarkers in these fluids in the past. Therefore improved technologies are needed. Fortunately, some progress has been made using quantitative shot-gun proteomics, recently.[378]

10.2 Metabolomics

Metabolomic studies use mass spectrometric technologies or nuclear magnetic resonance spectroscopy to measure derivatives of metabolism. The technique offers a snapshot of the physiological state of an organism at the level of body fluids (urine, tears, serum, plasma), cells or even tissues. Metabolomic analysis of AMD has great potential to uncover novel pathways in the disease that are reflective of the interaction between the genetic blueprint of an individual and environmental factors that influence the metabolites (for example diet and smoking). To date, only one metabolome-wide study was conducted in plasma samples of 26 nAMD patients and 19 controls. Pathway analysis pointed towards involvement of tyrosine metabolism, urea metabolism as well as vitamin-D related metabolism.[379]

10.3 Epigenomics

Whereas it is clear that both genetic components as well as environmental elements contribute to the risk of developing AMD, it is less clear how these two systems interact. This interaction is the domain of epigenetics, induced changes in the expression levels of genes controlled by outside influences. Epigenetics is a broad term, encompassing many possible regulatory mechanisms of gene expression. One type of epigenetic mark that has been explored in a number of studies is the difference in DNA methylation patterns between cases and controls.

Epigenetic changes can be observed in peripheral blood leukocytes, which are relatively easy to obtain. One study showed a decrease in methylation near the IL17RC promotor region, suggesting that this could serve as a potential biomarker for AMD.[380] However the finding could not be validated by an independent study with a sufficiently powered study design.[381] Based on these results, and also because epigenetic mechanisms are likely to be tissue-specific, the relationship between DNA methylation patterns in peripheral blood and retinal tissue was investigated in a recent study.[382] Although no epigenome-wide association peak was observed, the study did report consistent methylation changes across multiple samples near the *ARMS2* locus and near the protease serine 50 (*PRSS50*) gene.

Despite a limited sample size, the results provided some evidence that methylation patterns in blood leukocytes could serve as proxies for retinal changes, implying that such studies could deliver additional biomarkers for AMD.[382]

10.4 Circulating microRNAs

A microRNA (miRNA) is a small non-coding RNA molecule that regulates gene expression after transcription, thereby influencing biological processes. These miRNAs are present in circulation and could potentially serve as biomarkers.[196] Because we focus on compounds found in body fluids, only the studies that investigate circulating miRNAs (cmiRNAs) in serum or plasma are described here.

In a small study by Ertekin, plasma samples of 33 nAMD patients and 31 controls were analyzed for the expression of 384 miRNAs.[383] They found 16 miRNAs to be differentially expressed between the two groups and additionally discovered 10 miRNAs to be only expressed in nAMD patients.

Grassmann and colleagues identified 203 cmiRNAs in serum, of which three (hsa-mir-301-3p, hsa-mir-361-5p, hsa-mir-424-5p) were significantly altered in nAMD patients (n=129) compared to control individuals (n=147).[384] No significant association was found in GA patients (n=59), suggesting different mechanisms for advanced AMD subtypes. Pathway analysis of the genes that are likely regulated by the altered cmiRNAs implicated the mTOR and TGF-β pathways in nAMD and knockdown of these cmiRNAs *in vitro* resulted in increased angiogenesis, but only significantly for hsa-mir-361-5p.

Szemraj et al also reported significant differences in cmiRNA profiles between dry and neovascular AMD patients.[385] In this study, serum expression levels of 377 miRNA genes in 300 AMD patients (150 nAMD/150 dry AMD patients) and 200 control individuals were analyzed. This study identified 31 differentially expressed miRNAs between patients and controls, including two of the three previously associated[384] cmiRNAs (hsa-mir-301-5p and hsa-mir-424-5p). Of the differentially expressed miRNAs in this study, five were significantly different between patients with dry and neovascular AMD. Additionally, the correlation between these miRNAs and expression of *VEGF* and *VEGFR2* was assessed, and it was suggested that miRNA Let-7 is implicated in the neoangiogenesis in nAMD.

So far, limited studies on miRNA profiling in AMD have been performed and results need to be replicated in larger studies, however, these initial findings emphasize the potential of cmiRNAs as biomarkers in AMD.

In general, studies using hypothesis-free techniques demonstrate proof of concept that *omic* analyses are able to identify novel biomarkers for AMD; however, more are needed to validate results and to confirm the clinical utility of these biomarkers.

11. CONCLUSION AND FUTURE DIRECTIONS

In summary, numerous compounds have been analyzed in relation to AMD. However, only a few of these have potential as AMD biomarkers. The most promising biomarker candidates belong to the oxidative stress pathway, the complement system, and to a lesser extent, lipid metabolism. Finally, the use of hypothesis-free techniques in biomarker detection holds great promise. For summarized findings regarding factors belonging to the other biological pathways described in this review, we refer to the closing paragraphs of the respective chapters. As of yet, none of the biomarkers that we have reviewed here are used clinically.

Many studies reported decreased antioxidant levels and elevated levels of oxidized proteins or lipids indicating oxidative stress in AMD. MDA is often used as a marker for lipid peroxidation and increased levels of MDA have been very consistently observed in both wet and dry AMD (11 out of 11 studies, section 3.1). Additionally, most studies reported higher levels of homocysteine, an intermediate in the oxidative stress pathway, in AMD (12 out of 18 studies, section 3.3). Besides dysregulation of the oxidative stress pathway, many studies indicate the involvement of the complement system in AMD. Products of complement activation and levels of complement activation – described by the ratio of C3 and its degradation product C3d (C3d/C3) – were repeatedly associated with AMD (section 4.1). In addition, there is clear involvement of lipids in AMD from genetic and molecular studies. However, the role of systemic lipids in AMD is not fully elucidated and therefore they are not yet applicable as robust biomarkers for the disease.

In general, many inconsistencies exist between studies evaluating biomarkers and their association with AMD. The contradicting results are difficult to interpret due to a variety of differences between studies, including methodological differences (fasting versus nonfasting blood), different populations (Caucasian/Asian/Mediterranean) with different dietary habits, different study designs, different analytical methods and correction factors, but also types of AMD included in the studies. It must be noted that compiling and comparison of data deriving from different sources represent a major limitation. Therefore, large well-conducted prospective studies are needed to further clarify these results.

Although AMD represents a phenotype restricted to the eye, many studies have investigated systemic markers in relation to AMD. However, because of the presence of the blood-retinal barrier, biomarkers might be only locally dysregulated inside the eye without a measurable systemic effect. Additionally, some compounds are differently expressed between tissues, leading to different results when analyzing different matrices. One might therefore argue to measure markers only locally, however because of the invasive character and accompanying ethical issues systemic markers are preferred for implementation as clinical biomarkers.

Until now, most studies have targeted specific single biomarkers in a candidate-driven approach. *Omic* studies with an unbiased view are heavily outnumbered. Future biomarker

research should therefore combine hypothesis-free as well as candidate-driven approaches. Quantitative analytical approaches applied in an untargeted as well as targeted fashion, such as metabolomic or proteomic studies, are necessary to identify novel biomarker candidates. Once validated as robust and reliable markers, they can offer more insights into the etiology and pathogenesis of AMD, and support prediction, diagnosis, stratification, monitoring of treatment, and drug development for AMD.

Other biomarker types in AMD, such as genetic factors, imaging biomarkers or visual function measurements, are currently of key importance for proper clinical diagnosis, stratification and treatment of AMD. In the future, these established clinical examinations and diagnostic tests may well be applied in combination with molecular biomarkers, an area which is still in a nascent stage.

12. METHODS OF LITERATURE SEARCH

A review of literature was performed through a thorough PubMed search in November 2015. We used the following keywords and their synonyms in various combinations: age-related macular degeneration, serum, plasma, blood, urine, tear, aqueous, and vitreous. No limitations were set for the time range covered by our search, and therefore all articles published until our search were included.

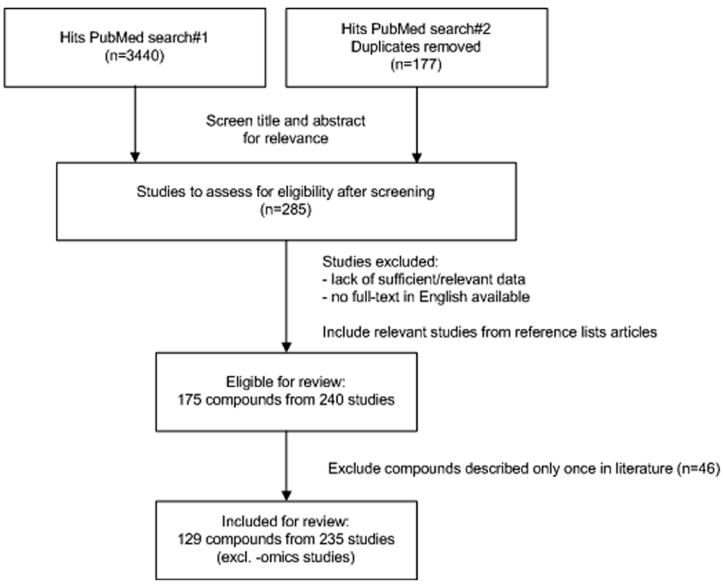


Figure 4. Flow diagram of literature search
The screening and selection process of studies included for this review is depicted in the flow diagram.

All abstracts were screened for relevance and full texts of the selected articles were studied. We included only papers written in English. Articles cited in the reference lists of articles obtained through this search were also included whenever relevant. Animal, *ex vivo* and *in vitro* studies were excluded. To include the most recent developments before submission, the search was repeated in June 2016. An overview of our selection process is detailed in figure 4. After the final article selection, all described compounds in these studies were grouped based on their common biological function or pathway and results were discussed accordingly. Of note, compounds that were only described once in literature were not mentioned in this review to reduce the effect of selective reporting.

13. REFERENCES

1. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89-95.
2. Zolg JW, Langen H. How industry is approaching the search for new diagnostic markers and biomarkers. *Mol Cell Proteomics.* 2004;3(4):345-354.
3. La Thangue NB, Kerr DJ. Predictive biomarkers: a paradigm shift towards personalized cancer medicine. *Nat Rev Clin Oncol.* 2011;8(10):587-596.
4. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health.* 2014;2(2):e106-116.
5. Chakravarthy U, Evans J, Rosenfeld PJ. Age related macular degeneration. *BMJ.* 2010;340:c981.
6. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. *N Engl J Med.* 2008;358(24):2606-2617.
7. Holz FG, Strauss EC, Schmitz-Valckenberg S, van Lookeren Campagne M. Geographic atrophy: clinical features and potential therapeutic approaches. *Ophthalmology.* 2014;121(5):1079-1091.
8. Wong TY, Chakravarthy U, Klein R, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. *Ophthalmology.* 2008;115(1):116-126.
9. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol.* 2010;10:31.
10. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. *Am J Ophthalmol.* 2004;137(3):486-495.
11. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. *Lancet.* 2012;379(9827):1728-1738.
12. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
13. Fritsche LG, Fariss RN, Stambolian D, Abecasis GR, Curcio CA, Swaroop A. Age-related macular degeneration: genetics and biology coming together. *Annu Rev Genomics Hum Genet.* 2014;15:151-171.
14. Tong JP, Chan WM, Liu DT, et al. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. *Am J Ophthalmol.* 2006;141(3):456-462.
15. dell'Omo R, Cassetta M, dell'Omo E, et al. Aqueous humor levels of vascular endothelial growth factor before and after intravitreal bevacizumab in type 3 versus type 1 and 2 neovascularization. A prospective, case-control study. *Am J Ophthalmol.* 2012;153(1):155-161 e152.
16. Sakurada Y, Nakamura Y, Yoneyama S, et al. Aqueous humor cytokine levels in patients with polypoidal choroidal vasculopathy and neovascular age-related macular degeneration. *Ophthalmic Res.* 2015;53(1):2-7.
17. Holekamp NM, Bouck N, Volpert O. Pigment epithelium-derived factor is deficient in the vitreous of patients with choroidal neovascularization due to age-related macular degeneration. *Am J Ophthalmol.* 2002;134(2):220-227.
18. Huber M, Wachtlin J. Vitreous levels of proteins implicated in angiogenesis are modulated in patients with retinal or choroidal neovascularization. *Ophthalmologica.* 2012;228(3):188-193.
19. Ambreen F, Ismail M, Qureshi IZ. Association of gene polymorphism with serum levels of inflammatory and angiogenic factors in Pakistani patients with age-related macular degeneration. *Mol Vis.* 2015;21:985-999.

20. Grierson R, Meyer-Rusenberg B, Kunst F, Berna MJ, Richard G, Thill M. Endothelial progenitor cells and plasma vascular endothelial growth factor and stromal cell-derived factor-1 during ranibizumab treatment for neovascular age-related macular degeneration. *J Ocul Pharmacol Ther.* 2013;29(6):530-538.
21. Lip PL, Blann AD, Hope-Ross M, Gibson JM, Lip GY. Age-related macular degeneration is associated with increased vascular endothelial growth factor, hemorheology and endothelial dysfunction. *Ophthalmology.* 2001;108(4):705-710.
22. Tsai DC, Charng MJ, Lee FL, Hsu WM, Chen SJ. Different plasma levels of vascular endothelial growth factor and nitric oxide between patients with choroidal and retinal neovascularization. *Ophthalmologica.* 2006;220(4):246-251.
23. Carneiro AM, Costa R, Falcao MS, et al. Vascular endothelial growth factor plasma levels before and after treatment of neovascular age-related macular degeneration with bevacizumab or ranibizumab. *Acta Ophthalmol.* 2012;90(1):e25-30.
24. Enders P, Muether PS, Hermann M, Ristau T, Fauser S. Long-term alterations of systemic vascular endothelial growth factor levels in patients treated with ranibizumab for age-related macular degeneration. *Retina.* 2015;35(3):454-458.
25. Goncalves FT, Cezario SM, Calastri MC, et al. Influence of VEGF-C936T genetic variant on age-related macular degeneration. *Arq Bras Oftalmol.* 2015;78(5):290-294.
26. Gu X, Yu X, Dai H. Intravitreal injection of ranibizumab for treatment of age-related macular degeneration: effects on serum VEGF concentration. *Curr Eye Res.* 2014;39(5):518-521.
27. Haas P, Steindl K, Aggermann T, et al. Serum VEGF and CFH in exudative age-related macular degeneration. *Curr Eye Res.* 2011;36(2):143-148.
28. Machalinska A, Safranow K, Dziedziejko V, et al. Different populations of circulating endothelial cells in patients with age-related macular degeneration: a novel insight into pathogenesis. *Invest Ophthalmol Vis Sci.* 2011;52(1):93-100.
29. Mo FM, Proia AD, Johnson WH, Cyr D, Lashkari K. Interferon gamma-inducible protein-10 (IP-10) and eotaxin as biomarkers in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2010;51(8):4226-4236.
30. Scotti F, Maestroni A, Palini A, et al. Endothelial progenitor cells and response to ranibizumab in age-related macular degeneration. *Retina.* 2014;34(9):1802-1810.
31. Wang X, Sawada T, Sawada O, Saishin Y, Liu P, Ohji M. Serum and plasma vascular endothelial growth factor concentrations before and after intravitreal injection of aflibercept or ranibizumab for age-related macular degeneration. *Am J Ophthalmol.* 2014;158(4):738-744 e731.
32. Zehetner C, Kirchmair R, Neururer SB, Kralinger MT, Bechrakis NE, Kieselbach GF. Systemic upregulation of PDGF-B in patients with neovascular AMD. *Invest Ophthalmol Vis Sci.* 2014;55(1):337-344.
33. Owen LA, Morrison MA, Ahn J, et al. FLT1 genetic variation predisposes to neovascular AMD in ethnically diverse populations and alters systemic FLT1 expression. *Invest Ophthalmol Vis Sci.* 2014;55(6):3543-3554.
34. Uehara H, Mamalis C, McFadden M, et al. The reduction of serum soluble Flt-1 in patients with neovascular age-related macular degeneration. *Am J Ophthalmol.* 2015;159(1):92-100 e101-102.
35. Hou HY, Liang HL, Wang YS, et al. A therapeutic strategy for choroidal neovascularization based on recruitment of mesenchymal stem cells to the sites of lesions. *Mol Ther.* 2010;18(10):1837-1845.
36. Bai Y, Liang S, Yu W, et al. Semaphorin 3A blocks the formation of pathologic choroidal neovascularization induced by transforming growth factor beta. *Mol Vis.* 2014;20:1258-1270.
37. Guymer RH, Tao LW, Goh JK, et al. Identification of urinary biomarkers for age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2011;52(7):4639-4644.

38. Bosevski M, Borozanov V, Peovska I, Georgievska-Ismail L. Endothelial dysfunction correlates with plasma fibrinogen and HDL cholesterol in type 2 diabetic patients with coronary artery disease. *Bratisl Lek Listy.* 2007;108(7):297-300.

39. Kakafika AI, Liberopoulos EN, Mikhailidis DP. Fibrinogen: a predictor of vascular disease. *Curr Pharm Des.* 2007;13(16):1647-1659.

40. Dasch B, Fuhs A, Behrens T, et al. Inflammatory markers in age-related maculopathy: cross-sectional analysis from the Muenster Aging and Retina Study. *Arch Ophthalmol.* 2005;123(11):1501-1506.

41. The Eye Disease Case-Control Study Group. Risk factors for neovascular age-related macular degeneration. The Eye Disease Case-Control Study Group. *Arch Ophthalmol.* 1992;110(12):1701-1708.

42. Michalska-Malecka K, Slowinska L, Dorecka M, Romaniuk W. Correlations in some pathogenetic factors and values of hemorheological parameters in age-related macular degeneration. *Clin Hemorheol Microcirc.* 2008;38(3):209-216.

43. Smith W, Mitchell P, Leeder SR, Wang JJ. Plasma fibrinogen levels, other cardiovascular risk factors, and age-related maculopathy: the Blue Mountains Eye Study. *Arch Ophthalmol.* 1998;116(5):583-587.

44. Colak E, Majkic-Singh N, Zoric L, Radosavljevic A, Kosanovic-Jakovic N. The impact of inflammation to the antioxidant defense parameters in AMD patients. *Aging Clin Exp Res.* 2012;24(6):588-594.

45. Inhoffen W, Nussgens Z. Rheological studies on patients with posterior subretinal neovascularization and exudative age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 1990;228(4):316-320.

46. Klein R, Klein BE, Marino EK, et al. Early age-related maculopathy in the cardiovascular health study. *Ophthalmology.* 2003;110(1):25-33.

47. Klein R, Knudtson MD, Klein BE, et al. Inflammation, complement factor h, and age-related macular degeneration: the Multi-ethnic Study of Atherosclerosis. *Ophthalmology.* 2008;115(10):1742-1749.

48. Rudnicka AR, MacCallum PK, Whitelocke R, Meade TW. Circulating markers of arterial thrombosis and late-stage age-related macular degeneration: a case-control study. *Eye (Lond).* 2010;24(7):1199-1206.

49. Schaumberg DA, Christen WG, Buring JE, Glynn RJ, Rifai N, Ridker PM. High-sensitivity C-reactive protein, other markers of inflammation, and the incidence of macular degeneration in women. *Arch Ophthalmol.* 2007;125(3):300-305.

50. Tan JS, Mitchell P, Smith W, Wang JJ. Cardiovascular risk factors and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology.* 2007;114(6):1143-1150.

51. Wang JJ, Ross RJ, Tuo J, et al. The LOC387715 polymorphism, inflammatory markers, smoking, and age-related macular degeneration. A population-based case-control study. *Ophthalmology.* 2008;115(4):693-699.

52. Wu KH, Tan AG, Rochtchina E, et al. Circulating inflammatory markers and hemostatic factors in age-related maculopathy: a population-based case-control study. *Invest Ophthalmol Vis Sci.* 2007;48(5):1983-1988.

53. Binder BR, Christ G, Gruber F, et al. Plasminogen activator inhibitor 1: physiological and pathophysiological roles. *News Physiol Sci.* 2002;17:56-61.

54. Bertelmann T, Spychalska M, Kohlberger L, et al. Intracameral concentrations of the fibrinolytic system components in patients with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2013;251(12):2697-2704.

55. Klein R, Cruickshanks KJ, Nash SD, et al. The prevalence of age-related macular degeneration and associated risk factors. *Arch Ophthalmol.* 2010;128(6):750-758.

56. Klein R, Deng Y, Klein BE, et al. Cardiovascular disease, its risk factors and treatment, and age-related macular degeneration: Women's Health Initiative Sight Exam ancillary study. *Am J Ophthalmol.* 2007;143(3):473-483.
57. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology.* 2003;110(6):1273-1280.
58. Cho BJ, Heo JW, Kim TW, Ahn J, Chung H. Prevalence and risk factors of age-related macular degeneration in Korea: the Korea National Health and Nutrition Examination Survey 2010-2011. *Invest Ophthalmol Vis Sci.* 2014;55(2):1101-1108.
59. Roh MI, Kim JH, Byeon SH, Koh HJ, Lee SC, Kwon OW. Estimated prevalence and risk factor for age-related maculopathy. *Yonsei Med J.* 2008;49(6):931-941.
60. Sadler JE. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem.* 1998;67:395-424.
61. Cai J, Nelson KC, Wu M, Sternberg P, Jr., Jones DP. Oxidative damage and protection of the RPE. *Prog Retin Eye Res.* 2000;19(2):205-221.
62. Nowak JZ. Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacol Rep.* 2013;65(2):288-304.
63. Ates O, Azizi S, Alp HH, et al. Decreased serum paraoxonase 1 activity and increased serum homocysteine and malondialdehyde levels in age-related macular degeneration. *Tohoku J Exp Med.* 2009;217(1):17-22.
64. Baskol G, Karakucuk S, Oner AO, et al. Serum paraoxonase 1 activity and lipid peroxidation levels in patients with age-related macular degeneration. *Ophthalmologica.* 2006;220(1):12-16.
65. Park DH, Shin JP, Kim IT. Association of plasma malondialdehyde with ARMS2 genetic variants and phenotypes in polypoidal choroidal vasculopathy and age-related macular degeneration. *Retina.* 2014;34(6):1167-1176.
66. Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. *Biochim Biophys Acta.* 2012;1818(10):2465-2475.
67. Gu X, Meer SG, Miyagi M, et al. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. *J Biol Chem.* 2003;278(43):42027-42035.
68. Ikeda K, Higashi T, Sano H, et al. N (epsilon)-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. *Biochemistry.* 1996;35(24):8075-8083.
69. Semba RD, Cotch MF, Gudnason V, et al. Serum carboxymethyllysine, an advanced glycation end product, and age-related macular degeneration: the Age, Gene/Environment Susceptibility-Reykjavik Study. *JAMA Ophthalmol.* 2014;132(4):464-470.
70. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol.* 2000;45(2):115-134.
71. Tokarz P, Kaarniranta K, Blasiak J. Role of antioxidant enzymes and small molecular weight antioxidants in the pathogenesis of age-related macular degeneration (AMD). *Biogerontology.* 2013;14(5):461-482.
72. Yildirim O, Ates NA, Tamer L, et al. Changes in antioxidant enzyme activity and malondialdehyde level in patients with age-related macular degeneration. *Ophthalmologica.* 2004;218(3):202-206.
73. Venza I, Visalli M, Oteri R, Teti D, Venza M. Combined effects of cigarette smoking and alcohol consumption on antioxidant/oxidant balance in age-related macular degeneration. *Aging Clin Exp Res.* 2012;24(5):530-536.
74. Totan Y, Cekic O, Borazan M, Uz E, Sogut S, Akyol O. Plasma malondialdehyde and nitric oxide levels in age related macular degeneration. *Br J Ophthalmol.* 2001;85(12):1426-1428.

75. Evereklioglu C, Er H, Doganay S, et al. Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration. *Doc Ophthalmol.* 2003;106(2):129-136.
76. Jia L, Dong Y, Yang H, Pan X, Fan R, Zhai L. Serum superoxide dismutase and malondialdehyde levels in a group of Chinese patients with age-related macular degeneration. *Aging Clin Exp Res.* 2011;23(4):264-267.
77. Shen XL, Jia JH, Zhao P, et al. Changes in blood oxidative and antioxidant parameters in a group of Chinese patients with age-related macular degeneration. *J Nutr Health Aging.* 2012;16(3):201-204.
78. Totan Y, Yagci R, Bardak Y, et al. Oxidative macromolecular damage in age-related macular degeneration. *Curr Eye Res.* 2009;34(12):1089-1093.
79. Yildirim Z, Ucgun NI, Yildirim F. The role of oxidative stress and antioxidants in the pathogenesis of age-related macular degeneration. *Clinics (Sao Paulo).* 2011;66(5):743-746.
80. Chou MY, Fogelstrand L, Hartvigsen K, et al. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J Clin Invest.* 2009;119(5):1335-1349.
81. Weismann D, Hartvigsen K, Lauer N, et al. Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. *Nature.* 2011;478(7367):76-81.
82. Raghavan S, Subramaniyam G, Shanmugam N. Proinflammatory effects of malondialdehyde in lymphocytes. *J Leukoc Biol.* 2012;92(5):1055-1067.
83. Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. *Prog Lipid Res.* 1983;22(2):79-131.
84. Gu J, Pauer GJ, Yue X, et al. Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers. *Mol Cell Proteomics.* 2009;8(6):1338-1349.
85. Ni J, Yuan X, Gu J, et al. Plasma protein pentosidine and carboxymethyllysine, biomarkers for age-related macular degeneration. *Mol Cell Proteomics.* 2009;8(8):1921-1933.
86. Wang H, Guo J, West XZ, et al. Detection and biological activities of carboxyethylpyrrole ethanolamine phospholipids (CEP-EPs). *Chem Res Toxicol.* 2014;27(12):2015-2022.
87. West XZ, Malinin NL, Merkulova AA, et al. Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. *Nature.* 2010;467(7318):972-976.
88. Ebrahem Q, Renganathan K, Sears J, et al. Carboxyethylpyrrole oxidative protein modifications stimulate neovascularization: Implications for age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2006;103(36):13480-13484.
89. Okamoto T, Tanaka S, Stan AC, et al. Advanced glycation end products induce angiogenesis in vivo. *Microvasc Res.* 2002;63(2):186-195.
90. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta.* 2003;329(1-2):23-38.
91. Zafrilla P, Losada M, Perez A, Caravaca G, Mulero J. Biomarkers of oxidative stress in patients with wet age related macular degeneration. *J Nutr Health Aging.* 2013;17(3):219-222.
92. Ugurlu N, Asik MD, Yulek F, Neselioglu S, Cagil N. Oxidative stress and anti-oxidative defence in patients with age-related macular degeneration. *Curr Eye Res.* 2013;38(4):497-502.
93. Ikeda T, Obayashi H, Hasegawa G, et al. Paraoxonase gene polymorphisms and plasma oxidized low-density lipoprotein level as possible risk factors for exudative age-related macular degeneration. *Am J Ophthalmol.* 2001;132(2):191-195.
94. Javadzadeh A, Ghorbanihaghjo A, Bahreini E, Rashtchizadeh N, Argani H, Alizadeh S. Plasma oxidized LDL and thiol-containing molecules in patients with exudative age-related macular degeneration. *Mol Vis.* 2010;16:2578-2584.

95. Javadzadeh A, Ghorbanihaghjo A, Bahreini E, Rashtchizadeh N, Argani H, Alizadeh S. Serum paraoxonase phenotype distribution in exudative age-related macular degeneration and its relationship to homocysteine and oxidized low-density lipoprotein. *Retina.* 2012;32(4):658-666.

96. Klein R, Klein BE, Knudtson MD, et al. Subclinical atherosclerotic cardiovascular disease and early age-related macular degeneration in a multiracial cohort: the Multiethnic Study of Atherosclerosis. *Arch Ophthalmol.* 2007;125(4):534-543.

97. Ebrahimi KB, Fijalkowski N, Cano M, Handa JT. Decreased membrane complement regulators in the retinal pigmented epithelium contributes to age-related macular degeneration. *The Journal of pathology.* 2013;229(5):729-742.

98. Yating Q, Yuan Y, Wei Z, et al. Oxidized LDL Induces Apoptosis of Human Retinal Pigment Epithelium Through Activation of ERK-Bax/Bcl-2 Signaling Pathways. *Curr Eye Res.* 2014:1-8.

99. Podrez EA, Poliakov E, Shen Z, et al. A novel family of atherogenic oxidized phospholipids promotes macrophage foam cell formation via the scavenger receptor CD36 and is enriched in atherosclerotic lesions. *J Biol Chem.* 2002;277(41):38517-38523.

100. Bhutto IA, Baba T, Merges C, McLeod DS, Lutty GA. Low nitric oxide synthases (NOSs) in eyes with age-related macular degeneration (AMD). *Exp Eye Res.* 2010;90(1):155-167.

101. Garcia A, Zanibbi K. Homocysteine and cognitive function in elderly people. *CMAJ.* 2004;171(8):897-904.

102. Schalinske KL, Smazal AL. Homocysteine imbalance: a pathological metabolic marker. *Adv Nutr.* 2012;3(6):755-762.

103. Coral K, Raman R, Rathi S, et al. Plasma homocysteine and total thiol content in patients with exudative age-related macular degeneration. *Eye (Lond).* 2006;20(2):203-207.

104. Axer-Siegel R, Bourla D, Ehrlich R, et al. Association of neovascular age-related macular degeneration and hyperhomocysteinemia. *Am J Ophthalmol.* 2004;137(1):84-89.

105. Ghosh S, Saha M, Das D. A study on plasma homocysteine level in age-related macular degeneration. *Nepal J Ophthalmol.* 2013;5(2):195-200.

106. Gopinath B, Flood VM, Rochtchina E, Wang JJ, Mitchell P. Homocysteine, folate, vitamin B-12, and 10-y incidence of age-related macular degeneration. *Am J Clin Nutr.* 2013;98(1):129-135.

107. Kamburoglu G, Gumus K, Kadayifcilar S, Eldem B. Plasma homocysteine, vitamin B12 and folate levels in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2006;244(5):565-569.

108. Manresa N, Mulero J, Losada M, Zafrilla P. Effect of Pegaptanib and Ranibizumab on Plasma and Vitreous Homocysteine in Patients with Exudative Age-Related Macular Degeneration. *Retina.* 2015;35(9):1765-1771.

109. Rochtchina E, Wang JJ, Flood VM, Mitchell P. Elevated serum homocysteine, low serum vitamin B12, folate, and age-related macular degeneration: the Blue Mountains Eye Study. *Am J Ophthalmol.* 2007;143(2):344-346.

110. Seddon JM, Gensler G, Klein ML, Milton RC. Evaluation of plasma homocysteine and risk of age-related macular degeneration. *Am J Ophthalmol.* 2006;141(1):201-203.

111. Vine AK, Stader J, Branham K, Musch DC, Swaroop A. Biomarkers of cardiovascular disease as risk factors for age-related macular degeneration. *Ophthalmology.* 2005;112(12):2076-2080.

112. Christen WG, Cook NR, Ridker PM, Buring JE. Prospective study of plasma homocysteine level and risk of age-related macular degeneration in women. *Ophthalmic Epidemiol.* 2015;22(2):85-93.

113. Heuberger RA, Fisher AI, Jacques PF, et al. Relation of blood homocysteine and its nutritional determinants to age-related maculopathy in the third National Health and Nutrition Examination Survey. *Am J Clin Nutr.* 2002;76(4):897-902.

114. Obeid R, Ninios K, Loew U, et al. Aqueous humor glycation marker and plasma homocysteine in macular degeneration. *Clin Chem Lab Med.* 2013;51(3):657-663.

115. The Eye Disease Case-Control Study Group. Antioxidant status and neovascular age-related macular degeneration. Eye Disease Case-Control Study Group. *Arch Ophthalmol.* 1993;111(1):104-109.

116. Plestina-Borjan I, Katusic D, Medvidovic-Grubisic M, et al. Association of age-related macular degeneration with erythrocyte antioxidant enzymes activity and serum total antioxidant status. *Oxid Med Cell Longev.* 2015;2015:804054.

117. Meister A. Glutathione metabolism and its selective modification. *J Biol Chem.* 1988;263(33):17205-17208.

118. Brantley MA, Jr., Osborn MP, Sanders BJ, et al. Plasma biomarkers of oxidative stress and genetic variants in age-related macular degeneration. *Am J Ophthalmol.* 2012;153(3):460-467.e461.

119. Delcourt C, Cristol JP, Tessier F, Leger CL, Descomps B, Papoz L. Age-related macular degeneration and antioxidant status in the POLA study. POLA Study Group. Pathologies Oculaires Liees a l'Age. *Arch Ophthalmol.* 1999;117(10):1384-1390.

120. Qin L, Mroczkowska SA, Ekart A, Patel SR, Gibson JM, Gherghel D. Patients with early age-related macular degeneration exhibit signs of macro- and micro-vascular disease and abnormal blood glutathione levels. *Graefes Arch Clin Exp Ophthalmol.* 2014;252(1):23-30.

121. Samiec PS, Drews-Botsch C, Flagg EW, et al. Glutathione in human plasma: decline in association with aging, age-related macular degeneration, and diabetes. *Free Radic Biol Med.* 1998;24(5):699-704.

122. Cohen SM, Olin KL, Feuer WJ, Hjelmeland L, Keen CL, Morse LS. Low glutathione reductase and peroxidase activity in age-related macular degeneration. *Br J Ophthalmol.* 1994;78(10):791-794.

123. De La Paz MA, Zhang J, Fridovich I. Red blood cell antioxidant enzymes in age-related macular degeneration. *Br J Ophthalmol.* 1996;80(5):445-450.

124. Evereklioglu C, Doganay S, Er H, Cekmen M, Ozerol E, Otlu B. Serum leptin concentrations are decreased and correlated with disease severity in age-related macular degeneration: a preliminary study. *Eye (Lond).* 2003;17(3):350-355.

125. Prashar S, Pandav SS, Gupta A, Nath R. Antioxidant enzymes in RBCs as a biological index of age related macular degeneration. *Acta Ophthalmol (Copenh).* 1993;71(2):214-218.

126. Delcourt C, Cristol JP, Leger CL, Descomps B, Papoz L. Associations of antioxidant enzymes with cataract and age-related macular degeneration. The POLA Study. Pathologies Oculaires Liees a l'Age. *Ophthalmology.* 1999;106(2):215-222.

127. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients.* 2014;6(2):466-488.

128. Stahl W, Sies H. Antioxidant activity of carotenoids. *Mol Aspects Med.* 2003;24(6):345-351.

129. Cardinault N, Abalain JH, Sairafi B, et al. Lycopene but not lutein nor zeaxanthin decreases in serum and lipoproteins in age-related macular degeneration patients. *Clin Chim Acta.* 2005;357(1):34-42.

130. Simonelli F, Zarrilli F, Mazzeo S, et al. Serum oxidative and antioxidant parameters in a group of Italian patients with age-related maculopathy. *Clin Chim Acta.* 2002;320(1-2):111-115.

131. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu Rev Nutr.* 2003;23:171-201.

132. Delcourt C, Carriere I, Delage M, Barberger-Gateau P, Schalch W, Group PS. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. *Invest Ophthalmol Vis Sci.* 2006;47(6):2329-2335.

133. Zhou H, Zhao X, Johnson EJ, et al. Serum carotenoids and risk of age-related macular degeneration in a chinese population sample. *Invest Ophthalmol Vis Sci.* 2011;52(7):4338-4344.

134. Gale CR, Hall NF, Phillips DI, Martyn CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2003;44(6):2461-2465.

135. Mares-Perlman JA, Brady WE, Klein R, et al. Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Arch Ophthalmol.* 1995;113(12):1518-1523.

136. Michikawa T, Ishida S, Nishiwaki Y, et al. Serum antioxidants and age-related macular degeneration among older Japanese. *Asia Pac J Clin Nutr.* 2009;18(1):1-7.

137. Sanders TA, Haines AP, Wormald R, Wright LA, Obeid O. Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. *Am J Clin Nutr.* 1993;57(3):428-433.

138. Lorenzo Y, Azqueta A, Luna L, Bonilla F, Dominguez G, Collins AR. The carotenoid beta-cryptoxanthin stimulates the repair of DNA oxidation damage in addition to acting as an antioxidant in human cells. *Carcinogenesis.* 2009;30(2):308-314.

139. Smith W, Mitchell P, Rochester C. Serum beta carotene, alpha tocopherol, and age-related maculopathy: the Blue Mountains Eye Study. *Am J Ophthalmol.* 1997;124(6):838-840.

140. West S, Vitale S, Hallfrisch J, et al. Are antioxidants or supplements protective for age-related macular degeneration? *Arch Ophthalmol.* 1994;112(2):222-227.

141. Albanes D, Heinonen OP, Taylor PR, et al. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst.* 1996;88(21):1560-1570.

142. Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst.* 1996;88(21):1550-1559.

143. Gajowik A, Dobrzynska MM. Lycopene - antioxidant with radioprotective and anticancer properties. A review. *Rocz Panstw Zakl Hig.* 2014;65(4):263-271.

144. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology.* 2008;115(2):334-341.

145. van Leeuwen R, Boekhoorn S, Vingerling JR, et al. Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA.* 2005;294(24):3101-3107.

146. Wu J, Cho E, Willett WC, Sastry SM, Schaumberg DA. Intakes of Lutein, Zeaxanthin, and Other Carotenoids and Age-Related Macular Degeneration During 2 Decades of Prospective Follow-up. *JAMA Ophthalmol.* 2015;133(12):1415-1424.

147. Age-Related Eye Disease Study 2 Research G. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA.* 2013;309(19):2005-2015.

148. Age-Related Eye Disease Study 2 Research G, Chew EY, Clemons TE, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA Ophthalmol.* 2014;132(2):142-149.

149. Age-Related Eye Disease Study Research G. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol.* 2001;119(10):1417-1436.

150. Hashizume K, Hirasawa M, Imamura Y, et al. Retinal dysfunction and progressive retinal cell death in SOD1-deficient mice. *Am J Pathol.* 2008;172(5):1325-1331.

151. Justilien V, Pang JJ, Renganathan K, et al. SOD2 knockdown mouse model of early AMD. *Invest Ophthalmol Vis Sci.* 2007;48(10):4407-4420.

152. Anand A, Sharma NK, Gupta A, Prabhakar S, Sharma SK, Singh R. Superoxide dismutase1 levels in North Indian population with age-related macular degeneration. *Oxid Med Cell Longev.* 2013;2013:365046.

153. Sharma NK, Sharma SK, Gupta A, Prabhakar S, Singh R, Anand A. Predictive model for earlier diagnosis of suspected age-related macular degeneration patients. *DNA Cell Biol.* 2013;32(9):549-555.

154. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. *Cell Mol Life Sci.* 2004;61(2):192-208.

155. Camelo S. Potential Sources and Roles of Adaptive Immunity in Age-Related Macular Degeneration: Shall We Rename AMD into Autoimmune Macular Disease? *Autoimmune Dis.* 2014;2014:532487.

156. Lad EM, Cousins SW, Van Arnam JS, Proia AD. Abundance of infiltrating CD163+ cells in the retina of postmortem eyes with dry and neovascular age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2015;253(11):1941-1945.

157. Fritsche LG, Chen W, Schu M, et al. Seven new loci associated with age-related macular degeneration. *Nat Genet.* 2013;45(4):433-439, 439e431-432.

158. Gasque P. Complement: a unique innate immune sensor for danger signals. *Mol Immunol.* 2004;41(11):1089-1098.

159. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol.* 2002;134(3):411-431.

160. Hageman GS, Mullins RF. Molecular composition of drusen as related to substructural phenotype. *Mol Vis.* 1999;5:28.

161. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308(5720):385-389.

162. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement System Part I- Molecular Mechanisms of Activation and Regulation. *Front Immunol.* 2015;6:262.

163. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. *Invest Ophthalmol Vis Sci.* 2009;50(12):5818-5827.

164. Scholl HP, Charbel Issa P, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS One.* 2008;3(7):e2593.

165. Silva AS, Teixeira AG, Bavia L, et al. Plasma levels of complement proteins from the alternative pathway in patients with age-related macular degeneration are independent of Complement Factor H Tyr(4)(0)(2)His polymorphism. *Mol Vis.* 2012;18:2288-2299.

166. Smailhodzic D, Klaver CC, Klevering BJ, et al. Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. *Ophthalmology.* 2012;119(2):339-346.

167. Hecker LA, Edwards AO, Ryu E, et al. Genetic control of the alternative pathway of complement in humans and age-related macular degeneration. *Hum Mol Genet.* 2010;19(1):209-215.

168. Ristau T, Ersoy L, Lechanteur Y, et al. Allergy is a protective factor against age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(1):210-214.

169. Ristau T, Paun C, Ersoy L, et al. Impact of the common genetic associations of age-related macular degeneration upon systemic complement component C3d levels. *PLoS One.* 2014;9(3):e93459.

170. Sivaprasad S, Adewoyin T, Bailey TA, et al. Estimation of systemic complement C3 activity in age-related macular degeneration. *Arch Ophthalmol.* 2007;125(4):515-519.

171. Smailhodzic D, van Asten F, Blom AM, et al. Zinc supplementation inhibits complement activation in age-related macular degeneration. *PLoS One.* 2014;9(11):e112682.

172. Ansari M, McKeigue PM, Skerka C, et al. Genetic influences on plasma CFH and CFHR1 concentrations and their role in susceptibility to age-related macular degeneration. *Hum Mol Genet.* 2013;22(23):4857-4869.

173. Sharma NK, Gupta A, Prabhakar S, et al. Association between CFH Y402H polymorphism and age related macular degeneration in North Indian cohort. *PLoS One.* 2013;8(7):e70193.

174. Hakobyan S, Harris CL, Tortajada A, et al. Measurement of factor H variants in plasma using variant-specific monoclonal antibodies: application to assessing risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2008;49(5):1983-1990.

175. Guymer R, Cipriani T, Rittenhouse KD, et al. Plasma levels of amyloid beta and other proinflammatory mediators in patients with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2015;253(8):1347-1354.

176. van de Ven JP, Nilsson SC, Tan PL, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. *Nat Genet.* 2013;45(7):813-817.

177. Stanton CM, Yates JR, den Hollander AI, et al. Complement factor D in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2011;52(12):8828-8834.

178. Haas P, Aggermann T, Nagl M, Steindl-Kuscher K, Krugluger W, Binder S. Implication of CD21, CD35, and CD55 in the pathogenesis of age-related macular degeneration. *Am J Ophthalmol.* 2011;152(3):396-399.e391.

179. Singh A, Faber C, Falk M, Nissen MH, Hviid TV, Sorensen TL. Altered expression of CD46 and CD59 on leukocytes in neovascular age-related macular degeneration. *Am J Ophthalmol.* 2012;154(1):193-199.e192.

180. Paun CC, Lechanteur YT, Groenewoud JM, et al. A Novel Complotype Combination Associates with Age-Related Macular Degeneration and High Complement Activation Levels in vivo. *Sci Rep.* 2016;6:26568.

181. Geerlings MJ, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2017;135(1):39-46.

182. Kavanagh D, Yu Y, Schramm EC, et al. Rare genetic variants in the CFI gene are associated with advanced age-related macular degeneration and commonly result in reduced serum factor I levels. *Hum Mol Genet.* 2015;24(13):3861-3870.

183. Triebwasser MP, Roberson ED, Yu Y, et al. Rare Variants in the Functional Domains of Complement Factor H Are Associated With Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(11):6873-6878.

184. Wagner EK, Raychaudhuri S, Villalonga MB, et al. Mapping rare, deleterious mutations in Factor H: Association with early onset, drusen burden, and lower antigenic levels in familial AMD. *Sci Rep.* 2016;6:31531.

185. Yu Y, Triebwasser MP, Wong EK, et al. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. *Hum Mol Genet.* 2014;23(19):5283-5293.

186. Geerlings MJ, de Jong EK, den Hollander AI. The complement system in age-related macular degeneration: A review of rare genetic variants and implications for personalized treatment. *Mol Immunol.* 2016.

187. Mauer J, Denson JL, Bruning JC. Versatile functions for IL-6 in metabolism and cancer. *Trends Immunol.* 2015;36(2):92-101.

188. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci (Lond).* 2012;122(4):143-159.

189. Haas P, Kubista KE, Krugluger W, Huber J, Binder S. Impact of visceral fat and pro-inflammatory factors on the pathogenesis of age-related macular degeneration. *Acta Ophthalmol.* 2015;93(6):533-538.

190. Klein R, Myers CE, Cruickshanks KJ, et al. Markers of inflammation, oxidative stress, and endothelial dysfunction and the 20-year cumulative incidence of early age-related macular degeneration: the Beaver Dam Eye Study. *JAMA Ophthalmol.* 2014;132(4):446-455.

191. Aoki A, Tan X, Yamagishi R, et al. Risk Factors for Age-Related Macular Degeneration in an Elderly Japanese Population: The Hatoyama Study. *Invest Ophthalmol Vis Sci.* 2015;56(4):2580-2585.

192. Klein R, Klein BE, Knudtson MD, Wong TY, Shankar A, Tsai MY. Systemic markers of inflammation, endothelial dysfunction, and age-related maculopathy. *Am J Ophthalmol.* 2005;140(1):35-44.

193. Nassar K, Grisanti S, Elfar E, Luke J, Luke M, Grisanti S. Serum cytokines as biomarkers for age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2015;253(5):699-704.

194. Seddon JM, George S, Rosner B, Rifai N. Progression of age-related macular degeneration: prospective assessment of C-reactive protein, interleukin 6, and other cardiovascular biomarkers. *Arch Ophthalmol.* 2005;123(6):774-782.

195. Faber C, Jehs T, Juel HB, et al. Early and exudative age-related macular degeneration is associated with increased plasma levels of soluble TNF receptor II. *Acta Ophthalmol.* 2015;93(3):242-247.

196. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A.* 2008;105(30):10513-10518.

197. Munch IC, Linneberg A, Larsen M. Precursors of age-related macular degeneration: associations with physical activity, obesity, and serum lipids in the inter99 eye study. *Invest Ophthalmol Vis Sci.* 2013;54(6):3932-3940.

198. Hwang HS, Lee SB, Jee D. Association between Blood Lead Levels and Age-Related Macular Degeneration. *PLoS One.* 2015;10(8):e0134338.

199. Zhao M, Bai Y, Xie W, et al. Interleukin-1beta Level Is Increased in Vitreous of Patients with Neovascular Age-Related Macular Degeneration (nAMD) and Polypoidal Choroidal Vasculopathy (PCV). *PLoS One.* 2015;10(5):e0125150.

200. Ross RJ, Zhou M, Shen D, et al. Immunological protein expression profile in Ccl2/Cx3cr1 deficient mice with lesions similar to age-related macular degeneration. *Exp Eye Res.* 2008;86(4):675-683.

201. Sennlaub F, Auvynet C, Calippe B, et al. CCR2(+) monocytes infiltrate atrophic lesions in age-related macular disease and mediate photoreceptor degeneration in experimental subretinal inflammation in Cx3cr1 deficient mice. *EMBO Mol Med.* 2013;5(11):1775-1793.

202. Takeda A, Baffi JZ, Kleinman ME, et al. CCR3 is a target for age-related macular degeneration diagnosis and therapy. *Nature.* 2009;460(7252):225-230.

203. Falk MK, Singh A, Faber C, Nissen MH, Hviid T, Sorensen TL. CX3CL1/CX3CR1 and CCL2/CCR2 chemokine/chemokine receptor complex in patients with AMD. *PLoS One.* 2014;9(12):e112473.

204. Grunin M, Burstyn-Cohen T, Hagbi-Levi S, Peled A, Chowers I. Chemokine receptor expression in peripheral blood monocytes from patients with neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2012;53(9):5292-5300.

205. Anand A, Sharma NK, Gupta A, et al. Single nucleotide polymorphisms in MCP-1 and its receptor are associated with the risk of age related macular degeneration. *PLoS One.* 2012;7(11):e49905.

206. Zhang R, Gascon R, Miller RG, et al. MCP-1 chemokine receptor CCR2 is decreased on circulating monocytes in sporadic amyotrophic lateral sclerosis (sALS). *J Neuroimmunol.* 2006;179(1-2):87-93.

207. Fauser S, Smailhodzic D, Caramoy A, et al. Evaluation of serum lipid concentrations and genetic variants at high-density lipoprotein metabolism loci and TIMP3 in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2011;52(8):5525-5528.

208. Yip JL, Khawaja AP, Chan MP, et al. Cross Sectional and Longitudinal Associations between Cardiovascular Risk Factors and Age Related Macular Degeneration in the EPIC-Norfolk Eye Study. *PLoS One.* 2015;10(7):e0132565.

209. Falk MK, Singh A, Faber C, Nissen MH, Hviid T, Sorensen TL. Blood expression levels of chemokine receptor CCR3 and chemokine CCL11 in age-related macular degeneration: a case-control study. *BMC Ophthalmol.* 2014;14:22.

210. Sharma NK, Prabhakar S, Gupta A, et al. New biomarker for neovascular age-related macular degeneration: eotaxin-2. *DNA Cell Biol.* 2012;31(11):1618-1627.

211. Angiolillo AL, Sgadari C, Taub DD, et al. Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo. *J Exp Med.* 1995;182(1):155-162.

212. Falk MK, Singh A, Faber C, Nissen MH, Hviid T, Sorensen TL. Dysregulation of CXCR3 expression on peripheral blood leukocytes in patients with neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(7):4050-4056.

213. Machalinska A, Klos P, Safranow K, et al. Neural stem/progenitor cells circulating in peripheral blood of patients with neovascular form of AMD: a novel view on pathophysiology. *Graefes Arch Clin Exp Ophthalmol.* 2011;249(12):1785-1794.

214. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv Immunol.* 2007;96:41-101.

215. Cohn AC, Busija L, Robman LD, et al. Younger siblings, C-reactive protein, and risk of age-related macular degeneration. *Am J Epidemiol.* 2013;177(9):933-943.

216. Colak E, Kosanovic-Jakovic N, Zoric L, Radosavljevic A, Stankovic S, Majkic-Singh N. The association of lipoprotein parameters and C-reactive protein in patients with age-related macular degeneration. *Ophthalmic Res.* 2011;46(3):125-132.

217. Hong T, Tan AG, Mitchell P, Wang JJ. A review and meta-analysis of the association between C-reactive protein and age-related macular degeneration. *Surv Ophthalmol.* 2011;56(3):184-194.

218. Kikuchi M, Nakamura M, Ishikawa K, et al. Elevated C-reactive protein levels in patients with polypoidal choroidal vasculopathy and patients with neovascular age-related macular degeneration. *Ophthalmology.* 2007;114(9):1722-1727.

219. Min JK, Kim J, Woo JM. Elevated Plasma Pentraxin3 Levels and Its Association with Neovascular Age-related Macular Degeneration. *Ocul Immunol Inflamm.* 2015;23(3):205-211.

220. Robman L, Baird PN, Dimitrov PN, Richardson AJ, Guymer RH. C-reactive protein levels and complement factor H polymorphism interaction in age-related macular degeneration and its progression. *Ophthalmology.* 2010;117(10):1982-1988.

221. Seddon JM, Gensler G, Milton RC, Klein ML, Rifai N. Association between C-reactive protein and age-related macular degeneration. *Jama.* 2004;291(6):704-710.

222. Seddon JM, Gensler G, Rosner B. C-reactive protein and CFH, ARMS2/HTRA1 gene variants are independently associated with risk of macular degeneration. *Ophthalmology.* 2010;117(8):1560-1566.

223. Ulas F, Balbaba M, Ozmen S, Celebi S, Dogan U. Association of dehydroepiandrosterone sulfate, serum lipids, C-reactive protein and body mass index with age-related macular degeneration. *Int Ophthalmol.* 2013;33(5):485-491.

224. Weiner DE, Tighiouart H, Reynolds R, Seddon JM. Kidney function, albuminuria and age-related macular degeneration in NHANES III. *Nephrol Dial Transplant.* 2011;26(10):3159-3165.

225. Boey PY, Tay WT, Lamoureux E, et al. C-reactive protein and age-related macular degeneration and cataract: the singapore malay eye study. *Invest Ophthalmol Vis Sci.* 2010;51(4):1880-1885.

226. Ho L, Witteman JC, Rohrer B, Hofman A, de Jong PT, Vingerling JR. Lipoprotein-associated phospholipase A2 and risk of age-related macular degeneration: the Rotterdam Study. *Arch Ophthalmol.* 2009;127(3):340-341.

227. Hogg RE, Woodside JV, Gilchrist SE, et al. Cardiovascular disease and hypertension are strong risk factors for choroidal neovascularization. *Ophthalmology.* 2008;115(6):1046-1052.e1042.

228. Jonasson F, Fisher DE, Eiriksdottir G, et al. Five-year incidence, progression, and risk factors for age-related macular degeneration: the age, gene/environment susceptibility study. *Ophthalmology.* 2014;121(9):1766-1772.

229. McGwin G, Hall TA, Xie A, Owsley C. The relation between C reactive protein and age related macular degeneration in the Cardiovascular Health Study. *Br J Ophthalmol.* 2005;89(9):1166-1170.

230. Singh A, Falk MK, Hviid TV, Sorensen TL. Increased expression of CD200 on circulating CD11b+ monocytes in patients with neovascular age-related macular degeneration. *Ophthalmology.* 2013;120(5):1029-1037.

231. Subramani S, Khor SE, Livingstone BI, Kulkarni UV. Serum uric acid levels and its association with age-related macular degeneration (ARMD). *Med J Malaysia.* 2010;65(1):36-40.

232. Boekhoorn SS, Vingerling JR, Witteman JC, Hofman A, de Jong PT. C-reactive protein level and risk of aging macula disorder: The Rotterdam Study. *Arch Ophthalmol.* 2007;125(10):1396-1401.

233. Mitta VP, Christen WG, Glynn RJ, et al. C-reactive protein and the incidence of macular degeneration: pooled analysis of 5 cohorts. *JAMA Ophthalmol.* 2013;131(4):507-513.

234. Chen H, Liu C, Sun S, Mei Y, Tong E. Cytokine-induced cell surface expression of adhesion molecules in vascular endothelial cells in vitro. *J Tongji Med Univ.* 2001;21(1):68-71.

235. Blumenkranz MS, Russell SR, Robey MG, Kott-Blumenkranz R, Penneys N. Risk factors in age-related maculopathy complicated by choroidal neovascularization. *Ophthalmology.* 1986;93(5):552-558.

236. Klein R, Klein BE, Franke T. The relationship of cardiovascular disease and its risk factors to age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology.* 1993;100(3):406-414.

237. Shankar A, Mitchell P, Rochtchina E, Tan J, Wang JJ. Association between circulating white blood cell count and long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Am J Epidemiol.* 2007;165(4):375-382.

238. Joachim ND, Mitchell P, Kifley A, Wang JJ. Incidence, Progression, and Associated Risk Factors of Medium Drusen in Age-Related Macular Degeneration: Findings From the 15-Year Follow-up of an Australian Cohort. *JAMA Ophthalmol.* 2015;133(6):698-705.

239. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. Association of emphysema, gout, and inflammatory markers with long-term incidence of age-related maculopathy. *Arch Ophthalmol.* 2003;121(5):674-678.

240. Ilhan N, Daglioglu MC, Ilhan O, et al. Assessment of Neutrophil/Lymphocyte Ratio in Patients with Age-related Macular Degeneration. *Ocul Immunol Inflamm.* 2014:1-4.

241. Juel HB, Faber C, Munthe-Fog L, et al. Systemic and Ocular Long Pentraxin 3 in Patients with Age-Related Macular Degeneration. *PLoS One.* 2015;10(7):e0132800.

242. Doni A, Garlanda C, Bottazzi B, Meri S, Garred P, Mantovani A. Interactions of the humoral pattern recognition molecule PTX3 with the complement system. *Immunobiology.* 2012;217(11):1122-1128.

243. Poletaev AB, Churilov LP, Stroev YI, Agapov MM. Immunophysiology versus immunopathology: Natural autoimmunity in human health and disease. *Pathophysiology.* 2012;19(3):221-231.

244. Cherepanoff S, Mitchell P, Wang JJ, Gillies MC. Retinal autoantibody profile in early age-related macular degeneration: preliminary findings from the Blue Mountains Eye Study. *Clin Experiment Ophthalmol.* 2006;34(6):590-595.

245. Gurne DH, Tso MO, Edward DP, Ripps H. Antiretinal antibodies in serum of patients with age-related macular degeneration. *Ophthalmology.* 1991;98(5):602-607.

246. Patel N, Ohbayashi M, Nugent AK, et al. Circulating anti-retinal antibodies as immune markers in age-related macular degeneration. *Immunology.* 2005;115(3):422-430.

247. Penfold PL, Provis JM, Furby JH, Gatenby PA, Billson FA. Autoantibodies to retinal astrocytes associated with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 1990;228(3):270-274.

248. Adamus G, Chew EY, Ferris FL, Klein ML. Prevalence of anti-retinal autoantibodies in different stages of Age-related macular degeneration. *BMC Ophthalmol.* 2014;14:154.

249. Kubicka-Trzaska A, Wilanska J, Romanowska-Dixon B, Sanak M. Circulating antiretinal antibodies predict the outcome of anti-VEGF therapy in patients with exudative age-related macular degeneration. *Acta Ophthalmol.* 2012;90(1):e21-24.

250. Kubicka-Trzaska A, Wilanska J, Romanowska-Dixon B, Sanak M. Circulating anti-retinal antibodies in response to anti-angiogenic therapy in exudative age-related macular degeneration. *Acta Ophthalmol.* 2014;92(8):e610-614.

251. Iannaccone A, Giorgianni F, New DD, et al. Circulating Autoantibodies in Age-Related Macular Degeneration Recognize Human Macular Tissue Antigens Implicated in Autophagy, Immunomodulation, and Protection from Oxidative Stress and Apoptosis. *PLoS One.* 2015;10(12):e0145323.

252. Joachim SC, Bruns K, Lackner KJ, Pfeiffer N, Grus FH. Analysis of IgG antibody patterns against retinal antigens and antibodies to alpha-crystallin, GFAP, and alpha-enolase in sera of patients with "wet" age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 2007;245(5):619-626.

253. Morohoshi K, Ohbayashi M, Patel N, Chong V, Bird AC, Ono SJ. Identification of anti-retinal antibodies in patients with age-related macular degeneration. *Exp Mol Pathol.* 2012;93(2):193-199.

254. Morohoshi K, Patel N, Ohbayashi M, et al. Serum autoantibody biomarkers for age-related macular degeneration and possible regulators of neovascularization. *Exp Mol Pathol.* 2012;92(1):64-73.

255. Ozkan B, Karabas LV, Altintas O, Tamer GS, Yuksel N, Caglar Y. Plasma antiphospholipid antibody levels in age-related macular degeneration. *Can J Ophthalmol.* 2012;47(3):264-268.

256. Honarmand H. Atherosclerosis Induced by Chlamydophila pneumoniae: A Controversial Theory. *Interdiscip Perspect Infect Dis.* 2013;2013:941392.

257. Kalayoglu MV, Galvan C, Mahdi OS, Byrne GI, Mansour S. Serological association between Chlamydia pneumoniae infection and age-related macular degeneration. *Arch Ophthalmol.* 2003;121(4):478-482.

258. Miller DM, Espinosa-Heidmann DG, Legra J, et al. The association of prior cytomegalovirus infection with neovascular age-related macular degeneration. *Am J Ophthalmol.* 2004;138(3):323-328.

259. Robman L, Mahdi OS, Wang JJ, et al. Exposure to Chlamydia pneumoniae infection and age-related macular degeneration: the Blue Mountains Eye Study. *Invest Ophthalmol Vis Sci.* 2007;48(9):4007-4011.

260. Faber C, Singh A, Kruger Falk M, Juel HB, Sorensen TL, Nissen MH. Age-related macular degeneration is associated with increased proportion of CD56(+) T cells in peripheral blood. *Ophthalmology.* 2013;120(11):2310-2316.

261. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res.* 2001;20(6):705-732.

262. Wang L, Clark ME, Crossman DK, et al. Abundant lipid and protein components of drusen. *PLoS One.* 2010;5(4):e10329.

263. Wu J, Uchino M, Sastry SM, Schaumberg DA. Age-related macular degeneration and the incidence of cardiovascular disease: a systematic review and meta-analysis. *PLoS One.* 2014;9(3):e89600.

264. Lusis AJ. Atherosclerosis. *Nature.* 2000;407(6801):233-241.

265. Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet.* 2009;10(2):109-121.

266. Butt AL, Lee ET, Klein R, et al. Prevalence and risks factors of age-related macular degeneration in Oklahoma Indians: the Vision Keepers Study. *Ophthalmology.* 2011;118(7):1380-1385.

267. Peiretti E, Mandas A, Abete C, et al. Age-related macular degeneration and cognitive impairment show similarities in changes of neutral lipids in peripheral blood mononuclear cells. *Exp Eye Res.* 2014;124:11-16.

268. Ambreen F, Khan WA, Qureshi N, Qureshi IZ. Assessment of serum lipids in patients with age related macular degeneration from Pakistan. *J Pak Med Assoc.* 2014;64(6):664-669.

269. Davari MH, Gheitasi H, Yaghobi G, Heydari B. Correlation between serum lipids and age-related macular degeneration: a case-control study. *J Res Health Sci.* 2013;13(1):98-101.

270. Fourgeux C, Dugas B, Richard F, et al. Single nucleotide polymorphism in the cholesterol-24S-hydroxylase (CYP46A1) gene and its association with CFH and LOC387715 gene polymorphisms in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2012;53(11):7026-7033.

271. Ghorbanihaghjo A, Javadzadeh A, Rashtchizadeh N, Sorkhabi R, Khalili H, Rahimi-Ardabili B. Osteoprotegerin and soluble receptor activator of nuclear factor-kappa B ligand in exudative age-related macular degeneration. *Acta Med Iran.* 2014;52(4):265-270.

272. Nowak M, Swietochowska E, Marek B, et al. Changes in lipid metabolism in women with age-related macular degeneration. *Clin Exp Med.* 2005;4(4):183-187.

273. Klein R, Myers CE, Buitendijk GH, et al. Lipids, lipid genes, and incident age-related macular degeneration: the three continent age-related macular degeneration consortium. *Am J Ophthalmol.* 2014;158(3):513-524 e513.

274. Cackett P, Wong TY, Aung T, et al. Smoking, cardiovascular risk factors, and age-related macular degeneration in Asians: the Singapore Malay Eye Study. *Am J Ophthalmol.* 2008;146(6):960-967. e961.

275. Chaker L, Buitendijk GH, Dehghan A, et al. Thyroid function and age-related macular degeneration: a prospective population-based cohort study--the Rotterdam Study. *BMC Med.* 2015;13:94.

276. Cougnard-Gregoire A, Delyfer MN, Korobelnik JF, et al. Elevated high-density lipoprotein cholesterol and age-related macular degeneration: the Alienor study. *PLoS One.* 2014;9(3):e90973.

277. Delcourt C, Michel F, Colvez A, Lacroux A, Delage M, Vernet MH. Associations of cardiovascular disease and its risk factors with age-related macular degeneration: the POLA study. *Ophthalmic Epidemiol.* 2001;8(4):237-249.

278. Jonas JB, Nangia V, Kulkarni M, Gupta R, Khare A. Associations of early age-related macular degeneration with ocular and general parameters. The Central India Eyes and Medical Study. *Acta Ophthalmol.* 2012;90(3):e185-191.

279. Kim EC, Cho E, Jee D. Association between blood cadmium level and age-related macular degeneration in a representative Korean population. *Invest Ophthalmol Vis Sci.* 2014;55(9):5702-5710.

280. Kim EC, Han K, Jee D. Inverse relationship between high blood 25-hydroxyvitamin D and late stage of age-related macular degeneration in a representative Korean population. *Invest Ophthalmol Vis Sci.* 2014;55(8):4823-4831.

281. La TY, Cho E, Kim EC, Kang S, Jee D. Prevalence and risk factors for age-related macular degeneration: Korean National Health and Nutrition Examination Survey 2008-2011. *Curr Eye Res.* 2014;39(12):1232-1239.

282. Park SJ, Lee JH, Woo SJ, et al. Age-related macular degeneration: prevalence and risk factors from Korean National Health and Nutrition Examination Survey, 2008 through 2011. *Ophthalmology.* 2014;121(9):1756-1765.

283. Seshasai S, Liao J, Toh QC, et al. Serum leptin and age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(3):1880-1886.

284. van Leeuwen R, Klaver CC, Vingerling JR, et al. Cholesterol and age-related macular degeneration: is there a link? *Am J Ophthalmol.* 2004;137(4):750-752.

285. Wang S, Xu L, Jonas JB, You QS, Wang YX, Yang H. Dyslipidemia and eye diseases in the adult Chinese population: the Beijing eye study. *PLoS One.* 2012;7(3):e26871.

286. Yang K, Wang FH, Liang YB, et al. Associations between cardiovascular risk factors and early age-related macular degeneration in a rural Chinese adult population. *Retina.* 2014;34(8):1539-1553.

287. Paun CC, Ersoy L, Schick T, et al. Genetic Variants and Systemic Complement Activation Levels Are Associated With Serum Lipoprotein Levels in Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(13):7766-7773.

288. Abalain JH, Carre JL, Leglise D, et al. Is age-related macular degeneration associated with serum lipoprotein and lipoparticle levels? *Clin Chim Acta.* 2002;326(1-2):97-104.

289. Ouweneel AB, Van Eck M. Lipoproteins as modulators of atherothrombosis: From endothelial function to primary and secondary coagulation. *Vascul Pharmacol.* 2016;82:1-10.

290. Lusis AJ, Pajukanta P. A treasure trove for lipoprotein biology. *Nat Genet.* 2008;40(2):129-130.

291. Javadzadeh A, Ghorbanihaghjo A, Rashtchizadeh N, Rafeey M, Rahimi-Ardabili B. Enhanced susceptibility of low-density lipoprotein to oxidation in wet type age-related macular degeneration in male patients. *Saudi Med J.* 2007;28(2):221-224.

292. Reynolds R, Rosner B, Seddon JM. Serum lipid biomarkers and hepatic lipase gene associations with age-related macular degeneration. *Ophthalmology.* 2010;117(10):1989-1995.

293. You QS, Xu L, Yang H, et al. Five-year incidence of age-related macular degeneration: the Beijing Eye Study. *Ophthalmology.* 2012;119(12):2519-2525.

294. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499-502.

295. Hyman L, Schachat AP, He Q, Leske MC. Hypertension, cardiovascular disease, and age-related macular degeneration. Age-Related Macular Degeneration Risk Factors Study Group. *Arch Ophthalmol.* 2000;118(3):351-358.

296. Emerging Risk Factors C, Erqou S, Kaptoge S, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA.* 2009;302(4):412-423.

297. Kassner U, Schlabs T, Rosada A, Steinhagen-Thiessen E. Lipoprotein(a)--An independent causal risk factor for cardiovascular disease and current therapeutic options. *Atheroscler Suppl.* 2015;18:263-267.

298. Ersoy L, Ristau T, Lechanteur YT, et al. Nutritional risk factors for age-related macular degeneration. *Biomed Res Int.* 2014;2014:413150.

299. Dashti N, McGwin G, Owsley C, Curcio CA. Plasma apolipoproteins and risk for age related maculopathy. *Br J Ophthalmol.* 2006;90(8):1028-1033.

300. Ouchi M, Ikeda T, Nakamura K, Harino S, Kinoshita S. A novel relation of fatty acid with age-related macular degeneration. *Ophthalmologica.* 2002;216(5):363-367.

301. Merle BM, Benlian P, Puche N, Bassols A, Delcourt C, Souied EH. Circulating omega-3 Fatty acids and neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(3):2010-2019.

302. Merle BM, Delyfer MN, Korobelnik JF, et al. High concentrations of plasma n3 fatty acids are associated with decreased risk for late age-related macular degeneration. *J Nutr.* 2013;143(4):505-511.

303. Barker FM, 2nd, Snodderly DM, Johnson EJ, et al. Nutritional manipulation of primate retinas, V: effects of lutein, zeaxanthin, and n-3 fatty acids on retinal sensitivity to blue-light-induced damage. *Invest Ophthalmol Vis Sci.* 2011;52(7):3934-3942.

304. Souied EH, Aslam T, Garcia-Layana A, et al. Omega-3 Fatty Acids and Age-Related Macular Degeneration. *Ophthalmic Res.* 2015;55(2):62-69.

305. Merle BM, Richard F, Benlian P, Puche N, Delcourt C, Souied EH. CFH Y402H and ARMS2 A69S Polymorphisms and Oral Supplementation with Docosahexaenoic Acid in Neovascular Age-Related Macular Degeneration Patients: The NAT2 Study. *PLoS One.* 2015;10(7):e0130816.

306. Kabasawa S, Mori K, Horie-Inoue K, et al. Associations of cigarette smoking but not serum fatty acids with age-related macular degeneration in a Japanese population. *Ophthalmology.* 2011;118(6):1082-1088.

307. Orban T, Johnson WM, Dong Z, et al. Serum levels of lipid metabolites in age-related macular degeneration. *Faseb j.* 2015;29(11):4579-4588.

308. Johnson LV, Anderson DH. Age-related macular degeneration and the extracellular matrix. *N Engl J Med.* 2004;351(4):320-322.

309. Nita M, Strzalka-Mrozik B, Grzybowski A, Mazurek U, Romaniuk W. Age-related macular degeneration and changes in the extracellular matrix. *Med Sci Monit.* 2014;20:1003-1016.

310. Chong NH, Keonin J, Luthert PJ, et al. Decreased thickness and integrity of the macular elastic layer of Bruch's membrane correspond to the distribution of lesions associated with age-related macular degeneration. *Am J Pathol.* 2005;166(1):241-251.

311. Coleman HR, Chan CC, Ferris FL, 3rd, Chew EY. Age-related macular degeneration. *Lancet.* 2008;372(9652):1835-1845.

312. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res.* 2006;69(3):562-573.

313. Ratnapriya R, Zhan X, Fariss RN, et al. Rare and common variants in extracellular matrix gene Fibrillin 2 (FBN2) are associated with macular degeneration. *Hum Mol Genet.* 2014;23(21):5827-5837.

314. Chau KY, Sivaprasad S, Patel N, Donaldson TA, Luthert PJ, Chong NV. Plasma levels of matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) in age-related macular degeneration. *Eye (Lond).* 2007;21(12):1511-1515.

315. Chau KY, Sivaprasad S, Patel N, Donaldson TA, Luthert PJ, Chong NV. Plasma levels of matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) in age-related macular degeneration. *Eye (Lond).* 2008;22(6):855-859.

316. Zeng R, Wen F, Zhang X, Su Y. Serum levels of matrix metalloproteinase 2 and matrix metalloproteinase 9 elevated in polypoidal choroidal vasculopathy but not in age-related macular degeneration. *Mol Vis.* 2013;19:729-736.

317. Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. *Birth Defects Res C Embryo Today.* 2007;81(4):229-240.

318. Hogan MJ. Bruch's membrane and disease of the macula. Role of elastic tissue and collagen. *Trans Ophthalmol Soc U K.* 1967;87:113-161.

319. Sivaprasad S, Chong NV, Bailey TA. Serum elastin-derived peptides in age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2005;46(9):3046-3051.

320. Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. *J Nutr.* 2001;131(2):369S-373S.

321. Belda JI, Roma J, Vilela C, et al. Serum vitamin E levels negatively correlate with severity of age-related macular degeneration. *Mech Ageing Dev.* 1999;107(2):159-164.

322. Tsang NC, Penfold PL, Snitch PJ, Billson F. Serum levels of antioxidants and age-related macular degeneration. *Doc Ophthalmol.* 1992;81(4):387-400.

323. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr.* 2008;88(2):491S-499S.

324. Itty S, Day S, Lyles KW, Stinnett SS, Vajzovic LM, Mruthyunjaya P. Vitamin D deficiency in neovascular versus nonneovascular age-related macular degeneration. *Retina.* 2014;34(9):1779-1786.

325. Parekh N, Chappell RJ, Millen AE, Albert DM, Mares JA. Association between vitamin D and age-related macular degeneration in the Third National Health and Nutrition Examination Survey, 1988 through 1994. *Arch Ophthalmol.* 2007;125(5):661-669.

326. Cougnard-Gregoire A, Merle BM, Korobelnik JF, et al. Vitamin D Deficiency in Community-Dwelling Elderly Is Not Associated with Age-Related Macular Degeneration. *J Nutr.* 2015;145(8):1865-1872.

327. Golan S, Shalev V, Treister G, Chodick G, Loewenstein A. Reconsidering the connection between vitamin D levels and age-related macular degeneration. *Eye (Lond).* 2011;25(9):1122-1129.

328. Millen AE, Voland R, Sondel SA, et al. Vitamin D status and early age-related macular degeneration in postmenopausal women. *Arch Ophthalmol.* 2011;129(4):481-489.

329. Morrison MA, Silveira AC, Huynh N, et al. Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. *Hum Genomics.* 2011;5(6):538-568.

330. Singh A, Falk MK, Subhi Y, Sorensen TL. The association between plasma 25-hydroxyvitamin D and subgroups in age-related macular degeneration: a cross-sectional study. *PLoS One.* 2013;8(7):e70948.

331. Bhattacharya PT, Misra SR, Hussain M. Nutritional Aspects of Essential Trace Elements in Oral Health and Disease: An Extensive Review. *Scientifica (Cairo).* 2016;2016:5464373.

332. Song D, Dunaief JL. Retinal iron homeostasis in health and disease. *Front Aging Neurosci.* 2013;5:24.

333. Wills NK, Ramanujam VM, Chang J, et al. Cadmium accumulation in the human retina: effects of age, gender, and cellular toxicity. *Exp Eye Res.* 2008;86(1):41-51.

334. Houston MC. Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. *J Clin Hypertens (Greenwich).* 2011;13(8):621-627.

335. Wills NK, Ramanujam VM, Kalariya N, Lewis JR, van Kuijk FJ. Copper and zinc distribution in the human retina: relationship to cadmium accumulation, age, and gender. *Exp Eye Res.* 2008;87(2):80-88.

336. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci.* 2000;57(13-14):1825-1835.

337. Nan R, Farabella I, Schumacher FF, et al. Zinc binding to the Tyr402 and His402 allotypes of complement factor H: possible implications for age-related macular degeneration. *J Mol Biol.* 2011;408(4):714-735.

338. Nan R, Gor J, Lengyel I, Perkins SJ. Uncontrolled zinc- and copper-induced oligomerisation of the human complement regulator factor H and its possible implications for function and disease. *J Mol Biol.* 2008;384(5):1341-1352.

339. Nan R, Tetchner S, Rodriguez E, et al. Zinc-induced self-association of complement C3b and Factor H: implications for inflammation and age-related macular degeneration. *J Biol Chem.* 2013;288(26):19197-19210.

340. Satarug S, Kikuchi M, Wisedpanichkij R, et al. Prevention of cadmium accumulation in retinal pigment epithelium with manganese and zinc. *Exp Eye Res.* 2008;87(6):587-593.

341. Park SJ, Lee JH, Woo SJ, Kang SW, Park KH. Five heavy metallic elements and age-related macular degeneration: Korean National Health and Nutrition Examination Survey, 2008-2011. *Ophthalmology.* 2015;122(1):129-137.

342. Wu EW, Schaumberg DA, Park SK. Environmental cadmium and lead exposures and age-related macular degeneration in U.S. adults: the National Health and Nutrition Examination Survey 2005 to 2008. *Environ Res.* 2014;133:178-184.

343. Junemann AG, Stopa P, Michalke B, et al. Levels of aqueous humor trace elements in patients with non-exsudative age-related macular degeneration: a case-control study. *PLoS One.* 2013;8(2):e56734.

344. Erie JC, Good JA, Butz JA, Hodge DO, Pulido JS. Urinary cadmium and age-related macular degeneration. *Am J Ophthalmol.* 2007;144(3):414-418.

345. Mayer MJ, van Kuijk FJ, Ward B, Glucs A. Whole blood selenium in exudative age-related maculopathy. *Acta Ophthalmol Scand.* 1998;76(1):62-67.

346. Wysokinski D, Danisz K, Blasiak J, et al. An association of transferrin gene polymorphism and serum transferrin levels with age-related macular degeneration. *Exp Eye Res.* 2013;106:14-23.

347. Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. *Int J Gen Med.* 2016;9:229-255.

348. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2002;99(23):14682-14687.

349. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. *Acta Biochim Pol.* 2003;50(4):1129-1146.

350. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. *J Biomed Sci.* 2000;7(6):444-458.

351. Kennaway DJ, Lushington K, Dawson D, Lack L, van den Heuvel C, Rogers N. Urinary 6-sulfatoxymelatonin excretion and aging: new results and a critical review of the literature. *J Pineal Res.* 1999;27(4):210-220.

352. Schmid-Kubista KE, Glittenberg CG, Cezanne M, Holzmann K, Neumaier-Ammerer B, Binder S. Daytime levels of melatonin in patients with age-related macular degeneration. *Acta Ophthalmol.* 2009;87(1):89-93.

353. Rosen R, Hu DN, Perez V, et al. Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. *Mol Vis.* 2009;15:1673-1679.

354. Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Front Neuroendocrinol.* 2009;30(1):65-91.

355. Tamer C, Oksuz H, Sogut S. Serum dehydroepiandrosterone sulphate level in age-related macular degeneration. *Am J Ophthalmol.* 2007;143(2):212-216.

356. Baulieu EE, Thomas G, Legrain S, et al. Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. *Proc Natl Acad Sci U S A.* 2000;97(8):4279-4284.

357. Defay R, Pinchinat S, Lumbroso S, Sutan C, Delcourt C. Sex steroids and age-related macular degeneration in older French women: the POLA study. *Ann Epidemiol.* 2004;14(3):202-208.

358. Duvvari MR, Paun CC, Buitendijk GH, et al. Analysis of rare variants in the C3 gene in patients with age-related macular degeneration. *PLoS One.* 2014;9(4):e94165.

359. Klein R, Knudtson MD, Lee KE, Klein BE. Serum cystatin C level, kidney disease markers, and incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Arch Ophthalmol.* 2009;127(2):193-199.

360. Liew G, Mitchell P, Wong TY, Iyengar SK, Wang JJ. CKD increases the risk of age-related macular degeneration. *J Am Soc Nephrol.* 2008;19(4):806-811.

361. Chong EW, Guymer RH, Klein R, et al. Is renal function associated with early age-related macular degeneration? *Optom Vis Sci.* 2014;91(8):860-864.

362. Arbor SC, LaFontaine M, Cumbay M. Amyloid-beta Alzheimer targets - protein processing, lipid rafts, and amyloid-beta pores. *Yale J Biol Med.* 2016;89(1):5-21.

363. Anderson DH, Talaga KC, Rivest AJ, Barron E, Hageman GS, Johnson LV. Characterization of beta amyloid assemblies in drusen: the deposits associated with aging and age-related macular degeneration. *Exp Eye Res.* 2004;78(2):243-256.

364. Dentchev T, Milam AH, Lee VM, Trojanowski JQ, Dunaief JL. Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. *Mol Vis.* 2003;9:184-190.

365. Johnson LV, Leitner WP, Rivest AJ, Staples MK, Radeke MJ, Anderson DH. The Alzheimer's A beta -peptide is deposited at sites of complement activation in pathologic deposits associated with aging and age-related macular degeneration. *Proc Natl Acad Sci U S A.* 2002;99(18):11830-11835.

366. Kim TW, Kang JW, Ahn J, et al. Proteomic analysis of the aqueous humor in age-related macular degeneration (AMD) patients. *J Proteome Res.* 2012;11(8):4034-4043.

367. Yao J, Liu X, Yang Q, et al. Proteomic analysis of the aqueous humor in patients with wet age-related macular degeneration. *Proteomics Clin Appl.* 2013;7(7-8):550-560.

368. Kannan R, Sreekumar PG, Hinton DR. Novel roles for alpha-crystallins in retinal function and disease. *Prog Retin Eye Res.* 2012;31(6):576-604.

369. Lee H, Choi AJ, Kang GY, et al. Increased 26S proteasome non-ATPase regulatory subunit 1 in the aqueous humor of patients with age-related macular degeneration. *BMB Rep.* 2014;47(5):292-297.

370. Kang GY, Bang JY, Choi AJ, et al. Exosomal proteins in the aqueous humor as novel biomarkers in patients with neovascular age-related macular degeneration. *J Proteome Res.* 2014;13(2):581-595.

371. Lyngholm M, Vorum H, Nielsen K, Ehlers N, Honore B. Attempting to distinguish between endogenous and contaminating cytokeratins in a corneal proteomic study. *BMC Ophthalmol.* 2011;11:3.

372. Park KH, Choi AJ, Yoon J, et al. Wnt modulators in the aqueous humor are associated with outer retinal damage severity in patients with neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(9):5522-5530.

373. Koss MJ, Hoffmann J, Nguyen N, et al. Proteomics of vitreous humor of patients with exudative age-related macular degeneration. *PLoS One.* 2014;9(5):e96895.

374. Nobl M, Reich M, Dacheva I, et al. Proteomics of vitreous in neovascular age-related macular degeneration. *Exp Eye Res.* 2016;146:107-117.

375. Xu XR, Zhong L, Huang BL, et al. Comparative proteomic analysis of plasma proteins in patients with age-related macular degeneration. *Int J Ophthalmol.* 2014;7(2):256-263.

376. Kim HJ, Ahn SJ, Woo SJ, et al. Proteomics-based identification and validation of novel plasma biomarkers phospholipid transfer protein and mannan-binding lectin serine protease-1 in age-related macular degeneration. *Sci Rep.* 2016;6:32548.

377. Kim HJ, Woo SJ, Suh EJ, et al. Identification of vinculin as a potential plasma marker for age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(11):7166-7176.

378. Geyer PE, Kulak NA, Pichler G, Holdt LM, Teupser D, Mann M. Plasma Proteome Profiling to Assess Human Health and Disease. *Cell Syst.* 2016;2(3):185-195.

379. Osborn MP, Park Y, Parks MB, et al. Metabolome-wide association study of neovascular age-related macular degeneration. *PLoS One.* 2013;8(8):e72737.

380. Wei L, Liu B, Tuo J, et al. Hypomethylation of the IL17RC promoter associates with age-related macular degeneration. *Cell Rep.* 2012;2(5):1151-1158.

381. Oliver VF, Franchina M, Jaffe AE, et al. Hypomethylation of the IL17RC promoter in peripheral blood leukocytes is not a hallmark of age-related macular degeneration. *Cell Rep.* 2013;5(6):1527-1535.

382. Oliver VF, Jaffe AE, Song J, et al. Differential DNA methylation identified in the blood and retina of AMD patients. *Epigenetics.* 2015;10(8):698-707.

383. Ertekin S, Yildirim O, Dinc E, Ayaz L, Fidanci SB, Tamer L. Evaluation of circulating miRNAs in wet age-related macular degeneration. *Mol Vis.* 2014;20:1057-1066.

384. Grassmann F, Schoenberger PG, Brandl C, et al. A circulating microrna profile is associated with late-stage neovascular age-related macular degeneration. *PLoS One.* 2014;9(9):e107461.

385. Szemraj M, Bielecka-Kowalska A, Oszajca K, et al. Serum MicroRNAs as Potential Biomarkers of AMD. *Med Sci Monit.* 2015;21:2734-2742.

14. SUPPLEMENTARY MATERIAL

Supplementary table 1. Factors involved in neovascularization

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Vascular Endothelial Growth Factor (VEGF)	(Lip et al., 2001)	Up	Any AMD	78	25	Cross-sectional	Plasma	
	(Holekamp et al., 2002)	No difference	Advanced: Neovascular	9	12	Case-control	Vitreous	
	(Tong et al., 2006)	Up	Advanced: Neovascular	12	10	Prospective Case-control	Aqueous	
	(Tsai et al., 2006)	Up	Early AMD/ Advanced: neovascular	17/ 60	42	Case-control	Plasma	VEGF levels were higher in nAMD vs. early AMD
	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Machalinska et al., 2011b)	No difference	Advanced: Neovascular	29	38	Case-control	Plasma	
	(Haas et al., 2011b)	No difference	Advanced: Neovascular	66	66	Case-control	Serum	
	(Huber and Wachtlin, 2012)	No difference	Advanced: Neovascular	12	11	Case-control	Vitreous	
	(Carneiro et al., 2012)	No difference	Advanced: Neovascular	43	19	Case-control	Plasma	
	(dell'Omo et al., 2012)	Up	Advanced: Neovascular	29	14	Prospective Case-control	Aqueous	VEGF levels were higher in type 3 neovascularizations compared to controls and type 1/2
	(Grierson et al., 2013)	Up	Advanced: Neovascular	31	10	Case-control	Plasma	
	(Wang et al., 2014b)	No difference	Advanced: Neovascular	32	12	Case-control	Plasma, serum	
	(Zehetner et al., 2014)	No difference	Advanced: Neovascular	30	12	Prospective Case-control	Plasma	Significant positive correlation with PDGF-B
	(Gu et al., 2014)	No difference	Advanced: Neovascular	39	39	Case-control	Serum	
	(Scotti et al., 2014)	No difference	Advanced: Neovascular	23	20	Case-control	Plasma	
	(Ambreen et al., 2015)	Up	Any AMD	90	100	Cross-sectional Case-control	Serum	VEGF was sign higher in wet vs dry AMD
	(Enders et al., 2015)	No difference	Advanced: Neovascular	61	68	Prospective observational	Plasma	Trend towards downregulation (P=0.06)
	(Goncalves et al., 2015)	No difference	Advanced: Neovascular	50	30	Case-Control	Serum	Patients received treatment, results might reflect treatment efficacy
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Soluble VEGF receptor 1	(Huber and Wachtlin, 2012)	Up	Advanced: Neovascular	12	11	Case-control	Vitreous	
	(Owen et al., 2014)	No difference	Advanced: Neovascular	341	198	Family-based cohort	Serum	Only analyzed in discovery cohort (n=322)
	(Uehara et al., 2015)	No difference/ Down	Early AMD/ Advanced: Neovascular	53/ 97	56	Case-control	Serum	sVEGF receptor 1 was lower in nAMD than in early
PEDF	(Holekamp et al., 2002)	Down	Advanced: Neovascular	9	12	Case-control	Vitreous	
	(Tong et al., 2006)	Up	Advanced: Neovascular	12	10	Case-control	Aqueous	
	(Huber and Wachtlin, 2012)	Down	Advanced: Neovascular	12	11	Case-control	Vitreous	
TGF-beta	(Guymer et al., 2011)	Up/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	51/ 19/ 33	54	Cross-sectional	Urine	No correlation between serum and urinary TGF-beta levels
	(Bai et al., 2014)	Up	Advanced: Neovascular	14	12	Case-control	Vitreous	
Fibrinogen	(Inhoffen and Nussgens, 1990)	No difference	Advanced: Neovascular	35	35	Case-control	Blood	
	(Eye-Disease-Case-Control-Study-Group, 1992)	Up	Advanced: Neovascular	421	615	Case-control	Serum	
	(Smith et al., 1998)	No difference/ Up	Early AMD/ Advanced: Any	240/ 72	3342	Population-Based Cross-sectional	Plasma	Blue Mountains Eye Study
	(Lip et al., 2001)	Up	Any AMD	78	25	Case-control	Plasma	
	(Klein et al., 2003a)	No difference	Early AMD	366	1995	Population-Based Cohort	Serum	Cardiovascular Health Study
	(Dasch et al., 2005)	Up/ Up	Early AMD/ Advanced: Any	422/ 270	181	Case-control	Plasma	Muenster Aging and Retina Study
	(Schaumberg et al., 2007)	No difference	Any AMD	150	27537	Population-Based Longitudinal	Plasma	Women's Health Study, only women included in study
	(Tan et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	?	?	Population-Based Cohort	Serum	Blue Mountains Eye Study, numbers are unclear from text (total n=2395)
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Plasma	Blue Mountains Eye Study
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Plasma	Multi-Ethnic Study of Atherosclerosis
	(Michalska-Malecka et al., 2008)	Up	Any AMD	52	42	Case-control	Plasma	

	(Wang et al., 2008)	No difference	Any AMD	278	557	Population-Based Case-control	Serum	Blue Mountains Eye Study
	(Rudnicka et al., 2010)	No difference	Advanced: Any	81	77	Case-control	Blood	
	(Colak et al., 2012)	No difference	Any AMD	84	84	Case-control	Plasma	Subgroup analyses showed higher risk of AMD when fibrinogen > 3.8 g/l (P=0.019)
Plasminogen activator inhibitor I (PAI-1)	(Wu et al., 2007)	Up/ Up	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Plasma	Blue Mountains Eye Study
	(Wang et al., 2008)	No difference	Any AMD	188	393	Population-Based Case-control	Serum	Blue Mountains Eye Study, conflict between text and table
	(Rudnicka et al., 2010)	No difference	Advanced: Any	81	77	Case-control	Blood	
	(Bertelmann et al., 2013)	No difference	Dry AMD/ Advanced: Neovascular	13/ 6	30	Case-control	Aqueous	PAI-1 was not detected in neither groups
Platelet count	(Inhoffen and Nussgens, 1990)	No difference	Advanced: Neovascular	35	35	Case-control	Blood	
	(Lip et al., 2001)	No difference	Any AMD	78	25	Cross-sectional	Blood	Borderline significant upregulation in AMD (P=0.055)
	(Klein et al., 2003c)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	?	?	Population-Based Cohort	Blood	Beaver Dam Eye Study, total n=3672, case-control ratios not presented
	(Klein et al., 2007a)	No difference/ No difference/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	866/ 39/ 14	3369	Observational	Blood	Women's Health Initiative Sight Examination, only women included
	(Roh et al., 2008)	Down	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study, not significant after adjustment for age
	(Klein et al., 2010)	No difference	Early AMD	96	2714	Cross-sectional	Blood	
	(Cho et al., 2014)	Down/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, only significant in univariate analyses, not in multivariate
Von Willebrand factor (VWF)	(Lip et al., 2001)	Up	Any AMD	78	25	Case-control	Plasma	
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Blood	Blue Mountains Eye Study
	(Wang et al., 2008)	No difference	Any AMD	188	393	Population-Based Case-control	Serum	Blue Mountains Eye Study, Conflict between text and table
	(Rudnicka et al., 2010)	No difference	Advanced: Any	81	77	Case-control	Blood	

Supplementary table 2. Factors involved in oxidative stress

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Malondialdehyde (MDA)	(Totan et al., 2001)	Up	Advanced: Neovascular	20	10	Case-control	Plasma	
	(Evereklioglu et al., 2003b)	Up	Any AMD	41	25	Cross-sectional	Plasma	Higher in advanced vs. early AMD Pos correlation NO, neg correlation SOD and GSHP
	(Yildirim et al., 2004)	Up	Advanced: Neovascular	30	60	Case-control	Plasma	
	(Baskol et al., 2006)	Up	Dry AMD	37	29	Case-control	Serum	Neg correlation PON1 activity
	(Ates et al., 2009)	Up	Advanced: Neovascular	40	40	Cross-sectional	Serum	Neg correlation PON1 activity
	(Totan et al., 2009)	Up	Advanced: Neovascular	47	25	Case-control	Serum	
	(Jia et al., 2011)	Up	Any AMD	56	34	Case-control	Serum	Higher in nAMD vs. early AMD Pos correlation SOD activity
	(Yildirim et al., 2011)	Up	Advanced: Neovascular	25	25	Case-control	Serum	
	(Shen et al., 2012)	Up/ Up/ Up	Early/ Advanced: GA/ Advanced: Neovascular	21/ 13/ 22	34	Case-control	Serum	Higher in nAMD vs. early AMD
	(Venza et al., 2012)	Up/ Up	Early AMD/ Advanced: Any	211/ 205	262	Case-control	Plasma, erythrocytes	
	(Park et al., 2014a)	Up	Advanced: Neovascular	42	84	Case-control	Plasma	Pos correlation ARMS2 risk genotype
Carboxyethyl-pyrrole (CEP) adducts	(Gu et al., 2009)	Up/ Up	Early/ Advanced: any	307/ 609	488	Case-control	Plasma	No significant differences between disease categories
	(Ni et al., 2009)	Up	Any AMD	54	32	Case-control	Plasma	Pos correlation with CML and pentosidine
	(Wang et al., 2014a)	Up	Any AMD	10	7	Case-control	Plasma	
Carboxyethyl-pyrrole (CEP) autoantibodies	(Gu et al., 2003)	Up	Any AMD	19	19	Case-control	Plasma	
	(Gu et al., 2009)	Up/ Up	Early/ Advanced: any	307/ 609	488	Case-control	Plasma	No significant difference between disease categories
	(Ni et al., 2009)	No difference	Any AMD	58	32	Case-control	Plasma	
N(6)-carboxymethyl-lysine (CML)	(Ni et al., 2009)	Up	Any AMD	58	32	Case-control	Plasma	Pos correlation with CEP adducts and pentosidine
	(Semba et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	1025/ 276	3606	Population-based Cross-sectional	Serum	Age, Gene/Environment Susceptibility–Reykjavik Study

Protein Carbonyl groups (PCG)	(Totan et al., 2009)	Up	Advanced: Neovascular	47	25	Case-control	Serum	
	(Zafrilla et al., 2013)	Up	Advanced: Neovascular	163	170	Case-control	Serum	
Total Oxidation Status (TOS)	(Totan et al., 2009)	Up	Advanced: Neovascular	47	25	Case-control	Serum	Neg correlation TAC
	(Ugurlu et al., 2013)	Up	Advanced: Neovascular	22	23	Case-control	Serum	
Oxidized LDL (Ox-LDL)	(Ikeda et al., 2001)	Up	Advanced: Neovascular	72	140	Case-control	Plasma	
	(Klein et al., 2007b)	No difference	Early AMD	221	5666	Longitudinal	Serum	Multi-Ethnic Study of Atherosclerosis
	(Javadzadeh et al., 2010)	Up	Advanced: Neovascular	45	45	Case-control	Plasma	Pos correlation Hcy
	(Javadzadeh et al., 2012)	Up	Advanced: Neovascular	45	45	Case-control	Plasma	Note: this is the same cohort as described by (Javadzadeh et al., 2010)
Nitric Oxide (NO)	(Totan et al., 2001)	Down	Advanced: Neovascular	20	10	Case-control	Plasma	
	(Evereklioglu et al., 2003b)	Up	Any AMD	41	25	Cross-sectional	Plasma	Higher in Advanced: any vs. early AMD Neg correlation GSHP and SOD Pos correlation MDA
	(Tsai et al., 2006)	No difference/ No difference	Early AMD/ Advanced: neovascular	17/ 60	42	Case-control	Plasma	
Homocysteine (Hcy)	(Heuberger et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	329/ 16	3182	Population-Based Cross-sectional	Serum	NHANES III
	(Axer-Siegel et al., 2004)	Up/ No difference	Advanced: Neovascular/ Dry AMD	59/ 58	56	Cross-sectional	Plasma	Higher in Advanced: Neovascular vs. Dry AMD
	(Vine et al., 2005)	Up	Any AMD	79	77	Case-control	Plasma	
	(Coral et al., 2006)	Up	Advanced: Neovascular	16	20	Case-control	Plasma	Neg correlation tSH and GSH
	(Kamburoglu et al., 2006)	Up/ Up	Advanced: Neovascular/ Dry AMD	30/ 30	30	Case-control	Plasma	
	(Seddon et al., 2006)	Up	Advanced: Any	222	184	Cross-sectional Case-control	Plasma	No statistical analysis were reported for early AMD (n=528)
	(Rochtchina et al., 2007)	Up	Advanced: Any	53	2910	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Plasma	Blue Mountains Eye Study
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
	(Wang et al., 2008)	No difference	Any AMD	278	557	Population-Based Case-control	Serum	Blue Mountains Eye Study, Conflict between text and table!
	(Ates et al., 2009)	Up	Advanced: Neovascular	40	40	Cross-sectional	Serum	Neg correlation PON1 activity
	(Javadzadeh et al., 2010)	Up	Advanced: Neovascular	45	45	Case-control	Plasma	Pos correlation Ox-LDL
	(Javadzadeh et al., 2012)	Up	Advanced: Neovascular	45	45	Case-control	Plasma	Note: this is the same cohort as described by (Javadzadeh et al., 2010)
	(Ghosh et al., 2013)	Up/ No difference	Advanced: Neovascular/ Dry AMD	12/ 20	32	Case-control	Plasma	
	(Gopinath et al., 2013)	Up	Any AMD	219	1171	Cohort	Serum	Blue Mountains Eye Study, Subgroup analysis showed significant upregulation in early AMD, but not in advanced AMD
	(Obeid et al., 2013)	No difference/ No difference	Advanced: Neovascular/ Dry AMD	31/ 38	48	Case-control	Plasma	All cataract subjects Neg correlation esRAGE in Dry AMD
	(Christen et al., 2015)	No difference	Any AMD	452	27479	Prospective cohort	Plasma	Women only
	(Manresa et al., 2015)	Up	Advanced: Neovascular	73	80	Case-control	Vitreous, Plasma	Treatment naïve patients, treatment did not alter Hcy levels
Total Antioxidant Capacity (TAC)	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	578	Case-control	Serum	
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Plasma	
	(Totan et al., 2009)	Down	Advanced: Neovascular	47	25	Case-control	Serum	Neg correlation TOS
	(Colak et al., 2012)	Down	Any AMD	84	84	Cross-sectional	Plasma	
	(Shen et al., 2012)	Down/ Down/ Down	Early/ Advanced: GA/ Advanced: Neovascular	21/ 13/ 22	34	Case-control	Serum	
	(Ugurlu et al., 2013)	No difference	Advanced: Neovascular	22	23	Case-control	Serum	
	(Zafrilla et al., 2013)	Down	Advanced: Neovascular	163	170	Case-control	Serum	
	(Plestina-Borjan et al., 2015)	Down	Any AMD	57	50	Case-control	Serum	
Thiol content (tSH)	(Coral et al., 2006)	Down	Advanced: Neovascular	16	20	Case-control	Plasma	Neg correlation Hcy
	(Javadzadeh et al., 2010)	Down	Advanced: Neovascular	45	45	Case-control	Plasma	

	(Ugurlu et al., 2013)	Down	Advanced: Neovascular	22	23	Case-control	Serum	
Glutathione (GSH)	(Samiec et al., 1998)	No difference	Any AMD	40	27	Case-control	Plasma	GSSG did not differ between AMD cases and age-matched controls
	(Delcourt et al., 1999b)	No difference	Advanced: Any	33	1895	Population-based	Erythrocytes	POLA, Same population as (Delcourt et al., 1999a)
	(Coral et al., 2006)	Down	Advanced: Neovascular	16	20	Case-control	Plasma	Neg correlation Hcy
	(Javadzadeh et al., 2010)	Down	Advanced: Neovascular	45	45	Case-control	Plasma	
	(Yildirim et al., 2011)	No difference	Advanced: Neovascular	25	25	Case-control	Serum	
	(Brantley et al., 2012)	No difference	Any AMD	69	67	Case-control	Plasma	
	(Qin et al., 2014)	No difference	Early	14	14	Case-control	Blood	GSSG (oxidized glutathione) higher in early AMD vs. controls
Glutathione Reductase (GSHR)	(Cohen et al., 1994)	Down	Any AMD	18	18	Case-control	Blood	
	(De La Paz et al., 1996)	No difference	Any AMD	54	12	Case-control	Erythrocytes	
	(Colak et al., 2012)	Down	Any AMD	84	84	Cross-sectional	Plasma	
	(Zafrilla et al., 2013)	Down	Advanced: Neovascular	163	170	Case-control	Serum	
Glutathione Peroxidase (GSHP)	(Prashar et al., 1993)	Down	Advanced: Neovascular	17	11	Case-control	Erythrocytes	
	(Cohen et al., 1994)	No difference	Any AMD	18	18	Case-control	Blood	
	(De La Paz et al., 1996)	No difference	Any AMD	54	12	Case-control	Erythrocytes	
	(Delcourt et al., 1999a)	No difference/ Up	Early/ Advanced: Any	642/ 38	1476	Population-Based Cross-sectional	Plasma	POLA
	(Evereklioglu et al., 2003b)	Down	Any AMD	41	25	Cross-sectional	Plasma, erythrocytes	Lower in advanced vs. early AMD Neg correlation NO and MDA
	(Yildirim et al., 2011)	No difference	Advanced: Neovascular	25	25	Case-control	Serum	
	(Colak et al., 2012)	No difference	Any AMD	84	84	Cross-sectional	Blood	
	(Venza et al., 2012)	Down/ Down	Early AMD/ Advanced: Any	211/ 205	262	Case-control	Plasma, erythrocytes	
	(Zafrilla et al., 2013)	No difference	Advanced: Neovascular	163	170	Case-control	Serum	
	(Plestina-Borjan et al., 2015)	Down	Any AMD	57	50	Case-control	Serum	
Carotenoids	(Eye-Disease-Case-Control-Study-Group, 1992)	Down	Advanced: Neovascular	414	606	Case-control	Serum	Sum of lutein/zeaxanthine, beta-carotene, a-carotene, cryptoxanthin and lycopene
	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	577	Case-control	Serum	

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Plasma	Lower in advanced vs early AMD
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting samples
Lutein	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	577	Case-control	Serum	Measured together with zeaxanthine
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	Correlation cholesterol
	(Mares-Perlman et al., 1995)	No difference	Any AMD	80	80	Case-control	Serum	
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Serum	Measured together with zeaxanthine
	(Gale et al., 2003)	No difference	Any AMD	78	302	Case-control	Plasma	
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting samples
	(Delcourt et al., 2006)	Down	Any AMD	41	599	Population-Based	Plasma	POLA
	(Michikawa et al., 2009)	No difference/ No difference	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	Measured together with zeaxanthine
	(Zhou et al., 2011)	No difference/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	
Zeaxanthin	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	577	Case-control	Serum	Measured together with lutein
	(Mares-Perlman et al., 1995)	No difference	Any AMD	80	80	Case-control	Serum	
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Serum	Measured together with lutein
	(Gale et al., 2003)	Down	Any AMD	78	302	Case-control	Plasma	
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting samples
	(Delcourt et al., 2006)	Down	Any AMD	41	599	Population-Based	Plasma	POLA
	(Michikawa et al., 2009)	No difference/ No difference	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	Measured together with luteine
	(Zhou et al., 2011)	No difference/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	

β-cryptoxanthin	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	577	Case-control	Serum	
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	Correlation cholesterol
	(Mares-Perlman et al., 1995)	No difference	Any AMD	167	167	Case-control	Serum	
	(Simonelli et al., 2002)	No difference/ Down	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Serum	Lower in advanced vs early AMD
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting samples
	(Delcourt et al., 2006)	No difference	Any AMD	41	599	Population-Based	Plasma	POLA
	(Michikawa et al., 2009)	No difference/ Down	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	
	(Zhou et al., 2011)	No difference/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	
α-carotene	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	577	Case-control	Serum	
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	
	(Mares-Perlman et al., 1995)	No difference	Any AMD	167	167	Case-control	Serum	
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting samples
	(Delcourt et al., 2006)	No difference	Any AMD	41	599	Population-Based	Plasma	POLA
	(Michikawa et al., 2009)	No difference/ No difference	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	
	(Zhou et al., 2011)	Up/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	
β-carotene	(Eye-Disease-Case-Control-Study-Group, 1993)	Down	Advanced: Neovascular	391	577	Case-control	Serum	
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	
	(West et al., 1994)	No difference	Any AMD	129	377	Case-control	Plasma	
	(Mares-Perlman et al., 1995)	No difference	Any AMD	167	167	Case-control	Serum	
	(Smith et al., 1997)	No difference/ No difference	Early AMD/ Advanced: Any	102/ 54	156	Case-control	Serum	Blue Mountains Eye Study, also no difference for pooled analysis of all AMD cases vs controls

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Serum	
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting samples
	(Delcourt et al., 2006)	No difference	Any AMD	41	599	Population-Based	Plasma	POLA
	(Michikawa et al., 2009)	No difference/ Down	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	
	(Zhou et al., 2011)	No difference/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	
Lycopene	(Eye-Disease-Case-Control-Study-Group, 1993)	No difference	Advanced: Neovascular	391	577	Case-control	Serum	
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	Correlation cholesterol
	(Mares-Perlman et al., 1995)	No difference	Any AMD	167	167	Case-control	Serum	Logistic regression of high vs low levels revealed higher AMD risk for very low levels of lycopene
	(Simonelli et al., 2002)	Down/ Down	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Serum	
	(Cardinault et al., 2005)	Down	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables
	(Delcourt et al., 2006)	No difference	Any AMD	41	599	Population-Based	Plasma	POLA
	(Michikawa et al., 2009)	No difference/ No difference	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	
	(Zhou et al., 2011)	No difference/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	
Super Oxide Dismutase (SOD)	(Prashar et al., 1993)	Down	Advanced: Neovascular	17	11	Case-control	Erythrocytes	
	(Cohen et al., 1994)	No difference	Any AMD	18	18	Case-control	Erythrocytes	
	(De La Paz et al., 1996)	No difference	Any AMD	54	12	Case-control	Erythrocytes	
	(Delcourt et al., 1999a)	No difference/ No difference	Early/ Advanced: Any	642/ 38	1476	Population-Based Cross-sectional	Erythrocytes	POLA
	(Everekliogl u et al., 2003b)	Down	Any AMD	41	25	Cross-sectional	Plasma, erythrocytes	Lower in advanced vs. early AMD Neg correlation NO and MDA
	(Jia et al., 2011)	Up	Any AMD	56	34	Case-control	Serum	Activity levels, Pos correlation MDA

	(Yildirim et al., 2011)	Down	Advanced: Neovascular	25	25	Case-control	Serum	
	(Colak et al., 2012)	No difference	Any AMD	84	84	Cross-sectional	Blood hemolysate	
	(Shen et al., 2012)	No difference/ No difference/ Up	Early/ Advanced: GA/ Advanced: Neovascular	21/ 13/ 22	34	Case-control	Serum	Activity levels
	(Venza et al., 2012)	Down/ Down	Early AMD/ Advanced: Any	211/ 205	262	Case-control	Plasma, erythrocytes	Activity levels
	(Anand et al., 2013)	Up	Any AMD	115	61	Case-control	Serum	
	(Sharma et al., 2013b)	Up	Any AMD	73	33	Case-control	Serum	
	(Zafrilla et al., 2013)	Down	Advanced: Neovascular	163	170	Case-control	Serum	
	(Plestina-Borjan et al., 2015)	No difference	Any AMD	57	50	Case-control	Serum	
Paraoxonase 1 (PON1)	(Baskol et al., 2006)	Down	Dry AMD	37	29	Case-control	Serum	Activity level, Neg correlation MDA
	(Ates et al., 2009)	Down	Advanced: Neovascular	40	40	Cross-sectional	Serum	Activity level, Neg correlation MDA and Hcy
	(Javadzadeh et al., 2012)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Comparison of three PON1 phenotypes showed weak PON1 activity in nAMD compared to controls
	(Ugurlu et al., 2013)	Down	Advanced: Neovascular	22	23	Case-control	Serum	
Catalase	(Prashar et al., 1993)	Down	Advanced: Neovascular	17	11	Case-control	Erythrocytes	
	(De La Paz et al., 1996)	No difference	Any AMD	54	12	Case-control	Erythrocytes	
	(Evereklioglu et al., 2003b)	No difference	Any AMD	41	25	Cross-sectional	Erythrocytes	
	(Yildirim et al., 2004)	Down	Advanced: Neovascular	30	60	Case-control	Erythrocytes	Activity levels
	(Venza et al., 2012)	Down/ Down	Early AMD/ Advanced: Any	211/ 205	262	Case-control	Plasma, erythrocytes	Downregulation only in plasma, no significant difference in erythrocytes
	(Plestina-Borjan et al., 2015)	No difference	Any AMD	57	50	Case-control	Serum	

Supplementary table 3: Factors involved in immunity

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
C3	(Scholl et al., 2008)	No difference	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	
	(Silva et al., 2012)	No difference	Any AMD	119	152	Case-control	Plasma	
	(Smailhodzic et al., 2012)	No difference	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
C3a	(Scholl et al., 2008)	Up	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	Up/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	
C3d	(Scholl et al., 2008)	Up	Any AMD	112	67	Case-control	Plasma	
	(Hecker et al., 2010)	Up	Any AMD	125	149	Case-control	Plasma	
	(Smailhodzic et al., 2012)	Up	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
C3a des Arg	(Sivaprasad et al., 2007)	Up	Early AMD/ Advanced: Neovascular	42/ 42	38	Case-control	Plasma	
	(Guymer et al., 2011)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Any	51/ 19/ 33	54	Case-control	Urine	No correlation between serum and urinary C3a des Arg levels
C3d/C3	(Smailhodzic et al., 2012)	Up	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
	(Ristau et al., 2014a)	Up/ Up	Any AMD/ Advanced: Any	864/ 495	1014	Case-control	Serum	EUGENDA, analysis for this marker only performed in subset of 1255 participants
	(Ristau et al., 2014b)	Up	Any AMD	1387	1268	Case-control	Serum	EUGENDA
C5a	(Scholl et al., 2008)	Up	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	Up/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	Trend to upregulation in nAMD (P=0.09)
	(Hecker et al., 2010)	No difference	Any AMD	125	149	Case-control	Plasma	
	(Smailhodzic et al., 2012)	Up	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
SC5b-9	(Scholl et al., 2008)	Up	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	
	(Smailhodzic et al., 2012)	No difference	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
FH	(Hakobyan et al., 2008)	Up	Any AMD	53	75	Case-control	Plasma	Only in AMD patients with a heterozygous Y402H variant

	(Scholl et al., 2008)	No difference	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	Down/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	Trend to downregulation in nAMD (P=0.06)
	(Silva et al., 2012)	No difference	Any AMD	119	152	Case-control	Plasma	
	(Smailhodzic et al., 2012)	No difference	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
	(Ansari et al., 2013)	Down	Any AMD	382	201	Case-control	Plasma	
	(Sharma et al., 2013a)	Down/ Down	Dry AMD/ Advanced: Neovascular	31/ 84	61	Case-control	Serum	
	(Sharma et al., 2013b)	Down	Any AMD	73	33	Case-control	Serum	
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
FI	(Reynolds et al., 2009)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	
	(Silva et al., 2012)	Up	Any AMD	119	152	Case-control	Plasma	
	(Smailhodzic et al., 2012)	No difference	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA, Trend to upregulation (P=0.068)
	(van de Ven et al., 2013)	No difference	Any AMD	100	97	Case-control	Plasma	Significant downregulation in patients with Gly119Arg or Gly188Ala variant in the *CFI* gene
FB	(Scholl et al., 2008)	Up	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	
	(Hecker et al., 2010)	Up	Any AMD	125	149	Case-control	Plasma	Not significant after correction and standardization
	(Silva et al., 2012)	No difference	Any AMD	119	152	Case-control	Plasma	Higher in women with AMD compared to men with AMD
	(Smailhodzic et al., 2012)	Up	Advanced: Neovascular	197	150	Case-control	Serum	EUGENDA
FD	(Scholl et al., 2008)	Up	Any AMD	112	67	Case-control	Plasma	
	(Reynolds et al., 2009)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	58/ 62	60	Case-control	Plasma	
	(Hecker et al., 2010)	Up	Any AMD	125	149	Case-control	Plasma	
	(Stanton et al., 2011)	Up	Any AMD	751	474	Case-control	Plasma	
	(Silva et al., 2012)	Down	Any AMD	119	152	Case-control	Plasma	
DAF/CD55	(Haas et al., 2011a)	No difference	Advanced: Neovascular	50	48	Case-control	Blood	
	(Singh et al., 2012)	No difference	Advanced: Neovascular	35	30	Case-control	Blood	

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
IL-1α	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	Up	Advanced: Neovascular	18	20	Case-control	Aqueous	Only in univariate analysis, not significant in multivariate analysis
IL-1β	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
	(Zhao et al., 2015)	Up	Advanced: Neovascular	10	6	Prospective	Vitreous	
IL-2	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis
	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
IL-4	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
IL-5	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
IL-6	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Serum	Blue Mountains Eye Study
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis, subgroup analysis in GA patients showed significant upregulation (n=18)
	(Wang et al., 2008)	No difference	Any AMD	188	393	Population-Based Case-control	Serum	Blue Mountains Eye Study, Conflict between text and table
	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	

	(Colak et al., 2012)	No difference	Any AMD	84	84	Cross-sectional	Serum	Subgroup analyses showed higher risk of AMD when IL-6>4.9 pg/ml (P=0.024)
	(Klein et al., 2014b)	Up	Early AMD	176	704	Longitudinal Population-Based Cohort	Serum	Beaver Dam Eye Study
	(Ambreen et al., 2015)	Up	Any AMD	90	100	Cross-sectional Case-control	Serum	IL-6 was sign higher in dry AMD vs nAMD
	(Aoki et al., 2015)	No difference	Any AMD	185	295	Cross-sectional	Serum	Hatoyama Cohort Study
	(Haas et al., 2015)	Up	Advanced: Neovascular	54	46	Case-control	Blood	
	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
IL-8	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Ambreen et al., 2015)	Up	Any AMD	90	100	Cross-sectional Case-control	Serum	IL-8 was sign higher in dry AMD vs nAMD
	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	Trend to downregulation (P=0.09)
IL-10	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
IL-12	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
IL-13	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	Down	Advanced: Neovascular	18	20	Case-control	Aqueous	P=0.05
IL-15	(Faber et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	30/ 16/ 90	74	Case-control	Plasma	
	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	Up	Advanced: Neovascular	18	20	Case-control	Aqueous	Only in univariate analysis, not significant in multivariate analysis

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
IL-17	(Nassar et al., 2015)	Up	Advanced: Neovascular	30	15	Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
IL-18	(Ijima et al., 2014)	Up/ No difference	Dry AMD/ Advanced: Neovascular	17/ 43	40	Case-control	Serum	
	(Faber et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	30/ 16/ 90	74	Case-control	Plasma	
CCL2	(Zhang et al., 2006)	Up	Any AMD	34	38	Case-control	Serum	
	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Guymer et al., 2011)	Up/ Up/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	51/ 19/ 33	54	Cross-sectional	Urine	No correlation between serum and urinary CCL2 levels
	(Anand et al., 2012)	Up	Any AMD	133	80	Case-control	Serum	
	(Grunin et al., 2012)	No difference	Advanced: Neovascular	30	27	Case-control	Serum	
	(Sharma et al., 2013b)	Up	Any AMD	73	33	Case-control	Serum	
	(Falk et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Neovascular	30/ 90	30	Case-control	Plasma	
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
	(Sakurada et al., 2015)	No difference	Advanced: Neovascular	18	20	Case-control	Aqueous	
CCR2 on Monocytes	(Zhang et al., 2006)	No difference	Any AMD	34	38	Case-control	Serum	
	(Anand et al., 2012)	Down	Any AMD	133	80	Case-control	Serum	
	(Grunin et al., 2012)	Up	Advanced: Neovascular	18	20	Case-control	Serum	
	(Falk et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Neovascular	30/ 90	30	Case-control	Plasma	
CCL11	(Mo et al., 2010)	Up/ Up/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Falk et al., 2014a)	No difference	Advanced: Neovascular	83	114	Case-control	Plasma	Significant association with age
CCL24	(Sharma et al., 2012)	Up	Advanced: GA/ Advanced: Neovascular	38/ 95	80	Case-control	Serum	Higher CCL24 levels in nAMD vs. GA

	(Sharma et al., 2013b)	Up	Any AMD	73	33	Case-control	Serum	
CXCL10	(Mo et al., 2010)	Up/ Up/ Up	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Grunin et al., 2012)	No difference	Advanced: Neovascular	20	20	Case-control	Serum	
	(Falk et al., 2014c)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	30/ 12/ 89	31	Case-control	Plasma	
	(Sakurada et al., 2015)	Up	Advanced: Neovascular	18	20	Case-control	Aqueous	
CXCL12	(Machalinska et al., 2011a)	Down	Advanced: Neovascular	46	46	Case-control	Plasma	
	(Machalinska et al., 2011b)	Down	Advanced: Neovascular	29	38	Case-control	Plasma	
	(Scotti et al., 2014)	Up	Advanced: Neovascular	23	20	Case-control	Plasma	
	(Grierson et al., 2013)	No difference	Advanced: Neovascular	31	10	Case-control	Plasma	
CX3CR1	(Grunin et al., 2012)	No difference	Advanced: Neovascular	18	20	Case-control	Serum	
	(Falk et al., 2014b)	Up/ Up	Early AMD/ Advanced: Neovascular	30/ 90	30	Case-control	Plasma	Only in CD8+ cells
TNF-α	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis
	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Zehetner et al., 2014)	No difference	Advanced: Neovascular	30	12	Prospective Case-control	Plasma	
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
	(Haas et al., 2015)	No difference	Advanced: Neovascular	54	46	Case-control	Blood	
	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
TNF-α R2	(Klein et al., 2014b)	No difference	Early AMD	179	708	Longitudinal Population-Based Cohort	Serum	Beaver Dam Eye Study, Trend to upregulation (P=0.06)
	(Faber et al., 2015)	Up/ No difference/ Up	Early AMD/ Advanced: GA/ Advanced: Neovascular	30/ 16/ 90	74	Case-control	Plasma	

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Interferon-gamma (IFN-y)	(Mo et al., 2010)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	39/ 20/ 19	18	Case-control	Serum	
	(Faber et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	30/ 16/ 90	74	Case-control	Plasma	
	(Nassar et al., 2015)	No difference	Advanced: Neovascular	30	15	Case-control	Serum	
CRP	(Klein et al., 2003a)	No difference	Early AMD	366	1995	Population-Based Cohort	Blood	Cardiovascular Health Study
	(Seddon et al., 2004)	No difference/ Up	Early AMD/ Advanced: Any	525/ 222	183	Case-control	Serum	
	(Dasch et al., 2005)	No difference/ Up	Early AMD/ Advanced: Any	422/ 270	181	Case-control	Serum	Muenster Aging and Retina Study, after correction this association was not significant
	(McGwin et al., 2005)	No difference	Any AMD	390	2365	Cross-sectional	Blood	Cardiovascular Health Study
	(Vine et al., 2005)	Up	Any AMD	79	77	Case-control	Serum	In model including both CRP and Hcy, CRP trends to upregulation (P=0.098)
	(Kikuchi et al., 2007)	Up	Advanced: Neovascular	176	262	Case-control	Serum	
	(Hogg et al., 2008)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	97/ 195	115	Cross-sectional Case-control	Serum	
	(Roh et al., 2008)	No difference	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study
	(Ho et al., 2009)	No difference	Any AMD	164	1484	Population-Based Case-cohort	Blood	Rotterdam Study
	(Boey et al., 2010)	No difference/ No difference	Early AMD/ Advanced: Any	155/ 22	2923	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study Highest vs lowest quartile CRP levels in non-diabetics were associated with advanced AMD
	(Robman et al., 2010)	No difference/ Up	Early AMD/ Advanced: Any	219/ 38	232	Case-control	Serum	
	(Seddon et al., 2010)	No difference/ Up	Early AMD/ Advanced: Any	175/ 69	209	Case-control	Serum	Age Related Eye Disease Ancillary Study
	(Subramani et al., 2010)	No difference	Any AMD	113	119	Case-control	Serum	Age-Related Macular Degeneration-Uric Acid Study
	(Colak et al., 2011)	Up	Any AMD	79	84	Cross-sectional	Serum	
	(Hong et al., 2011)	Up	Advanced: Any	-	-	Meta-analysis	Serum and plasma	Included 11 studies with a total of 41690 participants

	(Weiner et al., 2011)	Up	Any AMD	865	865	Case-control	Blood	
	(Colak et al., 2012)	No difference	Any AMD	84	84	Case-control	Serum	Subgroup analyses showed higher risk of AMD when CRP>3mg/l (P<0.05)
	(Silva et al., 2012)	No difference	Any AMD	119	152	Case-control	Plasma	
	(Cohn et al., 2013)	Up/ Up	Early AMD/ Advanced: Any	310/ 65	306	Cross-sectional	Blood	CHARM and ARMSS study, after adjustment only significant in advanced AMD
	(Singh et al., 2013a)	No difference	Advanced: Neovascular	62	44	Prospective Case-control	Serum	
	(Ulas et al., 2013)	Up	Advanced: Neovascular	142	141	Case-control	Serum	
	(Jonasson et al., 2014)	No difference	Any AMD	328	2540	Population-Based Cohort	Blood	Age Gene/Environment Susceptibility-Reykjavik Study
	(Semba et al., 2014)	Up	Early AMD/ Advanced: Any	1025/ 276	3606	Population-based cross-sectional	Serum	Age, Gene/Environment Susceptibility–Reykjavik Study
	(Ambreen et al., 2015)	Up	Any AMD	90	100	Cross-sectional Case-control	Serum	CRP was sign higher in wet vs dry
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
	(Min et al., 2015)	Up	Advanced: Neovascular	30	30	Case-control	Plasma	Positive correlation with PTx3
	(Sakurada et al., 2015)	Up	Advanced: Neovascular	18	20	Case-control	Aqueous	
	(Yip et al., 2015)	Up	Any AMD	673	4671	Prospective Cohort	Serum	EPIC Norfolk Eye Study
hsCRP	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Boekhoorn et al., 2007)	Up/ Up	Early AMD/ Advanced: Any	561/ 97	3946	Population-based Longitudinal	Serum	Rotterdam Study
	(Schaumberg et al., 2007)	Up	Any AMD	150	27537	Population-Based Longitudinal	Plasma	Women's Health Study, women only
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Serum	Blue Mountains Eye Study
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis, subgroup analysis in GA patients showed significant upregulation (n=18)
	(Wang et al., 2008)	No difference	Any AMD	188	393	Population-Based Case-control	Serum	Blue Mountains Eye Study, Conflict between text and table

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
	(Mitta et al., 2013)	Up	Any AMD	647	1480	Nested Case-control	Blood	Includes data from 5 studies
	(Klein et al., 2014b)	Up	Early AMD	178	697	Longitudinal Population-Based Cohort	Serum	Beaver Dam Eye Study
	(Aoki et al., 2015)	No difference	Any AMD	185	295	Cross-sectional	Serum	Hatoyama Cohort Study, Trend to upregulation in AMD (P=0.07)
	(Haas et al., 2015)	Up	Advanced: Neovascular	54	46	Case-control	Blood	
(soluble) ICAM	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Schaumberg et al., 2007)	Up	Any AMD	150	27537	Population-Based Longitudinal	Plasma	Women's Health Study, women only
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Serum	Blue Mountains Eye Study, borderline significant upregulation in nAMD (P=0.0541) vs controls
	(Hogg et al., 2008)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	97/ 195	115	Cross-sectional Case-control	Serum	
	(Wang et al., 2008)	No difference	Any AMD	188	393	Population-Based Case-control	Serum	Blue Mountains Eye Study
	(Klein et al., 2014b)	No difference	Early AMD	180	708	Longitudinal Population-Based Cohort	Serum	Beaver Dam Eye Study
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
(soluble) VCAM	(Hogg et al., 2008)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	97/ 195	115	Cross-sectional Case-control	Serum	
	(Klein et al., 2014b)	Up	Early AMD	180	710	Longitudinal Population-Based Cohort	Serum	Beaver Dam Eye Study, upregulation found in fully adjusted model
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
WBC Count	(Blumenkranz et al., 1986)	Up	Advanced: Neovascular	26	23	Case-control	Blood	
	(Inhoffen and Nussgens, 1990)	No difference	Any AMD	35	35	Case-control	Blood	

	(Klein et al., 1993)	No difference/ Up/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	? 	?	Population-Based	Blood	Beaver Dam Eye Study, total n=4771, case-control ratios not presented
	(Lip et al., 2001)	No difference	Any AMD	78	25	Cross-sectional	Blood	
	(Klein et al., 2003a)	No difference	Early AMD	366	1995	Population-Based Cohort	Blood	Cardiovascular Health Study
	(Klein et al., 2003c)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	? 	?	Population-Based Cohort	Blood	Beaver Dam Eye Study, total n=3674, case-control ratios not presented
	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Klein et al., 2007a)	No difference/ No difference/ Up	Early AMD/ Advanced: Neovascular/ Advanced: GA	866/ 39/ 14	3369	Observational	Blood	Women's Health Initiative Sight Examination, only women included
	(Shankar et al., 2007)	Up/ No difference	Early AMD/ Advanced: Any	249/ 63	3342	Population-based Cohort	Blood	Blue Mountains Eye Study
	(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Blood	Blue Mountains Eye Study
	(Roh et al., 2008)	No difference	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study
	(Wang et al., 2008)	No difference	Any AMD	278	557	Population-Based Case-control	Serum	Blue Mountains Eye Study, Conflict between text and table
	(Klein et al., 2010)	No difference	Early AMD	96	2714	Cross-sectional	Blood	
	(Weiner et al., 2011)	Up	Any AMD	865	865	Cross-sectional Case-control	Blood	NHANESIII
	(Gopinath et al., 2013)	No difference	Any AMD	219	1171	Cohort	Blood	Blue Mountains Eye Study
	(Singh et al., 2013a)	No difference	Advanced: Neovascular	62	44	Prospective Case-control	Blood	
	(Cho et al., 2014)	Down/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, only significant in univariate analyses, not in multivariate
	(Klein et al., 2014b)	Up	Early AMD	181	711	Longitudinal Population-Based Cohort	Serum	Beaver Dam Eye Study, not significantly associated in fully adjusted model (P=0.16)
	(Joachim et al., 2015)	No difference	Early AMD	281	1036	Population-Based Cohort	Blood	Blue Mountains Eye Study, trend to downregulation (P=0.06)
Pentraxin 3 (PTX3)	(Juel et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	26/ 17/ 81	118	Case-control	Plasma	
	(Min et al., 2015)	Up	Advanced: Neovascular	30	30	Case-control	Plasma	Positive correlation with CRP

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Anti-retinal autoantibodies (ARAs)	(Penfold et al., 1990)	Up	Any AMD	118	50	Case-control	Serum	Number of cases in text (128) does not match table (118). Most frequently observed staining pattern almost identical to anti-GFAP Ab.
	(Gurne et al., 1991)	Up	Any AMD	30	12	Case-control	Serum	
	(Patel et al., 2005)	Up/ Up	Early AMD/ Advanced: Neovascular	64/ 51	39	Case-control	Serum	
	(Cherepanoff et al., 2006)	Up	Early AMD	47	16	Longitudinal	Serum	Blue Mountains Eye Study, no association was found for progression to advanced AMD
	(Joachim et al., 2007)	Up & down	Advanced: Neovascular	39	101	Case-control	Serum	Different ARA profiles between cases and controls: upregulation of GFAP and α-enolase, downregulation of α-crystallin
	(Kubicka-Trzaska et al., 2012)	Up	Advanced: Neovascular	22	22	Longitudinal	Serum	ARAs decreased after anti-VEGF treatment
	(Morohoshi et al., 2012a)	Up	Any AMD	55	20	Case-control	Serum	Identification of 4 retinal antigens: Rbp3, ALDOC, PKM2, RLBP1
	(Adamus et al., 2014)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	41/ 28/ 33	26	Cross-sectional	Serum	Age-Related Eye Disease Study, specific ARAs associated with different severity stages
	(Kubicka-Trzaska et al., 2014)	Up	Advanced: Neovascular	98	50	Longitudinal	Serum	ARAs decreased after anti-VEGF treatment, correlated with lesion size and clinical outcomes anti-VEGF treatment
	(Iannaccone et al., 2015)	Up	Any AMD	131	231	Cross-sectional	Serum	Age-Related Maculopathy Ancillary Study, identification of HSPA8, HSPA9, CRYAA, ANXA5, S100A9
IgG anti-cardiolipin	(Ozkan et al., 2012)	Up/ Up	Dry AMD/ Advanced: Neovascular	19/ 23	25	Case-control	Plasma	
	(Morohoshi et al., 2012b)	No difference/ Up	Early AMD/ Advanced: Neovascular	35/ 20	20	Case-control	Serum	
Anti-Chlamydia pneumoniae	(Kalayoglu et al., 2003)	Up	Any AMD	25	18	Case-control	Serum	
	(Miller et al., 2004)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	36/ 47	67	Case-control	Serum	

	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Robman et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based	Plasma	Blue Mountains Eye Study
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis
Anti-cytomegalo-virus (CMV)	(Miller et al., 2004)	No difference/ Up	Dry AMD/ Advanced: Neovascular	36/ 47	67	Case-control	Serum	nAMD significantly higher than dry AMD
	(Faber et al., 2013)	No difference	Any AMD	117	106	Case-control	Plasma	
Antibodies to H.pylori	(Miller et al., 2004)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	36/ 47	67	Case-control	Serum	
	(Klein et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	235/ 29	5623	Prospective Cohort	Serum	Multi-Ethnic Study of Atherosclerosis, NB: analysis in random subset of 1000 participants

Supplementary table 4: Factors involved in lipid metabolism

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
(total) cholesterol	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	Fasting
	(Eye-Disease-Case-Control-Study-Group, 1992)	Up	Advanced: Neovascular	421	615	Case-control	Serum	
	(Tsang et al., 1992)	No difference	Any AMD	80	86	Case-control	Serum	Fasting
	(Klein et al., 1993)	No difference/ No difference/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	?	?	Population-Based	Serum	Beaver Dam Eye Study, down in female with early AMD, total n=4771, case-control ratios not presented
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	Nonfasting
	(Smith et al., 1998)	No difference/ No difference	Early AMD/ Advanced: Any	240/ 72	3342	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study, fasting
	(Hyman et al., 2000)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	227/ 182	235	Case-control	Serum	Fasting
	(Delcourt et al., 2001)	No difference/ No difference	Early AMD/ Advanced: Any	730/ 38	1372	Population-Based	Plasma	POLA, Fasting
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	fasting
	(Klein et al., 2003a)	Down	Early AMD	366	1995	Population-Based Cohort	Plasma	Cardiovascular Health Study
	(Klein et al., 2003b)	No difference/ No difference/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	?	?	Population-Based Cohort	Serum	Beaver Dam Eye Study, trend to downregulation in nAMD (P=0.06), total n=2764, case-control ratios not presented
	(van Leeuwen et al., 2004)	No difference	Any AMD	414	4362	Population-Based Cohort	Serum	Rotterdam Study
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting
	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(McGwin et al., 2005)	Down	Any AMD	390	2365	Cross-sectional	Serum	Cardiovascular Health Study
	(Nowak et al., 2005)	Up	Dry AMD	60	45	Case-control	Serum	Fasting, only women included
	(Dashti et al., 2006)	No difference/ No difference	Early AMD/ Advanced: Any	58/ 39	32	Cross-sectional	Plasma	
	(Javadzadeh et al., 2007)	No difference	Advanced: Neovascular	60	60	Case-control	Serum	Fasting, only males included in study
	(Klein et al., 2007b)	No difference	Early AMD	221	5666	Longitudinal	Serum	Multi-ethnic Study of Atherosclerosis, Fasting samples

(Tan et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	?	?	Population-Based Cohort	Serum	Blue Mountains Eye Study, total n=2395, case-control ratios not presented
(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Serum	Blue Mountains Eye Study, Fasting
(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
(Hogg et al., 2008)	No difference/ Up	Dry AMD/ Advanced: Neovascular	97/ 195	115	Cross-sectional Case-control	Serum	Nonfasting
(Roh et al., 2008)	No difference	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study
(Ho et al., 2009)	No difference	Any AMD	164	1484	Population-Based case cohort	Blood	Rotterdam Study, nonfasting
(Boey et al., 2010)	No difference	Any AMD	177	2923	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
(Javadzadeh et al., 2010)	Up	Advanced: Neovascular	45	45	Case-control	Serum	Fasting
(Klein et al., 2010)	No difference	Early AMD	96	2714	Cross-sectional	Serum	
(Reynolds et al., 2010)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	123/ 195	140	Case-control	Serum	Fasting
(Rudnicka et al., 2010)	No difference	Advanced: Any	81	77	Case-control	Serum	Nonfasting
(Butt et al., 2011)	Down	Any AMD	347	639	Cross-sectional	Serum	Fasting
(Colak et al., 2011)	Up	Any AMD	79	84	Cross-sectional	Serum	Fasting
(Fauser et al., 2011)	No difference	Any AMD	792	521	Case-control	Serum	Nonfasting
(Weiner et al., 2011)	No difference	Any AMD	865	865	Cross-sectional Case-control	Blood	
(Fourgeux et al., 2012)	Up	Advanced: Any	128	71	Case-control	Plasma	Fasting
(Javadzadeh et al., 2012)	Up	Advanced: Neovascular	45	45	Case-control	Serum	Fasting, note: this is the same cohort as described by (Javadzadeh et al., 2010)
(Jonas et al., 2012)	No difference	Early AMD	215	4319	Population-Based Cross-sectional	Serum	Central India Eyes and Medical Study, postprandial, only 8 late cases thus not analyzed
(Wang et al., 2012)	No difference	Any AMD	161	2704	Population-Based	Serum	Beijing Eye Study, fasting
(Davari et al., 2013)	Up	Any AMD	32	32	Case-control	Serum	
(Munch et al., 2013)	No difference	Early AMD	251	644	Cross-sectional	Serum	Inter99 Study, fasting
(Ortak et al., 2013)	No difference	Any AMD	144	172	Case-control	Serum	Fasting
(Ulas et al., 2013)	Up	Advanced: Neovascular	142	141	Cross-sectional	Serum	Fasting

Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
(Ambreen et al., 2014)	Up	Any AMD	90	100	Cross-sectional Case-control	Serum	
(Cho et al., 2014)	No difference/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
(Cougnard-Gregoire et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	238/ 47	540	Population-Based	Plasma	ALIENOR, fasting
(Ersoy et al., 2014)	No difference	Advanced: Any	1147	1773	Case-control	Serum	Nonfasting
(Ghorbanihaghjo et al., 2014)	Up	Advanced: Neovascular	45	45	Cross-sectional	Serum	Fasting
(Jonasson et al., 2014)	No difference	Any AMD	328	2540	Population-Based Cohort	Plasma	Age Gene/Environment Susceptibility-Reykjavik Study
(Kim et al., 2014a)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
(Kim et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Any	1163/ 115	15767	Population-Based Cross-sectional	Blood	KNHANES
(Klein et al., 2014a)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	?	?	Meta-analysis	Serum	Numbers are not clear from text, 3 population-based studies are included in meta-analysis (total n=6950)
(La et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	1034/95	13223	Population-Based Cross-sectional	Blood	KNHANES, fasting
(Merle et al., 2014)	No difference	Advanced: Neovascular	290	144	RCT	Plasma	NAT2, fasting
(Park et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Any	958/ 88	12667	Population-based Cross-sectional	Serum	KNHANES
(Peiretti et al., 2014)	Down	Any AMD	136	38	Case-control	Plasma	
(Qin et al., 2014)	No difference	Early AMD	14	14	Case-control	Plasma	Fasting
(Semba et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	1025/ 276	3606	Population-based cross-sectional	Serum	Age, Gene/Environment Susceptibility–Reykjavik Study
(Yang et al., 2014)	No difference	Early AMD	200	6377	Population-Based	Serum	Handan Eye Study, fasting
(Cezario et al., 2015)	No difference	Any AMD	30	30	Case-control	Serum	
(Chaker et al., 2015)	No difference	Any AMD	805	4768	Population-Based Cohort	Blood	Rotterdam Study
(Hwang et al., 2015)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
(Joachim et al., 2015)	No difference	Early AMD	281	1036	Population-Based Cohort	Serum	Blue Mountains Eye Study, Fasting

	(Min et al., 2015)	No difference	Advanced: Neovascular	30	30	Case-control	Plasma	
	(Paun et al., 2015)	No difference	Any AMD	1491	1579	Case-control	Serum	EUGENDA
	(Seshasai et al., 2015)	No difference	Any AMD	426	927	Population-Based Case-control	Serum	Singapore Indian Eye Study & Singapore Chinese Eye Study, nonfasting
	(Yip et al., 2015)	No difference	Any AMD	673	4671	Prospective Cohort	Serum	EPIC Norfolk Eye Study
Triglycerides (TG)	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	Fasting
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Tsang et al., 1992)	No difference	Any AMD	80	86	Case-control	Serum	Fasting
	(Smith et al., 1998)	No difference/ No difference	Early AMD/ Advanced: Any	240/ 72	3342	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study, fasting
	(Hyman et al., 2000)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	227/ 182	235	Case-control	Serum	Fasting
	(Delcourt et al., 2001)	No difference/ No difference	Early AMD/ Advanced: Any	730/ 38	1372	Population-Based	Plasma	POLA, fasting
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Klein et al., 2003a)	Down	Early AMD	366	1995	Population-Based Cohort	Plasma	Cardiovascular Health Study
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting
	(Nowak et al., 2005)	Up	Dry AMD	60	45	Case-control	Serum	Fasting, only women included in this study
	(Javadzadeh et al., 2007)	No difference	Advanced: Neovascular	60	60	Case-control	Serum	Fasting, only male included in this study
	(Klein et al., 2007b)	No difference	Early AMD	221	5666	Longitudinal	Serum	Multi-Ethnic Study of Atherosclerosis, Fasting
	(Tan et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	?	?	Population-Based Cohort	Serum	Blue Mountains Eye Study, total n=2395, case-control ratios not presented
	(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
	(Hogg et al., 2008)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	97/ 195	115	Cross-sectional Case-control	Serum	Nonfasting
	(Roh et al., 2008)	Down	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study
	(Boey et al., 2010)	Down	Any AMD	177	2923	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study

Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
(Javadzadeh et al., 2010)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Fasting
(Reynolds et al., 2010)	No difference/ No difference	Advanced: GA/ Advanced: Neovascular	123/ 195	140	Case-control	Serum	Fasting
(Colak et al., 2011)	No difference	Any AMD	79	84	Cross-sectional	Serum	Fasting
(Fauser et al., 2011)	No difference	Any AMD	780	521	Case-control	Serum	Nonfasting
(Weiner et al., 2011)	No difference	Any AMD	865	865	Cross-sectional Case-control	Not reported	
(Javadzadeh et al., 2012)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Fasting, note: this is the same cohort as described by (Javadzadeh et al., 2010)
(Wang et al., 2012)	No difference	Any AMD	161	2704	Population-Based	Serum	Beijing Eye Study, fasting
(You et al., 2012)	No difference	Early AMD	?	?	Population-Based	Blood	Beijing Eye Study, total n=3049, case-control ratios not presented
(Davari et al., 2013)	Up	Any AMD	32	32	Case-control	Serum	
(Munch et al., 2013)	Up	Early AMD	251	644	Cross-sectional	Serum	Inter99 Study, fasting, only significant higher levels in women
(Ortak et al., 2013)	No difference	Any AMD	144	172	Case-control	Serum	Fasting
(Ulas et al., 2013)	No difference	Advanced: Neovascular	142	141	Cross-sectional	Serum	Fasting
(Ambreen et al., 2014)	No difference	Any AMD	90	100	Cross-sectional Case-control	Serum	
(Cho et al., 2014)	No difference/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
(Cougnard-Gregoire et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	238/ 47	540	Population-Based	Plasma	ALIENOR, Fasting
(Ersoy et al., 2014)	No difference	Advanced: Any	1147	1773	Case-control	Serum	Nonfasting
(Ghorbanihaghjo et al., 2014)	No difference	Advanced: Neovascular	45	45	Cross-sectional	Serum	Fasting
(Kim et al., 2014a)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
(Kim et al., 2014b)	Down	Any AMD	1278	15767	Population-Based Cross-sectional	Blood	KNHANES
(La et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	1034/95	13223	Population-Based Cross-sectional	Blood	KNHANES, fasting
(Merle et al., 2014)	Down	Advanced: Neovascular	290	144	RCT	Plasma	NAT2, fasting

	(Park et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Any	958/ 88	12667	Population-based Cross-sectional	Serum	KNHANES
	(Qin et al., 2014)	No difference	Early AMD	14	14	Case-control	Plasma	Fasting
	(Semba et al., 2014)	Down/ Down	Early AMD/ Advanced: Any	1025/ 276	3606	Population-based cross-sectional	Serum	Age, Gene/Environment Susceptibility–Reykjavik Study
	(Yang et al., 2014)	Down	Early AMD	200	6377	Population-Based	Serum	Handan Eye Study, fasting
	(Cezario et al., 2015)	No difference	Any AMD	30	30	Case-control	Serum	
	(Hwang et al., 2015)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
	(Joachim et al., 2015)	No difference	Early AMD	281	1036	Population-Based Cohort	Serum	Blue Mountains Eye Study, fasting
	(Paun et al., 2015)	Down	Any AMD	1491	1579	Case-control	Serum	EUGENDA
	(Yip et al., 2015)	No difference	Any AMD	673	4671	Prospective Cohort	Serum	EPIC Norfolk Eye Study
Phospholipids	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	Nonfasting
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables, fasting
LDL-C	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	Fasting
	(Hyman et al., 2000)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	227/ 182	235	Case-control	Serum	Fasting
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Klein et al., 2003a)	Down	Early AMD	366	1995	Population-Based Cohort	Plasma	Cardiovascular Health Study
	(McGwin et al., 2005)	No difference	Any AMD	390	2365	Cross-sectional	Serum	Cardiovascular Health Study, Trend to downregulation (P=0.0679)
	(Nowak et al., 2005)	Up	Dry AMD	60	45	Case-control	Serum	Fasting, only women included in study
	(Javadzadeh et al., 2007)	Up	Advanced: Neovascular	60	60	Case-control	Serum	Fasting, only males included in study
	(Klein et al., 2007b)	No difference	Early AMD	221	5666	Longitudinal	Serum	Multi-ethnic Study of Atherosclerosis, fasting
	(Tan et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	?	?	Population-Based Cohort	Serum	Blue Mountains Eye Study, total n=2395, case-control ratios not presented
	(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
	(Roh et al., 2008)	No difference	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study

Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
(Boey et al., 2010)	No difference	Any AMD	177	2923	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
(Javadzadeh et al., 2010)	Up	Advanced: Neovascular	45	45	Case-control	Serum	Fasting
(Reynolds et al., 2010)	No difference/ Up	Advanced: GA/ Advanced: Neovascular	123/ 195	140	Case-control	Serum	Fasting
(Colak et al., 2011)	Up	Any AMD	79	84	Cross-sectional	Serum	Fasting sample
(Weiner et al., 2011)	No difference	Any AMD	865	865	Cross-sectional Case-control	Blood	
(Javadzadeh et al., 2012)	Up	Advanced: Neovascular	45	45	Case-control	Serum	Fasting, note: this is the same cohort as described by (Javadzadeh et al., 2010)
(Wang et al., 2012)	No difference	Any AMD	161	2704	Population-Based	Serum	Beijing Eye Study, fasting
(You et al., 2012)	No difference	Early AMD	?	?	Population-Based	Blood	Beijing Eye Study, total n=3049, case-control ratios not presented
(Davari et al., 2013)	Up	Any AMD	32	32	Case-control	Serum	
(Munch et al., 2013)	No difference	Early AMD	251	644	Cross-sectional	Serum	Inter99 Study, fasting
(Ortak et al., 2013)	No difference	Any AMD	144	172	Case-control	Serum	Fasting
(Ulas et al., 2013)	Up	Advanced: Neovascular	142	141	Cross-sectional	Serum	Fasting
(Ambreen et al., 2014)	No difference	Any AMD	90	100	Cross-sectional Case-control	Serum	
(Cho et al., 2014)	No difference/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
(Cougnard-Gregoire et al., 2014)	No difference/ No difference	Early AMD/ Advanced: Any	238/ 47	540	Population-Based	Plasma	ALIENOR, Fasting
(Ghorbanihaghjo et al., 2014)	Up	Advanced: Neovascular	45	45	Cross-sectional	Serum	Fasting
(Merle et al., 2014)	No difference	Advanced: Neovascular	290	144	RCT	Plasma	NAT2, fasting
(Qin et al., 2014)	Up	Early AMD	14	14	Case-control	Plasma	Fasting
(Semba et al., 2014)	No difference	Early AMD/ Advanced: Any	1025/ 276	3606	Population-based cross-sectional	Serum	Age, Gene/Environment Susceptibility–Reykjavik Study
(Yang et al., 2014)	No difference	Early AMD	200	6377	Population-Based	Serum	Handan Eye Study, fasting
(Cezario et al., 2015)	No difference	Any AMD	30	30	Case-control	Serum	
(Paun et al., 2015)	No difference	Any AMD	1491	1579	Case-control	Serum	EUGENDA
(Yip et al., 2015)	No difference	Any AMD	673	4671	Prospective Cohort	Serum	EPIC Norfolk Eye Study

nonHDL-C	(Colak et al., 2011)	Up	Any AMD	79	84	Cross-sectional	Serum	Fasting
	(Klein et al., 2014a)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	?	?	Meta-analysis	Serum	Numbers are not clear from text, 3 population-based studies are included in meta-analysis (total n=6950)
	(Paun et al., 2015)	No difference	Any AMD	1491	1579	Case-control	Serum	EUGENDA
HDL-C	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	Fasting
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Klein et al., 1993)	Up/ No difference/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	?	?	Population-Based	Serum	Beaver Dam Eye Study, association with HDL-D only found in male participants, total n=4771, case-control ratios not presented
	(Smith et al., 1998)	No difference/ No difference	Early AMD/ Advanced: Any	240/ 72	3342	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study, fasting
	(Hyman et al., 2000)	No difference/ Up	Dry AMD/ Advanced: Neovascular	227/ 182	235	Case-control	Serum	Fasting
	(Delcourt et al., 2001)	Up/ No difference	Early AMD/ Advanced: Any	730/ 38	1372	Population-Based	Plasma	POLA, higher levels of HDL-C in patients with soft drusen
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Klein et al., 2003b)	No difference/ No difference/ Up	Early AMD/ Advanced: Neovascular/ Advanced: GA	?	?	Population-Based Cohort	Serum	Beaver Dam Eye Study, Total n=2764, case-control ratios not presented
	(van Leeuwen et al., 2004)	Up	Any AMD	414	4352	Population-Based Cohort	Serum	Rotterdam Study
	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(McGwin et al., 2005)	No difference	Any AMD	390	2365	Cross-sectional	Serum	Cardiovascular Health Study
	(Nowak et al., 2005)	Down	Dry AMD	60	45	Case-control	Serum	Fasting, only women included
	(Javadzadeh et al., 2007)	No difference	Advanced: Neovascular	60	60	Case-control	Serum	Fasting, only males included in study
	(Klein et al., 2007b)	Up	Early AMD	221	5666	Longitudinal	Serum	Multi-Ethnic Study of Atherosclerosis, fasting, higher HDL-C in AMD only borderline significant in multivariate analysis (0.05<P<0.10)
	(Tan et al., 2007)	No difference/ Down	Early AMD/ Advanced: Any	?	?	Population-Based Cohort	Serum	Blue Mountains Eye Study, total n=2395, case-control ratios not presented

Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
(Wu et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Any	159/ 38	433	Population-Based Cross-sectional Case-control	Serum	Blue Mountains Eye Study, Fasting
(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
(Hogg et al., 2008)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	97/ 195	115	Cross-sectional Case-control	Serum	Nonfasting
(Roh et al., 2008)	No difference	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study
(Ho et al., 2009)	Up	Any AMD	164	1484	Population-Based case cohort	Blood	Rotterdam Study, nonfasting
(Boey et al., 2010)	No difference	Any AMD	177	2923	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
(Javadzadeh et al., 2010)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Fasting
(Klein et al., 2010)	Down	Early AMD	96	2714	Cross-sectional	Serum	
(Reynolds et al., 2010)	No difference/ Down	Advanced: GA/ Advanced: Neovascular	123/ 195	140	Case-control	Serum	Fasting
(Butt et al., 2011)	Up	Any AMD	347	639	Cross-sectional	Serum	Fasting
(Colak et al., 2011)	No difference	Any AMD	79	84	Cross-sectional	Serum	Fasting
(Fauser et al., 2011)	No difference	Any AMD	805	521	Case-control	Serum	Nonfasting
(Weiner et al., 2011)	Up	Any AMD	865	865	Cross-sectional Case-control	Blood	Only significant in Any AMD, not significantly associated with late AMD
(Javadzadeh et al., 2012)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Fasting, note: this is the same cohort as described by (Javadzadeh et al., 2010)
(Jonas et al., 2012)	No difference	Early AMD	215	4319	Population-Based Cross-sectional	Serum	Central India Eyes and Medical Study, Postprandial, only 8 late cases thus not analyzed
(Wang et al., 2012)	No difference	Any AMD	161	2704	Population-Based	Serum	Beijing Eye Study, fasting
(You et al., 2012)	No difference	Early AMD	?	?	Population-Based	Blood	Beijing Eye Study, total n=3049, case-control ratios not presented
(Davari et al., 2013)	No difference	Any AMD	32	32	Case-control	Serum	
(Munch et al., 2013)	No difference	Early AMD	251	644	Cross-sectional	Serum	Inter99 Study, fasting
(Ortak et al., 2013)	No difference	Any AMD	144	172	Case-control	Serum	Fasting

(Ulas et al., 2013)	No difference	Advanced: Neovascular	142	141	Cross-sectional	Serum	Trend to downregulation in nAMD (P=0.081), fasting
(Ambreen et al., 2014)	No difference	Any AMD	90	100	Cross-sectional Case-control	Serum	
(Cho et al., 2014)	Up/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
(Cougnard-Gregoire et al., 2014)	Up/ Up	Early AMD/ Advanced: Any	238/ 47	540	Population-Based	Plasma	ALIENOR, fasting
(Ersoy et al., 2014)	No difference	Advanced: Any	1147	1773	Case-control	Serum	Nonfasting
(Ghorbanihaghjo et al., 2014)	No difference	Advanced: Neovascular	45	45	Cross-sectional	Serum	Fasting
(Jonasson et al., 2014)	Up	Any AMD	328	2540	Population-Based Cohort	Plasma	Age Gene/Environment Susceptibility-Reykjavik Study
(Klein et al., 2014a)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	?	?	Meta-analysis	Serum	Numbers are not clear from text, 3 population-based studies are included in meta-analysis (total n=6950)
(Merle et al., 2014)	No difference	Advanced: Neovascular	290	144	RCT	Plasma	NAT2, fasting
(Park et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Any	626/ 58	9189	Population-based Cross-sectional	Serum	KNHANES, Trend to upregulation in early AMD (P=0.057)
(Peiretti et al., 2014)	No difference	Any AMD	136	38	Case-control	Plasma	
(Qin et al., 2014)	No difference	Early AMD	14	14	Case-control	Plasma	Fasting
(Semba et al., 2014)	No difference/ Up	Early AMD/ Advanced: Any	1025/ 276	3606	Population-based cross-sectional	Serum	Age, Gene/Environment Susceptibility–Reykjavik Study
(Yang et al., 2014)	No difference	Early AMD	200	6377	Population-Based	Serum	Handan Eye Study, fasting
(Aoki et al., 2015)	Up	Any AMD	185	295	Cross-sectional	Serum	Hatoyama Cohort Study
(Cezario et al., 2015)	Down?	Any AMD	30	30	Case-control	Serum	Conflict between abstract and text
(Joachim et al., 2015)	No difference	Early AMD	281	1036	Population-Based Cohort	Serum	Blue Mountains Eye Study, fasting
(Paun et al., 2015)	Up	Any AMD	1491	1579	Case-control	Serum	EUGENDA
(Seshasai et al., 2015)	No difference	Any AMD	426	927	Population-Based Case-control	Serum	Singapore Indian Eye Study & Singapore Chinese Eye Study, nonfasting, trend to upregulation (P=0.08)
(Yip et al., 2015)	Up	Any AMD	673	4671	Prospective Cohort	Serum	EPIC Norfolk Eye Study

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
lp(a)	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Klein et al., 2003a)	No difference	Early AMD	366	1995	Population-Based Cohort	Plasma	Cardiovascular Health Study
	(Nowak et al., 2005)	No difference	Dry AMD	60	45	Case-control	Serum	Fasting, only women included, trend to upregulation (P=0.065)
	(Colak et al., 2011)	No difference	Any AMD	79	84	Cross-sectional	Serum	Fasting
	(Fauser et al., 2011)	No difference	Any AMD	691	397	Case-control	Serum	Nonfasting
	(Ersoy et al., 2014)	No difference	Advanced: Any	1147	1773	Case-control	Serum	Nonfasting
apoA1	(Delcourt et al., 2001)	Up/ No difference	Early AMD/ Advanced: Any	730/ 38	1372	Population-Based	Plasma	POLA, Higher levels of ApoA1 in patients with soft drusen
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Nowak et al., 2005)	Down	Dry AMD	60	45	Case-control	Serum	Fasting, only women included
	(Dashti et al., 2006)	No difference/ No difference	Early AMD/ Advanced: Any	58/ 39	32	Cross-sectional	Plasma	
	(Colak et al., 2011)	No difference	Any AMD	79	84	Cross-sectional	Serum	Fasting
	(Fauser et al., 2011)	No difference	Any AMD	690	398	Case-control	Serum	EUGENDA, nonfasting, in paper ApoA2 but rectification to ApoA1
	(Ersoy et al., 2014)	No difference	Advanced: Any	1147	1773	Case-control	Serum	EUGENDA, nonfasting, in paper ApoA2 but rectification to ApoA1
	(Paun et al., 2015)	Up	Any AMD	1491	1579	Case-control	Serum	EUGENDA
apoB	(Delcourt et al., 2001)	No difference/ No difference	Early AMD/ Advanced: Any	730/ 38	1372	Population-Based	Plasma	POLA, fasting
	(Abalain et al., 2002)	No difference	Any AMD	84	62	Case-control	Serum	Fasting
	(Nowak et al., 2005)	Up	Dry AMD	60	45	Case-control	Serum	Fasting, only women included
	(Dashti et al., 2006)	No difference/ No difference	Early AMD/ Advanced: Any	58/ 39	32	Cross-sectional	Plasma	
	(Colak et al., 2011)	No difference	Any AMD	79	84	Cross-sectional	Serum	Fasting
	(Fauser et al., 2011)	Up	Any AMD	689	398	Case-control	Serum	Nonfasting
	(Ersoy et al., 2014)	No difference	Advanced: Any	1147	1773	Case-control	Serum	Nonfasting
	(Paun et al., 2015)	No difference	Any AMD	1491	1579	Case-control	Serum	EUGENDA
apoE	(Abalain et al., 2002)	Up	Any AMD	84	62	Case-control	Serum	Subgroup analyses showed significantly higher apoE levels in advanced AMD vs early AMD.

	(Klein et al., 2003a)	No difference	Early AMD	366	1995	Population-based Cohort	Plasma	Cardiovascular Health Study
Docosahexaenoic acid (DHA)	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma, erythrocytes	
	(Ouchi et al., 2002)	Up	Any AMD	11	10	Case-control	Plasma, erythrocytes	Only significant in erythrocytes
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Fasting
	(Merle et al., 2013)	Down	Advanced: Any	64	541	Population-Based	Plasma	ALIENOR, fasting, controls are defined as without late AMD
	(Merle et al., 2014)	Down	Advanced: Neovascular	290	144	RCT	RBCM, Serum	NAT2, fasting, only in RBCM
	(Orban et al., 2015)	Down	Advanced: Neovascular	21	22	Case-control	Serum	
Eicosapentaenoic acid (EPA)	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma, erythrocytes	
	(Ouchi et al., 2002)	No difference	Any AMD	11	10	Case-control	Plasma, erythrocytes	Trend to upregulation in erythrocytes (P=0.07)
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Trend to upregulation in nAMD (P=0.053)
	(Merle et al., 2013)	No difference	Advanced: Any	64	541	Population-Based	Plasma	ALIENOR, controls are defined as without late AMD, trend to downregulation (P=0.07), only significant in Advanced: GA (P=0.007)
	(Merle et al., 2014)	Down	Advanced: Neovascular	290	144	RCT	RBCM, Serum	NAT2
α-Linolenic acid (ALA)	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	
	(Merle et al., 2013)	Down	Advanced: Any	64	541	Population-Based	Plasma	ALIENOR, controls are defined as without late AMD
Docosapentaenoic acid (DPA)	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma, erythrocytes	
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	
	(Merle et al., 2013)	No difference	Advanced: Any	64	541	Population-Based	Plasma	ALIENOR, Trend to downregulation (P=0.05)
Arachidonic acid (AA)	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma, erythrocytes	
	(Ouchi et al., 2002)	Up	Any AMD	11	10	Case-control	Plasma, erythrocytes	Only significant in erythrocytes

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Fasting
	(Orban et al., 2015)	Up	Advanced: Neovascular	22	21	Case-control	Serum	Numbers are conflicting in text and figure
Linoleic acid (LA)	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma, erythrocytes	
	(Ouchi et al., 2002)	Down	Any AMD	11	10	Case-control	Plasma, erythrocytes	Only significant in erythrocytes
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Fasting
Oleic acid (OA)	(Ouchi et al., 2002)	Down	Any AMD	11	10	Case-control	Plasma, erythrocytes	Only significant in erythrocytes
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Fasting
Palmitic acid (PA)	(Ouchi et al., 2002)	Down	Any AMD	11	10	Case-control	Plasma, erythrocytes	Only significant in erythrocytes
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Fasting
Stearic acid (SA)	(Ouchi et al., 2002)	No difference	Any AMD	11	10	Case-control	Plasma, erythrocytes	
	(Kabasawa et al., 2011)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	31/ 166	143	Case-control	Serum	Fasting

Supplementary table 5: Factors involved in extracellular matrix remodeling

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
MMP1	(Zeng et al., 2013)	No difference/ No difference	Early AMD/ Advanced: Neovascular	75/ 89	80	Case-control	Serum	
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
MMP2	(Chau et al., 2007)	No difference/ No difference	Early AMD/ Advanced: Neovascular	15/ 18	17	Case-control	Plasma	
	(Zeng et al., 2013)	No difference/ No difference	Early AMD/ Advanced: Neovascular	75/ 89	80	Case-control	Serum	Associated with PCV
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	
MMP9	(Chau et al., 2007)	Up/ Up	Early AMD/ Advanced: Neovascular	15/ 18	17	Case-control	Plasma	
	(Zeng et al., 2013)	No difference/ No difference	Early AMD/ Advanced: Neovascular	75/ 89	80	Case-control	Serum	Associated with PCV
	(Guymer et al., 2015)	No difference/ No difference/ No difference	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Serum	

Supplementary table 6: Dietary factors

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Albumin	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Klein et al., 2003a)	Down	Early AMD	366	1995	Population-Based Cohort	Serum	Cardiovascular Health Study
	(Klein et al., 2003c)	No difference/ No difference/ Down	Early AMD/ Advanced: GA/ Advanced: Neovascular	?	?	Population-Based Cohort	Blood	Beaver Dam Eye Study, total n=3674, case-control ratios not presented
	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study, trend to downregulation (P=0.08)
Vitamin A (retinol)	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	
	(West et al., 1994)	No difference	Any AMD	129	377	Case-control	Plasma	
	(Delcourt et al., 1999b)	No difference	Advanced: Any	38	2119	Population-based	Plasma	POLA, same population as (Delcourt et al., 1999a)
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Plasma	
	(Michikawa et al., 2009)	No difference/ No difference	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	
	(Zhou et al., 2011)	No difference/ Down	Early AMD/ Advanced: Neovascular	92/ 82	89	Case-control	Serum	
Vitamin B12 (cobalamin)	(Heuberger et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	329/ 16	3182	Population-based Cross-sectional	Serum	NHANES III
	(Kamburoglu et al., 2006)	Down/ No difference	Advanced: Neovascular/ Dry AMD	30/ 30	30	Case-control	Plasma	Lower in nAMD vs. dry AMD
	(Rochtchina et al., 2007)	Down	Advanced: Any	53	2910	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study

	(Gopinath et al., 2013)	Down	Any AMD	219	1171	Cohort	Serum	Blue Mountains Eye Study, subgroup analysis showed significant downregulation in early and advanced AMD
	(Obeid et al., 2013)	No difference/ No difference	Advanced: Neovascular/ Dry AMD	31/ 38	48	Case-control	Plasma	All cataract subjects
Vitamin B9 (folate)	(Heuberger et al., 2002)	No difference	Early AMD	329	3182	Population-based Cross-sectional	Serum	NHANES III, Analysis for advanced AMD were not performed for folate because one or more quintiles had 0 cases
	(Klein et al., 2005)	No difference	Any AMD	188	195	Nested Case-control	Serum	Beaver Dam Eye Study
	(Kamburoglu et al., 2006)	No difference/ No difference	Advanced: Neovascular/ Dry AMD	30/ 30	30	Case-control	Plasma	
	(Rochtchina et al., 2007)	No difference	Advanced: Any	53	2910	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study
	(Gopinath et al., 2013)	No difference	Any AMD	219	1171	Cohort	Serum	Blue Mountains Eye Study, subgroup analysis also did not show significant differences
	(Obeid et al., 2013)	No difference/ No difference	Advanced: Neovascular/ Dry AMD	31/ 38	48	Case-control	Plasma	All cataract subjects
Vitamin C (ascorbic acid)	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Eye-Disease-Case-Control-Study-Group, 1993)	No difference	Advanced: Neovascular	390	577	Case-control	Serum	
	(West et al., 1994)	No difference	Any AMD	129	377	Case-control	Plasma	
	(Delcourt et al., 1999b)	No difference	Advanced: Any	38	2119	Population-based	Plasma	POLA, same population as (Delcourt et al., 1999a)
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Plasma	Lower in advanced vs early AMD
	(Yildirim et al., 2011)	No difference	Advanced: Neovascular	25	25	Case-control	Serum	
	(Shen et al., 2012)	Down/ No difference/ Down	Early/ Advanced: GA/ Advanced: Neovascular	21/ 13/ 22	34	Case-control	Serum	

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Vitamin D	(Parekh et al., 2007)	Down/ No difference	Early AMD/ Advanced: Any	823/ 54	6875	Population-based Cross-sectional	Serum	NHANES III
	(Golan et al., 2011)	No difference	Any AMD	1045	8124	Cross-sectional	Serum	
	(Millen et al., 2011)	No difference	Early AMD	241	1046	Observational	Serum	CAREDS, included women only, a significant interaction with age was found
	(Morrison et al., 2011)	No difference	Advanced: Neovascular	50	50	Family-based	Serum	
	(Singh et al., 2013b)	No difference	Any AMD	129	49	Cross-sectional	Plasma	
	(Cho et al., 2014)	No difference/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
	(Itty et al., 2014)	No difference/ Down	Dry AMD/ Advanced: Neovascular	216/ 146	100	Case-control	Serum	Lower in nAMD vs. dry AMD
	(Kim et al., 2014b)	Up	Any AMD	1278	15767	Population-Based Cross-sectional	Blood	KNHANES, after correction for possible confounders there was no significant association
	(Park et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: Any	959/ 88	12667	Population-based Cross-sectional	Serum	KNHANES
	(Cougnard-Gregoire et al., 2015)	No difference/ No difference	Early AMD/ Advanced: Any	269/ 63	365	Population-based	Plasma	ALIENOR
Vitamin E (a/y-tocopherol)	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Tsang et al., 1992)	No difference	Any AMD	80	86	Case-control	Serum	
	(Eye-Disease-Case-Control-Study-Group, 1993)	No difference	Advanced: Neovascular	391	578	Case-control	Serum	
	(Sanders et al., 1993)	No difference	Any AMD	65	65	Case-control	Plasma	Correlation cholesterol
	(West et al., 1994)	Down	Any AMD	129	377	Case-control	Plasma	
	(Mares-Perlman et al., 1995)	No difference/ Down	Any AMD/ Advanced: Neovascular	167/ 31	167	Case-control	Serum	Not significant after correction for serum cholesterol levels
	(Smith et al., 1997)	No difference/ No difference	Early AMD/ Advanced: Any	102/ 54	156	Case-control	Serum	Blue Mountains Eye Study, also no difference for pooled analysis of all AMD cases vs controls

	(Belda et al., 1999)	Down	Any AMD	25	15	Case-control	Serum	
	(Delcourt et al., 1999b)	No difference	Advanced: Any	38	2119	Population-based	Plasma	POLA, same population as (Delcourt et al., 1999a)
	(Simonelli et al., 2002)	No difference/ Down	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Plasma	Lower in advanced vs early AMD
	(Cardinault et al., 2005)	No difference	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables
	(Michikawa et al., 2009)	No difference/ No difference	Early AMD/ Advanced: Any	32/ 8	682	Case-control	Serum	Trend to downregulation of α-tocopherol in late AMD (P=0.056)
	(Shen et al., 2012)	No difference/ No difference/ No difference	Early/ Advanced: GA/ Advanced: Neovascular	21/ 13/ 22	34	Case-control	Serum	
Cadmium (Cd)	(Erie et al., 2007)	No difference	Any AMD	53	53	Prospective Case-control	Blood, urine	Positive association between urinary cadmium levels and AMD in smokers
	(Junemann et al., 2013)	Up	Dry AMD	12	11	Case-control	Aqueous	
	(Cho et al., 2014)	No difference/ Up	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, only significant in univariate analyses, not in multivariate
	(Kim et al., 2014a)	Up	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
	(Wu et al., 2014)	Up	Any AMD	426	4964	Cross-sectional	Blood, urine	NHANES, urinary samples were available in 1548 participants only
	(Park et al., 2015)	No difference/ Up	Early AMD/ Advanced: Any	243/ 11	3611	Population-Based Cross-sectional	Blood	KNHANES
Lead (Pb)	(Cho et al., 2014)	Up/ Up	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, only significant in univariate analyses, not in multivariate
	(Wu et al., 2014)	Up	Any AMD	426	4961	Cohort	Blood	NHANES, Not associated after correction
	(Hwang et al., 2015)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES, trend to upregulation (P=0.08)
	(Park et al., 2015)	Up/ Up	Early AMD/ Advanced: Any	243/ 11	3611	Population-Based Cross-sectional	Blood	KNHANES
Mercury (Hg)	(Cho et al., 2014)	No difference/ Up	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, only significant in univariate analyses, not in multivariate
	(Park et al., 2015)	No difference/ Up	Early AMD/ Advanced: Any	243/ 11	3611	Population-Based Cross-sectional	Blood	KNHANES

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Iron (Fe)	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Junemann et al., 2013)	Up	Dry AMD	12	11	Case-control	Aqueous	
	(Wysokinski et al., 2013)	No difference	Any AMD	493	171	Case-control	Serum	
Copper (Cu)	(Cardinault et al., 2005)	Up	Any AMD	37	24	Case-control	Serum	Numbers reported in abstract differ from text and tables
	(Junemann et al., 2013)	Down	Dry AMD	12	11	Case-control	Aqueous	
Manganese (Mn)	(Junemann et al., 2013)	No difference	Dry AMD	12	11	Case-control	Aqueous	
	(Park et al., 2015)	No difference/ Down	Early AMD/ Advanced: Any	100/ 4	1521	Population-Based Cross-sectional	Blood	KNHANES
Zinc (Zn)	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Belda et al., 1999)	Down	Any AMD	25	15	Case-control	Serum	
	(Simonelli et al., 2002)	No difference/ No difference	Early AMD/ Advanced: Any	19/ 29	46	Case-control	Serum	
	(Junemann et al., 2013)	No difference	Dry AMD	12	11	Case-control	Aqueous	
	(Park et al., 2015)	No difference/ Down	Early AMD/ Advanced: Any	71/ 3	1033	Population-Based Cross-sectional	Blood	KNHANES
Selenium (Se)	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Tsang et al., 1992)	Down	Any AMD	80	86	Case-control	Serum	Significant in univariate analysis only, borderline significant in multivariate (P=0.07)
	(Eye-Disease-Case-Control-Study-Group, 1993)	No difference	Advanced: Neovascular	390	578	Case-control	Serum	
	(Mayer et al., 1998)	Down	Advanced: Neovascular	10	9	Case-control	Blood	
	(Junemann et al., 2013)	No difference	Dry AMD	12	11	Case-control	Aqueous	

Supplementary table 7: Hormones

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Leptin	(Evereklioglu et al., 2003a)	Down/ Down	Early AMD/ Advanced: Any	16/ 16	20	Case-control	Serum	Advanced AMD patients had significantly lower levels compared to early AMD
	(Seshasai et al., 2015)	Down	Any AMD	426	927	Population-Based Case-control	Serum	Singapore Indian Eye Study & Singapore Chinese Eye Study
	(Haas et al., 2015)	No difference	Advanced: Neovascular	54	46	Case-control	Blood	
Melatonin	(Rosen et al., 2009)	Down	Any AMD	43	12	Case-control	Urine	measured 6-sulfatoxymelatonin
	(Schmid-Kubista et al., 2009)	Up	Any AMD	50	19	Prospective Cross-sectional Observational	Serum	measured N-acetyl-5-methoxytryptamine
DHEAS	(Defay et al., 2004)	Up	Early AMD	?	?	Population-Based	Plasma	POLA, only women included in study, total n=708, case-control ratios not presented
	(Tamer et al., 2007)	Down/ Down	Dry AMD/ Advanced: Neovascular	75/ 67	64	Case-control Prospective	Serum	
	(Ulas et al., 2013)	No difference	Advanced: Neovascular	142	141	Cross-sectional	Serum	

Supplementary table 8: Factors related to comorbidities

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
Cystatin C	(Klein et al., 2009)	Up/ Up/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	374/ 56/ 37	2286/ 3238/ 3243	Population-Based	Serum	Beaver Dam Eye Study
	(Chong et al., 2014)	No difference	Early AMD	221	5653	Population-Based Cross-sectional	Serum	Multi-Ethnic Study of Atherosclerosis, in normotensive participants the top 10% highest Cystatin C levels were associated with a higher risk of early AMD (P=0.049)
	(Aoki et al., 2015)	No difference/ No difference	Any AMD	185	295	Cross-sectional	Serum	Hatoyama Cohort Study
Creatinine	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	Fasting samples
	(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
	(Boey et al., 2010)	No difference	Any AMD	177	2923	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
	(Javadzadeh et al., 2010)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	
	(Javadzadeh et al., 2012)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Note: this is the same cohort as described by (Javadzadeh et al., 2010)
	(Obeid et al., 2013)	No difference/ No difference	Advanced: Neovascular/ Dry AMD	31/ 38	48	Case-control	Serum	All cataract subjects
	(Cho et al., 2014)	Down/ Up	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, Only significant in univariate analyses, not in multivariate
	(Itty et al., 2014)	No difference/ No difference	Dry AMD/ Advanced: Neovascular	216/ 146	100	Case-control	Serum	
	(Park et al., 2014b)	Down/ No difference	Early AMD Advanced: Any	958/ 88	12665	Population-based Cross-sectional	Serum	KNHANES
BUN	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Klein et al., 2009)	No difference/ No difference/ No difference	Early AMD/ Advanced: Neovascular/ Advanced: GA	392/ 62/ 39	2420/ 3424/ 3432	Population-Based	Serum	Beaver Dam Eye Study
	(Cho et al., 2014)	Down/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES , Only significant in univariate analyses, not in multivariate
	(Park et al., 2014b)	No difference/ No difference	Early AMD/ Advanced: any	958/ 88	12667	Population-based Cross-sectional	Serum	KNHANES

Glucose	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	Fasting samples
	(Eye-Disease-Case-Control-Study-Group, 1992)	No difference	Advanced: Neovascular	421	615	Case-control	Serum	
	(Smith et al., 1998)	No difference/ No difference	Early AMD/ Advanced: Any	240/ 72	3342	Population-Based Cross-sectional	Serum	Blue Mountains Eye Study, fasting
	(Delcourt et al., 2001)	No difference/ No difference	Early AMD/ Advanced: Any	730/ 38	1372	Population-Based	Plasma	POLA, Fasting samples
	(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
	(Boey et al., 2010)	No difference	Any AMD	177	2923	Population-Based Cross-sectional	Blood	Singapore Malay Eye Study
	(Javadzadeh et al., 2010)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	fasting
	(Javadzadeh et al., 2012)	No difference	Advanced: Neovascular	45	45	Case-control	Serum	Fasting Note: this is the same cohort as described by (Javadzadeh et al., 2010)
	(Jonas et al., 2012)	No difference	Early AMD	215	4319	Population-Based Cross-sectional	Serum	Central India Eyes and Medical Study, postprandial glucose, 8 late cases not analyzed
	(You et al., 2012)	No difference	Early AMD	?	?	Population-Based	Blood	Beijing Eye Study, total n = 3049, case-control ratios not presented
	(Kim et al., 2014a)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
	(La et al., 2014)	No difference/ Down	Early AMD/ Advanced: Any	1034/95	13223	Population-Based Cross-sectional	Blood	KNHANES, Fasting, conflicting results in text and tables
	(Yang et al., 2014)	No difference	Early AMD	200	6377	Population-Based	Plasma	Handan Eye Study, fasting
	(Hwang et al., 2015)	No difference	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
HbA1c	(Boey et al., 2010)	No difference	Any AMD	177	2923	Population-Based Cross-sectional	Blood	Singapore Malay Eye Study
	(Cackett et al., 2008)	No difference/ No difference	Early AMD/ Advanced: Any	169/ 21	3075	Population-Based Cross-sectional	Serum	Singapore Malay Eye Study
	(Jonas et al., 2012)	No difference	Early AMD	215	4319	Population-Based Cross-sectional	Serum	Central India Eyes and Medical Study, postprandial glucose, only 8 late cases thus not analyzed
	(Ulas et al., 2013)	No difference	Advanced: Neovascular	142	141	Cross-sectional	Serum	Trend to downregulation in nAMD (P=0.062), fasting
	(Cho et al., 2014)	Down/ Down	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES , Only significant in univariate analyses, not in multivariate

	Reference	Up/down/ no difference	Type of AMD	Cases (n)	Controls (n)	Study design	Matrix	Comment
	(Kim et al., 2014a)	Down	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
	(Kim et al., 2014b)	No difference/ Down	Early AMD/ Advanced: Any	1163/ 115	15767	Population-Based Cross-sectional	Blood	KNHANES
	(La et al., 2014)	Down/ Down	Early AMD/ Advanced: Any	1034/95	13223	Population-Based Cross-sectional	Blood	KNHANES, Fasting, conflicting results in text and tables
	(Zehetner et al., 2014)	No difference	Advanced: Neovascular	30	12	Case-control	Plasma	
	(Hwang et al., 2015)	Down	Any AMD	312	4621	Population-Based Cross-sectional	Blood	KNHANES
LDH	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Roh et al., 2008)	Up	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study, only significant in univariate analysis, not in multivariate
AST	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Cho et al., 2014)	Up/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES, Only significant in univariate analysis, not in multivariate
ALT	(Blumenkranz et al., 1986)	No difference	Advanced: Neovascular	26	23	Case-control	Serum	
	(Cho et al., 2014)	No difference/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
Hepatitis B surface antigen (HBsAg)	(Roh et al., 2008)	Up	Any AMD	235	9082	Case-control	Serum	Yonsei Eye Study
	(Cho et al., 2014)	Up/ No difference	Any AMD/ Advanced: Any	584/ 55	7315	Population-Based Cross-sectional	Serum	KNHANES
	(Park et al., 2014b)	Up/ No difference	Early AMD Advanced: Any	52/ 5	446	Population-based Cross-sectional	Serum	KNHANES
Amyloid beta (1-42)	(Obeid et al., 2013)	No difference/ No difference	Advanced: Neovascular/ Dry AMD	31/ 38	48	Case-control	Plasma	All cataract subjects
	(Guymer et al., 2015)	Up/ Up/ Up	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Plasma	trend to increasing plasma levels as AMD stage advances
	(Haas et al., 2015)	Up	Advanced: Neovascular	54	46	Case-control	Blood	
Amyloid beta (1-40)	(Guymer et al., 2015)	No difference/ No difference/ Up	Early AMD/ Advanced: GA/ Advanced: Neovascular	18/ 21/ 23	19	Cross-sectional Case-control	Plasma	Trend to upregulation in GA patients (P=0.064)
	(Haas et al., 2015)	No difference	Advanced: Neovascular	54	46	Case-control	Blood	

Chapter 3.2
Metabolomics in serum of patients with non-advanced age-related macular degeneration reveals aberrations in the glutamine pathway

Eveline Kersten*
Sascha Dammeier*
Soufiane Ajana
Joannes M.M. Groenewoud
Marius Codrea
Franziska Klose
Yara T. Lechanteur
Sascha Fauser
EYE-RISK Consortium
Marius Ueffing
Cécile Delcourt
Carel B. Hoyng
Eiko K. de Jong
Anneke I. den Hollander

*These authors contributed equally to this study

Accepted for publication in PLOSONE

ABSTRACT

Age-related macular degeneration (AMD) is a common, progressive multifactorial vision-threatening disease and many genetic and environmental risk factors have been identified. The risk of AMD is influenced by lifestyle and diet, which may be reflected by an altered metabolic profile. Therefore, measurements of metabolites could identify biomarkers for AMD, and could aid in identifying high-risk individuals. Hypothesis-free technologies such as metabolomics have a great potential to uncover biomarkers or pathways that contribute to disease pathophysiology. To date, only a limited number of metabolomic studies have been performed in AMD. Here, we aim to contribute to the discovery of novel biomarkers and metabolic pathways for AMD using a targeted metabolomics approach of 188 metabolites. This study focuses on non-advanced AMD, since there is a need for biomarkers for the early stages of disease before severe visual loss has occurred. Targeted metabolomics was performed in 72 patients with early or intermediate AMD and 72 control individuals, and metabolites predictive for AMD were identified by a sparse partial least squares discriminant analysis. In our cohort, we identified four metabolite variables that were most predictive for early and intermediate stages of AMD. Increased glutamine and phosphatidylcholine diacyl C28:1 levels were detected in non-advanced AMD cases compared to controls, while the rate of glutaminolysis and the glutamine to glutamate ratio were reduced in non-advanced AMD. The association of glutamine with non-advanced AMD corroborates a recent report demonstrating an elevated glutamine level in early AMD using a different metabolomics technique. In conclusion, this study indicates that metabolomics is a suitable method for the discovery of biomarker candidates for AMD. In the future, larger metabolomics studies could add to the discovery of novel biomarkers in yet unknown AMD pathways and expand our insights in AMD pathophysiology.

INTRODUCTION

Age-related macular degeneration (AMD) is a common vision-threatening disease affecting the elderly.[1-3] Visual loss in AMD occurs as a result of progressive degenerative events at the centre of the retina, known as the macula. Early AMD is characterized by the accumulation of waste products (drusen) in the macula. Usually, patients experience no or only mild complaints at this stage. As AMD progresses, visual loss occurs and two advanced subtypes of AMD are distinguished: geographic atrophy and choroidal neovascularization, also referred to as wet AMD. Targeting vascular endothelial growth factor, which is central to the disease process of wet AMD, has proven to be a highly effective treatment.[4] However, for the early, intermediate and atrophic stages of AMD, constituting over 80% of AMD patients, no effective treatment exists.

Many environmental and genetic risk factors for AMD have been discovered, including age, smoking, dietary factors (plasma lipids and anti-oxidant levels) and both common and rare genetic variants.[3,5-9] However, not all individuals with a high genetic risk develop AMD, while some low-risk individuals do develop AMD. Potentially, the disease risk in these AMD patients could be influenced by lifestyle and diet, which may be reflected by their metabolic profile. It has been described that metabolite levels can be influenced by many factors, including age, body-mass index (BMI) and nutrition,[10] factors that are also associated with AMD. Therefore, measurements of metabolites could identify biomarkers for AMD, which could aid in identifying high-risk individuals.

Metabolomics is an hypothesis-free approach that enables simultaneous analysis of large numbers of metabolites, and has the potential to uncover physiological pathways that differ between patients and controls.[11] To date, only a limited number of metabolomic studies have been performed in AMD.[12-17] Two small case-control studies involving a total of 45 and 40 individuals, respectively, showed that individual metabolites and metabolic pathways relevant for AMD pathogenesis can be identified using metabolomics.[13,15] Another larger study in 396 individuals concluded that, although metabolite changes related to AMD are of low magnitude, they seem to be specific to AMD and further studies are warranted.[12] More recently, a study in 120 individuals indicated that the most significant metabolites belong to the glycerophospholipid pathway.[16]

Here, we aim to contribute to the discovery of novel biomarkers for AMD and uncover clinically relevant metabolic pathways using a targeted metabolomics approach. Ideally, future AMD treatment should be initiated in early stages of the disease to prevent progression to advanced AMD with accompanying visual loss. To identify biomarkers for early stages of the disease, this study focuses on non-advanced AMD.

MATERIALS AND METHODS

Study design and study population

Individuals were selected from the European Genetic Database (EUGENDA), a large multicenter database for clinical and molecular analysis of AMD. Disease status was determined based on classification of color fundus photographs, and if available spectral domain optical coherence tomograms and fluorescein angiography by certified graders as described previously.[18] In this study, we included cases with non-advanced AMD defined as presence of at least 10 small drusen (<63µm) and pigmentary changes in at least one eye, and absence of central geographic atrophy or choroidal neovascularization in both eyes. Individuals having only pigmentary changes, less than 10 small drusen or without macular abnormalities were classified as control individuals.

Individuals were matched for age, sex, smoking status, body mass index (BMI), number or risk alleles of two prominent common genetic variants in *CFH* (p.Y402H; rs1061170) and *ARMS2* (p.A69S; rs10490924), and complement activation levels (measured as C3d/C3 ratio19) to minimize potential confounding effects. Interviewer-assisted questionnaires provided information on lifestyle, dietary habits and other environmental factors. For metabolomic analyses in this study, 72 AMD cases and 72 controls were selected (total n=144).

This study was approved by the local ethical committees at both sites of patient recruitment, the Radboud university medical center and the University Hospital of Cologne, and was performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was provided by all individuals.

Serum collection and genotyping

Venous blood samples were collected from all individuals in a non-fasting state at time of enrolment in EUGENDA. Serum was obtained using standardized coagulation and centrifugation procedures, and subsequently stored at-80°C within one hour after collection until analysis.

Genomic DNA was extracted from peripheral blood leukocytes, and genotyping of single nucleotide polymorphisms (SNPs) in the *CFH* (rs1061170) and *ARMS2* (rs10490924) genes was performed using competitive allele-specific PCR assays (KASPar SNP Genotyping System, KBiosciences).

Targeted metabolomics

Targeted identification and quantification of 188 metabolites (Supplementary Table 1) was achieved by executing the mass spectrometric acquisition methods as provided by the AbsoluteIDQ p180 kit (Biocrates Life Sciences, Innsbruck, Austria) with some modifications. Instead of using a conventional HPLC-MS system the analyses were performed on an Eksigent

200 microLC chromatography system (ABSciex, Darmstadt, Germany) coupled to a 6500 QTRAP (ABSciex, Darmstadt, Germany). To detect amino acids and biogenic amines, 50 µL of the metabolite extract were diluted in 350 µL of 50% methanol. Chromatography was performed using two running solvents (A: water, 0.2% formic acid; B: acetonitrile, 0.2% formic acid). Two µL of the diluted metabolite extracts were resolved on an Acquity UPLC BEH C18, 1.0 x 50 mm (120 A) reverse phase column (Waters, Eschborn, Germany) using a linear gradient from 2% B to 40% B in 3.5 minutes, from 40 % B to 80 % B in 1.5 minutes, and to 100% B in 0.1 min at 30 µL/min.

To determine the content of glycerophospholipids, hexoses and acylcarnitines, 50 µL of the metabolite extract were diluted with 450 µL methanol. Five µL of this dilution were analyzed in the mass spectrometer by direct infusion using the acquisition parameters as given by the manufacturer's manual. Two injections were done to acquire data in positive and negative mode separately.

Quality control

Technical quality control steps were undertaken before statistical analyses. Individual analytical batches were normalized to at least 3 replicates of the identical plasma quality control provided by the kit manufacturer to account for plate-to-plate variability. All metabolites that exhibited concentration values below limit of detection (as defined by the analytical specifications) in more than 50% of the measurements were omitted from the dataset (Supplementary Table 1).

Statistical analyses

To compare demographic characteristics of the two groups, one-way ANOVA and chi-squared tests were performed. After quality control 153 metabolites remained available for statistical analyses (Supplementary Table 1). Additionally, various derivative variables were created based on the metabolite levels (n=57; Supplementary Table 2). Due to the large number of variables to be evaluated, we used a sparse partial least squares discriminant analysis (sPLSda) to perform variable selection while taking into account the correlations between the variables. This approach aims at combining variable selection and dimension reduction in a one-step procedure.[20] We considered one latent dimension since we are predicting a univariate binary outcome and to facilitate interpretation of the model.[20] The optimal tuning parameters (i.e., number of selected predictors) were estimated using a leave one out cross-validation strategy. Thereafter, a logistic regression was performed on the selected predictors resulting from the sPLSda to reduce the bias induced by shrinkage.[21] We performed these analyses on the entire dataset (including the created variables) and on the crude metabolites only. Statistical tests were performed using R statistical software (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing,

Vienna, Austria. URL https://www.R-project.org/). We used the cran R package “mixOmics” to train and to test the sPLSda.

RESULTS

Serum samples of 72 cases and 72 control individuals were selected for metabolomic analyses. Demographic characteristics and other potential confounding factors in our cohort are provided in Table 1. No significant differences between the groups were detected for sex, age, smoking status, BMI, diabetic status, diet and complement activation levels.

Next, we performed sPLSda to select the most predictive variables from our dataset, including all 153 measured metabolites and 57 derivative variables. This approach selected four relevant predictors for non-advanced AMD: glutamine, glutamate:glutamine ratio, glutaminolysis, and phosphatidylcholine diacyl C28:1 (PC aa C28.1) (Table 2; Fig 1). Two of these predictors are derivative variables that were created from measured metabolite levels, which both involved glutamine: the rate of glutaminolysis was expressed by the ratio of the sum of the potential glutamine conversion products (aspartate, alanine, glutamate) to glutamine (Fig 2), and another measure of glutamine metabolism was defined as the ratio between glutamine and glutamate (Glu:Gln ratio).

The distributions of these variables in non-advanced AMD cases and control individuals are illustrated in Figure 1. A higher mean glutamine level was detected in non-advanced AMD cases (746.33 μM) compared to controls (695.0 μM). The mean rate of glutaminolysis and the Glu:Gln ratio were reduced in non-advanced AMD cases (0.73 and 0.08, respectively) compared to controls (0.80 and 0.10, respectively). The mean level of phosphatidylcholine diacyl C28:1 was elevated in non-advanced AMD cases (3.35 μM) compared to controls (3.04 μM).
When performing sPLSda on the measured metabolites only (excluding the derivative variables), glutamine levels were the most predictive for non-advanced AMD (OR 1.005).

Table 1. Patient characteristics of AMD cases and control individuals

		AMD cases (n=72)	Control individuals (n=72)	*P*-value
Sex	Male	26 (36.1%)	28 (38.9%)	
	Female	46 (63.9%)	44 (61.1%)	0.73
Age (mean years ±SD)		72.65±7.30	70.64±5.27	0.06
Smoking status	Never	34 (47.2%)	37 (51.4%)	
	Past	38 (52.8%)	35 (48.6%)	
	Current	0 (0%)	0 (0%)	0.62
BMI (kg/m²)	<20	3 (4.2%)	3 (4.2%)	
	20-25	35 (48.6%)	29 (40.3%)	
	25-30	28 (38.9%)	33 (45.8%)	
	>30	6 (8.3%)	7 (9.7%)	0.79
Diabetes Mellitus*	Present	64 (90.1%)	64 (91.4%)	
	Absent	7 (9.9%)	6 (8.6%)	0.79
Diet	Regular diet	65 (94.2%)	66 (95.7%)	
	Vegetarian diet**	4 (5.8%)	3 (4.3%)	0.70
Complement activation (mean±SD*)**	C3d/C3 ratio	1.54±0.40	1.47±0.44	0.37

*Self-reported diagnosis
**Vegetarian diet when participant indicated to (almost) never eat fish and red meat
***For the purpose of analyses data was transformed to the natural logarithm
Abbreviations: SD, standard deviation; BMI, body mass index.

Table 2. Metabolite predictors for non-advanced AMD from sPLSda

	Estimate	Odds ratio
Glutamine (µM)	0.0037	1.004
Glu:Gln ratio	-2.79	0.061
Glutaminolysis	-1.73	0.177
PC.aa.C28.1 (µM)	0.62	1.858

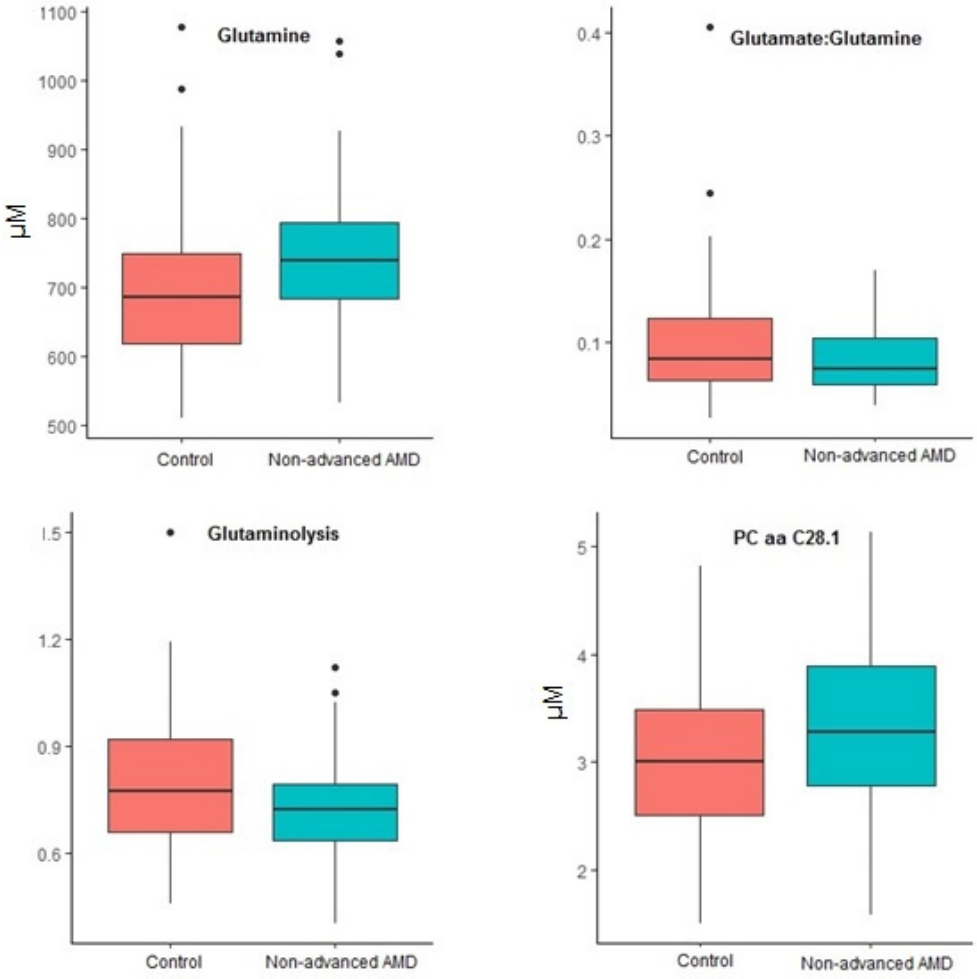


Figure 1. Boxplots of the four metabolite predictors for non-advanced AMD from sPLSda
All metabolites were measured in µM.

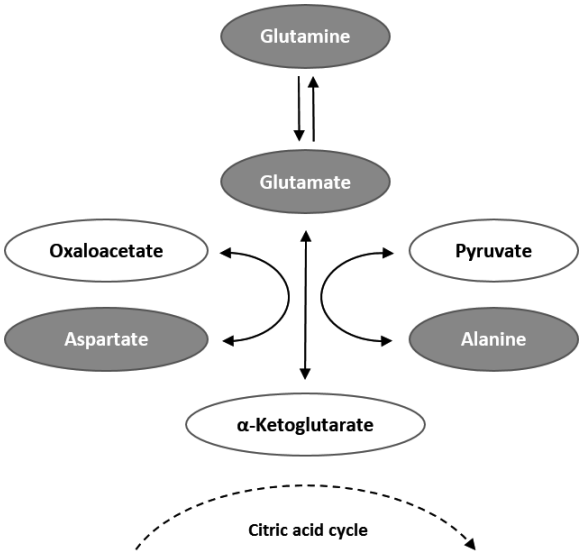


Fig 2. Metabolic conversion of glutamine
Glutaminolysis, the metabolic conversion of glutamine to glutamate, aspartate and alanine, represents an alternative pathway to supply the mitochondrial citric acid cycle with a surplus of α-ketoglutarate. As this pathway is preferentially used by proliferating tissue, glutaminolysis measured as $(c_{Ala}+c_{Asp}+c_{Glu})/c_{Gln}$ is increased in tumor tissue.[22] Metabolites determined in this study are marked in grey.

DISCUSSION

In the present study, we investigated potential differences of the metabolome between non-advanced AMD patients and control individuals using a targeted metabolomics approach. Four variables were identified by sPLSda as the most predictive features to discriminate between non-advanced AMD patients and control individuals, including glutamine, glutamine-related variables and a glycerophospholipid (PC aa C28.1). These results are in line with previous studies describing metabolic differences between AMD patients and controls.[12,13,15] Of particular interest is the slightly increased glutamine level in AMD patients, which independently corroborates a recent report demonstrating an elevated glutamine level in early AMD using a different metabolomics technique.[12] Also, other measures of glutamine metabolism in our study were indicative of a possible association between glutamine and AMD. In AMD patients, both glutaminolysis (the ratio of glutamate, aspartate and alanine to glutamine) as well as the Glu:Gln ratio were decreased, although these effects were driven mainly by elevated levels of glutamine. Glutamine is a nonessential amino acid necessary to sustain immune competence,[23] and immunological processes are at the heart of AMD pathology.[24,25] It remains to be investigated whether increased glutamine in serum is a

physiological response to a higher demand from the immune system or a result of increased protein catabolism, decreased clearance of glutamine, increased dietary intake of glutamine, or other mechanisms.

The observed association between a glycerophospholipid (PC aa C28.1) and non-advanced AMD is interesting because alterations in concentrations of these species have been implicated in a variety of metabolic diseases and pathomechanisms.[26-28] Glycerophospholipids and sphingomyelin are constituents of cell membranes and myelin sheaths, and phosphatidylcholines are the main constituents of lipoproteins,[29] which have been described to be relevant for AMD pathology.[30,31] Although literature is not entirely consistent, multiple studies have associated higher high-density lipoprotein cholesterol levels with increased risk of AMD.[8,25,31-34] Furthermore, it is conceivable that not only levels of lipoprotein classes, but also their molecular composition in terms of glycerophospholipid species could be potential biomarkers for AMD development, as has been reported for other conditions, such as arterial hypertension.[35] Therefore in-depth lipidomics studies that cover a wider range of lipoprotein subclasses and their molecular constituents are required to corroborate our findings and to further explore the relationship between lipids, lipoprotein dynamics and AMD pathology.

It must be noted that the effect sizes of the identified AMD-associated metabolites are small, consistent with slightly altered metabolic profiles of AMD patients compared to controls. Due to the limited sample size of the current study, we might have been unable to detect smaller associations and therefore larger studies are warranted. A strength of our study is that the study groups were carefully selected and matched on potential confounders including age, sex, smoking status and BMI. Due to this strict patient stratification, differences in metabolites likely reflect true differences in metabolic profiles between AMD cases and controls. Additionally, our study included patients with non-advanced AMD only, which allows for the identification of potentially relevant biomarkers already in an early stage of the disease.

Of note, the samples used for this study were collected at time of enrolment in EUGENDA and were not specifically collected for the current study. Because of possible influences of diet on the metabolome, the use of non-fasting samples in this study might not be ideal. However, although the reproducibility of metabolite levels over time was previously reported to be lower using non-fasting samples compared to fasting samples, in general the reliability of metabolites was not significantly different when comparing fasting versus non-fasting samples.[36]

In summary, the findings of this study indicate that metabolomics is a suitable method for the discovery of biomarkers in AMD. Using a targeted approach, several metabolites were identified as candidate biomarkers for AMD with glutamine being the most promising, which may serve as potential targets for future interventions. Larger metabolomic studies are needed to further elucidate the metabolic profile of AMD patients. Additionally, untargeted metabolomic studies could provide novel biomarkers in yet unknown AMD pathways and expand our insights in AMD pathophysiology.

REFERENCES

1. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health.* 2014;2(2):e106-116.
2. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology.* 2017;124(12):1753-1763.
3. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet.* 2018;392(10153):1147-1159.
4. Eandi CM, Alovisi C, De Sanctis U, Grignolo FM. Treatment for neovascular age related macular degeneration: The state of the art. *Eur J Pharmacol.* 2016;787:78-83.
5. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
6. Chakravarthy U, Wong TY, Fletcher A, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol.* 2010;10:31.
7. Saunier V, Merle BMJ, Delyfer MN, et al. Incidence of and Risk Factors Associated With Age-Related Macular Degeneration: Four-Year Follow-up From the ALIENOR Study. *JAMA Ophthalmol.* 2018;136(5):473-481.
8. Colijn JM, den Hollander AI, Demirkan A, et al. Increased High-Density Lipoprotein Levels Associated with Age-Related Macular Degeneration: Evidence from the EYE-RISK and European Eye Epidemiology Consortia. *Ophthalmology.* 2018.
9. Merle BMJ, Colijn JM, Cougnard-Gregoire A, et al. Mediterranean Diet and Incidence of Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium. *Ophthalmology.* 2018.
10. Kosmides AK, Kamisoglu K, Calvano SE, Corbett SA, Androulakis IP. Metabolomic fingerprinting: challenges and opportunities. *Crit Rev Biomed Eng.* 2013;41(3):205-221.
11. Kohler I, Hankemeier T, van der Graaf PH, Knibbe CAJ, van Hasselt JGC. Integrating clinical metabolomics-based biomarker discovery and clinical pharmacology to enable precision medicine. *Eur J Pharm Sci.* 2017;109S:S15-S21.
12. Lains I, Duarte D, Barros AS, et al. Human plasma metabolomics in age-related macular degeneration (AMD) using nuclear magnetic resonance spectroscopy. *PLoS One.* 2017;12(5):e0177749.
13. Osborn MP, Park Y, Parks MB, et al. Metabolome-wide association study of neovascular age-related macular degeneration. *PLoS One.* 2013;8(8):e72737.
14. Lauwen S, de Jong EK, Lefeber DJ, den Hollander A. Omics Biomarkers in Ophthalmology. *Invest Ophthalmol Vis Sci.* 2017;58(6):BIO88-BIO98.
15. Luo D, Deng T, Yuan W, Deng H, Jin M. Plasma metabolomic study in Chinese patients with wet age-related macular degeneration. *BMC Ophthalmol.* 2017;17(1):165.
16. Lains I, Kelly RS, Miller JB, et al. Human Plasma Metabolomics Study across All Stages of Age-Related Macular Degeneration Identifies Potential Lipid Biomarkers. *Ophthalmology.* 2018;125(2):245-254.
17. Lains I, Gantner M, Murinello S, et al. Metabolomics in the study of retinal health and disease. *Prog Retin Eye Res.* 2018.
18. Ristau T, Ersoy L, Lechanteur Y, et al. Allergy is a protective factor against age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(1):210-214.
19. Smailhodzic D, Klaver CC, Klevering BJ, et al. Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. *Ophthalmology.* 2012;119(2):339-346.

20. Le Cao KA, Boitard S, Besse P. Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. *BMC Bioinformatics.* 2011;12:253.
21. Hastie TT, R.; Friedman, R. The Elements of Statistical Learning: Data Mining, Inference, and Prediction. . *New York: Springer.* 2009.
22. Aledo JC. Glutamine breakdown in rapidly dividing cells: waste or investment? *Bioessays.* 2004;26(7):778-785.
23. Calder PC, Yaqoob P. Glutamine and the immune system. *Amino Acids.* 1999;17(3):227-241.
24. Camelo S. Potential Sources and Roles of Adaptive Immunity in Age-Related Macular Degeneration: Shall We Rename AMD into Autoimmune Macular Disease? *Autoimmune Dis.* 2014;2014:532487.
25. Kersten E, Paun CC, Schellevis RL, et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv Ophthalmol.* 2018;63(1):9-39.
26. Ma DW, Arendt BM, Hillyer LM, et al. Plasma phospholipids and fatty acid composition differ between liver biopsy-proven nonalcoholic fatty liver disease and healthy subjects. *Nutr Diabetes.* 2016;6(7):e220.
27. Jove M, Naudi A, Portero-Otin M, et al. Plasma lipidomics discloses metabolic syndrome with a specific HDL phenotype. *FASEB J.* 2014;28(12):5163-5171.
28. Rodriguez-Cuenca S, Pellegrinelli V, Campbell M, Oresic M, Vidal-Puig A. Sphingolipids and glycerophospholipids- The "ying and yang" of lipotoxicity in metabolic diseases. *Prog Lipid Res.* 2017;66:14-29.
29. Cole LK, Vance JE, Vance DE. Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim Biophys Acta.* 2012;1821(5):754-761.
30. Cheung CMG, Gan A, Fan Q, et al. Plasma lipoprotein subfraction concentrations are associated with lipid metabolism and age-related macular degeneration. *J Lipid Res.* 2017;58(9):1785-1796.
31. Burgess S, Davey Smith G. Mendelian Randomization Implicates High-Density Lipoprotein Cholesterol-Associated Mechanisms in Etiology of Age-Related Macular Degeneration. *Ophthalmology.* 2017;124(8):1165-1174.
32. Cougnard-Gregoire A, Delyfer MN, Korobelnik JF, et al. Elevated high-density lipoprotein cholesterol and age-related macular degeneration: the Alienor study. *PLoS One.* 2014;9(3):e90973.
33. Wang Y, Wang M, Zhang X, et al. The Association between the Lipids Levels in Blood and Risk of Age-Related Macular Degeneration. *Nutrients.* 2016;8(10).
34. Paun CC, Ersoy L, Schick T, et al. Genetic Variants and Systemic Complement Activation Levels Are Associated With Serum Lipoprotein Levels in Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(13):7766-7773.
35. Ozerova IN, Perova NV, Shchel'tsyna NV, Mamedov MN. Parameters of high-density lipoproteins in patients with arterial hypertension in combination with other components of metabolic syndrome. *Bull Exp Biol Med.* 2007;143(3):320-322.
36. Carayol M, Licaj I, Achaintre D, et al. Reliability of Serum Metabolites over a Two-Year Period: A Targeted Metabolomic Approach in Fasting and Non-Fasting Samples from EPIC. *PLoS One.* 2015;10(8):e0135437.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. List of metabolites

Metabolite name	Abbreviation	Passed QC?
Aminoacids (n=21)		
Alanine	Ala	Yes
Arginine	Arg	Yes
Asparagine	Asn	Yes
Aspartate	Asp	Yes
Citrulline	Cit	Yes
Glutamine	Gln	Yes
Glutamate	Glu	Yes
Glycine	Gly	Yes
Histidine	His	Yes
Isoleucine	Ile	Yes
Leucine	Leu	Yes
Lysine	Lys	Yes
Methionine	Met	Yes
Ornithine	Orn	Yes
Phenylalanine	Phe	Yes
Proline	Pro	Yes
Serine	Ser	Yes
Threonine	Thr	Yes
Tryptophan	Trp	Yes
Tyrosine	Tyr	Yes
Valine	Val	Yes
Biogenic amines (n=21)		
Acetylornithine	Ac-Orn	< LOD
Asymmetric dimethylarginine	ADMA	Yes
alpha-Aminoadipic acid	alpha-AAA	< LOD
cis-4-Hydroxyproline	c4-OH-Pro	< LOD
Carnosine	Carnosine	< LOD
Creatinine	Creatinine	Yes
DOPA	DOPA	< LOD
Dopamine	Dopamine	< LOD
Histamine	Histamine	< LOD
Kynurenine	Kynurenine	Yes
Methioninesulfoxide	Met-SO	< LOD
Nitrotyrosine	Nitro-Tyr	< LOD
Phenylethylamine	PEA	< LOD
Putrescine	Putrescine	Yes
Sarcosine	Sarcosine	Yes
Serotonin	Serotonin	Yes

Metabolite name	Abbreviation	Passed QC?
Spermidine	Spermidine	< LOD
Spermine	Spermine	< LOD
trans-OH-Pro	t4-OH-Pro	< LOD
Taurine	Taurine	Yes
Symmetric dimethylarginine	SDMA	Yes
Acylcarnitines (n=40)		
Carnitine	C0	Yes
Acetylcarnitine	C2	Yes
Propionylcarnitine	C3	Yes
Propenoylcarnitine	C3:1	< LOD
Hydroxypropionylcarnitine	C3-OH	< LOD
Butyrylcarnitine	C4	Yes
Butenylcarnitine	C4:1	Yes
Hydroxybutyrylcarnitine	C4-OH (C3-DC)	Yes
Valerylcarnitine	C5	Yes
Tiglylcarnitine	C5:1	Yes
Glutaconylcarnitine	C5:1-DC	Yes
Glutarylcarnitine (Hydroxyhexanoylcarnitine)	C5-DC (C6-OH)	Yes
Methylglutarylcarnitine	C5-M-DC	< LOD
Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	C5-OH (C3-DC-M)	< LOD
Hexanoylcarnitine (Fumarylcarnitine)	C6 (C4:1-DC)	Yes
Hexenoylcarnitine	C6:1	< LOD
Pimelylcarnitine	C7-DC	< LOD
Octanoylcarnitine	C8	Yes
Nonaylcarnitine	C9	Yes
Decanoylcarnitine	C10	Yes
Decenoylcarnitine	C10:1	Yes
Decadienylcarnitine	C10:2	< LOD
Dodecanoylcarnitine	C12	Yes
Dodecenoylcarnitine	C12:1	Yes
Dodecanedioylcarnitine	C12-DC	< LOD
Tetradecanoylcarnitine	C14	Yes
Tetradecenoylcarnitine	C14:1	Yes
Hydroxytetradecenoylcarnitine	C14:1-OH	< LOD
Tetradecadienylcarnitine	C14:2	Yes
Hydroxytetradecadienylcarnitine	C14:2-OH	< LOD
Hexadecanoylcarnitine	C16	Yes
Hexadecenoylcarnitine	C16:1	< LOD
Hydroxyhexadecenoylcarnitine	C16:1-OH	< LOD
Hexadecadienylcarnitine	C16:2	< LOD
Hydroxyhexadecadienylcarnitine	C16:2-OH	< LOD
Hydroxyhexadecanoylcarnitine	C16-OH	< LOD

Metabolite name	Abbreviation	Passed QC?
Octadecanoylcarnitine	C18	Yes
Octadecenoylcarnitine	C18:1	Yes
Hydroxyoctadecenoylcarnitine	C18:1-OH	< LOD
Octadecadienylcarnitine	C18:2	Yes
Glycerophospholipids (n=90)		
lysophosphatidylcholine acyl C14:0	lysoPC a C14:0	< LOD
lysophosphatidylcholine acyl C16:0	lysoPC a C16:0	Yes
lysophosphatidylcholine acyl C16:1	lysoPC a C16:1	Yes
lysophosphatidylcholine acyl C17:0	lysoPC a C17:0	Yes
lysophosphatidylcholine acyl C18:0	lysoPC a C18:0	Yes
lysophosphatidylcholine acyl C18:1	lysoPC a C18:1	Yes
lysophosphatidylcholine acyl C18:2	lysoPC a C18:2	Yes
lysophosphatidylcholine acyl C20:3	lysoPC a C20:3	Yes
lysophosphatidylcholine acyl C20:4	lysoPC a C20:4	Yes
lysophosphatidylcholine acyl C24:0	lysoPC a C24:0	Yes
lysophosphatidylcholine acyl C26:0	lysoPC a C26:0	Yes
lysophosphatidylcholine acyl C26:1	lysoPC a C26:1	Yes
lysophosphatidylcholine acyl C28:0	lysoPC a C28:0	Yes
lysophosphatidylcholine acyl C28:1	lysoPC a C28:1	Yes
Phosphatidylcholine diacyl C24:0	PC aa C24:0	Yes
Phosphatidylcholine diacyl C26:0	PC aa C26:0	< LOD
Phosphatidylcholine diacyl C28:1	PC aa C28:1	Yes
Phosphatidylcholine diacyl C30:0	PC aa C30:0	Yes
Phosphatidylcholine diacyl C30:2	PC aa C30:2	< LOD
Phosphatidylcholine diacyl C32:0	PC aa C32:0	Yes
Phosphatidylcholine diacyl C32:1	PC aa C32:1	Yes
Phosphatidylcholine diacyl C32:2	PC aa C32:2	Yes
Phosphatidylcholine diacyl C32:3	PC aa C32:3	Yes
Phosphatidylcholine diacyl C34:1	PC aa C34:1	Yes
Phosphatidylcholine diacyl C34:2	PC aa C34:2	Yes
Phosphatidylcholine diacyl C34:3	PC aa C34:3	Yes
Phosphatidylcholine diacyl C34:4	PC aa C34:4	Yes
Phosphatidylcholine diacyl C36:0	PC aa C36:0	Yes
Phosphatidylcholine diacyl C36:1	PC aa C36:1	Yes
Phosphatidylcholine diacyl C36:2	PC aa C36:2	Yes
Phosphatidylcholine diacyl C36:3	PC aa C36:3	Yes
Phosphatidylcholine diacyl C36:4	PC aa C36:4	Yes
Phosphatidylcholine diacyl C36:5	PC aa C36:5	Yes
Phosphatidylcholine diacyl C36:6	PC aa C36:6	Yes
Phosphatidylcholine diacyl C38:0	PC aa C38:0	Yes
Phosphatidylcholine diacyl C38:1	PC aa C38:1	Yes
Phosphatidylcholine diacyl C38:3	PC aa C38:3	Yes
Phosphatidylcholine diacyl C38:4	PC aa C38:4	Yes

Metabolite name	Abbreviation	Passed QC?
Phosphatidylcholine diacyl C38:5	PC aa C38:5	Yes
Phosphatidylcholine diacyl C38:6	PC aa C38:6	Yes
Phosphatidylcholine diacyl C40:1	PC aa C40:1	< LOD
Phosphatidylcholine diacyl C40:2	PC aa C40:2	Yes
Phosphatidylcholine diacyl C40:3	PC aa C40:3	Yes
Phosphatidylcholine diacyl C40:4	PC aa C40:4	Yes
Phosphatidylcholine diacyl C40:5	PC aa C40:5	Yes
Phosphatidylcholine diacyl C40:6	PC aa C40:6	Yes
Phosphatidylcholine diacyl C42:0	PC aa C42:0	Yes
Phosphatidylcholine diacyl C42:1	PC aa C42:1	Yes
Phosphatidylcholine diacyl C42:2	PC aa C42:2	Yes
Phosphatidylcholine diacyl C42:4	PC aa C42:4	Yes
Phosphatidylcholine diacyl C42:5	PC aa C42:5	Yes
Phosphatidylcholine diacyl C42:6	PC aa C42:6	Yes
Phosphatidylcholine acyl-alkyl C30:0	PC ae C30:0	Yes
Phosphatidylcholine acyl-alkyl C30:1	PC ae C30:1	Yes
Phosphatidylcholine acyl-alkyl C30:2	PC ae C30:2	Yes
Phosphatidylcholine acyl-alkyl C32:1	PC ae C32:1	Yes
Phosphatidylcholine acyl-alkyl C32:2	PC ae C32:2	Yes
Phosphatidylcholine acyl-alkyl C34:0	PC ae C34:0	Yes
Phosphatidylcholine acyl-alkyl C34:1	PC ae C34:1	Yes
Phosphatidylcholine acyl-alkyl C34:2	PC ae C34:2	Yes
Phosphatidylcholine acyl-alkyl C34:3	PC ae C34:3	Yes
Phosphatidylcholine acyl-alkyl C36:0	PC ae C36:0	Yes
Phosphatidylcholine acyl-alkyl C36:1	PC ae C36:1	Yes
Phosphatidylcholine acyl-alkyl C36:2	PC ae C36:2	Yes
Phosphatidylcholine acyl-alkyl C36:3	PC ae C36:3	Yes
Phosphatidylcholine acyl-alkyl C36:4	PC ae C36:4	Yes
Phosphatidylcholine acyl-alkyl C36:5	PC ae C36:5	Yes
Phosphatidylcholine acyl-alkyl C38:0	PC ae C38:0	Yes
Phosphatidylcholine acyl-alkyl C38:1	PC ae C38:1	Yes
Phosphatidylcholine acyl-alkyl C38:2	PC ae C38:2	Yes
Phosphatidylcholine acyl-alkyl C38:3	PC ae C38:3	Yes
Phosphatidylcholine acyl-alkyl C38:4	PC ae C38:4	Yes
Phosphatidylcholine acyl-alkyl C38:5	PC ae C38:5	Yes
Phosphatidylcholine acyl-alkyl C38:6	PC ae C38:6	Yes
Phosphatidylcholine acyl-alkyl C40:1	PC ae C40:1	Yes
Phosphatidylcholine acyl-alkyl C40:2	PC ae C40:2	Yes
Phosphatidylcholine acyl-alkyl C40:3	PC ae C40:3	Yes
Phosphatidylcholine acyl-alkyl C40:4	PC ae C40:4	Yes
Phosphatidylcholine acyl-alkyl C40:5	PC ae C40:5	Yes
Phosphatidylcholine acyl-alkyl C40:6	PC ae C40:6	Yes
Phosphatidylcholine acyl-alkyl C42:0	PC ae C42:0	< LOD
Phosphatidylcholine acyl-alkyl C42:1	PC ae C42:1	Yes

Metabolite name	Abbreviation	Passed QC?
Phosphatidylcholine acyl-alkyl C42:2	PC ae C42:2	Yes
Phosphatidylcholine acyl-alkyl C42:3	PC ae C42:3	Yes
Phosphatidylcholine acyl-alkyl C42:4	PC ae C42:4	Yes
Phosphatidylcholine acyl-alkyl C42:5	PC ae C42:5	Yes
Phosphatidylcholine acyl-alkyl C44:3	PC ae C44:3	Yes
Phosphatidylcholine acyl-alkyl C44:4	PC ae C44:4	Yes
Phosphatidylcholine acyl-alkyl C44:5	PC ae C44:5	Yes
Phosphatidylcholine acyl-alkyl C44:6	PC ae C44:6	Yes
Sphingolipids (n=15)		
Hydroxysphingomyelin C14:1	SM (OH) C14:1	Yes
Hydroxysphingomyelin C16:1	SM (OH) C16:1	Yes
Hydroxysphingomyelin C22:1	SM (OH) C22:1	Yes
Hydroxysphingomyelin C22:2	SM (OH) C22:2	Yes
Hydroxysphingomyelin C24:1	SM (OH) C24:1	Yes
Sphingomyelin C16:0	SM C16:0	Yes
Sphingomyelin C16:1	SM C16:1	Yes
Sphingomyelin C18:0	SM C18:0	Yes
Sphingomyelin C18:1	SM C18:1	Yes
Sphingomyelin C20:2	SM C20:2	Yes
Sphingomyelin C22:3	SM C22:3	< LOD
Sphingomyelin C24:0	SM C24:0	Yes
Sphingomyelin C24:1	SM C24:1	Yes
Sphingomyelin C26:0	SM C26:0	Yes
Sphingomyelin C26:1	SM C26:1	Yes
Monosaccharides (n=1)		
Sum of hexoses	H1	Yes

Abbreviations: LOD = limit of detection.
*Notation of fatty acid chains take the form “C x:y,” where “x” is the number of carbon atoms and “y” the number of double bonds in the fatty acid.

Supplementary Table 2. List of custom metabolic indicator variables

Custom metabolic indicator	Calculation
AAA	Sum of aromatic amino acids (Phe, Tyr, Trp)
ADMA/Arg	Ratio of Asymmetric dimethylarginine to Arginine
Arg/(Arg+Orn)	Ratio of Arginine to (Arginine+Ornithine)
BCAA	Sum of branched chain amino acids (Val, Leu, Ile)
Cit/Arg	Ratio of Citrulline to Arginine
Cit/Orn	Ratio of Citrulline to Ornithine
Essential AA	Total of essential aminoacids
Fisher ratio	Ratio of BCAA to AAA
Glu/Gln	Ratio of Glutamate to Glutamine
Glucogenic AA	Sum of selected amino acids (Ala, Gly, Ser)
Glutaminolysis	Ratio of (Asparagine+Alanine+Glutamate) to Glutamine
Gly/Arg	Ratio of Glycine to Arginine
Gly/Gln	Ratio of Glycine to Glutamine
Gly/His	Ratio of Glycine to Histidine
Gly/Ser	Ratio of Glycine to Serine
Glycolysis	Sum of selected amino acids (Ala, Gly, Ser)
Kynurenine/Trp	Ratio of Kynurenine to Tryptophan
Non essential AA	Total of non essential aminoacids
Orn/Arg	Ratio of Ornithine to Arginine
Orn/Ser	Ratio of Ornithine to Serine
Putrescine/Orn	Ratio of Putrescine to Ornithine
SDMA/Arg	Ratio of Symmetric dimethylarginine to Arginine
Serotonin/Trp	Ratio of Serotonin to Tryptophan
Thr/Ser	Ratio of Threonine to Serine
Total AA	Total of aminoacids
Total DMA/Arg	Ratio of (SDMA+ADMA) to Arginine
Tyr/Phe	Ratio of Tyrosine to Phenylalanine
(C2+C3)/C0	Ratio of (Acetylcarnitine+Propionylcarnitine) to Carnitine
C18/C18:1	Ratio of Octadecanoylcarnitine to Octadecenoylcarnitine
C2/C0	Ratio of Acetylcarnitine to Carnitine
C3/C4	Ratio of Propionylcarnitine to Butyrylcarnitine
C4/C0	Ratio of Butyrylcarnitine to Carnitine
C4/C5	Ratio of Butyrylcarnitine to Valerylcarnitine
CPT-I ratio	Ratio of (Hexadecanoylcarnitine+Octadecanoylcarnitine) to Carnitine
lysoPC a C16:0/lysoPC a C16:1	Ratio of lysophosphatidylcholine acyl C16:0 to lysophosphatidylcholine acyl C16:1
lysoPC a C20:4/lysoPC a C20:3	Ratio of lysophosphatidylcholine acyl C20:4 to lysophosphatidylcholine acyl C20:3
MUFA (PC)	Sum of mono-unsaturated glycerophosphocholins
MUFA (PC)/SFA (PC)	Ratio of MUFA (PC) to SFA (PC)
PUFA (PC)	Sum of poly-unsaturated glycerophosphocholins
PUFA (PC)/MUFA (PC)	Ratio of PUFA (PC) to MUFA (PC)
PUFA (PC)/SFA (PC)	PUFA (PC)/SFA (PC)

Custom metabolic indicator	Calculation
SFA (PC)	Sum of saturated glycerophosphocholins
Total (PC+SM)	Sum of choline-containing phospholipids
Total AC/C0	Ratio of esterified to free carnitine
Total AC-DC/Total AC	Fraction of dicarboxyacylcarnitines of the total acylcarnitines
Total AC-OH/Total AC	Fraction of hydroxylated acylcarnitines of the total acylcarnitines
Total lysoPC	Sum of lysoglycerosphosphocholines
Total lysoPC/Total PC	Ratio of lysoglycerosphosphocholines to glycerosphosphocholines
Total PC	Sum of glycerosphosphocholines
Total PC aa	Sum of diacyl-glycerosphosphocholines
Total PC ae	Sum of glycerosphosphocholines plasmalogens
Total SM	Sum of ceramide phosphocholines (sphingomyelins)
Total SM/Total (SM+PC)	Fraction of ceramide phosphocholines (sphingomyelins) of total phospholipid pool
Total SM/Total PC	Ratio of ceramide phosphocholines (sphingomyelins) to total glycerophosphocholines
Total SM-non OH	Sum of non-hydroxylated ceramide phosphocholines (sphingomyelins)
Total SM-OH	Sum of hydroxylated ceramide phosphocholines (sphingomyelins)
Total SM-OH/Total SM-non OH	Ratio of hydroxylated to non-hydroxylated ceramide phosphocholines (sphingomyelins)

-

Chapter 3.3
Geographical distribution of rare variants which are associated with age-related macular degeneration

Maartje J. Geerlings*
Eveline Kersten*
Joannes M.M. Groenewoud
Lars G. Fritsche
Carel B. Hoyng
Eiko K. de Jong
Anneke I. den Hollander

*These authors contributed equally to this study

Published in: Molecular Vision. 2018 Jan 27;24:75-82.

ABSTRACT

Purpose: A recent genome-wide association study by the International Age-related Macular Degeneration Genomics Consortium (IAMDGC) identified seven rare variants that are individually associated with age-related macular degeneration (AMD), the most common cause of vision loss in elderly. In literature, several of these rare variants have been reported with different frequencies and odds ratios across populations of Europe and North America. Here, we aim to describe the representation of these seven AMD-associated rare variants in different geographical regions based on 24 AMD studies.

Methods: We explored the occurrence of seven rare variants independently associated with AMD, namely (*CFH* rs121913059 (p.Arg1210Cys), *CFI* rs141853578 (p.Gly119Arg), *C3* rs147859257 (p.Lys155Gln), and *C9* rs34882957 (p.Pro167Ser) and three non-coding variants in or near the *CFH* gene (rs148553336, rs35292876, rs191281603), in 24 AMD case-control studies. We studied the difference in distribution, interaction and effect size for each of the rare variants based on the minor allele frequency within the different geographical regions.

Results: We demonstrate that two rare AMD-associated variants in the *CFH* gene (rs121913059 [p.Arg1210Cys] and rs35292876) deviate in frequency among different geographical regions (p=0.004 and p=0.001, respectively). The risk estimates of each of the seven rare variants were comparable across the geographical regions.

Conclusion: Our results emphasize the importance of identifying population-specific rare variants, for example by performing sequencing studies in case-control studies of various populations, because their identification may have implications for diagnostic screening and personalized treatment.

INTRODUCTION

Genetic diversity is observed among populations of different ancestries. Allele frequencies can exhibit large diversity among populations due to forces like genetic drift and natural selection. While most common variants are shared worldwide, rare variants (minor allele frequency [MAF] <1%) have the tendency to cluster in specific populations. Particularly population-specific rare variants tend to have a strong functional effect.[1]

In age-related macular degeneration (AMD), large variability in rare variant frequency has been reported in case-control studies of various populations, for instance for variant rs121913059 (p.Arg1210Cys) in complement factor H (*CFH*). *CFH* rs121913059 was first reported in a case-control study from the United States.[2] While some studies could replicate the finding,[3-5] other Caucasian studies[6-9] and Asian studies[10,11] were unable to replicate its strong association (Table 1). Another example, variant rs141853578 (p.Gly119Arg) in complement factor I (*CFI*) first reported in a European cohort,[7] was screened both in a British[12] and American[13] cohort (OR = 22.2; 8.5 and 2.6, respectively). However, while the variant was associated with AMD, its risk effect size was much weaker when compared to the first report.

Table 1. Minor allele frequencies of the *CFH* rs121913059 (p.Arg1210Cys) variant among different geographical regions reported in literature

	Source	Carriers (n)	Total Cases (n)	Total Controls (n)	MAF Cases (%)‡	MAF Controls (%)‡	Odds-Ratio	P-value
World	Fritsche2016 [3]	108	16144	17832	0.319	0.014	20.3	8.9×10^{-24}
Eastern USA	Raychaudhuri2011 [2#]	34	2414	1120	0.684	0.045	NA	8.0×10^{-5}
	Zhan2013 [5]	24	2268	2268	0.507	0.022	23.1	2.9×10^{-6}
European	Helgason2013 [8]	0	1143	51435	0.000	0.000	NA	NA
	Saksens2016 [9*]	0	1589	1386	0.000	0.000	NA	NA
	Recalde2016 [4]	5	259	330	0.965	0.000	NA	NA
Asian	Shen2012 [11]	0	258	426	0.000	0.000	NA	NA
	Miyake2015 [10]	1	1364	1208	0.037	0.000	NA	NA

Additional publications from: the Boston study[#13,14] and EUGENDA study[*6,7]. ‡ major allele C, minor allele T. NA = Not available or not reported.

In a recent genome-wide association study of the International Age-related Macular Degeneration Genomics Consortium (IAMDGC)[3] seven rare variants were observed to independently confer risk for AMD. All seven rare variants are localized in or near genes encoding components of the complement system, namely *CFH, CFI*, and complement components 3 and 9 (*C3* and *C9*).

The difference in association for rare variants among different AMD case-control studies may reflect the difference in distribution of such rare alleles across geographical regions. This observation raises the question if these variants identified by the IAMDGC are represented in all case-control studies or whether the association is driven by one or more studies from a specific geographical region. Therefore, we sought to evaluate the representation of these seven AMD-associated rare variants in 24 AMD case-control studies of different geographical regions.

MATERIALS AND METHODS

Data for this study were provided by the IAMDGC. The genotypes are in part available via dbGaP under accession number phs001039.v1.p1. The original dataset contained data from 40,633 individuals of European ancestry as described by Fritsche et al.[3] For analyses of the current study, participants from the Utah case-control study were excluded due to their mixed regions of origin. Also, the Jerusalem case-control study was excluded due to its small sample size compared to the other geographical regions. Final analyses were performed on 39,582 participants deriving from 24 of 26 studies.[3] The included studies were grouped in five geographical regions: eastern USA, western Europe, Britain, western USA and Australia (Supplementary Table 1). Data were collected by all study groups in accordance with the tenets of the Declaration of Helsinki; participants provided informed consent and study protocols were approved by local ethical committees.[3]

The MAF in each region was calculated and compared independently of AMD status. For comparison of effect sizes and interaction analyses, individuals were assigned “AMD” when exhibiting signs of (1) advanced AMD defined as geographic atrophy and/or choroidal neovascularization in at least one eye, or (2) non-advanced AMD defined as pigmentary changes in the macula and/or more than five macular drusen with a diameter ≥63 μm. Individuals without any reported signs of AMD were assigned “No AMD”.

Genotype data of seven rare genetic variants were selected from array based data generated by the IAMDGC.[3] Fritsche *et al*.[3] showed these seven rare variants to be independently associated with AMD: *CFH* rs121913059 (p.Arg1210Cys), *CFI* rs141853578 (p.Gly119Arg), *C3* rs147859257 (p.Lys155Gln), and *C9* rs34882957 (p.Pro167Ser) and three non-coding variants in or near *CFH* (rs148553336, rs35292876, rs191281603).

The software package SAS, (Statistical Analysis System Institute, V9.2) was used to compare MAFs between the different geographical regions in a logistic regression analysis with Firth correction.[15] Furthermore, we estimated the mean allele frequency of each rare genetic variant in each of the geographical regions including a 95% confidence interval. To study a potential difference in effect size of each variant between the geographical regions, interaction

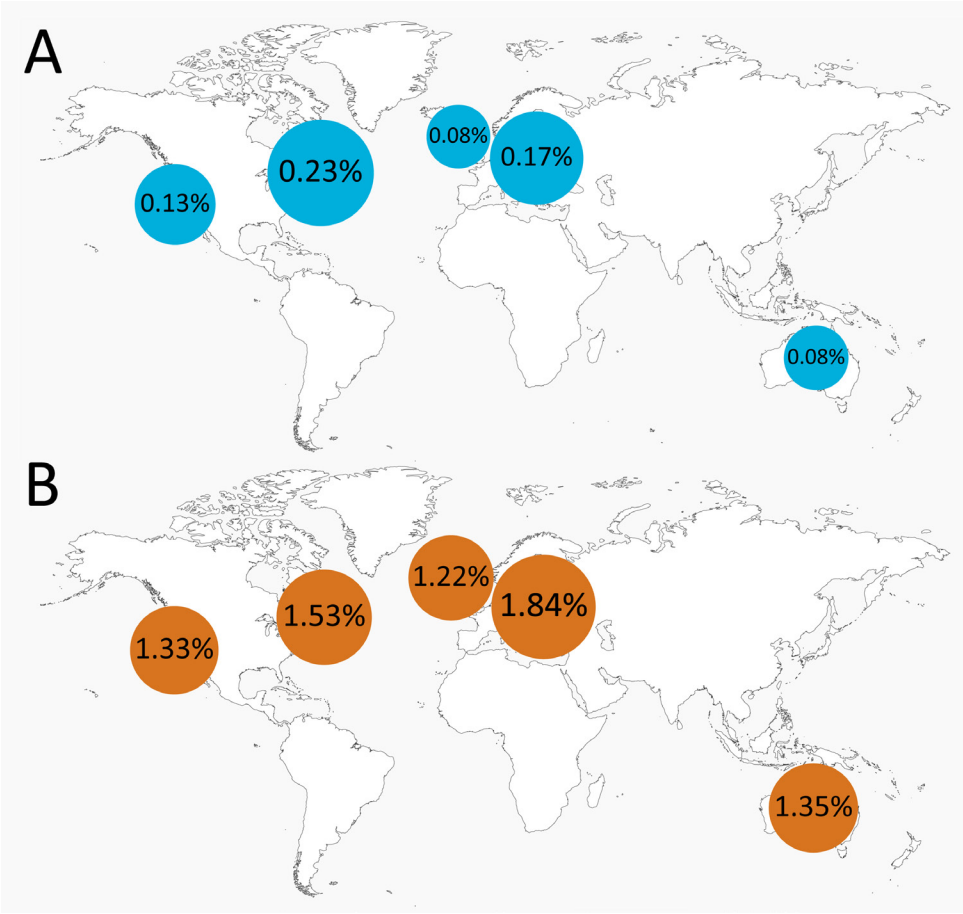


Figure 1. The two rare variants in *CFH* that are differently distributed variants among different geographical regions
Minor allele frequencies (in percentage) for *CFH* rs121913059 (A) and *CFH* rs35292876 (B). Variants mapped to geographical location (from left to right): western USA, eastern USA, Britain, western Europe, and Australia.

analyses were performed using binary logistic regression models with SPSS statistics software (IBM SPSS Statistics, V22.0).

RESULTS

Demographic characteristics for each of the five geographical regions are shown in Supplementary Table 1. The characteristics of participants from the different regions were comparable, although the British study samples were slightly younger than the others, and

the western European study samples included relatively more female participants compared to the remainder . These differences were comparable in both cases and controls.

We analyzed the difference in distribution of the seven rare variants among case-control studies from eastern USA, western Europe, Britain, western USA and Australia using logistic regression analysis with Firth correction (Table 2; Figure 1), and observed a difference in distribution of variants *CFH* rs121913059 (p.Arg1210Cys, p=0.004) and *CFH* rs35292876 (p=0.001) across the different geographical regions. *CFH* rs121913059 was found at a higher frequency in eastern USA, especially compared to Britain and Australia (p=0.011 and p=0.003, respectively). *CFH* rs35292876 was found at a higher frequency in western Europe, compared to all other regions (ranging from p<0.001 in Britain to p=0.012 in Eastern USA). The other five variants were found to have similar allele frequencies among all geographical regions.
The difference in distribution is also reflected by the estimated MAFs of each variant in the different geographical regions (Supplementary Table 2). The allele frequency of *CFH* rs121913059 is nearly three times higher in eastern USA than in Britain and Australia. Noteworthy is the near absence of this risk variant in control individuals without AMD, indicating that the difference in distribution appears to be driven solely by AMD individuals (Supplementary Table 3).
To determine if the effect size was influenced by geographical region we performed interaction analyses for each variant. We observed that the risk associated with each specific rare variant is independent of geographical region (Table 2). Overall effect sizes of the rare variants are comparable to the effect sizes reported in the IAMDGC study.[3]

Table 2. Distribution and interaction analysis of seven rare AMD-associated genetic variants across five geographical regions

	Difference in distribution between geographical regions#	**Interaction Analysis***	**Overall effect size‡**
	p-value	p-value	OR (95%CI)
CFH rs121913059 (p.Arg1210Cys)	**0.004**	0.665	24.2 (8.9-65.6)
CFI rs141853578 (p.Gly119Arg)	0.707	0.563	3.7 (2.5-5.7)
C3 rs147859257 (p.Lys155Gln)	0.665	0.680	2.8 (2.3-3.4)
C9 rs34882957 (p.Pro167Ser)	0.315	0.572	1.7 (1.5-2.0)
CFH rs148553336	0.053	0.015	0.5 (0.4-0.6)
CFH rs35292876	**0.001**	0.709	2.3 (2.0-2.6)
CFH rs191281603	0.735	0.980	0.9 (0.7-1.1)

#Logistic Regression with Firth correction. Individual Wald Chi-Square from likelihood ratio test for each of the variants across the geographical regions.
*Interaction Analysis: Effect sizes in entire study and interaction analysis to study potential differences in effect size between cohorts.
‡Overall effect size adjusted for geographical region.
Bold values: p-value considered significant after Bonferroni correction (p<0.007).

DISCUSSION

The distribution of rare *CFH* variants rs121913059 (p.Arg1210Cys) and rs35292876 was significantly different between several of the studied geographical regions. This confirms differences reported in previous studies for the *CFH* rs121913059 variant (Table 1).[2-11,13,14] *CFH* rs121913059 was first associated with AMD in study from the USA,[2] however the association was not consistently replicated in Dutch/German,[7] Icelandic,[8] Japanese[10] and Chinese[11] studies. In this study we confirmed the hypothesis that rare variants can be differently distributed among geographical regions but, as expected, the risk estimates are comparable across the geographical regions.

In AMD, a difference in geographical distribution has already been described for common risk haplotypes of *CFH* and *ARMS2* genes, which are the most prominent common genetic AMD risk factors.[3] While Asian populations report a lower frequency of *CFH* risk haplotypes, the opposite holds true for the *ARMS2/HTRA1* risk haplotype which is more prevalent in Asians compared to Caucasian populations.[16,17] These patient and population specific variations have implications for genetic counseling and carrier screening in both diagnostic and research settings.

Besides single variant associations, a significant burden of rare variants in the *CFH* and *CFI* genes has been reported for AMD.[3,14] The disease burden in these genes is attributed to the cumulative effect of rare coding variants, some of which are identified in multiple studies, while others are restricted to a single population or even a single patient.[18] Carriers of specific rare genetic variants in the complement genes that increase complement activation may benefit more from complement inhibiting therapy than those who do not carry such variants.[18] Personalized treatment aiming at complement activating rare variants in clinical trials may only be applicable to specific populations where these variants are sufficiently common.

It is likely that additional rare variants, other than *CFH* Arg1210Cys and rs35292876, fluctuate in frequency among geographical regions. To identify these variants, additional large sequencing studies will need to be performed in populations originating from diverse geographic regions. Up to now, large sequencing initiatives are predominantly of North American or European origin, and sample sizes for non-European-descent population are limited.[3,19] Recruiting case-control studies from other geographical regions and ancestries could allow for identification of novel highly penetrant rare variants implicated in AMD pathogenesis. These variants may be located in known AMD pathways, such as the complement system, or novel pathways.[20]

In conclusion, we demonstrated that rare AMD-associated variants *CFH* rs121913059 and rs35292876 are differently distributed among different geographical regions. These results emphasize the importance of identifying population-specific rare variants in AMD.

REFERENCES

1. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature.* 2015;526(7571):68-74.
2. Raychaudhuri S, Iartchouk O, Chin K, et al. A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat Genet.* 2011;43(12):1232-1236.
3. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
4. Recalde S, Tortajada A, Subias M, et al. Molecular Basis of Factor H R1210C Association with Ocular and Renal Diseases. *J Am Soc Nephrol.* 2016;27(5):1305-1311.
5. Zhan X, Larson DE, Wang C, et al. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. *Nat Genet.* 2013;45(11):1375-1379.
6. Duvvari MR, Saksens NT, van de Ven JP, et al. Analysis of rare variants in the CFH gene in patients with the cuticular drusen subtype of age-related macular degeneration. *Mol Vis.* 2015;21:285-292.
7. van de Ven JP, Nilsson SC, Tan PL, et al. A functional variant in the CFI gene confers a high risk of age-related macular degeneration. *Nat Genet.* 2013;45(7):813-817.
8. Helgason H, Sulem P, Duvvari MR, et al. A rare nonsynonymous sequence variant in C3 is associated with high risk of age-related macular degeneration. *Nat Genet.* 2013;45(11):1371-1374.
9. Saksens NT, Geerlings MJ, Bakker B, et al. Rare Genetic Variants Associated With Development of Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2016;134(3):287-293.
10. Miyake M, Saito M, Yamashiro K, Sekiryu T, Yoshimura N. Complement factor H R1210C among Japanese patients with age-related macular degeneration. *Jpn J Ophthalmol.* 2015;59(5):273-278.
11. Shen SK, Liu XQ, Lu F, Yang ZL, Shi Y. [Association study between age-related macular degeneration and R1210C mutation of CFH gene in Chinese population]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.* 2012;29(5):570-572.
12. Alexander P, Gibson J, Cree AJ, Ennis S, Lotery AJ. Complement factor I and age-related macular degeneration. *Mol Vis.* 2014;20:1253-1257.
13. Seddon JM, Yu Y, Miller EC, et al. Rare variants in CFI, C3 and C9 are associated with high risk of advanced age-related macular degeneration. *Nat Genet.* 2013;45(11):1366-1370.
14. Triebwasser MP, Roberson ED, Yu Y, et al. Rare Variants in the Functional Domains of Complement Factor H Are Associated With Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(11):6873-6878.
15. King G, Zeng L. Logistic Regression in Rare Events Data. *Political Analysis.* 2001;9:137–163.
16. Kopplin LJ, Igo RP, Jr., Wang Y, et al. Genome-wide association identifies SKIV2L and MYRIP as protective factors for age-related macular degeneration. *Genes Immun.* 2010;11(8):609-621.
17. Hu J, Yuan Y, Shen L, Zhang J, Hu N, Guan H. Age-Related Macular Degeneration-Susceptibility Single Nucleotide Polymorphisms in a Han Chinese Control Population. *Ophthalmic Epidemiology.* 2011;18(3):137-142.
18. Geerlings MJ, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2017;135(1):39-46.
19. Geerlings MJ, de Jong EK, den Hollander AI. The complement system in age-related macular degeneration: A review of rare genetic variants and implications for personalized treatment. *Mol Immunol.* 2016.
20. Huang LZ, Li YJ, Xie XF, et al. Whole-exome sequencing implicates UBE3D in age-related macular degeneration in East Asian populations. *Nat Commun.* 2015;6:6687.

SUPPLEMENTARY INFORMATION

Supplementary Table 1. Demographic characteristics of AMD cohorts grouped in five geographical regions

		Eastern USA[1]	Western Europe[2]	Britain[3]	Western USA[4]	Australia[5]
Participants (n)		18454	6590	4329	4226	5983
Mean Age (years ± SD)		74.0 ± 9.2	74.1 ± 8.2	69.6 ± 10.4	74.8 ± 10.0	74.3 ± 9.7
Gender	Male (%)	7934 (43.0%)	2512 (38.1%)	1743 (40.3%)	1875 (44.4%)	2531 (42.3%)
	Female (%)	10520 (57.0%)	4078 (61.9%)	2586 (59.7%)	2351 (55.6%)	3452 (57.7%)
AMD status	AMD (%)	11564 (62.7%)	3865 (58.6%)	2125 (49.1%)	2061 (48.8%)	2449 (40.9%)
	No AMD (%)	6890 (37.3%)	2725 (41.4%)	2204 (50.9%)	2165 (51.2%)	3534 (59.1%)

[1)]Eastern USA: AREDS, BDES, CWRU, Marshfield, Vanderbilt, Miami, Michigan, Pittsburgh, Pennsylvania, Baltimore. [2)]Western Europe: Regensburg, Rotterdam, Creteil, Paris, Bonn, Cologne, UMCN. [3)]Britain: Cambridge, Southampton, NHS_HPF, Edinburgh. [4)]Western USA: University California, UCSD, Oregon. [5)]Australia: Westmead, UWA/LEI/ Flinders and Melbourne.

Supplementary Table 2. Overall estimated mean MAF of seven rare AMD-associated genetic variants across five geographical regions

	Eastern USA	Western Europe	Britain	Western USA	Australia
CFH rs121913059	0.229 (0.185-0.283)	0.178 (0.119-0.267)	0.087 (0.042-0.177)	0.136 (0.076-0.242)	0.088 (0.048-0.161)
CFI rs141853578	0.197 (0.156-0.247)	0.163 (0.107-0.249)	0.249 (0.163-0.379)	0.184 (0.112-0.302)	0.213 (0.145-0.314)
C3 rs147859257	0.803 (0.717-0.900)	0.907 (0.758-1.084)	0.918 (0.738-1.143)	0.917 (0.734-1.144)	0.840 (0.691-1.020)
C9 rs34882957	1.196 (1.090-1.312)	1.286 (1.107-1.493)	1.276 (1.060-1.536)	1.366 (1.140-1.637)	1.065 (0.896-1.266)
CFH rs148553336	0.630 (0.554-0.716)	0.573 (0.457-0.717)	0.815 (0.645-1.027)	0.503 (0.373-0.679)	0.740 (0.601-0.910)
CFH rs35292876	1.535 (1.414-1.665)	1.840 (1.624-2.084)	1.230 (1.018-1.485)	1.331 (1.107-1.599)	1.350 (1.158-1.573)
CFH rs191281603	0.321 (0.268-0.384)	0.391 (0.297-0.513)	0.295 (0.200-0.434)	0.337 (0.234-0.486)	0.313 (0.228-0.431)

Calculated by SAS for each variant in percentage separated by geographical region including 95% confidence interval

Supplementary Table 3. Minor allele frequencies (%) of seven rare AMD-associated genetic variants across five geographical regions stratified by AMD status

		Eastern USA	Western Europe	Britain	Western USA	Australia
CFH rs121913059	AMD	0.359	0.272	0.165	0.243	0.204
	No AMD	0.007	0.037	0.000	0.023	0.000
CFI rs141853578	AMD	0.259	0.259	0.353	0.291	0.388
	No AMD	0.087	0.018	0.136	0.069	0.085
C3 rs147859257	AMD	1.020	1.216	1.365	1.431	1.450
	No AMD	0.435	0.459	0.476	0.416	0.410
C9 rs34882957	AMD	1.397	1.630	1.647	1.698	1.307
	No AMD	0.856	0.789	0.907	1.039	0.891
C3 rs147859257	AMD	1.020	1.216	1.365	1.431	1.450
	No AMD	0.435	0.459	0.476	0.416	0.410
CFH rs148553336	AMD	0.519	0.349	0.376	0.291	0.286
	No AMD	0.813	0.881	1.225	0.693	1.047
CFH rs35292876	AMD	1.889	2.393	1.765	1.868	2.103
	No AMD	0.936	1.046	0.703	0.808	0.821
CFH rs191281603	AMD	0.307	0.336	0.259	0.315	0.286
	No AMD	0.341	0.459	0.318	0.346	0.325

Chapter 3.4
Genetic screening for macular dystrophies in patients clinically diagnosed with dry age-related macular degeneration

Eveline Kersten
Maartje J. Geerlings
Marc Pauper
Jordi Corominas
Bjorn Bakker
Lebriz Altay
Sascha Fauser
Eiko K. de Jong
Carel B. Hoyng
Anneke I. den Hollander

Published in: Clinical Genetics. 2018 Dec;94(6):569-574.

Abstract

It can be clinically challenging to distinguish dry age-related macular degeneration (AMD) from AMD-mimicking dystrophies, and sometimes misdiagnosis occurs. With upcoming therapies for dry AMD it is important to exclude patients with a different retinal disease from clinical trials.

In this study we evaluated the occurrence of AMD-mimicking dystrophies in an AMD cohort. Whole-exome sequencing (WES) was performed in 218 patients with intermediate AMD or geographic atrophy secondary to AMD and 133 control individuals. WES data was analyzed for rare variants in nineteen genes associated with autosomal dominant and recessive macular dystrophies mimicking AMD.

In three (1.4%) of 218 cases we identified a pathogenic heterozygous variant (*PRPH2* c.424C>T; p.R142W) causal for autosomal dominant central areolar choroidal dystrophy (CACD). Phenotypically, these patients all presented with geographic atrophy. In twelve (5.5%) of 218 cases we identified a heterozygous variant of unknown clinical significance, but predicted to be highly deleterious, in genes previously associated with autosomal dominant macular dystrophies.

The distinction between AMD and AMD-mimicking dystrophies, such as CACD, can be challenging based on fundus examination alone. Genetic screening for genes associated with macular dystrophies, especially *PRPH2*, can be beneficial to help identify AMD-mimicking dystrophies.

INTRODUCTION

Age-related macular degeneration (AMD) is a common progressive retinal disorder affecting the elderly.[1] The early stages of AMD are characterized by drusen accumulation in the macula, and as disease progresses two types of advanced AMD can be distinguished: geographic atrophy (GA) and choroidal neovascularization (CNV).[2] Currently, no curative treatment exists for the early and atrophic stages of AMD, which affect the majority of patients (80-90%). However, therapies targeting AMD disease pathways are currently being evaluated in clinical trials.[3,4]

In order for clinical trials to be successful, it is crucial to select patients that will most likely benefit from the treatment. However, sometimes it is clinically challenging to distinguish AMD from inherited macular dystrophies.[5-7] Especially when a patient presents at older age and GA has already developed, it can be challenging to distinguish AMD from GA secondary to other macular diseases and potentially patients might be misdiagnosed. Before inclusion of patients in clinical trials for dry AMD, it may therefore be useful to perform genetic testing to exclude AMD-mimicking dystrophies. In this study, we evaluated the occurrence of rare genetic variants associated with autosomal dominant or autosomal recessive AMD-mimicking dystrophies in 218 cases diagnosed with dry AMD.

METHODS

Study population

For this study we selected patients with intermediate AMD (n=126) or advanced atrophic AMD (n=92) from the European Genetic Database (EUGENDA). For 33 cases one of more family members were included. In total 62 family members were included, of which 40 were diagnosed with AMD, and 22 did not have signs of AMD. Additionally, 133 control individuals aged 65 years and older without signs of AMD were included in this study. Color fundus photographs of both eyes, and if available spectral domain optical coherence tomograms and fluorescein angiograms, were evaluated by two independent reading center graders according to the Cologne Image Reading Center and Laboratory (CIRCL) classification protocol.[8] All individuals provided written informed consent for enrollment in EUGENDA. This research was approved by the local ethical committees at the Radboud university medical center and the University Hospital of Cologne and the study adhered to the tenets of the Declaration of Helsinki.

Whole-exome sequencing

Whole-exome sequencing (WES) was performed as previously described.[9] WES data were analyzed for rare variants in nineteen genes associated previously with autosomal dominant and recessive macular dystrophies mimicking AMD as described by Saksens et al.[5] and RetNet, the Retinal Information Network (Supplementary Table 1). Filtering of the data was performed to select protein-altering, nonsense, frameshift or splice-site variants with a minor allele frequency (MAF) ≤1% in European and Dutch population reference panels.[10,11] Additional filter criteria included coverage depth of >=20 reads, >=10 variant reads and >=20% variation of reads. A variation of reads between 20% and 80% was defined as heterozygous, and all variants with a variation of reads ≥90% were named homozygous. Individual variants that were seen on less than 25 variant reads were confirmed by Sanger sequencing. Literature and public archives (ClinVar[12] and LOVD[13]) were consulted to determine if a variant is described to be pathogenic or is of unknown clinical significance (including variants with conflicting interpretations of pathogenicity). We explored the deleteriousness of nonsynonymous missense variants of unknown clinical significance using scaled Combined Annotation Dependent Depletion (CADD phred) prediction scores.[14]

RESULTS AND DISCUSSION

Variants in genes associated with autosomal dominant macular dystrophies

We identified a heterozygous variant in the *PRPH2* gene (c.424C>T, p.Arg142Trp) in three (1.4%) of 218 patients. All three cases presented with geographic atrophy which could be secondary to AMD(Table 1, Figure 1), although the area of atrophy in two patients is somewhat larger than would be expected in a typical AMD patient. This variant causes a central cone dystrophy phenotype associated with autosomal dominant central areolar choroidal dystrophy (CACD), and represents a founder mutation in the southeast of the Netherlands.[15,16] CACD and atrophic AMD have strong phenotypic similarities and their age of onset overlaps.[6,15-17] CACD can be misdiagnosed with AMD based on ophthalmological examination alone, especially in families with incomplete penetrance, which may mask the autosomal dominant inheritance of CACD. Additional imaging, such as spectral-domain optical coherence tomography and fundus autofluorescence imaging, or genetic analyses could help distinguish these two diseases.[6]

In additional, 28 rare variants of unknown clinical significance were identified in other genes associated with autosomal dominant macular dystrophies, while they were not identified in 133 control individuals (Supplementary Table 2). Because of their uncertain significance further evaluation included only those variants leading to a premature nonsense codon or a frameshift, affecting the invariable splice donor or acceptor sites, and nonsynonymous

missense variants predicted to be the 1% most deleterious variants in the human genome (CADD score ≥ 20). We identified sixteen variants of unknown clinical significance predicted to be highly deleterious. In four cases, the variants of unknown clinical significance (*CTNNA1* c.536C>T; p.A179V, *FSCN2* c.1025G>A; p.R342Q, *OTX2* c.425C>G; p.P142R, *PROM1* c.2450A>G; p.K817R) did not segregate with the disease in available family members, and were therefore not considered to be pathogenic. The twelve remaining cases carried a variant of unknown clinical significance in the *BEST1*, *ELOVL4*, *FSCN2*, *IMPG1*, *OTX2*, *PRDM13*, *PROM1* or *RP1L1* gene (Table 1). All twelve cases had typical characteristics of intermediate AMD or GA with drusen (Table 1, Supplementary Figure 1; available online). We cannot rule out the possibility that these variants might be disease-causing. Therefore, one might consider to exclude patients carrying variants in genes associated with autosomal dominant macular dystrophies from clinical trials, in particular if the disease phenotype matches previously reported disease characteristics of these dystrophies.

Variants in genes associated with autosomal recessive retinal dystrophies

None of our cases carried homozygous or two heterozygous deleterious variants in genes associated with autosomal recessive macular dystrophies. However, thirteen (6.0%) out of 218 cases carried a single heterozygous variant, previously described to be pathogenic in literature or in public archives, in the *ABCA4*, *ABCC6*, *MFSD8* or *PROM1* gene (Table 2, Supplementary Figure 2; available online). Eight variants showed comparable MAFs with population reference panels, while seven variants were not detected in the 133 control individuals.

It has been suggested that carriers of a single *ABCA4* variant are at increased risk of developing AMD compared to noncarriers,[18,19] although a more recent study described that monoallelic *ABCA4* carriers do not result in retinal changes.[20] In this study, we identified seven (3.2%) of 218 cases that carried a heterozygous *ABCA4* variant previously reported to be pathogenic, compared to only one (0.8%) of 133 control individuals carrying a heterozygous pathogenic *ABCA4* variant. Larger studies are needed to evaluate the hypothesis that carriers of heterozygous variants associated with autosomal recessive macular dystrophies might be at increased risk for AMD development. The frequency of *ABCA4* variants in our control individuals is, however, lower than expected based on population frequencies, and could also be coincidentally low.

Clinical implications

It is increasingly important to correctly diagnose patients with macular degeneration with respect to inclusion in clinical trials and for future treatment. No curative treatment is currently available for dry AMD, though multiple clinical trials are ongoing.[4] For clinical trials and future therapies for AMD it is important to identify those patients that will benefit most

Table 1. Previously described pathogenic variants and variants of unknown clinical significance* in autosomal dominant macular dystrophy genes identified in cases diagnosed with dry AMD

	ExAC MAF (%)	Cases n (MAF[%])	CADD score	Disease association	Gender	Age	Phenotypic characteristics on retinal imaging
Known pathogenic variants associated with autosomal dominant macular dystrophy (Figure 1)							
PRPH2							
c.424C>T; p.R142W	0.001	3 (0.69%)	28.6	Central areolar choroidal dystrophy	F M M	67 64 76	GA with foveal sparing surrounded by drusen Central GA Extensive central GA and peripapillary atrophy
Variants of unknown clinical significance in autosomal dominant macular dystrophy (Supplementary Figure 1; available online)							
BEST 1				Adult-onset foveomacular vitelliform dystrophy Best vitelliform macular dystrophy			
c.1193C>T; p.S398F	0.08	1 (0.23%)	27.8		F	66	Central GA surrounded by small hard drusen extending to the periphery
ELOVL4				Stargardt-like macular dystrophy Autosomal dominant macular dystrophy			
c.145A>G; p.T49A	-	1 (0.23%)	24.3		F	59	Large soft drusen throughout the macula
FSCN2				Autosomal dominant macular degeneration Autosomal dominant retinitis pigmentosa			
c.1057G>A; p.V353M	0.04	1 (0.23%)	27.9		F	95	Reticular pseudodrusen and some soft drusen
IMPG1				Autosomal dominant benign concentric annular macular dystrophy Autosomal dominant vitelliform macular dystrophies			
c.1982G>A; p.R661H	-	1 (0.23%)	23.5		M	83	Multifocal GA and some intermediate drusen

c.1945C>T; p.L649F	0.40	1 (0.23%)	27.4		F	75	Few intermediate to large soft macular drusen
c.1738C>T; p.R580C	0.02	1 (0.23%)	34		F	70	Intermediate to large soft macular drusen
c.336TC>C; p.I112IX	-	1 (0.23%)	-		F	87	Intermediate to large soft macular drusen
OTX2				Autosomal dominant pattern dystrophy			
c.844T>A; p.C282S	0.003	1 (0.23%)	24.3		F	84	Extensive large soft drusen and calcified drusen throughout the macula and reticular pseudodrusen around the retinal arcades
PRDM13				North-Carolina macular dystrophy			
c.113C>T; p.S38L	0.07	1 (0.23%)	28.8		M	74	Numerous small hard (cuticular) drusen throughout the macula extending beyond the vascular arches
PROM1				Autosomal dominant bull's-eye macular dystrophy Autosomal dominant stargardt-like dystrophy			
c.1345G>A; p.V449M	0.20	1 (0.23%)	20.4		F	79	Central GA surrounded by intermediate to large drusen and some peripheral drusen
c.155T>C; p.I52T	0.003	1 (0.23%)	23.2		F	81	Drusen deposition throughout the macula
RP1L1				Autosomal dominant occult macular dystrophy			
c.553G>T; p.A185S	-	1 (0.23%)	26.2		F	70	Multifocal GA with foveal sparing surrounded by large soft drusen

Abbreviations: AMD, age-related macular degeneration; CADD score, Combined Annotation Dependent Depletion score; ExAC, Exome Aggregation Consortium; F, female; GA, geographic atrophy; MAF, minor allele frequency; M, male.
*This table includes variants of unknown clinical significance leading to a premature nonsense codon, frameshift, affecting the splice donor or acceptor sites (-1,-2, +1, +2), and nonsynonymous missense variants predicted to be the 1% most deleterious variants in the human genome (CADD score ≥ 20).

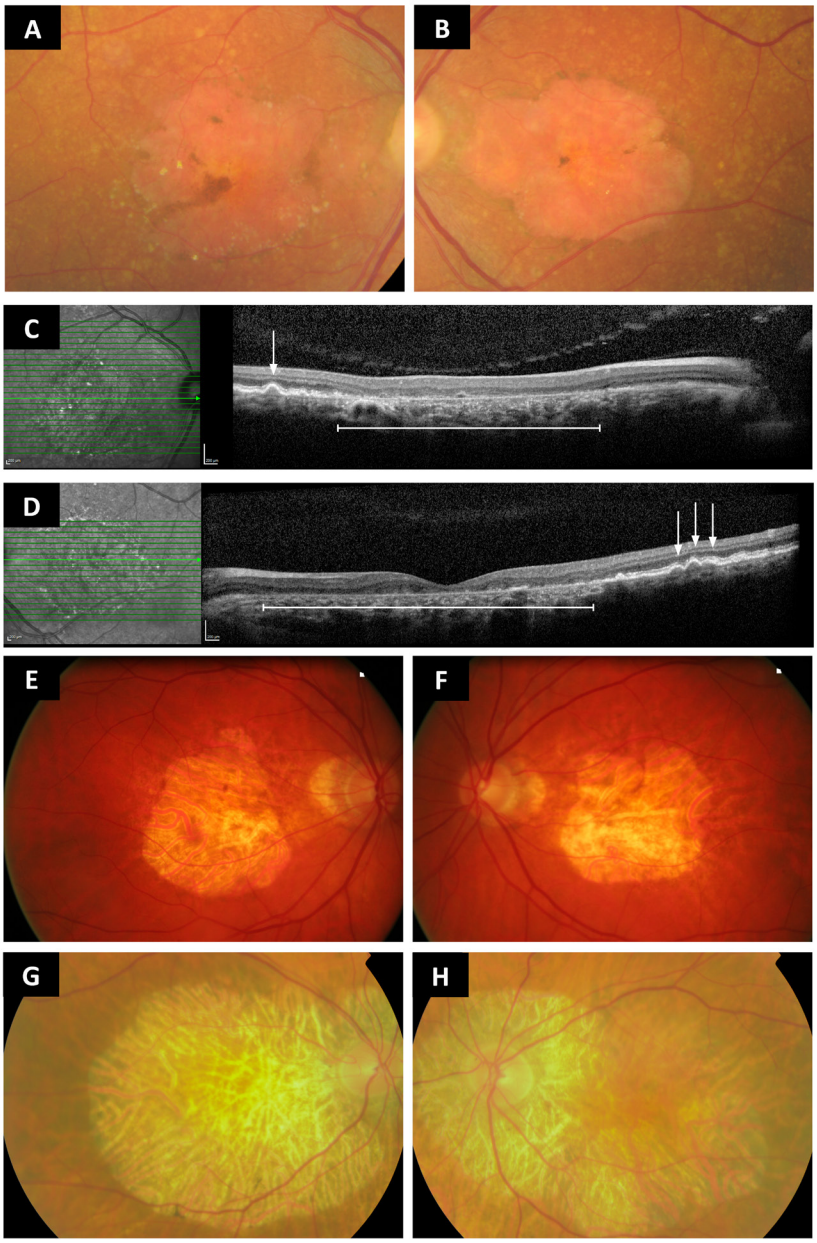


Figure 1. Retinal images of three patients with geographic atrophy secondary to autosomal dominant central areolar choroidal dystrophy (CACD) caused by a heterozygous variant in *PRPH2* (c.424C>T, p.Arg142Trp)
Patient 1 (A-D). Color fundus photographs of right (A) and left (B) eye of a 67-year-old female with geographic atrophy and foveal sparing surrounded by drusen secondary to CACD. On optical coherence tomography (OCT) images of both eyes (C+D) drusen are visible near the edges of the central atrophy. Drusen are indicated by arrows, atrophy is indicated by a continuous line with dashes just below the atrophic area. Patient 2 (E-F). A 64-year-old male with central atrophy in both eyes secondary to CACD. Patient 3 (G-H). A 76-year-old male with extensive geographic atrophy and peripapillary atrophy in both eyes.

Table 2. Variants in autosomal recessive macular dystrophy genes previously described as pathogenic

	ExAC MAF (%)	GoNL MAF (%)	Cases total n=218 n(MAF[%])	Controls total n=133 n(MAF[%])	Disease association	Gender	Age	Phenotypic characteristics on retinal imaging
ABCA4								
c.6089G>A; p.R2030Q	0.06%	0.10%	1 (0.23%)	-	Stargardt disease	F	35	Extensive drusen deposition, mainly located temporal to the fovea and pigmentary alterations
c.3113C>T; p.A1038V	0.20%	0.30%	1 (0.23%)	-	Stargardt disease	M	85	Central GA, no evident drusen
c.2947A>G; p.T983A	-	-	1 (0.23%)	-	Stargardt disease	F	72	Small to intermediate hard drusen and pigmentary alterations
c.2588G>C; p.G863A	0.81%	0.80%	4 (0.92%)	1 (0.38%)	Stargardt disease	M F M F	75 78 66 73	Multifocal GA with pigmentary alterations and drusen Extensive drusen deposition (some calcified), mainly located temporal to the fovea and central GA Pigmentary alteration with small to intermediate drusen Confluent soft drusen and minimal central GA
c.2546T>C; p.V849A	0.01%	-	1 (0.23%)	-	Stargardt disease	F	74	Intermediate to large soft drusen throughout the macula
ABCC6								
c.2787+1G>T; p.?	0.02%	-	2 (0.46%)	-	Pseudoxanthoma elasticum	F F	69 66	Central GA surrounded with reticular drusen Minimal pigmentary alterations and small hard drusen
MFSD8								
c.1006G>C; p.E336Q	0.33%	0.30%	2 (0.46%)	-	Nonsyndromic autosomal recessive macular dystrophy	M F	91 71	Multifocal GA with some drusen Central GA without evident drusen
PROM1								
c.1355A>TA; p.Y452YX	0.03%	-	1 (0.23%)	-	Autosomal recessive cone-rod dystrophy	F	91	Small to intermediate hard drusen extending beyond the vascular arcades into the periphery

Abbreviations: AMD, age-related macular degeneration; GA, geographic atrophy; GoNL, Genome of the Netherlands Consortium; ExAC, Exome Aggregation Consortium; MAF, minor allele frequency.

likely from the treatment and to exclude AMD-mimicking dystrophies. Detailed phenotyping is necessary for distinguishing different macular diseases, and multimodal imaging can be useful. Despite modern imaging technologies, however, it can be difficult to clinically differentiate AMD from AMD-mimicking dystrophies. Genetic screening of genes involved in AMD-mimicking dystrophies can aid in establishing an accurate diagnosis. Based on the findings of this study, genetic screening of the PRPH2 gene is recommended because of the significant clinical overlap between CACD and AMD.

REFERENCES

1. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology.* 2017.
2. Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. *N Engl J Med.* 2008;358(24):2606-2617.
3. Buschini E, Fea AM, Lavia CA, et al. Recent developments in the management of dry age-related macular degeneration. *Clin Ophthalmol.* 2015;9:563-574.
4. Li H, Chintalapudi SR, Jablonski MM. Current drug and molecular therapies for the treatment of atrophic age-related macular degeneration: phase I to phase III clinical development. *Expert Opin Investig Drugs.* 2017:1-12.
5. Saksens NT, Fleckenstein M, Schmitz-Valckenberg S, et al. Macular dystrophies mimicking age-related macular degeneration. *Prog Retin Eye Res.* 2014;39:23-57.
6. Smailhodzic D, Fleckenstein M, Theelen T, et al. Central areolar choroidal dystrophy (CACD) and age-related macular degeneration (AMD): differentiating characteristics in multimodal imaging. *Invest Ophthalmol Vis Sci.* 2011;52(12):8908-8918.
7. Westeneng-van Haaften SC, Boon CJ, Cremers FP, Hoefsloot LH, den Hollander AI, Hoyng CB. Clinical and genetic characteristics of late-onset Stargardt's disease. *Ophthalmology.* 2012;119(6):1199-1210.
8. Ristau T, Ersoy L, Lechanteur Y, et al. Allergy is a protective factor against age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(1):210-214.
9. Corominas J, Colijn JM, Geerlings MJ, et al. Whole-Exome Sequencing in Age-Related Macular Degeneration Identifies Rare Variants in COL8A1, a Component of Bruch's Membrane. *Ophthalmology.* 2018.
10. Genome of the Netherlands C. Whole-genome sequence variation, population structure and demographic history of the Dutch population. *Nat Genet.* 2014;46(8):818-825.
11. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* 2016;536(7616):285-291.
12. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* 2016;44(D1):D862-868.
13. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. *Hum Mutat.* 2011;32(5):557-563.
14. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet.* 2014;46(3):310-315.
15. Boon CJ, Klevering BJ, Cremers FP, et al. Central areolar choroidal dystrophy. *Ophthalmology.* 2009;116(4):771-782, 782.e771.
16. Hoyng CB, Deutman AF. The development of central areolar choroidal dystrophy. *Graefes Arch Clin Exp Ophthalmol.* 1996;234(2):87-93.
17. Klevering BJ, van Driel M, van Hogerwou AJ, et al. Central areolar choroidal dystrophy associated with dominantly inherited drusen. *Br J Ophthalmol.* 2002;86(1):91-96.
18. Shroyer NF, Lewis RA, Yatsenko AN, Wensel TG, Lupski JR. Cosegregation and functional analysis of mutant ABCR (ABCA4) alleles in families that manifest both Stargardt disease and age-related macular degeneration. *Hum Mol Genet.* 2001;10(23):2671-2678.
19. Zhang K, Kniazeva M, Hutchinson A, Han M, Dean M, Allikmets R. The ABCR gene in recessive and dominant Stargardt diseases: a genetic pathway in macular degeneration. *Genomics.* 1999;60(2):234-237.
20. Muller A, Keeffe JE, Taylor HR. Changes in eye care utilization following an eye health promotion campaign. *Clin Exp Ophthalmol.* 2007;35(4):305-309.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Genes associated with AMD-mimicking diseases

Gene	Chr.	**Associated disease**
Autosomal Dominant		
BEST1	11	Adult-onset foveomacular vitelliform dystrophy (AFVD) Best vitelliform macular dystrophy (BVMD)
C1QTNF5/MFRP	11	Late onset retinal degeneration (LORD)
CTNNA1	5	Butterfly-shaped pigment dystrophy
EFEMP1	2	Malattia Leventinese (ML)/Doyne honeycomb retinal dystrophy
ELOVL4	6	Stargardt-like macular dystrophy (STGD3) Autosomal dominant macular dystrophy
FSCN2	17	Autosomal dominant macular degeneration Autosomal dominant retinitis pigmentosa
GUCA1B	6	Autosomal dominant retinal degeneration
OTX2	14	Autosomal dominant pattern dystrophy
PRDM13	6	North-Carolina macular dystrophy (NCMD)
PRPH2	6	Central areolar choroidal dystrophy Adult-onset foveomacular vitelliform dystrophy Autosomal dominant pattern dystrophy Pseudo-Stargardt pattern dystrophy
RP1L1	8	Autosomal dominant occult macular dystrophy
TIMP3	22	Sorsby fundus dystrophy
Autosomal Recessive		
ABCA4	1	(late-onset) Stargardt disease
ABCC6	16	Pseudoxanthoma elasticum related dystrophy (angioid streaks)
DRAM2	1	Autosomal recessive macular dystrophy
MFSD8	4	Nonsyndromic autosomal recessive macular dystrophy
Autosomal Dominant or Autosomal Recessive		
IMPG1	6	Autosomal dominant benign concentric annular macular dystrophy Autosomal dominant and autosomal recessive vitelliform macular dystrophies
PROM1	4	Autosomal dominant bull's-eye macular dystrophy Autosomal dominant stargardt-like dystrophy Autosomal recessive cone-rod dystrophy

Based on Saksens et al. 2014 (Prog Retin Eye Res 39:23-57) and RetNet, the Retinal Information Network.

Supplementary Table 2. Variants of unknown clinical significance or conflicting interpretations of pathogenicity

Gene name	Inheritance	Chromosome	Start position	Reference	Variant	c.DNA change	Protein change	ExAC_NFE	AF_GoNL	CADD_phred	Reads	Variation reads	% Variation	Cases (n=218)	Controls (n=133)
ABCA4	AR	chr1	94467548	C	G	c.6148G>C	V2050L	0.0037	0.201	33	36	16	44.44	1	0
ABCA4	AR	chr1	94473287	G	A	c.5908C>T	L1970F	0.0042	0.502	26.4	42.33	20.67	48.83	1	2
ABCA4	AR	chr1	94476377	C	T	c.5693G>A	R1898H	0.0028	0.1	22.8	48.5	21	43.3	1	1
ABCA4	AR	chr1	94476874	C	T	c.5528G>A	R1843Q	1.50E-05	0	35	35	14	40	1	0
ABCA4	AR	chr1	94480221	G	C	c.5338C>G	P1780A	0.0002	0	26.2	54	35	64.81	1	0
ABCA4	AR	chr1	94487404	C	T	c.4771G>A	G1591R	0.0045	0.402	24.6	77.75	36.25	46.62	1	3
ABCA4	AR	chr1	94496039	C	T	c.4297G>A	V1433I	0.0019	0.502	20.9	31	13.5	43.55	1	0
ABCA4	AR	chr1	94506773	C	T	c.3514G>A	G1172S	1.50E-05	0	9.41	29	12	41.38	1	0
ABCA4	AR	chr1	94514466	T	C	c.2701A>G	T901A	0.0027	0.201	9.516	85	45	52.94	1	0
ABCA4	AR	chr1	94514477	G	A	c.2690C>T	T897I	0.0018	0.402	23.6	83	46	55.42	1	0
ABCA4	AR	chr1	94528142	A	C	c.1928T>G	V643G	0.0022	0.703	27.5	30.75	15.25	49.59	2	2
ABCA4	AR	chr1	94528774	C	T	c.1654G>A	V552I	0.0039	0.602	15.19	37.67	19	50.44	1	2
ABCA4	AR	chr1	94528818	C	T	c.1610G>A	R537H	0.0024	0.301	26.2	37	21	56.76	1	0
ABCA4	AR	chr1	94546094	C	A	c.1039G>T	A347S	.	0	25.4	152	67	44.08	1	0
ABCA4	AR	chr1	94568627	C	T	c.514G>A	G172S	0.0006	0	23.4	154	73	47.4	1	0
ABCA4	AR	chr1	94568675	T	C	c.466A>G	I156V	0.0017	0.1	5.706	162.5	70	43.08	2	2
ABCA4	AR	chr1	94568686	C	T	c.455G>A	R152Q	0.0031	0.402	21.5	158	78	49.37	1	0
ABCA4	AR	chr1	94574244	C	T	c.331G>A	E111K	.	0	21.4	74	38	51.35	1	0
ABCC6	AR	chr16	16271467	G	A	c.2432C>T	T811M	0	0	28.5	35	16	45.71	1	0
ABCC6	AR	chr16	16282693	C	T	c.1774G>A	V592I	1.73E-05	0	11.72	20	12	60	1	0
ABCC6	AR	chr16	16286695	G	C	c.1423C>G	H475D	1.52E-05	0	0.323	43	19	44.19	1	0
ABCC6	AR	chr16	16286750	G	C	c.1368C>G	I456M	0.0002	0	12.35	46.8	21	44.87	2	2
ABCC6	AR	chr16	16291993	T	A	c.1223A>T	D408V	.	0	23	20	10	50	1	0
BEST1	AD	chr11	61729819	C	T	c.1193C>T	S398F	0.0008	0.1	27.8	31	19	61.29	1	0
BEST1	AD	chr11	61730145	T	C	c.1339T>C	S447P	0.0018	0	2.919	47	19	40.43	1	0
CTNNA1	AD	chr5	138147939	C	T	c.536C>T	A179V	0.0018	0.301	23.2	270.25	126.5	46.81	3	0
CTNNA1	AD	chr5	138269759	A	G	c.2702A>G	K901R	.	0	15.99	43	16	37.21	1	0
EFEMP1	AD	chr2	56145171	T	G	c.146A>C	D49A	0.0013	0.301	15.49	28.8	13.4	46.53	4	1
ELOVL4	AD	chr6	80626470	A	G	c.800T>C	I267T	0.0068	0.904	8.833	102	49.72	48.75	5	2
ELOVL4	AD	chr6	80636054	T	C	c.145A>G	T49A	.	0	24.3	100	42	42	1	0
FSCN2	AD	chr17	79503213	G	A	c.1025G>A	R342Q	0.0002	0	23	49.5	28	56.57	1	0

FSCN2	AD	chr17	79503245	G	A	c.1057G>A	V353M	0.0004	0.602	27.9	36	15	41.67	1	0
IMPG1	AD/AR	chr6	76640695	G	A	c.2218C>T	L740F	0.0007	0.1	10.89	28	16	57.14	1	0
IMPG1	AD/AR	chr6	76640830	C	T	c.2083G>A	E695K	.	0	15.38	39	21	53.85	1	0
IMPG1	AD/AR	chr6	76657093	C	T	c.1982G>A	R661H	0	0	23.5	66	33	50	1	0
IMPG1	AD/AR	chr6	76657130	G	A	c.1945C>T	L649F	0.004	0.402	27.4	106	52	49.06	1	0
IMPG1	AD/AR	chr6	76660365	G	A	c.1738C>T	R580C	0.0002	0	34	21	12	57.14	1	0
IMPG1	AD/AR	chr6	76660397	T	C	c.1706A>G	K569R	0.0039	0.301	15.88	38.4	18.6	48.44	2	0
IMPG1	AD/AR	chr6	76731868	C	T	c.631G>A	D211N	0.0008	0	3.873	139	64	46.04	1	1
IMPG1	AD/AR	chr6	76744470	GA	G	c.336TC>C	I112IX	.	0	.	31	10	32.26	1	0
IMPG1	AD/AR	chr6	76751738	C	T	c.173G>A	R58K	0.0055	0.301	10.32	123.5	55.5	44.94	2	0
MFRP	AD	chr11	119214636	G	T	c.1014C>A	S338R	0.0015	0	7.075	25	11.5	46	1	1
MFRP	AD	chr11	119216274	G	C	c.497C>G	P166R	1.51E-05	0	23.6	20	10	50	1	1
MFSD8	AR	chr4	128842876	C	G	c.1153G>C	G385R	0.0005	0	6.644	138	58	42.03	1	0
MFSD8	AR	chr4	128842893	A	G	c.1136T>C	F379S	0.0003	0.201	15.18	137.5	66.5	48.36	1	1
MFSD8	AR	chr4	128843095	A	G	c.1022T>C	L341P	0	0	27.4	167	68	40.72	1	0
OTX2	AD	chr14	57268503	A	T	c.844T>A	C282S	3.00E-05	0	24.3	73	33	45.21	1	0
OTX2	AD	chr14	57268922	G	C	c.425C>G	P142R	0.0002	0	21.3	50	26	52	1	0
PRDM13	AD	chr6	100055023	C	T	c.113C>T	S38L	0.0007	0	28.8	49	22	44.9	1	0
PROM1	AD/AR	chr4	15982084	T	C	c.2450A>G	K817R	.	0	31	135	61	45.19	1	0
PROM1	AD/AR	chr4	15987578	A	G	c.2184+2T>C	?	.	0	18.29	29	18	62.07	1	0
PROM1	AD/AR	chr4	15992900	G	C	c.1928C>G	A643G	0.001	0.1	8.738	176	78	44.32	1	0
PROM1	AD/AR	chr4	16008270	C	T	c.1345G>A	V449M	0.002	0.201	20.4	30	11	36.67	1	0
PROM1	AD/AR	chr4	16040595	A	G	c.250T>C	Y84H	.	0	7.446	98	53	54.08	1	0
PROM1	AD/AR	chr4	16077375	A	G	c.155T>C	I52T	3.12E-05	0	23.2	58	24	41.38	1	0
PROM1	AD/AR	chr4	16077475	A	C	c.55T>G	S19A	0.0091	1.004	1.486	44.86	23	51.27	2	3
RP1L1	AD	chr8	10464616	G	A	c.6992C>T	T2331M	0.0023	0.402	1.738	42.67	19.67	46.1	3	0
RP1L1	AD	chr8	10465024	C	A	c.6584G>T	G2195V	1.50E-05	0	9.777	23	11	47.83	1	0
RP1L1	AD	chr8	10467629	T	TTTC	c.3979A>GAAA	T1327E	.	0	.	97.31	45.52	46.78	13	2
RP1L1	AD	chr8	10467637	T	*	**	E1324G	.	0	.	25.67	21	81.81	1	5
RP1L1	AD	chr8	10468963	C	T	c.2645G>A	R882Q	0	0	6.663	20	10	50	1	0
RP1L1	AD	chr8	10480144	G	A	c.568C>T	R190C	0.0027	0.904	17.97	43.4	19.4	44.7	4	1
RP1L1	AD	chr8	10480159	C	A	c.553G>T	A185S	.	0	26.2	38	21	55.26	1	0

* TCCTCTAACTGCACCCTCTCTTCTTGCAGCCCTTCTATTACTTTAGTCC

** c.3971A>GGACTAAAGTAATAGAAGGGCTGCAAGAAGAGAGGGTGCAGTTAGAGGA

Chapter 4

General discussion

The aim of this thesis was to increase our understanding of the clinical and molecular characteristics of AMD development and progression. In this chapter we elaborate on the main findings described in this thesis and their clinical relevance, and in particular for genetic and predictive testing and for treatment and personalized medicine for AMD.

GENETIC TESTING IN AMD

The contribution of genetic factors in the development of AMD has been well established and genetic studies have yielded important results that contribute to our understanding of AMD pathology. Since the discovery of the Y402H risk variant in the *CFH* gene in 2005[1-3] and rapid technological developments, our understanding of the genetics contributing to AMD development have increased significantly. To date more than half of the genomic heritability can be explained by both common and rare genetic variants in or near 34 genes.[4] The explained genomic heritability of AMD is considerably higher than for other multifactorial diseases, for which in general only a limited percentage of the genomic heritability has been explained.[5] AMD may therefore be among the first multifactorial diseases for which genetic testing can be offered in a clinical setting. In this section we discuss the value of genetic testing in the clinic, provide an overview of genetic tests that are offered for AMD, and provide recommendations for which individuals genetic testing for AMD would particularly be useful.

Value of genetic testing for risk reduction and treatment

Although one's genetic predisposition cannot be changed, an individual's AMD risk is based on multiple factors of which lifestyle factors, such as smoking and diet, are modifiable. Smoking cessation, regular physical activity, and intake of nutrients high in antioxidants and omega-3 fatty acids have been proven to reduce AMD risk.[6-10] Even in those at high genetic risk dietary adjustments seem beneficial.[11] Therefore, ophthalmologists should advise their patients to refrain from smoking, perform physical exercise regularly, increase the intake of dietary food groups such as green leafy vegetables and fatty fish, and dietary supplementation of the AREDS formulation.[12] Determining an individual's genetic risk might increase motivation for lifestyle adjustments in high-risk individuals. A recent study evaluating genetic testing for AMD showed that people made lifestyle adaptations after genetic testing.[13] However, it must be noted that most participants had one or more family members with AMD, which could also increase motivation to make lifestyle changes. In general, existing literature suggests that communicating genetic risk has little or no effect on risk-reducing health behavior, even in people with a high risk for disease.[14] The effect of genetic testing on risk reduction might be improved if combined with personalized motivational strategies, for example through a lifestyle coach. In 2019, measures to adopt a healthy lifestyle will be covered by the Dutch

health insurance, as such preventive measures are expected to reduce healthcare costs.[15] Genetic testing can also be helpful for selecting patients for future treatments. For example, as suggested in **chapter 2.4**, patients carrying rare variants in the *CFH* gene may respond better to treatment with complement inhibitors, and/or may benefit from *CFH* supplementation therapy. Selection of patients for treatments targeting the complement system is further discussed in section 'Towards treatment and personalized medicine'.

Genetic tests for AMD

Several genetic tests are commercially available for AMD risk prediction (Table 1). Some tests are offered as direct-to-consumer personal genetic tests, while others are recommended to be offered by a healthcare provider or ophthalmologist. Most of these currently available tests are not yet suitable for clinical application as prediction is not accurate and there seems to be considerable variation in predicted AMD risks between the tests.[16] Also, none of these tests include rare variants, while such variants have been reported to confer a very high risk for AMD.[4,17] The EYE-RISK consortium is therefore developing a genetic test for AMD that includes all AMD-associated genetic variants and sequence analysis of genes (e.g. *CFH*, *CFI*, *SLC16A8*, *TIMP3*) that have been reported to carry rare variants.[4]

Genetic testing for differential diagnosis of AMD-mimicking macular dystrophies

The genetic assay in development by the EYE-RISK consortium also contains a number of genes that are not associated with AMD, but cause inherited macular dystrophies (e.g. *ABCA4* and *PRPH2*). Sometimes it can be clinically challenging to distinguish AMD from other macular diseases presenting with geographic atrophy in the elderly patient. In such cases genetic testing could help the clinician rule out AMD-mimicking macular diseases, which will become more important as therapies are being developed. In **chapter 3.4** we identified a heterozygous variant in the *PRPH2* gene (p.Arg142Trp) in three patients originally diagnosed with dry AMD. However, this variant has previously been described to cause autosomal dominant central areolar choroidal dystrophy (CACD).[18,19] There are strong phenotypic similarities between CACD and AMD. The focal parafoveal RPE changes in the early stages, and atrophy of the retinal pigment epithelium and choriocapillaris as disease progresses, can very much resemble the AMD phenotype.[20,21] Additionally, CACD has been described in combination with the presence of drusen in some families.[22] Furthermore, CACD can be easily overlooked and misdiagnosed with AMD based on ophthalmological examination alone, especially in families with incomplete penetrance, which may mask the autosomal dominant inheritance of CACD. Although we did not discover any other macular dystrophies in our study, other reports also underline the importance of detailed phenotyping and genotyping to distinguish AMD from other macular diseases such as late-onset Stargardt disease and mitochondrial retinal dystrophy (associated with maternally inherited diabetes and deafness).[23,24]

Selecting individuals for genetic testing

Several groups of individuals would potentially benefit more from genetic testing for AMD than others. In clinical practice, ophthalmologists are regularly confronted by individuals who have family members with AMD, and would like to know what their risk is of developing the disease. In **chapter 2.1** we show that patients with a positive family history of AMD have a lower age of onset of first symptoms and more often progress to geographic atrophy compared to individuals with no family history of AMD. Several lifestyle factors, such as nutrition and physical activity, tended to play a less important role in familial AMD than in sporadic AMD, supporting a stronger genetic component in families. In **chapter 2.2** we demonstrate that in most families, AMD can be explained by a clustering of common genetic and environmental risk factors. However, in a subset of families the number of affected family members was much higher than expected based on these factors, and might be rather explained by other factors, such as rare genetic variants. Genetic testing could therefore be of added value for discovery of novel AMD variants and predicting risk in individuals with a family history of AMD. However, other factors such as lifestyle should also be taken into account in predictive tests for AMD, which will be further discussed in section 'Predictive testing in AMD'.

Chapter 2.4 suggests that phenotypic characteristics may be helpful to select individuals for genetic testing. In this chapter, we show that carriers of rare variants in the *CFH* gene have distinct disease characteristics compared to non-carriers. Patients carrying rare *CFH* variants have a more extensive drusen area, and present more often with drusen with crystalline appearance, and drusen nasal to the optic disc. These characteristics could therefore aid in the selection of patients for genetic testing for AMD.

As not all patients with early or intermediate AMD will progress to end-stage disease, it is important for these patients to assess their risk of developing end-stage AMD. In **chapter 2.3** we demonstrated that genetic variants in the *CFH* and *CETP* genes are associated with progression to advanced AMD. Other studies have identified additional genetic variants that are associated with AMD progression (reviewed in chapter 2.3), suggesting that genetic testing could provide some useful information on risk of progression. However, in chapter 2.3 we also show that clinical characteristics may be helpful for identifying patients who are at risk of developing end-stage AMD. Genetic testing alone is therefore not sufficient to predict AMD progression, and other parameters should be included as well. In the next section, we provide recommendations for factors that should be included in predictive tests for AMD.

Table 1. Commercially available genetic tests for AMD risk prediction

Test	Company (website)	Variants/genes covered	For who?	Specimen for DNA extraction	Available for	Costs	Outcome test
Macula Risk PGx	ArcticDx (https://www.macularisk.com/)	15 variants across 12 loci + age, BMI, smoking history, educational level, AMD status*	AMD patients	Buccal	Healthcare provider/ ophthalmologist	Not provided**	2-/5-/10-year risk for development advanced AMD Recommendation vitamin supplementation
Vita Risk	ArcticDx (https://www.macularisk.com/)	15 variants across 12 loci	AMD patients	Buccal	Healthcare provider/ ophthalmologist	Not provided**	Recommendation vitamin supplementation
Age-related macular degeneration NGS panel	Asper Biotech (http://www.asperbio.com/)	Complete coding regions of *ABCA4*, *ARMS2*, *C2*, *C3*, *C9*, *CFB*, *CFH*, *CFI*, *CST3*, *CX3CR1*, *ERCC6*, *FBLN5*, *HMCN1*, *HTRA1*, *RAX2*	People at risk	Blood	Direct-to-consumer	€ 995	Risk determination of at-risk individuals for early diagnosis and prediction of disease progression Risk assessment of individuals with family history of AMD
Targeted mutation analysis	Asper Biotech (http://www.asperbio.com/)	*ARMS2*, *CFH*	People at risk	Blood or saliva	Direct-to-consumer	€ 87	
EasyDNA – Genetic predisposition test	EasyDNA (https://www.easydna.co.uk/)	SNPs not further specified	People at risk	Buccal	Direct-to-consumer	£ 199 (~ € 228)	Genetic predisposition for 35 conditions including AMD
23andMe	23andMe (https://www.23andme.com/)	For AMD: 2 variants in *CFH* and *ARMS2*	People at risk	Saliva	Direct-to-consumer	€ 169,-	Genetic health risk on several diseases (including AMD), ancestry reports, carrier status for several diseases, traits reports
RetnaGene	Nicox/Sequenom (http://www.mynicox.com)	12 genetic variants (*CFH/CFH* region, *C2*, *CRFB*, *ARMS2*, *C3*)	AMD patients	Buccal	Unclear whether test is still available	Not provided	Progression to advanced neovascular disease within 2/5/10 years

*Based on Yu et al. and validated by Seddon et al. Variants included in this test: *CFH* (rs3766405, rs412852, rs1048663), *CFI* (rs10033900), *C3* (rs2230199), *C2* (rs9332739), *CFB* (rs541862), *LIPC* (rs10468017), *ABCA1* (rs1883025), *CETP* (rs3764261), *COL8A1* (rs13095226), *APOE* (rs429358, rs7412), *TIMP3* (rs9621532), *ARMS2* (372_815del443ins54)
** Macular degeneration testing is reimbursed by almost all insurances for patients who have findings of AMD. Most insurances pay 100% of the cost.

PREDICTIVE TESTING IN AMD

Clinicians are increasingly using prediction models for several purposes; in order to identify high-risk individuals, in order to make therapeutic decisions, recommendations and monitoring strategies, as well as selection for clinical trials. As AMD is a complex disease, one cannot rely on genetic variants alone for predictive testing. Ideally, risk assessment and patient profiling should include a combination of factors: clinical characteristics, genetic information and molecular biomarkers. In this section we discuss current prediction models for AMD, and provide recommendations for improving predictive tests using molecular and clinical biomarkers.

Prediction models for AMD

Many prediction models for development of (advanced) AMD have already been developed with different sets of variables.[25-52] The first prediction models published included only a few variables and were sometimes limited to genetic factors only.[29,31,34-36] The addition of more variables, and inclusion of non-genetic (demographic, environmental and phenotypic) risk factors has led to increased predictive performance.[25,28,40,44,46,53] It is striking though, that none of the published models include the same risk variables. This can be easily explained since studies use predefined datasets and only include variables that are actually measured in each study. Most studies include age, gender, smoking status and some AMD-associated common genetic variants in their prediction models.

Current risk prediction models could be improved by including more AMD-associated genetic variants,[4] and implementation of molecular biomarkers in blood, environmental and phenotypic risk factors. Until now, only few studies include systemic biomarkers or rare genetic variants in their prediction model.[41,46,47] The addition of plasma complement components in one study,[41] and the addition of a rare genetic variant in the *C3* gene in another study[46] led to small but significant improvements of the prediction models. However, adding systemic biomarkers and rare genetic variants also comes with limitations. Measurement of systemic biomarkers often require a strict sample handling protocol to avoid measurement errors and these measurements can be costly. Incorporation of rare genetic variants seems important since they are have a stronger impact on disease risk compared to most common genetic variants, however since occurrence might be limited to certain populations or geographic areas (**chapter 3.3**) models including rare genetic variants will not be applicable to all populations. Also, the addition of family history as a risk factor might be of additive value (**chapter 2.2**), since there is still a significant part of unexplained heritability for which family history may serve as substitute marker and aid in risk prediction.

Molecular biomarkers for AMD

Molecular biomarkers could be helpful in improving prediction models for AMD. In addition, they can also be useful for monitoring disease progression, evaluation of treatment efficacy, to unravel disease mechanisms, and for identification of new targets for treatment. In **chapter 3.1** we provide a comprehensive overview of all molecular compounds that have been evaluated in association to AMD and discussed their potential as AMD biomarker. Based on the existing literature, compounds belonging to the oxidative stress pathway, the complement system, and lipid metabolism seem the most promising biomarker candidates for AMD.[54]

From genetic as well as functional studies, it is known that dysregulation of the complement system plays an important role in AMD. However, the exact role of this complex system remains unclear because only a limited number of complement components have been investigated in relation to AMD and mainly include the central molecule of the complement system (C3), its activation products (C3a and C3d) and complement regulators (Factor H and Factor I). Future studies might benefit from evaluating the entire pathway in an unbiased manner rather than targeting one or more specific single biomarkers in a candidate-driven approach. By measuring all complement components simultaneously in one sample one has the opportunity to gain a detailed and unprecedented insight into the role of the complement system in AMD. Especially, when combining these functional measurements to genetic information our understanding of the role of the complement system in AMD could improve significantly. Apart from improving prediction models for AMD, molecular biomarkers of the complement system could be helpful to interpret genetic test results. For example, rare variants in the *CFH* and *CFI* gene can lead to either lower protein levels in serum, or can lead to a disturbed protein function.[17,55-57] Measuring Factor H, Factor I and complement activation levels in serum or plasma could help to interpret the effect of rare variants that are identified by genetic testing.

Various classes of lipids and lipid-related genes have been associated with AMD, and lipids are one of the main components of drusen, pointing towards an important role of the lipid metabolism in AMD. A recent study by the EYE-RISK consortium and the European Eye Epidemiology (E3) consortium investigated the association of lipids and lipoproteins in more than 30,000 individuals.[58] High density lipoprotein (HDL) cholesterol was associated with increased risk of AMD, while triglycerides were associated with a decreased risk of AMD. The study also evaluated lipid subfractions, which identified extra-large HDL to be the most prominent subfraction associated with AMD. Additional and larger lipidomics studies could provide more insight into which lipid subfractions are associated with AMD, how these relate to AMD-associated variants in the lipid metabolism genes, and whether their concentrations change during disease progression. In addition, a more detailed analysis of the composition of the lipoprotein particles of these subfractions, for example using proteome studies of lipoprotein particles, could potentially identify AMD-specific lipoprotein biomarkers.

Many studies reported decreased antioxidant levels and elevated levels of oxidized proteins or lipids in AMD. The most thoroughly investigated biomarkers of oxidative stress in AMD are malondialdehyde (MDA) and homocysteine (**chapter 3.1**). These biomarkers could be valuable to assess oxidative stress status, and could potentially improve predictive tests for AMD. In addition, measuring levels of antioxidants such as carotenes, vitamins and minerals in blood could help to assess the nutritional status and to design personalized prevention strategies by providing individuals advice on how to alter their diet or lifestyle in order to reduce their risk for AMD.

As described in **chapter 3.1**, omics studies with an unbiased view are heavily outnumbered in the AMD field. Future biomarker research should therefore combine hypothesis-free as well as candidate-driven approaches. Quantitative analytical approaches applied in an untargeted and targeted fashion, such as metabolomic or proteomic studies, are necessary to identify novel biomarker candidates. In **chapter 3.2** metabolomics identified an association of glutamine with early and intermediate AMD, and larger omics studies may identify additional biomarkers in the future. Once validated as robust and reliable markers, they can offer more insights into the etiology and pathogenesis of AMD and support prediction, diagnosis, stratification, monitoring of treatment, and drug development for AMD.

Although AMD represents a phenotype restricted to the eye, many studies have investigated systemic markers in relation to AMD. One might argue to measure biomarkers locally, they might be only locally dysregulated inside the eye without a measurable systemic effect because of the presence of the blood-retinal barrier. However, due to the invasive character and accompanying ethical issues, systemic markers are preferred for implementation as clinical biomarkers.

From a physiological perspective, it is unlikely that biological systems within the human body would operate independently from each other in health or in disease, like AMD. Therefore, integration of biological systems into a coherent and all-encompassing biological understanding is needed to identify the precipitating event(s) that lead to the development and progression of AMD. The possibility of an integrative approach is now at our doorstep with the deeper and better understanding of genetic, cellular and molecular processes and improved prediction.

Clinical biomarkers for AMD progression

Conversion to advanced AMD is a crucial moment for the management of AMD patients. As mentioned in section 'Genetic testing in AMD', identification of individuals at high-risk for progression to advanced AMD and its associated vision loss is highly desirable. Better knowledge about possible development of advanced AMD will contribute to more effective support and monitoring of those patients. Not all individuals with early or intermediate AMD will progress to end-stage, but progression rates in literature vary widely (**chapter 2.3**).

Additionally, studies use different definitions for "progression". Most commonly progression is defined as conversion to advanced AMD in one or both eyes, however, some studies also report progression to a more severe stage of AMD and not necessarily advanced AMD.
Clinical disease stage at baseline has been shown to be one of the strongest predictors of progression, with the more severe phenotype conferring the highest risk for progression.[40,46,59-62]
In **chapter 2.3** we evaluated a combination of genetic factors and phenotypic biomarkers on different imaging modalities for progression of early or intermediate AMD to end-stage disease. We identified pigment abnormalities, drusenoid pigment epithelial detachment, reticular pseudodrusen and hyperreflective foci as relevant imaging biomarkers for conversion to advanced AMD. The high predictive value of phenotypic characteristics for AMD progression is in concordance with previous studies.[37,40]
Large prospective studies in patients with early or intermediate AMD need to be performed to validate the identified clinical biomarkers and to search for new potential biomarkers using multimodal imaging. In the MACUSTAR project more than 700 patients with intermediate AMD will be followed over a period of 3 years in order to identify clinical biomarkers that can predict the rate of progression (https://www.imi.europa.eu/projects-results/project-factsheets/macustar).

Prediction websites
Although we are still far away from a clinically useful prediction model suitable for individual risk prediction, there are already online prediction websites available which integrate genotype and phenotype information for risk calculation (Table 2). However, in order to calculate risks using these websites one needs all requested information including genotypes of certain genetic variants, but genetic testing is not provided by these websites.
Currently, the EYE-RISK consortium is developing a user-friendly web application for personalized risk assessment of AMD based on integration of risk profiles derived from retinal imaging, molecular genetics, assessment of lifestyle, and biochemical testing from large-scale epidemiological data. The website also aims to offer a genetic test that can be ordered through the website, and to provide personalized advice on how altering diet and lifestyle can lead to risk reduction of AMD.

TOWARDS TREATMENT AND PERSONALIZED MEDICINE

The main preventive measure to reduce risk of progression to advanced AMD is through lifestyle interventions and use of nutritional supplements. Currently, the only treatment available for AMD consists of repeated intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF), aiming to reduce neovascularization. No treatment is yet available

for the early, intermediate and advanced atrophic stages of AMD, however, several clinical trials targeting different pathways have been performed or are ongoing (reviewed in chapter 1 of this thesis). In this section we provide advice on how the effectiveness and design of clinical trials can be improved, and discuss which factors can influence response to nutritional supplements and to anti-VEGF treatment.

Improving the design and effectiveness of clinical trials

Many clinical trials targeting the complement system – one of the main AMD disease pathways - have been performed and are still ongoing, however, they have not yet yielded groundbreaking results. To date, many trials focus on reducing atrophy growth, however, trials may need to target early stages of the disease rather than the atrophic end-stage, when apoptosis is already ongoing. To perform trials in early stages of the disease, adequate biomarkers are needed to detect progression and to measure the effectiveness of the treatment that is tested. In addition, biomarkers for progression could be helpful to select individuals who are at high risk of developing end-stage AMD (**chapter 2.3**). This will allow the selection of high-risk patients, which will reduce time and costs needed to run clinical trials.

Due to the heterogeneity in the AMD population, it is plausible that the effect of therapeutic interventions depends on the biological drivers of disease in each individual patient. Therefore, certain treatments might only be effective in a subgroup of AMD patients. One of the main drivers in AMD pathogenesis includes the complement system, and therapies targeting different components of the complement system are being developed. Carriers of rare variants in complement-regulating genes, as described in **chapter 2.4**, may benefit more from complement-inhibiting therapies than patients with AMD in general and therefore identification of these patients through genetic testing seems to be important.[55] Other studies have suggested other common genetic variants in genes of the complement system that could be used for selecting patients for trials with complement inhibitors.[63,64] Patients with rare loss-of-function variants in the *CFH* and *CFI* genes may also benefit from supplementation therapies for *CFH* and *CFI*, respectively.[17]

Additionally, to aid patient selection for clinical trials we recommend the use of genetic testing to exclude AMD-mimicking dystrophies (**chapter 3.4**). In particular screening of the *PRPH2* and *ABCA4* genes could be useful to exclude CACD and late-onset Stargardt disease, which both progress to chorioretinal atrophy similar to advanced atrophic AMD.[23,65,66]

Next to patient selection, choice of the target(s) of intervention is very important. Several pathways are known to be important in AMD pathogenesis, such as the complement system, the lipid metabolism and oxidative stress. However, the exact pathogenesis of AMD is not fully understood, and might represent a complex interplay of the different disease pathways. Targeting only one of these pathways might not be sufficient to treat or avoid AMD; more effective treatment might be reached by targeting multiple AMD disease pathways. In

Table 2. Online available risk calculators for development of (advanced) AMD.

Risk calculators (website)	Outcome	Demographic/ environmental factors	Phenotypic factors	Genetic factors
Advanced AMD Risk Calculation (caseyamdcalc.ohsu.edu)	Risk of development advanced AMD in 2-10 years	Age Family history Smoking status	AREDS simple scale Very large drusen (>250um) Advanced AMD (GA/CNV)	*CFH* (rs1061170) *ARMS2* (rs10490924)
AREDS risk calculator (www.myvisiontest.com/riskcalc.php)	Risk of development advanced AMD in 6 years	Age Gender Education Smoking status BMI Nutraceutical use	Intermediate drusen and/or noncentral GA in worst eye Neovascular/geographic atrophy in one eye	*CFH* (rs1061170, rs1410996) *ARMS2* (rs10490924) *C2* (rs9332739) *CFB* (rs64115) *C3* (rs2230199)
Canadian risk calculator (www.myvisiontest.com/riskcalc.php)	Risk of development advanced AMD	Age Smoking status	None	*CFH* (rs1048663, rs2274700, rs412852, rs11582939, rs1280514) *ARMS2* (rs10490924) *C3* (rs2230199) mDNA (MTND2*LHON4917G)
AMD risk calculator (seddonamdriskscore.org)	Predicted probability of AMD by projected age	Age Gender Race Height Weight Education Smoking status	Baseline AMD (0-4) Baseline drusen (0-5)	*CFH* (rs1061170, rs1410996, **rs121913059**) *ARMS2* (rs10490924) *C2* (rs9332739) *CFB* (rs641153) *C3* (rs2230199, **rs147859257**) *COL8A1* (rs13095226) *RAD51B* (rs8017034) *PELI3* (rs145732233) *CTRB1* (rs8056814) *HSPH1/B3GALTL* (rs9542236)

addition, the right targets within the disease pathways need to be chosen for effective intervention. For example, clinical trials with complement inhibitors have so far targeted complement factor D, C3 and C5 (reviewed in Chapter 1 of this thesis). Other approaches, such as supplementation therapy for *CFH* and *CFI*, might be more effective, in particular in patients carrying rare variants in the *CFH* and *CFI* genes. Other targets in the complement system might be identified by evaluating all components in the complement system simultaneously by proteomics, as mentioned in section 'Predictive testing in AMD'. Such studies and other types of omics studies might identify other possible therapeutic targets for effective interventions in AMD.

Response to AREDS supplementation

For the early and dry forms of AMD no curative treatment is available, but nutritional supplementation with anti-oxidants and zinc have been shown to reduce risk for progression to advanced AMD with 25% in high risk patients.[67,68] Since the publication of these results ophthalmologists should advise patients with unilateral advanced AMD or bilateral intermediate AMD to consider taking nutritional supplements according to the AREDS2 formula. However, ever since the introduction of nutritional supplements in AMD care there is an intense debate ongoing about the influence of genotype on the treatment outcome with AREDS supplementation. It has been suggested that response to AREDS supplements may be related to *CFH* genotype. Klein et al. (2008) were the first to publish a possible interaction between *CFH* genotype and response to nutritional supplements. They concluded that despite a reduced effect of supplementation in patients carrying *CFH* Y402H risk alleles, there is still a beneficial effect. Thereafter, multiple publications (all using data from the AREDS) followed with conflicting results. Several studies implicated that pharmacogenetic selection could be beneficial for nutritional supplementation, moreover, supplementation with zinc specifically could be harmful in patients carrying *CFH* risk alleles.[69-72] Other studies could not replicate these findings and argue that the statistical methods used and conclusions drawn by Awh et al. were not correct.[73-75] The intense debate on whether the effectiveness of antioxidant and zinc supplementation depends on a patient's genotype underscores the urgency for independent studies to resolve the controversy. Since it currently remains the only proven beneficial treatment for AMD, it is still recommended to continue AREDS supplementation for all patients at risk for progression to advanced AMD, regardless of genotype.

Response to anti-VEGF treatment

Treatment of neovascular AMD by targeting VEGF through intravitreal injections with anti-VEGF antibodies has resulted in significant improvements in visual acuity.[76] Nevertheless, anti-VEGF therapy has not proven beneficial for all patients with neovascular AMD, and a small proportion of patients continues to decline in vision despite treatment.[77] This

variability in treatment response might in part be influenced by genetic variations, and many pharmacogenetic studies have been published to date.[78] However, due to conflicting results and heterogeneity in study designs there is no clear consensus and therefore genetic testing is not (yet) warranted to personalize anti-VEGF treatment.[78]

FINAL REMARKS

AMD management warrants an integrative approach for risk assessment, combining lifestyle factors and phenotypic, genetic and molecular biomarkers (Figure 1). Future predictive tests should implement all these factors to provide personalized risks of development of AMD and to aid patient stratification for personalized treatment. The American Academy of Ophthalmology taskforce on genetic testing has recommended that routine genetic testing of patients with complex eye diseases such as AMD is not warranted until clinical trials can demonstrate that patients with specific genotypes benefit from specific therapies or monitoring.[79] However, one could argue that identification of high-risk individuals in an early phase could increase awareness and motivation for reducing AMD risk by adopting a healthy lifestyle. Offering individuals a genetic or predictive test alone may not be sufficient, but could potentially be more effective if combined with personalized motivational strategies, such as a lifestyle coach. After all, prevention is better than cure.

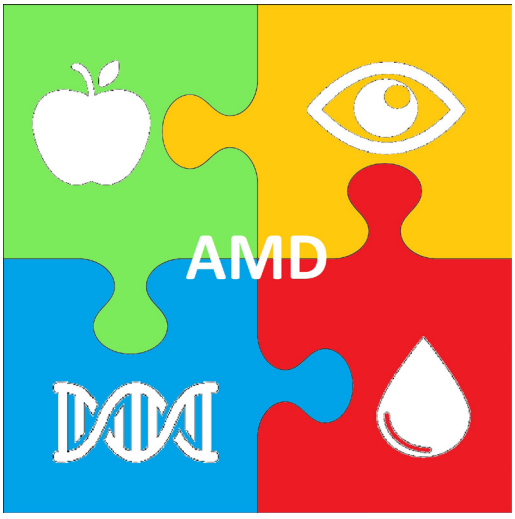


Figure 1. Integrative approach in AMD management, combining lifestyle factors (green), phenotypic (orange), genetic (blue) and molecular (red) biomarkers

REFERENCES

1. Edwards AO, Ritter R, 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005;308(5720):421-424.
2. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* 2005;308(5720):419-421.
3. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308(5720):385-389.
4. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet.* 2016;48(2):134-143.
5. Torkamani A, Wineinger NE, Topol EJ. The personal and clinical utility of polygenic risk scores. *Nat Rev Genet.* 2018;19(9):581-590.
6. Neuner B, Komm A, Wellmann J, et al. Smoking history and the incidence of age-related macular degeneration--results from the Muenster Aging and Retina Study (MARS) cohort and systematic review and meta-analysis of observational longitudinal studies. *Addict Behav.* 2009;34(11):938-947.
7. McGuinness MB, Le J, Mitchell P, et al. Physical Activity and Age-related Macular Degeneration: A Systematic Literature Review and Meta-analysis. *Am J Ophthalmol.* 2017;180:29-38.
8. van Leeuwen R, Boekhoorn S, Vingerling JR, et al. Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA.* 2005;294(24):3101-3107.
9. Tan JS, Wang JJ, Flood V, Rochtchina E, Smith W, Mitchell P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology.* 2008;115(2):334-341.
10. Merle BMJ, Colijn JM, Cougnard-Gregoire A, et al. Mediterranean Diet and Incidence of Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium. *Ophthalmology.* 2018.
11. Ho L, van Leeuwen R, Witteman JC, et al. Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and omega-3 fatty acids: the Rotterdam study. *Arch Ophthalmol.* 2011;129(6):758-766.
12. Gezelschap NO. Richtlijn Leeftijdsgebonden Macula Degeneratie. 2014.
13. McCarty CA, Fuchs MJ, Lamb A, Conway P. How Do Patients Respond to Genetic Testing for Age-related Macular Degeneration? *Optom Vis Sci.* 2018;95(3):166-170.
14. Hollands GJ, French DP, Griffin SJ, et al. The impact of communicating genetic risks of disease on risk-reducing health behaviour: systematic review with meta-analysis. *BMJ.* 2016;352:i1102.
15. Retrieved from https://nos.nl/artikel/2234580-gezondere-levensstijl-in-basispakket.html.
16. Buitendijk GH, Amin N, Hofman A, van Duijn CM, Vingerling JR, Klaver CC. Direct-to-consumer personal genome testing for age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(10):6167-6174.
17. Geerlings MJ, de Jong EK, den Hollander AI. The complement system in age-related macular degeneration: A review of rare genetic variants and implications for personalized treatment. *Mol Immunol.* 2016.
18. Boon CJ, Klevering BJ, Cremers FP, et al. Central areolar choroidal dystrophy. *Ophthalmology.* 2009;116(4):771-782, 782.e771.
19. Hoyng CB, Heutink P, Testers L, Pinckers A, Deutman AF, Oostra BA. Autosomal dominant central areolar choroidal dystrophy caused by a mutation in codon 142 in the peripherin/RDS gene. *Am J Ophthalmol.* 1996;121(6):623-629.
20. Hoyng CB, Deutman AF. The development of central areolar choroidal dystrophy. *Graefes Arch Clin Exp Ophthalmol.* 1996;234(2):87-93.

21. Smailhodzic D, Fleckenstein M, Theelen T, et al. Central areolar choroidal dystrophy (CACD) and age-related macular degeneration (AMD): differentiating characteristics in multimodal imaging. *Invest Ophthalmol Vis Sci.* 2011;52(12):8908-8918.
22. Klevering BJ, van Driel M, van Hogerwou AJ, et al. Central areolar choroidal dystrophy associated with dominantly inherited drusen. *Br J Ophthalmol.* 2002;86(1):91-96.
23. Lindner M, Lambertus S, Mauschitz MM, et al. Differential Disease Progression in Atrophic Age-Related Macular Degeneration and Late-Onset Stargardt Disease. *Invest Ophthalmol Vis Sci.* 2017;58(2):1001-1007.
24. Saksens NT, Fleckenstein M, Schmitz-Valckenberg S, et al. Macular dystrophies mimicking age-related macular degeneration. *Prog Retin Eye Res.* 2014;39:23-57.
25. Buitendijk GH, Rochtchina E, Myers C, et al. Prediction of age-related macular degeneration in the general population: the Three Continent AMD Consortium. *Ophthalmology.* 2013;120(12):2644-2655.
26. Chen Y, Zeng J, Zhao C, et al. Assessing susceptibility to age-related macular degeneration with genetic markers and environmental factors. *Arch Ophthalmol.* 2011;129(3):344-351.
27. Chiu CJ, Mitchell P, Klein R, et al. A risk score for the prediction of advanced age-related macular degeneration: development and validation in 2 prospective cohorts. *Ophthalmology.* 2014;121(7):1421-1427.
28. Ding Y, Liu Y, Yan Q, et al. Bivariate Analysis of Age-Related Macular Degeneration Progression Using Genetic Risk Scores. *Genetics.* 2017;206(1):119-133.
29. Farwick A, Dasch B, Weber BH, Pauleikhoff D, Stoll M, Hense HW. Variations in five genes and the severity of age-related macular degeneration: results from the Muenster aging and retina study. *Eye (Lond).* 2009;23(12):2238-2244.
30. Gibson J, Cree A, Collins A, Lotery A, Ennis S. Determination of a gene and environment risk model for age-related macular degeneration. *Br J Ophthalmol.* 2010;94(10):1382-1387.
31. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet.* 2006;38(4):458-462.
32. Grassmann F, Fritsche LG, Keilhauer CN, Heid IM, Weber BH. Modelling the genetic risk in age-related macular degeneration. *PLoS One.* 2012;7(5):e37979.
33. Hageman GS, Gehrs K, Lejnine S, et al. Clinical validation of a genetic model to estimate the risk of developing choroidal neovascular age-related macular degeneration. *Hum Genomics.* 2011;5(5):420-440.
34. Hughes AE, Orr N, Patterson C, et al. Neovascular age-related macular degeneration risk based on CFH, LOC387715/HTRA1, and smoking. *PLoS Med.* 2007;4(12):e355.
35. Jakobsdottir J, Conley YP, Weeks DE, Ferrell RE, Gorin MB. C2 and CFB genes in age-related maculopathy and joint action with CFH and LOC387715 genes. *PLoS One.* 2008;3(5):e2199.
36. Jakobsdottir J, Gorin MB, Conley YP, Ferrell RE, Weeks DE. Interpretation of genetic association studies: markers with replicated highly significant odds ratios may be poor classifiers. *PLoS Genet.* 2009;5(2):e1000337.
37. Klein ML, Francis PJ, Ferris FL, 3rd, Hamon SC, Clemons TE. Risk assessment model for development of advanced age-related macular degeneration. *Arch Ophthalmol.* 2011;129(12):1543-1550.
38. McCarthy LC, Newcombe PJ, Whittaker JC, et al. Predictive models of choroidal neovascularization and geographic atrophy incidence applied to clinical trial design. *Am J Ophthalmol.* 2012;154(3):568-578 e512.
39. McKay GJ, Dasari S, Patterson CC, Chakravarthy U, Silvestri G. Complement component 3: an assessment of association with AMD and analysis of gene-gene and gene-environment interactions in a Northern Irish cohort. *Mol Vis.* 2010;16:194-199.

40. Perlee LT, Bansal AT, Gehrs K, et al. Inclusion of genotype with fundus phenotype improves accuracy of predicting choroidal neovascularization and geographic atrophy. *Ophthalmology.* 2013;120(9):1880-1892.
41. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. *Invest Ophthalmol Vis Sci.* 2009;50(12):5818-5827.
42. Ristau T, Ersoy L, Hahn M, et al. Nongenetic risk factors for neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2014;55(8):5228-5232.
43. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci.* 2009;50(5):2044-2053.
44. Seddon JM, Reynolds R, Yu Y, Daly MJ, Rosner B. Risk models for progression to advanced age-related macular degeneration using demographic, environmental, genetic, and ocular factors. *Ophthalmology.* 2011;118(11):2203-2211.
45. Seddon JM, Reynolds R, Yu Y, Rosner B. Validation of a prediction algorithm for progression to advanced macular degeneration subtypes. *JAMA Ophthalmol.* 2013;131(4):448-455.
46. Seddon JM, Silver RE, Kwong M, Rosner B. Risk Prediction for Progression of Macular Degeneration: 10 Common and Rare Genetic Variants, Demographic, Environmental, and Macular Covariates. *Invest Ophthalmol Vis Sci.* 2015;56(4):2192-2202.
47. Sharma NK, Sharma SK, Gupta A, Prabhakar S, Singh R, Anand A. Predictive model for earlier diagnosis of suspected age-related macular degeneration patients. *DNA Cell Biol.* 2013;32(9):549-555.
48. Shin KU, Song SJ, Bae JH, Lee MY. Risk Prediction Model for Progression of Age-Related Macular Degeneration. *Ophthalmic Res.* 2017;57(1):32-36.
49. Spencer KL, Olson LM, Schnetz-Boutaud N, et al. Using genetic variation and environmental risk factor data to identify individuals at high risk for age-related macular degeneration. *PLoS One.* 2011;6(3):e17784.
50. Ying GS, Maguire MG, Complications of Age-related Macular Degeneration Prevention Trial Research G. Development of a risk score for geographic atrophy in complications of the age-related macular degeneration prevention trial. *Ophthalmology.* 2011;118(2):332-338.
51. Yu Y, Reynolds R, Rosner B, Daly MJ, Seddon JM. Prospective assessment of genetic effects on progression to different stages of age-related macular degeneration using multistate Markov models. *Invest Ophthalmol Vis Sci.* 2012;53(3):1548-1556.
52. Zanke B, Hawken S, Carter R, Chow D. A genetic approach to stratification of risk for age-related macular degeneration. *Can J Ophthalmol.* 2010;45(1):22-27.
53. Zhang M, Baird PN. A decade of age-related macular degeneration risk models: What have we learned from them and where are we going? *Ophthalmic Genet.* 2016:1-7.
54. Kersten E, Paun CC, Schellevis RL, et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv Ophthalmol.* 2017.
55. Geerlings MJ, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. *JAMA Ophthalmol.* 2017;135(1):39-46.
56. Triebwasser MP, Roberson ED, Yu Y, et al. Rare Variants in the Functional Domains of Complement Factor H Are Associated With Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci.* 2015;56(11):6873-6878.
57. Kavanagh D, Yu Y, Schramm EC, et al. Rare genetic variants in the CFI gene are associated with advanced age-related macular degeneration and commonly result in reduced serum factor I levels. *Hum Mol Genet.* 2015;24(13):3861-3870.

58. Colijn JM, Hollander AID, Demirkan A, et al. Increased High Density Lipoprotein-levels associated with Age-related Macular degeneration. Evidence from the EYE-RISK and E3 Consortia. *Ophthalmology.* 2018.

59. Joachim N, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. *Ophthalmology.* 2015;122(12):2482-2489.

60. Vitale S, Clemons TE, Agron E, et al. Evaluating the Validity of the Age-Related Eye Disease Study Grading Scale for Age-Related Macular Degeneration: AREDS2 Report 10. *JAMA Ophthalmol.* 2016;134(9):1041-1047.

61. Cheung CMG, Ong PG, Neelam K, et al. Six-Year Incidence of Age-Related Macular Degeneration in Asian Malays: The Singapore Malay Eye Study. *Ophthalmology.* 2017;124(9):1305-1313.

62. Chew EY, Clemons TE, Agron E, et al. Ten-year follow-up of age-related macular degeneration in the age-related eye disease study: AREDS report no. 36. *JAMA Ophthalmol.* 2014;132(3):272-277.

63. Lores-Motta L, Paun CC, Corominas J, et al. Genome-Wide Association Study Reveals Variants in CFH and CFHR4 Associated with Systemic Complement Activation: Implications in Age-Related Macular Degeneration. *Ophthalmology.* 2018;125(7):1064-1074.

64. Paun CC, Lechanteur YT, Groenewoud JM, et al. A Novel Complotype Combination Associates with Age-Related Macular Degeneration and High Complement Activation Levels in vivo. *Sci Rep.* 2016;6:26568.

65. Westeneng-van Haaften SC, Boon CJ, Cremers FP, Hoefsloot LH, den Hollander AI, Hoyng CB. Clinical and genetic characteristics of late-onset Stargardt's disease. *Ophthalmology.* 2012;119(6):1199-1210.

66. Kersten E, Geerlings MJ, Pauper M, et al. Genetic screening for macular dystrophies in patients clinically diagnosed with dry age-related macular degeneration. *Clin Genet.* 2018;94(6):569-574.

67. Age-Related Eye Disease Study 2 Research G. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA.* 2013;309(19):2005-2015.

68. Age-Related Eye Disease Study Research G. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol.* 2001;119(10):1417-1436.

69. Awh CC, Hawken S, Zanke BW. Treatment response to antioxidants and zinc based on CFH and ARMS2 genetic risk allele number in the Age-Related Eye Disease Study. *Ophthalmology.* 2015;122(1):162-169.

70. Awh CC, Lane AM, Hawken S, Zanke B, Kim IK. CFH and ARMS2 genetic polymorphisms predict response to antioxidants and zinc in patients with age-related macular degeneration. *Ophthalmology.* 2013;120(11):2317-2323.

71. Vavvas DG, Small KW, Awh CC, Zanke BW, Tibshirani RJ, Kustra R. CFH and ARMS2 genetic risk determines progression to neovascular age-related macular degeneration after antioxidant and zinc supplementation. *Proc Natl Acad Sci U S A.* 2018;115(4):E696-E704.

72. Seddon JM, Silver RE, Rosner B. Response to AREDS supplements according to genetic factors: survival analysis approach using the eye as the unit of analysis. *Br J Ophthalmol.* 2016;100(12):1731-1737.

73. Assel MJ, Li F, Wang Y, Allen AS, Baggerly KA, Vickers AJ. Genetic Polymorphisms of CFH and ARMS2 Do Not Predict Response to Antioxidants and Zinc in Patients with Age-Related Macular Degeneration: Independent Statistical Evaluations of Data from the Age-Related Eye Disease Study. *Ophthalmology.* 2017.

74. Chew EY, Klein ML, Clemons TE, Agron E, Abecasis GR. Genetic testing in persons with age-related macular degeneration and the use of the AREDS supplements: to test or not to test? *Ophthalmology.* 2015;122(1):212-215.

75. Chew EY, Klein ML, Clemons TE, et al. No clinically significant association between CFH and ARMS2 genotypes and response to nutritional supplements: AREDS report number 38. *Ophthalmology.* 2014;121(11):2173-2180.

76. Campbell JP, Bressler SB, Bressler NM. Impact of availability of anti-vascular endothelial growth factor therapy on visual impairment and blindness due to neovascular age-related macular degeneration. *Arch Ophthalmol.* 2012;130(6):794-795.

77. Group CR, Martin DF, Maguire MG, et al. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med.* 2011;364(20):1897-1908.

78. Lores-Motta L, de Jong EK, den Hollander AI. Exploring the Use of Molecular Biomarkers for Precision Medicine in Age-Related Macular Degeneration. *Mol Diagn Ther.* 2018;22(3):315-343.

79. Stone EM, Aldave AJ, Drack AV, et al. Recommendations for genetic testing of inherited eye diseases: report of the American Academy of Ophthalmology task force on genetic testing. *Ophthalmology.* 2012;119(11):2408-2410.

Chapter 5

Epilogue

Chapter 5.1
Summary/Samenvatting

SUMMARY

Age-related macular degeneration (AMD) is a complex, multifactorial disease affecting the central retina and the leading cause of visual impairment among the elderly in Western countries. Both environmental and genetic risk factors have been described to be associated with AMD, but the exact pathophysiology is not yet completely understood. Only a small proportion of patients is eligible for treatment with intravitreal anti-VEGF injections, while no treatment is available yet for the majority of patients. The aim of this thesis is to increase our understanding of the clinical and molecular characteristics of AMD development and progression. A general introduction to AMD and the outline of this thesis are provided in **chapter 1**.

In **chapter 2**, we focus on clinical characteristics of AMD. Familial aggregation of AMD has been described and is evaluated in *chapter 2.1* and *chapter 2.2*. Differences in clinical and phenotypic characteristics between familial and sporadic AMD patients are described in *chapter 2.1*. We report an earlier onset of first symptoms in patients with a family history of AMD compared to those without family history. While increasing age and smoking were associated with AMD risk in both familial and sporadic AMD, other environmental factors (such as physical activity and consumption of red meat) seemed less important in familial AMD compared to sporadic AMD. With regard to phenotype, geographic atrophy and cuticular drusen were more prevalent in familial AMD. These findings support the hypothesis of a stronger genetic component and smaller contribution of environmental factors in familial AMD. Although the majority of AMD families can be explained by clustering of common environmental and genetic risk factors, some densely affected AMD families cannot be fully explained by known risk factors and may harbor rare genetic variants (*chapter 2.2*). Risk prediction in AMD families therefore remains complex and should encompass evaluation of multiple familymembers for accurate risk prediction.

Identification of predictive factors for progression to advanced AMD is important as it may contribute to a more efficient and personalized approach in monitoring and support of patients with early or intermediate AMD. *Chapter 2.3* presents a prediction model for conversion to advanced AMD including phenotypic characteristics and genetic, environmental, and demographic risk factors. In our cohort progression to advanced AMD was associated with advanced age, genetic variants in the *CFH* gene, and several phenotypic characteristics: pigment abnormalities, drusenoid pigment epithelial detachment, reticular pseudodrusen and hyperreflective foci.

Rare genetic variants in the *CFH* gene are among the variants that confer the highest risk for AMD,. Patients carrying these variants seem an ideal patient group for upcoming therapies targeting the complement system. In c*hapter 2.4* we show that patients with an extensive drusen area, drusen with crystalline appearance, and drusen nasal to the optic disc are more

likely to carry a rare *CFH* variant. These phenotypical characteristics in combination with other patient characteristics could aid selection of patients for genetic testing and upcoming therapies.

Chapter 3 contains several studies focusing on molecular characteristics of AMD. Biomarkers can help unravel mechanisms of disease and identify targets for treatment. Additionally, in clinical practice they can be useful for detecting disease, monitoring disease progression, evaluation of treatment efficacy, and risk assessment. In *chapter 3.1* we review all literature on potential non-genetic biomarkers and their applicability in AMD. Compounds belonging to the oxidative stress pathway, the complement system, and lipid metabolism seem to be the most promising biomarker candidates for AMD. Also, the use of hypothesis-free 'omics' techniques have great potential to uncover biomarkers or pathways that contribute to AMD pathophysiology, but so far only limited studies have been performed. In *chapter 3.2* we aim to contribute to the discovery of novel biomarkers and metabolic pathways for AMD using a targeted metabolomics approach. In this study, we found glutamine among the metabolites most predictive for AMD. However, larger studies are needed to further resolve the metabolic profile of AMD patients.

Much progress has been made in the discovery of rare genetic variants contributing to AMD risk, and carriers of rare variants in genes of the complement system may be a suitable group for upcoming therapies. In *chapter 3.3* we explored the occurrence of seven rare genetic variants that were reported to be independently associated with AMD by the International AMD Genomics Consortium. We demonstrate that two rare variants in the *CFH* gene deviate in frequency among different geographical regions. Our findings emphasize that personalized treatment targeting rare variants with functional effects may only be applicable to specific populations where these variants are sufficiently common. With upcoming therapies it is also important to exclude patients with a different retinal disease from clinical trials. In *chapter 3.4* we evaluated the occurrence of AMD-mimicking dystrophies in a cohort with patients diagnosed with intermediate or atrophic AMD. In three of 218 cases we identified a pathogenic heterozygous variant in the *PRPH2* gene causal for autosomal dominant central areolar choroidal dystrophy (CACD). The distinction between AMD and AMD-mimicking dystrophies, such as CACD, can be challenging based on fundus examination alone. Genetic screening of genes involved in AMD-mimicking dystrophies can be beneficial in establishing an accurate diagnosis.

Finally, **chapter 4** elaborates on the main findings described in this thesis and their clinical relevance. Particularly, implications and recommendations for genetic and predictive testing and for treatment and personalized medicine for AMD are discussed.

SAMENVATTING

Leeftijdsgebonden maculadegeneratie (LMD) is een complexe, multifactoriële aandoening die de centrale retina aantast, en is de belangrijkste oorzaak van visusverlies onder ouderen in westerse landen. Zowel omgevingsfactoren als genetische factoren zijn geassocieerd met LMD, maar het exacte ziektemechanisme is nog niet geheel opgehelderd. Slechts een deel van de patiënten met LMD komt in aanmerking voor behandeling met intravitreale anti-VEGF injecties; voor het merendeel van de patiënten is er nog geen therapie beschikbaar. Het doel van dit proefschrift is om onze kennis van de klinische en moleculaire kenmerken van de ontwikkeling en progressie van LMD te vergroten. Een algemene introductie over LMD en de opzet van dit proefschrift worden beschreven in **hoofdstuk 1**.

In **hoofdstuk 2** focussen wij op de klinische kenmerken van LMD. Het is beschreven dat LMD kan voorkomen in families, en dit wordt geëvalueerd in *hoofdstuk 2.1* en *hoofdstuk 2.2*. Verschillen in klinische en oogheelkundige eigenschappen tussen familiaire en sporadische LMD patiënten worden beschreven in *hoofdstuk 2.1*. We vonden dat patiënten met een familievoorgeschiedenis van LMD eerder symptomen vertonen vergeleken met patiënten zonder LMD in de familie. Toenemende leeftijd en roken waren geassocieerd met zowel familiaire als sporadische LMD, echter andere omgevingsfactoren (zoals lichaamsbeweging en consumptie van rood vlees) leken minder belangrijk in familiaire LMD vergeleken met sporadische LMD. Met betrekking tot het oogheelkundige ziektebeeld zagen wij dat geografische atrofie en cuticulaire drusen vaker voorkwamen bij familiare LMD patiënten. Deze bevindingen ondersteunen de hypothese dat er een grotere genetische component speelt en er een kleinere bijdrage van omgevingsfactoren is in familiaire LMD. Hoewel een groot deel van de LMD families verklaard kan worden op basis van clustering van veelvoorkomende omgevings- en genetische risicofactoren, kunnen sommige sterk belaste families niet volledig door bekende risicofactoren verklaard worden. Mogelijk spelen zeldzame genetische varianten een rol in deze families (*hoofdstuk 2.2*). Risicovoorspelling in LMD families blijft daardoor complex en zou evaluatie van meerdere familieleden moeten omvatten om een accurate risicovoorspelling te kunnen doen.

Naast het risico op het ontwikkelen van LMD is de identificatie van voorspellende factoren voor progressie naar late LMD belangrijk. Zulke factoren kunnen bijdragen aan een efficiëntere en meer persoonsgerichte benadering in het opvolgen en ondersteunen van patiënten met beginnende of intermediaire LMD. *Hoofdstuk 2.3* beschrijft een predictiemodel voor de conversie naar late LMD waarin oogheelkundige kenmerken, genetische, omgevings- en demografische risicofactoren worden meegenomen. In ons cohort was progressie naar late LMD geassocieerd met toenemende leeftijd, genetische varianten in het *CFH* gen, en verscheidene oogheelkundige kenmerken: pigmentveranderingen, drusenoïde pigment epitheel loslating, reticulaire pseudodrusen en hyperreflectieve foci.

Zeldzame genetische varianten gelokaliseerd in het *CFH* gen behoren tot de varianten die het grootste risico voor LMD geven, en patiënten die deze varianten dragen lijken een ideale patiëntgroep voor opkomende therapieën die gericht zijn op het complement systeem. In *hoofdstuk 2.4* laten we zien dat patiënten met een uitgebreid drusen oppervlakte, kristallijne drusen en drusen nasaal van de papil meer kans hebben om een zeldzame *CFH* variant te dragen. Deze oogheelkundige kenmerken kunnen, in combinatie met andere patiëntkarakteristieken, waardevol zijn om patiënten te selecteren voor aanvullend genetisch onderzoek en opkomende therapieën.

Hoofdstuk 3 omvat meerdere studies die focussen op moleculaire kenmerken van LMD. Biomarkers kunnen helpen bij het ontrafelen van het ziektemechanisme en het identificeren van targets voor therapieën. Daarnaast kunnen ze in de klinische praktijk nuttig zijn om ziekte op te sporen en te monitoren, om het effect van therapie te beoordelen en voor risico bepaling. In *hoofdstuk 3.1* geven wij een overzicht van alle literatuur over potentiële niet-genetische biomarkers en hun toepasbaarheid in LMD.

Moleculaire factoren behorende tot het oxidatieve stress mechanisme, het complement systeem en het lipiden metabolisme lijken de meest veelbelovende biomarkers voor LMD te zijn. Ook heeft het gebruik van hypothese-vrije 'omics' technieken grote potentie om biomarkers of ziektemechanismen te ontdekken die een rol spelen bij LMD, maar tot nu toe zijn er slechts een klein aantal studies uitgevoerd. In *hoofdstuk 3.2* hebben we gebruikt gemaakt van een grootschalige analyse van metaboliten (metabolomics) om nieuwe biomarkers en metabole mechanismen te ontdekken die een rol spelen bij LMD. In deze studie vonden we dat glutamine een van de metabolieten is die het meest voorspellend is voor LMD. Er zijn echter grotere studies nodig om het metabole profiel van LMD patiënten beter in kaart te brengen.

Verscheidene studies hebben aangetoond dat zeldzame genetische varianten een verhoogd risico geven op LMD. Patiënten die een zeldzame genetische variant dragen zijn mogelijk goede kandidaten voor nieuwe behandelingen gericht op het complement systeem. In *hoofdstuk 3.3* hebben we zeven zeldzame genetische varianten onderzocht die geassocieerd zijn met LMD. We laten zien dat twee zeldzame varianten in het *CFH* gen in frequentie variëren in populaties van verschillende werelddelen. Onze bevindingen benadrukken dat persoongerichte behandelingen gericht op zeldzame varianten mogelijk alleen toepasbaar zijn in bepaalde populaties waar deze varianten voorkomen. Met de opkomst van nieuwe behandelingen is het ook belangrijk om patiënten met andere retinale aandoeningen te excluderen van klinische trials. In *hoofdstuk 3.4* evalueren we het voorkomen van op LMD lijkende macula dystrofieën in een cohort van patiënten gediagnosticeerd met intermediaire of atrofische LMD. In drie van de 218 patiënten vonden we een pathogene heterozygote variant in het *PRPH2* gen dat autosomaal dominante centrale areolaire choroidale dystrofie (CACD) veroorzaakt. Het kan moelijk zijn om op basis van alleen fundusonderzoek onderscheid

te maken tussen LMD en op LMD lijkende dystrofieën, zoals CACD. Genetische screening van genen betrokken bij op LMD lijkende dystrofieën kan nuttig zijn bij het vaststellen van de juiste diagnose.

Tot slot gaat **hoofdstuk 4** dieper in op de belangrijkste bevindingen beschreven in dit proefschrift en de klinische relevantie daarvan. In het bijzonder worden implicaties en aanbevelingen voor genetische en voorspellende testen voor LMD besproken, en voor de behandeling en persoonsgerichte zorg van LMD.

Chapter 5.2
List of publications

Burden of common genetic and environmental risk factors in families affected with age-related macular degeneration

Kersten E, Lechanteur YT, Saksens NT, Geerlings MJ, Schick T, Fauser S, de Jong EK, Boon CJ, den Hollander AI, Klaver CC, Hoyng CB

Manuscript submitted

Risk factors for conversion from early and intermediate to late age-related macular degeneration: 5-year follow-up in the EUGENDA cohort

Sitnilska V, Kersten E, Altay L, Schick T, Enders P, de Jong EK, Langmann T, Hoyng CB, den Hollander AI, Fauser S.

Manuscript submitted

Metabolic profiling in serum of patients with non-advanced age-related macular degeneration reveals aberrations in glutamine pathway

Kersten E, Dammeier S, Ajana S, Groenewoud JM, Codrea M, Klose F, Lechanteur YT, Fauser S, EYE-RISK Consortium, Ueffing M, Delcourt C, Hoyng CB, de Jong EK, den Hollander AI.

Accepted for publication in PLOSONE

Acute metamorfopsie bij senioren vraagt om snel handelen.

Kersten E, Hoyng CB, Keunen JE.

Accepted for publication in NTvG

Macular dystrophy and cone-rod dystrophy caused by mutations in the RP1 gene: extending the RP1 disease spectrum.

Verbakel SK, van Huet RAC, den Hollander AI, Geerlings MJ, Kersten E, Klevering BJ, Klaver CCW, Nikopoulos K, Rivolta C, Ikeda Y, Sonoda K, Wada Y, Boon CJF, Nakazawa T, Hoyng CB, Nishiguchi KM

Invest Ophthalmol Vis Sci. 2019 Mar 1;60(4):1192-1203. doi: 10.1167/iovs.18-26084.

Increased High Density Lipoprotein-levels associated with Age-related Macular degeneration. Evidence from the EYE-RISK and E3 Consortia.

Colijn JM, Hollander AID, Demirkan A, Cougnard-Grégoire A, Verzijden T, Kersten E, Meester MA, Merle BMJ, Papageorgiou G, Ahmad S, Mulder MT, Costa MA, Benlian P, Bertelsen G, Bron A, Claes B, Creuzot-Garcher C, Erke MG, Fauser S, Foster PJ, Hammond CJ, Hense HW, Hoyng CB, Khawaja AP, Korobelnik J, Piermarocchi S, Segato T, Silva R, Souied EH, Williams KM, van Duijn CM, Delcourt C, Klaver CCW; E3 Consortium and EYE-RISK Consortium.

Ophthalmology. 2019 Mar;126(3):393-406. doi: 10.1016/j.ophtha.2018.09.045.

Genetic screening for macular dystrophies in patients clinically diagnosed with dry age-related macular degeneration.

Kersten E, Geerlings MJ, Pauper M, Corominas J, Bakker B, Altay L, Fauser S, de Jong EK, Hoyng CB, den Hollander AI.

Clin Genet. 2018 Dec;94(6):569-574. doi: 10.1111/cge.13447.

Mediterranean Diet and Incidence of Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium.

Merle BMJ, Colijn JM, Cougnard-Grégoire A, de Koning-Backus APM, Delyfer MN, Kiefte-de Jong JC, Meester-Smoor M, Féart C, Verzijden T, Samieri C, Franco OH, Korobelnik JF, Klaver CCW, Delcourt C; EYE-RISK Consortium.

Ophthalmology. 2019 Mar;126(3):381-390. doi: 10.1016/j.ophtha.2018.08.006.

A new perspective on lipid research in age-related macular degeneration.

van Leeuwen EM, Emri E, Merle BMJ, Colijn JM, Kersten E, Cougnard-Gregoire A, Dammeier S, Meester-Smoor M, Pool FM, de Jong EK, Delcourt C, Rodrigez-Bocanegra E, Biarnés M, Luthert PJ, Ueffing M, Klaver CCW, Nogoceke E, den Hollander AI, Lengyel I.

Prog Retin Eye Res. 2018 Nov;67:56-86. doi: 10.1016/j.preteyeres.2018.04.006.

Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population.

Mauschitz MM, Bonnemaijer PWM, Diers K, Rauscher FG, Elze T, Engel C, Loeffler M, Colijn JM, Ikram MA, Vingerling JR, Williams KM, Hammond CJ, Creuzot-Garcher C, Bron AM, Silva R, Nunes S, Delcourt C, Cougnard-Grégoire A, Holz FG, Klaver CCW, Breteler MMB, Finger RP; European Eye Epidemiology (E3) Consortium.

Ophthalmology. 2018 Oct;125(10):1526-1536. doi: 10.1016/j.ophtha.2018.03.026

The European Eye Epidemiology spectral-domain optical coherence tomography classification of macular diseases for epidemiological studies.

Gattoussi S, Buitendijk GH, Peto T, Leung I, Schmitz-Valckenberg S, Oishi A, Wolf S, Deák G, Delcourt C, Klaver CCW, Korobelnik JF; European Eye Epidemiology (E3) consortium.

Acta Ophthalmol. 2018 Sep 22. doi: 10.1111/aos.13883. [Epub ahead of print]

Whole-Exome Sequencing in Age-Related Macular Degeneration Identifies Rare Variants in *COL8A1*, a Component of Bruch's Membrane.

Corominas J, Colijn JM, Geerlings MJ, Pauper M, Bakker B, Amin N, Lores Motta L, Kersten E, Garanto A, Verlouw JAM, van Rooij JGJ, Kraaij R, de Jong PTVM, Hofman A, Vingerling JR, Schick T, Fauser S, de Jong EK, van Duijn CM, Hoyng CB, Klaver CCW, den Hollander AI.

Ophthalmology. 2018 Sep;125(9):1433-1443. doi: 10.1016/j.ophtha.2018.03.040.

The Decreasing Prevalence of Nonrefractive Visual Impairment in Older Europeans: A Meta-analysis of Published and Unpublished Data.
Delcourt C, Le Goff M, von Hanno T, Mirshahi A, Khawaja AP, Verhoeven VJM, Hogg RE, Anastosopoulos E, Cachulo ML, Höhn R, Wolfram C, Bron A, Miotto S, Carrière I, Colijn JM, Buitendijk GHS, Evans J, Nitsch D, Founti P, Yip JLY, Pfeiffer N, Creuzot-Garcher C, Silva R, Piermarocchi S, Topouzis F, Bertelsen G, Foster PJ, Fletcher A, Klaver CCW, Korobelnik JF; European Eye Epidemiology Consortium.
Ophthalmology. 2018 Aug;125(8):1149-1159. doi: 10.1016/j.ophtha.2018.02.005.

Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration.
Kersten E, Paun CC, Schellevis RL, Hoyng CB, Delcourt C, Lengyel I, Peto T, Ueffing M, Klaver CCW, Dammeier S, den Hollander AI, de Jong EK.
Surv Ophthalmol. 2018 Jan - Feb;63(1):9-39. doi: 10.1016/j.survophthal.2017.05.003.

Geographic distribution of rare variants associated with age-related macular degeneration.
Geerlings MJ, Kersten E, Groenewoud JMM, Fritsche LG, Hoyng CB, de Jong EK, den Hollander AI.
Mol Vis. 2018 Jan 27;24:75-82.

Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future.
Colijn JM, Buitendijk GHS, Prokofyeva E, Alves D, Cachulo ML, Khawaja AP, Cougnard-Gregoire A, Merle BMJ, Korb C, Erke MG, Bron A, Anastasopoulos E, Meester-Smoor MA, Segato T, Piermarocchi S, de Jong PTVM, Vingerling JR; EYE-RISK consortium, Topouzis F, Creuzot-Garcher C, Bertelsen G, Pfeiffer N, Fletcher AE, Foster PJ, Silva R, Korobelnik JF, Delcourt C, Klaver CCW; European Eye Epidemiology (E3) consortium.
Ophthalmology. 2017 Dec;124(12):1753-1763. doi: 10.1016/j.ophtha.2017.05.035.

Phenotype Characteristics of Patients With Age-Related Macular Degeneration Carrying a Rare Variant in the Complement Factor H Gene.
Kersten E, Geerlings MJ, den Hollander AI, de Jong EK, Fauser S, Peto T, Hoyng CB.
JAMA Ophthalmol. 2017 Oct 1;135(10):1037-1044. doi: 10.1001/jamaophthalmol.2017.3195.

Differential Disease Progression in Atrophic Age-Related Macular Degeneration and Late-Onset Stargardt Disease.
Lindner M, Lambertus S, Mauschitz MM, Bax NM, Kersten E, Lüning A, Nadal J, Schmitz-Valckenberg S, Schmid M, Holz FG, Hoyng CB, Fleckenstein M; Foveal sparing Atrophy Study Team (FAST).
Invest Ophthalmol Vis Sci. 2017 Feb 1;58(2):1001-1007. doi: 10.1167/iovs.16-20980.

Clinical characteristics of familial and sporadic age-related macular degeneration: differences and similarities.

Saksens NT, Kersten E, Groenewoud JM, van Grinsven MJ, van de Ven JP, Sánchez CI, Schick T, Fauser S, den Hollander AI, Hoyng CB, Boon CJ.

Invest Ophthalmol Vis Sci. 2014 Oct 9;55(11):7085-92. doi: 10.1167/iovs.14-14659.

Chapter 5.3
Data management page

Type of data	Subject to privacy (yes/ no)	Way of anonymization	Storage
Informed consents of patients included in EUGENDA	Yes	All patients have been assigned a study-ID number and the key is stored in a password protected SPSS file	Written informed consents of patients included at the Ophthalmology department in Nijmegen are stored in a locked archive. The key file can be found on the Ophthalmology H-drive and is stewarded by the database manager: *H:\Onderzoek\Data files EUGENDA database*
Clinical and questionnaire data of patients included in EUGENDA	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Both data can be found on the Ophthalmology H-drive: *H:\Onderzoek\ Data files EUGENDA database*
Images of patients included in EUGENDA	Yes	All patients have been assigned a study-ID number and data is stored by study-ID	Images are stored in an online accessible password-protected secured database *https://www.eugenda-database.org*
Contact details of patients enrolled in EUGENDA	Yes	NA	This information is stored in an online password-protected secured database *https://www.eugenda-database.org*. Access is limited to a few clinicians responsible for ophthalmologic examinations of the patients, all other researchers can only access anonymized data
DNA of patients included in EUGENDA	Yes	A DNA number is assigned to each individual by the cell culture facility of the Department of Human Genetics	The key is stored in the MCCD database and is only accessible by clinicians and members of the cell culture facility. DNA samples are stored at the Department of Human Genetics. Contact person for the DNA samples is Saskia van der Velde-Visser; *saskia.vandervelde-visser@radboudumc.nl*
Serum and plasma samples of patients included in EUGENDA	Yes	Anonymized with a sample-ID number and corresponding clinical information is stored in a password protected excel file	Stored in the-80°C freezers at the department of Ophthalmology. Contact person where to find the samples is Bjorn Bakker; *bjorn.bakker@radboudumc.nl* Clinical information of the samples can be found on the H-drive of Ophthalmology: *H:\Onderzoek\Data files EUGENDA database*

Type of data	Subject to privacy (yes/ no)	Way of anonymization	Storage
Genotype data of individual SNPs of patients included in EUGENDA	No	Data is already anonymized	Genotype information is stored in an SPSS file in the following folder on the Ophthalmology H-drive: *H:\Onderzoek\ Data files EUGENDA database*
Exome sequencing data of patients included in EUGENDA	No	Data is already anonymized	Exome sequencing data can be found in the following folder on the Ophthalmology T-drive: *\\umcsanfsclp01\PIgroup-Anneke-denHollander\ExomeData*
Genotype data of patients included in IAMDGC	No	Data is already anonymized	Genotype information is stored in an SPSS file in the following folder on H-drive: *H:\Onderzoek\ Eveline Kersten\Projecten\IAMDGC\ Bronbestanden (Caucasian ONLY)\ Genotypes_8SNPs_20160322_LF.txt*
Files for publications presented in this thesis	No	NA	All files can be found on H-drive of Ophthalmology: *H:\Onderzoek\Eveline Kersten\Projecten*

Chapter 5.4
PhD portfolio

Institute for Health Sciences
Radboudumc

Name PhD candidate: Eveline Kersten
Department: Department of Ophthalmology
Graduate school: Radboud institute for Health Sciences

PhD period: 1-9-2014 to 31-8-2017
Promotors: Prof. dr. C.B. Hoyng, Prof. dr. A.I. den Hollander
Co-promotor: Dr. E.K. de Jong

	Year(s)	ECTS
TRAINING ACTIVITIES		
Courses & Workshops		
SPSS Statistics course	2014	0.2
RIHS introduction course for PhD students	2015	0.5
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (eBROK)	2015	1.25
Biometrics	2015-2016	2
Adobe InDesign and Illustrator workshop	2016	0.4
How to write a medical scientific paper	2016	0.2
Scientific integrity for PhD students	2016	0.5
Course "R"	2016	0.2
BBMRI-omics: introduction	2017	0.5
Dissertation first aid: How to prepare and print your thesis	2017	0.1
Seminars & Lectures		
Radboud Research Round Sensory Disorders (n=4)	2015-2017	0.4
Refereeravonden Oogheelkunde (n=4)	2015-2016	0.4
PhD defences (n=8)	2014-2017	0.8
Orations (n=2)	2015-2016	0.2
Seminar "Selective publication and the replicability crisis"	2016	0.1
Seminar Lars Fritsche "GWAS of AMD…"	2016	0.1
Donders Lecture John O'Keefe	2016	0.1
Lecture Bioinformatics	2017	0.1
BORA lecture^	2017	0.1
Symposia & Conferences		
Macula symposium, Rotterdam	2014	0.25
OOG/ZOG (n=3)	2014-2016	0.3
Dutch Ophthalmology PhD Students conference^ (n=3)	2015-2017	1.5
ARVO conference^ (n=2)	2015-2016	3
International symposium on AMD, Baden-Baden^	2015	1
Nederlands Oogheelkundig Gezelschap conference^ (n=2)	2016-2017	1
Big data symposium, Rotterdam^	2016	0.5
Cybergenetics symposium, KNAW	2016	0.1
Young researcher vision camp^	2017	1

Other		
EUGENDA Retreat (n=3)^	2014-2017	1.5
EYERISK/E3-consortium meeting (n=6)^	2015-2017	6
TEACHING ACTIVITIES (LECTURING, SUPERVISION AND OTHER)		
Radboud Science Award- Education primary school students and teachers	2014-2015	3
Lecture MD-patient organization (n=2)	2014-2015	0.5
Supervision research internship Master students Medicine	2015-2016	1
5KMP1 Medical Biotechnology- Education Bachelor students	2016	0.2
President & Organizing committee DOPS 2016	2016	2
50+ Beurs- Education patients	2015	0.5
TOTAL		31.5

^Indicates oral or poster presentation

Radboud Universiteit Radboudumc

Chapter 5.5
Acknowledgements/Dankwoord

Het moment is daar, eindelijk ben ik aangekomen bij het schrijven van het laatste en leukste gedeelte van mijn proefschrift: het dankwoord! Een promotietraject kent pieken en dalen, en soms had ik het gevoel alsof ik er helemaal alleen voor stond, maar dit proefschrift had nooit tot stand kunnen komen zonder een heleboel mensen die ik hier graag wil bedanken.

Allereerst wil ik graag alle personen bedanken die hebben meegedaan aan ons onderzoek naar leeftijdsgebonden maculadegeneratie, want zonder jullie medewerking kunnen wij geen onderzoek verrichten, hartelijk dank!

Beste Carel, onder jouw leiding deed ik mijn wetenschappelijk stage tijdens mijn studie geneeskunde. Ik heb het erg naar mijn zin gehad tijdens deze stage en jij was degene die me geënthousiasmeerd heeft voor het wetenschappelijk onderzoek. Gelukkig voor mij melde je op mijn laatste stagedag dat er een promotieplek zou komen, dus ik heb direct die maandag een sollicitatiebrief ingeleverd. Dankjewel voor je vertrouwen in mij, waardoor ik mijn promotie onderzoek kon starten bij de Oogheelkunde!

Beste Anneke, dankjewel voor je begeleiding en kritische blik op mijn projecten en artikelen. Je hield alles goed in de gaten en zorgde voor duidelijke afspraken en deadlines. Ik heb veel respect voor jou en je werk. Mede dankzij jouw kennis en kunde zijn veel van mijn artikelen net even wat beter geworden!

Beste Eiko, als co-promotor ben jij wat later bij mijn promotie betrokken geraakt. Ik ben heel erg blij dat ik bij jou terecht kon voor een luisterend oor naar mijn ongezouten mening, die ik niet bij iedereen kwijt kon. Ook bedankt voor een fijne samenwerking met name bij het tot stand komen van het biomarker review en het metabolomics project wat een lange adem heeft gekost. Veel succes met je carrière buiten de oogheelkunde!

Iedereen die heeft meegeholpen met de AMD-dagen: ontzettend bedankt! Bjorn, Constantin, Laura en Bert, dank voor alle labgerelateerde zaken waar ik helemaal geen verstand van had. Asha, bedankt voor je enthousiasme en onvermoeibare inzet voor onder andere mijn onderzoek maar ook al het andere onderzoek waar je bij betrokken bent. Hacer, Michel, Jack, Maartje, Stanley, Roos, Dyon, Vivian, Sanne, Mireille en studenten (Brett, Roel, Enna), dankjewel voor het afnemen van saaie vragenlijsten, het eindeloos druppelen van patiënten, het maken van allerhande beeldvorming en het bloedprikken. Hierdoor hebben we mooie data kunnen verzamelen!

Ook Hans Groenewoud bedankt, voor al je statistische hulp bij mijn projecten en je positieve houding!

Also thanks to all people I got the chance to collaborate with during my PhD-time, especially the researchers in Cologne, Sascha, Lebriz, Tina and Vasilena, for our joint EUGENDA research, and all wonderful people involved in the EYERISK project, in particular Tunde, Cécile, Caroline, and Sascha for collaboration on several projects, and of course Annemarie, Fran, Soufiane and the other "young researchers" for all great moments during meetings, dinners, ARVO and Vision Camp.

Aan alle promovendi waar ik mee heb samen mogen werken (Yara, Nicole, Myrte, Constantin, Michel, Freekje, Laura, Maartje, Nathalie, Stanley, Roos, Sanne, Vivian, Dyon, Tom, Anita, Esmée, Susette, Erkin, Sarah), bedankt voor de superleuke tijd die ik heb gehad tijdens mijn promotie, de gezellige momenten in de pauzes, noodzakelijke koffiebreaks, de fantastische tijd tijdens congressen, de badminton- en beachvolleybaltoernooitjes, de spelletjesavonden, alle lol met het maken van filmpjes en liedjes voor andere promovendi, pubquizen, kleding-swap-avonden en alles wat ik vergeten ben. In het bijzonder bedankt: Nicole, voor je begeleiding tijdens mijn wetenschappelijk stage dat mijn eerste artikel opleverde; Yara, voor je nette overdracht van de EUGENDA database en alle moeite die je gestoken in de database; Freekje, voor je hulp op statistisch en epidemiologisch vlak en vooral alle Zwelgjesmomenten samen met Maartje en Nathalie; Nathalie, voor je vrolijke aanwezigheid en steun waar ik altijd op kan rekenen, fijn om zo'n maatje te hebben; Stanley, bedankt dat je het hebt volgehouden met mij en Nathalie op een onvergetelijke vakantie na ARVO; Roos, dank voor de topsamenwerking bij de organisatie van DOPS2016 en ons hilarische avond evenement. En natuurlijk Maartje, mijn onderzoeksmaatje en paranimf, ontzettend bedankt voor alles! Het was fijn om met nog zo'n perfectionist samen te werken, database problemen op te lossen, projecten te delen, en ons motto "We doen het wel zelf!" heeft mooie resultaten opgeleverd;)
Natuurlijk kan een bedankje aan mijn huidige collega's niet uitblijven: alle AIOS bedankt dat jullie de ellende van alle promovendi over het afronden van onze proefschriften nog kunnen aanhoren, maar vooral bedankt dat ik het zo naar mijn zin heb in de opleiding! Dat laatste woord van dank geldt ook voor alle stafleden, optometristen, orthoptisten, verpleegkundigen, OK-medewerkers, dames van het secretariaat, de administratie en het planbureau.

Ook al lijkt het soms alsof je alleen maar met werk bezig bent, tijd voor ontspanning, familie en vrienden is ook nodig. In mijn oude huisje "de Houthof" had ik gelukkig altijd een ontspannen en fijne thuisbasis waar ik menig waardevolle herinneringen aan heb met Saskia, Bart, Paul en later Yannic. Dank dat jullie er altijd waren, ook al was ik vaak druk met mijn opleiding of

promotie. Ook de Smashinghal is voor mij een plek van ontspanning waar ik meermaals per week even helemaal mijn gedachten kan verzetten. Ik heb wel jaren iedere training de vraag moeten aanhoren “En Eef, is ’t al af?” maar nu kan ik eindelijk vol trots zeggen “Yes Yan, het is af!”.

Lieve familie en vrienden, soms waren er tijden dat ik niets van me liet horen, gelukkig maakte dat voor jullie nooit uit en kan ik altijd op jullie rekenen.

Lieve Saskia, wat begon als een kennis van badminton groeide uit tot een hele goede vriendin waar ik een heel aantal jaren veel samen mee heb gedeeld. Sorry dat ik er de laatste tijd niet voor je ben geweest, nadat de verdediging van mijn proefschrift is geweest ga ik betere prioriteiten stellen.

Lieve Sarah en Meike, dank jullie voor jullie vriendschap sinds onze co-schappen, voor mooie herinneringen aan onder andere onze vakantie naar Chersonissos, de weekendjes weg, uitstapjes, escaperooms, Q-music foute party, en nog veel meer, maar ook voor de gewone chillavonden of-middagen op de bank bij een van ons thuis waar we onze harten konden luchten over werk/opleiding/promotie. Hopelijk volgen er nog veel meer mooie momenten samen!

Lieve familie en schoonfamilie, dankjewel dat jullie mij de tijd geven om aan mijn carrière te werken in tijd die ik ook met jullie had kunnen doorbrengen. Toen ik aan mijn promotie begon had ik nog twee opa’s en een oma die trots mijn werk volgde, helaas kunnen zij dit bijzondere moment alle drie niet meer meemaken. Gelukkig kan ik altijd op mijn familie rekenen en jullie op mij.

Lieve Madelon, dankjewel dat je er bent voor alles. Ik ben niet van de vele woorden, maar jij zegt het wel eens tegen mij en daarom zeg ik het nu tegen jou: ik ben trots op je!

Lieve Papa en Annamarie, dankjewel dat jullie me stimuleren te excelleren. Jullie zijn een voorbeeld voor me, ook al zeggen jullie me niet zo te worden als papa. Bedankt voor alle ondersteuning en alle leuke momenten samen, want ik kan nergens zo van genieten als een lekker glas wijn, heerlijk eten, maar vooral jullie aanwezigheid.

Lieve Mama, dankjewel dat je er altijd bent. Je hebt je altijd ingezet voor Madelon en mij, alles zodat wij het maar goed hadden, op school, sport, studie of werk, het maakt niet uit. Zelfs nu zorg je er voor dat ik af en toe wat vitaminen binnen krijg op het werk;) Dankjewel dat je zoveel voor ons gedaan hebt en dat ik onvoorwaardelijk bij je terecht kan.

Lieve Paul, het klinkt cliché maar zonder jou had ik het niet gered. Behalve dat je een enorme steun en toeverlaat voor me bent, zorg jij er de laatste tijd meer dan ooit voor dat ik alle ballen in de lucht kan houden: ik hoef me niet druk te maken over boodschappen, eten koken, etc, jij regelt het. Dat geeft me enorm veel rust en ruimte om mijn werk goed te kunnen doen. Dankjewel voor al je begrip en alle keren dat ik stoom bij je kon afblazen. Liefje, ik hou van je, of zoals jij zou zeggen “de tram”;)

Appendix

Appendix
Acute metamorfopsie bij senioren vraagt om snel handelen

Eveline Kersten
Carel B. Hoyng
Jan E.E. Keunen

Accepted for publication in Nederlands Tijdschrift voor Geneeskunde

Dames en Heren,

Leeftijdsgebonden natte maculadegeneratie is een belangrijke oorzaak van slechtziendheid op oudere leeftijd in de westerse wereld en geassocieerd met een negatieve invloed op de kwaliteit van leven van senioren. Het doel van deze klinische les is het belang van vroege herkenning en tijdige behandeling van deze veelvoorkomende oogaandoening te verduidelijken.

CASUSBESCHRIJVINGEN

Patiënte A is een heer van 75 jaar en belt de praktijk van zijn huisarts met als klacht minder goed te kunnen zien. Toen hij gisteren de boodschappen wilde afrekenen in de supermarkt merkte hij een vlek in het midden van zijn blikveld en stonden de cijfers van het pinapparaat schuin. Het baart hem zorgen dat de klachten na 1 nacht slapen nog steeds bestaan. De assistente vraagt naar de oogheelkundige voorgeschiedenis, die blanco is. Meneer krijgt eerst het advies zijn bril te laten nakijken, maar op nadrukkelijk verzoek van de patiënt mag hij diezelfde dag langskomen. De huisarts bepaalt de gezichtsscherpte van beide ogen. De visus van het rechteroog is 0.6 en links 1.0. De Amsler test geeft met het rechteroog beeldvervorming (metamorfopsie) aan en een wazige vlek in het centrale gedeelte van het gezichtsveld (relatief centraal scotoom). Links is geen sprake van metamorfopsie. Hierop besluit de huisarts meneer de volgende werkdag door te verwijzen naar een oogarts met de verdenking op de natte vorm van een leeftijdsgebonden maculadegeneratie. De oogarts bevestigt de diagnose en dezelfde week wordt een behandeling gestart met vaatgroeiremmende injecties in het rechteroog. Als meneer een jaar later terugkomt voor een reguliere controle bedraagt de visus rechts 0.8 en vertelt hij blij te zijn dat hij nog goed kan zien na een serie van twee keer drie ooginjecties.

Patiënte B, een vitale dame van 86 jaar, merkt bij de bushalte dat ze het cijfer van de buslijn op de bus niet meer goed kan lezen. Dezelfde avond valt bij het skypen met haar kleindochter op dat het linkeroog niet goed ziet. De volgende dag lijkt het onveranderd en na twee dagen ziet ze met het linkeroog gelukkig weer wat beter. Maar dezelfde dag wordt ‘s avonds plotseling de visus links snel minder. Ze gaat de volgende dag meteen naar de opticien, waar men haar bril niet kan aanpassen om de visus te verbeteren en wordt doorverwezen naar de huisarts. Daar kan ze na twee weken terecht. Bij onderzoek blijkt de visus rechts 1.0 en links 0.2. Er wordt niet gevraagd naar metamorfopsie. Patiënte wordt doorverwezen naar de oogarts, waar ze na drie weken terecht kan. Deze stelt als diagnose een natte leeftijdsgebonden maculadegeneratie links in een gevorderd stadium en start onmiddellijk een behandeling

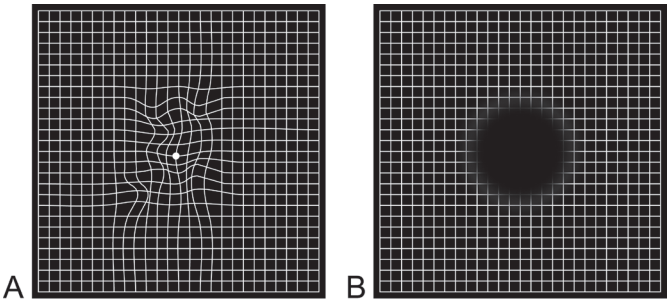
met vaatgroeiremmende injecties in het linkeroog. Een jaar later en 9 ooginjecties verder is de visus links niet verder verslechterd dan de uitgangswaarde. De oogarts vertelt desgevraagd dat de kans op een betere visus dan 0.2 minimaal is. Er wordt een Amsler kaart meegegeven als hulpmiddel ter zelfcontrole mee en hij waarschuwt: als ze metamorfopsie of een visusdaling rechts ervaart, moet ze direct de huisarts bellen.

BESCHOUWING

Klinisch beeld en diagnostiek

Leeftijdsgebonden maculadegeneratie (LMD) is een veelvoorkomende oogaandoening boven de leeftijd van 50 jaar. De incidentie bedraagt ongeveer 1.8 per 1000 personen per jaar in Nederland,[1] en de prevalentie van vroege LMD is 13.2% bij personen boven de 70 jaar in Europa.[2] Het beginstadium van LMD verloopt meestal asymptomatisch en wordt gekenmerkt door drusen, dit zijn ophopingen onder het retinaal pigment epitheel die zichtbaar zijn als gelige vlekjes in de retina. Naarmate de ziekte voorschrijdt kunnen er twee vormen onderscheiden worden: een atrofische ("droge") en een exsudatieve ("natte") vorm waarbij choroidale neovascularisaties zijn ontstaan. Beide vormen kunnen leiden tot ernstige slechtziendheid, het beloop is echter verschillend. Droge LMD kenmerkt zich door een geleidelijke, meestal bilaterale afname van de centrale gezichtsscherpte. Patiënten hebben moeite met lezen, herkenning van gezichten en zien minder goed in schemerige omstandigheden. Natte maculadegeneratie daarentegen kenmerkt zich meestal door een eenzijdige visusdaling ontstaan binnen enkele dagen en metamorfopsie (beeldvervorming). Hierbij staan rechte lijnen schijnbaar krom en dit is vaak één van de eerste klachten bij natte LMD.

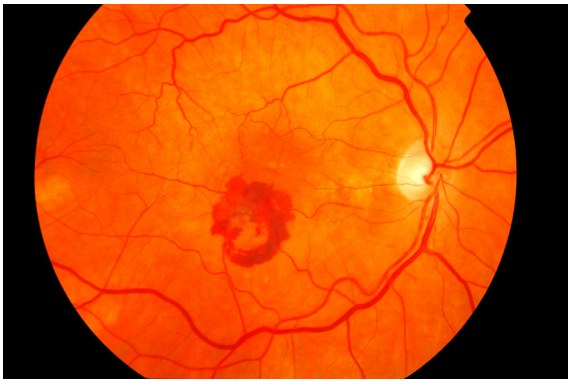
Het is belangrijk om metamorfopsie vroegtijdig te signaleren, zowel door de patiënt als de arts want in de dagelijkse praktijk blijkt nogal eens dat patiënten metamorfopsie zelf niet aangeven, maar wel bevestigen als er expliciet naar gevraagd wordt. De Zwitserse oogarts Marc Amsler (1891-1968) schreef hier al in 1953 over en introduceerde daarom de Amsler testkaart.[3] Patiënten A en B klaagden beide over minder goed zien, niet over beeldvervorming. Met behulp van de Amsler test kwam bij patient A de metamorfopsie naar voren en werd patiënt direct met een verdenking op neovascularisatie doorverwezen. Echter bij patiënt B is er niet gevraagd naar metamorfopsie danwel een Amsler test uitgevoerd, waardoor een belangrijk symptoom van natte LMD niet meteen aan het licht gekomen is. Vraag dus bij visusklachten bij ouderen altijd expliciet naar vervorming van het beeld (bijvoorbeeld "Ziet u de deurpost als een rechte lijn?") en test hierop met behulp van de Amslerkaart (zie figuur 1). De Amsler test is ook zeer geschikt voor de patiënt als zelfcontrole. Het verdient aanbeveling dat zowel de oogarts als de huisarts dit met patiënten bespreekt. Ook bij droge LMD ontstaat uiteindelijk metamorfopsie, maar het beloop daarvan is geleidelijk en beiderzijds.



Figuur 1. De Amslerkaart
Instructies aan de patiënt: houd de kaart op leesafstand, dek één van beide ogen af en focus op de stip in centrum van de kaart. Wat is het verloop van de lijnen (recht of schijnbaar krom)? Zijn er plaatsen waar de hokjes minder of helemaal niet zichtbaar zijn? Herhaal de test voor het andere oog. Indien de lijnen vervormd (A), wazig of verminderd (B) worden waargenomen is het van belang om snel het netvlies te laten beoordelen door een oogarts.

Behandeling

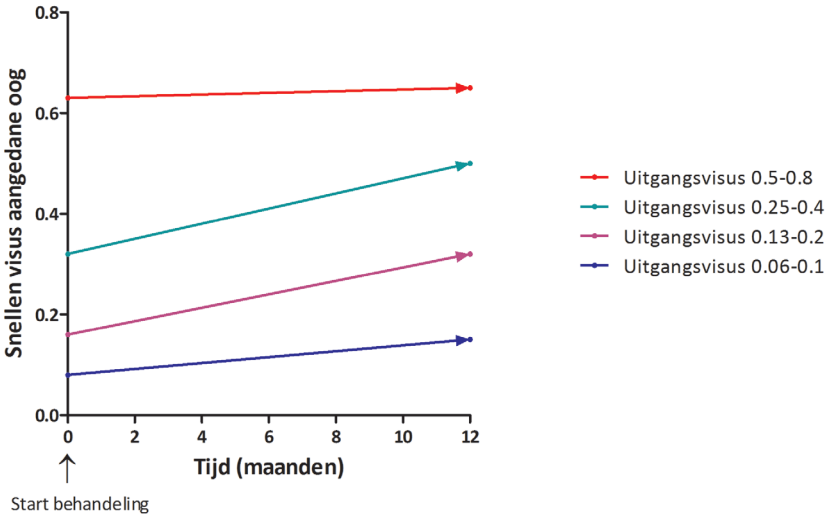
Bij natte LMD is er in de macula sprake van vaatnieuwvormingen met fragiele vaatwanden, waaruit makkelijk lekkage en bloedingen kunnen ontstaan die een acute visusdaling veroorzaken (figuur 2). Sinds 2007 is het is mogelijk om hiervoor intravitreale injecties met vaatgroeiremmende medicatie te geven, beter bekend als anti-VEGF medicatie, en daarmee de visus te stabiliseren of verbeteren. De belangrijkste anti-VEGF medicijnen zijn bevacizumab (Avastin), ranibizumab (Lucentis) en aflibercept (Eylea). Deze medicijnen hebben een vergelijkbare effectiviteit en verschillen in veiligheid konden niet worden aangetoond.[4] Hoewel bij gebruik van Eylea minder injecties nodig zijn, is in verband met het grote verschil in kosten Avastin het middel van eerste keuze in Nederland.



Figuur 2. Natte LMD
Een kleurenopname van de macula bij natte LMD. Er is een zichtbare bloeding in de macula door een onderliggende subretinale neovascularisatie.

Er is veel onderzoek gedaan naar de effectiviteit van deze vaatgroeiremmers. Verschillende factoren zijn geassocieerd met een betere uitkomst van de behandeling onafhankelijk van het soort vaatgroeiremmer: de duur van de klachten, de gezichtsscherpte bij aanvang van de therapie, en de uitgebreidheid van de neovascularisatie. Hierbij geldt dat een kortere klachtenduur, een kleinere vaatnieuwvorming en een betere visus bij aanvang van de behandeling (zie figuur 3) de kans vergroten op een betere visus één jaar na start van de behandeling.[5] Om de laatste reden, meer kans op een betere visus bij een hogere uitgangsvisus, is snel handelen door alle betrokkenen (patiënt, huisarts *en* oogarts) van het grootste belang zo opdat therapie zo vroeg mogelijk gestart kan worden. Bij patiënt A was de behandeling succesvol mede dankzij de snelle interventie door alle betrokkenen. Daarentegen was de kans op visuswinst bij patiënt B beperkt doordat de therapie pas werd gestart na enkele weken.

De behandeling van natte LMD met vaatgroeiremmers heeft geleid tot een sterke vermindering van het aantal ernstig slechtzienden.[6] LMD en visusverlies door LMD is geassocieerd met moeilijkheden bij het uitvoeren van dagelijkse activiteiten, verhoogde emotionele distress en depressie. Het heeft een negatieve invloed op de kwaliteit van leven, niet alleen van de patiënt zelf maar vaak ook van de nabije omgeving.[7] Tijdige herkenning van LMD en daarmee



Figuur 3. Gezichtsscherpte vóór de behandeling als voorspeller van de visus één jaar na de behandeling met vaatgroeiremmende injecties
Hoe beter de gezichtsscherpte bij aanvang van behandeling (uitgangsvisus) hoe beter de gezichtsscherpte één jaar na start van de behandeling. Deze belangrijke figuur is gebaseerd op onderzoeksresultaten van Ying en collega's.[5]

samenhangend vroege behandeling kan de kwaliteit van leven gunstig beïnvloeden. Vroege herkenning heeft tevens een bewezen gunstig effect op de zorgkosten.[8] Voor de droge vorm van LMD is tot op heden geen curatieve behandeling mogelijk.

Voorlichting en leefstijladvies

Goede voorlichting en leefstijladviezen kunnen het beloop van LMD positief beïnvloeden en progressie naar een gevorderd stadium vertragen. Stoppen met roken is daarbij één van de belangrijkste maatregelen (ook op hoge leeftijd!),[9] alsmede het zorgen voor een gezond lichaamsgewicht[10] en voldoende beweging.[11] Een voedingspatroon met veel groenten en fruit (antioxidanten) wordt geadviseerd.[12] Resultaten van een belangrijke studie in de Verenigde Staten tonen aan dat het gebruik van speciale voedingssupplementen kan leiden tot een milde verlaging van het risico op progressie van de ziekte. Deze zogenaamde AREDS2 formule voedingssupplementen bestaan uit een hoge dosering zink (25-80 mg), luteïne (10 mg), zeaxantine (2 mg), vitamine C (500 mg) en vitamine E (400 IE). Ze worden geadviseerd aan LMD patiënten met gevorderde stadia van droge of natte LMD aan één oog of beide ogen.[13] LMD patiënten die roken of de afgelopen 5 jaar gerookt hebben mogen geen voedingssupplement met bètacaroteen gebruiken vanwege een verhoogde kans op de ontwikkeling van longkanker.

Hoewel voor natte LMD een goede behandeling voorhanden is, bestaat er nog steeds een aanzienlijk patient- en doctor's delay.[14] Ouderen zien vaak slechter dan ze zelf denken. "Het zal wel bij de leeftijd horen" lijken ouderen vaak te veronderstellen. Daarnaast is er vaak sprake van co-morbiditeit en/of verminderde mobiliteit waardoor men afhankelijk van anderen is om een bezoek aan de oogarts te brengen. Naarmate de leeftijd toeneemt wordt het patient delay groter.[14] Daar komt bij dat LMD voor veel mensen een onbekende oogaandoening is; slechts 4-30% van de mensen in westerse landen zijn in meer of mindere mate bekend met LMD.[7] Voorlichting is dan ook erg belangrijk. Aan patient empowerment bij LMD wordt in Nederland veel aandacht gegeven door de maculadegeneratie patiëntenvereniging en de WHO preventieprojectgroep VISION 2020 *Netherlands*.

CONCLUSIE

Dames en Heren,

Natte LMD is een aandoening die in de westerse wereld veel voorkomt, waarbij de vroegdiagnostiek nog te wensen overlaat terwijl een adequate behandeling voorhanden is. Dit klemt te meer daar ouderen metamorfopsie vaak zelf niet aangeven, de resultaten van de kosteneffectieve behandeling bij een hogere uitgangsvisus beter zijn en de kwaliteit van leven van ouderen sterk gecorreleerd is aan een goede visus. Actieve voorlichting aan senioren

en huisartsen speelt dan ook een belangrijke rol en kan de kwetsbaarheid van ouderen structureel verminderen. Het verdient aanbeveling om bij ouderen met visusklachten expliciet te vragen naar metamorfopsie en dit te testen met behulp van een Amslerkaart. Bij acute metamorfopsie is het wenselijk de patiënt binnen enkele werkdagen door te verwijzen naar een oogarts.

REFERENTIES

1. van Leeuwen, R., et al., The risk and natural course of age-related maculopathy: follow-up at 6 1/2 years in the Rotterdam study. *Arch Ophthalmol*, 2003. 121(4): p. 519-26.
2. Colijn, J.M., et al., Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology*, 2017.
3. Amsler, M., Earliest symptoms of diseases of the macula. *Br J Ophthalmol*, 1953. 37(9): p. 521-37.
4. Comparison of Age-related Macular Degeneration Treatments Trials Research Group, et al., Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology*, 2012. 119(7): p. 1388-98.
5. Ying, G.S., et al., Baseline predictors for one-year visual outcomes with ranibizumab or bevacizumab for neovascular age-related macular degeneration. *Ophthalmology*, 2013. 120(1): p. 122-9.
6. Campbell, J.P., S.B. Bressler, and N.M. Bressler, Impact of availability of anti-vascular endothelial growth factor therapy on visual impairment and blindness due to neovascular age-related macular degeneration. *Arch Ophthalmol*, 2012. 130(6): p. 794-5.
7. Mitchell, J. and C. Bradley, Quality of life in age-related macular degeneration: a review of the literature. *Health Qual Life Outcomes*, 2006. 4: p. 97.
8. Butt, T., et al., The cost-effectiveness of initiating ranibizumab therapy in eyes with neovascular AMD with good vision: an economic model using real-world outcomes. *BMJ Open*, 2015. 5(5): p. e006535.
9. Neuner, B., et al., Smoking history and the incidence of age-related macular degeneration--results from the Muenster Aging and Retina Study (MARS) cohort and systematic review and meta-analysis of observational longitudinal studies. *Addict Behav*, 2009. 34(11): p. 938-47.
10. Zhang, Q.Y., et al., Overweight, Obesity, and Risk of Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci*, 2016. 57(3): p. 1276-83.
11. McGuinness, M.B., et al., Physical Activity and Age-related Macular Degeneration: A Systematic Literature Review and Meta-analysis. *Am J Ophthalmol*, 2017. 180: p. 29-38.
12. van Leeuwen, R., et al., Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA*, 2005. 294(24): p. 3101-7.
13. Age-Related Eye Disease Study 2 Research, G., Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA*, 2013. 309(19): p. 2005-15.
14. Schalnus, R., et al., Time between symptom onset and assessment in age-related macular degeneration with subfoveal choroidal neovascularization. *Ophthalmologica*, 2010. 224(3): p. 176-82.