# What Does Genetics Tell Us About Age-Related Macular Degeneration?

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## Abstract

Age-related macular degeneration (AMD) is a chronic degenerative disease of the central retina and a major cause of vision impairment and blindness with millions of people affected in the elderly population. In recent years, considerable efforts have been made to understand disease pathology with the long-term goal of designing novel and effective treatment options for this devastating disease. Although striking advances in treating the neovascular stage of late AMD have occurred, no therapy is available for almost half of all AMD patients, specifically those who are affected by the atrophic form of the disease. This review highlights current knowledge on the genetic factors associated with early- and late-stage forms of the disease. It also summarizes the findings regarding the extent to which these factors may play a role in the transition from one disease stage to another, and it emphasizes the need to explore further the underlying mechanisms for both development and progression of this disease as a starting point for designing innovative therapies for it.

# INTRODUCTION

## Genetic Contribution to Complex Traits and Diseases

Most human traits, such as height, body mass index, or metabolic turnover, as well as common diseases such as type II diabetes or Alzheimer's disease, are complex in nature, implying that environmental and genetic factors influence both disease risk and disease severity. The degree of genetic contribution to a complex disease is commonly reflected by an estimated heritability. This measure is derived from twin studies and indicates the portion of disease risk attributable to genetic factors. Knowledge of the genetic basis of disease is likely to provide valuable insight into both understanding underlying disease mechanisms and identifying novel targets for treatment.

## Methodology for Identification of Disease-Associated Genetic Variants

Initial approaches in genetic studies of complex diseases drew conclusions from the aggregation of genetic variants in affected compared with unaffected siblings in families (genetic linkage analysis). In contrast, current designs rely predominantly on the frequency of association of genetic markers with disease in unrelated subjects. Patients and controls are genotyped for genetic variants [known as single nucleotide polymorphisms (SNPs)], and differences in allele frequencies between cases and controls are measured and statistically evaluated. The number of variants analyzed can range from fewer than one hundred to as many as hundreds of thousands of SNPs, provided they are sufficiently uniform in their distribution across the genome. Current statistical methods require appropriate measures to account for multiple testing or multiple comparisons in order to reduce false positive signals. These measures usually do so by correcting the obtained (raw) $p$-value using various algorithms (Aickin & Gensler 1996) or by defining a conservative genome-wide significance threshold of $p \leq 5 \times 10^{-8}$. Genome-wide searches for associated genetic variants require particularly large sample sizes; several thousand patients and controls are needed to obtain sufficient statistical power in such studies. High-throughput technologies, reduced costs in genotyping assays, and increased computational power have greatly increased the available association data, facilitating rapid identification of novel associated loci. In addition, multiple genome-wide studies are frequently combined in meta-analyses, allowing sample sizes to eventually include as many as 300,000 individuals (Randall et al. 2013). As of September 2014, 15,121 SNPs were identified in 2,079 studies as being associated with a human trait or disease (**http://www.ebi.ac.uk/fgpt/gwas/**) (Welter et al. 2014).

## Genome-Wide Association Studies of Age-Related Diseases

Common age-related diseases frequently exhibit complex etiologies, and genome-wide association studies (GWAS) have become a widely used approach to delineate the extent of the genetic contributions to such diseases (Kraft & Cox 2008). A crucial hurdle still to be overcome is the often-encountered phenotypic heterogeneity of disease manifestations, eventually accompanied by varying degrees of disease severity with distinct (sub)phenotypes. Importantly, disease-associated changes may at times be difficult to distinguish from nonpathologic age-related alterations. In addition, some have suggested that the contribution of environmental factors (i.e., disease variance not explained by genetics) can confound the association of genetic factors, specifically in instances in which strong interactions may exist between environmental and genetic risk factors.

Nevertheless, aggregation of phenotypes into a single disease status has yielded a tremendous number of risk-associated genetic variants (**http://www.ebi.ac.uk/fgpt/gwas/**), likely owing to increased statistical power for detecting associations. The data might not sufficiently reflect the complexity of the respective disease entity, however. GWAS of age-related macular degeneration (AMD) are currently conducted in international settings involving collaboration of multiple research laboratories and include tens of thousands of samples. For example, the International AMD Genomics Consortium (IAMDGC), an association of more than 26 research groups worldwide, has collected over 50,000 DNA samples from healthy and diseased individuals. In the following sections, we discuss AMD phenotypes in detail and contrast them with normal age-related changes in the retina.

## AGE-RELATED MACULAR DEGENERATION PHENOTYPES

In principle, AMD is broadly characterized in two main disease categories, early and late-stage AMD. Refined grading systems are based on characteristic disease alterations involving the retina, the retinal pigment epithelium (RPE), and the choroid, and these systems place particular emphasis on lesion severity, size, and location. The clinical grading is based on findings from ophthalmoscopy and color fundus photography, which have been the gold standard for many decades and which still are easily accessible methods for examining and classifying AMD patients (Bird et al. 1995; Ferris et al. 2005, 2013; Klein et al. 1997; Seddon et al. 2006). Technical improvements in the quality and nature of imaging techniques, such as fundus autofluorescence, scanning laser ophthalmoscopy with and without adaptive optics, and functional diagnostics (e.g., dark adaptation, contrast sensitivity), have further advanced the characterization of subphenotypes of both early and late-stage AMD (Bindewald et al. 2005, Godara et al. 2010, Mrejen et al. 2014, Suzuki et al. 2014). On a cellular and subcellular level, histological study by light and electron microscopy spawned different grading systems (Sarks 1976), and AMD classifications also exist for eye bank tissue and donor eyes (Curcio et al. 1998, Olsen & Feng 2004).

In this section, we explore the histological basis for identifying and classifying changes in the retina, RPE, and choroid with age, as well as its consequence for AMD pathology. We further describe the advantages and disadvantages of macroscopic phenotype grading and elucidate possible discrepancies between grading based on microscopy versus macroscopy.

### Histological Classification

By definition, the risk of being affected with an age-related disease increases with age. Therefore, distinguishing normal aging from disease-associated processes is crucial. In particular, understanding AMD pathology requires differentiating between normal aging and (early) AMD (**Figure 1**). Any age-related changes in the retina involve multiple layers of the outer retina (photoreceptors and/or RPE) and adjacent tissue [Bruch's membrane (BrM) and/or the choroid].

**Normal aging.** The changes presented in this section are associated with normal aging and, as such, should not be associated with vision impairment. Cells of each layer of the photoreceptor-RPE-BrM-choriocapillaris complex exhibit unique features to enable visual function and to ensure an intact outer retina (**Figure 1*a***). Photoreceptors are integral to phototransduction and the visual cycle. Cells in the RPE phagocytose outer segments that have been shed by photoreceptors, recycle visual cycle metabolites, maintain nutrient flow to the outer retina, and secrete cytokines and lipoproteins (Strauss 2005). BrM is a multilayered extracellular matrix embedded between the RPE and the choriocapillaris, and, similar to a vessel wall (Jackson et al. 2002), it regulates

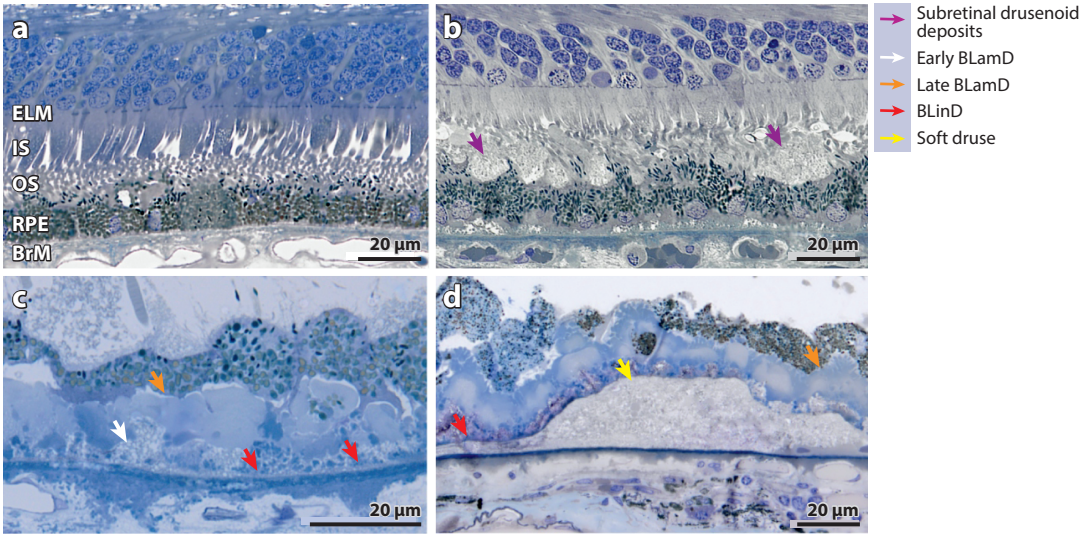


**Figure 1**

Histological changes in the outer retinal neurovascular unit [photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane (BrM), choroid] in age-related macular degeneration. (*a*) Histology of healthy layers of the outer retina: external limiting membrane (ELM), photoreceptor outer segment (OS), and inner segment (IS), RPE, and BrM in a normal eye. OS compaction is a result of tissue preparation. (*b–d*) Early AMD retinal changes. (*b*) Subretinal drusenoid deposits (*purple arrows*) at the apical side of the RPE. (*c*) Basal laminar deposits (BLamD) are a thickening of the basal lamina of the RPE in early (*white arrow*) and late-stage (*orange arrow*) forms. Basal linear deposits (BlinD; *red arrows*) are extracellular deposits at the basal side of RPE cells. (*d*) A BLinD (*red arrow*) is continuous with a soft druse (*yellow arrow*); BLamD (*orange arrow*) sit on top of the BLinD. Artificial detachment of photoreceptors. Toluidine blue stain. Scale bar: 20 µm. All images are courtesy of J.D. Messinger and C.A. Curcio. **http://projectmacula.cis.uab.edu**.

Erratum

metabolic transport between these two tissues (Booij et al. 2010, Ramrattan et al. 1994, Sivaprasad et al. 2005). Finally, the choriocapillaris is a fenestrated tissue responsible for providing nutrition and oxygen to support the outer retina (Ramrattan et al. 1994). This tissue complex develops characteristic changes with aging (see below), but it still maintains its integrity, as age-related changes normally do not significantly alter physiological cell and tissue metabolism.

Rod photoreceptor density diminishes with age in both the extrafoveal and the foveal retina, whereas cone density in the fovea remains stable (Curcio et al. 1993). RPE cell density in the macula remains stable (Ach et al. 2014), and the foveal photoreceptor–RPE ratio does not change with age (Gao & Hollyfield 1992). Cells of the RPE accumulate significant amounts of autofluorescent material, which are stored as lipofuscin and melanolipofuscin granules in lysosomal compartments. Simultaneously, the number of melanosomes per cell decreases with age (Feeney 1978, Feeney-Burns et al. 1984). Both mechanisms significantly increase total autofluorescence per cell, without affecting RPE cell density (Ach et al. 2014).

BrM in the macula thickens with age (Feeney-Burns & Ellersieck 1985, Ramrattan et al. 1994) owing to abundant accumulation of lipoproteins (Rudolf & Curcio 2009, Wang et al. 2009), mineralization (Spraul et al. 1999), and hyalinization (Sarks 1976). Additional age-related changes in BrM structure result from modulated expression of proteins such as collagen, elastin, and matrix metallopoteinases in all layers of the membrane (Kamei & Hollyfield 1999, Marshall et al. 1992). The thickening of BrM leads to decreased permeability in anterograde or retrograde flux (Moore

et al. 1995), which in turn can result in further accumulation of deposits and lead to a delicate transition between age-related changes and early AMD pathology.

The thicknesses of the choriocapillaris and choroid also decrease with age, as confirmed by histology and spectral domain optical coherence tomography (SD-OCT) (Ramrattan et al. 1994, Spaide 2009). All aforementioned changes are by definition age-related, not pathological, although some may also precede AMD.

**Early age-related macular degeneration.** Clinically, early AMD has a heterogenous manifestation and should be carefully distinguished from normal aging processes. Characteristic changes in early AMD include deposition of extracellular material into the sub-RPE space (drusen) and RPE pigmentary changes (hypopigmentation and/or hyperpigmentation). Deposits include basal laminar deposits (BLamD) and basal linear deposits (BLinD), as well as drusen (deposits between the basal lamina of the RPE and the inner collagenous layer of BrM) and subretinal drusenoid deposits (SDD; also called reticular drusen) at the apical side of the RPE (**Figure 1*b–d***).

BLamD comprise individual deposits or a continuous layer with a thickness of up to a few micrometers located between the RPE and its basal membrane (**Figure 1*c,d***) (Curcio & Millican 1999). The deposits consist of various proteins such as metalloproteinases, tissue inhibitors of metalloproteinases, laminin and collagen, and esterified and unesterified cholesterols (Curcio et al. 2005b, van der Schaft et al. 1994). BLamD are thought to increase the risk of advanced AMD (Sarks et al. 2007).

BLinD are composed of membranous material and lakes of lipids and lipoproteins (Curcio et al. 2005b, Malek et al. 2003). They are located between the basal lamina of the RPE and the inner collagenous layer of BrM, which is thickest in the fovea (**Figure 1*c,d***) (Curcio et al. 2010). Several authors have suggested that BLinD and soft drusen may interchange (Curcio & Millican 1999, Sarks 1982).

Drusen consist mostly of unesterified and esterified cholesterols and phospholipids, along with proteins (Coleman et al. 2008, Crabb et al. 2002, Malek et al. 2003, Mullins et al. 2000, Sarks 1982); the latter have been shown to be part of inflammatory processes (Anderson et al. 2002, Johnson et al. 2000). Drusen are described as being either hard or soft, in relation to their characteristic edges. Soft drusen are restricted to the macula, have abundant BlamD (Rudolf et al. 2008), and are a risk factor for AMD progression (Magnusson et al. 2006). Hard drusen, in contrast, are not restricted to the fovea and macula, are also found in normal aging but do not predict AMD disease (Klein et al. 2007).

SDD are extracellular lesions located between the outer segments of photoreceptors and the apical sides of the RPE cells (**Figure 1*b***). These deposits consist of unesterified cholesterol and are preferentially detectable at the perifovea (Curcio et al. 2005a). Occurrence of SDD is associated with a higher risk of geographic atrophy (GA) (Curcio et al. 2005a, Rudolf et al. 2008, Schmitz-Valckenberg et al. 2011).

These characteristic changes in the photoreceptor-RPE-BrM-choriocapillaris complex can be evaluated with refined imaging techniques such as OCT or magnetic resonance tomography (MRT) and provide useful insights into the disease. Further, both fine mapping of the early stages of the disease, including novel disease categories such as drusen area, drusen type, and dyspigmentation area, and genetic association testing can shed new light on underlying (eventually subtype-specific) disease processes. Note, however, that there are limitations to how these techniques can be used in epidemiological settings. We discusses these limitations in later sections.

**Late-stage age-related macular degeneration.** Although pathological changes associated with early AMD do not affect the function of the inner retina and thus typically are not associated with

visual impairment, late-stage AMD is categorized into nonexudative atrophic AMD and exudative neovascular (NV) AMD. Both of these manifestations lead to severe vision loss. In NV AMD, activated endothelial cells are stimulated by the release of vascular endothelial growth factors (VEGFs) (Pe'er et al. 1995) secreted from the RPE (Adamis et al. 1993). The endothelial cells originate in the choriocapillaris and extend through BrM into the sub-RPE and/or subretinal space (Killingsworth 1995). Breaks in a calcified and fragmented BrM otherwise not observed in age-matched controls or patients with atrophic AMD foster the passage of these activated endothelial cells through BrM (Spraul & Grossniklaus 1997), although their specific path through the membrane might be related to the presence of soft drusen (Sarks et al. 1997). In more advanced stages of NV AMD, vessels appear in the fibrous tissue with or without exudation, and choricapillary atrophy can be observed. A review by Spaide and colleagues summarizes different theories of choroidal neovascularization (Spaide et al. 2003).

GA is the late stage of nonexudative AMD and is characterized by advanced RPE cell loss and depigmentation (hyperpigmentation and hypopigmentation) (Coleman et al. 2008). Hyperpigmented cells can also be found in the subretinal space. In the area of RPE atrophy, photoreceptors may or may not be present, and these cells may be accompanied by a few abnormal RPE cells (Sarks et al. 1988). The progression from early to late-stage AMD and the underlying risk factors can be evaluated with longitudinal studies involving observation of elderly individuals for years or decades. Several studies suggest that the same genetic and environmental factors identified in case-control studies also impact disease progression (Buitendijk et al. 2013, Seddon et al. 2009).

## Clinical Classification

Several clinical classifications describe a number of phenotypes of different AMD stages and/or risk of AMD progression (Ferris et al. 2005, Klein et al. 1991, Seddon et al. 2006). More recently, an international expert panel separated normal aging changes from early AMD [characterized by medium drusen (63 to 125 μm in diameter) and absence of AMD-related pigmentary abnormalities], intermediate AMD [characterized by large drusen (more than 125 μm in diameter) and/or presence of any AMD-related pigmentary abnormalities], and advanced AMD (Ferris et al. 2013). The advanced stage was further dichotomized into NV AMD versus any GA AMD. The presence of small drusen (63 μm in diameter or less, called druplets) and the absence of pigmentary abnormalities were classified as normal aging changes. This classification did not consider subretinal drusenoid deposits (SDD, reticular pseudodrusen), although several studies provide evidence for a relation of such deposits with AMD and AMD progression (Alten & Eter 2015, Curcio et al. 2013, Hogg et al. 2014).

## Disease Classification and Histology

Comparing clinical disease classification with histological findings in early and late-stage AMD uncovers inconsistencies that should be considered in future study designs. Histological findings are based on mostly ex vivo histopathological imaging, and such findings generally occur before clinically visible manifestations (Sarks 1976). For instance, hard drusen in the Age-Related Eye Disease Study (AREDS) are defined as deposits with diameters that are less than or equal to 63 μm. Drusen are visible in clinical fundus images only if their diameters exceed 30 μm (Sarks et al. 1999). Therefore, although they might influence RPE physiology early and adversely, smaller drusen or deposits are not visible in standard clinical images. Lesions at the fovea that are 30 μm in diameter affect at least four to nine RPE cells (Ach et al. 2014), and changes in late-stage AMD (e.g., GA AMD) extend over a much larger area, involving hundreds or thousands of RPE cells.

It becomes apparent that subtle changes in early phases of AMD pathology are likely missed. Nevertheless, recruitment of large cohorts in early AMD studies mostly does not include in vivo imaging methods with adequate resolution.

In the next sections, we describe the current knowledge of the genetics of AMD, emphasizing study designs and their limitations. Further, we suggest future study designs that should be useful to genetically characterize in depth early and late-stage disease.

## THE GENETICS OF LATE-STAGE AGE-RELATED MACULAR DEGENERATION

The genetics of late-stage AMD is well understood compared with that of early AMD (Fritsche et al. 2013), keeping in mind the necessary caution with regard to phenotyping issues as elaborated above. Often, both late-stage forms (GA and NV AMD) are analyzed jointly as one disease status (termed late-stage AMD). The heritability of late-stage AMD has been estimated to range between 45% and 71% (Seddon et al. 2005), well in line with the estimated heritability of other complex diseases such as Alzheimer's disease, Crohn's disease, psoriasis, obesity, and epilepsy (Gatz et al. 2006, Kjeldsen et al. 2001, Tysk et al. 1988, Walley et al. 2006). The first findings regarding genetic risk factors for AMD were reported in 1997 and implicated mutations in *ABCA4*, a gene initially associated with autosomal recessive Stargardt disease (Allikmets et al. 1997), although this association was controversial, as several studies failed to replicate the initial finding (Rivera et al. 2000, Stone et al. 1998). Only later were monoallelic changes in *ABCA4* significantly associated with a relatively rare subphenotype of atrophic AMD defined by fundus autofluorescence, namely a fine granular RPE pattern with peripheral punctate spots (Fritsche et al. 2012). In 1998, a significant association between late-stage AMD and genetic variants in the apolipoprotein E (*APOE*) gene was reported (Klaver et al. 1998). This finding was subsequently replicated in several independent studies, thereby implicating lipid metabolism in disease pathology (Baird et al. 2006, McKay et al. 2011, Zareparsi et al. 2004). Heralding the era of GWAS, Klein et al. (2005) identified a common variant (rs1061170, p.Y402H) in the *CFH* gene strongly associated with AMD. A second, similarly strong signal was later observed in the *ARMS2/HTRA1* locus on 10q26 (Jakobsdottir et al. 2005, Rivera et al. 2005), and additional candidate gene studies identified AMD risk variants in *C3* and *CFB* (Hageman et al. 2005, Spencer et al. 2008). A recent meta-analysis that included 17,000 cases and 60,000 controls implicated a total of 19 loci in AMD (Fritsche et al. 2013). More detailed reviews of genetic studies and identified variants in AMD are given in articles by Fritsche et al. (2014) and Gorin (2012).

Notably, the estimated odds ratios (ORs) per adverse allele for some of these variants exceed 2.0 (i.e., more than double the risk for late-stage AMD), making the genetics of late-stage AMD rather unique among complex diseases. In general, the effect sizes for common variants associated with complex diseases are low (ORs less than1.2), although the odds ratios for rare variants (those with allele frequencies below 1%) may be strikingly high. **Figure 2** shows the relationship between the variant frequency and the observed effect size for risk variants associated with AMD. Variants associated with Alzheimer's disease and psoriasis are given for comparison underscoring several AMD-associated variants that exceed their expected effect size by large margins (e.g., *ARMS2/HTRA1*, *CFH*, *CFB*, and *C3*). The reason underlying this unique feature of AMD genetics is unknown.

Because only a few variants explain a large proportion of its heritability, late-stage AMD has become a paradigm for the genetic dissection of complex diseases. The 19 risk loci currently implicated in AMD pathology explain 10–30% of the disease variance, corresponding to 15–65% of the heritability, depending on the estimated age prevalence of late-stage disease

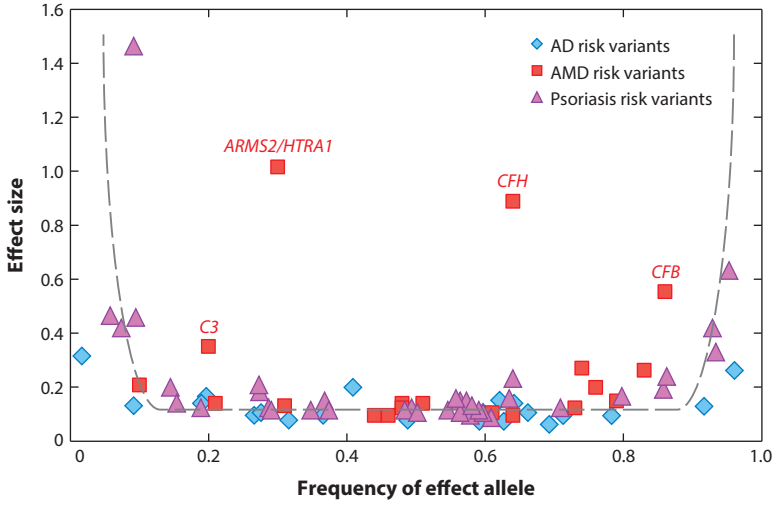


**Figure 2**

Plot of effect size versus effect-allele frequency of early and late-stage age-related macular degeneration (AMD), Alzheimer's disease (AD), and psoriasis risk variants. For complex diseases, the observed effect sizes usually depend on the frequency of the variant. Common variants generally have low effect sizes, whereas rare variants have increased effect sizes. For AMD-associated variants, several loci exceed this expectation (*ARMS2/HTRA1*, *CFH*, *CFB*, and *C3*). Data are based on studies by Fritsche et al. (2013), Lambert et al. (2013), and Tsoi et al. (2012).

pathology (Fritsche et al. 2013). This observation has led to efforts to predict disease risk for late-stage AMD purely on the basis of genetic factors. Such genetic risk models are based on strongly associated variants and demonstrate good classification accuracies that are useful for categorizing individuals as having a high or low risk of developing AMD (Buitendijk et al. 2013, Grassmann et al. 2014), although the clinical utility of such models for prediction has yet to be shown.

## THE GENETICS OF EARLY AGE-RELATED MACULAR DEGENERATION

The genetics of early stage AMD is still in its infancy despite an urgent medical need for a better understanding of early disease processes and early treatment options. One reason for the lack of knowledge is that early AMD usually is clinically asymptomatic and therefore observed less frequently in ophthalmology clinics. In addition, general population-based studies recruit individuals from all age ranges, with recruitment of large cohorts with signs of early AMD a cumbersome task. From twin studies, the heritability of early AMD was estimated to be 35–55%, lower than the estimated heritability of late-stage AMD (Hammond et al. 2002).

A recent meta-analysis of five GWAS implicated the *CFH* and the *ARMS2/HTRA1* loci in early AMD (Holliday et al. 2013). Additionally, the authors found association of early AMD with gene loci previously not associated with late-stage AMD, although these findings failed to reach a genome-wide level of significance. If replicated, the novel loci could provide guidance for understanding novel pathways in early disease pathology. Notably, the risk variants in *CFH* and *ARMS2/HTRA1* showed genetic association in the same direction for early and

late-stage AMD (i.e., alleles associated with increased risk of late-stage AMD were also associated with increased risk of early AMD), although the effect sizes were strikingly lower in early disease. This result is startling but could be explained by a situation in which risk variants simply confer small effect sizes in early disease. Alternatively, assuming clinical heterogeneity in early AMD would result in groups of subphenotypes, each possibly having its own set of risk-associated gene variants. Such heterogeneity in early AMD may arise in several ways and could result from (*a*) inconsistencies in grading schemes across studies; (*b*) intergrader variability (Klein et al. 2014); (*c*) lack of a defined disease variable (e.g., cumulative drusen area); or (*d*) true underlying heterogeneity, namely, phenocopies within early AMD not progressing to the late-stage form. Understanding both the spectrum of risk factors underlying early disease events and the processes of disease progression may lead to interesting options in preventive treatment.

## CURRENT STUDY DESIGNS: THE GOOD, THE BAD, AND THE SAMPLE SIZE

Currently, ocular imaging is limited and depends on the underlying technique (Spaide et al. 2010, Zweifel et al. 2010), suggesting that although recent developments in imaging technology are promising, current patient recruitment settings are not able to represent the wide spectrum of AMD phenotypes, particularly those for early signs of the disease. For example, SD-OCT revealed changes in the outer plexiform layer (OPL) and in the inner nuclear layer, along with the development of hyporeflective wedge-shaped bands in the OPL in areas of drusen-associated atrophy (termed nascent GA) in patients with early AMD (Wu et al. 2014). Such clinical findings can be used as novel variables to search for genetic variants associated with AMD, but they may also be helpful as early surrogate end points in interventional trials for early AMD. Similarly, adaptive optics scanning laser ophthalmoscopy (Godara et al. 2010, Zarbin et al. 2014, Y. Zhang et al. 2014) could broaden the phenotypic spectrum and thus lessen the clinical heterogeneity of patient samples used to identify genetic susceptibilities.

The need for large sample sizes may have once led to collapsing phenotypes (GA and NV for late-stage AMD, and all subtypes for early AMD) and to including phenotypes ascertained by heterogeneous imaging technologies (e.g., fundus exam, fundus photography, OCT). However, future genetic studies that include refined phenotypes and standardized ascertainment are anticipated to substantially further our knowledge about detailed mechanisms in disease pathology. For instance, dissection of the genetic landscape of drusen etiology will be feasible, and identification of the causes underlying an increased likelihood for progression to late AMD may be possible. In addition, large sample sizes will allow the dissection of the contribution of environmental factors in conjunction with genetic factors on disease risk (i.e., gene × environment analysis).

Another aspect of study design also warrants consideration. Many current studies are hospital-based case-control studies, and only a few of them include the general aged population. Only studies that include this population allow the investigation of phenotype development and progression in a longitudinal setting (AREDS 2001, Buitendijk et al. 2013), although they necessitate elaborate logistics because of the advanced age of participants and considerable financial commitments. In addition, comorbidities may interfere with data acquisition, posing further difficulties.

Future studies into the genetics of early and late-stage AMD are anticipated to include large sample sizes and to use a standardizable and easily applicable technology to assess a wide spectrum of phenotypes. Such studies are likely to not only uncover the full genetic spectrum of early or

late-stage AMD, but also allow a refined view of defined aspects of early AMD pathology (e.g., many small drusen versus few large drusen) and how they progress to late stages of the disease. Issues to be addressed in studies of late-stage AMD could include identification of factors that influence therapy success, the recurrence of wet or dry AMD in the treated eye, or the progression of dry AMD once late-stage disease has already developed.

In many cases, AMD patients enrolled in clinical trials are stratified for genetic markers, fostering targeted and personalized drug development. For example, the aim of clinical trial NCT01363570 is to identify an association between genetic risk factors and treatment response. Another trial, NCT02051998, seeks to elucidate factors associated with the growth of GA lesions. This trial also considers subphenotypes of GA AMD (Holz et al. 2007). Finally, NCT01115387, a study on the genetics of age-related maculopathy, is interested in evaluating the presence or absence of early retinal changes with age-related maculopathy by imaging techniques such as OCT, fundus autofluorescence (FAF), and fundus images.

## IN THE LONG RUN: IDENTIFICATION OF GENES UNDERLYING PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION

Although GWAS have proven most successful in identifying disease-associated genetic variants (Edwards et al. 2013), the resolution of this approach is insufficient to reveal the causal variant and the underlying gene in most instances. Nevertheless, these studies often point to a candidate gene close to a lead signal (the variant with the strongest signal of association) (Edwards et al. 2013). Notably, the signal with the highest association is not necessarily the causal variant, owing to the design of genotyping platforms that are meant to assay an underlying haplotype structure by genotyping a minimum set of DNA variants. Strategies to identify the responsible gene, and possibly the causal variant, have been covered extensively in an article by Edwards et al. (2013). In the following subsections, we selectively focus on some crucial steps and pitfalls in this process.

### Statistical and Bioinformatics Methods

Following a GWAS, the initial step to determine a functional variant aims to refine the mapping of a broadly identified gene locus. This step includes the imputation of missing or nongenotyped variants based on a large reference sample set, such as that of the 1000 Genomes Project (**http://www.1000genomes.org/**). Subsequently, forward conditional logistic regression models are applied to separate independent signals at a given locus. If an independent signal results from a single associated haplotype carrying the responsible variant, this approach identifies not only the responsible variant, but also identifies other highly correlated variants of this same haplotype. Signals with a single associated haplotype can be further subjected to annotation and functional characterization. Previous studies have shown that many independent signals are the result of two or more distinct variant associations from different haplotypes, however, and the signal thus represents the joint effect of those haplotypes (Gold et al. 2006, Hageman et al. 2005). Haplotypes can reliably be estimated by phasing the genotypes, which is done routinely prior to imputation (Gorski et al. 2014).

Owing to meiotic recombination, several distinct haplotypes are observed at each gene locus, with frequencies varying from common (>5%) to unique. So far, there is no unified statistical approach to account for the number of possible haplotypes. The classic approach uses all genetic variants at a locus to construct haplotypes while excluding rare ones from analysis, but these rare haplotypes might be the ones that carry the responsible variant. Another approach is to cluster

similar haplotypes and to perform statistical analyses with the resulting canonical or consensus haplotypes (Teo & Small 2010). However, it is not obvious a priori how many distinct canonical haplotypes exist. Thus, this and similar approaches have limitations and need to be tested using existing data sets. Moreover, the results of these approaches need to be compared with those of classical haplotype analyses (Boyle et al. 2012, Sifrim et al. 2012).

Subsequent to the analyses described above, variants of associated haplotypes are subjected to in silico annotation with biological features (called biofeatures), which are widely available from public data sets (Coetzee et al. 2012), and ranked according to their potential impact (**Table 1** and **Figure 3**). Ultimately, top-ranked variants can be subjected to additional experimental approaches designed to test for functional implication in a biological context.

## Functional Annotation of Risk Variants Associated with Age-Related Macular Degeneration

To demonstrate the power of bioinformatic approaches for functional annotation, we have extracted all genetic variants highly correlated with the 19 lead variants ($R^2 > 0.7$) as given in an article by Fritsche et al. (2013). Highly correlated variants were then annotated in an effort to identify candidate functional variants by integrating information from tag variant locations, lists of linked variants from the 1000 Genomes Project, and locations of chromatin features with possible functional significance (Coetzee et al. 2012). This process identified coding variants in the *C3*, *CFH*, *ARMS2*, *HTRA1*, *COL10A1*, *APOE*, and *AGPAT3* genes (**Table 1** and **Figure 3**). Variants in the 3′ untranslated region of the *TGFBR1* gene were also identified, as were several variants previously described as being expressed quantitative trait loci that regulate transcript expression in lymphoblastoid cell lines (Liang et al. 2013, Zou et al. 2012). Notably, by analyzing high-throughput RNA sequencing data from human total retinas, ARPE-19 cells, human choroid tissue, and RPE cells derived from human induced pluripotent stem cells (hiPSCs) (Brandl et al. 2014), we established three additional candidate functional variants in novel exons of two possible AMD genes. In other AMD loci, we compared the locations of candidate variants with those of putative regulatory regions predicted by ENCODE (Encyclopedia of DNA Elements) (Kellis et al. 2014) and FANTOM 5 (Functional Annotation of the Mammalian Genome 5) (Andersson et al. 2014, Forrest et al. 2014) and found overlap with sites of DNase hypersensitivity. We also found overlap with putative transcription factor binding sites in two loci, as estimated from high-throughput DNA sequencing of chromatin immunoprecipitation (ChIP-Seq) (Lefrançois et al. 2010).

## Experimental Methods

An often repeated criticism of current findings in AMD genetics is that the vast knowledge about associated genetic variants has not yet resulted in defining appropriate mouse models for AMD and, more importantly, has not led to novel treatment options. Although several clinical trials addressing the complement cascade have been initiated in the past few years, therapeutic efficacy in AMD has yet to be shown (Weber et al. 2014, Williams et al. 2014). Similarly, the authors of a comprehensive overview of suitable mouse models for AMD note that "In spite of the large number of models developed, no one model yet recapitulates all of the features of human AMD." (Pennesi et al. 2012, p. 487).

This recognition has redirected attention from animal models to cell culture–based models for analyzing the cellular physiology of AMD. These models involve reprogramming adult somatic cells into a pluripotent state via overexpression of four defined transcription factors, an approach

**Table 1 Functional annotation of age-related macular degeneration (AMD)-associated variants[a]**

| Location | Locus name[b] | Lead variant[b] | Candidate variant | Correlation with top hit ($R^2$) | Annotation(s) | Gene implicated |
|---|---|---|---|---|---|---|
| chr1 | *CFH* | rs10737680 | rs2274700 | 0.99 | Coding exon | *CFH* |
| chr3 | *COL8A1/FILIP1L* | rs13081855 | rs62283492 | 0.97 | RNASeq exon iRPE2 | *COL8A1* |
| chr3 | *COL8A1/FILIP1L* | rs13081855 | rs6808963 | 0.75 | RNASeq exon ARPE1; RNASeq exon ARPE2; RNASeq exon iRPE2 | *COL8A1* |
| chr3 | *ADAMTS9/MIR548A2* | rs6795735 | | | | |
| chr4 | *CFI* | rs4698775 | rs13846 | 0.90 | Coding exon | *AGPAT3* |
| chr6 | *IER3/DDR1* | rs3130783 | rs116487320 | 0.73 | DNase I hypersensitivity (ENCODE V2); FANTOM5 enhancers | |
| chr6 | *C2/CFB* | rs429608 | rs149176277 | 0.70 | RefSeq promotor; Lymphocyte eQTL (Liang et al. 2013) | *DOM3Z* |
| chr6 | *COL10A1* | rs3812111 | rs1064583 | 0.99 | Coding exon | *COL10A1* |
| chr6 | *VEGFA* | rs943080 | | | | |
| chr8 | *TNFRSF10A* | rs79037040 | rs13255394 | 0.77 | ncRNA gene; lymphocyte eQTL (Liang et al. 2013) | *LOC389641* |
| chr8 | *TNFRSF10A* | rs79037040 | rs11777697 | 0.76 | Lymphocyte eQTL (Liang et al. 2013) | *LOC389641* |
| chr8 | *TNFRSF10A* | rs79037040 | rs13254617 | 0.78 | Lymphocyte eQTL (Liang et al. 2013) | *LOC389641* |
| chr9 | *TGFBR1* | rs334353 | rs1590 | 0.94 | 3′ UTR | *TGFBR1* |
| chr9 | *TGFBR1* | rs334353 | rs334348 | 0.94 | 3′ UTR | *TGFBR1* |
| chr9 | *TGFBR1* | rs334353 | rs334349 | 0.93 | 3′ UTR | *TGFBR1* |
| chr10 | *ARMS2/HTRA1* | rs10490924 | rs1049331 | 0.96 | Coding exon | *HTRA1* |
| chr10 | *ARMS2/HTRA2* | rs10490924 | rs2293870 | 0.89 | Coding exon | *HTRA1* |
| chr10 | *ARMS2/HTRA3* | rs10490924 | rs10490924 | 1.00 | Coding exon | *ARMS2* |
| chr13 | *B3GALTL* | rs9542236 | rs9542236 | 1.00 | Brain eQTL (Zou et al. 2012) | *B3GALTL* |
| chr14 | *RAD51B* | rs8017304 | rs3784099 | 0.72 | DNase I hypersensitivity (ENCODE V2); FANTOM5 enhancers | |
| chr15 | *LIPC* | rs920915 | | | | |
| chr16 | *CETP* | rs1864163 | | | | |
| chr19 | *C3* | rs2230199 | rs1047286 | 0.83 | Coding exon | *C3* |
| chr19 | *C3* | rs2230199 | rs2230199 | 1.00 | Coding exon | *C3* |
| chr19 | *C3* | rs2230199 | rs2230203 | 0.72 | Coding exon | *C3* |
| chr19 | *APOE* | rs4420638 | rs429358 | 0.74 | Coding exon | *APOE* |

(*Continued*)

**Table 1** (*Continued*)

| Location | Locus name[b] | Lead variant[b] | Candidate variant | Correlation with top hit ($R^2$) | Annotation(s) | Gene implicated |
|---|---|---|---|---|---|---|
| chr22 | *SLC16A8* | rs8135665 | rs67460670 | 0.86 | RNASeq exon hChoroid; RNASeq exon iRPE1; RNASeq exon iRPE2 | *SLC16A8* |
| chr22 | *TIMP3* | rs5749482 | | | | |

[a]Analyses were done based on the algorithms implemented in the FunciSNP R/Bioconductor package.
[b]Locus names and variants are included according to Fritsche et al. (2013).
Empty cells indicate no data are available. Abbreviations: ENCODE, Encyclopedia of DNA Elements; eQTL, expression quantitative trait locus; FANTOM5, Functional Annotation of the Mammalian Genome; ncRNA, noncoding RNA; RefSeq, NCBI Reference Sequence Database; RNASeq, RNA Sequencing/Whole Transcriptome Shotgun Sequencing; UTR, untranslated region.

that has become widely used to routinely differentiate such cells into any somatic cell type (Takahashi et al. 2007, Yu et al. 2007) and as such is now appreciated as a valuable cell-based system for disease modeling, drug screening, and testing transplantation therapy for human degenerative diseases (Yamanaka 2012).

hiPSC technology is gaining momentum in the era of personalized medicine because it offers the prospect of establishing individual, patient-specific cell lines. Otherwise inaccessible adult donor cells can regularly be obtained noninvasively from accessible somatic cell sources such as skin samples, blood lymphocytes, or endothelial cells from urine collection (Kim 2014, Zhou et al. 2012). Moreover, advanced methods allow the generation of transgene- or integration-free hiPSCs, thus ensuring safety and suitablity for clinical application (Zhou & Zeng 2013). For a more detailed description of reprogramming methods, the reader is referred to a review by Zhou et al. (2012). Prospects and pitfalls of the iPSC technology were summarized in a recent article by Brandl et al. (2015).

Cell types of interest in AMD pathology include the vascular endothelium, the photoreceptors, and the RPE, all of which are not readily accessible from the patient but can be generated via hiPSC differentiation (Adams et al. 2013, Brandl et al. 2014, Buchholz et al. 2013, Krohne et al. 2012, Parameswaran et al. 2010, Singh et al. 2013b). As the RPE is suspected to be the cellular origin of primary AMD pathology, it has attracted particular interest in this field. RPE differentiation from hiPSCs is straightforward, and several protocols for direct differentiation of these cells have been established (Brandl et al. 2014, Buchholz et al. 2013, Krohne et al. 2012, Singh et al. 2013a). These stem cell–derived RPE cultures were reported to yield pure populations of highly functional cells that display many physical features of true native RPEs, such as cell morphology and pigmentation and gene and protein expression of mature RPE markers, in addition to functional capabilities such as transepithelial resistance, photoreceptor outer segment phagocytosis, or polarized secretion of known biological factors including PEDF (pigment epithelium–derived factor) and VEGF (Brandl et al. 2014, Buchholz et al. 2013, Krohne et al. 2012, Singh et al. 2013a). An important aspect in terms of establishing cell repositories is the ability of hiPSC-derived RPE cells to regain viability and appropriate functional properties after cryopreservation (Brandl et al. 2014).

The CRISPR (clustered regularly interspaced short palindromic repeats)-associated nuclease Cas9 system provides another emerging tool to study variant functionality by modifying a genomic locus of choice using an RNA-guided nuclease (Cas9) and a guide RNA that is complementary to a target sequence (Shalem et al. 2014, Wang et al. 2014). Combining this system with hiPSC technology is currently revolutionizing research into disease mechanisms, as it allows

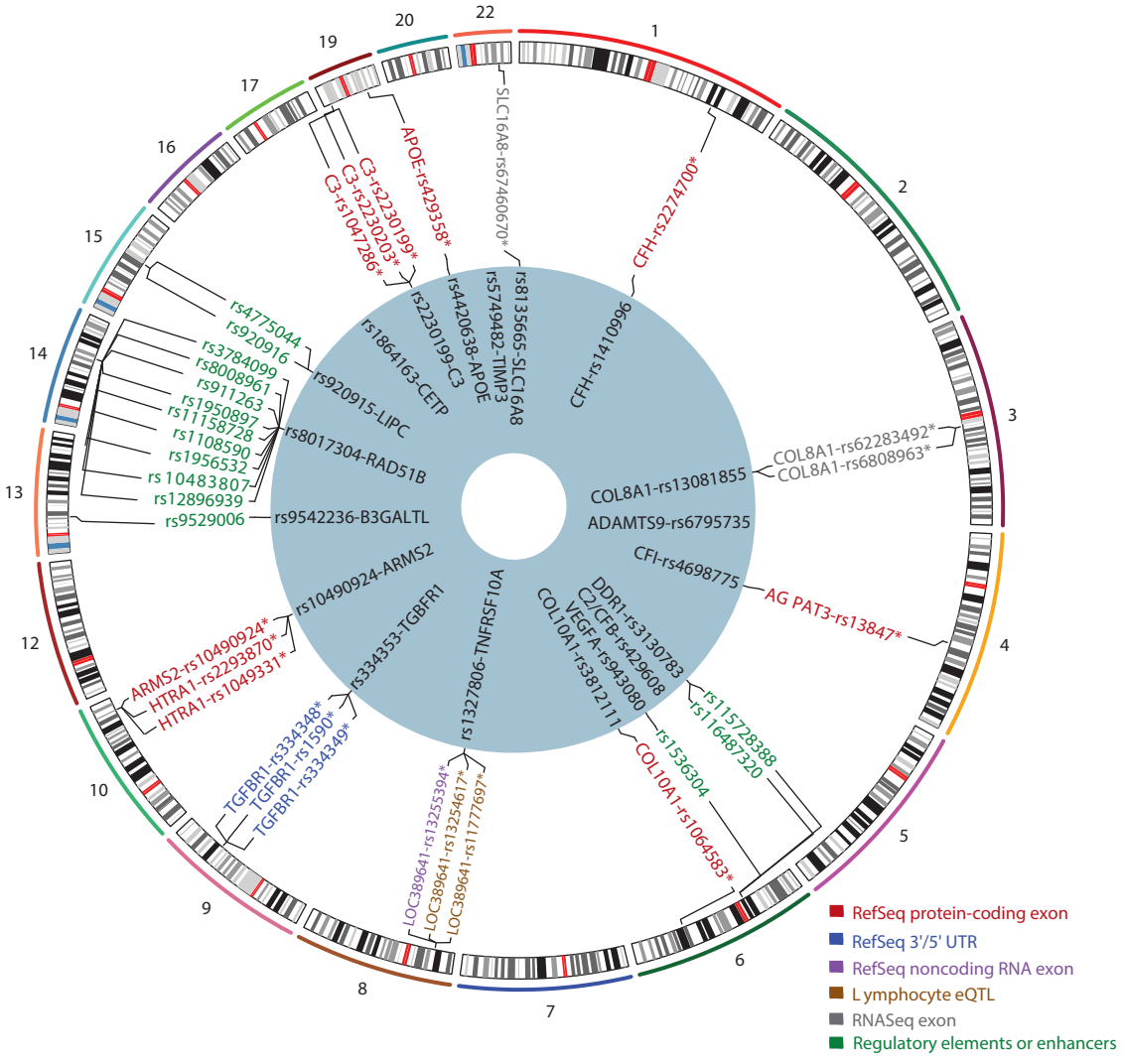


**Figure 3**

Functional annotation of age-related macular degeneration (AMD)-associated candidate variants. Top variants as well as variants highly correlated with the top variant ($R^2 > 0.7$) in 19 AMD-associated loci were annotated based on biofeatures implemented in the R/Bioconductor package FunciSNP, and a detailed map showing the locations and annotations of AMD-associated candidate variants and regions in the genome was plotted. Candidate variants are color coded by functional category. Asterisks indicate transcripts expressed in the human (h) retina, human retinal pigment epithelium (RPE), or human RPE derived from induced pluripotent stem cells (iPSCs). Several previously published functional variants are not shown; these variants had poor correlations ($R^2 > 0.7$) with the top single-nucleotide polymorphism and thus were not detected by simple correlation analyses. Abbreviations: eQTL, expression quantitative trait locus; RefSeq, NCBI Reference Sequence Database; RNASeq, RNA sequencing/whole transcriptome shotgun sequencing; UTR, untranslated region.

targeted manipulation of cell lines within a background of defined genetic risk and nonrisk factors (F. Zhang et al. 2014). In addition, such cellular models can be challenged by chronic stressors that mimic environmental or endogenous factors known to influence disease pathology. In AMD, such challenges may include rod photoreceptor outer segment (ROS) feeding (Birch & Liang 2007), cigarette extracts and nicotine (Chakravarthy et al. 2010), or activation of the complement cascade via human sera (Skerka et al. 2007).

## PATHWAYS INVOLVED IN PATHOGENESIS OF AGE-RELATED MACULAR DEGENERATION

We conducted a pathway-enrichment analysis using WebGestalt (Wang et al. 2013) and included AMD-associated genes with functional variants established either by previous experimental evidence or by bioinformatics studies, as detailed above. The genes we used included the following: *CFH*, *C3*, *CFB*, *C2*, *CFHR1*, *CFHR3*, *TGFBR1*, *HTRA1*, *ARMS2*, *AGPAT3*, *APOE*, *COL10A1*, *COL8A1*, *SLC16A8*, *LOC389641*, *DOM3Z*, and *B3GALTL*. This approach allows identification of a single significant pathway or biological process represented by the complement activation pathway (in this case, GO:0006956 and KEGG:hsa04610), which has raw *p*-values that are below the genome-wide significance level of $5 \times 10^{-8}$. Although other pathways, for example, lipid metabolism and extracellular matrix pathways, have been implicated in disease etiology (Fritsche et al. 2013, Yu et al. 2011), little evidence exists to support their involvement, other than an observed association near genes within them (e.g., near *TIMP3* or near *LIPC*). Until the associated causal variant at each locus is experimentally verified, any GWAS-deduced pathway possibly involved in AMD pathogenesis, with the exception of the complement cascade, is hypothetical and should be treated with caution.

## TREATMENT FOR AGE-RELATED MACULAR DEGENERATION

Recent developments in the treatment of AMD for late-stage neovascular and geographic atrophy have been reviewed extensively (Bowes Rickman et al. 2013, Holz et al. 2014, Smith & Kaiser 2014, Weber et al. 2014). In addition, the effects and safety of complement inhibitors in the prevention or treatment of advanced AMD were systematically reviewed in a recent article by Williams et al. (2014), although the results are currently inconclusive owing to the lack of randomized controlled trials. An overview of preclinical data demonstrating the potential of transplanted (stem cell–derived or iPSC-derived) RPE cells to functionally rescue photoreceptors is given in an article by Westenskow et al. (2014).

### Treatment of Neovascular Age-Related Macular Degeneration

Nine out of ten ongoing clinical trials aim to attenuate neovascular complications in AMD by anti-VEGFA treatment. A single study is testing an anti-C3 antibody (POT-4) to suppress inflammation in the eye, but no data on the outcome of this trial are public thus far. Most anti-VEGFA studies have reported successful treatment of neovascular AMD, although longer-term follow-up data suggest that 98% of patients treated with anti-VEGFA antibodies develop macular atrophy similar to GA, and the affected area correlates with poor visual outcome (Rofagha et al. 2013).

VEGFA is expressed in two isoforms, $VEGFA_{165a}$ and $VEGFA_{165b}$. Although both are neuroprotective, $VEGFA_{165a}$ also has proangiogenic properties, whereas $VEGFA_{165b}$ lacks these features. It was hypothesized that neuroprotective effects of VEGFA could be lost during treatment, eventually resulting in a pronounced degeneration of the RPE and photoreceptors (Beazley-Long

et al. 2013). Novel anti-VEGFA treatment may therefore focus on reducing the levels of the $VEGFA_{165a}$ isoform rather than globally inhibiting VEGFA in a non-isoform-specific fashion. Little is known about the effects of genetic factors on the size and severity of neovascular lesions.

### Treatment of Geographic Atrophy Age-Related Macular Degeneration

Diverse options for treatment of GA AMD are being explored and target several distinct pathways. Current preclinical studies focus on visual cycle inhibitors, inhibitors of (local) inflammation, enhanced neuroprotection of photoreceptors and transplantation of RPE cells (Holz et al. 2014). Thus far, treatment efficacy has not been reported for any of these approaches.

As discussed above, the complement cascade appears to be a promising target for treatment of AMD and, eventually, the atrophic form specifically. Note, however, that the genetic evidence indicates that genetic variants in several complement genes appear to be associated with increased risk of developing AMD (e.g., *CFH*, *C3*, *CFI*, and *C9*). So far, variants in the complement pathway have not been implicated as markers for progression of AMD lesions once the disease already has developed (Caire et al. 2014, Klein et al. 2010, Scholl et al. 2009). Hence, targeting the complement system therapeutically may be more suited to preventing the development of late-stage AMD than for treating late-stage AMD to avoid further progression to severe sight-threatening stages. Thus, future studies correlating the growth rate of GA lesions with genetic factors are crucial for learning more about possible treatment targets for AMD once the disease already has developed to an advanced stage.

### Treatment of Early Age-Related Macular Degeneration

Vitamin supplementation to prevent progression from early to late-stage AMD was evaluated in the AREDS, and the results of this study showed that such supplementation, especially with fish oil–based fatty acids (DHA and EPA), has promising effects. In addition, the Laser Intervention in Early AMD (LEAD) Study recently reported a reduced drusen area after treating drusen with the Ellex 2RT nanosecond laser. Whether this approach influences the progression to late-stage AMD remains to be shown. We are not aware of additional trials treating early AMD, although promising treatment agents are available, for example, to reduce drusen in the macula (Nociari et al. 2014).

## WHAT DOES GENETICS TELL US ABOUT AGE-RELATED MACULAR DEGENERATION?

In this review, we have discussed the results of past and current genetic studies that greatly helped to drive forward efforts to shed light on certain aspects of AMD pathogenesis. Despite these tremendous advances, specifically those made within the past decade, it has also become obvious that many of the genetic studies suffer from insufficient, maybe even inadequate, clinical data. It should be emphasized again that the genetic data depend on the clinical data and can reflect only the accuracy and depth of clinical phenotyping. In this regard, most of the work still lies ahead.

A refined phenotyping, specifically of the early stages of the disease, will be mandatory to further dissect the genetics of the complexities of the AMD phenotype. High-resolution noninvasive imaging technologies are in development or have recently become available but may not yet be part of routine clinical diagnosis. Thus, major efforts need to be directed toward recruiting large patient cohorts with state-of-the-art imaging technologies. Such recruitment will likely be achievable only in a multicenter setting, requiring a high level of standardization and harmonization of clinical

data across centers. To this end, addressing early AMD genetics is going to pose a particular challenge. As early AMD has few if any consequences for regular vision, recruitment needs to be population based, and the number of people included in an initial phenotype screening will likely need to exceed tens if not hundreds of thousands of individuals.

Given a stable foundation of large cohorts with well-differentiated subphenotypes, many open issues regarding the genetics of AMD remain to be addressed. First, we would like to understand the initial steps in the transition from a healthy retina to a retina revealing first pathologic signs of AMD. We need to appreciate the molecular processes and pathways underlying these early disease manifestations. Second, there is a medical need to understand the molecular mechanisms of progression from one disease stage to the next, as these phases may indicate crucial time points for pharmacological intervention. Third, once the full architecture of AMD subphenotype genetics is established and both the responsible variant and affected gene are known, adequate animal and cellular models for experimental research need to be developed. Finally, AMD model systems will make the development of advanced drug testing platforms feasible and will even allow researchers to address drug responses on a defined genetic background. These research environments will also favor direct investigations into gene function, gene × gene interactions, and gene × environment dependencies of disease development.

In summary, these are exciting times to approach a fundamental understanding of AMD pathogenesis. The technologies for a refined clinical assessment and for the required experimental approaches are mostly in place, and clinicians and scientists have learned to collaborate intensively to tackle the complexities of a complex disease such as AMD. Our current state of knowledge may simply reflect only the tip of the iceberg, however; there may be much more to discover than we presently anticipate.

## SUMMARY POINTS

1. Genetic association studies statistically compare the frequencies of genetic variation between affected and nonaffected individuals. In the past decade, tremendous knowledge on the risk of complex diseases has been obtained from GWAS.
2. Normal, age-related changes in the outer retina include a reduction in cell density, thickening of BrM, and accumulation of autofluorescent material. Clinical and histological classification schemes need to distinguish these features from similar disease-associated manifestations.
3. In contrast to the vast knowledge regarding the genetics of late-stage AMD, little is known about the genetic contribution to early AMD. This relative scarcity of knowledge is in opposition to the urgent medical need to identify processes involved in early disease manifestation.
4. Identification of the genes and pathways underlying AMD pathogenesis requires further fine-mapping strategies of known disease-associated loci and suitable animal and in vitro models of AMD.
5. Although the neovascular form of AMD has become treatable during the past few years, most patients ultimately will develop pronounced photoreceptor and RPE atrophy. Currently, there is no approved treatment for either the early or the atrophic late-stage form of the disease.

### FUTURE ISSUES

1. Current study designs are well-suited to identifying genetic markers associated with risk for late-stage disease, but novel study types are required to investigate processes involved in disease progression and severity.
2. Advanced imaging and grading platforms will be needed to investigate the genetic and environmental contributions leading to the full spectrum of disease.
3. Applications of iPSC technology and advances in gene editing via approaches such as the CRIPSR/Cas9 system promise to become excellent tools to understand the processes involved in AMD pathology.
4. Advances in the in vitro and in silico characterization of genes and pathways involved in AMD pathogenesis will provide novel targets for treatment and prevention of the disease.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## LITERATURE CITED


Ach T, Huisingh C, McGwin G, Messinger JD, Zhang T, et al. 2014. Quantitative autofluorescence and cell density maps of the human retinal pigment epithelium. *Investig. Ophthalmol. Vis. Sci.* 55(8):4832–41

Adamis AP, Shima DT, Yeo KT, Yeo TK, Brown LF, et al. 1993. Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. *Biochem. Biophys. Res. Commun.* 193(2):631–38

Adams WJ, Zhang Y, Cloutier J, Kuchimanchi P, Newton G, et al. 2013. Functional vascular endothelium derived from human induced pluripotent stem cells. *Stem Cell Rep.* 1(2):105–13

Aickin M, Gensler H. 1996. Adjusting for multiple testing when reporting research results: the Bonferroni versus Holm methods. *Am. J. Public Health* 86(5):726–28

Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, et al. 1997. Mutation of the Stargardt disease gene (*ABCR*) in age-related macular degeneration. *Science* 277(5333):1805–7

Alten F, Eter N. 2015. Current knowledge on reticular pseudodrusen in age-related macular degeneration. *Br. J. Ophthalmol.* 99:717–22

Anderson DH, Mullins RF, Hageman GS, Johnson L V. 2002. A role for local inflammation in the formation of drusen in the aging eye. *Am. J. Ophthalmol.* 134(3):411–31

Andersson R, Gebhard C, Miguel-Escalada I, Hoof I, Bornholdt J, et al. 2014. An atlas of active enhancers across human cell types and tissues. *Nature* 507(7493):455–61

AREDS (Age-Related Eye Dis. Study Res. Group). 2001. 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.* 119(10):1417–36

Baird PN, Richardson AJ, Robman LD, Dimitrov PN, Tikellis G, et al. 2006. Apolipoprotein (APOE) gene is associated with progression of age-related macular degeneration (AMD). *Hum. Mutat.* 27(4):337–42

Beazley-Long N, Hua J, Jehle T, Hulse RP, Dersch R, et al. 2013. VEGF-$A_{165}$b is an endogenous neuroprotective splice isoform of vascular endothelial growth factor A in vivo and in vitro. *Am. J. Pathol.* 183(3):918–29

Bindewald A, Schmitz-Valckenberg S, Jorzik JJ, Dolar-Szczasny J, Sieber H, et al. 2005. Classification of abnormal fundus autofluorescence patterns in the junctional zone of geographic atrophy in patients with age related macular degeneration. *Br. J. Ophthalmol.* 89(7):874–78

Birch DG, Liang FQ. 2007. Age-related macular degeneration: a target for nanotechnology derived medicines. *Int. J. Nanomed.* 2(1):65–77

Bird AC, Bressler NM, Bressler SB, Chisholm IH, Coscas G, et al. 1995. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv. Ophthalmol.* 39(5):367–74

Booij JC, Baas DC, Beisekeeva J, Gorgels TGMF, Bergen AAB. 2010. The dynamic nature of Bruch's membrane. *Prog. Retin. Eye Res.* 29(1):1–18

Bowes Rickman C, Farsiu S, Toth CA, Klingeborn M. 2013. Dry age-related macular degeneration: mechanisms, therapeutic targets, and imaging. *Investig. Ophthalmol. Vis. Sci.* 54(14):ORSF68–80

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, et al. 2012. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res.* 22(9):1790–97

Brandl C, Grassmann F, Riolfi J, Weber BHF. 2015. Tapping stem cells to target AMD - challenges and prospects. *J. Clin. Med.* 4:282–303

Brandl C, Zimmermann SJ, Milenkovic VM, Rosendahl SMG, Grassmann F, et al. 2014. In-depth characterisation of Retinal Pigment Epithelium (RPE) cells derived from human induced pluripotent stem cells (hiPSC). *NeuroMol. Med.* 16(3):551–64

Buchholz DE, Pennington BO, Croze RH, Hinman CR, Coffey PJ, Clegg DO. 2013. Rapid and efficient directed differentiation of human pluripotent stem cells into retinal pigmented epithelium. *Stem Cells Transl. Med.* 2(5):384–93

Buitendijk GHS, Rochtchina E, Myers C, van Duijn CM, Lee KE, et al. 2013. Prediction of age-related macular degeneration in the general population: the Three Continent AMD Consortium. *Ophthalmology* 120(12):2644–55

Caire J, Recalde S, Velazquez-Villoria A, Garcia-Garcia L, Reiter N, et al. 2014. Growth of geographic atrophy on fundus autofluorescence and polymorphisms of *CFH*, *CFB*, *C3*, *FHR1-3*, and *ARMS2* in age-related macular degeneration. *JAMA Ophthalmol.* 2014:528–34

Chakravarthy U, Wong TY, Fletcher A, Piault E, Evans C, et al. 2010. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol.* 10(1):31

Coetzee SG, Rhie SK, Berman BP, Coetzee GA, Noushmehr H. 2012. FunciSNP: an R/bioconductor tool integrating functional non-coding data sets with genetic association studies to identify candidate regulatory SNPs. *Nucleic Acids Res.* 40(18):e139

Coleman HR, Chan C-C, Ferris FL, Chew EY. 2008. Age-related macular degeneration. *Lancet* 372(9652):1835–45

Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, et al. 2002. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *PNAS* 99(23):14682–87

Curcio CA, Johnson M, Huang J-D, Rudolf M. 2010. Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. *J. Lipid Res.* 51(3):451–67

Curcio CA, Medeiros NE, Millican CL. 1998. The Alabama Age-Related Macular Degeneration Grading System for donor eyes. *Investig. Ophthalmol. Vis. Sci.* 39(7):1085–96

Curcio CA, Messinger JD, Sloan KR, McGwin G, Medeiros NE, Spaide RF. 2013. Subretinal drusenoid deposits in non-neovascular age-related macular degeneration: morphology, prevalence, topography, and biogenesis model. *Retina* 33(2):265–76

Curcio CA, Millican CL. 1999. Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch. Ophthalmol.* 117(3):329–39

Curcio CA, Millican CL, Allen KA, Kalina RE. 1993. Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. *Investig. Ophthalmol. Vis. Sci.* 34(12):3278–96

Curcio CA, Presley JB, Malek G, Medeiros NE, Avery DV, Kruth HS. 2005a. Esterified and unesterified cholesterol in drusen and basal deposits of eyes with age-related maculopathy. *Exp. Eye Res.* 81(6):731–41

Curcio CA, Presley JB, Millican CL, Medeiros NE. 2005b. Basal deposits and drusen in eyes with age-related maculopathy: evidence for solid lipid particles. *Exp. Eye Res.* 80(6):761–75

Edwards SL, Beesley J, French JD, Dunning AM. 2013. Beyond GWASs: illuminating the dark road from association to function. *Am. J. Hum. Genet.* 93(5):779–97

Feeney L. 1978. Lipofuscin and melanin of human retinal pigment epithelium. Fluorescence, enzyme cytochemical, and ultrastructural studies. *Investig. Ophthalmol. Vis. Sci.* 17(7):583–600

Feeney-Burns L, Ellersieck MR. 1985. Age-related changes in the ultrastructure of Bruch's membrane. *Am. J. Ophthalmol.* 100(5):686–97
Feeney-Burns L, Hilderbrand ES, Eldridge S. 1984. Aging human RPE: morphometric analysis of macular, equatorial, and peripheral cells. *Investig. Ophthalmol. Vis. Sci.* 25(2):195–200
Ferris FL, Davis MD, Clemons TE, Lee L-Y, Chew EY, et al. 2005. A simplified severity scale for age-related macular degeneration: AREDS Report No. 18. *Arch. Ophthalmol.* 123(11):1570–74
Ferris FL, Wilkinson CP, Bird A, Chakravarthy U, Chew E, et al. 2013. Clinical classification of age-related macular degeneration. *Ophthalmology* 120(4):844–51
Forrest ARR, Kawaji H, Rehli M, Baillie JK, de Hoon MJL, et al. 2014. A promoter-level mammalian expression atlas. *Nature* 507(7493):462–70
Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, et al. 2013. Seven new loci associated with age-related macular degeneration. *Nat. Genet.* 45(4):433–39
Fritsche LG, Fariss RN, Stambolian D, Abecasis GR, Curcio CA, Swaroop A. 2014. Age-related macular degeneration: genetics and biology coming together. *Annu. Rev. Genomics Hum. Genet.* 15:151–71
Fritsche LG, Fleckenstein M, Fiebig BS, Schmitz-Valckenberg S, Bindewald-Wittich A, et al. 2012. A subgroup of age-related macular degeneration is associated with mono-allelic sequence variants in the *ABCA4* gene. *Investig. Ophthalmol. Vis. Sci.* 53(4):2112–18
Gao H, Hollyfield JG. 1992. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. *Investig. Ophthalmol. Vis. Sci.* 33(1):1–17
Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, et al. 2006. Role of genes and environments for explaining Alzheimer disease. *Arch. Gen. Psychiatry* 63(2):168–74
Godara P, Siebe C, Rha J, Michaelides M, Carroll J. 2010. Assessing the photoreceptor mosaic over drusen using adaptive optics and SD-OCT. *Ophthalmic Surg. Lasers Imaging* 41:S104–8
Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, et al. 2006. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. *Nat. Genet.* 38(4):458–62
Gorin MB. 2012. Genetic insights into age-related macular degeneration: controversies addressing risk, causality, and therapeutics. *Mol. Aspects Med.* 33(4):467–86
Gorski M, Winkler TW, Stark K, Müller-Nurasyid M, Ried JS, et al. 2014. Harmonization of study and reference data by PhaseLift: saving time when imputing study data. *Genet. Epidemiol.* 38(5):381–88
Grassmann F, Heid IM, Weber BHF. 2014. Genetic risk models in age-related macular degeneration. *Adv. Exp. Med. Biol.* 801:291–300
Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, et al. 2005. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. *PNAS* 102(20):7227–32
Hammond CJ, Webster AR, Snieder H, Bird AC, Gilbert CE, Spector TD. 2002. Genetic influence on early age-related maculopathy: a twin study. *Ophthalmology* 109(4):730–36
Hogg RE, Silva R, Staurenghi G, Murphy G, Santos AR, et al. 2014. Clinical characteristics of reticular pseudodrusen in the fellow eye of patients with unilateral neovascular age-related macular degeneration. *Ophthalmology* 121(9):1748–55
Holliday EG, Smith AV, Cornes BK, Buitendijk GHS, Jensen RA, et al. 2013. Insights into the genetic architecture of early stage age-related macular degeneration: a genome-wide association study meta-analysis. *PLOS ONE* 8(1):e53830
Holz FG, Bindewald-Wittich A, Fleckenstein M, Dreyhaupt J, Scholl HPN, Schmitz-Valckenberg S. 2007. Progression of geographic atrophy and impact of fundus autofluorescence patterns in age-related macular degeneration. *Am. J. Ophthalmol.* 143(3):463–72
Holz FG, Schmitz-Valckenberg S, Fleckenstein M. 2014. Recent developments in the treatment of age-related macular degeneration. *J. Clin. Investig.* 124(4):1430–38
Jackson GR, Owsley C, Curcio CA. 2002. Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. *Ageing Res. Rev.* 1(3):381–96
Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. 2005. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am. J. Hum. Genet.* 77(3):389–407
Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. 2000. A potential role for immune complex pathogenesis in drusen formation. *Exp. Eye Res.* 70(4):441–49

Kamei M, Hollyfield JG. 1999. TIMP-3 in Bruch's membrane: changes during aging and in age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 40(10):2367–75
Kellis M, Wold B, Snyder MP, Bernstein BE, Kundaje A, et al. 2014. Defining functional DNA elements in the human genome. *PNAS* 111(17):6131–38
Killingsworth MC. 1995. Angiogenesis in early choroidal neovascularization secondary to age-related macular degeneration. *Graefes Arch. Clin. Exp. Ophthalmol.* 233(6):313–23
Kim C. 2014. Disease modeling and cell based therapy with iPSC: future therapeutic option with fast and safe application. *Blood Res.* 49(1):7–14
Kjeldsen MJ, Kyvik KO, Christensen K, Friis ML. 2001. Genetic and environmental factors in epilepsy: a population-based study of 11,900 Danish twin pairs. *Epilepsy Res.* 44(2–3):167–78
Klaver CCW, Kliffen M, van Duijn CM, Hofman A, Cruts M, et al. 1998. Genetic association of apolipoprotein E with age-related macular degeneration. *Ophthalmic Res.* 200–206
Klein ML, Ferris FL III, Francis PJ, Lindblad AS, Chew EY, et al. 2010. Progression of geographic atrophy and genotype in age-related macular degeneration. *Ophthalmology* 117(8):1554–1559.e1
Klein R, Davis MD, Magli YL, Segal P, Klein BEK, Hubbard L. 1991. The Wisconsin age-related maculopathy grading system. *Ophthalmology* 98(7):1128–34
Klein R, Klein BEK, Jensen SC, Meuer SM. 1997. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 104(1):7–21
Klein R, Klein BEK, Knudtson MD, Meuer SM, Swift M, Gangnon RE. 2007. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology* 114(2):253–62
Klein R, Meuer SM, Myers CE, Buitendijk GHS, Rochtchina E, et al. 2014. Harmonizing the classification of age-related macular degeneration in the Three-Continent AMD Consortium. *Ophthalmic Epidemiol.* 21(1):14–23
Klein RJ, Zeiss C, Chew EY, Tsai J-Y, Sackler RS, et al. 2005. Complement factor H polymorphism in age-related macular degeneration. *Science* 308(5720):385–89
Kraft P, Cox DG. 2008. Study designs for genome-wide association studies. *Adv. Genet.* 60:465–504
Krohne TU, Westenskow PD, Kurihara T, Friedlander DF, Lehmann M, et al. 2012. Generation of retinal pigment epithelial cells from small molecules and *OCT4* reprogrammed human induced pluripotent stem cells. *Stem Cells Transl. Med.* 1(2):96–109
Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, et al. 2013. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat. Genet.* 45(12):1452–58
Lefrançois P, Zheng W, Snyder M. 2010. ChIP-Seq: using high-throughput DNA sequencing for genome-wide identification of transcription factor binding sites. *Methods Enzymol.* 470:77–104
Liang L, Morar N, Dixon AL, Lathrop GM, Abecasis GR, et al. 2013. A cross-platform analysis of 14,177 expression quantitative trait loci derived from lymphoblastoid cell lines. *Genome Res.* 23(4):716–26
Magnusson KP, Duan S, Sigurdsson H, Petursson H, Yang Z, et al. 2006. *CFH* Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLOS Med.* 3(1):e5
Malek G, Li C-M, Guidry C, Medeiros NE, Curcio CA. 2003. Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. *Am. J. Pathol.* 162(2):413–25
Marshall GE, Konstas AGP, Reid GG, Edwards JG, Lee WR. 1992. Type IV collagen and laminin in Bruch's membrane and basal linear deposit in the human macula. *Br. J. Ophthalmol.* 76(10):607–14
McKay GJ, Silvestri G, Chakravarthy U, Dasari S, Fritsche LG, et al. 2011. Variations in apolipoprotein E frequency with age in a pooled analysis of a large group of older people. *Am. J. Epidemiol.* 173(12):1357–64
Moore DJ, Hussain AA, Marshall J. 1995. Age-related variation in the hydraulic conductivity of Bruch's membrane. *Investig. Ophthalmol. Vis. Sci.* 36(7):1290–97
Mrejen S, Sato T, Curcio CA, Spaide RF. 2014. Assessing the cone photoreceptor mosaic in eyes with pseudodrusen and soft drusen in vivo using adaptive optics imaging. *Ophthalmology* 121(2):545–51
Mullins RF, Russell SR, Anderson DH, Hageman GS. 2000. 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.* 14(7):835–46
Nociari MM, Lehmann GL, Perez Bay AE, Radu RA, Jiang Z, et al. 2014. Beta cyclodextrins bind, stabilize, and remove lipofuscin bisretinoids from retinal pigment epithelium. *PNAS* 111(14):E1402–8

Olsen TW, Feng X. 2004. The Minnesota Grading System of eye bank eyes for age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 45(12):4484–90

Parameswaran S, Balasubramanian S, Babai N, Qiu F, Eudy JD, et al. 2010. Induced pluripotent stem cells generate both retinal ganglion cells and photoreceptors: therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration. *Stem Cells* 28(4):695–703

Pe'er J, Shweiki D, Itin A, Hemo I, Gnessin H, Keshet E. 1995. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. *Lab. Investig.* 72(6):638–45

Pennesi ME, Neuringer M, Courtney RJ. 2012. Animal models of age related macular degeneration. *Mol. Aspects Med.* 33(4):487–509

Ramrattan RS, van der Schaft TL, Mooy CM, de Bruijn WC, Mulder PG, de Jong PT. 1994. Morphometric analysis of Bruch's membrane, the choriocapillaris, and the choroid in aging. *Investig. Ophthalmol. Vis. Sci.* 35(6):2857–64

Randall JC, Winkler TW, Kutalik Z, Berndt SI, Jackson AU, et al. 2013. Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLOS Genet.* 9(6):e1003500

Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, et al. 2005. 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.* 14(21):3227–36

Rivera A, White K, Stöhr H, Steiner K, Hemmrich N, et al. 2000. A comprehensive survey of sequence variation in the *ABCA4* (*ABCR*) gene in Stargardt disease and age-related macular degeneration. *Am. J. Hum. Genet.* 67(4):800–13

Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K. 2013. Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology* 120(11):2292–99

Rudolf M, Clark ME, Chimento MF, Li C-M, Medeiros NE, Curcio CA. 2008. Prevalence and morphology of druse types in the macula and periphery of eyes with age-related maculopathy. *Investig. Ophthalmol. Vis. Sci.* 49(3):1200–9

Rudolf M, Curcio CA. 2009. Esterified cholesterol is highly localized to Bruch's membrane, as revealed by lipid histochemistry in wholemounts of human choroid. *J. Histochem. Cytochem.* 57(8):731–39

Sarks JP, Sarks SH, Killingsworth MC. 1988. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye* 2:552–77

Sarks JP, Sarks SH, Killingsworth MC. 1997. Morphology of early choroidal neovascularisation in age-related macular degeneration: correlation with activity. *Eye* 11:515–22

Sarks S, Cherepanoff S, Killingsworth M, Sarks J. 2007. Relationship of basal laminar deposit and membranous debris to the clinical presentation of early age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 48(3):968–77

Sarks SH. 1976. Ageing and degeneration in the macular region: a clinico-pathological study. *Br. J. Ophthalmol.* 60(5):324–41

Sarks SH. 1982. Drusen patterns predisposing to geographic atrophy of the retinal pigment epithelium. *Aust. J. Ophthalmol.* 10(2):91–97

Sarks SH, Arnold JJ, Killingsworth MC, Sarks JP. 1999. Early drusen formation in the normal and aging eye and their relation to age related maculopathy: a clinicopathological study. *Br. J. Ophthalmol.* 83(3):358–68

Schmitz-Valckenberg S, Alten F, Steinberg JS, Jaffe GJ, Fleckenstein M, et al. 2011. Reticular drusen associated with geographic atrophy in age-related macular degeneration. *Investig. Ophthalmol. Vis. Sci.* 52(9):5009–15

Scholl HPN, Fleckenstein M, Fritsche LG, Schmitz-Valckenberg S, Göbel A, et al. 2009. *CFH*, *C3* and *ARMS2* are significant risk loci for susceptibility but not for disease progression of geographic atrophy due to AMD. *PLOS ONE* 4(10):e7418

Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. 2005. The US Twin Study of Age-Related Macular Degeneration: relative roles of genetic and environmental influences. *Arch. Ophthalmol.* 123(3):321–27

Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. 2009. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Investig. Ophthalmol. Vis. Sci.* 50(5):2044–53

Seddon JM, Sharma S, Adelman RA. 2006. Evaluation of the clinical age-related maculopathy staging system. *Ophthalmology* 113(2):260–66

Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, et al. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 343(6166):84–87

Sifrim A, Van Houdt JKJ, Tranchevent L-C, Nowakowska B, Sakai R, et al. 2012. Annotate-it: a Swiss-knife approach to annotation, analysis and interpretation of single nucleotide variation in human disease. *Genome Med.* 4(9):73

Singh R, Phillips MJ, Kuai D, Meyer J, Martin JM, et al. 2013a. Functional analysis of serially expanded human iPS cell-derived RPE cultures. *Investig. Ophthalmol. Vis. Sci.* 54(10):6767–78

Singh R, Shen W, Kuai D, Martin JM, Guo X, et al. 2013b. iPS cell modeling of Best disease: insights into the pathophysiology of an inherited macular degeneration. *Hum. Mol. Genet.* 22(3):593–607

Sivaprasad S, Bailey TA, Chong VNH. 2005. Bruch's membrane and the vascular intima: Is there a common basis for age-related changes and disease? *Clin. Exp. Ophthalmol.* 33(5):518–23

Skerka C, Lauer N, Weinberger AAWA, Keilhauer CN, Sünhel J, et al. 2007. Defective complement control of Factor H (Y402H) and FHL-1 in age-related macular degeneration. *Mol. Immunol.* 44:3398–3406

Smith AG, Kaiser PK. 2014. Emerging treatments for wet age-related macular degeneration. *Expert Opin. Emerg. Drugs.* 19(1):157–64

Spaide RF. 2009. Age-related choroidal atrophy. *Am. J. Ophthalmol.* 147(5):801–10

Spaide RF, Armstrong D, Browne R. 2003. Choroidal neovascularization in age-related macular degeneration—what is the cause? *Retina* 23(5):595–614

Spaide RF, Curcio CA, Zweifel SA. 2010. Drusen, an old but new frontier. *Retina* 30(8):1163–65

Spencer KL, Olson LM, Anderson BM, Schnetz-Boutaud N, Scott WK, et al. 2008. C3 R102G polymorphism increases risk of age-related macular degeneration. *Hum. Mol. Genet.* 17(12):1821–24

Spraul CW, Grossniklaus HE. 1997. Characteristics of drusen and Bruch's membrane in postmortem eyes with age-related macular degeneration. *Arch. Ophthalmol.* 115(2):267–73

Spraul CW, Lang GE, Grossniklaus HE, Lang GK. 1999. Histologic and morphometric analysis of the choroid, Bruch's membrane, and retinal pigment epithelium in postmortem eyes with age-related macular degeneration and histologic examination of surgically excised choroidal neovascular membranes. *Surv. Ophthalmol.* 44:S10–32

Stone EM, Webster AR, Vandenburgh K, Streb LM, Hockey RR, et al. 1998. Allelic variation in *ABCR* associated with Stargardt disease but not age-related macular degeneration. *Nat. Genet.* 20(4):328–29

Strauss O. 2005. The retinal pigment epithelium in visual function. *Physiol. Rev.* 85(3):845–81

Suzuki M, Sato T, Spaide RF. 2014. Pseudodrusen subtypes as delineated by multimodal imaging of the fundus. *Am. J. Ophthalmol.* 157(5):1005–12

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5):861–72

Teo YY, Small KS. 2010. A novel method for haplotype clustering and visualization. *Genet. Epidemiol.* 34(1):34–41

Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, et al. 2012. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat. Genet.* 44(12):1341–48

Tysk C, Lindberg E, Järnerot G, Flodérus-Myrhed B. 1988. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 29(7):990–96

van der Schaft TL, Mooy CM, de Bruijn WC, Bosman FT, de Jong PT. 1994. Immunohistochemical light and electron microscopy of basal laminar deposit. *Graefes Arch. Clin. Exp. Ophthalmol.* 232(1):40–46

Walley AJ, Blakemore AIF, Froguel P. 2006. Genetics of obesity and the prediction of risk for health. *Hum. Mol. Genet.* 15:R124–30

Wang J, Duncan D, Shi Z, Zhang B. 2013. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. *Nucleic Acids Res.* 41:W77–83

Wang L, Li C-M, Rudolf M, Belyaeva OV, Chung BH, et al. 2009. Lipoprotein particles of intraocular origin in human Bruch membrane: an unusual lipid profile. *Investig. Ophthalmol. Vis. Sci.* 50(2):870–77

Wang T, Wei JJ, Sabatini DM, Lander ES. 2014. Genetic screens in human cells using the CRISPR-Cas9 system. *Science* 343(6166):80–84

Weber BHF, Charbel Issa P, Pauly D, Herrmann P, Grassmann F, Holz FG. 2014. The role of the complement system in age-related macular degeneration. *Dtsch. Ärztebl. Int.* 111(8):133–38

Welter D, MacArthur J, Morales J, Burdett T, Hall P, et al. 2014. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res.* 42:D1001–6

Westenskow PD, Kurihara T, Friedlander M. 2014. Utilizing stem cell-derived RPE cells as a therapeutic intervention for age-related macular degeneration. *Adv. Exp. Med. Biol.* 801:323–29

Williams MA, McKay GJ, Chakravarthy U. 2014. Complement inhibitors for age-related macular degeneration. *Cochrane Database Syst. Rev.* 1:CD009300

Wu Z, Luu CD, Ayton LN, Goh JK, Lucci LM, et al. 2014. Optical coherence tomography–defined changes preceding the development of drusen-associated atrophy in age-related macular degeneration. *Ophthalmology* 121:2415–22

Yamanaka S. 2012. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 10(6):678–84

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318(5858):1917–20

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

Zarbin MA, Casaroli-Marano RP, Rosenfeld PJ. 2014. Age-related macular degeneration: clinical findings, histopathology and imaging techniques. *Dev. Ophthalmol.* 53:1–32

Zareparsi S, Reddick AC, Branham KEH, Moore KB, Jessup L, et al. 2004. Association of apolipoprotein E alleles with susceptibility to age-related macular degeneration in a large cohort from a single center. *Investig. Ophthalmol. Vis. Sci.* 45(5):1306–10

Zhang F, Wen Y, Guo X. 2014. CRISPR/Cas9 for genome editing: progress, implications and challenges. *Hum. Mol. Genet.* 23(R1):R40–46

Zhang Y, Wang X, Rivero EB, Clark ME, Witherspoon CD, et al. 2014. Photoreceptor perturbation around subretinal drusenoid deposits as revealed by adaptive optics scanning laser ophthalmoscopy. *Am. J. Ophthalmol.* 158(3):584–96.e1

Zhou T, Benda C, Dunzinger S, Huang Y, Ho JC, et al. 2012. Generation of human induced pluripotent stem cells from urine samples. *Nat. Protoc.* 7(12):2080–89

Zhou Y, Zeng F. 2013. Integration-free methods for generating induced pluripotent stem cells. *Genomics Proteomics Bioinform.* 11(5):284–87

Zou F, Chai HS, Younkin CS, Allen M, Crook J, et al. 2012. Brain expression genome-wide association study (eGWAS) identifies human disease-associated variants. *PLOS Genet.* 8(6):e1002707

Zweifel SA, Spaide RF, Curcio CA, Malek G, Imamura Y. 2010. Reticular pseudodrusen are subretinal drusenoid deposits. *Ophthalmology* 117(2):303–12.e1