Highly Penetrant Alleles in Age-Related Macular Degeneration

Anneke I. den Hollander and Eiko K. de Jong

Department of Ophthalmology and Department of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands

Correspondence: Anneke.denHollander@radboudumc.nl

Age-related macular degeneration (AMD) is a complex disease caused by a combination of genetic and environmental factors. Genome-wide association studies have identified several common genetic variants associated with AMD, which together account for 15%–65% of the heritability of AMD. Multiple hypotheses to clarify the unexplained portion of genetic variance have been proposed, such as gene–gene interactions, gene–environment interactions, structural variations, epigenetics, and rare variants. Several studies support a role for rare variants with large effect sizes in the pathogenesis of AMD. In this work, we review the methods that can be used to detect rare variants in common diseases, as well as the recent progress that has been made in the identification of rare variants in AMD. In addition, the relevance of these rare variants for diagnosis, prognosis, and treatment of AMD is highlighted.

Age-related macular degeneration (AMD) is a complex, multifactorial disease caused by a combination of genetic and environmental factors. The most commonly associated environmental risk factor is smoking (Thornton et al. 2005). Evidence for a strong genetic component in AMD comes from familial and twin-based studies (Seddon et al. 1997, 2005). The proportion of variability in the risk of AMD that is attributed to genetic makeup, or heritability, is ~45%–70% (Seddon et al. 2005).

Genome-wide association studies and candidate gene approaches have identified several common genetic variants (with a minor allele frequency [MAF] >5%) that are associated with AMD (Fig. 1). A significant breakthrough in the AMD field came with the identification of

a strong association between AMD and a coding variant (rs1061170; p.Tyr402His) and several noncoding variants in the complement factor H (*CFH*) gene, which encodes a primary regulator of the alternative complement pathway (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005). A strong association has also been identified between AMD and a haplotype on chromosome 10q26, which contains the *ARMS2* and *HTRA1* genes (Jakobsdottir et al. 2005; Rivera et al. 2005; Dewan et al. 2006). In addition, several genetic variants with smaller effect sizes have been identified in other complement factor genes (*CFB/C2*, *C3*, and *CFI*) (Gold et al. 2006; Yates et al. 2007; Fagerness et al. 2009) as well as in genes encoding members of the high-density lipoprotein cholesterol pathway

Editors: Eric A. Pierce, Richard H. Masland, and Joan W. Miller
Additional Perspectives on Retinal Disorders: Genetic Approaches to Diagnosis and Treatment available at www.perspectivesinmedicine.org

Copyright © 2015 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a017202
Cite this article as *Cold Spring Harb Perspect Med* 2015;5:a017202

A.I. den Hollander and E.K. de Jong

Figure 1. Effect sizes (odds ratios) of common and rare variants associated with AMD. Common variants with relatively small effect sizes at loci recently described in (Fritsche et al. 2013) are indicated in green. Rare variants with low population allele frequencies and large effect sizes are indicated in red: *CFI* Gly119Arg (MAF 0.001, OR 22) (van de Ven et al. 2013); *CFH* Arg1210Cys (MAF 0.0002, OR 18.8) calculated from (Raychaudhuri et al. 2011); *C3* Lys155Gln (MAF 0.004, OR 3.7) (Helgason et al. 2013); *C9* Pro167Ser (MAF 0.008, OR 2.2) (Seddon et al. 2013). MAF calculated from NHLBI exome sequencing project, alleles from European ancestry.

(*APOE*, *LIPC*, *CETP*), components of the extracellular collagen matrix (*TIMP3*, *COL8A1*, *COL10A1*), and proteins promoting angiogenesis (*VEGFA*, *TGFBR1*) (Yu et al. 2011; Fritsche et al. 2013). The most comprehensive genome-wide association study performed thus far involved 18 international research groups, and included >17,000 advanced AMD cases and >60,000 controls of European and Asian ancestry (Fritsche et al. 2013).

Although common variants in the *CFH* gene and at the *ARMS2/HTRA1* locus exert a relatively large effect, increasing disease risk by two- to threefold, most common variants individually confer relatively small increments in risk (Fig. 1) (Fritsche et al. 2013). Together, the common variants identified thus far account for 15%–65% of the heritability of AMD (Manolio et al. 2009). Although genome-wide association studies with even larger samples sizes might elucidate additional common variants with smaller effect sizes, it is clear that a significant proportion of the missing heritability remains unexplained. Multiple hypotheses to clarify the

unexplained portion of genetic variance have been proposed, such as gene–gene interactions, gene–environment interactions, structural variations, epigenetics, and rare variants (Liu and Leal 2012; Liu et al. 2012). Current efforts in complex disease genetics are shifting to investigate the “common disease, rare variant” hypothesis to determine whether rare variants (with a $MAF \leq 1\%$) and low-frequency variants (with a $MAF 1\%–5\%$) explain the remaining fraction of the missing heritability (Pritchard 2001; Bodmer and Bonilla 2008). Such rare and low-frequency variants would need to have large effect sizes to be detected, because those with small effect sizes will be very difficult or impossible to detect by genetic means (Manolio et al. 2009).

Several studies support a role for rare variants with large effect sizes in the pathogenesis of AMD. First of all, the risk of AMD is greatly increased (by 16-fold) by having an affected first-degree relative (Shahid et al. 2012). Second, relatives of AMD patients do not only have an increased risk, but also have an earlier onset of the disease (Klaver et al. 1998b). Finally,

a recent study reported that the aggregation of AMD in densely affected families is not sufficiently explained by the genotypic load of the common genetic risk variants for AMD. Simulation studies suggested that rare, highly penetrant variants are likely to explain the heritability in these families (Sobrin et al. 2010). In this article, we review the methods that can be used to detect rare variants in common disease, as well as the recent progress that has been made in the identification of rare variants in AMD. In addition, the relevance of these rare variants for diagnosis, prognosis, and treatment of AMD is highlighted.

METHODS TO IDENTIFY RARE VARIANTS IN COMMON DISEASE

Linkage Analysis in Families

For some common diseases, such as Alzheimer disease, rare familial forms exist that are caused by rare, highly penetrant alleles. The genes involved in such inherited (or Mendelian) diseases have traditionally been mapped using linkage analysis in families with several affected family members. Linkage analysis attempts to identify a region (or locus) in the genome associated with the disease by identifying alleles that are present only in affected members of the family (Mayeux 2005). A genome-wide segregation analysis is performed with polymorphic markers spread throughout the genome. In the past, sets of variable number of tandem repeats (VNTR) or microsatellite markers have been used, whereas more recently, microarrays containing 6000–10,000 single nucleotide polymorphisms (SNPs) have efficiently been used for genome-wide mapping.

A way to determine whether the identified locus is potentially relevant to the disease is to calculate the logarithm of odds (LOD) score, which compares the probability of the observation assuming a degree of linkage to the probability of obtaining the same observation without any linkage. For significant locus assignment by genome-wide genotyping, a minimum LOD score of 3.3 must be obtained, and a LOD score of 1.86 is suggestive for linkage (Lander and

Kruglyak 1995). A locus of interest can be mapped in more detail to narrow down the region, and subsequently the genes in the identified chromosomal interval can be analyzed for the causative mutation.

A benefit of linkage analysis in families is that the technique is suitable for identifying causal mutations in large families that show clear and penetrant Mendelian inheritance patterns. A potential drawback, especially in the case of age-related complex disorders, is that the disease is not always fully penetrant in some individuals. Furthermore, there may be a considerable contribution of other genetic and environmental factors to the development of the disease in other individuals. Thus, incomplete cosegregation of an allele within a family does not exclude the possibility that the allele can be causal in some individuals (Cirulli and Goldstein 2010).

Candidate Gene Analysis

Another approach that can be used to identify rare variants in common diseases is by sequencing one or more candidate genes in a case-control setting. A critical step in the study design is the selection of appropriate candidate genes for the disease of interest. Potential candidate genes are those that have been identified in familial forms of the disease, genes at or near common variants that have been identified to be associated with the disease by genome-wide association studies, or genes known to be involved in the biology of the disease based on biochemical and physiological studies (Bodmer and Bonilla 2008; Cirulli and Goldstein 2010).

In the past decades, DNA sequence analysis was based on the chain termination method described by Sanger (Sanger et al. 1977). Sanger sequencing can be used to sequence one or a limited number of genes in a limited number of samples, and is therefore not the most suitable approach to identify rare variants in common diseases. In the past five years, new sequence technologies (next-generation sequencing) have been developed, allowing the analysis of larger amounts of sequence data with higher speed and at much lower costs compared with

A.I. den Hollander and E.K. de Jong

Sanger sequencing. Various target enrichment strategies exist that can enrich a specific target (targeted next-generation sequencing of one or more genes), the exons of all human genes (exome sequencing), or even the entire genome (whole-genome sequencing).

A challenge in the design of candidate gene studies is the extremely large sample sizes that are required to implicate single genes in a complex disease. This can be overcome by target enrichment using molecular inversion probes, which allows candidate resequencing in very large cohorts at ultra-low costs per sample (O’Roak et al. 2012).

Exome Chip Analysis

Genome-wide association studies with SNP microarrays can successfully identify associations with common variants with a MAF of $>5\%$ in the general population, whereas exome sequencing can identify very rare and even unique coding variants. Because exome sequencing of very large numbers of samples is currently very expensive, an intermediate experiment can be designed for a fraction of the cost using exome chips containing $>100,000$ rare and low-frequency variants. These arrays contain coding variants that have been identified several times in existing sequence data sets. This line of thought implicitly underlies the 1000 Genomes Project, which is extending the catalogue of known human variants down to a frequency of near 1%. Exome chips will allow a new wave of genome-wide association studies that can identify rare and low-frequency variants contributing to common traits (Cirulli and Goldstein 2010; Huyghe et al. 2013). In an ongoing worldwide effort to identify rare and low-frequency variants in AMD, $>50,000$ samples are currently being genotyped with exome chips containing 103,947 rare and low-frequency variants by the International AMD Genomics Consortium.

Exome and Whole-Genome Sequencing

The most comprehensive study to identify rare variants in complex diseases involves exome sequencing or, preferably, whole-genome se-

quencing of very large sample sizes. Because these technologies are currently very expensive to apply to large numbers of samples, two designs can be used to enrich the study population for rare variants, which would require smaller sample sizes. The first design involves family-based sequencing, in which selected families with multiple affected individuals are analyzed. The second approach involves selecting individuals that are at the extreme ends of a trait distribution (Cirulli and Goldstein 2010).

When designing an exome or whole-genome sequencing study aimed to identify rare variants, important considerations are sufficient coverage (at least 40-fold), and preferably analyzing all samples (both cases and controls) together as a set. This ensures a high-quality data set, which is of utmost importance for downstream analyses. Exome and whole-genome sequencing generate a tremendous amount of data, which can make association analyses complex. To discern relevant from irrelevant effects, bioinformatics software can be used to predict the effect of amino acid changes, allowing researchers to distinguish whether a variant is or is not likely to be deleterious. Because deleterious variants are often rare, it is difficult to detect association using single-variant analyses. Statistical tests (e.g., burden tests) are available that collapse variants across a gene or pathway, resulting in a gene-based or pathway-based test. The burden test compares frequencies of damaging variants in a gene or pathway between cases and controls. Variants can be weighted using external information, such as pathogenicity prediction scores. Other statistical tests (such as the C-alpha test, the sequence kernel association test, and the estimated regression coefficient test) can also take the direction of the effect (i.e., deleterious or protective) into account (Kiezun et al. 2012; Goldstein et al. 2013).

RARE VARIANTS IN AMD

Complement Component C3

C3 is a central component of the complement cascade. Cleavage of C3 into C3a and C3b is the central step in complement activation. In a feed-

forward mechanism, C3b activation amplifies further cleavage of C3 and leads to cleavage of C5 via the generation of C3 and C5 convertase complexes, respectively. Subsequently, components C6 through C9 are recruited to form a large molecular pore (the membrane attack complex) on target membranes, resulting in cell lysis (Walport 2001a,b). Because these activation fragments are nondiscriminatory, the complement system is tightly regulated by several inhibitory proteins, including CFH and complement factor I (CFI), which protect the host cells from complement attack.

A common variant (Arg102Gly; rs2230199) in the C3 gene has been significantly associated with AMD (Yates et al. 2007). To search for rare and low-frequency variants in the previously identified AMD loci, whole-genome sequencing of 2230 Icelanders was performed and identified sequence variants were predicted (imputed) for a larger cohort of Icelanders genotyped with SNP microarrays. A rare missense variant (Lys155Gln; rs14785925) was identified that is highly associated with AMD ($p = 8.8 \times 10^{-16}$, odds ratio = 3.65 [95% CI 2.66–5.00]) (Fig. 1). The association of the rare Lys155Gln variant with AMD is independent of the common Arg102Gly variant (Helgason et al. 2013). The Lys155Gln variant was also identified by next generation sequencing of AMD genes and related pathways in two independent studies ($p = 5.2 \times 10^{-9}$, odds ratio = 3.8 [95% CI 2.3–6.1], and $p = 2.8 \times 10^{-5}$, odds ratio = 2.68]) (Seddon et al. 2013; Zhan et al. 2013).

The Lys155Gln variant localizes to the C3b fragment of the C3 protein. The presence of the Lys155Gln variant in the C3b protein causes reduced binding to the complement inhibitor molecule CFH. C3b becomes resistant to decay, which in turn leads to a gain of function of the C3 convertase activity (Miller 2012). A lower ability to degrade C3b, resulting in sustained complement attack in the macula, is the proposed disease mechanism for this rare variant.

Complement Component C9

Sequence analysis of 681 genes within all reported AMD loci and related pathways in 2493 cases

and controls identified a rare missense variant in the C9 gene (Pro167Ser) to be associated with AMD ($p = 6.5 \times 10^{-7}$, odds ratio 2.2 [95% CI 1.6–3.0]) (Seddon et al. 2013). Variants in the C9 gene have not previously been associated with AMD, and this therefore for the first time links a component of the membrane attack complex with the pathogenesis of AMD. The functional effect of the Pro167Ser variant on the activity of the membrane attack complex remains to be determined.

CFH

CFH is the primary inhibitor of the alternative complement pathway. Genetic studies identified strong associations between AMD and a coding variant (Tyr402His; rs1061170) and several noncoding variants in the *CFH* gene (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005). In addition to these common genetic variants, rare variants with an even larger effect have been identified in the *CFH* gene (Fig. 2A).

Heterozygous nonsense and frameshift mutations have been identified in the *CFH* gene among family members with cuticular drusen, a clinical subtype of AMD (Boon et al. 2008; van de Ven et al. 2012a). These mutations are predicted to lead to reduced levels of functional CFH protein. A recessive disease model was initially suggested in which patients were thought to develop cuticular drusen in the presence of a severe *CFH* mutation on one allele and the *CFH* Tyr402His risk variant on the other allele (Boon et al. 2008). A second study found that single heterozygous loss-of-function mutations in the *CFH* gene are sufficient for developing cuticular drusen, even in the absence of the Tyr402His risk allele (van de Ven et al. 2012a).

Another study identified a rare, highly penetrant missense variant (Arg1210Cys, rs121913059) in 40 out of 2423 AMD patients, compared with only one out of 1122 control individuals ($p = 8.0 \times 10^{-5}$) (Fig. 1) (Raychaudhuri et al. 2011). The Arg1210Cys variant conferred risk independently of the common Tyr402His variant. The AMD patients who carried the Arg1210Cys variant had a significantly younger age of disease onset than did AMD patients

A.I. den Hollander and E.K. de Jong

Figure 2. Overview of rare variants identified in complement factor H (*CFH*) and fibulin-5 (*FBLN5*) in AMD. (A) Location of rare variants in *CFH*. After an initial signal peptide, *CFH* consists of 20 adjacently positioned complement control protein (CCP, or sushi) domains that have differential affinity for C3 and/or cell surface structures. Underlined in green is the common variant Tyr402His. Underlined in red are rare variants in *CFH* that have been identified in AMD. GAG, glycosaminoglycans. (B) Location of rare variants in *FBLN5*. After an initial signal peptide, *FBLN5* has one cell adhesion or integrin-binding motif (RGD, Arginyl-glycyl-aspartic acid), followed by six epidermal growth factor (EGF)-like domains.

without this mutation. The *CFH* Arg1210Cys amino acid substitution causes reduced binding to C3d, C3b, heparin, and endothelial cells (Manuelian et al. 2003; Ferreira et al. 2009). In addition, the Arg1210Cys mutant protein forms a covalent interaction with human serum albu-

min, which is likely caused by the introduction of an additional cysteine residue at this position (Sanchez-Corral et al. 2002).

These highly penetrant variants lead to impaired function and/or decreased levels of *CFH*, subsequently leading to a decreased ability to

inhibit the complement system. Because CFH is expressed by the retinal pigment epithelium and the choroid (Anderson et al. 2010), the protection of these cells against complement activation is compromised in individuals carrying these rare variants. Several factors have been proposed to trigger complement activation in the macula, such as photooxidative stress caused by high sunlight exposure (Anderson et al. 2010). Individuals with insufficient complement-modulating activity can experience sustained complement attack, resulting in injury of the retinal pigment epithelium and subsequent formation of drusen (Anderson et al. 2010).

CFI

Similar to CFH, CFI is also required to maintain complement homeostasis and to restrict complement activation. Recently, rare variants in the *CFI* gene were found to confer a high risk of the development of AMD (van de Ven et al. 2013). Sequence analysis of a cohort of AMD patients identified two missense variants, Gly119Arg (rs141853578) and Gly188Ala. The Gly188Ala variant was found in four affected family members of one family. The Gly119Arg variant was identified in 20 out of 3567 unrelated AMD cases compared with only one out of 3937 controls, consistent with Gly119Arg conferring a high risk for developing AMD ($p = 3.79 \times 10^{-6}$, odds ratio = 22.20 [95% CI = 2.98–164.49]) (Fig. 1). Fundus fluorescein angiography revealed that carriers of the Gly119Arg and Gly188Ala variants had the cuticular drusen subtype of AMD (Boon et al. 2013). In addition, an increased burden of rare missense variants in *CFI* was observed in cases compared with controls. Rare missense variants were detected in 7.8% of AMD compared with 2.3% of controls ($p = 2 \times 10^{-8}$, odds ratio = 3.6) (Seddon et al. 2013).

The Gly119Arg and Gly188Ala variants severely impair the expression and secretion of the mutant CFI protein (van de Ven et al. 2013). As a result, patients carrying the CFI Gly119Arg or Gly188Ala variant have significantly lower plasma CFI levels than both controls and AMD patients who do not carry this CFI variant. Con-

sequently, these mutation carriers have a significantly lower ability to degrade C3b, leading to sustained complement activation (van de Ven et al. 2013). In individuals with impaired CFI levels, a similar disease mechanism is proposed as for individuals carrying highly penetrant variants in the *CFH* gene, in which insufficient complement-modulating activity results in sustained complement attack and injury of the macula.

ATP-Binding Cassette A4 (ABCA4)

The *ABCA4* gene encodes an ATP-binding cassette transporter that is expressed in retinal photoreceptor cells. ABCA4 actively flips *N*-retinylidene-phosphatidylethanolamine from the lumen to the cytoplasmic leaflet of disc membranes, thereby facilitating the removal of potentially toxic retinoid compounds from photoreceptors (Quazi et al. 2012). Mutations in the *ABCA4* gene are found in Stargardt disease, an autosomal recessive retinal disorder characterized by a juvenile-onset macular dystrophy, delayed dark adaptation, and subretinal deposition of lipofuscin-like material (Allikmets 1997). Because of the phenotypic similarities between Stargardt disease and AMD, the *ABCA4* gene was considered a candidate gene for AMD.

Several rare *ABCA4* variants were detected in AMD patients (Allikmets et al. 1997), but the finding was criticized by several researchers (Dryja et al. 1998; Klaver et al. 1998a). Two rare variants (Gly1961Glu and Asp2177Asn), which lead to altered ATPase activity of the ABCA4 protein (Sun et al. 2000), were found to be significantly associated with AMD (Allikmets 2000). The association of *ABCA4* alleles with AMD was not confirmed by others (De La Paz et al. 1999; Rivera et al. 2000; Guymer et al. 2001; Webster et al. 2001), but this does not rule out that *ABCA4* may play a role in some cases of AMD (De La Paz et al. 1999).

In recent studies, a subgroup of AMD patients with a fine granular pattern with peripheral punctate spots, also referred to as late-onset Stargardt disease, was found to be significantly associated with rare *ABCA4* variants (Burke et al. 2012; Fritsche et al. 2012; Westeneng-van

A.I. den Hollander and E.K. de Jong

Haaften et al. 2012). The differential diagnosis between this group of patients and AMD may be challenging, and it is plausible that such patients have been randomly included in some AMD cohorts, possibly explaining the previously described associations (Fritsche et al. 2012).

Fibulin-5 (FBLN5)

The *FBLN5* gene encodes a member of the fibulin family of extracellular matrix proteins. The fibulin proteins are involved in the formation and stabilization of supramolecular extracellular matrix complexes and are widely expressed in the basement membrane of epithelia and blood vessels. The fibulin genes were considered attractive candidate genes for AMD because a mutation in the fibulin-3 gene (also known as *EFEMP1*) causes Doyme honeycomb retinal dystrophy/malattia Leventinese, a Mendelian macular disease with an age of onset in early to mid-adulthood that shares phenotypic similarities with AMD (Stone et al. 1999).

Sequence analysis of all fibulin genes in a cohort of 402 AMD patients identified heterozygous amino acid variants in the *FBLN5* gene in seven patients, whereas none were observed among 429 control subjects ($p < 0.01$) (Fig. 2B) (Stone et al. 2004). Interestingly, all seven of these patients had cuticular drusen, a clinical subtype of AMD (Stone et al. 2004). In a follow-up study, an analysis of the *FBLN5* gene in 805 AMD cases and 279 controls identified two heterozygous amino acid variants in three patients (Fig. 2B). Two of these patients also had cuticular drusen (Lotery et al. 2006).

In the eye, fibulin-5 is located in Bruch's membrane (Mullins et al. 2007), a pentalamellar elastin- and collagen-rich extracellular matrix located between the retinal pigment epithelium and the choroid. Fibulin-5 is believed to serve a key role in elastogenesis by interacting with fibrillin, tropoelastin, and LOX-like protein, thereby bringing these proteins in close proximity (Schneider et al. 2010). Fibulin-5 also contains an amino-terminal Arg-Gly-Asp (RGD) motif that binds to cell-surface integrins and may facilitate the anchoring of elastic fibers to the cell surface (Schneider et al. 2010).

Four variants (Gly412Glu, Gly267Ser, Ile169Thr, Gln124Pro) in fibulin-5 that have been associated with AMD dramatically reduce the protein's secretion, and a similar effect was observed for mutations that have been linked to cutis laxa, a rare connective tissue disorder that is characterized by loose, sagging, inelastic skin that can present together with developmental emphysema and major defects in the systemic and pulmonary arteries (Lotery et al. 2006; Schneider et al. 2010). The Gly412Glu variant causes aggregation of the protein (Jones et al. 2010), and the Gly267Ser variant causes extensive misfolding (Schneider et al. 2010), which may explain the reduced secretion of these mutant proteins. Two other variants (Val60Leu and Arg71Gln) affecting residues close to the integrin-binding motif have insignificant effects on fibulin-5 aggregation (Jones et al. 2010); these variants might disrupt integrin binding or they may interfere with other intermolecular interactions.

The pathogenic effects of AMD-associated variants may stem from the long-term cumulative effects of subtle changes in Bruch's membrane over the individual's lifetime (Jones et al. 2010). A restricted availability of functional fibulin-5 during elastogenesis may lead to changes in the biophysical and biochemical properties of Bruch's membrane (Mullins et al. 2007; Schneider et al. 2010). Elastin is a principal component of Bruch's membrane, and reduced levels of functional fibulin-5 protein in the extracellular matrix may impair the normal assembly of elastin within Bruch's membrane (Stone et al. 2004). Interestingly, overexpression of HTRA1, which has been associated with AMD pathogenesis (Yang et al. 2010), has been shown to alter elastogenesis in Bruch's membrane through cleaving fibulin-5 (Yang et al. 2010; Vierkotten et al. 2011). Alternatively, interference with another function of fibulin-5 (for example, integrin-mediated cell attachment) may contribute to the pathogenic mechanism underlying fibulin-5 variants (Stone et al. 2004). Impeding normal elastic lamina formation in Bruch's membrane and/or attachment of the RPE to Bruch's membrane could make this structure susceptible to deposit formation (Mullins et al. 2007).

Hemicentin-1 (HMCN1)

The *HMCN1* gene encodes an extracellular matrix protein with similarities to fibulins, and is therefore also referred to as fibulin-6. Linkage analysis in a large family with AMD identified a locus on chromosome 1q25-q31 encompassing 50 genes with a LOD score of 3.0 (Klein et al. 1998). Sequence analysis of 20 genes in the interval identified 29 exonic variants, with only one rare variant (Gln5345Arg) in the *HMCN1* gene that exclusively segregates with the disease in the family. The glutamine at position 5345 in hemicentin-1 is highly conserved among mammalian species, suggesting that it may have an important functional role (Schultz et al. 2003). It has been suggested that mutations in fibulins may alter the assembly or stability of elastin fibers and could disturb normal adhesion of the retinal pigment epithelium to Bruch's membrane (Schultz et al. 2005), which would fit with the disease pathogenesis of AMD.

However, the rare Gln5345Arg variant in the *HMCN1* gene was not found to be significantly associated with AMD in several case-control studies, even if the results of all studies are combined (Hayashi et al. 2004; McKay et al. 2004; Schultz et al. 2005). In this light, it should be noted that in the large AMD family, besides the Gln5345Arg variant in the *HMCN1* gene, 10 exonic variants were also identified in the *CFH* gene, which resides in the same linkage interval (Schultz et al. 2003). Considering the well-recognized role of *CFH* in the pathogenesis of AMD, it is plausible that one or more of the *CFH* variants, rather than the *HMCN1* Gln5345Arg variant, underlie the disease in this large AMD family. Even if these *CFH* variants do not completely segregate in the family, this does not exclude them as the cause of AMD in at least some of the affected individuals.

RELEVANCE OF RARE VARIANTS IN AMD

Predictive Testing

The first predictive tests for AMD based on a small number of common variants have been

offered directly to consumers via the internet (23andMe, deCODEme, Navigenics, Sequenom, ArcticDx, Genetic Testing Laboratories, EasyDNA) (Kalf et al. 2014). However, the reliability and predictive value of these tests have been debated. The predictive ability of genetic risk models can be quantified by the area under the receiver operating characteristic curve (AUC). An AUC of 0.5 means that the test has no predictive value (like flipping a coin), whereas a model with an AUC of 1 provides a perfect prediction. The predictive tests offered by 23andMe (based on 3 common variants), deCODEme and Navigenics (both based on 6 common variants) had an AUC value around 0.80 (Kalf et al. 2014). Therefore, the predictive value of these tests was intermediate, performing better than flipping a coin but far from a perfect prediction. The predicted risks reported to individual consumers differed substantially among the three companies. For 20% of individuals, opposing risks for AMD were predicted by at least two companies (Kalf et al. 2014). Therefore, the results of the predictive tests that have been offered to consumers should be interpreted with great caution.

Because these predictive tests were based on a small number of common variants, they were not reliable in individuals carrying rare variants conferring high risk of AMD. It is essential to understand the role of rare variants in the genetic architecture of AMD before reliable genetic tests can be developed. To provide timely and effective treatments, reliable predictive tests are necessary to determine who is at high risk of developing AMD. These tests should not only be based on a small number of common variants, but also need to address rare, highly penetrant variants (van de Ven et al. 2013), for example, by including sequence analysis of all coding exons of the *C3*, *C9*, *CFH*, *CFI*, *ABCA4*, and *FBLN5* genes. This is particularly true for AMD cases in densely affected families, which are likely to be caused by rare, highly penetrant variants (Sobrin et al. 2010), and for whom this question is most urgent.

Genetic testing for AMD is currently not recommended by professional organizations, such as The American Academy of Ophthalmology.

A.I. den Hollander and E.K. de Jong

mology Task Force on Genetic Testing (Stone et al. 2012), because the assessment of a small number of common genetic variants cannot reliably predict the development of the disease. Genetic testing for complex diseases will become relevant to routine clinical practice as soon as clinical trials can show that patients with specific genotypes benefit from specific types of therapy or surveillance. Until such benefits can be shown, routine genetic testing of patients with AMD or their family members is not recommended (Stone et al. 2012). Recently, companies offering direct-to-consumer tests have been directed by the U.S. Food and Drug Administration to stop marketing and selling direct-to-consumer genetic testing owing to concerns about public health consequences of inaccurate results (Park 2013; Annas and Elias 2014).

Recognition of Clinical Subtypes of AMD

Interestingly, several patients carrying rare variants in the *CFH*, *CFI*, and *FBLN5* genes have been reported to have cuticular drusen (Stone et al. 2004; Lotery et al. 2006; Boon et al. 2008, 2013; van de Ven et al. 2012a). This clinical subtype of AMD presents at an earlier age than typical AMD, has a strong familial component, and it has been proposed that genetic factors may play a more important role in its development than in the general AMD population (Grassi et al. 2007; van de Ven et al. 2012b; Boon et al. 2013). Cuticular drusen can be visualized by fluorescein angiography, even in individuals as young as 18 yr old, before any visual complaints have occurred. Patients with variants in the *ABCA4* gene can be distinguished by a fine granular pattern with peripheral punctate spots on fundus autofluorescence, also described as late-onset Stargardt disease (Fritsche et al. 2012; Westeneng-van Haften et al. 2012). This implies that predictive tests may be improved by integrating results of clinical and genetic analyses, and that these tests can facilitate the recognition of clinical subtypes of AMD.

Development of Personalized Treatments for AMD

There is a need for treatment options that can stop the progression of AMD to advanced stages, rather than attempting to control end-stage complications. Elucidating rare, causal variants, for example in genes encoding components of the complement cascade, will enable a more-targeted therapeutic strategy in individual patients. The strong involvement of the complement system in AMD has prompted researchers to target complement activation in AMD. Complement-based therapeutics may have the potential to allow clinicians to intervene earlier in the disease process than current treatment options. Several compounds that target the complement pathway are currently being investigated, some of which are already in clinical trials. However, a phase 2 clinical trial with eculizumab, a systemic inhibitor of complement component C5, appeared not to effectively restrict geographic atrophy in AMD patients (Yehoshua et al. 2014). It has been speculated that complement inhibitors are more likely to be effective in individuals carrying rare variants conferring a large effect on complement activation as opposed to AMD patients in general (van de Ven et al. 2013).

CONCLUDING REMARKS

Genome-wide association studies have identified a number of common genetic variations to be associated with AMD, which together are estimated to account for 15%–65% of the genetic variance of AMD (Manolio et al. 2009; Fritsche et al. 2013). Recent studies suggest that at least a fraction of the “missing heritability” of AMD may be explained by rare variants with large effect sizes (Fig. 1) (Raychaudhuri et al. 2011; Seddon et al. 2013; van de Ven et al. 2013; Zhan et al. 2013). This shapes a new genetic architecture for AMD, encompassing both common genetic variants with relatively small effect sizes and rare genetic variants with large effect sizes. Further identification of such rare variants is important to improve predictive testing, to unravel the pathogenic mechanisms in different subgroups

of AMD patients, and to develop personalized medicine for individual AMD patients tailored to his or her genetic background.

ACKNOWLEDGMENTS

This work was supported by research grants from the Macula Vision Research Foundation and the Netherlands Organisation for Scientific Research (016.096.309).

REFERENCES

- Allikmets R. 1997. A photoreceptor cell-specific ATP-binding transporter gene (*ABCR*) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* **17**: 122.
- Allikmets R, The International ABCR Screening Consortium. 2000. Further evidence for an association of *ABCR* alleles with age-related macular degeneration. *Am J Hum Genet* **67**: 487–491.
- Allikmets R, Shroyer NE, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, et al. 1997. Mutation of the Stargardt disease gene (*ABCR*) in age-related macular degeneration. *Science* **277**: 1805–1807.
- Anderson DH, Radeke MJ, Gallo NB, Chapin EA, Johnson PT, Curletti CR, Hancox LS, Hu J, Ebright JN, Malek G, et al. 2010. The pivotal role of the complement system in aging and age-related macular degeneration: Hypothesis re-visited. *Prog Retin Eye Res* **29**: 95–112.
- Annas GJ, Elias S. 2014. 23andMe and the FDA. *N Engl J Med* **370**: 985–988.
- Bodmer W, Bonilla C. 2008. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet* **40**: 695–701.
- Boon CJ, Klevering BJ, Hoyng CB, Zonneveld-Vrieling MN, Nabuurs SB, Blokland E, Cremers FP, den Hollander AI. 2008. Basal laminar drusen caused by compound heterozygous variants in the *CFH* gene. *Am J Hum Genet* **82**: 516–523.
- Boon CJ, van de Ven JP, Hoyng CB, den Hollander AI, Klevering BJ. 2013. Cuticular drusen: Stars in the sky. *Prog Retin Eye Res* **37**: 90–113.
- Burke TR, Fishman GA, Zernant J, Schubert C, Tsang SH, Smith RT, Ayyagari R, Koenekoop RK, Umfress A, Ciccarelli ML, et al. 2012. Retinal phenotypes in patients homozygous for the G1961E mutation in the *ABCA4* gene. *Invest Ophthalmol Vis Sci* **53**: 4458–4467.
- Cirulli ET, Goldstein DB. 2010. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet* **11**: 415–425.
- De La Paz MA, Guy VK, Abou-Donia S, Heinis R, Bracken B, Vance JM, Gilbert JR, Gass JD, Haines JL, Pericak-Vance MA. 1999. Analysis of the Stargardt disease gene (*ABCR*) in age-related macular degeneration. *Ophthalmology* **106**: 1531–1536.
- Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, Zhao C, Tam PO, Chan WM, Lam DS, Snyder M, et al. 2006. *HTRA1* promoter polymorphism in wet age-related macular degeneration. *Science* **314**: 989–992.
- Dryja TP, Berson EL, Rosenfeld PJ, Abitbol M. 1998. *ABCR* gene and age-related macular degeneration. *Science* **279**.
- Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farrer LA. 2005. Complement factor H polymorphism and age-related macular degeneration. *Science* **308**: 421–424.
- Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. 2009. Variation near complement factor I is associated with risk of advanced AMD. *Eur J Hum Genet* **17**: 100–104.
- Ferreira VP, Herbert AP, Cortes C, McKee KA, Blau BS, Esswein ST, Uhrin D, Barlow PN, Pangburn MK, Kavanagh D. 2009. The binding of factor H to a complex of physiological polyanions and C3b on cells is impaired in atypical hemolytic uremic syndrome. *J Immunol* **182**: 7009–7018.
- Fritsche LG, Fleckenstein M, Fiebig BS, Schmitz-Valckenberg S, Bindewald-Wittich A, Keilhauer CN, Renner AB, Mackensen F, Mossner A, Pauleikhoff D, et al. 2012. A subgroup of age-related macular degeneration is associated with mono-allelic sequence variants in the *ABCA4* gene. *Invest Ophthalmol Vis Sci* **53**: 2112–2118.
- Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, Thorleifsson G, Zack DJ, Arakawa S, Cipriani V, Ripke S, et al. 2013. Seven new loci associated with age-related macular degeneration. *Nat Genet* **45**: 433–439.
- Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, Barile GR, et al. 2006. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. *Nat Genet* **38**: 458–462.
- Goldstein DB, Allen A, Keebler J, Margulies EH, Petrou S, Petrovski S, Sunyaev S. 2013. Sequencing studies in human genetics: Design and interpretation. *Nat Rev Genet* **14**: 460–470.
- Grassi MA, Folk JC, Scheetz TE, Taylor CM, Sheffield VC, Stone EM. 2007. Complement factor H polymorphism p.Tyr402His and cuticular drusen. *Arch Ophthalmol* **125**: 93–97.
- Guymer RH, Heon E, Lotery AJ, Munier FL, Schorderet DF, Baird PN, McNeil RJ, Haines H, Sheffield VC, Stone EM. 2001. Variation of codons 1961 and 2177 of the Stargardt disease gene is not associated with age-related macular degeneration. *Arch Ophthalmol* **119**: 745–751.
- Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, et al. 2005. A common haplotype in the complement regulatory gene factor H (*HF1/CFH*) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci* **102**: 7227–7232.
- Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, et al. 2005. Complement factor H variant increases the risk of age-related macular degeneration. *Science* **308**: 419–421.
- Hayashi M, Merriam JE, Klaver CC, Zernant J, Bergen AA, Smith RT, Chang S, Merriam JC, Allikmets R. 2004. Evaluation of the *ARMD1* locus on 1q25–31 in patients with age-related maculopathy: Genetic variation in laminin

A.I. den Hollander and E.K. de Jong

- genes and in exon 104 of *HEMICENTIN-1*. *Ophthalmol Genet* **25**: 111–119.
- Helgason H, Sulem P, Duvvari MR, Luo H, Thorleifsson G, Stefansson H, Jonsdottir I, Masson G, Gudbjartsson DE, Walters GB, et al. 2013. A rare nonsynonymous sequence variant in *C3* is associated with high risk of age-related macular degeneration. *Nat Genet* **45**: 1371–1374.
- Huyghe JR, Jackson AU, Fogarty MP, Buchkovich ML, Stanekova A, Stringham HM, Sim X, Yang L, Fuchsberger C, Cederberg H, et al. 2013. Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. *Nat Genet* **45**: 197–201.
- 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**: 389–407.
- Jones RP, Ridley C, Jowitt TA, Wang MC, Howard M, Bobola N, Wang T, Bishop PN, Kieley CM, Baldock C, et al. 2010. Structural effects of fibulin 5 missense mutations associated with age-related macular degeneration and cutis laxa. *Invest Ophthalmol Vis Sci* **51**: 2356–2362.
- Kalf RR, Mihaescu R, Kundu S, de Knijff P, Green RC, Janssens AC. 2014. Variations in predicted risks in personal genome testing for common complex diseases. *Genet Med* **16**: 85–91.
- Kiezun A, Garimella K, Do R, Stitzel NO, Neale BM, McLaren PJ, Gupta N, Sklar P, Sullivan PF, Moran JL, et al. 2012. Exome sequencing and the genetic basis of complex traits. *Nat Genet* **44**: 623–630.
- Klaver CC, Bergen AA, van Duijn CM. 1998a. *ABCR* gene and age-related macular degeneration. *Science* **279**.
- Klaver CC, Wolfs RC, Assink JJ, van Duijn CM, Hofman A, de Jong PT. 1998b. Genetic risk of age-related maculopathy. Population-based familial aggregation study. *Arch Ophthalmol* **116**: 1646–1651.
- Klein ML, Schultz DW, Edwards A, Matisse TC, Rust K, Berselli CB, Trzuppek K, Weleber RG, Ott J, Wirtz MK, et al. 1998. Age-related macular degeneration. Clinical features in a large family and linkage to chromosome 1q. *Arch Ophthalmol* **116**: 1082–1088.
- Lander E, Kruglyak L. 1995. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nat Genet* **11**: 241–247.
- Liu DJ, Leal SM. 2012. Estimating genetic effects and quantifying missing heritability explained by identified rare-variant associations. *Am J Hum Genet* **91**: 585–596.
- Liu MM, Chan CC, Tuo J. 2012. Genetic mechanisms and age-related macular degeneration: Common variants, rare variants, copy number variations, epigenetics, and mitochondrial genetics. *Hum Genom* **6**: 13.
- Lotery AJ, Baas D, Ridley C, Jones RP, Klaver CC, Stone E, Nakamura T, Luff A, Griffiths H, Wang T, et al. 2006. Reduced secretion of fibulin 5 in age-related macular degeneration and cutis laxa. *Hum Mutat* **27**: 568–574.
- Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, et al. 2009. Finding the missing heritability of complex diseases. *Nature* **461**: 747–753.
- Manuelian T, Hellwege J, Meri S, Caprioli J, Noris M, Heinen S, Jozsi M, Neumann HP, Remuzzi G, Zipfel PF. 2003. Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. *J Clin Invest* **111**: 1181–1190.
- Mayeux R. 2005. Mapping the new frontier: Complex genetic disorders. *J Clin Invest* **115**: 1404–1407.
- McKay GJ, Clarke S, Hughes A, McConnell V, Schultz DW, Klein ML, Silvestri G, Simpson DA. 2004. A novel diagnostic test detects a low frequency of the hemicentin Gln5345Arg variant among Northern Irish age related macular degeneration patients. *Mol Vis* **10**: 682–687.
- Miller E. 2012. Characterization of complement C3 dysregulation predisposing to two human disease states. PhD thesis, Washington University, St Louis.
- Mullins RE, Olvera MA, Clark AF, Stone EM. 2007. Fibulin-5 distribution in human eyes: Relevance to age-related macular degeneration. *Exp Eye Res* **84**: 378–380.
- O’Roak BJ, Vives L, Fu W, Egerton JD, Stanaway IB, Phelps IG, Carvill G, Kumar A, Lee C, Ankenman K, et al. 2012. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* **338**: 1619–1622.
- Park A. 2013. FDA halts 23andMe genetic tests. *Time* **182**: 18.
- Pritchard JK. 2001. Are rare variants responsible for susceptibility to complex diseases? *Am J Hum Genet* **69**: 124–137.
- Quazi F, Lenevich S, Molday RS. 2012. ABCA4 is an *N*-retinylidene-phosphatidylethanolamine and phosphatidylethanolamine importer. *Nat Commun* **3**: 925.
- Raychaudhuri S, Iartchouk O, Chin K, Tan PL, Tai AK, Ripke S, Gowrisankar S, Vemuri S, Montgomery K, Yu Y, et al. 2011. A rare penetrant mutation in *CFH* confers high risk of age-related macular degeneration. *Nat Genet* **43**: 1232–1236.
- Rivera A, White K, Stohr H, Steiner K, Hemmrich N, Grimm T, Jurklics B, Lorenz B, Scholl HP, Apfelstedt-Sylla E, 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**: 800–813.
- Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, Meitinger T, Weber BH. 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**: 3227–3236.
- Sanchez-Corral P, Perez-Caballero D, Huarte O, Simckes AM, Goicoechea E, Lopez-Trascasa M, de Cordoba SR. 2002. Structural and functional characterization of factor H mutations associated with atypical hemolytic uremic syndrome. *Am J Hum Genet* **71**: 1285–1295.
- Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci* **74**: 5463–5467.
- Schneider R, Jensen SA, Whiteman P, McCullagh JS, Redfield C, Handford PA. 2010. Biophysical characterisation of fibulin-5 proteins associated with disease. *J Mol Biol* **401**: 605–617.
- Schultz DW, Klein ML, Humpert AJ, Luzier CW, Persun V, Schain M, Mahan A, Runckel C, Cassera M, Vittal V, et al. 2003. Analysis of the *ARMD1* locus: Evidence that a mu-

- tation in *HEMICENTIN-1* is associated with age-related macular degeneration in a large family. *Hum Mol Genet* **12**: 3315–3323.
- Schultz DW, Weleber RG, Lawrence G, Barral S, Majewski J, Acott TS, Klein ML. 2005. *HEMICENTIN-1* (FIBULIN-6) and the 1q31 AMD locus in the context of complex disease: Review and perspective. *Ophthalmol Genet* **26**: 101–105.
- Seddon JM, Ajani UA, Mitchell BD. 1997. Familial aggregation of age-related maculopathy. *Am J Ophthalmol* **123**: 199–206.
- 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**: 321–327.
- Seddon JM, Yu Y, Miller EC, Reynolds R, Tan PL, Gowrisankar S, Goldstein JL, Triebwasser M, Anderson HE, Zerbib J, et al. 2013. Rare variants in *CFI*, *C3* and *C9* are associated with high risk of advanced age-related macular degeneration. *Nat Genet* **45**: 1366–1370.
- Shahid H, Khan JC, Cipriani V, Sepp T, Matharu BK, Bunce C, Harding SP, Clayton DG, Moore AT, Yates JR, et al. 2012. Age-related macular degeneration: The importance of family history as a risk factor. *Br J Ophthalmol* **96**: 427–431.
- Sobrin L, Maller JB, Neale BM, Reynolds RC, Fageress JA, Daly MJ, Seddon JM. 2010. Genetic profile for five common variants associated with age-related macular degeneration in densely affected families: A novel analytic approach. *Eur J Hum Genet* **18**: 496–501.
- Stone EM, Lotery AJ, Munier FL, Heon E, Piguet B, Guymer RH, Vandenberg K, Cousin P, Nishimura D, Swiderski RE, et al. 1999. A single *EFEMP1* mutation associated with both Malattia Leventinese and Doyme honeycomb retinal dystrophy. *Nat Genet* **22**: 199–202.
- Stone EM, Braun TA, Russell SR, Kuehn MH, Lotery AJ, Moore PA, Eastman CG, Casavant TL, Sheffield VC. 2004. Missense variations in the fibulin 5 gene and age-related macular degeneration. *N Engl J Med* **351**: 346–353.
- Stone EM, Aldave AJ, Drack AV, Maccumber MW, Sheffield VC, Traboulsi E, Weleber RG. 2012. Recommendations for genetic testing of inherited eye diseases: Report of the American Academy of Ophthalmology task force on genetic testing. *Ophthalmology* **119**: 2408–2410.
- Sun H, Smallwood PM, Nathans J. 2000. Biochemical defects in ABCR protein variants associated with human retinopathies. *Nat Genet* **26**: 242–246.
- Thornton J, Edwards R, Mitchell P, Harrison RA, Buchan I, Kelly SP. 2005. Smoking and age-related macular degeneration: A review of association. *Eye* **19**: 935–944.
- van de Ven JP, Boon CJ, Fauser S, Hoefsloot LH, Smailhodzic D, Schoenmaker-Koller F, Klevering J, Klaver CC, den Hollander AI, Hoyng CB. 2012a. Clinical evaluation of 3 families with basal laminar drusen caused by novel mutations in the complement factor H gene. *Arch Ophthalmol* **130**: 1038–1047.
- van de Ven JP, Smailhodzic D, Boon CJ, Fauser S, Groenewoud JM, Chong NV, Hoyng CB, Klevering BJ, den Hollander AI. 2012b. Association analysis of genetic and environmental risk factors in the cuticular drusen subtype of age-related macular degeneration. *Mol Vis* **18**: 2271–2278.
- van de Ven JP, Nilsson SC, Tan PL, Buitendijk GH, Ristau T, Mohlin FC, Nabuurs SB, Schoenmaker-Koller FE, Smailhodzic D, Campochiaro PA, et al. 2013. A functional variant in the *CFI* gene confers a high risk of age-related macular degeneration. *Nat Genet* **45**: 813–817.
- Vierkotten S, Muether PS, Fauser S. 2011. Overexpression of HTRA1 leads to ultrastructural changes in the elastic layer of Bruch's membrane via cleavage of extracellular matrix components. *PLoS ONE* **6**: e22959.
- Walport MJ. 2001a. Complement. First of two parts. *N Engl J Med* **344**: 1058–1066.
- Walport MJ. 2001b. Complement. Second of two parts. *N Engl J Med* **344**: 1140–1144.
- Webster AR, Heon E, Lotery AJ, Vandenberg K, Casavant TL, Oh KT, Beck G, Fishman GA, Lam BL, Levin A, et al. 2001. An analysis of allelic variation in the *ABCA4* gene. *Invest Ophthalmol Vis Sci* **42**: 1179–1189.
- Westeneng-van Haaften SC, Boon CJ, Cremers FP, Hoefsloot LH, den Hollander AI, Hoyng CB. 2012. Clinical and genetic characteristics of late-onset Stargardt's disease. *Ophthalmology* **119**: 1199–1210.
- Yang Z, Tong Z, Chen Y, Zeng J, Lu F, Sun X, Zhao C, Wang K, Davey L, Chen H, et al. 2010. Genetic and functional dissection of *HTRA1* and *LOC387715* in age-related macular degeneration. *PLoS Genet* **6**: e1000836.
- Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, Clayton DG, Hayward C, Morgan J, Wright AF, et al. 2007. Complement C3 variant and the risk of age-related macular degeneration. *N Engl J Med* **357**: 553–561.
- Yehoshua Z, de Amorim Garcia Filho CA, Nunes RP, Gregori G, Penha FM, Moshfeghi AA, Zhang K, Sadda S, Feuer W, Rosenfeld PJ. 2014. Systemic complement inhibition with eculizumab for geographic atrophy in age-related macular degeneration: The COMPLETE study. *Ophthalmology* **121**: 693–701.
- Yu Y, Bhangale TR, Fageress J, Ripke S, Thorleifsson G, Tan PL, Souied EH, Richardson AJ, Merriam JE, Buitendijk GH, et al. 2011. Common variants near *FRK/COL10A1* and *VEGFA* are associated with advanced age-related macular degeneration. *Hum Mol Genet* **20**: 3699–3709.
- Zhan X, Larson DE, Wang C, Koboldt DC, Sergeev YV, Fulton RS, Fulton LL, Fronick CC, Branham KE, Bragg-Gresham J, et al. 2013. Identification of a rare coding variant in complement 3 associated with age-related macular degeneration. *Nat Genet* **45**: 1375–1379.

Cold Spring Harbor Perspectives in Medicine

Highly Penetrant Alleles in Age-Related Macular Degeneration

Anneke I. den Hollander and Eiko K. de Jong

Cold Spring Harb Perspect Med 2015; doi: 10.1101/cshperspect.a017202 originally published online November 6, 2014

Subject Collection   [Retinal Disorders: Genetic Approaches to Diagnosis and Treatment](#)

Trial by "Firsts": Clinical Trial Design and Regulatory Considerations in the Development and Approval of the First AAV Gene Therapy Product in the United States

Kathleen Z. Reape and Katherine A. High

Immunology of Retinitis Pigmentosa and Gene Therapy–Associated Uveitis

Paul Yang, Debarshi Mustafi and Kathryn L. Pepple

Developing New Vectors for Retinal Gene Therapy

Emilia A. Zin, Bilge E. Ozturk, Deniz Dalkara, et al.

Beyond the NEI-VFQ: Recent Experience in the Development and Utilization of Patient-Reported Outcomes for Inherited Retinal Diseases

Todd Durham, Judit Banhazi, Francesco Patalano, et al.

Electronic Retinal Prostheses

Daniel Palanker

Alternative RNA Splicing in the Retina: Insights and Perspectives

Casey J. Keuthan, Sadik Karma and Donald J. Zack

X-Linked Retinoschisis

Cristy A. Ku, Lisa W. Wei and Paul A. Sieving

A Systematic Review of Optogenetic Vision Restoration: History, Challenges, and New Inventions from Bench to Bedside

Antonia Stefanov and John G. Flannery

Lessons Learned from the Development of the First FDA-Approved Gene Therapy Drug, Voretigene Neparvovec-rzyl

Jean Bennett and Albert M. Maguire

Therapeutic Gene Editing in Inherited Retinal Disorders

Jinjie Ling, Laura A. Jenny, Ashley Zhou, et al.

Cell-Based Therapies: Strategies for Regeneration

Marina Pavlou and Thomas A. Reh

The Importance of Natural History Studies in Inherited Retinal Diseases

Allison Ayala, Janet Cheetham, Todd Durham, et al.

Photoreceptor Cell Replacement Using Pluripotent Stem Cells: Current Knowledge and Remaining Questions

Christelle Monville, Olivier Goureau and Karim Ben M'Barek

iPSC-RPE in Retinal Degeneration: Recent Advancements and Future Perspectives

Tadao Maeda and Masayo Takahashi

Retinal Degeneration Animal Models in Bardet–Biedl Syndrome and Related Ciliopathies

Clarisse Delvallée and Hélène Dollfus

Mobility Testing and Other Performance-Based Assessments of Functional Vision in Patients with Inherited Retinal Disease

Daniel Chung, Colas Authié and Laure Blouin

For additional articles in this collection, see <http://perspectivesinmedicine.cshlp.org/cgi/collection/>

For additional articles in this collection, see <http://perspectivesinmedicine.cshlp.org/cgi/collection/>