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REVIEW ARTICLE

Genetics and genetic testing for age-related macular degeneration

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Abstract

Considerable advances have been made in our understanding of age-related macular degeneration (AMD) genetics over the past decade. The genetic associations discovered to date are estimated to account for approximately half of AMD heritability, and functional studies of these variants have revealed new insights into disease pathogenesis, leading to the development of potential novel therapies. There is furthermore growing interest in genetic testing for predicting an individual's risk of AMD and offering personalised preventive or therapeutic treatments. We review the progress made so far in AMD genetics and discuss the possible applications for genetic testing.

Introduction

Age-related macular degeneration (AMD) is the commonest cause of blindness in the developed world, affecting 5% of those aged >75 years old and an estimated 150-million people worldwide [1, 2]. Disease onset is in later life, typically after the age of 60 years, and is characterised clinically by retinal pigmentary change and the appearance of drusen at the macula. Central vision may subsequently decline either gradually with progressive geographic retinal atrophy (GA), or acutely due to retinal haemorrhage and fluid exudation from choroidal neovascularisation (CNV) if neovascular AMD (nvAMD) develops. While the latter may now be effectively treated with intravitreal anti-vascular endothelial growth factor (VEGF) injections, there is currently no therapy for GA available in clinical practice [3].

Over the past decade, considerable progress has been made in elucidating the genetic architecture of AMD. The discovery of multiple genetic associations and studies of their downstream functional consequences has helped reveal new underlying pathophysiological mechanisms in AMD, affording the potential to identify molecular targets for novel therapies. The complement pathway in particular, an important component of the innate immune system, has been consistently implicated [4]. Furthermore, there is growing interest in genetic testing to predict either an individual's risk of developing AMD or how well they will respond to treatment. Ultimately, this could mean that in the future, patients are offered personalised preventive or therapeutic treatments, tailored to their individual genetic makeup. In this review, we summarise the current literature and possible future directions for AMD genetics and genetic testing.

Genetics of AMD

AMD is a complex disease with multiple environmental and genetic risk factors. The most consistently associated environmental risk factors are age and smoking, although gender, race, cardiovascular disease, and diet have also been implicated [5]. Evidence for a genetic component was supported by family aggregation studies and twin studies. In family aggregation studies, the prevalence of AMD was higher in first-degree relatives of AMD patients (23.7%) compared with relatives of controls (11.6%), with an odds ratio (OR) of 2.4 [6]. By comparing disease concordance rates between monozygotic and dizygotic twins, the heritability of early and advanced AMD has been estimated at 46% and 71%, respectively, implying that 46–71% of AMD variation may be explained by genetic factors [7].

In early efforts to explore AMD genetics, association studies were performed for candidate genes known to cause Mendelian macular diseases such as Best's disease,

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Stargardt's disease, and Sorsby's fundus dystrophy. However, no consistent significant associations were made [8–11]. Other groups undertook genetic linkage studies of patient families to identify genomic regions containing susceptibility loci for AMD. A meta-analysis of these results showed that the most replicated findings were on chromosomes 1q25-31 and 10q26 [12]. Their importance was then validated by subsequent findings of specific AMD-associated common genetic variants at these two loci—the complement factor H (*CFH*) gene on chromosome 1 and the age-related maculopathy susceptibility 2/HtrA serine peptidase (*ARMS2/HTRA1*) genes on chromosome 10.

Common variants

Technological advancements enabling the analysis of whole genomes, rather than individual genes, have greatly accelerated the discovery of new genetic associations with common complex genetic diseases such as AMD. Genome-wide association studies (GWAS) examine a genome-wide set of genetic variants, typically single-nucleotide polymorphisms (SNPs), for associations with the disease of interest. While genetic linkage studies are effective for investigating high-penetrance single-gene defects underlying rare monogenic disorders, GWASs are better able to detect low-penetrance common genetic variants (with a minor allele frequency (MAF) >5%) associated with complex genetic diseases.

In 2005, landmark studies associated a common polymorphism (Tyr402His or Y402H) in the *CFH* gene on chromosome 1 with AMD [13–16]. One of these was the first GWAS performed for AMD, showing that the Y402H risk allele had a large effect size (4.6 and 7.4 increased the likelihood of AMD in heterozygous and homozygous individuals, respectively) [16]. Of note, this was the first successful GWAS of a 'complex disease', that is, one with both genetic and environmental factors contributing significantly to the disease. As such, it represented a major success for genetic approaches to studying common diseases. Subsequent GWASs and candidate gene studies have since associated several other common variants in complement-related genes with AMD, including *C2/CFB* [17], *C3* [18], *C7* [19], *CFI* [20], and *SERPING* [21].

The complement cascade encompasses a family of more than 30 circulating proteins and their regulators which form an important part of the innate immune system. Activation of the complement cascade ultimately results in formation of the membrane attack complex (MAC), which induces cell lysis. While the liver is the major source of systemic complement, retinal cells synthesise their own complement [22], and complement proteins have been detected in drusen from human eyes [23–25]. Raised systemic levels of complement have also been reported in AMD patients *vs* controls [26], and interestingly, patients with dense deposit disease, a renal condition associated with systemic complement dysregulation and glomerular C3 deposition, often develop drusen [27, 28]. However, a locally produced, rather than systemic, complement likely appears to be more important in AMD [26, 29].

Chronic intraocular complement-mediated inflammation in genetically susceptible individuals exposed to environmental triggers, such as cigarette smoke and oxidative stress, may contribute to the progressive retinal changes observed in AMD. While the functional relevance of most common AMD-associated genetic variants remains unknown, a common missense polymorphism in *C3* has been shown to result in reduced binding to CFH, a plasma regulator of complement, and increased activity of the alternative complement pathway [30]. Furthermore, a number of studies have looked at the functional effects of the non-synonymous *CFH* Y402H polymorphism. The minor allele, encoding a histidine amino acid at residue 402 of the CFH protein, alters its affinity for CRP [31], glycosaminoglycans in Bruch's membrane [32, 33], malondialdehyde [34], and zinc [35]. These changes are thought to decrease its ability to regulate complement. Reduced complement regulation may lead to increased MAC deposition and choroidal endothelial cell death, impairing the ability of the choriocapillaris to remove debris and allowing the accumulation of waste products in drusen [4]. In support of this theory, eyes homozygous for the *CFH* Y402H risk variant have thinner choroids [36] and increased MAC deposition [37] at the choriocapillaris compared with eyes with low-risk *CFH* genotypes.

Genes not involved in the complement pathway have also been associated with AMD. The *ARMS2/HTRA1* locus on chromosome 10q26 has been strongly associated to risk alleles conferring an OR of 5.0 and a population-attributable risk up to 57% [38, 39]. As both genes at this region are in strong linkage disequilibrium with each other and both harbour functional variants which could plausibly be relevant to AMD, dissecting out which is responsible for the observed association with AMD has proved to be challenging [40]. Recently, however, analysis of the largest data set for AMD genetics to date suggested that genetic variants at *ARMS2*, but not *HTRA1*, are responsible for AMD susceptibility at the 10q26 locus [41]. Further functional analysis of *ARMS2* is required to understand its role in AMD pathogenesis. Other non-complement associations with AMD include genes implicated in angiogenesis (*TGFBR1*, *VEGFA*), the extracellular collagen matrix (*COL10A1*, *COL8A1*), the high-density lipoprotein cholesterol pathway (*APOE*, *CETP*, and *LIPC*), and immune regulation (*PILRB*) [42–44].

Rare variants

In 2013, the AMD Gene Consortium published the largest GWAS for AMD conducted up until that time, evaluating >2.4 million SNPs in >17,100 cases and >60,000 controls. However, they estimated that only 15–65% of AMD heritability was explained by the 19 loci discovered [42]. Several theories seeking to explain the missing heritability exist, including gene–environment interactions, gene–gene interactions, epigenetics, copy number variation, and rare variants [45]. In particular, the focus of genetic research for complex diseases in general has shifted towards identifying low-frequency (MAF 1–5%) and rare variants (MAF≤1%) with relatively large effect sizes [46]. Furthermore, a recent simulation study demonstrated that the clustering of AMD in densely affected families was insufficiently explained by the genotypic load of common genetic risk variants and that rare variants may be more important [47].

Various approaches have been successful in discovering new rare genetic variants associated with AMD. Next-generation sequencing technology can be used to comprehensively analyse variation within candidate genes between cases and controls. The first rare variant to be associated with AMD, *CFH* R1210C was identified in this way [48]. It demonstrated high penetrance (present in 40 cases *vs* 1 control, $P=7.0 \times 10^{-6}$, and OR 18.8) and was associated with an earlier onset of the disease. Similar studies have also identified rare variants in *CFI*, *C3*, and *C9* [49–51]. Whole-exome or whole-genome sequencing is expensive to carry out in large numbers but has been performed in an Icelandic case–control cohort [52], as well as in large AMD families, thought to be enriched for rare variants [53–59]. In 2016, the International AMD Genomics Consortium (IAMDGC) identified 52 independently associated common and rare variants distributed across 34 loci [44]. The group performed a GWAS using an exome chip, customised to analyse >12 million variants (including >163,700 directly genotyped, mostly rare, protein-altering variants) in >16,100 patients and >17,800 controls. This is the largest study of AMD genetics performed to date, more than doubling the number of known associated variants and identifying the first variant specific to one advanced AMD phenotype (*MMP9* and nvAMD). Rare variants were discovered in *CFH* and *CFI*, as well as the non-complement genes *TIMP3* and *SLC16A8* [44].

Compared with common variants, highly penetrant rare variants often have clearer functional effects [60]. For example, in a large advanced AMD cohort, rare *CFH* variants tended to be located in functional domains and resulted in low CFH serum levels [61]. Rare variants in *CFI*, another regulator of the complement system, have also been associated with decreased serum CFI levels [54], and carriers of rare variants in both *CFH* and *CFI* have impaired ability to regulate complement activation [54, 59]. Furthermore, the rare *C3* Lys155Gln variant has been shown to impair C3b regulation by CFI with bound CFH [49]. Overall, these findings support the hypothesis that increased complement activity may contribute to AMD pathogenesis.

Carriers of rare variants appear to have differing phenotypes compared with non-carriers. A number of studies have shown rare variants to be associated with earlier onset of advanced AMD [48, 58, 59, 62, 63]. Carriers of rare *CFH* variants have increased drusen load, are more likely to have extramacular drusen, drusen nasal to the optic disc, and crystalline or calcified drusen [62, 63, 64]. In addition, rare variants in *CFH*, *CFI*, *C9*, and *C3* have been more frequently observed in patients with GA than those with nvAMD [58, 63]. Interestingly, a rare missense mutation in *TIMP3* (C1113G), identified by the IAMDGC, is associated with earlier age of disease onset (average age 65 years) and bilateral CNV [65]. Other mutations in *TIMP3* cause Sorsby's fundus dystrophy, an autosomal dominant fundus dystrophy characterised by a similar clinical phenotype to AMD, although typically, age of onset is in the fourth decade of life. Further, genotype–phenotype correlations are needed to determine whether the phenotype associated with this mutation more closely resembles AMD or Sorsby's fundus dystrophy.

The IAMDGC estimates that the 52 currently known variants account for approximately half the genomic heritability of AMD [44]. Despite this significant progress, there is still therefore a large portion of missing heritability. The IAMDGC highlights the need for very large sample sizes and extensive genome coverage in population studies looking for novel rare variants in complex diseases. Future studies looking at other potential sources of missing heritability and functional studies of known associated variants are also needed.

Genetic testing for AMD

As the list of known AMD-associated genetic variants continues to grow, so too does interest in developing predictive risk models incorporating these alleles. The ability to accurately predict a person's risk of developing AMD would be an important step towards personalised medicine, allowing appropriate preventive measures to be taken in high-risk individuals. Pharmacogenetic testing could furthermore help identify which AMD patients are most likely to benefit from certain treatments. For example, it is conceivable that novel therapies modulating the complement system may be most effective in patients harbouring complement-related risk alleles.

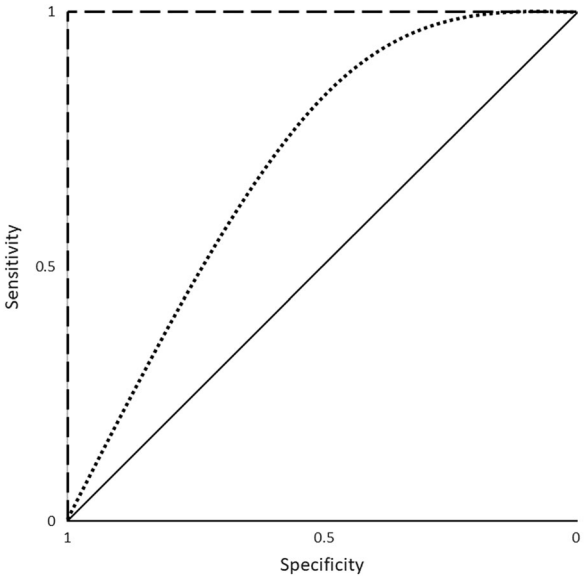

Fig. 1 An example of a receiver-operating characteristic (ROC) curve. The solid black line represents an area under the ROC (AUC) of 0.5, which indicates random chance. The dashed black line indicates an AUC of 1.0, a perfect model. The dotted grey line represents an ROC curve with an AUC between 0.5 and 1.0, as may be generated by a predictive risk model. Specificity decreases with increasing sensitivity.

Genetic risk models for AMD

In contrast to rare monogenic disorders, complex genetic diseases such as AMD are associated with multiple environmental and genetic risk factors. Possession of an allele known to be associated with the disease increases an individual's risk of developing the condition and possessing multiple risk alleles, or exposure to certain environmental triggers may further increase this risk. Such individuals could however either remain healthy or only ever progress to a mild asymptomatic stage. Risk models for complex diseases therefore aim to accurately predict risk by incorporating multiple alleles or environmental risk factors.

A common method for describing the accuracy of a risk model is to calculate the area under the receiver-operating characteristic (ROC) curve (AUC), the ROC being a plot of all possible (sensitivity, specificity) pairs for the model (Fig. 1). The AUC can theoretically take values between 0 and 1, but in practice varies from 0.5 to 1. A score of 1 means perfect accuracy, whereas a score of 0.5 indicates that the predictive ability of a model is equivalent to random chance or zero accuracy. A model with a score between 0.5 and 1.0 therefore has greater ability than chance to discriminate between cases and controls. It has been suggested that an AUC>0.75 is acceptable and >0.9 is excellent [66].

Existing risk models for AMD predict either the risk of developing AMD or of progressing from the early and intermediate stages to advanced disease [66]. Models incorporating only genetic information have achieved AUCs>0.8 [67, 68], and models combining both genetic and environmental risk factors have reported AUCs>0.9 [69, 70, 71]. Interestingly, a model which only included environmental risk factors predicted the risk of developing advanced AMD with a similar AUC of 0.88 [72], leading some to question the usefulness of genetic data in predictive models [73]. Baseline macular phenotype in particular is a strong predictor of AMD progression. While some groups have shown improved risk model accuracy for disease progression when the genotype is included with fundus phenotype [69, 70], a recent study found only a small addition to predictive power [74]. Future studies looking at which genetic variants associate specifically with progression of AMD may be useful.

While the AUC broadly measures the accuracy of a model to distinguish between cases and controls, additional factors need to be considered when evaluating a model's ability to estimate the risk for individuals. One method is to use the reclassification approach, which classifies individuals into risk strata. In a worked example, Jakobsdottir et al. [75] applied a genetic risk model for AMD with an AUC of 0.79 to a theoretical population with an AMD prevalence of 5.5% (corresponding approximately to AMD prevalence in patients older than 65 years). For a sensitivity of 74 and specificity of 31%, the model classified individuals with >4% risk of AMD as cases ('high risk') and those with <4% risk as controls ('low risk'). The corresponding positive predictive value was only 12%, meaning that 88% of those patients classified as 'high risk' should actually have been in the 'low risk' group. The authors also demonstrated how the model's predictive ability is heavily influenced by disease prevalence, which is highly age dependent for AMD. Applying their model with the same sensitivity and specificity to a population with a 15% AMD prevalence (the approximate prevalence for patients older than 80 years) classified individuals as >10% risk (cases) or <10% risk (controls) of AMD, with an improved positive predictive value of 30% [75].

As illustrated by the example above, a risk model with a seemingly acceptable AUC may therefore not be useful clinically as a predictor of individual risk. In addition, some genetic risk variants discovered in one population may not be as important in others. To date, the majority of AMD genetic associations have been studied in populations of European ancestry. The 19 risk loci discovered by the AMD Gene Consortium in a non-Amish Caucasian population appear to account for a lower proportion of AMD in Amish individuals, and a rare *CFH* variant (P503A) associated with AMD in the Amish population was absent in non-Amish cases and controls [42, 55]. The common *CFH* Y402H risk variant is present in ~30% of Utah residents of Northern and Western European ancestry, but only ~5% of

Japanese and Chinese individuals [76]. A recent study showed both the *CFH* Y402H and *ARMS2* A69S variants to be associated with AMD in European Americans but not in African Americans, Mexican Americans, or Singaporeans [77]. Furthermore, in another study, the common *ARMS2* A69S variant was associated with increased risk of AMD in non-Hispanic whites and Mexican Americans but was found to be protective in non-Hispanic black individuals [78]. This implies that differing genetic loci underlie AMD in different populations, and predictive risk models should account for these ethnic variations accordingly.

Despite the limitations of predictive testing in AMD, a number of commercial companies now provide genetic AMD tests. Improved accuracy of these tests could in the future aid the detection of high-risk individuals who may benefit from early preventive measures. However, there is currently no evidence that changes to the management of such patients beyond current practice are beneficial. For these reasons, the current recommendations from the American Academy of Ophthalmology (AAO) include avoidance of routine genetic testing for complex disorders like AMD, until prospective clinical trials have shown specific surveillance or treatment strategies to be of benefit [79].

Pharmacogenetics and AMD

Another potential use for genetic testing is to identify which AMD patients may respond best to specific treatments. The Age-Related Eye Disease Study (AREDS) antioxidant and zinc formulation is the only therapeutic intervention that has been shown prospectively to significantly reduce the risk of progression to advanced AMD [80]. Genetic testing to guide preventive treatment with the AREDS formulation has proved to be controversial. Awh et al. [81, 82] recently published retrospective analyses on patients from the AREDS trial, reporting that certain *CFH* and *ARMS2* genotypes were associated with potentially harmful responses to dietary antioxidant and zinc supplementation. The authors therefore concluded that favourable outcomes may be achieved by assigning nutritional supplementation based on *CFH* and *ARMS2* genotypes. Subsequent analyses by the original AREDS investigators, however, found the AREDS formulation to be beneficial for all genotypes, as well as pointing out statistical errors made by Awh et al. [83, 84]. Other studies have shown the effectiveness of nutritional supplementation to differ by genotype, but no harmful effects were observed [85, 86]. Prospective clinical trials are needed to more definitively address this issue, but at present, there is insufficient evidence to support genetic testing prior to recommending AREDS nutritional supplementation [87, 88].

A number of studies have also examined whether AMD-associated risk variants and VEGF-related gene polymorphisms affect response to anti-VEGF therapy in nvAMD. Although the majority of patients do well with anti-VEGF treatment, 10–15% respond poorly and lose vision [89]. Variation in treatment outcomes is related to clinical characteristics, such as baseline visual acuity and central retinal thickness, and may also be influenced by genetic factors. A single GWAS identified an association between a variant in the *OR52B4* gene and response to anti-VEGF; however, this is yet to be replicated [90]. Small retrospective studies have found statistically significant associations between variants in candidate genes, either related to angiogenesis or known to confer AMD risk, and response to anti-VEGF [91]. In contrast, no such genetic associations were observed in the major Comparison of AMD Treatments Trial (CATT) and Inhibit VEGF in Patients with Age-Related CNV Study (IVAN) randomised control trials [92–96]. Recent meta-analyses pooling data from these smaller studies with the major trials have reported positive associations between anti-VEGF treatment response and the *CFH* Y402H and *AMRS2* A69S variants, as well as polymorphisms in *VEGF-A* and *VEGFR-2*, but all acknowledge the need for large prospective trials to validate their findings [97–100].

Genetic testing in AMD research

Routine genetic testing for AMD is therefore currently not advised in clinical practice, and further supportive evidence from prospective clinical trials is needed before recommending genetic testing to guide treatment with AREDS nutritional supplementation or anti-VEGF [79, 87, 88]. Genetic testing may however be useful as a research tool, for example, to select suitable patients for future clinical trials of novel therapeutics designed to prevent AMD. Restricting patient recruitment to those individuals at the highest risk of developing advanced AMD is likely to decrease the sample size requirements for adequate powering of such studies. Reconsidering the predictive model with an AUC of 0.79 by Jakobsdottir et al. [75] discussed earlier, while a positive predictive value of 12–30% may be poor in the clinical setting, it might prove cost-effective to help improve the proportion of participants at high risk of developing AMD in a clinical trial.

Genetic testing could also be useful in therapeutic clinical trials. To date, the complement system has been most frequently implicated in AMD, and consequently, several trials of complement inhibitors have been initiated [60]. AMD is genetically heterogeneous and it is possible that patients with certain risk variants in complement-related genes may respond differentially to complement inhibition.

Recently reported findings from the phase II MAHALO trial of lampalizumab, a monoclonal antibody inhibitor of complement factor D, in GA support this concept. Progression of GA lesion size over 18 months follow-up was significantly less in patients treated with monthly intravitreal injections of lampalizumab; however, this response was only observed in those patients carrying a common *CFI* risk allele (rs17440077) [101]. Results from phase III trials are currently awaited, although preliminary results from one of these phase III trials, Spectri, showed no benefit of lampalizumab therapy overall. It will be interesting to see full results including results in genetic subgroups [102].

Summary and conclusions

The proportion of AMD heritability now explained by known genetic risk variants is estimated at ~50%. Identification of disease-associated common and rare variants has implicated various biological pathways in AMD pathogenesis. The complement system in particular appears to play an important role, and functional studies are beginning to elucidate how complement dysregulation may contribute to AMD. These discoveries have furthermore led to the development of potential novel therapies modulating complement activity. Promising results have now been reported in a phase II trial of the anti-complement factor D antibody lampalizumab in a genetic subgroup [101], (although early results from the phase III Spectri trial showed no benefit overall) 102], and also in a phase II trial inhibiting C3 in AMD patients [103]. Further work is needed to uncover the remaining heritable component of AMD and better understand how genetic and environmental factors interact to cause the disease.

An improved understanding of AMD genetics will also aid the development of risk models that can more accurately predict disease occurrence and progression. It is possible that such predictive tests may guide AMD patient management in the future if effective preventive therapies and pharmacogenetic associations are discovered, although, for now, there is insufficient evidence to recommend genetic testing in clinical practice [79, 87, 88]. Genotype-restricted sampling for clinical trials, however, may help accelerate progress in translational AMD research.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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