Genetic Factors Associated with Age-Related Macular Degeneration

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Key Words

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Abstract

Age-related macular degeneration (AMD) is a complex, multifactorial disease associated with environmental and genetic factors. This review emphasizes the clinical impact of the major genetic factors mainly located in the complement factor H gene and on the 10q26 locus, and their current and future implications for the management of AMD.

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Introduction

Age-related macular degeneration (AMD) is the main cause of visual loss among elderly people in developed countries. Indeed, the prevalence of age-related maculopathy, defined by drusen and pigment alteration located in the macular area, and late AMD, defined by atrophic or exudative AMD, is 9.8% among individuals aged ≥ 65 years and 12.0% (8.7–15.4) among individuals aged ≥ 80 years [1, 2]. Many factors, including environmental and genetic factors, are associated with the disease. Indeed, several studies have established a link between some modifiable environmental factors (mainly cigarette smoking, higher body mass index, increased plasma fibrinogen levels, poor carotenoid, omega-3 and fish consumption and higher *trans*-unsaturated fat intake) and AMD [1, 3–6]. On the other hand, multiple genetic factors play a major role in the disease, leading to a population-attributable risk >50% if 1 complement factor H (CFH) risk allele is involved to 87% with a combination of 6 risk single-nucleotide polymorphisms (SNPs) [7–9]. Moreover, some of these genetic susceptibility factors could influence disease progression [10, 11], and esti-

mates of the combined effects of major genetic factors with modifiable environmental or biological factors are consistent with a multiplicative or an additive effect that may be considered as a public health concern [7, 12–14]. The aim of this review is to analyze the influence of different genetic study designs and to investigate the impact of major genetic factors in AMD.

Because AMD occurs late in life, the parents are usually deceased and the offspring are often too young to be affected by the disease. Since only one informative generation is usually available for familial genetic studies of AMD, the design and the interpretation of these studies can be problematic. Under these conditions, familial aggregation studies, twin studies and segregation analyses are obviously difficult to perform because parental data on the index cases and because the offspring are not old enough to suffer from the disease.

Genetic Studies

Familial Aggregation Analysis and Twin Studies

Family and twin studies have been useful as a first step to establish genetic determinants in AMD. Indeed, a higher concordance rate of AMD has been demonstrated in both study designs including monozygotic twins or relatives of AMD patients. Twin studies have strongly suggested underlying genetic factors in AMD, showing that the concordance of the disease was higher among monozygotic than dizygotic twins [15–20]. In a study based on 406 twin pairs with 226 monozygotic and 280 dizygotic twins, Hammond et al. [18] calculated a heritability of age-related maculopathy and late AMD of 0.45 and 0.81, respectively. This was confirmed by Seddon et al. [20] based on the analysis of 840 elderly male twins including 210 monozygotic and 181 dizygotic complete twin pairs and 58 singletons, showing heritability rates for grade 3 and 5 AMD of 0.67 and 0.71, respectively.

Similarly, the first familial aggregation studies have consistently demonstrated familial aggregation in AMD [21–26]. Gass wrote that ‘more careful questioning of the patient and investigation of the few relatives available during this study has revealed a significant incidence of familial involvement for drusen and disciform detachment’ [22]. The risk of AMD in relatives of AMD patients based on these studies is presented in supplementary table 1 (www.karger.com/doi/10.1159/000328981). However, because environmental and/or genetic factors can explain any familial aggregation, the results of familial aggregation studies should be interpreted cautiously.

Segregation Studies

These studies investigate the mode of inheritance of a disease by comparing the transmission patterns of inheritance observed within families with genetic and nongenetic models. A segregation approach can also be used to analyze the effect of any SNP among relatives of multiplex families with affected cases to measure the segregation rate between the SNP and the disease trait.

In a segregation study including 546 sibships with an average size of 2.43 (2–9), Heiba et al. showed that a major gene effect in age-related maculopathy could be expected from their data [27]. In another study, Souied et al. analyzed the segregation of 6 heterozygous missense substitutions in different families, some members of which were affected with exudative AMD [28]. This approach enabled to conclude that the P940R and L1970F codon changes in the ATP-binding cassette transporter (ABCA4) gene could be implicated in a small proportion of cases with exudative AMD. In a study that successively used a candidate gene approach with 689 cases and 544 controls and a segregation approach in 5 multiplex Australian families, Guymer et al. analyzed the implications of the G1961E or D2177N variants of the ABCA4 gene in AMD [29]. This dual approach led to the exclusion of an association of these variants with AMD. In order to identify the causative genes of AMD, potentially located between LAMB2 and D1S3469 in the 1q25–31 region, Schultz et al. analyzed 49 variants of 20 genes for segregation with the disease haplotype in a multiplex family (4 generations and 40 subjects) [30]. They identified the G5345R variant of hemicentin-1 that exclusively segregated with the disease haplotype, showing that this variant might be associated with AMD in some cases.

Linkage Analysis

Linkage studies focused on the inheritance of loci in family pedigrees using polymorphic markers located across the genome. Because these studies require multiplex families and have less power in case of complex inheritance with incomplete penetrance, it was initially expected that they would lead to relatively weak results related to screening of genetic factors in AMD, considered to be a complex-trait disease.

However, in most of these studies, consistent evidence for linkage was established for the 1q [31–36] and 10q26 [34–37] loci. These converging results may be explained by the fact that although AMD is polygenic in nature, two major genetic factors are mainly involved in AMD. Other genes sometimes associated with the disease do not seem to play a major role because of their relatively small

allele frequency in the general population or because of their weak genetic effect. The results of the main linkage analyses are presented in supplementary table 2.

Candidate Gene Approach

This approach is based on a hypothesis-driven pathway in contrast to genome-wide association studies (GWAS), generally considered as more 'agnostic' in the field of genetic studies [38].

The candidate gene approach is a useful study design to validate previous associations or to demonstrate associations of a gene with a disease in different populations.

Because previous experimental studies, particularly immunochemical analyses of soft drusen, have generated strong hypotheses on specific biochemical pathways potentially involved in AMD, this approach has been useful to establish associations between genetic susceptibility factors and AMD. The genetic susceptibility factors associated with AMD in more than 2 studies using this hypothesis-driven pathway approach are listed in supplementary table 3.

Some studies published years or months before the first publications that have emphasized the implication of the complement cascade genes in AMD pointed out the potential key role of the complement pathway in the biogenesis of drusen and AMD [39–45]. Moreover, 6 linkage studies published before 2006 showed a consistent association between the 1q31 locus and AMD [46]. Using a candidate gene approach, Hageman et al. [47] identified a common haplotype in 8 most informative SNPs located in the CFH and studies based on previous GWAS also identified the CFH gene as a major genetic factor for AMD the same year [48–50]. This example emphasizes the need for candidate gene analyses to combine biochemical data with previously identified loci obtained through linkage studies in order to select ideal SNPs for analysis. However, it seems more useful to screen millions of SNPs in a GWAS than to screen few SNPs in a candidate gene approach, because a higher rate of positive association is obviously more frequently observed in the first case. In such studies, functional SNPs with a minor allele frequency cutoff usually >0.05 and potentially associated with modifications of the protein activity profile are usually preferred. Because a tagging-haplotype approach, facilitated by the International HapMap project (<http://www.hapmap.org/>), allows wide coverage of functional SNPs in a gene using few tagging SNPs, the choice of SNPs for a candidate gene approach can be improved. This deductive approach for targeted potential functional SNPs based on biochemical data, potentially crossed

with physical data obtained from previous linkage analysis, provides advantages over GWA studies as it also offers the ability of studying smaller populations in case of a rare disease, and lower minor allele frequencies (MAFs) or SNPs associated with minor genetic effects, with a cost-efficiency advantage.

Genome-Wide Association Studies

The classical stepwise approach with familial aggregation/twin studies – segregation – linkage association studies and candidate gene approach, as successive steps to identify genetic factors, has been useful, although time consuming in the case of AMD.

Advances in genotyping technologies allowing the performance of scans of up to one million SNPs and in genome analyses with the completion of the Human Genome Project have led to large-scale GWAS. If most of the common variants identified through GWAS in common complex-trait diseases individually confer small increments in risk and usually leave a large proportion of missing heritability [51], these studies have been successful in AMD mainly because few high-effect common variants [CFH, age-related maculopathy susceptibility 2 (ARMS2)/LOC387715-HtrA serine peptidase 1 (HTRA1) and hepatic lipase (LIPC) genes], characterize the genetics of this disease. Moreover, the putative causative role of these genes significantly associated with AMD in GWAS is strengthened when expression data confirm that they are also expressed in the retina [49, 52].

Genetic Factors Associated with AMD

Numerous genetic factors associated with AMD have been described since the identification of ApoE in 1998. Some of them might be involved in sporadic AMD cases or play a minor role as a risk factor, and few of them are responsible for a greater proportion of AMD cases. Indeed, the effect sizes of the two major risk genes for AMD (CFH Y402H and ARMS2/LOC387715) are dramatically larger than for other risk genes identified in most late-onset complex diseases [53–55], leading to potential primary preventive perspectives by genetic screening.

Numerous studies in different ethnic groups mainly emphasize the role of genetic factors as susceptibility factors for AMD, but only few studies have analyzed their effect on the incidence, progression, treatment response, or clinical features of the disease.

The pathogenesis of AMD seems to involve different biological pathways, such as inflammation, lipid, apopto-

sis and oxidation. These pathways can obviously share common networks and cannot be considered as completely dissociated.

Genes Involved in the Inflammatory Pathway

Previous immunohistochemical studies focusing on drusen or choroidal neovascular membranes implicated the inflammatory pathway and specifically the complement pathways in AMD [56, 57]. Since 2005, this hypothesis has been demonstrated by numerous genetic studies.

The CFH Gene

The four activation pathways of the complement cascade lead to formation of the cytolytic membrane attack complex (MAC). The CFH gene encodes a protein acting as a regulator of the basal activation of the alternative pathway of the complement cascade. The Y402H polymorphism of the CFH gene (rs1061170) has been associated with all forms of AMD in different populations worldwide and the odds ratios (ORs) in large case-control studies are summarized in supplementary figure 1. This SNP, which is located in the short consensus repeat 7, results in an amino acid substitution of histidine for tyrosine in a particular domain of factor H that contains binding sites for C-reactive protein, heparin, and streptococcal M6 protein [58].

Differences in binding properties on various cellular surfaces have been reported between the risk variant 402H and the wild variant 402Y [59, 60], but no differences in either CFH or CRP immunolabeling in drusen were detected between homozygous carriers for both variants [61].

The Y402H SNP of CFH as a genetic risk factor for AMD is not consistently replicated in some case-control studies in the Asian population [62–72]. This lack of a consistent association could be explained by the lower MAF in this population compared with Caucasian populations. Indeed, the C allele frequency is 0.282 in Europeans, 0.067 in Han Chinese in Beijing and 0.057 in Japanese subjects in Tokyo (<http://hapmap.ncbi.nlm.nih.gov/biomart>). However, other SNPs of the CFH gene are associated with AMD in Asians. Furthermore, some of these SNPs are also associated with polypoidal choroidal vasculopathy, which is sometimes considered as a frontier form of AMD (same age of onset, frequent association with occult choroidal neovascularization (CNV) in Asian patients) [62, 73–75]. The main results of case-control studies analyzing SNPs of the CFH gene in AMD in Chinese and Japanese populations are summarized in supplementary table 4.

Complement Component 2 and Factor B Genes

Activation of the alternative pathway is initiated by cleavage of C3b-bound factor B, leading to the formation of the C3Bb complex. Factor B (BF) and complement component 2 (C2), an activator of the classical complement pathway, are paralogous genes located only 500 bp apart on human chromosome 6p21. Risk haplotype and protective haplotypes have been identified [76]. Indeed, the H10 (L9H with E318D) and H7 (ISV10 with R32Q) haplotypes independently exert a protective effect in Caucasian populations with respective ORs of 0.45 (95% CI 0.33–0.61; $p < 0.0001$) and 0.36 (95% CI 0.23–0.56; $p < 0.0001$), whereas the H1 haplotype is associated with an increased risk of AMD with an OR of 1.32; $p = 0.0013$) in the study by Gold et al. Moreover, taking into account only 3 genes, C2, BF and CFH, Gold et al. consider that this could predict the clinical outcome in 74% of affected individuals [76].

The protective effect of some variants of C2 and BF has been confirmed in other studies [77, 78]. The protective effect of the 32Q variant of the BF could be linked to its decreased affinity for C3b, leading to a decreased amplification of complement activation due to lower production of convertase [79].

Complement C3

In two replicated case-control studies on Scottish ($n = 244$ cases and 351 controls) and English ($n = 603$ cases and 350 controls) individuals, Yates et al. showed that the rs2230199 (R80G) functional polymorphism in exon 3 of the C3 gene is a risk factor for both exudative and atrophic AMD, with ORs of 1.7 (95% CI 1.3–2.1) and 2.6 (95% CI 1.6–4.1) for heterozygous or homozygous carriers of the risk allele, respectively [80]. Similar results were also published by Maller et al. confirming that rs2230199 is associated with both forms of the disease, and excluding an association of C5 gene polymorphisms with the disease [81].

The MAF of rs2230199 (R80G) is 0.2 in Europeans, and this SNP is in almost complete linkage disequilibrium (LD) with rs1047286 (P292L) in exon 9 of the C3 gene ($r^2 = 0.8$; $D' = 1$) (<http://www.broadinstitute.org/mpg/snap/ldsearch.php>). However, in the studies by Yates et al. [80] and Spencer et al. [82], stepwise logistic-regression analyses confirmed that the R80G SNP is more likely to be the causative SNP of AMD than the P292L SNP.

In a study based on participants of the Rotterdam study ($n = 6,418$ individuals) and an independent case-control cohort ($n = 357$ cases and 173 controls), haplotype analysis showed that carriers of the 80G and the 292L

variants exhibited the highest difference in frequency between cases and controls (0.245 vs. 0.207; $p = 0.006$) compared with the 3 other haplotypes. Moreover, this study demonstrated that these risk alleles in LD are independent of the major risk alleles of CFH and LOC387715; they are also independent of smoking, with a population-attributable risk of 14.6% [83]. This independent effect from other known genetic factors has been confirmed in other studies [84–87].

Similarly to the Y402H allele of the CFH gene, the 80G variant of rs2230199 is not associated with exudative AMD in the Chinese population, likely because of the lower MAF – almost 0 for the C allele in the HapMap of Han Chinese in Beijing and 0.175 of the Centre d'Etude du Polymorphisme Humain (CEPH) population. However, the C3 IVS2 rs2250656 polymorphism appeared to be protective for exudative AMD in the Chinese population, the G allele conferring an OR of 0.58 (95% CI 0.35–0.96; $p = 0.033$) [88]. As mentioned by the authors, the low frequencies of the risk alleles of the rs2230199 of C3 and the rs1061170 of CFH could, to some extent, partly explain the lower prevalence of AMD in the Chinese population.

In a recent study combining immunochemical and case-control genetic analyses (478 AMD cases and 300 controls), the authors have identified other SNPs located in the C3 (MRD_4273), the C9 (rs476569), and the ficolin (collagen/fibrinogen domain containing) 1 (FCN1) genes (rs10117466, rs10120023, MRD_4502) that showed borderline associations with AMD [61].

The Complement Factor I Gene

The complement factor I (CFI) gene encodes a molecule containing a serine protease domain that cleaves and inactivates C3b and C4b. Based on a case-control analysis ($n = 1,228$ cases and 825 controls) of 1,500 SNPs selected among the complement pathway genes and on the meta-analysis of previous whole-genome linkage studies Fagerness et al. identified rs10033900 and rs13117504, and their combined haplotype of the CFI gene that are associated with AMD [89]. However, because these SNPs are unlikely to be functional, it is possible that these SNPs tag an undiscovered functional variant as mentioned by the authors. The role of a putative functional variant of the CFI gene associated with AMD is also supported by other studies [90, 91].

The VEGFA Gene

In a recent meta-analysis of GWAS for advanced AMD with follow-up replication of most significant signals in

5,640 cases and 52,174 controls, the rs4711751 SNP located nearby VEGFA and the rs1999930 SNP located nearby FRK/COL10A1 were associated with AMD with ORs of 1.15 (95% CI 1.1–1.21; $p = 8.7 \times 10^{-9}$) and 0.87 (95% CI 0.83–0.91; $p = 1.1 \times 10^{-8}$), respectively. These variants suggest that VEGFA involved in angiogenesis/inflammatory process genes involved in extracellular collagen matrix could contribute to AMD development [92].

The 10q26 Locus

Based on previous whole-genome analyses that have identified this locus associated with AMD [34–37], several studies focused more thoroughly on this region, which led to the identification of the other major genetic factor(s) of AMD, the LOC387715/age-related maculopathy susceptibility 2 (ARMS2) [93, 94], and the HTRA serine peptidase 1 (HTRA1) genes [52, 95]. In the 10q26 locus, a total of 15 variants are in strong LD and tag a single-risk haplotype, leaving statistical analyses with insufficient power to obtain enough discrimination between ARMS2 and HTRA1 variants [96]. Both associations of ARMS2 have been replicated through different population case-control studies, and the main results of these studies are presented in supplementary table 4. For the time being, it seems difficult to identify the causal variant associated with AMD because both variants are in strong LD in different populations ($r^2 = 1$ and $D' = 1$ in CEU; $r^2 = 0.88$ and $D' = 1$ in Africans, and $r^2 = 0.863$ and $D' = 0.929$ in Asians; <http://www.broadinstitute.org/mpg/snap/ldsearch.php>).

ARMS2 mRNA was detected in the human retina and could encode a putative protein whose expression and cellular location are still under debate. Indeed, the putative protein was initially observed in the mitochondrial outer membrane [97], and later in the cytosol and extracellular compartment [98, 99]. In a recent study, the authors showed that the risk haplotype in 10q26 is associated with a dramatic effect on ARMS2 but not on HTRA1 expression levels. Moreover, the rs2736911 variant also leads to significant reduction in ARMS2 transcript levels and is not associated with AMD. From these results, it seems unlikely that ARMS2 protein deficiency could be the direct pathogenic mechanism responsible for AMD [100]. The main results of case-control studies on ARMS2 are summarized in supplementary figure 2.

HTRA1 encodes a 50-kDa secreted protein belonging to the high-temperature requirement A family of serine proteases. Although the first studies associated the rs11200638 promoter variant of the HTRA1 gene with

increased expression of the protein [95, 52], other studies have not replicated these results [97, 101]. However, a recent study showed that HTRA1 mRNA expression is higher in cultured retinal pigment epithelial cells homozygous for the risk allele of HTRA1, and that some molecules involved in the complement pathway, such as clusterin, vitronectin and fibromodulin, are substrates for HTRA1 serine protease [102]. Other results have reinforced the idea that HTRA1 could be the causal gene [103, 104]. The main results of case-control studies on HTRA1 are summarized in supplementary figure 3.

Considering the interaction between major risk variants of the CFH and the ARMS2/LOC387715-HTRA1 genes, different studies suggest an independent multiplicative joint effect in AMD [13, 105].

The Lipid Pathway

The putative role of the lipid pathway in AMD has been suggested by the presence of cholesterol and esterified cholesterol accumulating in Bruch's membrane and drusen in AMD patients [106, 107]. Different genes involved in the lipid pathway are associated with AMD.

ApoE

ApoE is located at locus 19q13.2 and encodes a glycoprotein of 34.2 kDa. ApoE is located on lipoproteins and interacts with the cellular receptors of ApoE, the LDL receptors and other LDL receptor-related proteins (i.e. LRP1, LRP5 and LRP8), whose interactions enable a turnover of cellular lipids and the clearance of different lipoproteins from the circulation [108, 109]. Three main isoforms of ApoE, i.e. E3, E4, and E3, are described. The $\epsilon 3$ allele is most frequently observed in different populations leaving on agriculture or on agriculture-derived occupations (70–80%), whereas the frequency of the $\epsilon 4$ allele, the ancestral allele, is higher among Pygmies (0.407), Malaysian aborigines (0.240), Australian aborigines (0.260), Khoi San (0.370), Papuans (0.368), some Native Americans (0.280) and Lapps (0.310) [110].

Apo- ϵ is the first genetic factor identified in AMD through candidate gene approaches [111]. These results have been replicated in other studies [112–122] and confirmed by a meta-analysis [123]. However, likely because of the weak allele effects of Apo- ϵ , and because of the low allele frequencies of both the $\epsilon 2$ and $\epsilon 4$ alleles, some studies could not demonstrate any association between this gene and AMD [124–129]. The $\epsilon 4$ allele is associated with a reduced risk of developing different subtypes of the disease (exudative or atrophic forms) [115, 116], with sometimes a gender-specific protective effect reported for men [117],

whereas a gender effect of the $\epsilon 2$ isoform might confer an increased risk mainly for men [115]. These gender effects are not yet precisely clarified. Other specific gender effects have been observed for the Apo- $\epsilon 4$ allele in late-onset familial Alzheimer's disease with a higher risk for women [130] and in cardiovascular diseases with a higher risk for men [131]. Moreover, the relative rate of Apo- ϵ expression, in conjunction with functional differences of the respective isoforms, might also be associated with AMD [122].

The Scavenger Receptor Class B Type 1 Gene

The scavenger receptor class B type 1 (SCARB1) gene located on 12q24.31 encodes a protein (SRB1) of 509 amino acids that mediates the transfer of cholesterol between cells and high-density lipoproteins (HDL) and is also involved in the metabolism of vitamin E and lutein [132, 133].

In a collaborative case-control study including 1,241 + 1,732 AMD cases compared with 297 + 1,257 controls, the CT heterozygotes for the rs5888 SNP of SCARB1 were at increased risk of developing exudative AMD, with an OR of 3.6 (95% CI 1.7–7.6; $p < 0.0015$) compared with the CC genotype [134]. Although this genetic finding has not been widely replicated up to now, this association is interesting when considering the underlying role of cholesterol, lutein and vitamin E in AMD established by epidemiological studies.

The LIPC and Other Genes Associated with Lipid Metabolism

Hepatic triglyceride lipase (HL) and the cholesteryl ester transfer protein (CETP) are proteins that play a major role in the regulation of plasma lipids. HL is encoded by the LIPC gene located at 15q21–q23, and the CETP gene is encoded on 16q21.

Two GWAS based on large independent cohorts of 2,157 AMD cases/1,150 controls (Michigan-Mayo Clinic-AREDS-Pennsylvania GWAS) and of 821 AMD cases/1,709 controls (Tufts/MGH GWAS) identified 30 SNPs with consistent evidence for association. These SNPs were genotyped in additional samples of 7,749 AMD cases/4,625 controls. Among them, rs9621532 and nearby markers of the inhibitor of metalloproteinase 3 (TIMP3) gene, and both common rs493258 SNP located 35 kb upstream of the LIPC gene and rs3764261 SNP near the CETP gene were associated with an increased risk of AMD with ORs of 1.41 (95% CI 1.27–1.57; $p = 1.1 \times 10^{-11}$), 1.14 (95% CI 1.09–1.20; $p = 1.3 \times 10^{-7}$) and 1.19 (95% CI 1.12–1.27; $p = 7.4 \times 10^{-7}$), respectively [135]. As mentioned in the Discussion of that paper, it is noteworthy that the two latter risk SNPs for AMD are also associated with higher HDL

cholesterol levels [136, 137]. Among other SNPs associated with modifications of HDL cholesterol levels also investigated in that study, rs12678919 near the LPL gene and rs1883025 near the ABCA1 gene were associated with an increased risk of AMD, with ORs of 1.26 ($p = 0.003$) and 1.15 ($p = 5.6 \times 10^{-4}$). Other SNPs, i.e. rs173539 near the CETP gene, and rs10468017, near the LIPC gene, also revealed evidence for an association with AMD [132].

The Tufts/MGH GWAS with 979 AMD cases/1,709 controls and replication cohorts of 5,789 AMD cases/4,234 controls showed that rs493258 SNP, near the LIPC gene, is unlikely to be the causative SNP, but that a functional variant, rs10768017, located on the proximal promoter of the LIPC gene and in LD with the previous rs493258 SNP, might be the causative variant [138]. The rs10768017 SNP previously associated with reduced LIPC expression and higher HDL demonstrates a protective effect for both forms of AMD with an OR of 0.82 (95% CI 0.77–0.88). In the latter study, immunoprecipitation and real-time PCR showed that both LIPC protein and mRNA are present in human retinas.

A recent study investigated serum lipids and LIPC rs10768017 in 318 advanced AMD cases/140 controls [139]. In this study, HDL cholesterol is lower in AMD cases than in controls (49 vs. 53 mg/dl; $p = 0.05$), LDL cholesterol is higher in AMD cases than in controls (144 vs. 135 mg/dl; $p = 0.03$) and the protective T allele of LIPC rs10768017 is associated with increased HDL levels ($p = 0.05$). As mentioned by the authors, because of the independent associations of LIPC and HDL when considered simultaneously, the HDL level unlikely mediates the association between LIPC and AMD [139]. No other interaction of LIPC has been demonstrated up to now between other environmental factors, such as smoking, body mass index and lutein intake [140].

Other Genetic Mechanisms Potentially Involved in AMD

Most of the genetic studies on AMD focused on SNPs, but this mechanism probably does not explain the entire genetic component of this disease. Indeed, other mechanisms including epigenomic (i.e. DNA methylation, histone methylation, acetylation status and transcription factors) variants of mitochondrial DNA (mtDNA), mtDNA alterations [141] and copy number variation might be potentially involved in the pathophysiology of the disease.

mtDNA Variants

The maternally inherited mtDNA is 16,569 pb in size and is composed of 37 genes encoding 13 protein subunits involved in oxidative phosphorylation, 2 ribosomal RNAs and 22 transfer RNAs. Inherited variants located in the mtDNA T2 haplogroup and characterized by 2 variants in complex I gene (A11812G of MT-ND4 and A14233G of MT-ND6) have been associated with advanced AMD, with an OR of 2.54 (95% CI 1.36–4.80; $p \leq 0.004$) [142]. Other variants associated with mitochondrial haplogroups J (T16126C, G13708A and C16069T SNP), T (A4917G, G13368A and A73G SNPs) and U (A12308G, G9055A_SNP) have also been associated with AMD [143]. This latter study seems to confirm the findings of a previous study showing that haplogroups J and U are connected with some clinical features associated with age-related maculopathy, with ORs of 1.80 (95% CI 1.18–2.73) and 1.45 (95% CI 1.11–1.91), respectively [144].

Copy Number Variation

Copy number variation, including duplications, tandem repeats and deletions of 1 kb or more of genomic DNA could be associated with AMD [145, 146] through increased or decreased gene expression [147].

Complement factor H-related 1 (CFHR1) and complement factor H-related 3 (CFHR3) share significant amino acid sequence homology, and similar binding properties with CFH. However, unlike CFH that regulates C3 convertase, CFHR1 modulates the activity of C5 convertase and inhibits the formation of MAC. A protective effect of a haplotype carrying deletions of CFHR1 and CFHR3, with an OR of 0.4 (95% CI 0.3–0.5), independent of rs1061170 of the CFH gene, has been described. These deletions were associated with a lack of the proteins encoded by these genes among homozygotes for these deletions [148]. These results were replicated in other independent cohorts [149, 150]. However, this common CNV could be associated with other protective haplotypes located in the CFH gene as suggested by some authors [151]. The protective effect of deletions located in the CFHR1/CFHR3 genes could be mediated by removal of the C5a blockade and disinhibition of MAC formation [152].

Genetic Factors and Progression of AMD

Some genetic susceptibility factors for AMD have also demonstrated an impact upon the progression from intermediate to advanced AMD. Based on the AREDS cohort, Seddon et al. have analyzed the impact of CFH and

LOC387715/ARMS2 risk SNPs and environmental factors on the progression of AMD [153]. Among 1,466 participants, 281 progressed from grades 2 or 3 (drusen and/or pigment anomalies and/or noncentral geographic atrophy) to grades 4 (unilateral exudative or atrophic AMD) or 5 (bilateral exudative or atrophic AMD) or from grade 4 to grade 5. For the Y402H SNP of CFH, the ORs for AMD progression were 1.6 (95% CI 1.1–2.4) and 2.6 (95% CI 1.7–3.9) for the heterozygous and homozygous risk genotypes, respectively. For the A69S SNP of LOC387715/ARMS2, the ORs for AMD progression were 2.7 (95% CI 1.9–3.7) and 4.1 (95% CI 2.7–6.3) for the heterozygous and homozygous risk genotypes, respectively. In this same study, the risks of progression attributable to these genotypes were 71.8% for both SNPs and 81.2% for both SNPs combined with smoking and body mass index. Moreover, the rate of progression to the exudative form in homozygotes with the risk allele of LOC387715/ARMS2 was higher than the rate of progression to the atrophic form (ORs 6.1 and 3, respectively). This effect was not observed with the CFH risk allele, with similar rates of progression for both forms of the disease [153].

In another study also based on 1,446 individuals from the AREDS cohort of whom 279 progressed during a 6.3-year follow-up, multivariate analysis between demographic, genetic and environmental factors showed that age >70, baseline grade 3, current smoking and body mass index >25 were significantly associated with progression, with ORs of 1.5, 11, 3.1 and 1.6, respectively. In this study, rs1061170 of CFH, rs10490924 of ARMS2 and rs2230199 of C3 were also associated with a higher risk of progression, with ORs of 2 (1.1–3.5; $p = 0.019$), 4 (2.6–6.1; $p < 0.001$) and 1.8 (1–3.2; $p = 0.044$) for homozygous risk alleles. rs9332739 of C2 was associated with a lower risk of progression, with an OR of 0.4 (0.2–0.8; $p = 0.01$) [10].

In a study based on 3 different cohorts, Francis et al. analyzed progression from grade 3 (intermediate AMD) to advanced AMD (grade 4), or from advanced AMD in one eye (grade 4) to advanced AMD in both eyes among 889 patients from the AREDS cohort [121]. In this study, the protective alleles rs9332739/rs4151667 of C2/CFB (both in high LD) were associated with a reduced progression rate from intermediate AMD to atrophic or exudative forms, with an OR of 0.32 (95% CI 0.14–0.73; $p = 0.004$) for heterozygotes and the risk allele rs2230199 of the C3 gene was associated with an increased progression rate, with an OR of 3.32 (95% CI 1.46–7.59; $p = 0.004$) in homozygotes [121].

The effect of variants of CFH, LOC387715/ARMS2, C2, C3, APOE and TLR3 on the progression of the area

of geographic atrophy (GA) has been recently investigated by Klein et al. in a cohort of 114 participants from the AREDS [154]. Whereas the mean growth rate of GA was 1.79 mm²/year (0.17–4.76), extension of GA was higher among homozygotes with the LOC387715/ARMS2 risk genotype (2.34 mm²/year) compared with homozygotes with the nonrisk genotype (1.51 mm²/year) ($p = 0.014$).

Genetic Factors and Clinical Features of AMD

An earlier age of onset of AMD is the most common phenotypic manifestation associated with the LOC387715/ARMS2 genetic predisposition reported in few studies [155–159].

Studies also established that a larger size of the choroidal neovascular lesion is associated with the LOC387715/ARMS2 or HTRA1 risk alleles [155, 159, 160]. We previously analyzed the clinical features of AMD correlated to CFH and ARMS2 after genotypic selection. Significant associations were found for earlier disease onset ($p < 0.014$), fibrovascular scar ($p < 0.001$), bilateral CNV and lower visual acuity at presentation ($p = 0.02$) among patients homozygous for both risk alleles. An association was also found between ARMS2 and classical CNV ($p < 0.026$) [161]. These results have been partly confirmed by a recent study showing that progression of CNV and bilaterality of CNV are more consistently associated with the HTRA1/ARMS2 SNPs than with CFH SNP [162]. On the contrary, other groups established a positive association between homozygosity for the risk allele of rs1061170 SNP of CFH and predominantly classical CNV [163–165]. However, these latter studies did not include the major ARMS2 or HTRA1 gene in their analyses. In a recent publication, the ARMS2 gene conferred differential susceptibility to exudative and atrophic forms of the disease, with the risk allele of the rs10490924 SNP being more frequently observed for patients with exudative AMD ($n = 3,139$) than for patients with geographic atrophy ($n = 731$), with an OR of 1.37 (95% CI 1.21–1.51; $p = 4.2 \times 10^{-7}$). In this study, other genes, such as CFH, C2/CFB, C3, CFI, LIPC and TIMP3, did not confer differential susceptibility to both forms of the disease [166].

When considering the rs2230199 SNP of the C3 gene, it is rather associated with GA than with exudative AMD in a study including 341 patients with GA, 994 patients with CNV and 509 controls [162]. Indeed, the ORs for GA and for exudative AMD were, respectively, 3.86 (95% CI 1.77–8.40; $p = 0.001$) and 2.39 (95% CI 1.23–4.63; $p = 0.01$) for homozygous individuals. Considering the Y402H

variant of the CFH gene, no difference was observed between ORs for exudative or GA AMD (3.75 vs. 3.12), but the rs2274700 of the CFH gene was significantly associated with bilateral forms of GA compared with unilateral forms. As to the ARMS2 gene, the OR for CNV was 2-fold higher than the OR for GA in that study (13.49 vs. 6.57) [162]. Similar results with a higher effect of ARMS2 on exudative forms compared with atrophic forms have also been reported elsewhere [153].

Other particular clinical features or borderline forms of AMD have also been associated with genetic factors. Indeed, in a study comparing 62 cases of polypoidal choroidal vasculopathy (PCV) with 93 controls in the Chinese population, PCV was associated with rs3753394 of the CFH promoter and the rs800292 of the CFH gene, with individuals homozygous for the risk genotypes having ORs of 4.05 (95% CI 1.38–13.13; $p = 0.0055$) and of 4.49 (95% CI 1.29–20.15; $p = 0.011$) for both SNPs, respectively. ARMS2 and HTRA1 common risk alleles were also associated with a higher risk of PCV, with ORs of 3.97 (1.50–11.06; $p = 0.0025$) and 4.53 (95% CI 1.69–12.87; $p = 0.0011$) for homozygosity for both SNPs, respectively [73]. In the Japanese population, PCV has been found to be associated with ARMS2 and the Y402H SNP of the CFH gene [167]. In another Japanese study (100 PCV and 190 controls), the rs2241394 of the C3 gene, the rs800292 of the CFH gene and the rs10490924 of the ARMS2 gene were associated with an increased risk of PCV with ORs of 3.47 (1.48–8.38), 2.00 (1.37–2.92), and 4.16 (2.89–5.99). Other SNPs of the CFH gene were also associated with a higher risk of PCV in this genome-wide screening [75].

Other phenotypes, such as peripheral anomalies including reticular pigment changes and peripheral drusen, have been associated with both rs1061170 and rs1410996 of the CFH gene [168, 169].

Basal laminar drusen (also called cuticular drusen) are characterized by a typical ‘stars in the sky’ appearance in fluorescein angiography. This particular clinical feature has been associated with the 402H variant of the CFH gene [170] and with compound heterozygosity for the R1078S and R567G missense variants or the Q408X nonsense mutation with the Y402H variant [171].

Relation between Genetic and Environmental Factors

In the classical model of complex diseases, numerous genes and environmental factors with small effects contribute to overall risk. In AMD, this model has been com-

plicated by potential dominant or negative effects of some genes, potential gene-environment and gene-gene interactions, and by large genetic heterogeneity.

As smoking is the major environmental factor of exudative and atrophic AMD, accounting for 20% of the population-attributable risk, interaction analyses have been performed between this factor and various genetic factors [13]. Joint effects of the CFH gene and smoking, both independent from each other, are consistent with a multiplicative model [13, 172], whereas conflicting data for an interaction between smoking and ARMS2/HTRA1 have been reported, some studies showing a strong interaction [13, 105] while others found no interaction between ARMS2/HTRA1 and smoking [10, 173–175]. However, most of these studies described a strong synergistic effect of smoking with these variants [10, 13, 105, 174–176].

A higher body mass index has been associated with a higher incidence, prevalence and progression rate of AMD when combined with genetic factors [177–180]. Furthermore, although no interaction has been established between any particular genetic risk factor and body mass index up to now, this modifiable environmental factor increases the incidence and prevalence of AMD when combined with genetic factors [174, 181].

Genetic Factors and Response to Preventive Medication or Treatments

It is likely that the ‘standard of care’ concept for AMD treatment or prevention in which all patients receive similar interventions might rapidly be replaced by a more personalized approach, based on the molecular characteristics of individual patients as developed in cancer therapy [182, 183]. Predictive biomarkers including genomic, demographic and environmental parameters for increased risks of developing the disease can be used in primary prevention, might be used to evaluate the severity of the disease, and might also be used for secondary and tertiary prevention. Other biomarkers might be used to reflect sensitivity or resistance to existing therapies.

Preventive Medication and Genetic Biomarkers

The AREDS study demonstrated that a combination of zinc and antioxidants (β -carotene, vitamin C and vitamin E) leads to a 25% reduction in the development of advanced AMD over 5 years [184]. An ancillary study of the AREDS investigated whether genetic factors might influence the effect of the AREDS nutritional supplements on AMD [185]. This study is based on 876 indi-

viduals randomized to different subgroups (placebo, $n = 204$; antioxidants, $n = 219$; zinc, $n = 217$; antioxidants + zinc, $n = 236$) with a mean duration of treatment of 6.3 years. A greater reduction in AMD progression in the antioxidant + zinc group compared with the placebo group was observed in homozygous individuals with the wild allele (TT) compared with homozygous individuals with the risk allele (CC) of the CFH gene (reduction in the progression of 68 vs. 11%; $p = 0.004$). If the results of this study are confirmed, the preventive strategy using antioxidants and zinc could be considered as pointless in patients homozygous for the C risk allele, and very effective in patients homozygous for the T wild allele of rs1061170 of the CFH gene.

AMD Treatments and Genetic Biomarkers

In a recent study based on the analysis of baseline and posttreatment logMAR best corrected visual acuity of 110 Japanese patients with exudative AMD treated with photodynamic therapy (PDT) with a follow-up of 12 months, the authors demonstrated that the risk allele of rs11200638 of HTRA1 was associated with worse visual acuity outcomes ($p = 1.10 \times 10^{-3}$) and a 6-fold greater risk of CNV recurrence at 12 months ($p = 5.58 \times 10^{-3}$). Furthermore, both rs1410996 and rs2274700 of the CFH gene were also associated with a shorter interval between PDT treatment and CNV recurrence ($p = 8.5 \times 10^{-3}$) [186].

In another study including 309 eyes with exudative AMD of 267 patients treated with ranibizumab, patients carrying the CT or TT genotypes of rs1061170 of the CFH gene (Y402H variant) had better visual acuity outcomes at 12 months than those with the CC genotype, with an OR of 3.42 (95% CI 1.4–9.42; $p = 0.006$) [187].

In a study based on 168 eyes of 168 patients with exudative AMD treated with intravitreal injections of ranibizumab only or with a combination of ranibizumab and bevacizumab, the ApoE4 allele was associated with better visual acuity outcomes at 3 months, with an OR of 4.04 (95% CI 1.11–14.70; $p = 0.03$). Although not significant, a similar trend was reported between better visual acuity outcomes at 6 and 12 months and ApoE4 carriers, with an OR of 3.26 (95% CI 0.76–13.90; $p = 0.11$) and of 2.54 (95% CI 0.61–10.52; $p = 0.2$), respectively [188].

Toward Personalized Medicine for AMD

The era of personalized medicine has just begun, with commercialization of genomics testing along with genetic profiling for a wide panel of disorders, with the aim of

obtaining individualized disease risk estimates in apparently healthy people with potential genetic risks of developing a disease (e.g. Parkinson's disease, Alzheimer's disease, type 2 diabetes or AMD). Presymptomatic risk assessment for AMD and personalized care to extend a healthy life span are now global priorities. Indeed, unraveling the genetics of AMD could lead to personalized treatment/preventive procedures or follow-up as in cancer therapy [189].

The area under the ROC curve, widely used as a usefulness measure of the discrimination power for a classifier, should be greater than 0.75 for an individual at increased risk of disease, and greater than 0.99 for the general population to predict the risk of developing the disease [190]. As regards AMD, because risk genotypes for both CFH and ARMS2/HTRA1 are strong ($OR \geq 2$ for 1 risk allele in most studies) and common (with a MAF of 0.28 and 0.2/0.2, respectively, in the CEU population), good discriminative accuracy should be expected. Other proteomic or genomic biomarkers could perhaps increase the discrimination rate between AMD and controls with higher accuracy [191]. However, one should keep in mind that because of the lack of long-term prospective investigations in genetic epidemiology, genetic profiling is up to now mainly based on hypothetical models of simulation studies.

Conclusion

Genetic factors of AMD are now considered as reliable genomic biomarkers to predict the risk of developing the disease and to predict both disease severity and progression. Hitherto a binary therapeutic approach to exudative AMD has been available with two anti-VEGF therapies available. However, other therapeutic strategies will be developed and the response to each treatment should be considered in further studies taking into account genomic markers to define personalized treatment for each individual.

Disclosure Statement

There are no conflicts of interest.

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