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Genetic markers and biomarkers for age-related macular degeneration

Robert J Ross,

National Eye Institute Intramural Research Training Award Fellow, Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, USA, Tel.: +1 301 435 4563, Fax: +1 301 480 1122, rossr@nei.nih.gov

Varun Verma,

Howard Hughes Medical Institute – National Institutes of Health Research Scholar, Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, USA, Tel.: +1 301 496 7694, Fax: +1 301 480 1122, vermav@mail.nih.gov

Kevin I Rosenberg,

Summer Research Fellow, Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, USA, Tel.: +1 248 894 0293, krosenbe@med.umich.edu

Chi-Chao Chan, and

Section Chief, Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, USA, Tel.: +1 301 496 0417, Fax: +1 301 402 8664, chanc@nei.nih.gov

Jingsheng Tuo[†]

Staff Scientist, Section of Immunology, 10/10N103, 10 Center Drive, Bethesda, MD 20892-1857, USA, Tel.: +1 301 435 4577, Fax: +1 301 480 1122, tuoj@nei.nih.gov

Abstract

Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in the USA. Although the treatment of AMD has evolved to include laser photocoagulation, photodynamic therapy, surgical macular translocation and antiangiogenesis agents, treatment options for advanced AMD are limited. Furthermore, the dry form of AMD, albeit less devastating than the wet form, has even fewer viable treatment options. This review summarizes the various biomarkers of AMD and analyzes whether or not they may one day be exploited to determine risks of disease onset, measure progression of disease or even assess the effects of treatment of AMD. Potential biomarkers are important to identify since some might be utilized to reflect the disease state of a particular patient and to individualize therapy. Although studies have yielded promising results for nutrient and inflammatory biomarkers, these results have been inconsistent. At present, the best available markers of AMD risk are single nucleotide polymorphisms (SNPs). SNPs in complement factor H (*CFH*) and *PLEKHA1/ARMS2/HtrA1* capture a substantial fraction of AMD risk and permit the identification of individuals at high risk of developing AMD.

Keywords

age-related macular degeneration; biomarker; genetic marker; inflammation; nutrient; risk assessment; single nucleotide polymorphism; target molecules

[†] Author for correspondence 10/10N103, 10 Center Drive, Bethesda, MD 20892-1857, USA, Tel.: +1 301 496 0417, Fax: +1 301 402 8664, tuoj@nei.nih.gov

Age-related macular degeneration (AMD) is a disease characterized by chronic and progressive degeneration of photoreceptors, the underlying retinal pigment epithelium (RPE), Bruch's membrane and, possibly, the choriocapillaries in the macula. Clinical and pathological features of AMD include thickened Bruch's membrane, drusen formation, pathological changes of the RPE, photoreceptor atrophy, choroidal neovascularization (CNV) and/or fibroglial tissue in the macula. These alterations result in the loss of central visual acuity [1].

AMD is the leading cause of visual impairment and blindness in the USA [2]. To date, there is no proven treatment of advanced or early AMD. Low-intensity laser treatment in patients with bilateral large drusen has not been shown to be effective in the prevention of vision loss [3]. Medical therapy of AMD has focused mainly on treatments of CNV. Of these treatments, most target vascular endothelial growth factor (VEGF), which is recognized as the major angiogenic factor.

The etiology of AMD remains elusive. AMD pathogenesis involves environmental factors and varying susceptibilities to these external factors based upon different genetic backgrounds [4]. Many efforts have been made to identify genetic markers or biological features that can be used to predict the risk of disease onset and measure the progression of AMD or the effects of treatment. Given that serum and tissue levels of select nutrients, such as xanthophylls, have shown to be associated with a decreased risk of AMD, using nutrients as biomarkers of AMD seems worthy of future consideration. An additional benefit of examining nutrients is that they can be replaced or supplemented in patients much more easily than any genetic aberration can be corrected. Several genetic biomarkers of AMD risk have been discovered, with the best established being SNPs at the complement factor H (*CFH*) and *PLEKHA1/ARMS2* (alternate name: *LOC387715/HtrA1*) loci. SNPs in vascular endothelial growth factor (*VEGF*) and the complement proteins factor B and C2 are also promising. The individual profile obtained by measuring several parameters can be used to develop recommendations for lifestyle modifications, such as smoking cessation, diet, exercise and blood pressure control. The results may even suggest potential molecules for drug targeting. Well-established biomarkers will probably be important tools for AMD clinical trials by providing a scale for evaluating disease progression. This review will summarize recent developments in identifying AMD-associated molecules from clinically accessible biological samples.

Genetic markers

AMD-associated SNPs may eventually serve to identify at-risk individuals and separate AMD patients into homogenous groups for preventive and therapeutic studies. These genetic biomarkers also serve as powerful tools in the elucidation of the underlying etiology of AMD. We limit our discussion predominantly to those SNPs that are consistently associated with AMD in multiple case-control studies and, thus, have the strongest potential to serve as genetic biomarkers. Some more recent findings are also discussed. These markers include:

- *CFH* (Chromosome 1q32, Entrez Gene ID 3075)
- *Complement factor B* (chromosome 6p21.3, Entrez Gene ID 629)
- *Complement Component 2* (chromosome 6p21.3, Entrez Gene ID 717)
- *PLEKHA1/ARMS2/HtrA1* (chromosome 10q26, Entrez Gene ID 387715/5654/59338)
- *Excision repair cross-complementing rodent repair deficiency, complementation group 6* (chromosome 10q11.23, Entrez Gene ID 2074)
- *VEGF* (chromosome 6p12, Entrez Gene ID 7422)

Associations have been found for many additional SNPs, including *APOE*, *ELOVL4* and *TLR4*, but have not been consistently replicated across multiple populations. Further studies will be necessary before these SNPs can be recommended as biomarkers. They are reviewed elsewhere [5].

Complement factor H

Several AMD-associated SNPs and haplotypes within the *CFH* gene have been identified and are summarized in TABLE 1. In a convergence of approaches, three independent research groups simultaneously reported a *CFH* Y402H (rs1061170, 1277T to C) polymorphism, which appears to be a strong indicator of AMD predisposition. AMD risk for individuals bearing one variant allele increases by approximately 2.5-fold, while odds ratios (ORs) as high as 7.4 were reported for those homozygous for the risk allele [6–8]. Several additional studies have confirmed a strong association between the Y402H SNP and AMD in the USA and have yielded population attributable risk estimates ranging from 43 to 68% [9–13]. A recent meta-analysis of 5451 cases and 3540 controls yielded an OR of 2.43 for heterozygotes and 6.22 for homozygotes [14].

The Y402H marker has been shown to be a genetic risk factor for AMD worldwide. Specific associations have been identified in the populations of France, The Netherlands, Iceland, India, Germany, UK, China, Russia and Australia [12,15–22]. The Y402H polymorphism does not appear to be an AMD risk factor in the Japanese population [23]. Recent reports have shown that the Y402H SNP is not the only AMD-associated marker within the *CFH* gene [24,25]. One report identified a second association at rs1410996. This SNP is located within a *CFH* intron and harbors a stronger AMD association than the Y402H SNP ($p = 2.65 \times 10^{-61}$ vs $p = 1.79 \times 10^{-59}$) [25]. A second report identified eight major haplotypes defined by the alleles of five *CFH* SNPs. Of these haplotypes, two were protective and the remaining six were associated with an increased risk of AMD [24].

CFH is a critical regulator of the complement cascade in innate immunity and associated inflammatory processes. CFH acts upon the C3 cleavage product C3b. When C3b binds to the surface of cells, it forms C3b–Bb, which acts as an activating enzyme, leading to further production of C3b. If unchecked, this process would be harmful to the host tissue. CFH inhibits this alternative pathway cascade by binding to C3b, accelerating the decay of C3b–Bb and acting as a cofactor for the factor I-mediated proteolytic cleavage inactivation of C3b [26]. CFH is able to preferentially bind to and prevent complement activation on host tissue in part by binding to heparin and sialic acid. The polymorphism could potentially impact the affinity of CFH for heparin [8]. The functional significance of the recently discovered *CFH* AMD-associated SNPs and haplotypes is less clear; Li and colleagues have proposed the possibility that the variants influence the expression of CFH or of a neighboring functionally similar gene [24,25].

Complement factor B & complement component 2

Further evidence implicating the complement system in AMD pathogenesis has come from two studies showing the existence of protective and risk haplotypes within the region containing *BF* and *C2* [25,27]. BF plays an essential role in alternative pathway complement activation, whereas C2 is essential to activation of the classical complement pathway. A study by Gold and colleagues suggested that the most common haplotype at the *C2/BFH* locus, H1, leads to increased risk of AMD (OR: 1.32). Two haplotypes H7 and H10, which were tagged by four variants (H7: rs9332739 at *C2* and rs4151667 at *BF*; H10: rs547154 at *C2* and rs641153 at *BF*), yielded protective effects with ORs of 0.45 and 0.36, respectively. Stepwise logistic regression using a larger sample set suggested that the signal from rs641153 was the true susceptibility SNP in the H10 haplotype but was unable to differentiate between rs9332739

and rs4151667 [25]. Thus, the protective effect at C2/BF can be evaluated by genotyping rs641153 and either rs9332739 or rs4151667. While the SNP in *C2* does not appear to have a biological function, the SNPs in *BF* may functionally impact BF activity and modulate BF secretion [27]. The variant allele frequencies of each of these SNPs are under 0.10 [25]. Thus, although these SNPs are strongly associated with AMD and provide insights into the molecular pathogenesis of the disease, these SNPs do not influence the AMD susceptibility of a substantial fraction of the population.

PLEKHA1/ARMS2/HtrA1

Chromosome 10q26 has been implicated in AMD in several linkage studies (TABLE 2) [5, 28]. Considerable linkage disequilibrium in the region makes it difficult to resolve the true source of AMD linkage at 10q26 [11]. A study by Jakobsdottir and colleagues identified strong association signals at pleckstrin homology domain-containing family A, member 1 (*PLEKHA1*), the hypothetical gene *ARMS2* and HtrA serine peptidase 1 (*HtrA1*; alternative name: *PRSS11*). However, the study was not sufficiently powered to distinguish between the genes [11]. Several subsequent reports strongly suggested that the true AMD susceptibility SNP was a S69A polymorphism (rs10490924) in *ARMS2*. Since the strongest association signals appeared to be centered around *PLEKHA1* and *ARMS2*, SNPs in these genes were the most thoroughly investigated [14,15,25,29,30]. Maller and colleagues noted that a SNP in high linkage disequilibrium with S69A did exist in *HtrA1* [25]. Two recent reports identified a variant in the regulatory region of *HtrA1* (rs11200638) that appears to be the true source of the association signal found at *PLEKHA1/ARMS2/HtrA1* [31,32].

The association between AMD and the *HtrA1* SNP is highly significant ($p = 1 \times 10^{-9}$). ORs for individuals heterozygous and homozygous for the risk alleles in the Caucasian population are 1.86 and 6.56, respectively. The population attributable risk (PAR) for the *HtrA1* SNP is 49.3% in the Caucasian population [31].

Additional functional studies will be required to confirm that *HtrA1* rs11200638 is indeed the causal variant. Unlike *ARMS2*, a body of biological evidence supports the AMD involvement of *HtrA1*. Although this SNP is not found in the coding region of *HtrA1*, it appears to alter a transcription factor-binding site, leading to increased *HtrA1* expression [31,32]. *HtrA1* is expressed in the human retina [32]. Its expression in human fibroblasts increases with advancing age [33]. Moreover, immunohistochemistry analysis of the eyes of Caucasian patients diagnosed with wet AMD showed positive staining for HtrA1 in the drusen [31]. *HtrA1* is downregulated in human melanoma and breast cancer [34,35]. It is upregulated in osteo-arthritic cartilage and Duchenne muscular dystrophy and probably plays a role in the regulation of cell growth [36,37]. The aberrant expression of HtrA1 near the chick eye suppresses eye development [38]. Thus, overexpressed HtrA1 could play a role in AMD pathogenesis.

Similar to *CHF*, the *PLEKHA1/ARMS2/HtrA1* locus appears to be a marker of AMD worldwide. Notably, the same *HtrA1* SNP was associated with AMD in case-control groups from Utah (USA) and Hong Kong (China) [31,32]. The ORs for the Hong Kong population were similar to those of the Caucasian population. Studies have also found associations between the *ARMS2* SNP and AMD in Russian [21], German [15] and Japanese populations [39].

The SNP in *HtrA1* appears to be a marker for progression to advanced AMD. Both studies that implicate *HtrA1* used AMD patients with the neovascular (NV) form of the disease [31,32]. Other major studies of association in the *PLEKHA1/ARMS2/HtrA1* region also found that the association was much stronger when only advanced AMD patients were considered, which is defined as patients suffering from CNV/geographic atrophy [11,14,15,25,29].

Interactions between the *PLEKHA1/ARMS2/HtrA1* region and other genetic and environmental factors have been the subject of considerable investigation. Several studies have tested for an interaction between the Y402H SNP in *CFH* and the SNP in *ARMS2*. The effects of the variants at each gene appear to be independent and the joint effect of the two genes is best described by an additive model [11,14,15,25,29]. Interaction between cigarette smoking and *PLEKHA1/ARMS2/HtrA1* is uncertain. One study found strong evidence of interaction between cigarette smoking and AMD, which was shown using two independent statistical methods, logistic regression with cases and control, and a case-only analysis using pack-years of smoking as a continuous variable. As an example of the effect, individuals homozygous for risk alleles at *CFH* and *PLEKHA1/ARMS2/HtrA1* who did not smoke had a disease OR of 10.21 (CI: 3.27–31.94) while those who did smoke had an OR of 34.51 (CI: 11.87–100.32) [29]. However, another study yielded no evidence of statistical epistasis between the two risk factors [14].

Excision repair cross-complementing rodent repair deficiency, complementation group 6 (ERCC6)

An association between a SNP in the untranslated region of *ERCC6* (rs3793784, C-6530>G) and AMD has been demonstrated in clinically diagnosed patients and controls, as well as by using DNA extracted from archived tissue [40]. The association between *ERCC6* and AMD is relatively modest. Heterozygosity for the risk allele results in an OR of 1.51 and homozygosity for the risk allele results in an OR of 1.60. However, the SNP in *ERCC6* demonstrated statistical epistasis with the SNP in *CFH* (rs380390), yielding a combined disease risk OR of 23.05 for individuals homozygous for risk alleles at both the *CFH* SNP and the SNP in *ERCC6*. Higher expression of *ERCC6* is also detected in ocular AMD tissues.

ERCC6 functions in transcription coupled and base excision DNA repair as a partner in protein complexes [41]. The disease-associated SNP in *ERCC6* appears to cause increased *ERCC6* expression. Overexpression of a single element in an enzyme complex can adversely impact complex functionality [42]. Thus, the disease-associated SNP may lead to overexpression of *ERCC6*, potentially impacting DNA repair and transcription. The interaction between *ERCC6* and *CFH* may be a result of *ERCC6* functioning in universal transcription as a component of the RNA pol I complex [40].

Vascular endothelial growth factor

VEGF is postulated to be involved in the pathogenesis of AMD, especially in the NV form. VEGF is a major regulator of angiogenesis. VEGF inhibitors have been shown to aid in AMD treatment [43]. Several studies have suggested that VEGF polymorphisms may serve as effective biomarkers of AMD. One study found an association between AMD and a *VEGF* SNP (rs2010963), which was most strongly associated with NV AMD in independent case–control samples ($p = 0.16$ for all AMD; $p = 0.02$ comparing NV AMD and controls) [28]. A different *VEGF* SNP (rs833070) was also associated with both early and late AMD in family-based analysis [28]. Since no current genetic biomarkers are available for predicting progression to NV AMD, further investigation into whether rs2010963 is specific for NV may be of value. A recent case–control study by Churchill and colleagues found a single *VEGF* SNP and several *VEGF* haplotypes associated with all AMD cases compared with controls [44]. Specific *VEGF* polymorphisms and haplotypes may contain transcription factor-binding sites important for VEGF expression [44].

Inflammatory biomarkers

Various immunological molecules and inflammatory mediators have been identified at the site of AMD lesions [45]. Proinflammatory cytokines are released from immune cells during an inflammatory response. These cytokines mediate distant inflammatory effects.

C-reactive protein

C-reactive protein (CRP), a marker of nonspecific inflammation, has been shown to be a risk factor in cardiovascular disease [46]. CRP may be involved in the pathogenesis of AMD through chronic inflammation leading to oxidative damage, endothelial dysfunction, drusen development or the degeneration of Bruch's membrane [47]. CRP may also have a direct role in AMD development through its ability to induce complement activation [47].

Several studies show an association between CRP levels and AMD. Seddon and colleagues conducted a study, ancillary to the Age-Related Eye Disease Study (AREDS), which measured CRP serum levels of 1027 patients [48]. The study enrolled participants at two sites and divided them into four groups based on AMD severity. CRP levels were significantly increased in the group with the most AMD symptoms compared with the group with the least (2.7 vs 3.4 mg/l; $p = 0.02$). Stricter division of the groups based upon CRP levels led to even greater ORs (CRP greater than 90th percentile vs baseline; OR: 1.75). Moreover, elevated CRP levels were associated with AMD independent of smoking status, which is a known AMD risk factor [48]. Another case-control study by Vine and colleagues showed a significant association between elevated CRP levels and AMD ($p = 0.043$) [49].

Elevated CRP levels may serve as a marker for AMD progression. Seddon and colleagues performed a prospective cohort study examining the relationship between plasma CRP levels and AMD progression [50]. The study found an association between high CRP levels and likelihood of AMD progression (mean follow-up time: 4.6 years), with an adjusted OR of 2.10 ($p = 0.046$) [50].

However, Klein and colleagues demonstrated no significant association between CRP plasma levels and AMD or AMD progression in both case-control and prospective studies [51]. Another study by Klein and colleagues also found no association between early AMD and elevated plasma CRP levels [52]. In a case-control study using patients recruited from Muenster (Germany), researchers found significantly elevated plasma CRP levels as the degree of AMD severity increased compared with controls [53]. However, when cardiovascular risk factors were taken into consideration, no statistically significant increases were found in the ORs of AMD patients compared with controls [53].

Similarly, McGwin and colleagues reported no significant association between plasma CRP levels and AMD in a case-control study [54]. However, only 42 cases met the highest cut-off value for advanced AMD used in the Seddon study and CRP was only associated with advanced AMD in previous studies [50,54].

A prospective cohort study by Despret and colleagues showed that elevated plasma CRP levels in individuals homozygous for the *CFH* Y402H SNP have an increased risk of developing late AMD [18]. This study does not show an increased risk of late AMD development due to increased plasma CRP levels in individuals without this SNP, suggesting that plasma CRP levels interact with the SNP to induce the increased late AMD risk [18]. Another study showed that *CFH* Y402H homozygotes have increased CRP deposition in the choroids, which may contribute to AMD development [55]. However, another study failed to find an association between the *CFH* Y402H SNP and AMD [47]. It is postulated that the Y402H variant of *CFH* limits the ability of *CFH* to bind and suppress complement-mediated damage by CRP [47]. It is possible that the Y402H polymorphism, coupled with an increased plasma CRP level, may contribute to AMD development.

AMD & interleukin-6

IL-6 is a marker for systemic inflammation, such as acute pancreatitis, chronic arthritis and geriatric syndromes. Seddon and colleagues performed a prospective cohort study to see if

plasma IL-6 levels could predict progression of AMD [50]. The group found a correlation between the level of IL-6 and chances of AMD progression (mean follow-up time: 4.6 years; $p = 0.03$) [50]. This study shows that elevated IL-6 levels may serve as a marker for progression of AMD. However, Klein and colleagues found no significant association between IL-6 plasma levels and AMD or AMD progression [51]. Though few studies have been accessed to implicate IL-6 as a biomarker for AMD, the fact that a prospective study could predict AMD progression based on IL-6 levels coupled with the fact that IL-6 levels influence CRP and fibrinogen levels (other potential AMD biomarkers) points to a significant role for IL-6 in the development of AMD.

AMD & fibrinogen

Fibrinogen is an established biomarker of acute and chronic inflammation [56,57]. Fibrinogen is responsible for erythro-sedimentation rate elevation, which indicates the inflammatory state of the patient. A case-control study by Lip and colleagues recently found elevated levels of plasma fibrinogen in AMD cases compared with controls [56]. A case-control analysis from the large Blue Mountains Eye Study in Australia detected significantly elevated plasma fibrinogen levels in 'late' AMD patients compared with controls ($p < 0.05$) [58]. Late AMD patients included those with the wet form of AMD, as well as those with geographic atrophy. The relative risk of late AMD was 6.7 for a fibrinogen level of higher than 4.5 g/l (highest quartile) compared with the lowest quartile [58].

In another study using patients recruited from the Muenster Aging and Retina Study population in Muenster (Germany), the researchers found elevated plasma fibrinogen levels as the degree of AMD severity increased. The unadjusted comparison of means between plasma fibrinogen levels in late AMD compared with controls and comparing early AMD patients and controls, were both statistically significant, but after adjusting for cardiovascular risk factors there was no longer a significant difference [53]. Another study by Klein and colleagues found no association between plasma fibrinogen levels and AMD (specific data not presented in paper). Thus, data on the role of serum fibrinogen as a potential AMD biomarker are promising based on the cited studies yet inconclusive to date. Prospective studies of fibrinogen levels and AMD development, as well as fibrinogen genetic haplotypes, are needed to strongly link fibrinogen with AMD and justify its use as an AMD biomarker.

AMD & vascular endothelial growth factor

As discussed in the genetics section, there is strong evidence suggesting that VEGF is a good candidate AMD biomarker. VEGF is also elevated in RPE cells of AMD patients and in the AMD patient post-mortem eyes [56]. This evidence points to a role for elevated VEGF levels in AMD. A study by Lip found increased plasma VEGF levels in 78 AMD patients compared with 25 age-matched controls ($p = 0.0196$) [56]. Interestingly, no significant difference was found between dry and wet AMD cases in a comparison of plasma VEGF values [56].

In a recent study, Tsai and colleagues found increased plasma VEGF levels in 77 AMD patients compared with 42 controls ($p < 0.001$) [60]. Interestingly, these researchers found significantly higher plasma VEGF levels in wet AMD patients compared with dry AMD patients ($p < 0.05$) [59]. These results suggest that high VEGF levels may play a role in predisposing individuals to NV AMD. Substantial evidence, including specific polymorphisms and haplotype studies, as well as case-control plasma level studies, implicates an important role for VEGF in AMD. Additional studies must be undertaken to establish its role as a biomarker for this disease.

Nutrient biomarkers

Various nutrients in the literature have demonstrated risk reduction for AMD after supplementation. Given the ever-increasing studies on *in vivo* effects of nutrients on AMD pathogenesis, the following nutrients show some promise in becoming important biomarkers. The fact that nutrients have the potential to be replaced much easier than tackling genetic aberrations in patients makes them an attractive avenue to explore in the realm of biomarkers.

AMD & omega-3 fatty acids

Omega-3 fatty acids are polyunsaturated fatty acids that are vital components in the phospholipids of cell membranes. They cannot be synthesized by mammals and must be obtained from the diet, from sources such as fish. Omega-3 fatty acids, particularly docosahexaenoic acid (DHA), are highly concentrated in retinal tissue, especially in the photoreceptor outer segments [60]. In addition to structural roles, omega-3 fatty acids have been shown to exert *in vivo* and *in vitro* anti-inflammatory effects [61]. In light of the increasingly evident role of inflammation in AMD, they may encompass one mechanism of protection against AMD.

Association studies between omega-3 intake and AMD have been inconsistent. A case-control study, the US Twin Study of Age-Related Macular Degeneration, revealed dietary omega-3 fatty intake was inversely associated with AMD (OR: 0.55; 95% CI: 0.32–0.95) when comparing the highest versus lowest quartile. Furthermore, the reduction in risk was seen primarily among subjects with low levels (below median) of linoleic acid intake, an omega-6 fatty acid ($p < 0.001$). Other studies also highlight the need to maintain a certain ratio between omega-6 and omega-3 fatty acids [62]. It is interesting to note that the American diet has an undesirably high omega-6 to omega-3 ratio. The Blue Mountain Eye Study showed the highest versus lowest quintiles of omega-3 polyunsaturated fat intake had lower risk of incident early AMD (OR: 0.41; 95% CI: 0.22–0.75) [63].

In contrast to the previous studies, the Beaver Dam Eye Study did not find any association between omega-3 intake and AMD, whereas the third National Health and Nutrition Examination Survey (NHANES) found a protective effect of omega-3, but the result was not statistically significant [64]. The Dietary Ancillary Study of the Eye Disease Case Control Study (EDCCS) reported on neurovascular (late) AMD [62], revealing an inverse relationship between AMD and increasing amounts of omega-3 or fish intake, but this trend was nonsignificant when adjusted for confounding variables. In response to these rather inconsistent association studies between omega-3 fatty acids and AMD, it has been hypothesized that omega-3 may play the greatest role for patients who have high levels of inflammatory biomarkers, such as CRP or genotypes associated with increased risk of AMD [65].

Despite established knowledge that omega-3 fatty acids (particularly DHA) exist in high concentrations in the retina [60], we found no studies comparing omega-3 levels in the retina of AMD patients against that in controls. However, deprivation studies on rats revealed that the brain and retina are resistant to alterations in omega-3 fatty acid levels and two to three generations of deprivation were required to produce maximal effect on tissue levels [66]. A recent study on rhesus monkeys examining the effects of prenatal depletion of omega-3 revealed that in newborns, phospholipids of the prefrontal cerebral cortex and retina, normally highly enriched in DHA, had substantially reduced levels of DHA and other omega-3 fatty acids. Slowed visual acuity development and abnormalities of the electro-retinogram were also found. Furthermore, plasma n-3 fatty acids were reduced by 86% relative to control neonates from mothers who were fed normal diet [67].

Although studies in humans have revealed evidence for the efficacy of omega-3 fatty acids in slowing the progression of diseases such as retinitis pigmentosa [68], no such studies examine the possible therapeutic effect in AMD. AREDS II is currently seeking participants to determine if omega-3 fatty acids will decrease the risk of progression to advanced AMD.

Despite the putative role of omega-3 fatty acids and protection against AMD, studies thus far have captured the inverse association between omega-3 intake and AMD, while leaving in question the relation between serum levels, macular levels and effects on disease progression. Therefore, further studies are required before omega-3 can be considered an optimal biomarker.

AMD & α -tocopherol (vitamin E)

The most predominant form of vitamin E, α -tocopherol, is found in high concentrations within the RPE and retina [69]. Macular and peripheral retina, and macular RPE levels are observed to increase until the fifth decade, and decrease after the seventh decade [70].

As the major lipid-soluble antioxidant present in all cellular membranes, α -tocopherol has been shown to protect against lipid peroxidation [71]. Supplementation with α -tocopherol significantly reduces oxidative damage from H_2O_2 or hyperoxia in cultured human RPE cells [72]. Since RPE dysfunction is hypothesized to be a major disease process, this has many implications on the pathogenesis of AMD.

Unlike xanthophylls (lutein and zeaxanthin), no studies exist that compare α -tocopherol levels in AMD retinas against the normal retinas of age-matched controls. Furthermore, there is no evidence that supplementation with α -tocopherol can increase its concentration in the retina of AMD patients. Studies on associations between plasma concentrations or intake of vitamin E and AMD have been inconclusive and sometimes contradictory. The possible protective effect of vitamin E was illustrated by the prospective Pathologies Oculaires Liees à l'Age (POLA) study, on the 2584 inhabitants of Sète (France), which revealed that lipid-standardized plasma α -tocopherol levels showed a significant negative association with both early ($p = 0.04$) and late AMD ($p = 0.003$). The inverse association with signs of early AMD showed an OR of 0.72 (95% CI: 0.53–0.98; $p = 0.04$), while the risk of late AMD was reduced by 82% in the highest quintile compared with the lowest [73].

By contrast, a randomized controlled trial on 1193 Australian patients showed that daily supplementation with 500 mg vitamin E for 4 years did not alter the incidence or progression of AMD [74]. Of importance is the fact that this result was despite the fact that the mean serum concentrations of vitamin E in the treatment group were double that of the placebo group at both 2 and 4 years. Furthermore, outcomes including masked comparison of photographs from baseline and at 4 years, analysis of best corrected visual acuity and visual function data, showed no difference between the two groups. Studies of vitamin E supplementation combined with other antioxidants have shown a more promising effect. The randomized placebo controlled AREDS trial revealed that people at high risk of developing advanced stages of AMD lowered their risk by approximately 25% when treated for 5 years with a high-dose combination of vitamin C, vitamin E, β -carotene and zinc. The risk of vision loss caused by advanced AMD was reduced by approximately 19% [75]; however, serum vitamin E levels were not reported.

AMD & xanthophylls

It has been established that the xanthophylls lutein and zeaxanthin are responsible for the yellow color of the macular lutea and, hence, are the primary components of macular pigment [76]. Xanthophylls are a subclass of carotenoids, a large group of organic pigments that animals cannot synthesize and must obtain through their diet from sources such as dark green leafy vegetables.

There is evidence that lutein and zeaxanthin play multiple roles to protect against AMD. In addition to quenching reactive oxygen species, xanthophylls function in filtering short wavelength (blue) light [77] and, hence, may serve to protect components of the retina from oxidative damage. Furthermore, lutein and zeaxanthin may play a role in curbing the amount of lipofuscin formed, as revealed in RPE cell culture studies [78]. This has many implications for the pathogenesis of AMD, as lipofuscin deposits in the RPE, and has been shown to be phototoxic, a source of reactive oxygen species and retard phagocytosis by RPE cells [78].

The retina has the highest concentration of xanthophylls of any tissue in the human body. A 10,000-fold higher concentration of xanthophyll pigment in the retina compared with the blood is the result of a regulated active transport mechanism [79]. Noninvasive methods for assessing retinal xanthophyll levels *in vivo* include heterochromatic flicker photometry, Raman spectroscopy and fundus autofluorescence imaging.

Normal individuals have varying degrees of macular levels of xanthophylls, as measured by macular pigment optical density (MPOD), yet the maculae of AMD patients reveal lower levels of macular pigment than age-matched controls [80]. Although Raman signal intensity declined with age in normal eyes, average MPOD was 32% lower in AMD eyes versus normal elderly control eyes as long as the subjects were not consuming high-dose lutein supplements ($p = 0.001$) [80]. Quantitative studies on donor eyes using high-powered liquid chromatography have also confirmed this finding. Lutein and zeaxanthin levels in 112 donor eyes with AMD and in 112 donor eyes without AMD revealed that maculae with AMD had 62% of the lutein and zeaxanthin levels compared with eyes of control subjects [81]. Furthermore, levels of lutein and zeaxanthin decreased in magnitude from the inner to outer regions centered about the fovea, which is interesting since AMD is a disease that affects central vision. It remains difficult to distinguish whether lower macular pigment densities are a cause or an effect of the disease process.

Studies have shown that a diseased macula can accumulate and stabilize lutein and/or zeaxanthin; a statistically significant rise in MPOD in eyes with AMD was observed and was statistically similar to the healthy eyes [82]. Complementing these findings, long-term studies on monkeys revealed that feeding xanthophyll-free diets from birth until 7–16 years of age led to both undetectable serum concentrations and macular levels of lutein and zeaxanthin. After the deprivation period, when the animals were provided lutein or zeaxanthin supplements for 24–56 weeks, serum levels increased rapidly. Perhaps more importantly, MPOD was also seen to increase to a relatively steady level by 24–32 weeks [83]. Dietary supplementation studies in humans have shown some increase in macular pigment after long-term supplementation. The rate of accumulation is slow but, nonetheless, apparently associated with serum concentration and may remain stable after supplementation is stopped for a long period of time.

Studies have shown that increased serum xanthophylls provide a protective factor against AMD. In the EDCCS of 421 cases and 615 control subjects, individuals with high serum levels of lutein plus zeaxanthin reduced the risk of NV or wet AMD by a factor of two [84]. In a study of 380 men and women from Sheffield (UK) aged 66–75 years, a statistically significant correlation between plasma concentrations of zeaxanthin and risk of AMD was found, while plasma lutein was not statistically significantly correlated. Compared with those whose plasma concentrations of zeaxanthin were in the highest third of the distribution, individuals whose plasma concentration was in the lowest third had an OR for risk of AMD of 2.0 (95% CI: 1.0–4.1), after adjustment for age and other risk factors [85]. The POLA study, in which plasma levels of either lutein or zeaxanthin showed a significantly inverse association with AMD (OR: 0.31 and 0.07, respectively), subjects with high levels of both plasma lutein and zeaxanthin had a 79% reduced risk of AMD compared with subjects with low total plasma lutein and

zeaxanthin [86]. The POLA study results reveal that plasma zeaxanthin, more so than plasma lutein, was associated with a marked reduced risk of AMD.

However, there also exist a number of studies that have failed to reveal the association between serum levels of xanthophylls and AMD [87]. The Carotenoids in Age-Related Eye Disease Study (CAREDS), an ancillary study of the Women's Health Initiative, is an example. Despite strong correlations between serum xanthophyll levels and macular pigment density, serum levels of lutein and zeaxanthin were not associated with intermediate AMD [88].

In light of this data, it appears that using serum levels of xanthophylls as a biomarker for AMD may prove to be less fruitful than using macular levels as a biomarker. Since there exist a number of noninvasive methods of measuring macular levels of lutein and zeaxanthin levels *in vivo*, this method would allow adequate assessment of risk of disease onset, progress of AMD or the effects of treatment. Over the other nutrients reviewed, the xanthophylls show most promise in being developed as biomarkers for AMD.

Other biomarkers

AMD & homocysteine levels

Numerous hypotheses exist relating elevated homocysteine levels to the pathogenesis of AMD [89,90]. Plasma homocysteine may lead to AMD development by causing damage to vascular endothelium [91]. A case-control study by Vine and colleagues (79 participants with AMD vs 77 controls) found a significant association between AMD and elevated homocysteine levels [49].

A very large case-control study gave strong support of an association between elevated homocysteine levels and AMD [92]. This study revealed steadily increased levels of plasma homocysteine as the degree of AMD severity increased [93]. In addition, a study by Kamburoglu and colleagues found significantly increased levels of plasma homocysteine in both wet and dry AMD cases compared with levels in controls [93]. Another study by Coral and colleagues showed a significant increase in plasma homocysteine levels in wet AMD patients compared with controls [94]. Moreover, a study by Sieger-Axel and colleagues found a significant increase in the plasma homocysteine levels in wet AMD patients compared with both dry AMD patients and matched controls [95]. This suggests that elevated homocysteine levels may play a specific role in wet AMD pathogenesis.

However, a study by Heuberger and colleagues found no significant association between plasma homocysteine levels and AMD [96]. These authors realized possible limitations in their study including variability in fasting levels when cases and controls had blood drawn, as well as the exclusion of people with missing homocysteine values, who were older and more likely to have AMD [96]. Current research implicates elevated serum homocysteine levels with the development of both wet and dry AMD, and correlates increased levels of plasma homocysteine with the severity of AMD development. Though future prospective studies following individuals with various homocysteine levels and the development of AMD, as well as studies with homocysteine genetic variants and AMD development would lend evidence to this assertion, current studies show that homocysteine is a biomarker of AMD.

AMD & von Willebrand factor

Von Willebrand factor (VWF) is released during endothelial cell damage and, thus, serves as a marker for it [56,97]. A recent study by Lip and colleagues found increased plasma levels of VWF in 77 AMD cases compared with 25 controls [56]. Therefore, endothelial cell dysfunction may play a role in the pathogenesis of AMD. In addition, a study by Malukiewicz and colleagues found significantly increased levels of plasma VWF in AMD patients compared

with controls [98]. Prospective studies of VWF levels and AMD development and genetic haplotypes are needed to implicate VWF as a biomarker of AMD.

AMD & plasma leptin levels

A recent case-control study by Evreklioglu and colleagues (32 participants with AMD and 20 age-matched controls) found an inverse association between serum leptin levels and AMD onset and severity (6.01 ± 2.55 ng/ml in AMD patients vs 13.21 ± 2.27 ng/ml in controls; $p < 0.001$; and 3.81 ± 0.58 ng/ml; $p < 0.001$ in late-AMD patients vs 8.21 ± 1.68 ng/ml in early-AMD patients; $p < 0.001$) [99]. A possible mechanism may be linked to the fact that leptin decreases fatty acid and triglyceride synthesis, which accumulate in the eyes of individuals with AMD [99]. Thus, increased leptin levels may have a protective effect in the development and progress of AMD, and the leptin levels may serve as an effective biomarker for AMD development [99].

Expert commentary & five-year view

Association studies have been highly successful in identifying genetic biomarkers of AMD. SNPs in the genes *CFH* and *HtrA1* capture as much as 71.4% of the population attributable risk [31]. Genotyping five SNPs in *CFH*, *BF-C2* and *PLEKHA1/ARMS2/HtrA1* appears to identify individuals whose lifetime risk of AMD ranges from 1% to greater than 50%; however, intermediate risk is observed for most common allele combinations [25]. The ability to stratify individual risk will further improve as more AMD-associated SNPs are identified. Further examination of ways in which these genetic risk factors interact with environmental and lifestyle factors may lead to public health policies that decrease the incidence of this blinding disease.

Effective biomarkers must be standardized, logistically simple to analyze and clinically useful. Impact of sex, age, ethnicity and comorbid conditions on biomarkers is also of importance. Unfortunately, many of the candidate markers have yet to satisfy these requirements. In general, most studies of genetic and biological markers reported simply the disease association, few reflected disease classification, severity, stage or response to therapy. Moreover, those case-control studies can often overestimate the effectiveness of a particular biomarker while potentially more effective prospective cohort studies are rare and expensive. AMD biomarker studies have also yielded conflicting results in some reports. Since AMD is definitely an age-related disease, examining advanced AMD patient groups may provide the best chance of identifying effective biomarkers, both in DNA and in serum. Unknown relationships between a specific biomarker levels in plasma or serum and ocular tissue is another problem. In nutrient studies, the relationship between diet-plasma levels of micronutrients and antioxidants (e.g., carotenoids), and macular levels remains uncertain. Currently there are few ongoing studies, which will help to clarify the matter.

We predict that genetic markers will be most effective in determining AMD risk and the likelihood of progression to advanced AMD. As more AMD-associated genetic markers are identified, a panel of them in conjunction with select lifestyle factors and/or serum biomarkers should be effective in identifying high-risk individuals. There is a necessity for large-scale epidemiological studies incorporating gene-gene and gene-environment models.

Although large-scale studies should be more informative with respect to gene-gene and gene-environment interactions, no interaction has been replicated in multiple studies thus far. Serum and inflammatory markers are also of uncertain value at the present time. Fortunately, it is already possible to identify many individuals who are at high risk of developing AMD. The *CFH* Y402H SNP and *PLEKHA1/ARMS2/HtrA1* SNPs have population attributable risks of 43–68% and 27–57%, respectively [10–14,29]. The recently discovered noncoding marker at

CFH should further help to identify high-risk individuals and, using CFH genotype alone, individuals can be stratified from baseline to 15-fold increased risk of AMD development [25].

The ability to identify high-risk individuals should be useful for designing more informative clinical trials of potential AMD therapeutics. Individuals of similar genetic backgrounds can be randomized to treatment and nontreatment groups. Clinical trials with potentially high-risk procedures and therapeutics should target only those individuals who are most likely to develop advanced AMD.

Although genetic markers are currently effective predictors of AMD risk and advanced AMD risk, there are still limited biomarkers for predicting the development of NV AMD. Further research into the effects of the various *VEGF* haplotypes will be necessary to determine if these SNPs are effective. It remains to be seen whether subtype-specific genetic markers will be discovered. If identified, these genetic markers will aid in selecting a timely therapeutic intervention for such patients. Since therapeutic options are currently limited, the most effective function of these genetic markers at present is to suggest pathways involved in the pathogenesis of AMD, and lead to earlier monitoring of high-risk patients. Ultimately, we are hopeful that knowledge of the involvement of *PLEKHA1/ARMS2/HtrA1* and *CFH* in AMD will lead to the development of effective therapeutics.

Key issues

- Genetic markers can successfully identify individuals whose lifetime risk of age-related macular degeneration ranges from 1% to greater than 50%.
- The most important genetic markers to date are single nucleotide polymorphisms at *CFH* and *PLEKHA1/ARMS2/HtrA1*.
- Few effective nutrient biomarkers have been identified, which is primarily owing to the difficulty in measuring macular levels of these nutrients.
- Xanthophylls are the most promising nutrient biomarkers due to the existence of noninvasive methods to measure macular concentrations.
- Inflammatory biomarkers are still controversial as age-related macular degeneration predictors.

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Table 1

CFH SNP association studies.

Study	Cases	Controls	Results	Ref.
Klein <i>et al.</i> (2005)	96 unrelated subjects with large drusen, GA or NV AMD	50 unrelated subjects with	Being homozygous for the risk allele of a noncoding SNP in <i>CFH</i> (rs380390) is associated with an OR_{hom} of 7.4. This SNP is in LD with Y402H (rs1061170)	[8]
Edwards <i>et al.</i> (2005)	224 subjects for initial investigation, 176 additional cases used for replication	134 subjects for initial investigation, 68 additional subjects for replication	Having one risk allele at rs1061170 (S69A) leads to a 2.7-fold AMD risk increase. PAR for the Y402H SNP was 50%	[6]
Haines <i>et al.</i> (2005)	495 unrelated cases and cases taken from 182 families	185 unrelated controls and unaffected individuals from the 182 study families	PAR was 43% for Y402H. Being heterozygous for the risk allele (OR_{het}) yielded an OR of 2.45 (1.41, 4.25); $OR_{\text{hom}} = 3.33$ (1.79, 6.20)	[7]
Conley <i>et al.</i> (2006)	126 cases taken from the CHS, 701 cases taken from the AREDS	1051 controls taken from the CHS, 175 controls taken from the AREDS	Y402H showed significant association with AMD in both cohorts. It was not a subtype-specific AMD risk factor. Population attributable risk estimates derived from the CHS were 27% (CT genotype) and 25% (CC genotype). Meta-analysis yielded $OR_{\text{het}} = 2.43$ and $OR_{\text{hom}} = 6.22$	[14]
Hageman <i>et al.</i> (2005)	404 patients from the University of Iowa (IO, USA), 550 from Columbia	131 age- and ethnicity-matched subjects from the University of Iowa, 275 from Columbia	Identified eight CFH SNPs with strong AMD association, including Y402H. Determined one risk haplotype defined by presence of Y402H and I62V resulted in a disease OR_{het} of 2.46 and an OR_{hom} of 3.51 for individuals homozygous for this haplotype. Also found several protective haplotypes defined by SNPs in <i>CFH</i> exons 2 and 11	[10]
Jakobsdottir <i>et al.</i> (2005)	594 affected families genotyped for 684 SNPs on chromosome 1q, 196 unrelated cases genotyped for additional SNPs	179 unrelated subjects with no evidence of macular changes or with <10 hard drusen without other RPE changes	OR for bearing a risk allele at Y402H was 5.29. Noted that the strongest association was for rs1853883 and not Y402H itself	[11]
Magnusson <i>et al.</i> (2006)	581 Icelandic subjects with advanced AMD and 435 subjects with early AMD, 322 subjects from Utah (USA) with advanced AMD and 109 subjects with early AMD	Icelandic subjects: 891 population control subjects and 171 longevous controls aged 90 years or older who had no signs of AMD; Utah subjects: 203 age-matched controls	Y402H confers similar risk of soft drusen, $OR = 2.52$; geographic AMD, $OR = 2.27$; and NV AMD, $OR = 2.32$	[12]
Zarepari <i>et al.</i> (2005)	616 subjects from the Kellog Eye Center (MI, USA) consisting of 283 with NV AMD and 143 with GA AMD, 133 with both NV and GA AMD and 120 with large macular drusen	275 subjects at least 68 years old with no evidence of AMD	$OR_{\text{het}} = 2.44$, $OR_{\text{hom}} = 5.93$; PAR estimated at 39%	[13]
Souied <i>et al.</i> (2005)	141 unrelated French subjects with exudative AMD	91 healthy subjects with no signs of AMD, average age within 0.6 years of the cases	$OR_{\text{het}} = 3.0$, $OR_{\text{hom}} = 6.9$	[17]
Despriet <i>et al.</i> (2006)	3619 individuals in the Rotterdam population-based study with either no or small, hard drusen	78 cases with GA or NV AMD; 71 with soft indistinct drusen and pigmentary irregularities (stage 3); 357 with either small, soft distinct drusen or pigmentary irregularities (stage 2)	Significant results were obtained for heterozygous and homozygous <i>CFH</i> risk allele carriers when comparing stage 3 AMD and advanced AMD with controls. OR_{het} for stage 3 AMD was 1.67 and OR_{hom} was 4.58. OR_{het} for advanced AMD was 2.66 and OR_{hom} was 11.02	[18]
Kaur <i>et al.</i> (2006)	100 unrelated AMD patients recruited from all seven states of India	120 ethnically matched normal control subjects	The Y402H SNP conferred similar risks in the Indian population to those of the Caucasian population. $OR_{\text{het}} = 1.51$, $OR_{\text{hom}} = 11.52$	[16]
Rivera <i>et al.</i> (2005)	794 nonfamilial German AMD patients, with the largest fraction of patients	612 unrelated German controls who were free of macular changes. A	Y402H was significantly associated with AMD in the German population. Pooling the two German case-control groups	[15]

Study	Cases	Controls	Results	Ref.
Sepp <i>et al.</i> (2006)	having advanced AMD. Replication sample of 373 patients. 443 unrelated subjects from the UK population having either GA or NV AMD	replication set of 335 control subjects 262 spouses from the UK population were used as controls	yielded the following values: $OR_{het} = 1.99$; $OR_{hom} = 6.72$ Confirmed that Y402H was associated with both GA and NV AMD. $OR_{het} = 3.1$; $OR_{hom} = 6.3$. Showed that there was no interaction between smoking and AMD	[20]
Lau <i>et al.</i> (2006)	163 Chinese patients diagnosed with NV AMD	232 age-matched controls of Chinese descent	Shown a significant association between the <i>CFH</i> SNP in a Chinese population. The case and control group risk allele frequencies were noticeably lower (11.3% in AMD patients and 2.8% in controls). Bearing the C allele resulted in an 4.4-fold increased AMD risk	[19]
Gotoh <i>et al.</i> (2006)	146 Japanese patients diagnosed with NV AMD	105 normal controls of Japanese origin	No association was found between <i>CFH</i> Y402H and AMD ($p = 0.423$). The same was true of neighboring SNPs. The frequency of the risk allele in controls was much lower than controls taken from the Caucasian population (0.04 vs 0.45)	[23]
Okamoto <i>et al.</i> (2006)	96 unrelated Japanese cases having NV AMD	89 age-matched controls of Japanese descent	The haplotype containing Y402H was not significantly associated with AMD ($p = 0.802$). Two other <i>CFH</i> risk haplotypes were found, resulting in 1.9- and 2.5-fold risk increases.	[99]
Tuo <i>et al.</i> (2006)	460 unrelated individuals of Caucasian descent having advanced AMD	268 normal controls of Caucasian descent	One protective haplotype, which decreased AMD risk 1.6-fold, was also found C allele of <i>CFH</i> rs380390 was associated with advanced AMD with an OR of 7.4. It strongly interacted with ERCC6 C-6530>G	[39]
Fisher <i>et al.</i> (2006)	155 unrelated patients of Russian descent. 92 cases presented with NV or GA AMD while 63 had early signs of AMD including confluent drusen and areas with hyperplasia	151 age-matched controls of Russian descent	The Y402H SNP was associated with all AMD subgroups examined. Representative ORs were $OR_{het} = 2.01$; $OR_{hom} = 2.71$	[21]
Maller <i>et al.</i> (2006)	1238 unrelated individuals of European descent having either GA or choroidal neovascularization	934 unrelated individuals of European descent; age and gender matched; clinical age-related maculopathy staging system stage 1	Found an additional SNP in <i>CFH</i> which contributes to AMD risk (rs1410996) independently of Y402H. The association at this SNP is stronger than that at Y402H ($p = 2.65 \times 10^{-61}$ vs $p = 1.79 \times 10^{-59}$)	[28,105]
Baird <i>et al.</i> (2006)	236 Australian cases having early, NV, or geographic AMD	144 Australian control subjects, having an older average age than the cases	The Y402H SNP was associated with AMD. $OR_{het} = 1.86$; $OR_{hom} = 9.26$	[22]
Li <i>et al.</i> (2006)	726 subjects recruited from the Kellog Eye Center. 544 of the subjects were unrelated. All cases were of Western European descent	268 unrelated controls of Western European descent recruited from the Kellog Eye Center	Found 20 <i>CFH</i> polymorphisms with a stronger AMD association than Y402H. Defined eight <i>CFH</i> haplotypes based upon the genotypes at five SNPs (rs1048663, rs2274700, rs412852, rs11582939, rs1280514). Two of these haplotypes were protective, while the remaining six increased AMD risk	[24]

AMD: Age-related macular degeneration; AREDS: Age-Related Eye Disease Study; CFH: Complement factor H; CHS: Cardiovascular Health Study; GA: Geographic atrophy; LD: Linkage disequilibrium; NV: Neovascular; OR: Odds ratio; PAR: Population attributable risk; RPE: Retinal pigment epithelium; SNP: Single nucleotide polymorphism.

Table 2

PLEKHA1/ARMS2/HtrA1 SNP association studies.

Study	Cases	Controls	Results	Ref.
Jakobsdottir <i>et al.</i> (2005)	594 affected families genotyped for 684 SNPs on chromosome 1q. 196 unrelated cases genotyped for additional SNPs	179 unrelated subjects with no evidence of macular changes or with <10 hard drusen without other RPE changes	Found a strong association between <i>PLEKHA1/ARMS2/HtrA1</i> with the strongest signal being found at rs10490924 of <i>ARMS2</i> . Estimated the population attributable risk of the SNP at rs10490924 at 57%.	[11]
Rivera <i>et al.</i> (2005)	794 nonfamilial German AMD patients, with the largest fraction of patients having advanced AMD. Replication sample consisting of 373 patients	612 unrelated German controls who were free of macular changes. A replication set of 335 controls was also used	Determined that <i>ARMS2</i> rs10490924 was the source of the association signal at <i>PLEKHA1/ARMS2/HtrA1</i> . The OR for GT heterozygous individuals was 2.69. The OR for TT homozygous individuals was 8.21. Found that the combined risk of <i>CFH</i> and <i>ARMS2</i> is best described by an additive model	[15]
Schmidt <i>et al.</i> (2006)	610 nonfamilial patients, 64% of whom had advanced AMD. 391 subjects from families, of whom 57% had advanced AMD	259 nonfamilial controls and 85 controls from families. Controls were notably younger than cases (average age = 66.7 vs 76.8 years for the independent cases)	Added further evidence that rs10490924 was the source of the association signal at <i>PLEKHA1/ARMS2/HtrA1</i> . Demonstrated statistical epistasis between smoking history and the rs10490924 SNP. Smokers homozygous for the <i>ARMS2</i> and <i>CFH</i> risk alleles had an OR of 34.51	[29]
Maller <i>et al.</i> (2006)	1238 unrelated individuals of European descent having either GA or choroidal neovascularization	934 unrelated individuals of European descent; age and gender matched; clinical age-related maculopathy staging system stage 1	Added further confirmation that the source of the association signal was at rs10490924 but noted the presence of strong linkage disequilibrium with <i>HtrA1</i>	[25]
Conley <i>et al.</i> (2006)	126 cases taken from the CHS, 701 cases taken from the AREDS	1051 controls taken from the CHS, 175 controls taken from the AREDS	Added further support to rs10490924 as the association signal. Did not find statistical epistasis between smoking and AMD. Performed a meta-analysis of all SNP association studies focusing on the <i>PLEKHA1/ARMS2/HtrA1</i> region, yielding an OR_{het} of 2.48 and an OR_{hom} of 7.33	[14]
Fisher <i>et al.</i> (2006)	155 unrelated patients of Russian descent. 92 cases presented with NV or GA AMD while 63 had early signs of AMD, including confluent drusen/areas with hyperplasia	151 age-matched controls of Russian descent	Found an association between <i>ARMS2</i> and AMD that was most pronounced for advanced AMD patients. $OR_{het} = 2.55$; $OR_{hom} = 3.47$	[21]
DeWan <i>et al.</i> (2006)	96 NV AMD patients from a cohort of Southeast Asians in Hong Kong (China)	130 age-matched control subjects from the Hong Kong cohort who were AMD free	Found a SNP in complete linkage disequilibrium ($D' > 0.99$) with rs10490924 in the gene <i>HtrA1</i> . The SNP (rs11200638) may be the true source of the association in the region. <i>In vivo</i> evidence suggested that this SNP leads to increased expression of <i>HtrA1</i>	[32]
Yang <i>et al.</i> (2006)	448 AMD patients taken from a Caucasian cohort in Utah (USA)	309 controls who were taken from the Caucasian cohort in Utah	Found a highly significant association for rs10490924, ($p = 8.1 \times 10^{-8}$; $OR_{het} = 1.35$; $OR_{hom} = 6.09$); but found that rs11200638 was more significantly associated with AMD ($p = 1 \times 10^{-9}$; $OR_{het} = 1.86$; 1.35, 2.56, $OR_{hom} = 6.56$). Demonstrated an additive effect between this SNP and the Y402H SNP in <i>CFH</i>	[31]

AMD: Age-related macular degeneration; AREDS: Age-Related Eye Disease Study; CFH: Complement factor H; CHS: Cardiovascular Health Study; GA: Geographic atrophy; OR: Odds ratio; RPE: Retinal pigment epithelium; SNP: Single nucleotide polymorphism.