Eye (2016) 30, 1–14
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www.nature.com/eye

REVIEW

# Complement pathway biomarkers and age-related macular degeneration

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## Abstract

**In the age-related macular degeneration (AMD) 'inflammation model', local inflammation plus complement activation contributes to the pathogenesis and progression of the disease. Multiple genetic associations have now been established correlating the risk of development or progression of AMD. Stratifying patients by their AMD genetic profile may facilitate future AMD therapeutic trials resulting in meaningful clinical trial end points with smaller sample sizes and study duration.**

*Eye* (2016) **30,** 1–14; doi:10.1038/eye.2015.203; published online 23 October 2015

## Introduction

Based on the pioneering work of Dr Judah Folkman, novel research into 'angiogenesis' generated the commercial development of drugs to inhibit the growth of new blood vessels. This approach was developed as a strategy to 'treat' cancer as well as eye diseases that lead to progressive, irreversible visual loss. Pegaptanib was designed and approved by the FDA in 2004 as a valid treatment for age-related macular degeneration (AMD),[1] followed by the publication of the landmark phase 3 ranibizumab data in 2006,[2] and the published results of the short-term safety and efficacy of intravitreal bevacizumab, the 'parent' compound of ranibizumab, in patients with neovascular AMD (nAMD), by Rich *et al*.[3]

Since then, anti-vascular endothelial growth factor (VEGF) therapy has monopolized the treatment of nAMD.

However, AMD is a multifactorial, complex disease. Thus it seems unlikely that a single therapy that targets only the final result of a highly complicated pathogenetic process, will remain as the only viable treatment option in the future. Moreover, geographic atrophy (GA), the advanced non-neovascular form of AMD that accounts for 35% of all cases of late AMD and 20% of legal blindness attributable to AMD,[4,5] cannot be treated or prevented at the moment and indeed may be increased by anti-VEGF therapy.[6,7]

In this review, we present and comment on the response to both complement and non-complement-based treatments, in relation to complement pathway mechanisms and complement gene regulation of these mechanisms. We discuss current and potential treatments for both wet and dry AMD in relation to complement pathway pathogenetic mechanisms.

## The complement system

The innate immune system is composed of immunological effectors that provide robust, immediate, and nonspecific immune responses. These include evolutionarily primitive humoral, cellular, and mechanical processes that have a vital role in the protection of the host from pathogenic challenge. The complement system is a vital component of innate immunity and represents one of the major effector mechanisms of the innate immune system. It was so named for its ability to 'complement' the antibacterial properties of antibody in the heat-stable fraction of serum.[8]

The complement is a complex network of plasma and membrane-associated serum proteins, which are organized into a hierarchy of proteolytic cascades that start with the identification of pathogenic surfaces and lead to the generation of potent proinflammatory mediators (anaphylatoxins), opsonization ('coating') of the pathogenic surface through various complement opsonins (eg, C3b), and targeted lysis of the pathogenic surface through the assembly of membrane-penetrating pores known as the membrane attack complex (MAC).[9,10]

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Received: 9 April 2015
Accepted in revised form: 3 September 2015
Published online: 23 October 2015

The alternative pathway is in a continuous low-level state of activation characterized by the spontaneous hydrolysis of C3 into C3a and C3b fragments. C3b binds complement factor B (CFB) and, once bound, factor B is cleaved by complement factor D (CFD) into Ba and Bb, thereby forming the active C3 convertase (C3bBb). The convertase cleaves additional C3 molecules, generating more C3a and C3b, thereby promoting further amplification of the cascade.[11]

Activation of all complement pathways results in a proinflammatory response including generation of MACs, which mediate cell lysis, release of chemokines to attract inflammatory cells to the site of damage, and enhancement of capillary permeability.[12,13] Complement regulation occurs predominantly at two steps within the cascades, at the level of the convertases, both in their assembly and in their enzymatic activity, and during assembly of the MAC.[14]

There is an 'interplay' between adaptive and innate immunity. The adaptive immune system is organized around two classes of specialized lymphocytes, T and B cells, which display an extremely diverse repertoire of antigen-specific recognition receptors that enable specific identification and elimination of pathogens, as well as adaptive immune measures that ensure tailored immune responses, as well as long-lived immunological memory against re-infection.[8] The ability of complement not only to affect robust innate immune responses but also to interface with, and influence T- and B-cell biology and adaptive responses, has become increasingly appreciated. However, the exact mechanism(s) by which complement mediates T-cell immunity has yet to be determined.[15]

## AMD heritability

To accelerate progress in the discovery of macular degeneration genetics, 18 research groups from across the world formed the AMD Gene Consortium in the early 2010 and organized a meta-analysis of genome-wide association studies (GWAS). The meta-analysis evaluated the evidence for association at 2.442.884 single-nucleotide polymorphisms (SNPs) coming from $>17\,100$ cases with advanced disease (GA, neovascularization, or both) and $>60\,000$ controls.[16] Joint analysis of discovery and follow-up studies[17] resulted in the identification/confirmation of 19 AMD susceptibility loci reaching $P<5\times10^{-8}$. This included all susceptibility loci previously reaching $P<5\times10^{-8}$ and an additional seven new loci reaching $P<5\times10^{-8}$ for the first time. Two genes showed differential expression between post-mortem retina of young (ages 17–35) and elderly (ages 75 and 77 years) individuals: *CFH* ($P=0.009$) and *VEGFA* ($P=0.003$), both with increased expression in older individuals. Increased *C3* expression was revealed in adult retinal pigment epithelium (RPE) compared with fetal RPE ($P=0.0008$). *CFH*, *VEGFA*, and *C3* are thus upregulated with aging, and their role in AMD may indicate an accelerated aging process. In addition to *C3* and *CFH*, all the complement genes with detectable expression in the retina or RPE experiments showed higher expression levels in older tissue. *CFH* risk alleles were preferentially associated with risk of GA. Variants near *CFH* exhibited a stronger evidence for association among Europeans ($P=0.0000001$).

## *CFH* gene variants associated with AMD

A number of complement system proteins, complement activators, and complement regulatory proteins were identified as molecular constituents of drusen, the hallmark extracellular deposits associated with early AMD.[18,19] In this 'inflammation' model, local inflammation accompanied by complement activation, bystander cell lysis, and immune responsiveness are important facets of AMD pathogenesis and progression.[20–22]

Macrophages and the related giant cells have been demonstrated in histologic specimens from patients with AMD, especially in regions of RPE atrophy, breakdown of Bruch's membrane, and choroidal neovascularisation.[23–26] Moreover, as with uveitis, chemokines that mediate macrophage recruitment to the retina have also been associated with AMD.

Patel and Chan's study, as well as prior studies showing increased oxidized lipoprotein levels with age in normal eyes and AMD eyes, suggests that the macrophages seen in AMD and in choroidal neovascularization (CNV) lesions might serve to ingest oxidized low-density lipoprotein that accumulates with age.[15,27,28] The study did not clarify whether these macrophages serve maladaptive functions leading to CNV development or adaptive responses to the accumulation of oxidized protein and/or other pathological processes.[15] In other words, before AMD develops, macrophages function as housekeepers in removing waste products from the retina and RPE. After AMD develops, especially wet AMD, macrophages function as inflammatory stimulators, thereby exacerbating the lesion.

Any genetic polymorphism or environmental stressor, which activates complement or impairs complement regulation leads to overactivity of the complement system. This results in inflammatory damage to the retina.[29–36] Complement activation also leads to VEGF upregulation, promoting CNV.[37,38] Influx of tissue-destructive M1 macrophages also potentiates ocular inflammation, leading to further tissue damage. At this point, M2 macrophages involved in tissue remodeling

might also serve pro-angiogenic functions.[15] M1 macrophages have considerable proinflammatory activities that promotes tissue damage; the release of proinflammatory cytokines and production of reactive oxygen and nitrogen species. M2 macrophages are less inflammatory and they promote scavenging, immunoregulation, and tissue remodeling.[39]

Therefore, new therapeutic approaches can be envisioned, designed to restore the complement-modulating activity that is deficient in genetically susceptible individuals.

### *The CFH Y402H risk variant*

A SNP (T1277C) in one of the short consensus repeat domains (SCR7) of the *CFH* gene results in an amino acid substitution of histidine for tyrosine at position 402 in a CFH domain that contains binding sites for C-reactive protein (CRP), heparin, and streptococcal M6 protein.[40]

Clark *et al* demonstrated that the AMD-associated 402H variant binds less well to heparan sulfate and dermatan sulfate glycosaminoglycans within Bruch's membrane when compared with the 402Y form, although there was a similar level of binding of the two variants within the RPE. They proposed that the impaired binding of the 402H variant to Bruch's membrane results in an overactivation of the complement pathway leading to local chronic inflammation and thus contributes directly to the development and/or progression of AMD.[41]

Despite differences in binding properties, there are no detectable differences in either CFH or CRP immunolabeling in drusen between *CFH402HH* and *CFH402YY* homozygotes. However, *CFH402HH* homozygotes do show elevated levels of CRP in the choroid that is apparently systemic in origin, as there is no evidence for CRP transcription in either the RPE, neural retina, or choroid *in vivo.*[42]

This is supported by a case-control study of Seddon *et al*' of 1026 participants at two centers in the Age-Related Eye Disease Study (AREDS), which showed that CRP levels were significantly higher among patients with advanced AMD than among those without AMD and suggested that elevated CRP level is an independent risk factor for AMD and may implicate the role of inflammation in its pathogenesis.[43]

These results suggest that CRP is most likely extravasated from the choroidal capillaries, in response to local signals generated by the RPE–choroid. The recruitment of circulating CRP to the choroid is most likely an indication of cellular injury that may be exacerbated by the reduced binding of CRP to *CFH402HH*, diminished complement regulatory activity, and heightened complement activation in predisposed 'risk' individuals. Presence of the *CFH Y402H* polymorphism increases the risk of AMD significantly, with an odds ratio (OR) of 2.5 and 6.3 for the heterozygous CT (YH) and homozygous CC (HH) genotypes, respectively.[44]

### Other complement regulatory components linked to AMD

Vitronectin and clusterin are two negative regulators of the complement cascade that are present in drusen, including drusen in patients with AMD.[19,22] RPE cells near drusen show increased levels of cytoplasmic vitronectin, perhaps a compensatory response to complement attack.[22] Another complement inhibitor, cluster of differentiation 46 (CD46; also known as membrane cofactor protein), is similarly present in drusen and expressed at high levels in RPE cells adjacent to drusen.[22,33]

Gold *et al* screened *CFB* and complement component 2 (*C2*) genes in two independent cohorts of patients with or without AMD and found that two haplotypes—*L9H BF/E318D C2* and *R32Q BF*/a variant in intron 10 of *C2*—were protective for AMD.[45,46] Combined analyses of the *CFH* and *CFB/C2* variants indicated that they can account for nearly 75% of all AMD cases in the European and North American populations.[45]

Two non-synonymous polymorphisms in the *C3* gene were also reported to be associated with AMD.[47–50] *C3* is an acute phase reactant and is among the most highly expressed complement-related genes in the choroid, relative to adult liver.

Amyloid-beta, an aggregate found in drusen, is also found in the brains of patients with Alzheimer's disease, where it has been demonstrated *in vitro* to activate complement.[51] Co-localization of amyloid-beta with *C3* activation products in human AMD eyes suggests that amyloid-beta in AMD lesions might similarly activate complement.[52]

Accumulation of lipofuscin and A2E, the bis-retinoid component of lipofuscin, are early pathologic features of AMD, and *in vitro* photooxidation of A2E in RPE cells leads to complement activation.[36]

Activation products C3a, C5a, and C5b-9 are systemically elevated in patients suffering from AMD.[53–56] Locally, C5a and C3a accumulate in drusen and are shown to promote CNV.[37] Liu *et al* showed that C5a protected human CD4+ T cells from undergoing apoptosis and C5a promoted IL-22 and IL-17 expression from CD4+ T cells of AMD patients and normal subjects as well.[57] Consistent with the previous observation of elevated C5a expression in the serum of AMD patients, they found significantly increased levels of IL-22 and IL-17 in the sera of AMD patients, suggesting possible roles of IL-22 and IL-17 in the inflammation that

contributes to AMD. Targeting the adaptive immune system, more specifically the Th17 family of cytokines, may have a beneficial effect on the course of AMD.

AMD-associated variations in a region in close proximity to another alternative pathway gene *(CFI)* on chromosome 4 have also been reported. The rs10033900 variant of the *CFI* gene has been shown to be independently associated with AMD.[58,59] CFI is a serine protease that has a role in the complement pathway as it cleaves and inactivates C4b and C3b.

Genetic evidence from GWAS as well as from common and rare variant analyses show the engagement of mainly the alternative complement pathway in AMD pathogenesis.[60] However, there is a suggestion that the classical pathway may be important as well: an inverse relationship between AMD and a non-coding variant in intron 6 of the classical pathway gene *SERPING1* (C1 inhibitor) was reported in two cohorts.[61] This protective effect was not replicated in another two independent studies[62,63] but *SERPING1* was again shown to have a modest effect on the risk of nAMD in a study by Lee *et al*.[64] A recent study and meta-analysis suggested that the *SERPING1* is not a major genetic component of AMD in East Asians but is a genetic risk factor for AMD in Caucasians, providing evidence for ethnic diversity in the genetic etiology of AMD.[65]

Table 1 displays the most common complement pathway biomarkers that have been investigated for an association with the pathogenesis of AMD. Evidence for and against the association are also displayed.

### Intraocular *vs* systemic complement activity

Most complement components and many circulating complement regulatory proteins are synthesized primarily by liver hepatocytes and then released into the bloodstream.[11] However, in some tissues with limited access to circulating plasma proteins such as the brain, an extrahepatic system of complement biosynthesis also exists.[84–86]

According to Anderson *et al*, for the alternative pathway-related genes, differential expression in adult liver relative to the other cells and tissues was not as striking as it was for the classical pathway-related genes.[11] Expression of the alternative pathway inhibitors was also widespread in the various tissues/cells

**Table 1** Significant complement pathway biomarkers and evidence of association with AMD

| *Genes/variants* | *Confirming association* | *Not confirming association* |
|---|---|---|
| *CFH* | Edwards *et al*[66]<br>Hageman *et al*[67]<br>Haines *et al*[31]<br>Klein *et al*[29] | |
| *rs1061170/Y402H* | Li *et al*[68]<br>Zareparsi *et al*[69]<br>Johnson *et al*[42]<br>Seddon *et al*[43]<br>Clark *et al*[70]<br>Lauer *et al*[71]<br>Wegscheider *et al*[72]<br>Grassi *et al*[73]<br>Scott *et al*[74]<br>Bergeron-Sawitzke[75]<br>Ho *et al*[76]<br>Weismann *et al*[77] | Gotoh *et al*[78]<br>(no association between the *Y402H* polymorphism and exudative AMD among 146 Japanese patients and 105 Japanese controls)<br>Tadechi-Blok *et al*[79]<br>(using a population-based study among Latinos, they found that the *Y402H* polymorphism was not a major risk factor for overall early AMD, but may have an important role in susceptibility to bilateral early AMD) |
| *CFB/C2:*<br>*L9H of CFB and E318D (rs9332739) of C2*<br>*R32Q* of *CFB and the intron 10 of C2(rs547154)* | Gold *et al*[45]<br>Jakobsdottir *et al*[46]<br>Maller[80] | |
| *C3: rs2230199* | Despriet *et al*[47]<br>Park *et al*[50]<br>Francis *et al*[81]<br>Maller *et al*[48]<br>Yates *et al*[50]<br>Heurich *et al*[82] | Wu *et al*[83] (not replicated in Asian populations) |
| *CFI: rs10033900* | Ennis *et al*[37]<br>Fagerness *et al*[57] | |
| *SERPING1* | Ennis *et al*[61]<br>Lee *et al*[64]<br>Liu *et al*[65] | Allikmets *et al*[62]<br>Park *et al*[63] |

examined, but somewhat less prevalent in adult liver. Analysis of neural retina, RPE, and choroid isolates indicated that choroidal cells are the predominant local source of most alternative pathway components and regulators. For those complement-related genes that showed relatively high transcription levels in the neural retina, RPE, and/or choroid, they localized some of the corresponding proteins to specific cells and extracellular compartments within these tissues. A number of tissues including lung, RPE–choroid, and vein showed significant expression of the shared complement component C3. C3 levels in RPE–choroid were ~5% of those measured in adult liver. These findings were consistent with previous reports, most notably in the kidney,[87] showing that at least 10% of the circulating pool of C3 cannot be attributed to hepatic synthesis. C3 expression in the neural retina was 16-fold lower than in the RPE–choroid.

Khandahdia *et al* showed that in liver transplant patients, AMD was associated with recipient rather than donor CFH Y402H genotype.[88] Results by Khandhadia *et al* suggest that local intraocular complement activity in the eye may be more important in AMD pathogenesis and that any therapeutic intervention will thus need to be given intraocularly. This hypothesis was supported by results of the study by Bomback *et al*. They reported that intravenous administration of eculizumab (a monoclonal antibody that binds complement C5) over 12 months had no effect on retinal drusen in two patients with C3 glomerulopathy.[89]

On the contrary, Scholl *et al* performed a comprehensive investigation of the alternative pathway of complement protein plasma concentrations in a cohort of AMD patients and controls and correlated their findings with polymorphisms in the *CFH*, *BF-C2*, and *C3* genes. They showed that several parameters that reflect systemic complement activation were significantly elevated in the circulation of AMD patients as compared with controls. Moreover, the discriminatory ability of complement proteins appeared superior or at least similar to the discriminatory ability of genetic markers of complement genes for the prediction of AMD.[90]

It is not yet clear whether AMD is a systemic disease with local disease manifestation at the aging macula or an intraocular disease where local complement activity is more important in its pathogenesis. This is worth investigating further.

Further comparisons between isolated preparations of RPE, choroid, and neural retina reveal that the predominant cellular source(s) for most classical and alternative pathway gene expression, including the shared C3 component, resides in the choroid rather than in the RPE or neural retina. In short, the results indicate that cells in the choroid have the capacity to synthesize an extensive array of classical and alternative activation pathway components and regulatory molecules, most of which are apparently not produced by cells in the RPE or neural retina.[11]

Such studies suggest that the choroid may be the target of choice for the development of new therapeutic agents to treat AMD, uveitis, and other posterior segment diseases with an inflammatory component.

## Drug response related to complement pathway mechanisms

### *Genetic variation and anti-VEGF treatment*

Approximately 1800 patients were treated for nAMD with anti-VEGF drugs in the CATT and the IVAN trials. Phenotypic data were collected in a rigorous manner in these study populations. This made these patients ideal for evaluating the effects of a number of genetic polymorphisms on treatment response to anti-VEGF therapy in nAMD.

In the CATT study, a pharmacogenetic relationship was tested between response to treatment and SNPs rs1061170 (*CFH Y402H*), rs10490924 (*ARMS2 A69S*), rs11200638 (*HTRA1* promoter), and rs2230199 (*C3 R80G*) as these four SNPs had consistently been shown to have the strongest associations with the development and progression of AMD and have been postulated to influence the response to therapy.[91] *CFH* and *C3* encode genes involved in the complement cascade. Clinical measures of the response to treatment were based on visual acuity, anatomical features of AMD assessed by OCT and FA, and the total number of injections given in 1 year. For each of the three visual measures and for each of the five anatomic outcomes evaluated there was no association between genotype and either mean change in VA or mean change in total foveal thickness from baseline. Among the participants in the two PRN groups, no statistically significant difference was found in the number of injections among the different genotypes for any of the four SNPs, or for the total number of risk alleles. There were two instances where borderline significance was present. First, better visual acuity was seen in patients who were homozygous for the risk allele at C3 ($P=0.03$), which is the opposite of what would be expected. Second, the lowest mean change in total foveal thickness (less clinical response) was seen in patients who were homozygous for the *CFH* risk allele ($P=0.03$). However, patients who were heterozygous for the *CFH* risk allele had the highest mean change in total foveal thickness (best clinical response), which would again not be expected if the presence of the risk allele truly influences clinical response. Overall, according to the CATT trial, there is little biological evidence to support that the

complement pathway, or at least these SNPs in the complement pathway, strongly influence the response to therapy.

In the IVAN trial,[92] Lotery *et al* tested for replication of the three previously reported pharmacogenetic associations of response to VEGF inhibition in nAMD at the CFH, FZD4, and HTRA1/ARMS2 loci[93,94] as well as an additional 482 SNPs for pharmacogenetic associations using a candidate gene approach. They defined responder status based on the OCT metric of total retinal thickness (TRT) and they computed the change in TRT from baseline to the latest time point for which OCT data were available (3, 6, 9, or 12 months). None of the three SNPs previously associated with the *CFH*, *FZD4*, and *HTRA1/ARMS2* genes were found to be significantly associated with responder status in this cohort. None of the associations between SNP and responder status for the remaining 482 SNPs was statistically significant.

### *CFH polymorphisms and anti-VEGF treatment*

Chen *et al* performed a literature-based meta-analysis including 10 published association studies involving 1510 patients to investigate the association between polymorphism rs1061170 (T1277C, Y402H) of the *CFH* gene and treatment response of nAMD.[95] They identified 10 eligible studies for their systematic review. Their results showed that polymorphism rs1061170 was a predictor of treatment response of nAMD, especially for anti-VEGF agents. Individuals homozygous for the variant risk C-allele showed a decreased response to treatment by ~1.6-fold when compared with patients homozygous for the ancestral T allele. When they divided the patients according to ethnicity (Caucasians or not), they found that CC genotype was associated with a reduced response to treatment of nAMD with an OR of 1.73 (95% CI, 1.05–2.86) in Caucasians. As only one study performed in Asians was reported and included in the meta-analysis, they could not conduct a sub-meta-analysis in Asians. Further validation in larger studies is needed of course, but this was, to the best of our knowledge, the first meta-analysis confirming a genetic marker predictive for AMD treatment response.

Lee *et al* collected mouthwash samples for genotyping from 178 patients with a diagnosis of exudative AMD who were undergoing treatment for an active neovascular lesion with intravitreal ranibizumab.[96] Genomic DNA was prepared from buccal cells and genotyping for a SNP in the *CFH* gene (Y402H, rs1061170 T/C) was performed. The association between genotype and post-treatment visual acuities were assessed using two generalized linear modeling techniques at 6 and 9 months separately. For the CFH Y402H polymorphism, 37 patients (24%) were TT, 71 (45%) were TC, and 48 (31%) were CC. The prevalence of the C risk allele was 54%. The CFH genotypes were not found to significantly affect the post-treatment VA at 6 months ($P=0.38$) or 9 months ($P=0.70$). However, when the mean number of injections over a 9-month period was examined, they did observe a trend in the number of injections required over a 9-month period among the three genotypes, with the TT group receiving the fewest injections ($P=0.09$), and that patients homozygous for the variant genotype for CFH (CC) were more likely to require reinjection. They found a stepwise increase in risk of additional injections, as patients with the TC genotype had a 25% increased risk ($P=0.12$), and patients with the CC genotype had a 37% statistically significant higher risk ($P=0.04$), suggesting a possible genotype dose-dependent pharamacogenetic effect.

McKibbin *et al* identified preliminary evidence of associations between visual acuity outcome after 6 months of intravitreal ranibizumab and polymorphisms in the *CFH*, *VEGF*, and *HTRA1* genes. In their study, they did not find an association between CFH genotype and the number of injections in the first 6 months, but they did find a trend toward a better visual acuity response with the higher risk TC or CC genotypes. A gain of $>5$ ETDRS letters was seen in 34.6, 56.6, and 56% of eyes with the TT, TC, and CC genotypes ($P=0.04$, 0.4, and 0.06 for TC, CC, or combined TC or CC genotypes compared with TT).[97]

Findings by Smailhodzic *et al* suggest an additive effect of high-risk alleles in *CFH*, *ARMS2*, and *VEGFA*, leading to a younger age of nAMD onset in combination with poor response to intravitreal ranibizumab treatment. After ranibizumab treatment, patients with 6 high-risk alleles demonstrated a mean VA loss of 10 ETDRS letters ($P<0.0001$).[98]

Brantley *et al* collected mouthwash samples for genotyping from 86 Caucasian patients diagnosed with exudative AMD who were undergoing treatment with intravitreal bevacizumab.[99] Genomic DNA was prepared from buccal cells and genotyping for *CFH Y402H* and *LOC387715 A69S* was performed. They identified a significant association between the *CFH Y402H* genotype and response to the treatment with bevacizumab: post-bevacizumab VA was significantly worse in patients with the *CFH CC* genotype compared with VA for the *CFH TT* and *CFH TC* genotype ($P=0.016$). Conversely, presence of the *LOC387715 TT* genotype had no significant effect on the response to treatment with bevacizumab.

Nischler *et al* included 197 patients with exudative AMD in a prospective study in which patients were treated with 1.25 mg intravitreal bevacizumab at 6-week intervals until CNV was no longer active.[100] SNP genotyping was performed using restriction fragment length polymorphism analysis of PCR products. Their results showed that the *CFH402HH* genotype correlates with lower visual acuity outcome after treatment with

bevacizumab. They observed a significant worse outcome for distance and reading visual acuity in the *CFH402HH* genotype group ($P=0.042$; statistical power = 88% and $P=0.0039$; statistical power = 78%)

Despite the number of studies that did detect a statistically significant association between certain genetic variants and response to anti-VEGF treatment, the CATT trial in the US and the IVAN trial in the UK were the ones with the most rigorous follow-up and phenotyping and their outcomes did not identify any clear pharmacogenetic associations. If a pharmacogenetic association does exist, it may require much larger sample sizes to detect.

### *CFH polymorphisms and nutritional supplements*

Klein *et al* were the first ones that showed a potential pharmacogenetic interaction between variations in the Complement Factor H gene (*CFH*) and zinc therapy on the progression of AMD.[101] A significant interaction was found between the high-risk genotype (CC) for *CFH* Y402H and supplementation with a combination of zinc and antioxidants *vs* placebo ($P=0.03$), but not with the zinc only or the antioxidants only groups. When the genotypes of *CFH* Y402H were examined for zinc (zinc only combined with the zinc and antioxidants group) *vs* no zinc (antioxidants only combined with the placebo group), a significant interaction was found with the homozygous high-risk genotype (CC) compared with the homozygous low-risk (TT) variant ($P=0.004$). Specifically, when receiving zinc therapy, a 5% reduction in AMD progression was seen in the CC group, compared with a 22% reduction in AMD progression in the TT group.

The AREDS showed that antioxidants and zinc reduce progression rates of moderate to advanced AMD.[102] Awh *et al*, in their pharmacogenetic analysis of the AREDS patients,[103] performed genotyping of all AREDS white patients with category 3 disease in at least one eye (intermediate AMD, at least one drusen $>125\,\mu m$, extensive intermediate drusen or GA not involving the center of the macula). Among the genetic risk predictors for AMD that they selected, there was a set of five common *CFH* polymorphisms: rs1048663, rs3766405, rs412852, rs11582939, and rs1066420, as well as the *372_815de1443ins54 ARMS2* marker. Their data supported a deleterious interaction between *CFH* risk alleles and high-dose zinc supplementation, suggesting that individuals with one or two *CFH* risk alleles and $<2$ *ARMS2* alleles would benefit maximally from supplementation with antioxidants only, because they also found that the beneficial effect of antioxidants completely disappears in the presence of two *ARMS2* alleles. This allowed the identification of subgroups, who would benefit more than the average AMD patient from nutritional treatment. These findings were consistent with current understanding of how CFH interacts with zinc. Zinc binds CFH, inducing multimeric large forms that lose complement component 3b inhibitory activity as a function of zinc concentration.[104] Decreased targeting of CFH protein to sites of active complement activation as a functional consequence of *CFH* risk genotypes, may thus be exacerbated through zinc supplementation.[105,106]

However recently, Chew *et al* used statistical models, adjusted for age, gender, smoking status, and baseline AMD severity, to examine the influence of genotypes on the response to therapy with four randomly assigned arms of AREDS supplement components: placebo, antioxidants (vitamin C, vitamin E, β-carotene), zinc, or a combination. They analyzed the influence of *CFH RS1061170* and *rs1410996* combined with *ARMS2 rs10490924* and the combination of *CFH rs412852* and *rs3766405* with *ARMS2 c.372_815del443ins54* on progression to late AMD and they found no statistically significant difference for the benefits of AREDS supplements among different genotype groups.[107]

As demonstrated above, associations between anti-VEGF therapy or nutritional supplements and genotypes vary markedly between different studies. This may be because study designs are flawed, phenotypes are imprecise or the associations are actually false. Therefore replication of such results in independent cohorts is essential in order to confirm the validity of such correlations.

## Dry AMD potential treatments

Dry AMD emerging treatments, in addition to stem cell–based treatment, lasers (micropulse), implantable miniature telescopes and low vision rehabilitation, have basically focused on two strategies: prevention of photoreceptor and RPE cell loss, and suppression of inflammation. The former may be achieved by neuroprotection induction, oxidative damage prevention, and visual cycle modification.[108]

Potential therapies that decrease inflammation and oxidative stress include the AREDS vitamins, complement inhibitors, steroids (fluocinolone acetate), OT-551, prostaglandins, and hydroxychloroquine.[109]

A randomized, placebo controlled, clinical trial of high dose supplementation of vitamins C, E, beta-carotene, zinc, and copper was the first effective treatment to slow the progression of the disease for patients at high risk for developing advanced AMD as oxidative stress and depletion of essential micronutrients are important factors for AMD progression.[101] In 2006, the same research group began a second study called AREDS2 to determine whether they could improve the AREDS formulation.

In this study, which was completed in 2013, they tried adding omega-3 fatty acids, as well as the antioxidants lutein and zeaxanthin, which are in the same family of

nutrients as beta-carotene. The researchers also tried substituting lutein and zeaxanthin for beta-carotene, which prior studies had associated with an increased risk of lung cancer in smokers. The study found that while omega-3 fatty acids had no effect on the formulation, lutein, and zeaxanthin together appeared to be a safe and effective alternative to beta-carotene.[110]

Neuroprotective drugs aim to preserve macular function by preventing apoptosis of viable RPE cells and photoreceptors. The main drugs under investigation have been: Ciliary neurotrophic factor (NT-501, Neurotech Pharmaceuticals, Cumberland, RI, USA),[111] brimonidine tartrate (Allergan, Inc., Irvine, CA, USA),[112] tandospirone (Alcon Laboratories, Inc., Fort Worth, TX, USA),[113] anti-amyloid β antibodies (Glatiramer acetate, Copaxone; Teva Pharmaceutical, Petah Tikva, Israel and RN6G, Pfizer Inc., New York, NY, USA)[114,115] and doxycycline.[116]

Visual cycle modifiers include ACU-4429,[117] fenretidine, and anti-amyloid b antibodies. [118,119]

Therapies that increase oxygenation and affect blood flow include rheopheresis,[120–122] ozonated autohemotherapy,[123] and vasodilators.[124,125]

Therapies that are currently in clinical trials include brimonidine, doxycycline, anti-amyloid antibodies (GSK933776 and RN6G), RPE65 inhibitor (ACU-4429, Acucela Inc., Seattle, WA, USA), complement inhibitors (ARC1905, FCFD4514S), hydroxychloroquine, intravitreal fluocinolone acetate, and vasodilators like sildenafil, moxaverine, and MC-1101.[106] Therapies that have not been shown to be effective include POT-4, eculizumab, tandospirone, anecortave acetate, the antioxidant OT-551, sirolimus, and vitamin E.[109]

### *Complement-based treatments*

Complement-based treatments are optimized to specific components of the pathway and differ in their mechanism of action, facilitating inhibition, replacement, or modulation of their target. They must also regulate complement activity without adversely affecting defence and immunomodulatory function of the cell. In contrast to the anti-VEGF agents that form the mainstay of conventional treatment, as complement-based therapeutics have the potential to intervene earlier in the disease process, perhaps even before an AMD phenotype is distinguishable.[126]

POT-4 (Potentia Pharmaceuticals, Crestwood, KY, USA) is a synthetic peptide that reversibly binds complement factor C3 and inhibits activation of the complement cascade. Inhibition of C3 shuts down all downstream complement activation that could otherwise lead to local inflammation, tissue damage, and upregulation of angiogenic factors.

As with VEGF inhibitors, POT-4 may be administered intravitreally, limiting possible unwanted systemic effects. At higher dosages, an intravitreal gel forms creating a drug depot, with the potential to sustain therapeutic levels of the compound within the eye for several months following a single injection. (ClinicalTrials.gov number, NCT00473928).

ARC1905 is an aptamer-based C5 inhibitor, blocking the cleavage of C5 into C5a and C5b fragments. Nucleic acid aptamers are synthetically derived and demonstrate desirable therapeutic properties largely owing to their three-dimensional structure; namely high target specificity and binding affinity. It has been tested for both dry and nAMD (being tested in combination with ranibizumab for the latter; ClinicalTrials.gov number, NCT 00950638).

Eculizumab (Soliris), like ARC1905, is another antibody-based complement inhibitor. Eculizumab binds complement component C5, preventing cleavage, downstream activation, and the formation of MAC. The drug is administered intravenously over 6 months; weekly dosing during the initial induction period followed by two weekly maintenance doses. (ClinicalTrials.gov number, NCT00935883).

C5a, a product of proteolytic cleavage of C5 in the complement pathway, has been observed to have potent proinflammatory properties in its own right. Compared with complement component inhibitors that prevent C5a formation, receptor antagonists competitively bind to the C5a receptor neutralizing interaction. JSM-7717 and JPE-137539 are two peptidomimetic C5a receptor antagonists currently in preclinical assessment for AMD.[127]

Lampalizumab or anti-factor D is an antigen-binding fragment (Fab) of a humanized, monoclonal antibody directed against CFD. CFD is a rate-limiting enzyme involved in the activation of the alternative complement pathway. In the phase 2 MAHALO study, a reduction in disease progression was observed in patients with GA treated with lampalizumab.[128] The MAHALO results also suggest that a *CFI* biomarker is prognostic for GA progression and predictive of lampalizumab treatment response; 57% of the samples selected in this study were positive for the *CFI* biomarker. In patients with AMD with GA, the complement inhibitor lampalizumab reduced disease progression by 20% and by 44% in the *CFI* biomarker-defined subset. This biomarker was identified by the International AMD Gene Consortium. Arguable it is the most effective example to date of a useful biomarker in an AMD treatment trial.[129]

### Comments/conclusions

There is strong evidence that genetic variations in genes that regulate the alternative complement pathway are

associated with an increased risk of developing AMD. However, the role of complement in the manifestation of the more advanced forms of the disease, particularly GA and CNV, is still unclear.

Clinical presentation of neovascular membranes in advanced wet AMD represents only the final stage of a complex cascade of events and therefore monotherapies, which are increasingly utilized to manage neovascular membranes, offer limited treatment options to patients.[130] Recent clinical trials support this view. Combination therapy with anti-VEGF and anti-PDGF antibodies demonstrated improved visual acuity outcomes *cf.* monotherapy.[131]

Differences in individual patients' responses to anti-VEGF treatment and possible correlations between this and SNPs of several genes, as well as the impact of pro-angiogenic mechanisms implicated in the pathogenesis of the disease, suggest that the complex cascade of events, which lead to the development of advanced AMD, can be modulated at different steps of the pathogenetic process. Perhaps, clinical drug resistance could be reasonably determined by upregulation of pro-angiogenic factors other than VEGFA.

As the authors of the results of the IVAN trial noted, there is no consensus of what constitutes response to treatment *vs* non-response. Correlations between vision and retinal morphologic features are modest at best, even when using OCT parameters as the metric of choice.[92] Conversely, visual acuity may improve despite absence of morphologic change. Thus, using it as a marker of treatment responsiveness can lead to erroneous classifications. VA improvement is maximal after three injections and pharmacogenetic studies that were statistically significant used this as a primary outcome in contrast to the CATT and IVAN trials.

Even if new therapeutics or diagnostics could be easily and efficiently constructed, based on the knowledge of disease-associated genetic variations, these therapeutics and diagnostics would still need to be tested for their safety and efficacy, and these often come at great expense and over long periods of time, thereby making it unclear what immediate clinical benefits genetic association study results might have. Simon and Maitournam have already proposed the use of genetic variations to restrict entry into clinical trials.[132] Their motivation was to assess potential efficiency gains in clinical trials, limited to participants possessing a genetic profile that is known to respond better to the drug in question. Schork and Topol also showed, that the increase in the incidence of outcomes in trials restricted to individuals with specific genotypic profiles can result in substantial reductions in requisite sample sizes for such trials.[133]

If the results of the MAHALO study were confirmed in the phase III trials, they would highlight the potential of genotype-restricted sampling for trials in AMD. This would set an example of how such trials could be designed. This approach may lead to quicker development of novel treatments. It may also reduce the risk to patients by stratifying therapy more appropriately to those most likely to respond effectively. Such approaches are already being increasing adopted in other therapeutic areas such as oncology.[134]

In summary, the tremendous advancement of knowledge into the genetic basis of AMD is now starting to generate novel biomarkers for patients affected with AMD. These biomarkers are likely to become increasingly important in the management of patients in the future.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

We searched the NCBI databases for 'complement factor pathway and age related macular degeneration' and we found results in 17 of them for the term above. We focused on results found in PubMed (142 medical abstracts/citations) and OMIM (On line Mendelian Inheritance in Men, 19 results). Headlines used to locate related articles and restrict our search were 'the role of complement pathway in the pathogenesis of age related macular degeneration', 'genetic variation and age related macular degeneration', '*CFH* gene in age related macular degeneration', 'inflammation and neurodegenerative disease', 'pharmagenomics in AMD'. A manual search was also based on references from articles on the subjects above as well as review articles.

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