

Neovascular age-related macular degeneration: disease pathogenesis and current state of molecular biomarkers predicting treatment response—a scoping review

Nikolaos Dervenis ,^{1,2} Panagiotis Dervenis,³ Eleftherios Agorogiannis ⁴

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ABSTRACT

Age-related macular degeneration is a major cause of blindness, and the development of anti-vascular endothelial growth factor (VEGF) intravitreal treatments has revolutionised the management of the disease. At the same time, new challenges and unmet needs arose due to the limitations of the current therapeutic options. Neovascularisation development during the course of the disease has a complex pathogenetic mechanism, and several biomarkers and their association with treatment outcomes have been investigated. We reviewed the relevant literature about neovascularisation development and biomarkers related to response to treatment. Improving our knowledge on the field can improve patient outcomes and offer personalised care.

INTRODUCTION

Age-related macular degeneration (AMD) is a major cause of blindness worldwide.¹ Neovascular AMD, despite accounting for a small percentage of total AMD cases,² is responsible for a large percentage of vision impairment and blindness and may present with a dramatic onset and progression, if left untreated, which heavily impacts patients' vision and related quality of life.³ Treatment options have been limited for a long period of time, and prognosis has been poor. An unknown factor, named factor X, has long been proposed as the key factor for the development of macular neovascularisation in neovascular AMD.

The identification of vascular endothelial growth factor (VEGF) and its relationship with the development of macular neovascularisation have been a major breakthrough in the management of the disease.⁴ Nine different isoforms of VEGF have been identified in humans. Although all VEGF isoforms can induce endothelial cell proliferation, the most abundant isoform found in the eye is VEGF165.⁵ Factor X could now be named and

targeted for treatment with the development of specific molecules that could be administered as intravitreal injections. A previously untreatable condition could now be treated, and patients' vision could be stabilised or even improved.

However, as old challenges have been overcome, new challenges are arising. Repeated injections have become a huge burden both for health systems and patients.⁶ Real-world outcomes often fall short of randomised trial results.⁷ Poor response to existing treatment options can be seen in a percentage of patients. Newer treatment modalities have been developed to overcome these limitations, and new drugs have been targeted against molecules in addition to VEGF, such as the placental growth factor (PlGF) or angiopoietin-2 (Ang-2).⁸ Carefully reviewing the literature about the exact pathogenetic mechanisms and molecules involved in the development of macular neovascularisation can provide insight into additional molecules/pathways that could represent novel therapeutic targets for the disease.

Besides understanding disease pathogenesis, ongoing research has improved our knowledge about related biomarkers.⁹ A biomarker is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'.¹⁰ With regard to neovascular AMD, as available treatment options increase, more accurate prognosis and treatment guidance can be facilitated by ongoing research on AMD biomarkers. Reviewing existing literature about the degree of response to AMD treatments can provide insight into the condition and improve our treatment protocols.



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¹Aristotle University of Thessaloniki, Thessaloniki, Greece

²Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK

³Colchester Hospital University NHS Foundation Trust, Colchester, UK

⁴Uveitis and Medical Retina, Manchester Royal Eye Hospital, Manchester, UK

Correspondence to

Dr Nikolaos Dervenis; nikosdervenis@gmail.com

The aim of this review is to summarise the level of current knowledge about the pathogenesis of macular neovascularisation in AMD and the molecular biomarkers that have been assessed as anti-VEGF treatment outcome predictors and discuss the future challenges and research questions to be answered in the field as available treatment options are increasing.

METHODS

A prespecified review protocol was written before literature search and evidence acquisition.

Eligibility criteria

The studies included in our analysis met the following inclusion criteria:

- ▶ Publication before 31 August 2023
- ▶ Involve subjects who suffered from neovascular AMD and analyse potential molecular biomarkers as outcome predictors

The study exclusion criteria included the following:

- ▶ Reports not published in English
- ▶ Conference abstracts
- ▶ Retracted papers

Search method

A meticulous literature search was conducted across the MEDLINE, COCHRANE and ClinicalTrials.gov databases to identify all relevant studies to the study aim from inception until the present. The last literature search was conducted on 31 August 2023. Furthermore, for the retrieved studies, a manual search was performed in their references to find possible past reports. The search strategy included terms such as AMD, pathogenesis, biomarkers and anti-VEGF. Specifically, for MEDLINE, the following search strategies using the Boolean operators 'OR' and 'AND' were used:

1. (Age-related macular degeneration OR AMD) AND (anti-vascular endothelial growth factor or anti-VEGF)
2. (Age-related macular degeneration OR AMD) AND biomarkers
3. (Age-related macular degeneration OR AMD) AND pathogenesis

All titles and abstracts that were retrieved were reviewed for eligibility by ND and PD. In case of disagreement, the third author, EA, was asked about study eligibility. For titles and abstracts of potentially eligible studies, the full texts were screened. Eligible studies were listed in a spreadsheet.

RESULTS

Literature search yielded 1132 potentially eligible studies. After screening for duplicates, we ended up with 998 studies. We excluded 103 studies not written in English, thus ending up with 895 potentially relevant studies. After screening the abstracts for eligibility, we excluded 713 studies, and thus, we retrieved 182 full texts. We also excluded 102 studies that were not relevant to our review aim (ie, did not analyse any molecular biomarkers or any

predictors of neovascular AMD treatment outcome), and thus, we included 80 studies in our review.

Neovascular AMD pathogenesis

Pathogenesis of macular neovascularisation in AMD is the final angiogenic step of a multilevel pathway that includes the formation of drusen, the accumulation of lipofuscin and the development of localised inflammation.¹¹ All the pathogenetic mechanisms are related to the ageing of the retina, the retinal pigment epithelium (RPE) and Bruch's membrane.¹²

Angiogenesis is the development of new blood vessels from pre-existing vessels. It has a role to play not only in several physiological conditions but also in disease. Both malignant (cancer) and benign diseases (such as chronic inflammation) are associated with abnormal angiogenesis.^{13 14} Several molecules are involved by either stimulating or inhibiting angiogenesis. Angiogenesis activators identified include the following: VEGF, nitric oxide, integrins ($\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$), transforming growth factor beta 1 and its receptors, growth factors (acidic fibroblast growth factor, basic fibroblast growth factor (bFGF), hepatocyte growth factor, insulin-like growth factor I, platelet-derived growth factor (PDGF) and epidermal growth factor), hypoxia-inducible factor 1 alpha, interleukin (IL)-8, IL-1, prostaglandins (PGE 1, PGE 2 and PGF), erythropoietin, histamine, bradykinin and tumour necrosis factor alpha.¹⁵ VEGF is a member of the PDGF family. The VEGF gene family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF.^{16 17}

Neovascular AMD is associated with the development of macular neovascularisation which represents the growth of new abnormal vessels within the choroid or the retina. The following types of angiogenesis have been identified:

- ▶ Type 1 neovascularisation arises from choroidal neovascularisation (CNV) proliferating below the RPE and corresponds to the occult poorly defined pattern of leakage described on fluorescein angiography. In this type, it is typical for the fluid to be mainly subretinal. In some cases, however, intraretinal fluid accumulation may occur following the breakdown of the external limiting membrane, or VEGF expression may induce intraretinal leakage independently.¹⁸
- ▶ Type 2 neovascularisation refers to CNV proliferating above the RPE in the subretinal space and corresponds to the classic pattern of intense fluorescein leakage described on fluorescein angiography.
- ▶ Type 3 neovascularisation (or retinal angiomatous proliferation) occurs when the retinal circulation is involved, with an anastomosis between the choroidal and retinal circulations.¹⁹

A further subclassification of type 1 neovascularisation involves polypoidal choroidal vasculopathy, which consists of a large aneurysmal component and is observed more commonly in African and Asian people, with a reported frequency of 22%–62% among people with AMD in Asian populations (two to four times higher than in European populations).^{20 21}

Angiogenesis in the macula follows the disturbance between proangiogenic (eg, VEGF) and antiangiogenic (eg, pigment epithelium-derived factor, PEDF) factors. This may result either in an unbalanced increase in proangiogenic activity or a decrease in antiangiogenic (eg, PEDF) activity.¹¹ While the primary stimulus for retinal neovascularisation (eg, in proliferative diabetic retinopathy) is considered to be ischaemia or hypoxia, the primary stimulus for CNV (the main form of macular neovascularisation) is presumed to be local inflammation and immune reactivity.^{22–23} Loss of choroidal vasculature, presumably due to a reduction in blood supply secondary to stenosis of large vessels, may generate a proinflammatory milieu allowing accumulation of proinflammatory mediators during earlier stages of AMD progression.^{24–25} Drusen deposits developing in the macular area during the course of the disease may contain complement components (such as C3a and C5a) that may induce VEGF expression. Recruitment of immune cells to areas of macular damage and atrophy results in the secretion of proinflammatory and proangiogenic cytokines.^{26–28} Neutrophils, macrophages, mast cells and activated microglia have all been shown to produce and release an array of proangiogenic factors, including VEGF. At the same time, VEGF induces the expression of intercellular adhesion molecule-1 on vascular endothelial cells and regulates leucocyte adhesion to these.⁵ Several retinal cell types, as well as RPE cells, also can express and secrete VEGF.⁵ VEGF activates endothelial cells by binding VEGFR-1 and VEGFR-2 endothelial cell receptors, which in turn activate intracellular signal transduction cascades.⁵ VEGFR-2 is thought to be principally responsible for VEGF signalling in angiogenesis. Apart from endothelial cell proliferation, VEGF prevents endothelial cell apoptosis.⁵

Basic FGF2 might also be involved in macular neovascularisation as it is detectable in RPE cells in surgically excised CNV membranes.²⁹ It is also overexpressed in RPE cells, choroidal vascular endothelial cells and fibroblasts in laser-induced CNV. It has been postulated that bFGF2 has an angiogenic action only in the setting of cellular injury.³⁰

The cytolytic membrane attack complex, the final product of complement system activation, has been associated with the angiogenesis process.^{31–32} In laser-induced CNV, it has been shown to induce the release of several growth factors, such as bFGF, VEGF and PDGF, from various cells.³³ Moreover, experimental data have shown that pharmacological blockage of the complement system reduces the development of CNV.³³ Similarly, experimental findings suggest that complement factor 3 (C3) is associated with VEGF expression and induces both blood vessel leakage and endothelial cell proliferation.³⁴

RPE cells also produce PEDF, a neurotrophic growth factor for photoreceptors which has antiangiogenic activity.³⁵ Its reaction to oxygen is reciprocal to that of VEGF.³⁶ Experimental models have shown that PEDF inhibits ischaemia-induced retinopathy, VEGF-induced

leakage and laser-induced CNV formation.^{37–38} However, endogenous PEDF is not capable of preventing the development of CNV during AMD progression. Oxidative stress may be contributing to the altered balance between RPE-derived VEGF and PEDF.³⁹

Thrombospondin-1 (TSP1) represents another endogenous inhibitor of angiogenesis in ocular vascular homeostasis.³⁵ Experimental models suggest that TSP1 alterations may contribute to the pathogenesis of AMD and may, therefore, represent an additional therapeutic target.⁴⁰

Overall, angiogenic factors act on the endothelial cells lining blood vessels which are normally resistant to neovascular stimuli.³⁶ In particular, VEGF-A and PlGF have been shown to activate quiescent endothelial cells and promote cell proliferation, migration and vascular permeability.^{35–41} The newly growing blood vessels leak fluid, disrupting and damaging the layer of photoreceptors and impairing vision.

Tie-1 and Tie-2 are tyrosine kinase endothelial cell receptors which are later involved during the angiogenesis pathway in the retina.⁴¹ Angiopoietins interact with Tie-2 receptors of the endothelial cells and promote neovascularisation.³⁵ Ang-1 interacts with Tie-2 receptors leading to pericyte recruitment and formation of multicellular structures from simple endothelial tubes.⁴¹ This process also induces endothelial cells to associate with the extracellular matrix (ECM) and mesenchyme, promoting vascular integrity and maintenance of adult vasculature while stabilising vascular tight junctions at the same time. Therefore, it forms an increased density and calibre of non-leaking vessels and at the same time modulates VEGF-induced expansion of existing vessels. Ang-2 blocks Ang-1 function and, in this manner, may allow vascular remodelling and angiogenesis by proangiogenic signals (such as VEGF). Therefore, Ang-1 results in maturation and stabilisation of forming vessels, while Ang-2 may allow endothelial cells to respond to angiogenic signals.^{42–43} Cultured RPE cells express Ang-1 and Ang-2 messenger RNA (mRNA), and VEGF upregulates RPE Ang-1 mRNA translation and Ang-1 protein secretion.⁴⁴ Ang-1 probably modulates the effect of VEGF on endothelial cells during CNV formation, while Ang-2 acts as a competitive antagonist by inhibiting Tie-2 phosphorylation and therefore causing vascular destabilisation.⁴¹ Ang-2 blocking is thought to improve vascular stability and desensitise the vessels to the actions of VEGF. It has been proposed that an important mechanism of resistance to anti-VEGF therapy is the activation of alternative angiogenic pathways involving PDGF, FGF, Ang2 or other mediators.⁴⁵

ECM is an area of dynamic changes both in early and advanced AMD which is associated with the activity of each regulator, metalloproteinase and their tissue inhibitors.^{46–47} ECM components may participate in several ways in the regulation of angiogenesis as degradation of ECM releases and/or activates proangiogenic factors.⁴⁸ ECM molecules are capable of binding to integrins

Table 1 Stimulators and inhibitors involved in ocular neovascularisation

Stimulators	Inhibitors
Vascular endothelial growth factor	Transforming growth factor- β
Fibroblast growth factor	Pigment epithelium-derived factor
Tumour necrosis factor α	Peroxisome proliferator-activator receptor- γ ligands
Insulin-like growth factor 1	Angiopoietin-2
Hepatocyte growth factor	
Angiopoietin-1 and angiopoietin-2	
Hypoxia	

upregulating and downregulating various intracellular signalling pathways. At the same time, proangiogenic factors may act in part by altering integrin expression on endothelial cells.³⁹ Breakdown of ECM during angiogenesis is facilitated by two proteolytic systems, namely, urokinase-type plasminogen activator and matrix metalloproteinases. Both systems are present in excised CNV specimens and are upregulated in laser-induced CNV experimental models.^{49 50} In addition, proteolytic enzymes, such as collagenase and elastase, which can degrade Bruch's membrane, can be produced by activated macrophages and other inflammatory cells which become active during the early inflammatory phase of AMD development.

Table 1 shows stimulators and inhibitors reported to be involved in ocular neovascularisation.^{39 51–54}

Biomarkers associated with response to anti-VEGF treatment

Anti-VEGF injections remain the gold standard treatment for neovascular AMD. Nevertheless, many patients have a poor or no response to injections.⁴⁵ The Comparison of Age-related Macular Degeneration Treatments Trials (CATT) study revealed that more than half of the patients receiving ranibizumab and bevacizumab had evidence of persistent fluid on optical coherence tomography (OCT).⁵⁵ Similarly, 19.7%–36.6% of patients on aflibercept may have exudation on either OCT or angiography.⁵⁶ As a result, there is increasing interest in the identification of biomarkers associated with response to anti-VEGF treatment. However, their use has not been adopted yet on routine clinical practice. Identifying predictors of anti-VEGF treatment will help understand disease prognosis, individualise treatment plans and guide future research towards patients with poor treatment response.

While most studies scrutinise the use of genomic biomarkers, proteomic and metabolomic biomarkers can also be useful and their use might be clinically meaningful. Besides these, demographic, lifestyle, ophthalmic, systemic or imaging factors might be predictive of treatment response, but their review is beyond the scope of this article.⁹

Most genomic studies focus on the use of single nucleotide polymorphisms (SNPs) and analyse genes that may be associated with the risk of AMD development. DNA extraction for SNP analysis can be obtained from saliva, whole blood, plasma, blood mononuclear cells or even the aqueous humour. The major molecular biomarkers associated with response to anti-VEGF treatment are described below.

VEGF polymorphisms

VEGFA SNPs rs3025000 (with at least one T allele present) and rs699946 (with the G allele present) have been associated with improved outcomes to anti-VEGF treatment.^{57 58} More specifically, in a prospective cohort study including 201 patients with AMD treated over a period of 1 year with a loading phase of ranibizumab or bevacizumab followed by pro re nata (PRN), for *VEGFA* SNP rs3025000, the presence of at least one T allele appears to be advantageous in anti-VEGF treatment as either TT or TC genotypes are associated with greater visual acuity improvement compared with the CC genotype. Also, fewer injections were needed for the T allele patients.⁵⁷ Similarly, for *VEGFA* SNP rs699946, patients with the G allele responded better to bevacizumab therapy compared with patients carrying the A allele.⁵⁹

VEGFA SNPs rs833069 and rs2071559 were associated with an increased risk of AMD.⁶⁰ Similarly, for *VEGFA* SNP rs833068, genotypes GG received an average of 2.67 ranibizumab injections over a period of 12 months following a PRN treatment regimen compared with AG and AA genotypes which received an average of 6.57 injections and 6.40 injections, respectively.⁶¹

A trend for a higher probability of not responding to treatment has been observed for variants rs699947 CC, rs833061 TT and rs1570360 GG, although these results did not reach statistical significance.⁶²

For *VEGFA* rs3025039, the TT genotype was associated with a higher probability of improvement of visual acuity by ≥ 15 letters in a prospective, Korean study of newly diagnosed wet patients with AMD treated with ranibizumab with a loading phase followed by PRN.⁶³ In another study, ranibizumab treatment was found to be significantly more effective in patients harbouring the *VEGFA*2578C allele, whereas patients carrying the *VEGFA*2578AA genotype showed an absence of an early functional response.⁶⁴

Finally, no association was found between *VEGFA* polymorphisms rs1413711, rs3025039, rs2010963, rs833061, rs699947, rs3024997, rs833069 and rs1005230 and visual outcomes after anti-VEGF treatment.⁶²

VEGFR polymorphisms

The rs4576072 and rs6828477 polymorphisms in the *VEGFR2* gene were independently associated with a significantly improved visual acuity compared with the control group after 3 months and 12 months of treatment with ranibizumab.⁶⁵ However, more recent findings from CATT and IVAN trials analysing data of 512 participants treated with ranibizumab or bevacizumab do not support

pharmacogenetic associations between rs4576072 and rs6828477 or change in visual acuity after anti-VEGF treatment.⁶⁶

On the other hand, *VEGFR1* variants rs7993418 TC and TT were associated with a better anatomic response as indicated by central foveal thickness reduction after 12 months of treatment following a PRN regimen, while the *VEGFR2/KDR* (kinase insert domain receptor) SNP rs2071559 was not related to ranibizumab response.^{67–69}

Complement factor H polymorphisms

Complement factor H (CFH) polymorphisms, more particularly the *CFH* Y402H (rs1061170) genotype (with alleles T and C), have been strongly associated with anti-VEGF treatment (ranibizumab or bevacizumab) outcomes.^{68 69} Homozygosity for the C allele has been associated with poor response to anti-VEGF treatments, while patients with at least one T allele appear to respond more favourably to ranibizumab treatment.^{70 71} Several studies have confirmed this association, and in a relevant meta-analysis, 6 out of 10 studies included showed the C allele to be a predictor of poor response to anti-VEGF treatment.^{68 69 72–75} However, inconsistencies between different studies have been reported. Of note, the CC Y402H genotype is strongly associated with AMD development as well.⁷⁶ It is likely that ethnicity might be an important confounder regarding the Y402H polymorphism as the association with response to treatment has been identified in Caucasian populations but not in Asian ones. Treatment regimen variations among studies might also be influencing outcomes.

The CATT study did not identify any statistically significant difference for different allelic genotypes in patients with *CFH* SNP rs1061170.⁷⁷ Other SNPs investigated are the rs1048663, rs3766405, rs412852, rs11582939 and rs1066420, and associations with worse visual outcomes have been reported. The rs800292 AA carriers presented with a better baseline VA, but the outcome after treatment was better in those with the risk allele (GG and GA).⁶⁷ On the other hand, rs800292, rs1329428 and rs1410996 have been associated with poor anti-VEGF response in a different study.^{78 79}

Complement factor 3 polymorphisms

Complement factor 3 (*C3*) polymorphisms have been associated with response to anti-VEGF treatment. More particularly, the GG genotype in SNP rs2230199 may indicate a better response to ranibizumab treatment following a PRN regimen.^{71 72} Similarly, patients with SNP rs12614 showed a tendency for improved visual outcomes after treatment.⁶⁷ On the contrary, the GG genotype for rs2230199, tested in blood mononuclear cell samples of patients with AMD, was significantly associated with the phenotype of large vascularised pigment epithelial detachment poorly responding to ranibizumab therapy.⁸⁰

ARMS2 polymorphisms

Research findings about *ARMS2* SNP rs10490924(A69A) regarding response to anti-VEGF treatment have been contradictory. While this SNP has been strongly associated with the development of late AMD, its presence has not been found to be a predictor of anti-VEGF treatment response.^{67 69 71 72 81} However, in other studies, the rs10490924 TT genotype was associated with no response to treatment. Similarly, a trend for worse treatment response was identified in patients with this risk genotype compared with patients with genotypes TG and GG.⁷⁰ No correlation has been identified between other *ARMS2* SNPs (rs3750848 and rs1061170) and response to ranibizumab treatment.^{82 83}

HTRA1 polymorphisms

High-temperature requirement factor (*HTRA1*) genotypes have been associated with improved outcomes after anti-VEGF treatment with bevacizumab using a PRN regimen. The T allele of *HTRA1* LOC387715 was associated with a lower average number of bevacizumab injections and a greater improvement in visual acuity at 6 months after treatment.⁸⁴ Contradictory results have been described for *HRTA1* SNP rs11200638, as no correlation was identified in some studies and poor treatment response was found in others.^{57 85–87}

Apolipoprotein E polymorphisms

The apolipoprotein E (*APOE*) polymorphisms have been evaluated for possible association with anti-VEGF treatment response, and the *APOE4* allele was associated with significantly better visual acuity improvement compared with *APOE2* allele following PRN ranibizumab/bevacizumab.⁸⁸ On the contrary, no association was identified for the *APOE* rs4420638.⁸⁹

PLAG12A polymorphisms

The SNP rs2285714 from the phospholipase A2 group XII A (*PLA2G12A*) gene has been assessed for possible association with response to anti-VEGF treatment, but no statistical significance was reached.⁹⁰

SERPINF1 polymorphisms

The *SERPINF1* (serpin family F member 1) gene encodes an antiangiogenic protein secreted by the RPE cells.⁶⁷ The rs12603486 and rs1136287 polymorphisms have been associated with response to ranibizumab injections for neovascular AMD. Patients carrying the A allele of rs12603486 were more likely to have poor treatment response. With regard to rs1136287, genotypes CT and CC were associated with worse anatomical outcomes after ranibizumab treatment.⁶⁸

OR52B4

The *OR52B4* gene encodes the olfactory receptor 52B4 protein, and the SNPs associated with AMD treatment are rs4910623, rs323085 and rs10158937. The *OR52B4* rs4910623 and rs10158937 were associated with worse treatment outcomes, while rs323085 was associated with

Table 2 Main predictive biomarkers for response to anti-VEGF

GENE	Polymorphism	Anti-VEGF response
VEGFA	rs3025000 TT/TC	Positive
	rs699946 G	Positive
	rs833068 GG	Positive
	rs3025039 TT	Positive
	2578AA	Negative
VEGFR1	rs7993418 TC/TT	Positive
CFH	rs1061170 CC	Negative
	rs800292	Negative
	rs1329428	Negative
	rs1410996	Negative
HRTA1	LOC387715 T	Positive
	rs12614	Positive
APOE	ε4	Positive
SERPINF1	rs12603486 A	Negative
	rs1136287 CT/CC	Negative

anti-VEGF, anti-vascular endothelial growth factor; APOE, apolipoprotein E; CFH, complement factor H; HRTA1, high-temperature requirement factor A1; SERPINF1, serpin family F member 1.

better anti-VEGF response (PRN ranibizumab/bevacizumab).^{78 91}

The main molecular biomarkers associated with response to anti-VEGF treatment are analysed in [table 2](#).

Discussion

Neovascular AMD is a disease with a wide phenotypic variation. Macular neovascularisation can originate from and penetrate through different areas of the choroid or retina.²⁰ Several different molecular pathogenic pathways may be involved in neovascularisation development; however, pathophysiological mechanisms may not be equally contributory and responsible in all patients.^{20 21} VEGF blockage is not always adequate to halt disease progression and/or recurrence. Prognosis and optimal treatment regimens may also vary among different patients.⁶⁷ Continuous research efforts have increased the arrows in the ophthalmologist's quiver, allowing emerging treatments to target different molecules and pathogenic pathways.

Although many biomarkers have already been described as predictors of AMD risk, response to AMD treatment cannot be forecasted yet using genetic markers. The incorporation of biomarker use in AMD treatment protocols can improve disease management in many ways. Prognosis can be accurately defined, and treatment can be individualised based on specific, non-modifiable, molecular patient characteristics. At the same time, follow-up planning can be personalised based on underlying disease phenotypes and predicted treatment

outcomes, thereby minimising the need for unnecessary visits and any attendant burden on patients, carers and the overall healthcare system.⁹²

While no clinical use of molecular biomarkers has been implemented in AMD yet, there is growing evidence to suggest that some of the biomarkers investigated can provide additional prognostic information for patients. More specifically, among the biomarkers analysed, VEGF, CFH and C3 polymorphisms have been shown in several studies to be associated with treatment outcomes. As additional treatments are getting approved, biomarker analysis can provide personalised information about the best management plan for patients.

As already mentioned, despite the perceived benefits of using predictors of prognosis and treatment response in AMD management, current everyday clinical practice does not benefit from such biomarkers. This may be related to the fact that available literature mainly comprises small retrospective studies with limited follow-up periods and often conflicting results. Additional compounding factors, including differences in ethnicity, patient demographics and clinical characteristics, implemented treatment protocols and endpoint definitions, may further contribute to the increased heterogeneity in available published evidence.^{93 94} Widespread point-of-care implementation will necessitate the development of robust biomarkers relatable to specific disease phenotypes and treatment strategies, while scrutinising the interaction and relationship among different (molecular and non-molecular) indicators will help identify biomarker signatures with optimal prediction performance. Progress in retinal imaging and artificial intelligence can be of additional help as it can provide additional information about different AMD phenotypes and differentiation among these.

The development of newer treatments targeting alternative pathways involved in neovascular AMD holds promise for future optimisation of disease management.²⁰ At the same time, this necessitates the need to identify additional biomarkers, enabling patient stratification depending on predicted therapeutic responses and allowing the implementation of individualised treatment regimens. In addition, biomarker characterisation can provide insight into disease pathogenesis and guide future research on the development of novel treatment options, including gene therapy.²⁰

We extensively reviewed the literature about AMD pathogenesis and the molecular biomarkers analysed in relation to response to treatment. Some limitations of our study include the lack of robustness in eligibility criteria for the literature search and the inability to perform data synthesis. However, by using broad terms in our search strategy, we believe that our study provides a good overview of the current level of evidence and future directions for research in the field.

In conclusion, AMD is a disease with complex pathogenesis and resultant phenotypic presentation. Current evidence holds promise, especially about the use of

VEGF, CFH and C3 polymorphisms as prognostic biomarkers for AMD. Appropriately designed research on biomarkers related to neovascular AMD prognosis and therapeutic outcomes can improve treatment protocols and allow tailored patient management. This is essential as additional pathways responsible for macular neovascularisation can be targeted and treatment options for patients with AMD progressively increase.

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ORCID iDs

Nikolaos Dervenis <http://orcid.org/0000-0002-7269-2785>

Eleftherios Agorogiannis <http://orcid.org/0000-0001-9353-9379>

REFERENCES

- Kocur I, Resnikoff S. Visual impairment and blindness in Europe and their prevention. *Br J Ophthalmol* 2002;86:716–22.
- Li JQ, Welchowski T, Schmid M, et al. Prevalence and incidence of age-related macular degeneration in Europe: a systematic review and meta-analysis. *Br J Ophthalmol* 2020;104:1077–84.
- Finger RP, Fleckenstein M, Holz FG, et al. Quality of life in age-related macular degeneration: a review of available vision-specific psychometric tools. *Qual Life Res* 2008;17:559–74.
- Churchill AJ, Carter JG, Lovell HC, et al. VEGF polymorphisms are associated with neovascular age-related macular degeneration. *Hum Mol Genet* 2006;15:2955–61.
- Penn JS, Madan A, Caldwell RB, et al. Vascular endothelial growth factor in eye disease. *Prog Retin Eye Res* 2008;27:331–71.
- Pina Marín B, Gajate Paniagua NM, Gómez-Baldó L, et al. Burden of disease assessment in patients with neovascular age-related macular degeneration in Spain: Results of the AMD-MANAGE study. *Eur J Ophthalmol* 2022;32:385–94.
- Mehta H, Kim LN, Mathis T, et al. Trends in real-world neovascular AMD treatment outcomes in the UK. *Clin Ophthalmol* 2020;14:3331–42.
- Tan CS, Ngo WK, Chay IW, et al. Neovascular Age-Related Macular Degeneration (nAMD): a review of emerging treatment options. *Clin Ophthalmol* 2022;16:917–33.
- Lambert NG, ElShelmani H, Singh MK, et al. Risk factors and biomarkers of age-related macular degeneration. *Prog Retin Eye Res* 2016;54:64–102.
- Biomarkers Definitions Working. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001;69:89–95.
- Bressler SB. Introduction: understanding the role of angiogenesis and antiangiogenic agents in age-related macular degeneration. *Ophthalmology* 2009;116(10 Suppl):S1–7.
- Ardeljan D, Chan CC. Aging is not a disease: distinguishing age-related macular degeneration from aging. *Prog Retin Eye Res* 2013;37:68–89.
- Aguilar-Cazares D, Chavez-Dominguez R, Carlos-Reyes A, et al. Contribution of angiogenesis to inflammation and cancer. *Front Oncol* 2019;9:1399.
- Nishida N, Yano H, Nishida T, et al. Angiogenesis in cancer. *Vasc Health Risk Manag* 2006;2:213–9.
- Felmeden DC, Blann AD, Lip GYH. Angiogenesis: basic pathophysiology and implications for disease. *Eur Heart J* 2003;24:586–603.
- Mattei MG, Borg JP, Rosnet O, et al. Assignment of vascular endothelial growth factor (VEGF) and placenta growth factor (PLGF) genes to human chromosome 6p12-p21 and 14q24-q31 regions, respectively. *Genomics* 1996;32:168–9.
- Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer* 2011;2:1097–105.
- Rosenfeld PJ. Optical coherence tomography and the development of antiangiogenic therapies in neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2016;57.
- Spaide RF, Jaffe GJ, Sarraf D, et al. Consensus nomenclature for reporting neovascular age-related macular degeneration data: consensus on neovascular age-related macular degeneration nomenclature study group. *Ophthalmology* 2020;127:616–36.
- Flores R, Carneiro A, Vieira M, et al. Age-related macular degeneration: pathophysiology, management, and future perspectives. *Ophthalmologica* 2021;244:495–511.
- Corvi F, Chandra S, Invernizzi A, et al. Multimodal imaging comparison of polypoidal choroidal vasculopathy between asian and caucasian populations. *Am J Ophthalmol* 2022;234:108–16.
- Arden GB, Sivaprasad S. Hypoxia and oxidative stress in the causation of diabetic retinopathy. *Curr Diabetes Rev* 2011;7:291–304.
- Kauppinen A, Paterno JJ, Blasiak J, et al. Inflammation and its role in age-related macular degeneration. *Cell Mol Life Sci* 2016;73:1765–86.
- Boltz A, Luksch A, Wimpfing B, et al. Choroidal blood flow and progression of age-related macular degeneration in the fellow eye in patients with unilateral choroidal neovascularization. *Invest Ophthalmol Vis Sci* 2010;51:4220–5.
- Lains I, Wang J, Providência J, et al. Choroidal changes associated with subretinal drusenoid deposits in age-related macular degeneration using swept-source optical coherence tomography. *American Journal of Ophthalmology* 2017;180:55–63.
- Campbell M, Doyle SL. An eye on the future of inflammasomes and drug development in AMD. *J Mol Med (Berl)* 2013;91:1059–70.
- Doyle SL, Campbell M, Ozaki E, et al. NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. *Nat Med* 2012;18:791–8.
- Nozaki M, Raisler BJ, Sakurai E, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A* 2006;103:2328–33.
- Frank RN, Amin RH, Elliott D, et al. Basic fibroblast growth factor and vascular endothelial growth factor are present in epiretinal and choroidal neovascular membranes. *Am J Ophthalmol* 1996;122:393–403.
- Matsuda Y, Nonaka Y, Futakawa S, et al. Anti-angiogenic and anti-scarring dual action of an anti-fibroblast growth factor 2 aptamer in animal models of retinal disease. *Mol Ther Nucleic Acids* 2019;17:819–28.
- Anderson DH, Mullins RF, Hageman GS, et al. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 2002;134:411–31.
- Kijlstra A, La Heij E, Hendrikse F. Immunological factors in the pathogenesis and treatment of age-related macular degeneration. *Ocul Immunol Inflamm* 2005;13:3–11.
- Bora PS, Sohn J-H, Cruz JMC, et al. Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. *J Immunol* 2005;174:491–7.
- Cashman SM, Desai A, Ramo K, et al. Expression of complement component 3 (C3) from an adenovirus leads to pathology in the murine retina. *Invest Ophthalmol Vis Sci* 2011;52:3436–45.
- Farnoodian M, Sorenson CM, Sheibani N. Negative regulators of angiogenesis, ocular vascular homeostasis, and pathogenesis and treatment of exudative AMD. *J Ophthalmic Vis Res* 2018;13:470–86.
- Nowak JZ. Age-related macular degeneration (AMD): pathogenesis and therapy. *Pharmacol Rep* 2006;58:353–63.
- Mori K, Gehlbach P, Ando A, et al. Regression of ocular neovascularization in response to increased expression of

- pigment epithelium-derived factor. *Invest Ophthalmol Vis Sci* 2002;43:2428–34.
- 38 Mori K, Gehlbach P, Yamamoto S, *et al*. AAV-mediated gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization. *Invest Ophthalmol Vis Sci* 2002;43:1994–2000.
- 39 Ohno-Matsui K, Morita I, Tombran-Tink J, *et al*. Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. *J Cell Physiol* 2001;189:323–33.
- 40 Wang S, Sorenson CM, Sheibani N. Lack of thrombospondin 1 and exacerbation of choroidal neovascularization. *Arch Ophthalmol* 2012;130:615–20.
- 41 Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. *Arch Ophthalmol* 2004;122:598–614.
- 42 Hanahan D. Signaling vascular morphogenesis and maintenance. *Science* 1997;277:48–50.
- 43 Maisonnier PC, Suri C, Jones PF, *et al*. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 1997;277:55–60.
- 44 Hangai M, Murata T, Miyawaki N, *et al*. Angiopoietin-1 upregulation by vascular endothelial growth factor in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 2001;42:1617–25.
- 45 Yang S, Zhao J, Sun X. Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. *Drug Des Devel Ther* 2016;10:1857–67.
- 46 García-Onrubia L, Valentin-Bravo FJ, Coco-Martin RM, *et al*. Matrix metalloproteinases in age-related macular degeneration (AMD). *Int J Mol Sci* 2020;21:5934.
- 47 Nita M, Strzalka-Mrozik B, Grzybowski A, *et al*. Age-related macular degeneration and changes in the extracellular matrix. *Med Sci Monit* 2014;20:1003–16.
- 48 Sarkis JP, Sarkis SH, Killingsworth MC. Morphology of early choroidal neovascularisation in age-related macular degeneration: correlation with activity. *Eye (Lond)* 1997;11 (Pt 4):515–22.
- 49 Kvant A, Shen WY, Sarman S, *et al*. Matrix metalloproteinase (MMP) expression in experimental choroidal neovascularization. *Curr Eye Res* 2000;21:684–90.
- 50 Steen B, Sejersen S, Berglin L, *et al*. Matrix metalloproteinases and metalloproteinase inhibitors in choroidal neovascular membranes. *Invest Ophthalmol Vis Sci* 1998;39:2194–200.
- 51 Asahara T, Chen D, Takahashi T, *et al*. Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. *Circ Res* 1998;83:233–40.
- 52 Campochiaro PA, Soloway P, Ryan SJ, *et al*. The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration. *Mol Vis* 1999;5:34.
- 53 Murata T, He S, Hangai M, *et al*. Peroxisome proliferator-activated receptor-gamma ligands inhibit choroidal neovascularization. *Invest Ophthalmol Vis Sci* 2000;41:2309–17.
- 54 Seo MS, Kwak N, Ozaki H, *et al*. Dramatic inhibition of retinal and choroidal neovascularization by oral administration of a kinase inhibitor. *Am J Pathol* 1999;154:1743–53.
- 55 Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group, Martin DF, Maguire MG, *et al*. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology* 2012;119:1388–98.
- 56 Heier JS, Brown DM, Chong V, *et al*. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. *Ophthalmology* 2012;119:2537–48.
- 57 Abedi F, Wickremasinghe S, Richardson AJ, *et al*. Variants in the VEGFA gene and treatment outcome after anti-VEGF treatment for neovascular age-related macular degeneration. *Ophthalmology* 2013;120:115–21.
- 58 Fauser S, Lambrou GN. Genetic predictive biomarkers of anti-VEGF treatment response in patients with neovascular age-related macular degeneration. *Surv Ophthalmol* 2015;60:138–52.
- 59 Agosta E, Lazzeri S, Orlandi P, *et al*. Pharmacogenetics of antiangiogenic and antineovascular therapies of age-related macular degeneration. *Pharmacogenomics* 2012;13:1037–53.
- 60 Galan A, Ferlin A, Caretti L, *et al*. Association of age-related macular degeneration with polymorphisms in vascular endothelial growth factor and its receptor. *Ophthalmology* 2010;117:1769–74.
- 61 Francis PJ, Klein ML. Update on the role of genetics in the onset of age-related macular degeneration. *Clin Ophthalmol* 2011;5:1127–33.
- 62 Smailhodzic D, Klaver CCW, Klevering BJ, *et al*. Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. *Ophthalmology* 2012;119:339–46.
- 63 Park UC, Shin JY, McCarthy LC, *et al*. Pharmacogenetic associations with long-term response to anti-vascular endothelial growth factor treatment in neovascular AMD patients. *Mol Vis* 2014;20:1680–94.
- 64 Lazzeri S, Figus M, Orlandi P, *et al*. VEGF-A polymorphisms predict short-term functional response to intravitreal ranibizumab in exudative age-related macular degeneration. *Pharmacogenomics* 2013;14:623–30.
- 65 Hermann MM, van Asten F, Muether PS, *et al*. Polymorphisms in vascular endothelial growth factor receptor 2 are associated with better response rates to ranibizumab treatment in age-related macular degeneration. *Ophthalmology* 2014;121:905–10.
- 66 Hagstrom SA, Ying G, Maguire MG, *et al*. VEGFR2 gene polymorphisms and response to anti-vascular endothelial growth factor therapy in age-related macular degeneration. *Ophthalmology* 2015;122:1563–8.
- 67 Cobos E, Recalde S, Anter J, *et al*. Association between CFH, CFB, ARMS2, SERPINF1, VEGFR1 and VEGF polymorphisms and anatomical and functional response to ranibizumab treatment in neovascular age-related macular degeneration. *Acta Ophthalmol* 2018;96:e201–12.
- 68 Kitchens JW, Kassem N, Wood W, *et al*. A pharmacogenetics study to predict outcome in patients receiving anti-VEGF therapy in age related macular degeneration. *Clin Ophthalmol* 2013;7:1987–93.
- 69 Chang W, Noh DH, Sagong M, *et al*. Pharmacogenetic association with early response to intravitreal ranibizumab for age-related macular degeneration in a Korean population. *Mol Vis* 2013;19:702–9.
- 70 Brantley MA Jr, Edelstein SL, King JM, *et al*. Clinical phenotypes associated with the complement factor H Y402H variant in age-related macular degeneration. *Am J Ophthalmol* 2007;144:404–8.
- 71 Piermarocchi S, Miotto S, Colavito D, *et al*. Combined effects of genetic and non-genetic risk factors affect response to ranibizumab in exudative age-related macular degeneration. *Acta Ophthalmol* 2015;93:e451–7.
- 72 Chaudhary V, Brent M, Lam W-C, *et al*. Genetic risk evaluation in wet age-related macular degeneration treatment response. *Ophthalmologica* 2016;236:88–94.
- 73 Fauser S, Schwabecker V, Muether PS. Suppression of intraocular vascular endothelial growth factor during aflibercept treatment of age-related macular degeneration. *Am J Ophthalmol* 2014;158:532–6.
- 74 Lee AY, Raya AK, Kymes SM, *et al*. Pharmacogenetics of complement factor H (Y402H) and treatment of exudative age-related macular degeneration with ranibizumab. *Br J Ophthalmol* 2009;93:610–3.
- 75 Chen H, Yu KD, Xu GZ. Association between variant Y402H in age-related macular degeneration (AMD) susceptibility gene CFH and treatment response of AMD: a meta-analysis. *PLoS ONE* 2012;7:e42464.
- 76 Babanejad M, Moein H, Akbari MR, *et al*. Investigating the CFH gene polymorphisms as a risk factor for age-related macular degeneration in an Iranian population. *Ophthalmic Genet* 2016;37:144–9.
- 77 Hagstrom SA, Ying G-S, Pauer GJT, *et al*. Pharmacogenetics for genes associated with age-related macular degeneration in the Comparison of AMD Treatments Trials (CATT). *Ophthalmology* 2013;120:593–9.
- 78 Klein RJ, Zeiss C, Chew EY, *et al*. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308:385–9.
- 79 Wang Z, Zou M, Chen A, *et al*. Genetic associations of anti-vascular endothelial growth factor therapy response in age-related macular degeneration: a systematic review and meta-analysis. *Acta Ophthalmol* 2022;100:e669–80.
- 80 Moullem-Beziere A, Blanco-Garavito R, Richard F, *et al*. Genetics of large pigment epithelial detachments in neovascular age-related macular degeneration. *Retina* 2020;40:663–71.
- 81 Brantley MA Jr, Edelstein SL, King JM, *et al*. Association of complement factor H and LOC387715 genotypes with response of exudative age-related macular degeneration to photodynamic therapy. *Eye (Lond)* 2009;23:626–31.
- 82 Orlin A, Hadley D, Chang W, *et al*. Association between high-risk disease loci and response to anti-vascular endothelial growth factor treatment for wet age-related macular degeneration. *Retina* 2012;32:4–9.
- 83 Teper SJ, Nowinska A, Pilat J, *et al*. Involvement of genetic factors in the response to a variable-dosing ranibizumab treatment regimen for age-related macular degeneration. *Mol Vis* 2010;16:2598–604.
- 84 Kang HK, Yoon MH, Lee DH, *et al*. Pharmacogenetic influence of LOC387715/HTRA1 on the efficacy of bevacizumab treatment for age-related macular degeneration in a Korean population. *Korean J Ophthalmol* 2012;26:414–22.
- 85 Hadziahmetovic M, Malek G. Age-related macular degeneration revisited: from pathology and cellular stress to potential therapies. *Front Cell Dev Biol* 2020;8:612812.

- 86 Mohamad NA, Ramachandran V, Mohd Isa H, *et al.* Association of HTRA1 and ARMS2 gene polymorphisms with response to intravitreal ranibizumab among neovascular age-related macular degenerative subjects. *Hum Genomics* 2019;13:13.
- 87 Rodríguez FJ, Rios HA, Aguilar MC, *et al.* Genetic association with intravitreal ranibizumab response for neovascular age-related macular degeneration in hispanic population. *Taiwan J Ophthalmol* 2019;9:243–8.
- 88 Wickremasinghe SS, Sandhu SS, Amirul-Islam FM, *et al.* Polymorphisms in the APOE gene and the location of retinal fluid in eyes with neovascular age-related macular degeneration. *Retina* 2014;34:2367–75.
- 89 Yamashiro K, Mori K, Honda S, *et al.* A prospective multicenter study on genome wide associations to ranibizumab treatment outcome for age-related macular degeneration. *Sci Rep* 2017;7:9196.
- 90 Wang VM, Rosen RB, Meyerle CB, *et al.* Suggestive association between PLA2G12A single nucleotide polymorphism rs2285714 and response to anti-vascular endothelial growth factor therapy in patients with exudative age-related macular degeneration. *Mol Vis* 2012;18:2578–85.
- 91 Riaz M, Lorés-Motta L, Richardson AJ, *et al.* GWAS study using DNA pooling strategy identifies association of variant rs4910623 in OR52B4 gene with anti-VEGF treatment response in age-related macular degeneration. *Sci Rep* 2016;6:37924.
- 92 Stone EM. Genetic testing for age-related macular degeneration. *JAMA Ophthalmol* 2015;133:598.
- 93 Ross RJ, Verma V, Rosenberg KI, *et al.* Genetic markers and biomarkers for age-related macular degeneration. *Expert Rev Ophthalmol* 2007;2:443–57.
- 94 Farinha C, Barreto P, Coimbra R, *et al.* Common and rare genetic risk variants in age-related macular degeneration and genetic risk score in the Coimbra eye study. *Acta Ophthalmol* 2023;101:185–99.