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Precision medicine for age-related macular degeneration: current developments and prospects

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Abstract

Introduction: With the ageing population, diseases such as age-related macular degeneration (AMD) will become more prevalent. This will increase demand for provision of care on affected individuals, society and the healthcare system. To develop the best, individually tailored treatment for every patient, however, remains challenging.

Areas covered: Recent identifications of interactions between environmental, lifestyle, genetic and non-genetic factors opened the potential for developing personalised approaches for the prevention and treatment of AMD. In this review, we will discuss the implications of these interactions for early to late disease stage conversion, for neovascularization and cell atrophy. We will put the findings of recent studies within the context of the regulatory framework requirements surrounding the development of personalised medicine approach to AMD.

Expert Opinion: Precision medicine is now at a stage that it has its theoretical framework in place for the management of risk for patients with AMD requiring early diagnosis and timely treatment as several key components for such an approach are now clearly identified and are being applied to clinical developments successfully.

1. Introduction

1.1 Epidemiology

Age-related macular degeneration (AMD) is the primary cause of irreversible blindness in developed countries [1]. The worldwide prevalence of early stages of AMD in patients between 45 - 85 years is 8.01% (95% credible interval -CrI-, 3.98-15.49) and that of late AMD is 0.37% (95% CrI, 0.18-0.77) [2]. Prevalence rises steeply with age, reaching 30% for early and 7% for late AMD amongst those aged 85 years old of European ancestry [2]. Given the increase in life expectancy, globally nearly 200 million people is expected to have AMD by 2020 and 288 million by 2040 [2]. The disease has a significant economic impact, approaching $30 billion/year in the United States alone [3].

Apart from increasing age, other risk factors include being of Caucasian origin, smoking and family history [2, 4]. In fact, genetic factors explain 45% to 70% of the variation in the severity of AMD [5] (see section "1.3 Disease pathogenesis").

1.2 Phenotype

Light must enter the eye and cross the transparent structures of the eye before reaching the retina, the posterior and innermost aspect of the globe. The same biological aspect makes the retina accessible for non-invasive imaging; these have expanded in the past 25 years and led to better understanding of the disease due to defined disease phenotyping.

The earliest classification of AMD was based on phenotyping relevant abnormalities on color fundus images [6, 7]. Since then, several AMD classifications have been in use, somewhat limiting the comparability of studies [8]. Recently a classification system developed by the Beckman Initiative's is gaining momentum[8] as it is probably the easiest to incorporate into clinical practice (**Table 1**). The clinical hallmark of early AMD is drusen (from the German "druse", geode), yellowish extracellular deposits which, based on transmission electron microscopic investigations are typically located between the basal lamina of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch's membrane [9, 10]. These ultrastructural features are seen in the clinical course of AMD using eye tracked OCT, for example [11]. They range from small, innocuous deposits (≤63 µm, called drupelets), to medium (>63 µm to 125 µm) or large (>125 µm) drusen. Large drusen signal increased risk of progression to late stages of AMD. Drusen may be accompanied by RPE abnormalities,

identified by hyperpigmentation (brownish areas in the fundus representing vertical superimposed RPE cells) or hypopigmentation (areas devoid of pigmentation in the RPE) **(Fig. 1)**; these are major progression risk factors for AMD [7, 12]. Visual acuity in the early stages of AMD is not necessarily affected, though patients often report difficulties with dark adaptation (caused by a decrease in the supply of the chromophores from the RPE to the photoreceptors, which can be confirmed by a dark adaptation test) [13] or slight metamorphopsia (image deformation) [14, 15].

Patients with time may progress to the late stages of the disease: neovascular AMD (nAMD) and/or geographic atrophy (GA). In nAMD, new blood vessel growth from either the underlying choroid or within the retina, causing haemorrhage and exudation leading ultimately to disorganization of retinal architecture and potential cell death **(Fig. 1)**. Visual acuity loss is usually rapid and severe if treatment is not initiated on time. Clinically three types of nAMD are described, the classification of which depends on the location of new blood vessels in relation to the RPE: type 1 (exterior to the RPE), type 2 (interior to the RPE) and type 3 (of retinal vessel origin) [16]. The natural history, specific treatment and response to therapy vary according to lesion type [16, 17, 18, 19, 20, 21, 22, 23], therefore careful phenotyping of neovascular lesion is an absolute necessity if effective personalized treatment is to be initiated.

Geographic atrophy is characterized by RPE and adjacent photoreceptors atrophy, which results in slow but relentless vision loss [24]. The GA area tends to enlarge with time, with a wide inter-subject variability in growth rates. The use of fundus auto fluorescence (FAF) and optical coherence tomography imaging (SD-OCT) improves the phenotypic classification **(Fig. 1)** [24]. Three GA phenotypes have been reported using cluster analysis with different progression rates [25], but our current understanding of the disease does not lend itself to appropriate individual treatment to be initiated.

1.3 Disease pathogenesis

The exact pathogenesis of AMD is still elusive due to its multifactorial etiology, which includes a complex interplay between genetic and environmental factors. More than 50 single nucleotide polymorphisms in 34 loci have been linked to AMD [26], the most relevant being CFH (in the complement pathway) and ARMS2 (unknown function). Other molecular

pathways involve lipid metabolism, angiogenesis, remodeling of the extracellular matrix and apoptosis [27, 28].

Overall, several processes related to senescence are involved in the pathogenesis of AMD. Thickening of Bruch's membrane and decreased permeability (in which lipid deposition and peroxidation play a major role) [29], progressive loss of choroidal vascular supply and local hemodynamic changes [30] and increased accumulation of intracellular lipofuscin [31] create a milieu prone to oxidative stress and inflammation. This low grade, chronic inflammation in the outer retina in susceptible individuals (smokers, low antioxidant intake, genetic predisposition, etc.) tips the balance towards the incidence of AMD [32]).

Of note, SD-OCT can identify many of the abnormalities found on histology [33, 34], providing the ability to capture *in vivo* longitudinal images of patients with AMD and track disease progression, changes that were not recognizable on color fundus images. In fact, AMD is being redefined based on SD-OCT findings by the CAM (Classification of Atrophy) Group that incorporates changes in the outer retinal layers in addition to the RPE layer [35, 36]. The Project MACULA (MACulopathy Unveiled by Laminar Analysis, available at http://projectmacula.cs.uab.edu/) is an online resource for correlating SD OCT findings with histopathology, which has contributed to improve the interpretation of this imaging technology [37].

1.4 Current treatment

The current clinical management of AMD depends on its stage. In the early/intermediate forms, treatment is aimed at preventing the progression to the late stages to preserve visual function.

The Age-Related Eye Disease Study (AREDS) found that oral supplementation of antioxidants and zinc in patients with intermediate AMD (defined as patients with extensive intermediate drusen, ≥1 large druse, extrafoveal GA, or late AMD or vision loss due to AMD in at least one eye) reduced the risk of progression to nAMD by 25%. Unfortunately, supplementation had no effect on the progression to GA [38]. Due to the association of beta-carotene with increased risk of lung cancer in smokers, the follow-up study, AREDS2, substituted it in the original formulation by lutein and zeaxanthin, and also included omega-3 docosahexaenoic acid and eicosapentaenoic acid. [39]. None of the newly added constituents

increased the efficacy of the formulation, but inclusion of lutein and zeaxanthin instead of beta-carotene is now recommended for safety reasons. Supplementation in the USA is popular but in Europe it did not gain the same momentum [40].

The mainstay of nAMD treatment is anti-angiogenic therapy delivered by intravitreal injection targeting vascular endothelial growth factor (VEGF), a protein that stimulates proliferation and permeability of new blood vessels [41]. In the Western World there are currently 3 anti-VEGF treatments used: Ranibizumab (Lucentis®, Novartis) [42] and aflibercept (Eylea®, Regeneron/Bayer) [43] were approved by the Food and Drug Administration in 2006 and 2011 respectively, and bevacizumab (Avastin®, Roche) is used off-label since 2005. While previous treatment strategies such as laser photocoagulation [44] and photodynamic therapy [45] with verteporfin resulted in slower vision loss than those without treatment [45], anti-angiogenic therapy improved visual acuity for the first time [42, 46]. However, the need for multiple injections, significant numbers of non-responders, incident macular atrophy and high costs limit the potential benefits of anti-VEGF therapy in the real-world setting [47], and has slowed its introduction in the developing world.

There is currently no treatment to prevent, slow or recover the visual loss caused by GA. Approaches targeting different disease pathways such as oxidative stress [39], neuroprotection [48], visual cycle modulation [49], immunosuppression [50] or inflammation [51] have all failed in clinical trials in recent years. One potential explanation for the lack of success might be that the target population was inappropriately phenotyped and/or genotyped [52]. Another is that trials were designed based on inadequate or outdated molecular information [8, 53] or histopathology [54, 55, 56]. The need for more precise ultrastructural and molecular understanding of GA had recently been address raised [12, 13, 57, 58]. Excellent pathological descriptions do exist [59, 60] paving the way for further detailed investigations. It is also possible that GA represents a spectrum of diseases resulting in clinically similar RPE atrophy. For example, GA could develop by primary RPE damage or as a secondary insult caused by primary, adjacent photoreceptor loss. In fact, the latter (called "outer retinal atrophy") [61] occurs in the presence of reticular pseudodrusen, a special type of extracellular deposit located interior to the RPE. The existence of different mechanisms could explain the lack of efficacy of treatment on the overall GA population.

Given that there is only limited success in treating AMD patients, it signals that we still need to improve our understanding of interactions between phenotype, genotype, biomarker and

environmental factors so we begin to understand individual affection rather than that of population.

2. Precision medicine

2.1 Definitions and relevant regulations

Precision medicine can be defined as tailoring medical treatment to the individual characteristics of each patient [62]. It is an emerging approach aimed at using genetic and other biomarkers (e.g. proteins, ribonucleic acids, metabolites) in addition to clinical examination to make a precise assessment of the individuals regarding the susceptibility to a disease, the diagnostic and prognosis of a disease, and enable treatment decisions based on the knowledge about biological processes of the disease pathogenesis for the individual.

The diagnosis and staging of AMD is based, as described in section 1, on anatomical characteristics of the retina (phenotypes) assessed by imaging modalities such as fundus photography and more recently by SD-OCT. Similarly, the assessment of AMD prognosis, especially for the risk of conversion from early/intermediate AMD to late AMD (neovascular AMD or geographic atrophy) is mainly based on demographics and the fundus phenotype [12] in the absence of suitable genetic marker. However, recent research shows that soon biomarkers may enable more accurate clinical assessment and diagnosis leading to better disease prognostication in an individual patient [63, 64].

Two large international consortia are in the position to contribute significantly to advance precision medicine in AMD: The International AMD Gene Consortium (IAMDGC) [65] is supported by the United States National Eye Institute (NEI), a part of the National Institutes of Health, and the EYE-RISK consortium funded by the European Union's Horizon 2020 program. IAMDGC focuses on the analysis of AMD's genetic architecture, bridging the gap between association studies of common variants and sequencing studies of rare variants. However, phenotyping for IAMDGC is currently based on colour fundus images and, as explained in 1.3., phenotyping of AMD is being reclassified based on new imaging modalities and pathology. EYE-RISK consortium focuses on a broader range of both clinical and basic science topics, including but not limited to – new biomarker identification for patient stratification, development of new algorithms measuring the personalized risk for progression to advanced AMD, elucidating AMD pathology pathways and finally, the

potential combination of these. One of the goals of Eye-Risk is to devise more advance criteria for phenotype based on advancements in imaging as well as emerging molecular information like metabolomics [66, 67, 68]. In addition, the pharmaceutical industry is increasingly applying precision medicine strategies for the discovery and development of the new treatment modalities and paradigms for AMD.

One essential component for implementing precision medicine in clinical practice is the development of *in vitro* diagnostics (IVD) assays to analyse biomarkers with sufficiently high quality for appropriate clinical decision-making. Therefore, it is essential to understand the constantly evolving regulatory principles governing the development and use of IVDs both in the USA and in the EU (including prospective changes in EU legislation). For more in-depth review of the subject including worldwide regulations we recommend the excellent recent article by Pettitt et al. [69].

In summary, *in vitro* diagnostics (IVD) are typically regulated by national authorities. In the USA, IVDs' applications and oversight are the responsibility of the Food and Drug Administration [70]. IVDs are "medical devices" as defined in section 210(h) of the Federal Food, Drug and Cosmetic Act, and may also be biological products subject to section 351 of the Public Health Service Act and in addition are also subject to the Clinical Laboratory Improvement Amendments (CLIA '88) of 1988. In Europe, the directive 98/79/EC of the European Parliament and of the Council [71] constitutes the EU's regulatory framework for IVDs, but the new EU Directive 2017/746 (released on April 2017) will considerably change the regulation once it comes into force in 2022. The ongoing research strategies in AMD will need to be ready for the new regulation [72].

What is common between Europe and the USA is that approval and commercialisation of IVDs are based on risk classification. For the FDA regulation, risk is determined by the intended use of the IVD test. Class I devices (lowest risk) are subject to the least stringent control regulations including device registration, adverse event reporting and Good Manufacturing Practice (GMP) practice requirement; while Class II IVDs are required to include post-market surveillance activities as well the submission to FDA many premarket data demonstrating safety and effectiveness. Such data may include assessment of bias, analytical sensitivity and specificity together with information for the clinical samples analysed by the device. Clinical study data are usually not required for Class II devices but

they are obligatory for Class III to assess device performance. The FDA's Center for Devices and Radiological Health (CDRH) is responsible for all IVDs applications.

EU has a similar system, assigning commercial IVDs to four classes based on their risk assessment (class I, IIa, IIb and III). Class I is the lowest and class III is the highest risk. Contrary to US where a single federal agency reviews IVDs (the FDA), all Notified Bodies are European Commission accredited independent organizations and are responsible for assigning the CE ("Conformité Européene") mark to all diagnostic products which fulfil the appropriate legal, safety and quality criteria. As most attempts to apply precision medicine principles in AMD also involve IVD diagnostic methods, such as analysis of genetic variants, it is essential to understand the regulatory principals governing their usage.

2.2 Regulation of companion diagnostic in US and EU (present status and perspectives).

According to the FDA, companion diagnostics "is a medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product" [73]. The goal of a companion diagnostics (CDx) is to identify patients who will most likely benefit from certain therapeutic product, or identify patients who may exhibit adverse effects because of the treatment, or monitor the response to certain therapy with ultimate goal to improve effectiveness and safety. Typical CDx example are the diagnostics devices used to identify breast cancers overproducing the protein HER2 (Human Epidermal growth factor Receptor 2), the result is essential for making the decision on using the therapeutic antibody Trastuzumab (Herceptin®).

Several additional regulatory aspects are important for an IVD to be developed as a CDx device. The essential aspects is the coordinated development of both the therapeutic agent and of the CDx device, as they might have co-dependency for approval of both (FDA's draft guidance: "Principles for Co-development of an In Vitro Companion Diagnostic Device with a Therapeutic Product", 2016). [74]. Probably the most challenging part of a CDx development is to plan and execute the clinical trial demonstrating the "clinical validity" of such device. Such demonstration requires a prospective clinical trial where patients are allocated to treatment arms according to the pre-specified definition of CDx result, and the final analysis demonstrates that the CDx correctly assigns patients according to the predicted response to the drug. In Europe, the legislations covering the marketing of medicinal products

and IVD medical devices are not directly linked. On 28 July 2017, the European Medicines Agency (EMA, http://www.ema.europa.eu/ema/) released a concept paper for public consultation on the development and lifecycle of personalised medicines and CDx [75].

The development of a CDx device is a considerable challenge which demands careful planning and execution, and considerable resources. These challenges explain the relatively low number of Companion Diagnostic Devices approved by the regulatory authorities (the FDA's website list only 41 approved CDx so far).

3. Genetic tests and genotyping in AMD - prediction, progression and treatment

3.1 Genetic testing of AMD

Initial studies on monozygotic and dizygotic twins identified significant hereditary contribution of AMD especially for the intermediate and advance forms (67% and 71% respectively) [76]. However, further studies looking for association between well-known Mendelian macular diseases genes and AMD failed to produce further relevant results [77, 78]. Only through the introduction of whole genome screening techniques with the ability to interrogate millions of variants in the individual genome a real breakthrough in understanding AMD genetics was achieved.

A genome-wide association study (GWAS) utilizes specially designed chips to screen for millions of SNPs (single nucleotide polymorphisms) variants in the genome. As it is technically impossible to load every known SNP on a chip, a pre-selection is carried out based on the available haplotype information (SNPs that tend to always occur together). That is, if the presence of certain SNP is confirmed by the chip, adjacent SNP or SNPs can be imputed by the analysis software, and so an almost complete coverage of SNPs can be achieved. It must be emphasised that haplotype structure often varies between different populations based on their different ethnical and/or geographic origin, an important point to consideration so errors in the analysis or interpretation of results are avoided.

The most common approach in GWAS is the case-control study design when a population of individuals affected by the disease are compared to healthy controls. During the analysis only variants with p-value lower than 10^{-8} are considered significant; such stringent condition is necessary to avoid false positive results during the multi-million hypothesis testing procedure

[79]. The risk altering properties of variant are expressed as odds ratios (ORs) and corresponding confidence interval (CI). An OR of 1 suggests no change in the risk associated with a certain variant while OR above one signals increased and below 1 signals a decreased risk for a given disease. Common variants associated with complex diseases like AMD usually have low OR (<1.2); alternatively, rare variants (<1%) usually have a high OR>2. Recently GWAS results are often combined with complete sequencing of genes of interest leading to the discovery of not only new common but also new rare variants associated with higher OR values (see below).

While family-based genetic linkage studies are appropriate in discovering high-penetrance, low-frequency single gene defects typical for Mendelian diseases. GWAS is particularly well suited for identifying low-penetrance high-frequency genetic variants associated with complex diseases. As such, AMD is well suited for this approach as demonstrated by the discovery of increased AMD risk in individuals with Y420H substitution in the complement related complement factor H (*CFH*) [80] . In 2013, using 13000 advance stage AMD patients and 60 000 controls from European and Asian descent a study evaluated 2.4 million SNPs and identified 23 loci, 7 of which were novel (*COL8A1-FILIP1L*, *IER3-DDR1*, *SLC16A8*, *TGFBR1*, *RAD51B*, *ADAMTS9* and *B3GALTL*). The two known loci in CFH and ARMS2 had the highest ORs (, 2.4 and 2.7, respectively), while the novel loci had a modest OR of 1.1-1.2 [27]. The International Age-related Macular Degeneration Genomics Consortium (IAMDGC) interrogated more than 12 million SNPs and more than 163 000 directly genotyped (sequenced), mainly rare, protein-altering variants and identified 52 variants in 34 loci in total, including 7 rare variants with ORs between 1.5 and 47.6. Unfortunately, apart from some protein structure altering variants, it is not always possible to establish direct causal relationship between the loci identified, the adjacent gene (or genes) and the disease itself. Variants localized in non-coding gene expression regulatory sequences (enhancers) may influence the expression level of a gene localized a significant distance away [81]. Further non-genomic experimental research in such cases is essential to establish the relationship between suspected gene/locus and the disease of interest.

Using a variety of bioinformatics tools, the top scoring 34 genes were further analysed. Amongst the 15 newly identified loci with the highest gene priority score (GPS) were the matrix metalloproteinases, *COL4A3*, an immune function modulator (*PILRB*), and genes involved in lipid metabolism and inhibitor of the complement system [26].

When nAMD and GA were compared, four variants showed different associations (*ARMS2-HTRA1*, *CETP*, *MMP9* and *SYN-TIMP3*), but only *MMP9* showed exclusive association with nAMD [26]. Comparison of intermediate and advanced AMD showed a significant overlap in genetic determinants (correlation of 0.78 (95% CI=0.69-0.87). Most of those variants were exclusively associated with nAMD and these were related to extracellular matrix remodelling (*COL15A1*, *COL8A1*, *MMP9*, *PCOLCE*, *MMP19*, *CTRB1-CTRB2* and *ITGA7*), paving the way for a theory that patients with such variants may progress rapidly to nAMD and may have maximum benefit from future genetic diagnostics and preventive treatment [26].

3.2 Genetic tests as predictive tools for development and progression of AMD

A fundamental prerequisite of precision medicine is the availability of tests that can correctly predict personalized risk for both development and progression of given diseases. The rapid growth in the number of genetic variants associated with AMD and better understanding of the interactions of genetics and environmental means that such models often incorporate both genetic and non-genetic determinants of the disease for improved accuracy.

The most common method to quantify the accuracy of a risk model is to calculate the area under the receiver-operating characteristic (ROC) curve (area under the curve or AUC). The ROC curve is generated by plotting the true positive rate (TPR) versus the false positive rate (FPR) at various threshold settings for a given criterion (**Fig. 2**). TPR is also known as sensitivity (proportion of positives that are correctly identified as such) and FPR as fall-out or probability of false alarm and can be calculated by subtracting specificity from one, where specificity is the ratio of true negative and the sum of false positives and true negatives. Ideally, AUC must have the value of one (perfect accuracy) so all individuals are correctly assigned to the affected or to the control group. In reality, AUC curves typically acquire values between 0.5 and 1. For screening individuals with increased risk of developing a disease an AUC>0.75 is recommended (tests with AUC>0.9 are considered to be excellent) [82].

Most tests utilizing only genetic information and trying to predict the risk of development of AMD (or its variants) usually have AUC of around 0.8 both in the initial and validation samples [27, 83, 84]. Combination of genetic and clinical data led to AUC of around 0.9 [85, 86] in studies aiming to predict the progression to advanced AMD and even AUC of 0.94 and

0.96 in a study aiming to predict conversion from early-stage to nAMD or GA [87]. Surprisingly, a model relying only on clinical/environmental data could predict progression to advanced AMD with high AUC (0.88 in initial sample and 0.91 in validation sample) [88]. The reason for such excellent performance of a non-genetic model is likely to be related to the predictive power of accurate baseline AMD phenotype predicting progression. Other applications of refined phenotyping include a better understanding of the natural course of the disease [89] or improved genotype-phenotype correlations [90]. These results highlight that precise phenotyping must underpin any future prediction algorithms.

High AUC confers excellent discrimination properties, but it does not guarantee that the model also has good prediction properties of the actual risk of developing disease in the future (good calibration). For prognostic tests both good discrimination (AUC) and calibration indices (measure of how well the predicted probabilities match the actual observed risk) are necessary for accurate risk assessment, the details of which is beyond this current review, but can be studied elsewhere [91].

Differences in population genetics may limit the application of current prognostic genetic tests as the majority of AMD genetic associations so far have been studied in populations of European ancestry. This is illustrated by the fact that in Caucasians the common *CFH* Y402H risk variant is present in approximately 34% but in 7% of Japanese individuals [92]. In addition, it has been found recently that two of the most common *CFH* (rs1061170) and *ARMS2* (rs10490924) risk variants present in Caucasian population do not confer statistically significant risk in Asian and African populations [93]. Furthermore, the A69S variant within *ARMS2* gene is associated with increased risk (OR ~2.1 and 2.45) in Americans of European and Mexican descent respectively, but has protective function (OR 0.45) in Americans with African descent [94]. These findings suggest the need of additional gene variants studies in AMD extending to different population and signal that in addition to genetic profiling, appropriate individual demographic data is required if individual risk profile is to be appropriately generated.

3.3 Genetic tests as CDx/ Role of genetic tests in Pharmacogenomics

Currently there is no approved medicine for the treatment of AMD involving the use a companion diagnostics device (CDx). Several studies attempted to identify biomarkers,

mainly genetic polymorphisms, associated with the response to anti-VEGF therapy for nAMD (pharmacogenetic studies reviewed by [95]). The introduction of anti-VEGF therapy had a tremendous positive impact for visual outcomes in patients with nAMD. Taking one of the many trials as an example, in the MARINA trial, on average visual acuity improved by 7.2 letters with monthly intravitreal injections of 0.5 mg ranibizumab after 12 months, while in the sham-injection group there was an average loss of 10.4 letters [42]. In the same trial, visual acuity improved by 15 or more letters in 33.8% of the 0.5-mg ranibizumab group compared with 5.0% of the sham-injection group. However, at the individual patient level a considerable heterogeneity was observed in the response to anti-VEGF therapy, evidenced by the fact that while most people gained vision, 4.5% of nAMD patients lost more than 15 letters after 12 months treatment. This heterogeneity in response led to search of genetic markers possibly associated with response (pharmacogenetics). Common genetic variants in genes of *VEGF*, *VEGFR2*, *CFH*, *IL-8*, *PLA2G12A*, *ARMS2*, *FZD4*, *LRP5*, and others have been described to be associated with response to anti-VEGF therapy in nAMD. However, none of the associations were confirmed either in the Comparison of AMD Treatments Trials (CATT) [96] or the Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularisation (IVAN) [97] trials. In CATT, the common variants in genes of *CFH*, *ARMS2*, *HTRA1* and *C3* were all strongly associated with AMD prevalence, but were ultimately not associated with anti-VEGF response [96] and in IVAN none of 485 common variants, including in *CFH*, *FZD4*, and *HTRA1/ARMS2* loci, was associated with response to anti-VEGF [97]. Therefore, there is currently no genetic variants or any other biomarker that could guide the development of a CDx device for decision making in the use of anti-VEGF therapy in nAMD.

Development of a CDx device for the recent lampalizumab trial for treating GA was carried out. Lampalizumab, an antigen-binding fragment (Fab) of a humanized monoclonal antibody that targets complement factor D was developed as intravitreal treatment to slow the progression of GA. In the Phase 2 MAHALO trial, a targeted, exploratory pharmacogenetic analysis was performed assessing the possibility that 4 common variants within the alternative complement pathway (*CFH*, *C2/CFB*, *CFI*, and *C3*) may affect GA progression and lampalizumab treatment response [98]. In the all-comer population, the lampalizumab monthly arm showed a 20% reduction in mean change in GA area progression relative to the pooled sham group at month 18. In the exploratory pharmacogenetics analysis, patients carrying the CFI risk-allele had 44% reduction in GA area progression at month 18 in the

monthly lampalizumab-treated subgroup relative to the CFI pooled sham subgroup, although the difference did not reach conventional statistical significance. In the CFI non-risk-allele carrier patients there was no apparent lampalizumab treatment effect compared to sham. The results of MAHALO trial led to the launch of 2 phase 3 trials (SPECTRI and CHROMA trials) to test whether 10 mg lampalizumab administered intravitreally 4- or 6-weekly for approximately 96 weeks would decrease GA progression rate, including an assessment whether the CFI risk allele have an effect on treatment response. These studies use the so called Biomarker-Stratified Design, which is recommended to test the effect of a CDx on the treatment response [74]. The interim results released for the two phase-3 trials announced that lampalizumab did not meet its primary endpoint of reducing mean change in GA lesion area in patients treated with lampalizumab compared with sham treatment independent of the CFI genotype. The reasons for the discrepancy between the phase 2 and phase 3 trials are not clear and await further analysis. While the initial results were promising, the final outcome demonstrate that our current understanding of AMD is not detailed enough to allow for such individualised treatment decisions to take place just yet.

4. Potential use of non-genomic tests and modalities as biomarkers for patient stratification and/or as Companion Diagnostic

In an ideal world a combination of phenotype, genotype and non-genomic biomarkers, together with environmental and lifestyle factors would give the most comprehensive information set for personalized medicine [99]. With the widespread use and the continuous improvement of ocular imaging and image analysis [100], ‘full AMD phenotyping’ is becoming achievable in a clinical setting. Continuous refinements of phenotype [101, 102] allow the clustering of patients into ever-more refined and clinically meaningful groups [25]. The interplay between phenotype, genotype [103, 104], environmental [5] or lifestyle [105] are all being considered, but combination of these factors result in a complex interactions. In addition, recent reviews summarized the available potential biomarkers from serum, plasma, aqueous humour, vitreous, and urine of AMD patients [63, 64]. However, none of these has been clinically validated and routinely used just as yet, and as such are awaiting to be included in patient stratification and/or CDx models. Herein, we are focusing on promising biomarkers, suggested in the most recent reviews [63, 64], and summarize many studies which use combinational modelling for patient stratification.

In nAMD, clinical features such as age, baseline visual acuity and lesion size showed strong association with anti-VEGF treatment efficiency [106, 107]. However, despite the success of the anti-VEGF therapy, level of VEGF or its related receptors in fluids and tissues do not appear to give reliable indication for therapeutical success. VEGF levels in aqueous humour [108, 109, 110], sera/plasma [111, 112, 113, 114, 115, 116, 117] have been reported to be associated with AMD, however, the results in general are contradictory [63]. It was hypothesized that subgrouping populations for specific genotypes might help identify more significant association of systemic/ocular VEGF fluid level with AMD and help in prediction of therapy response efficiency. However, serum VEGF levels did not correlate with *CFH* Y402H polymorphism in a case-control study [112]. Other pro-neovascular factors like PEDF and TGF-B1 could be considered as further non-genomic biomarkers, but again, there is a lack of comparison with genotype. TGF-B1 urinary levels showed significant associations with early AMD and can become a candidate non-genomic biomarker. However, there was no correlation with *CFH* genotype [118].

Antioxidant capacity of AMD patients' serum has been reported to be associated with the disease. In the AREDS study, antioxidants and zinc supplementation interaction was observed between *CFH* Y402H genotype only when zinc or antioxidants plus zinc were taken but not if antioxidants only were administered [119]. However, comparing the number of risk alleles on *CFH* and *ARMS2* genes, in the presence of 0 or 1 *CFH* risk alleles and no *ARMS2* risk alleles, treatment with antioxidants showed more favourable response upon progression, and neutral or unfavourable responses in 3 genotype groups [120].

The most promising non-genomic biomarker candidates in the oxidative stress pathway according to Kersten et al. are malondialdehyde (MDA) and homocysteine. Increased systemic levels of MDA is strongly associated with nAMD and GA. In addition, CFH binds to MDA resulting in protective effect against oxidative stress. This binding efficiency is decreased in the presence of Y402H polymorphism [121]. Homocysteine also showed strong correlation with AMD at systemic and vitreal level. Homocysteine is an intermediate molecule in the conversion of the amino acid methionine to cysteine and glutathione. In a prospective case-control study Brantley et al. determined plasma levels of cysteine, cystine, glutathione, isoprostane and isofuran, and made comparative analysis to controls taking their *CFH* and *ARMS2* genotype status into consideration. Only cystine levels showed elevation and this appeared to be *CFH* polymorphisms dependent [122]. Lambert et al. paid attention to

carboxyethyl pyrrole (CEP) and its end products [64] and noted that serum CEP level was distinguishable between AMD and control subjects with 72% accuracy, and this increased to 92% when CEP and pentosidine were measured simultaneously.

Complement activation level (C3d/C3 ratio) has also been associated with AMD, but so far, no direct correlations was found between such level and the progression of AMD [63, 123, 124, 125, 126, 127] . The activation of the complement system was affected by Y402H *CFH* and *ARMS2* polymorphism in some studies [126, 127]. Guymer et al. reported, that there is a significant correlation among urinary level of MCP-1 and early AMD as well as GA, and that individuals with one or more *CFH* risk alleles are more likely to have urinary MCP-1 level above median levels [118]. Correlation of systemic-ocular interleukin-6 level and AMD is controversial, however strong association with AMD were found in subgroups in a cross-sectional study [128]. Also, consistently increased IL-6 levels were observed in GA [129]. To strengthen this observation, a correlation among IL-6 systemic level and *CFH* Y402H genotyped AMD patients was observed in a separate study [130]. Lambert et al suggested eotaxin as potential biomarker for early diagnosis of AMD, and reported that eotaxin serum levels are higher in nAMD than in GA or in controls [131, 132]. Eotaxin levels did not reliably predict the results of anti-VEGF therapy [133]. Several studies reported changes in titres of antiretinal antibodies (ARA) in AMD patients with or without anti-VEGF therapy [134, 135], but neither of that studies were conclusive enough for these to be used for disease or treatment predictions. Serum IgG/IgM ratio levels were elevated in both GA and nAMD [64] and so they do not differentiate enough for these to be used alone clinically.

Lipid metabolism components as potential biomarkers for AMD are very well studied and summarized recently [63, 136]. Omega-3 polyunsaturated fatty acid intake has been shown strong association with AMD [136]. Amongst circulating lipoproteins, HDL-C, LDL-C and Lipoprotein(a) level were shown in association with risk for AMD, but the observations are controversial in most cases and need more clarification [136]. Combinational model studies have also been performed, and showed association between circulating lipid levels and several genetic variants [137], including AMD-related genetic variants, such as *ABCA1, APOE, CETP, LIPC*, however specific mechanisms require further investigation [26]. However, there is evidence for intraocular (retinal and RPE) expression of many plasma lipoproteins genes, which is consistent with both histochemical and ultrastructural evidence of lipoprotein-like particles in Bruch's membrane [138, 139, 140] - these and other authors

suggested that relationships with plasma levels may be unrelated or even opposite to those in the eye [141]. The local lipoprotein hypothesis has received important experimental confirmation by a demonstration of drusen formation in culture by functional RPE [142] and proof-of-concept clinical [143, 144] and pre-clinical studies on a treatment [145].

Increased serum level of elastin peptide fragment showed strong correlation with neovascular AMD [146, 147]. Recent high throughput plasma proteomics study identified vinculin as potential plasma biomarker for exudative AMD [148]. Analysis of plasma vinculin levels in combination with *ARMS2* and *CFH* gene variants led to further improvement of discriminatory power of the assay (AUC = 0.916) [148]. In a follow-up study [149] the plasma levels of two additional proteins (phospholipid transfer protein (PLTP) and mannan-binding lectin serine protease (MASP)-1) were found to be significantly increased in AMD patients. Metabolomics studies are more helpful for pathway identification than identifying biomarker candidates. A recent study analysing plasma samples of neovascular AMD patients and controls identified tyrosine metabolism, amino acids related to urea metabolism and sulphur amino acid metabolism pathways to be significantly affected [150].

MicroRNA profile of AMD patients also, can provide new information for defined diagnosis and or therapy development. Mir23, Dicer and AluRNA has been associated with different stages of AMD [151, 152, 153].

Imaging modalities as non-genomic biomarkers are another good source for defined diagnosis and therapy development/follow-up. Because of excellent depth resolution, reflectivity can be localized to the subcellular level in OCT images [154]. It has been recently published highly reflective outer retinal tabulation prominent in late stage age-related macular degeneration [155]. Introduction of cellular-level imaging identifying the activity of single cells in living people [156, 157] allowed more accurate pathology of the disease which was made complex by over-reliance on CFP and fundus auto fluorescence. Optical coherence tomography angiography (OCTA) has been recently added to the imaging armamentarium (**Fig. 3**). This technology provides OCT-based, depth-resolved images of retinal and choroidal blood flow in a non-invasive manner [158], and promises to increase our understanding of disease pathogenesis and progression [159]. Drawbacks of the technique include longer acquisition times than SD-OCT, artifact identification and removal and limited quantitative information, but advances in these areas are taking place rapidly.

Finally, specific methods of artificial intelligence (machine learning and deep learning) are being increasingly used for automated image analyses in color fundus images and SD-OCT [160]. Their scope is wide, and include disease detection [161]. Deep learning is effective for classifying normal versus age-related macular degeneration OCT images [162], classification [163], quantification of specific disease features [164], and identification of hidden patterns that can be used to improve the prognosis [165] or response to treatment more efficiently [159, 166]. In conclusion, the continuing identification of potential non-genomic biomarkers is promising for establishing personalised treatment paradigms for AMD. The recently established -omics techniques might produce novel results that can lead to better treatment strategies [148].

5. Conclusion

Prediction models are improving with the inclusion of new scientific data, and significant progress made in understanding the complex molecular mechanisms associated with AMD pathogenesis. We predict that developing precision medicine for sufferers of AMD will be achieved when clinical and basic scientific information is coupled with advanced bioinformatics and, possibly, appropriate use of artificial intelligence. Combination of phenotype, genotype, environmental factors and yet undefined set(s) of biomarkers will achieve acceptable risk profiling and allow more precise response to treatment prediction.

In nAMD, new drugs with a different mechanism of action to anti-VEGF antibodies could help those who do not respond to regular injections. This requires identification of relevant pathways for specific geno- and phenotypes so selection of intervention could be better tailored. Personalised therapies will have to cater for the large patient population with GA. As progression and visual loss is slow in GA and not imminent in early AMD, personalised medicine will have to rely on new biomarkers to achieve high diagnostic accuracy and low complication rates, allowing good response in those who have a chance to respond, but not treating anyone for whom such treatment might be detrimental.

Our understanding of precise clinical phenotypes for AMD appears to be far from complete. New imaging modalities and careful analysis of these can lead to visualisation of new phenotypes, potentially leading to identification of important clinical features, is was the case for reticular pseudo-drusen. This phenotype is seriously under-represented in earlier studies

but recently with a consensus in their definition, classification and approaches to their valid and reproducible quantification is helping to determine their role as independent factor in prognostic modelling [167]. It appears, that re-classification of AMD features and stages might be appropriate to take the first steps towards precision medicine approaches. The establishment of large data- and image-sets organized in searchable databases, together with appropriate reporting of prospective studies leading to reliable meta-analyses [168] will significantly accelerate the identification of relevant clinical, genetic and environmental factors. In turn, these will lead to personalised medicine being appropriately applied in clinical practice for AMD. This will reduce the financial and societal burden of AMD-related blindness, help to refine treatment approaches tailored to the individual. With this, the future appears to be brighter to those with different forms of AMD.

Expert opinion

Precision medicine has a real potential to revolutionise the care provided to AMD patients. The promise that new precision medicine approaches will allow the reduction of AMD related visual loss, despite the exponential growth in the number of aged individuals around the World, is very appealing. The benefit delivered by personalised approached is shared between affected individuals, their care providers as well as the wider society. Retaining independence longer by reducing or delaying the onset of visual loss and the consequent comorbidities will deliver very significant financial benefit for the health service sector too. It is clear that the need to deliver existing therapies to individuals who would definitely benefit from these is paramount. Then, designing new therapeutic approaches to those who could not yet be treated is both an exciting and a daunting task at present. The co-development of new therapeutic agents with companion diagnostic devices with demonstrable clinical utility will undoubtedly require new approaches, new knowledge and new ways to analyse the information generated.

In nAMD, new drugs with a different mechanism of action to anti-VEGF antibodies will help those who do not respond to the current regular injections. There are several new molecular pathways and molecular targets interrogated at present, raising the hope that this most aggressive form of and stage AMD will benefit from new approaches. The progression of GA and early AMD is slow compared to nAMD, therefore, personalised medicine approach will have to rely on new imaging and molecular biomarkers to achieve high diagnostic accuracy

and a low complication rates, allowing good response in those who have a chance to respond, but not treating anyone for whom such treatment might be detrimental.

Our better understanding of the varied clinical phenotype of the different forms of AMD is advancing, but it is far from complete. New imaging modalities are introduced to visualize previously not appreciated phenotypes, some of which can subsequently led to identification of important clinical features, such as the recently identified reticular pseudo-drusen. This phenotype is seriously under-represented in clinical studies to date due to the lack of consensus in their definition, classification and approaches to their valid and reproducible quantification. In addition, a concerted effort will be required to define the molecular composition and the cellular processes behind the development of this, and any other new phenotype(s) before we could use these as independent prognostic indicators and a druggable target. The ongoing close multidisciplinary collaboration between clinical and basic scientists is proving to be successful in tackling the complex problems of multifactorial diseases like AMD, raising the hope that sooner rather than later we will be in the position to deliver precision medicine for more and more AMD sufferers.

The improvement and diversification of new clinical and basic research information come with challenges. While the establishment of large data- and image-sets are being organized into searchable databases processing of these information will require the use of deep learning and artificial intelligence approaches. These, together with the planning of registered, prospective studies and a cooperative environment with sharing of individual patient data to ease meta-analyses will significantly accelerate the identification of most relevant clinical, genetic and environmental factors and in turn, will lead to better precision and as such, personalised medicine practices for AMD.

While progress is somewhat currently held back by our rudimental understanding of molecular mechanisms underpinning the initiation and progression of AMD, there is every chance that this will change rapidly. Naturally, we hope to completely alleviate AMD, however, it is important to consider that we might not need to be able to stop the disease completely. It might be just as beneficial to slow the progression of the disease to the point that it is unlikely that it would lead to significant visual loss, a potentially more achievable target.

Five-year view

With the decreasing cost of whole genome sequencing in the next five years there will be a significantly better understanding of the genetic background of AMD. Illumina recently announced that whole genome technology will be available at $100 per patient. In addition, with broader approaches identification and integration of new biomarkers and molecular pathways into disease stratification will be driving patient selection for clinical trials. The better stratification will allow significantly improved precision in study design. With the ever-increasing precision and availability of clinical imaging modalities and emergence of big data sets for every participant, machine learning and artificial intelligence will help faster and more precise phenotyping. The introduction of artificial intelligence will also enable development of significantly improved prediction models leading to discoveries of prognostic factors (demographic, biological, clinical, etc.), assessment of their individual role in multivariable prognostic model research and validation in an external, independent cohort of patients. These will lead to the introduction of reliable commercialized genetic tests for risk assessment of developing AMD and subsequent safe genetic profiling of patients for optimizing AMD treatment. As a consequence of the improved precision, new, significantly more specific therapeutic agents will be tested for both the early and late stages of AMD.

Key issues:

- Harmonization of EU regulation is underway to streamline the future application of CDx (genetic tests for example) in diagnosis and treatment of AMD patients.
- Need a better understanding and integration of the underlying molecular mechanisms of the heterogeneous disease processes.
- Need to improve the design, conduct, analysis and reporting of prognostic factor and prognostic model research to minimize bias.
- Need to develop and provide access to very large datasets collected across Europe and the Globe to help development of machine learning and artificial intelligence approaches.

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●● **A review that summarises the magnitude of the blindness sand the expected disease burden in the near future.**

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Figures and Tables

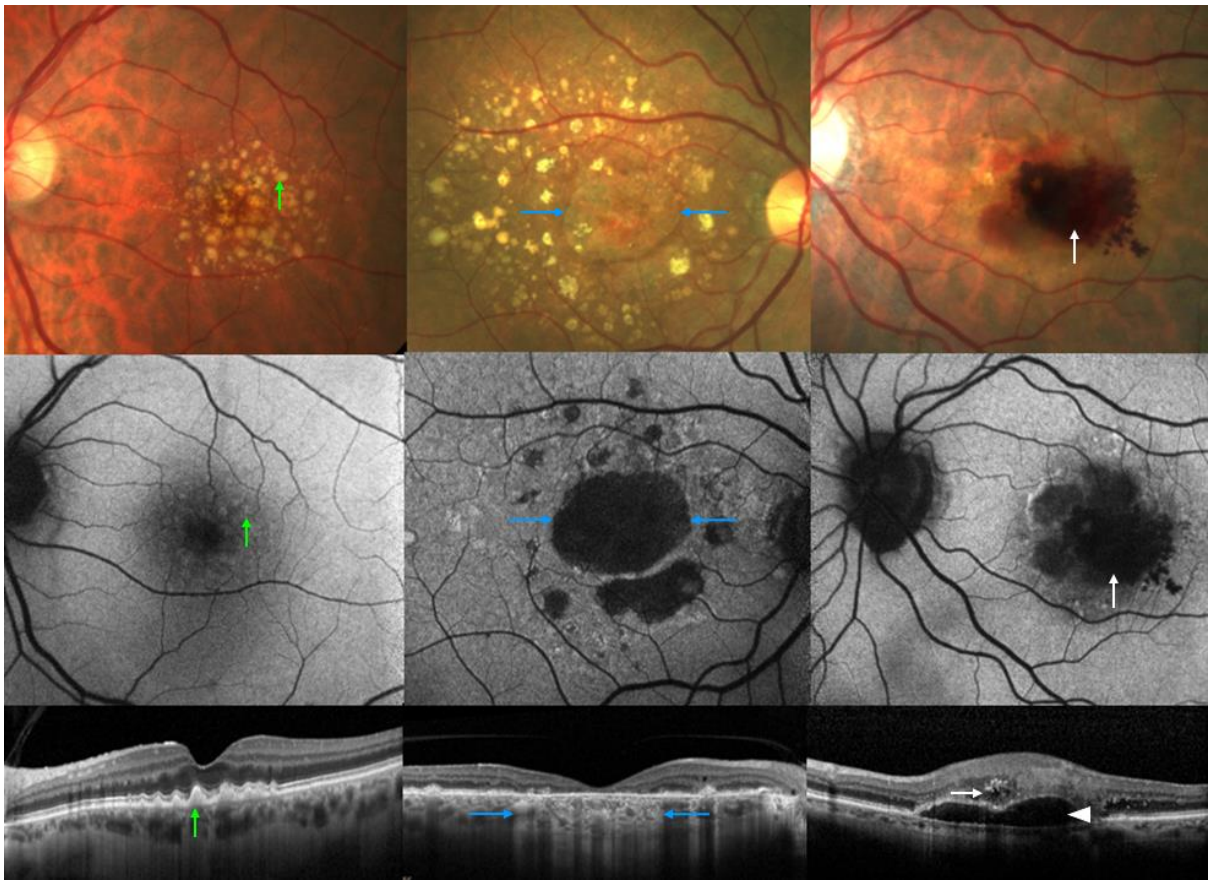

Figure 1. Examples of different AMD stages. Left, drusen and RPE abnormalities in intermediate AMD. Center, geographic atrophy. Right, neovascular AMD. Top row images are color fundus photographs; second row show FAF images; and third row, SD-OCT images. First column, the green arrow points to drusen, yellowish deposits on color fundus photography which are barely visible on FAF; on cross-sectional SD OCT, drusen appear as small elevations of the RPE. Second column, the blue arrows point to the borders of RPE atrophy, more readily visible on FAF (black area) than on color fundus images; blue arrows on SD-OCT point to an area of increased signal penetration into the choroid due to loss of overlying retinal tissue caused by RPE atrophy. Third column, white vertical arrows point a retinal haemorrhage on color fundus photography and FAF; white arrowhead on SD-OCT points to an RPE detachment and small white arrow to intraretinal fluid, signs of active neovascular AMD. AMD: age-related macular degeneration; FAF: fundus autofluorescence; RPE: retinal pigment epithelium; SD-OCT: spectral domain optical coherence tomography.

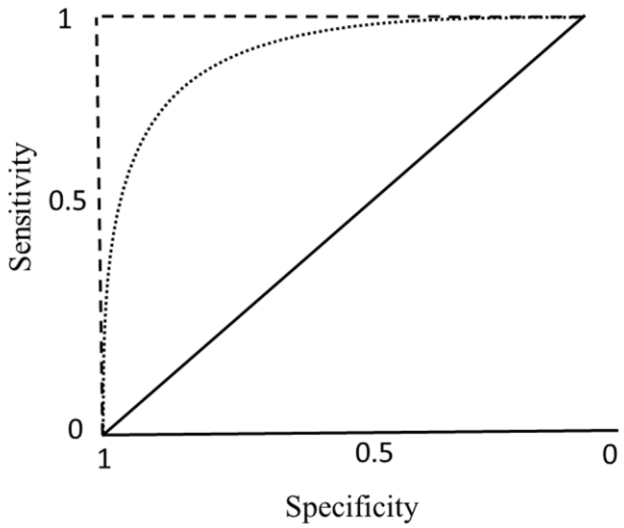

Figure 2. Receiver-operating characteristic curve (ROC) – an example. The solid black line represents an area under the curve (AUC) of 0.5 – random chance. The dash black line represents AUC of 1 – perfect model. The dotted line is ROC with AUC between 0.5 and 1 representing real predictive risk model.

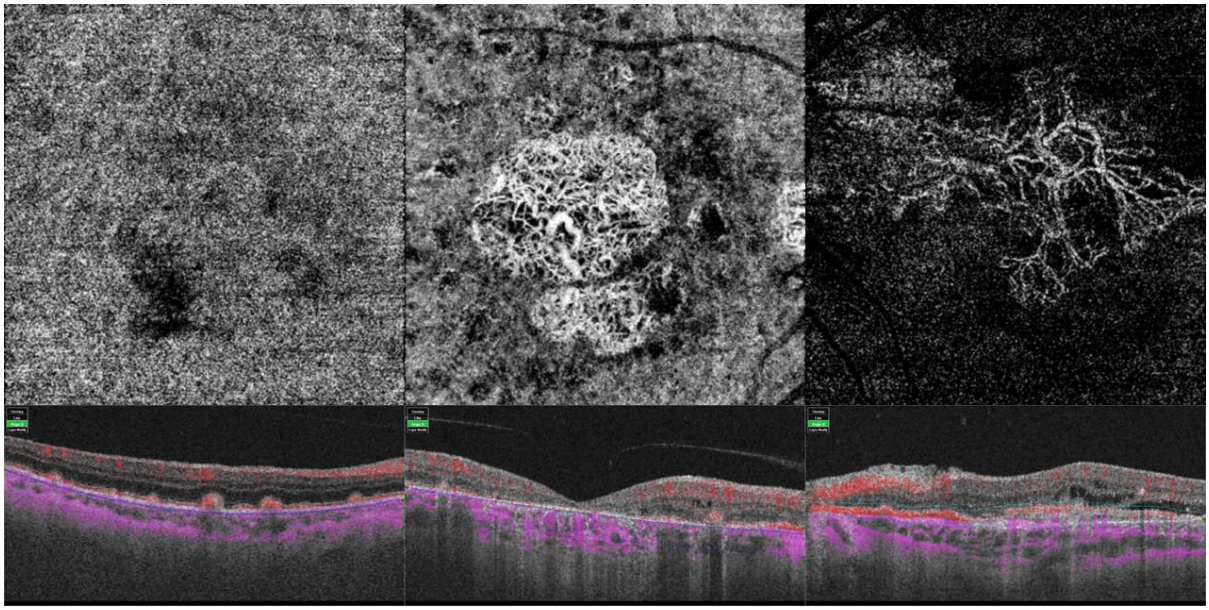

Figure 3. Examples of OCTA images in AMD. Left, drusen in intermediate AMD. Center, geographic atrophy. Right, neovascular AMD. First row shows *en face* OCTA images; second row shows B-scans with flow overlay; the thin blue lines represent the slab of tissue shown in a coronal perspective on top images. First column, apparent decreased choroidal perfusion below drusen. Second column, the area of GA is clearly delimited, through which the choroidal vessels can be clearly seen. Third column, the neovascular AMD vessels are clearly identifiable in the *en face* projection of the photoreceptor layer, while fluid is identified as black intraretinal spaces in the B-scan. AMD: age-related macular degeneration; OCTA: Optical coherence tomography angiography.

Classification of AMD	Definition
No apparent aging changes	No drusen and no AMD pigmentary abnormalities
Normal aging changes	Only drupelets and no AMD pigmentary abnormalities
Early AMD	Medium drusen >63 µm to 125 µm and no AMD pigmentary abnormalities
Intermediate AMD	Large drusen (>125 µm) and/or any AMD pigmentary abnormalities
Late AMD	Neovascular AMD and/or any geographic atrophy (GA)

Table 1. Classification of AMD