Omics Biomarkers in Ophthalmology

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"Omics" refers to high-throughput analyses of genes, proteins, or metabolites in a biological system, and is increasingly used for ophthalmic research. These system-based approaches can unravel disease-related processes and are valuable for biomarker discovery. Furthermore, potential therapeutic targets can be identified based on omics results, and targeted follow-up experiments can be designed to gain molecular understanding of the disease and to test new therapies. Here, we review the application of omics techniques in eye diseases, focusing on age-related macular degeneration (AMD), diabetic retinopathy (DR), retinal detachment (RD), myopia, glaucoma, Fuchs’ corneal dystrophy (FCD), cataract, keratoconus, and dry eyes. We observe that genomic analyses were mainly successful in AMD research (almost half of the genomic heritability has been explained), whereas large parts of disease variability or risk remain unsolved in most of the other diseases. Other omics studies like transcriptomics, proteomics, and metabolomics provided additional candidate proteins and pathways for several eye diseases, although sample sizes in these studies were often very small and replication is lacking. In order to translate omics results into clinical biomarkers, larger sample sizes and validation across different cohorts would be essential. In conclusion, omics-based studies are increasing in ophthalmology, and further application to the clinic might develop in the years to come. Integration of genomics with other types of omics data has the potential to improve the accuracy of predictive tests. Moreover, in the future, omics may lead to stratification of patients into subgroups based on molecular profiles, enabling the development of personalized treatments.

Keywords: omics, genomics, proteomics, transcriptomics, metabolomics

Common eye diseases are often complex, multifactorial diseases without classic Mendelian inheritance. Several genetic and environmental risk factors have been associated with these diseases, but their combinatorial effects and implication in pathogenic mechanisms remain poorly understood.¹ Low-throughput genetic and subsequent functional testing of candidate genes and proteins provide only limited information for these type of diseases. Therefore, there is a need for high-throughput analyses, so that a whole system can be evaluated in a hypothesis-free way.² Omics is the large-scale characterization and quantification of biological molecules, which went through rapid advancements over the last years due to technological improvements in sequencing techniques, mass spectrometry, and bioinformatic analyses. However, due to the very large scope of the datasets and disease heterogeneity, it might be challenging to extract biologically meaningful information from omics studies. Here, we discuss the use of omics techniques in ophthalmic research, focusing on age-related macular degeneration (AMD), diabetic retinopathy (DR), retinal detachment (RD), myopia, glaucoma, Fuchs’ corneal dystrophy (FCD), cataract, keratoconus, and dry eyes. We describe the omics technologies used in ophthalmology, provide an overview of the achievements in each of these diseases so far, and discuss the potential for the future.

Omics Techniques
Omics experiments can be performed on different layers within a biological system (Fig. 1). On the genomic level, the majority of discoveries have been made by genome-wide association studies (GWAS), in which DNA samples of large case-control cohorts are genotyped using microarrays, targeting a large number of single nucleotide polymorphisms (SNPs) distributed over the genome. Subsequent statistical analysis determines for each variant whether the frequency is significantly different in cases compared to controls.³ This approach is designed to detect associations of genetic variants that are mostly common in the population, which in general have small or modest effect sizes. In order to identify rare variants with relatively larger effect sizes, microarrays targeting rare variants (exome chips), whole exome sequencing (WES), or even whole genome sequencing (WGS) is more effective.⁴,⁵ Although WES and WGS have not been used very extensively yet in common eye diseases because of the still relatively high costs, an increase in their use is expected in the upcoming years. Furthermore,
relevant information can be extracted by developing and applying algorithms to detect gene–gene and gene–environment interactions. These higher-order interaction effects may explain additional genetic variability in common eye diseases.6–8

The genetic code alone only partially contributes to the development of a complex disease. Environmental factors influence gene expression levels and metabolism that ultimately triggers pathogenic mechanisms (Fig. 1). To explore actual disease-causing processes, genomics should therefore be complemented with other omics techniques, such as epigenomics, transcriptomics, proteomics, and metabolomics. Since the regulation of gene expression is specific to tissues and cell subsets, these types of omics studies face challenges in acquiring appropriate tissues when they are not readily accessible, like the eye. The sample sizes used in genomic studies performed so far compared to other omics studies reflect this limitation: Recent GWAS contain up to 45,711 subjects in total,9 whereas the number of subjects did not exceed 455 in any of the other omics disciplines10 (Supplementary Tables S1–S9).

Epigenomics refers to the large-scale analysis of functionally relevant changes to the genome that do not involve a change in the DNA sequence. Gene expression is regulated by various epigenetic mechanisms, including DNA methylation and different types of histone modifications (e.g., methylations and acetylations). These modifications affect the accessibility of DNA to bind transcriptional activators and to elongate messenger RNA (mRNA). Over the past years, several techniques have been developed to profile epigenetic marks on a genome-wide scale and to determine the spatial organization of chromatin.11 Until now, ophthalmic research has been restricted to investigations on DNA methylation profiles, which are commonly assessed using DNA methylation arrays or bisulfitesequencing.12

Transcriptomics refers to the large-scale analysis of the transcriptome—the set of all mRNA molecules transcribed from the genome. Evaluating the transcriptome in eye diseases has so far mainly been done by RNA microarray experiments (Supplementary Tables S1–S5, S5, S7, S8), but this is likely to increasingly shift toward RNA sequencing (RNA seq). RNA seq is currently more expensive than microarrays, but has the advantage to detect not-annotated sequences and alternative splice variants next to expression levels.13 Recently, studies investigating the expression of noncoding, regulatory RNAs like micro RNAs (miRNAs) and long-noncoding RNAs (lncRNAs) have also been reported in ophthalmology. miRNAs specifically bind to target mRNAs and affect the level of these targets and subsequent protein production.14 lncRNAs regulate both transcription and translation via different mechanisms.15 Disease-specific miRNA and lncRNA profiles have been extensively studied in several diseases over the last few years and are interesting biomarker candidates since miRNAs and lncRNAs are, unlike most other RNAs, stable in the circulation.16,17 In addition, evaluating their target mRNAs could provide insight into pathogenic mechanisms.18

Proteomics refers to the large-scale study of produced proteins and their modifications, and metabolomics analyzes a wide range of small molecules in a biological sample. Analysis of proteomes and metabolomes is generally performed with mass spectrometry technologies. Mass spectrometry is based on ionization of proteins or metabolites, followed by mass spectrometric analysis of the mass/charge (m/z) ratios of a peptide or metabolite. For identification of proteins or metabolites, public databases can be used, enabling the analysis of complex protein or metabolite mixtures with high sensitivity.19,20 However, improvements are still being made in protein and metabolite identification protocols and databases, application of different ionization techniques, and mass spectrometry instrumentation. In addition, specific methods exist for systems-level analysis of protein glycosylation (glycomics) and lipids (lipidomics), although these have so far rarely been used in ophthalmology (Supplementary Tables S1, S2, S9).

Novel data analysis algorithms are continuously developed and improved to facilitate the interpretation of omics data, as relevant information might be hidden or masked in the large datasets. Instead of merely considering individual up- or downregulated genes or proteins, network- or pathway-based analyses (interactomics) are increasingly applied in ophthal-
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AGE-RELATED MACULAR DEGENERATION (AMD)

Since the first GWAS was reported for AMD in 2005, a number of GWAS have been performed in different populations (Supplementary Table S1). The largest GWAS so far, which analyzed DNA samples of 16,144 patients and 17,832 controls using exome chips, identified 52 AMD-associated genetic variants at 34 genomic loci. Additionally, two relatively small case-control WES studies have been reported for AMD. Examining the biological pathways in which the associated genes function suggests the complement cascade, lipid metabolism, and extracellular matrix (ECM) remodeling to be the major pathways implicated in AMD pathogenesis (Supplementary Table S1). Although these genomics studies improved the biological insights into AMD, still many questions remain unsolved. The majority of the associated variants are in non-coding regions and their functional effects still need to be determined. Therefore, in the years to come the field will shift toward postgenomic research to understand the effect of the identified genetic variants on the disease pathogenesis of AMD.

Several efforts have been made to profile AMD-specific transcriptomes, proteomes, metabolomes, and DNA methylation patterns (Supplementary Table S1). Most apparent in these datasets is an enhanced activity of the immune system, appearing in all types of omics and in varying eye tissues and fluids (Supplementary Table S1). In a number of these studies, elevated levels of complement proteins were detected, congruent with the genetic data. Also proteins associated with ECM pathways were differentially expressed in AMD patients compared to controls. Following the indications for altered lipid metabolism, AMD research would benefit from lipidomics studies, of which one has been reported so far. In this study, analysis of lipid profiles in patients with a polypoidal choroidal vasculopathy subtype of AMD proposed platelet-activating factor (PAF) as a key indicator of altered lipid metabolism. Besides the predicted AMD pathways from genomic studies, oxidative stress-related factors such as antioxidant genes and genes involved in stress-induced protein unfolding and aggregation have regularly been found to be differentially expressed, as detected on the DNA methylation, mRNA, and protein level. Furthermore, enrichment of oxidative protein modifications was found in proteomic analysis of drusen. These findings support a role of increased oxidative stress as an important environmental risk factor for AMD.

Similar to the findings in eye materials, upregulation of immune response-related miRNAs and proteins has been found in white blood cells and plasma, potentially indicating systemic inflammation in AMD. Furthermore, based on selection of candidate proteins by proteomic analyses and subsequent targeted experiments, vinculin, phospholipid transfer protein (PLTP), and mannann-binding lectin serine protease 1 (MASP1) have been proposed as plasma biomarkers for AMD. Besides proteins, miRNAs are interesting biomarker candidates for AMD, since AMD-specific miRNA profiles have been identified in plasma and serum. However, still little is known about the role of these differentially expressed miRNAs in AMD, and their use as biomarkers requires further validation.

In conclusion, genomics has revealed a large part of the genomic heritability of AMD. The AMD-associated variants identified in the largest GWAS together account for 46.7% of the total genetic variability in European-ancestry subjects. The remaining heritability may reside in rare genetic variation and structural variation of the genome as well as gene–gene and gene–environment interactions. Several epistatic effects have been identified in AMD datasets, mainly involving genetic interactions with complement factor H (CFH), and the effect of several variants has been found to be modified by smoking. Combining genomic information with environmental factors or other types of omics data might improve the predictive accuracy to AMD susceptibility. It has already been reported that certain proteomic biomarkers (carboxyethylpyrrole [CEP] oxidative protein modifications and CEP autoantibodies) could distinguish the plasma of AMD patients from control samples with 76% accuracy, whereas these proteins together with genomic markers (ARMS2, HTRAI, CFH, C3) reached 80% discrimination accuracy. In addition, plasma measurements of complement components (C3a, Bb, and C5a) combined with genetic factors further increased the discrimination accuracy up to 94%. Applying these types of integrations on large-scale omics datasets will potentially further improve prediction models and expand biological insights.

DIABETIC RETINOPATHY (DR)

Although a considerable heritability for DR has been proposed (25%–52%), the detection of DR-associated variants has been challenging—only two out of six GWAS performed so far identified loci with genome-wide significance (Supplementary Table S2). Candidate genes identified in these studies have roles in endothelial cell angiogenesis, capillary permeability, and insulin signaling, among others (Supplementary Table S2). Strikingly, the top SNPs identified in these GWAS are hardly ever replicated in follow-up studies. This suggests that DR etiology might be very heterogeneous, and that larger sample sizes and meta-analyses are required to advance our understanding of the role of genetics in DR.

Temporary hyperglycemia disturbs metabolic homeostasis and is thought to contribute to DR development. Therefore, analyzing metabolic signatures may give valuable insights into this disease. Three metabolomic studies have been reported so far in DR research, suggesting enhanced lactate and glucose metabolism, and overactivity of the arginine-to-proline pathway. In addition, L-aspartic acid and linoelic acid have been found to distinguish different stages of DR. Further investigations may reveal the use of these signatures as biomarkers or even therapeutic targets. Furthermore, hyperglycemia has been linked to alterations in epigenetic modifications, which is a relatively new field of interest in DR research. Even if hyperglycemia is short lasting, changes in the epigenome could persist due to metabolic memory. Three genome-wide methylation studies revealed DR-specific DNA methylation profiles, and identified the natural killer cell-mediated cytotoxicity pathway as potentially involved in DR pathogenesis. Interestingly, an increase in global DNA methylation levels in blood has been correlated to the severity of DR, independent of common DR risk factors. This suggests DNA methylation patterns as potent candidate biomarkers for DR, although these findings warrant replication in larger cohorts.

Since proliferative DR (PDR) is characterized by neovascularization and fibrovascular proliferation, targeted studies have focused on detecting proteins related to these processes. In a transcriptomic analysis of fibrovascular membranes, angiogenesis appeared to be differentially expressed in PDR patients, along with ECM pathways. Furthermore, altered levels of pigment epithelium-derived factor (PEDF) and
clusterin (apolipoprotein J), which are both regulators of angiogenesis, have been identified frequently in proteomic studies.\textsuperscript{70,72–78} In addition, other members of the apolipoprotein family are regularly identified (Supplementary Table S2). Caosse et al.\textsuperscript{79} listed all potential biomarkers for DR in different eye tissues, and noticed that apolipoprotein A1 (ApoA1) is the only DR-associated protein present in vitreous and aqueous humor, as well as in tears. In a search for early plasma DR biomarkers, Jin et al.\textsuperscript{78} performed data mining on previously reported biomarker candidates, followed by extensive replication and validation experiments. This approach yielded a biomarker panel of four proteins (APOA4, clusterin, complement component 7 \textsuperscript{[C7]}, and inter-alpha-trypsin inhibitor heavy chain 2 \textsuperscript{[ITH2]}) that was able to discern DR status between patients and controls.\textsuperscript{78} A variety of other processes have been detected in proteomic studies (Supplementary Table S2). Besides the different tissues that are used (e.g., vitreous, fibrovascular membranes, blood samples), also the different type of controls used in these studies (e.g., diabetic controls, patients with macular holes, healthy controls) might explain the observed diversity in the results.

RETINAL DETACHMENT (RD)
Retinal detachment (RD) has some well-known risk factors such as myopia, lattice degeneration, and trauma (e.g., cataract surgery), but very little is known about the mechanisms behind nonsyndromic RD.\textsuperscript{80} To date, one GWAS has been reported on rhegmatogenous RD (RRD), in which one genome-wide significant signal was identified (after meta-analysis) in ceramide synthase 2 \textsuperscript{(CERS2)}.\textsuperscript{81} This result, in combination with a number of suggestive associations, points toward a potential role for cell adhesion and migration and apoptosis of photoreceptors and RPE cells in the pathology of RRD (Supplementary Table S3). In addition to this GWAS, only a limited number of transcriptome, proteome, and metabolome analyses have been performed on RRD (Supplementary Table S3). Altered inflammatory responses have been detected in all of these studies, and several potential biomarkers have been proposed. However, the number of subjects in these studies is very low (8–24 cases), so additional research with larger sample sizes is necessary to expand the molecular understanding of RD development and to identify valid biomarkers.

MYOPIA
The heritability for refractive error has been calculated to be larger than 50%, and some studies even estimated the heritability of myopia to be as high as 90% to 98%.\textsuperscript{82–86} To unravel genetic risk factors, several large GWAS and meta-analyses have been performed through international collaborations on myopia or refractive phenotypes relating to myopia (Supplementary Table S4). Additionally, a number of WES studies has been performed, mainly in families. These studies have shed light on possible myopia-associated pathways; however, still only 3.4% of all genetic variability of refractive errors can be explained by the identified variants.\textsuperscript{87} The high prevalence and variability in refractive error among the population suggest that this phenotype is mainly caused by common variants.\textsuperscript{87} Considering the small fraction of the explained genomic heritability, a large part could reside in gene–gene interactions of common variants with low effect sizes, which are difficult to identify. Additionally, gene–environment interactions involving, for example, near work, spending time outdoors, and education, have been suggested to contribute to myopia development.\textsuperscript{88,89}

Although these findings suggest that myopia is a genetically heterogeneous disease, the pathways that have been identified in myopia are quite reproducible across studies.\textsuperscript{85} It is thought that transmission of visual signals regulates eye development via ECM remodeling of the sclera, and that disruptions affecting this cascade may contribute to the development of refractive errors.\textsuperscript{82,90,91} The majority of myopia-associated pathways are possibly involved in this process (Supplementary Table S4), although the specific disease mechanisms and influences from environmental factors have not been uncovered in detail. In conclusion, GWAS results have been valuable in myopia research, but will probably not succeed in solving a large part of the genetic variability. Analyses of gene regulatory networks and signaling pathways, rather than investigating genes one by one, would possibly be a more effective way to gain understanding in this phenotype.\textsuperscript{91} Moreover, transcriptome, proteome, and metabolome analyses, which have rarely been reported for myopia, might provide additional information on disease-associated pathways.

GLAUCOMA
In glaucoma, elevated intraocular pressure (IOP) often precedes degeneration of retinal ganglion cells, resulting in irreversible damage to the optic nerve. Both the increase of IOP and development of glaucoma depend on genetic as well as environmental risk factors, but our understanding of the molecular disease mechanisms remains limited.\textsuperscript{92} Primary open angle glaucoma (POAG) is the most extensively studied glaucoma subtype. Despite a number of GWAS and WES studies in POAG patients, still only 1% to a few percent of the genomic variability can be explained by the identified variants, which have only moderate effect sizes.\textsuperscript{93,94} POAG-associated loci have not been shown to cluster clearly into pathways, but point toward a wide variety of potential disease pathways in POAG development (Supplementary Table S5). Omics-based research in the field of primary angle closure glaucoma (PACG) and primary exfoliation glaucoma (PEXG) is still very new—on the genome level, only two and three GWAS have been performed for PACG and PEXG, respectively (Supplementary Table S5). Whereas most of the identified variants explain only a very small portion of the heritability, genetic variants in the lysyl oxidate-like protein 1 (LOXL1) have been identified as important genetic risk factors for PEXG development. The high-risk haplotype has been reported to increase the chance of PEXG 27 times relative to the low-risk haplotype.\textsuperscript{95} POAG, PACG, and PEXG are suggested to each have their own genetic risk profile; however, indications of ECM and cell adhesion alterations have been found in all three subtypes.

Additional genomic research to unravel the genetic causes of glaucoma has been done using GWAS in which known anatomic risk factors were taken as quantitative traits (e.g., see Refs. 94, 96–101). With these studies, genetic factors involved in IOP, disc area, vertical cup–disc ratio, cup area, anterior chamber depth, and central corneal thickness have been identified, which are potentially relevant for glaucoma development.\textsuperscript{102,103} Taking this information into account with regard to possible POAG-causing mechanisms, the major pathways suggested to be involved include ECM, TGF-β and -β signaling, vascular tone, RhoA/Rho-associated kinase pathways, and eye development, as reviewed by Iglesias et al. in 2015.\textsuperscript{104} Although these mechanisms remain largely speculative, a first step has been made toward molecular understanding of POAG.

In order to gain insight into pathogenic events, other types of studies might complement the limited genetic knowledge of glaucoma. Mainly for POAG, disease-specific transcriptomes

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and proteomes have been investigated in specific cell types involved in glaucomatous degeneration. These cell types include the trabecular meshwork and Schlemm’s canal, since they contribute to the resistance of aqueous humor drainage.95–101 Furthermore, lamina cribrosa cells of the optic nerve are also useful, as they experience the mechanical stress caused by elevated IOP.95 Based on differentially expressed mRNAs and proteins, cell adhesion, ECM, and cell motility have been identified as candidate pathways involved in POAG consistently in all three of these cell types, agreeing with genetic findings.104–108 Additionally, similar results have been obtained in optic nerve head astrocytes, which are the most common cells in the optic nerve head and might also contribute to degeneration of retinal ganglion cells.109,110

Proteomic analyses in aqueous humor and serum mainly found proteins related to inflammation and oxidative stress to be differentially expressed in POAG and PEXG patients.111–113 The higher presence of immune-related proteins in serum of glaucoma patients was already known, and serum antibodies with target antigens in eye tissues were the main proteins screened for biomarker discovery in the past.114 Recently, proteomic studies characterized glaucoma-specific proteomes in tears, which might be an interesting source to further explore in the future, particularly because of the noninvasive nature of tear collection.115–118 Furthermore, mass spectrometric analyses of oxidative modifications might be valuable, since these modifications are common in glaucoma patients and could function as biomarkers.114 Overall, several biomarkers have been proposed following proteomic analysis but still require further validation in larger cohorts, and their clinical usefulness needs to be confirmed.114 Especially early-stage biomarkers would make a valuable contribution to the clinic, since glaucoma is often not noticed until irreversible damage to the optic nerve has already occurred.92

ANTERIOR SEGMENT DISORDERS: FUCHS’ CORNEAL DYSTROPHY, CATARACT, KERATOCONUS, AND DRY EYES

Compared to the most extensively studied retinal disorders, the number of omics experiments performed for corneal diseases is relatively small (Fig. 2). Although multiple family studies have been performed for congenital forms of cataract, only two GWAS have been reported (Supplementary Table S7). Likewise, FCD and keratoconus have been investigated by GWAS only once and twice, respectively (Supplementary Tables S6, S8). For FCD, a strong genetic association was identified in the transcription factor 4 (TGF4) gene—homozygous variants in this gene increase the risk for FCD 30 times.110

Proteomic research might be an effective way of investigating corneal diseases, since cell type-specific information can be obtained from fresh corneas or lenses collected during surgery. Furthermore, it is thought that cataract develops as a consequence of long-lived proteins in the lens that are subject to degradation or undergo posttranslational modifications (PTMs).120–122 Proteomic approaches may reveal the susceptible proteins and the attachment sites for these detrimental PTMs. A common finding in several studies is altered expression of crystallins (Supplementary Table S7), which are constituents of the lens with very low protein turnover.121 Moreover, elevated oxidation of cysteine residues has been found in crystallins of lenses from cataract patients.123 Additionally, several other proteins have been associated with cataracts, which are not very consistent across different studies, and their contribution to the disease etiology remains largely unknown (Supplementary Table S7). For keratoconus, associations with several cytoskeletal and ECM-related proteins have been identified, but similarly, the results are quite divergent, and no reproducible biomarker has been identified yet (Supplementary Table S8).124

Dry eyes are characterized by alterations in the composition of the tear film, resulting in deteriorated maintenance of the ocular surface.125 Therefore, analyzing the tear proteome is highly relevant for understanding this disease and for identification of biomarkers.126 The majority of omics studies performed so far for dry eyes are proteomic analyses in tears, along with some metabolomics and lipidomics studies, whereas no genomic and transcriptomic analyses have been performed (Fig. 2). A number of proteins have been identified and replicated in independent proteomics studies, including proline-rich protein 4, pro lactin-inducible protein, lipocalin-1, lysozyme, enolase, and proteins of the S100 family (Supplementary Table S9), suggesting that these are potential biomarkers. The main mechanisms underlying dry eye development are related to decreased tear secretion (aqueous-deficient subtype) and increased tear evaporation (lipid-deficient subtype).127 It is well known that the lipid layer of the tear film is essential for maintaining ocular surface homeostasis and for limiting tear evaporation, which makes lipidomics studies interesting for dry eye research.127 Lam et al.127 in 2014 reported no differences in total lipid levels between the tears of patients and controls, but structural-specific changes related to molecular weights and fatty acid chain saturation were identified in tear lipids. Interestingly, on protein, metabolite, and lipid levels, specific expressions for subgroups of dry eyes based on disease severity have been identified.128–130 These markers might therefore function as indicators of disease progression, although further validation is required to confirm these results.

DISCUSSION

The number of omics studies performed in ophthalmology has increased substantially over the past decade, and sample sizes have been expanding, especially in genomic studies (Supplementary Tables S1–S9). AMD, glaucoma, and DR have been most extensively studied with omics techniques, while the number of studies performed in other eye diseases is limited (Fig. 2). GWAS studies have been commonly used in ophthalmology and have yielded considerable insights, although the results vary between different diseases. Particularly the understanding of AMD has significantly improved due to GWAS, since almost half of the genomic heritability has been revealed, which is unrivaled by any other multifactorial disease.22,131 Interestingly, the identified genes in AMD cluster quite well into disease-related pathways. Subsequent functional work on the complement system, one of the major pathways predicted from the genetic data, has laid the foundation for ongoing human clinical trials exploring the effect of complement inhibition in AMD.4,22 Furthermore, strong genetic associations have been identified for PEXG in LOX11, and for FCD in TGF4.35,110 Other ocular diseases have not benefited extensively yet from GWAS, possibly due to the large heterogeneity and/or too small sample sizes, emphasizing the need for large consortia and multicenter studies. Also more detailed phenotyping and classification into subgroups, which could be accomplished by practicing standard operating procedures (SOPs) for phenotyping and grading, may help to unravel a larger portion of the genetic variability in these diseases.132 Additionally, WES and/or WGS, as well as analyses of gene–gene and gene–environment interactions, might potentially clarify more of the missing heritability.
Omics studies on the epigenome, transcriptome, proteome, metabolome, and lipidome have been mainly performed with relatively small sample sizes (Supplementary Tables S1–S9). These studies identified a number of potential biomarkers, but larger sample sizes and replication in different cohorts are necessary to acquire biomarkers usable for screening and prediction. Currently, development of prediction models for complex diseases mainly consists of genetic information combined with known environmental risk factors. Incorporation of other types of omics data in prediction models for eye diseases has not been done yet on a systems level; however, targeted studies in AMD suggest that there is potential to improve the prediction accuracy by combining genomic and proteomic data.\textsuperscript{4,51,52}

Omics research could be valuable in classification of patients in order to predict their response to treatment. For example, since the complement cascade is only one of the affected pathways in AMD, it is possible that patients with alterations in complement regulatory genes would benefit more from complement-inhibiting therapies than others. Omics experiments could play a role in determining whether a patient falls into this category.\textsuperscript{133} The concept of personalized medicine is already in a more advanced stage in the field of cancer research. For instance, genomic and transcriptomic profiles are being used for prognosis and treatment decisions in breast cancer, and several clinical trials in this field are ongoing.\textsuperscript{134,135} In ophthalmology, predictive tests for common eye diseases are not recommended as long as there is no
clinical intervention to improve disease outcome. This might change in the future, if, for example, a genetic test could predict the efficiency of complement-inhibiting treatment for AMD patients.

While clinically useful biomarkers are preferentially detectable in blood or eventually in tears, omics studies for basic research ideally use specific eye tissues or fluids. Human studies on inaccessible parts of the eye usually rely on postmortem material, which has the disadvantage that it is of lower quality than fresh samples, especially on the RNA level. Several types of omics experiments have been performed in numerous cell and animal models of eye diseases or disease-related phenotypes, in order to search for candidate pathways involved in the disease and to test treatment responses (e.g., see Refs. 137–144). In the future, model systems for further omics-based analyses might be obtained through differentiation of patient-derived induced pluripotent stem cells into eye-specific cell types relevant to the disease of interest.

Deeper understanding of a biological system could be obtained by integrating different layers of information like genomics, proteomics, transcriptomics, and metabolomics. Considering the complex nature of multifactorial diseases, investigating the whole system might be more relevant than focusing on only one of these processes. Although this is challenging and requires advanced bioinformatics, systems approaches have already been proven to be useful in other complex diseases like cancers (e.g., see Refs. 147–149). Since relatively little is known about multifactorial eye diseases, we would expect the field of ophthalmology to benefit from omics technologies in the near future, both in terms of understanding disease pathology and development of personalized therapies.

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