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Investigating the genetic complexity of glaucoma

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CHAPTER 7

General discussion



Glaucoma is the second leading cause of blindness worldwide and primary open-angle glaucoma (POAG) accounts for the majority of cases. Many studies have shown that glaucoma has a genetic component, but the identification of all the causative genetic factors has proven to be a challenge since each one contributes only little to the pathogenesis of the disease. For example, a variety of private mutations in genes, like *MYOC* and *OPTN*, are causative in only a few familial cases segregating monogenic forms of glaucoma. Together, these mutations explain only 6% of cases in the general glaucomatous population.¹² GWASs have identified associations with common genetic variation in multiple loci that, when taken together, explain approximately 10% of the heritability. As the total heritability of glaucoma is estimated to be 0.8, a large proportion (approximately more than 70%) of the heritability is still missing. Consequently, it is still necessary to identify more genetic factors to explain this missing heritability.³

The studies presented in this thesis, aimed to provide further insight into the aetiology of glaucoma through multiple approaches and by identifying additional genetic factors potentially associated with this condition. We aimed to find and analyse these genetic factors in nuclear genes, genomic rearrangements, and in the mitochondrial DNA. In this chapter, the main findings, clinical implications, and future directions are discussed.

Main Findings

Monogenic diseases can provide insights into more common forms of glaucoma

Glaucoma is a condition more commonly observed in adults. However, there are also forms of glaucoma that develop in the first few years. These are usually inherited forms of the disease segregating in an autosomal recessive or dominant mode of inheritance. Indeed, congenital glaucoma segregates in an autosomal recessive inheritance, while developmental glaucoma, juvenile and adult onset in a dominant inheritance. In Chapter 3 of this thesis, we performed candidate gene sequencing to identify causative mutations in patients and their families affected by Axenfeld-Rieger syndrome (ARS), a rare dominant disorder characterized by systemic features (craniofacial dysmorphism, microdontia, sensory hearing loss, hypertelorism and congenital heart defects) that leads to the development of glaucoma in approximately 50% of cases.

ARS is a genetically heterogeneous syndrome, which implies that mutations in different genes can lead to similar clinical manifestation.⁴ Rare mutations with large biological effects in the *FOXC1* and *PITX2* genes are known genetic causes of this syndrome.⁵⁶ These genes are involved in the regulation of the embryonic retinal development, through specific temporal and spatial expression patterns.⁷ Looking at their functions, *FOXC1* and *PITX2* are transcription factors of the forkhead and homeodomain families, respectively.⁸ These transcription factors regulate gene expression, so, mutations in these genes can cause pathological conditions and differences in the phenotypic classification of ARS. Indeed, mutations in the *PITX2* gene cause ocular, dental, and umbilical anomalies, while mutations in the *FOXC1* gene cause mainly isolated ocular or combined ocular, heart, and/or hearing defects.⁹ Experimental studies conducted in mouse models have shown that

FOXC1 and *PITX2* and a multitude of other genes work together in the regulation of anterior segment development.¹⁰

Further research of mutations in patients affected by **ARS** is warranted, because in the majority of patients with **ARS** (approximately 60%) the underlying mutations are still undiscovered.¹¹ In Chapter 3, novel heterozygous variations were identified in the coding region of the *PITX2* gene, extending the spectrum of mutations in individuals with **ARS** and enabling an improved diagnosis. In **ARS**, studying potential genotype–phenotype associations remain challenging because a direct relationship between position and nature of the mutation and the severity of the phenotype is not clear. Although it could be that novel mutations or genes remain to be identified, it is also likely that mutations within non-coding regulatory elements of the already known genes can explain cases without a genetic diagnosis.¹² Because most of the variation in the human genome is in non-coding DNA, looking at regulatory elements can contribute to supply new clues about the genetic causes of the disease.¹³ However, unless there is a shift to whole genome sequencing, deleterious mutations in the non-coding regions will remain undetectable.

Among all aforementioned symptomatology in **ARS** patients, one aspect that can benefit from early treatment is glaucoma, since it can prevent vision loss. In fact, when glaucoma arises, the first treatment is to prescribe medical therapy. If this does not prove to be efficacious, surgery is recommended to correct the angle defect in the eyes.¹⁴ When treatment starts early, patients usually respond well with a prolonged **IOP** control over the years and gain normal visual acuity.¹⁵ Genetic analysis in combination with clinical evaluation can help to give a precise diagnosis, and direct further clinical management, useful to inform families and relieve anxiety surrounding this disease. Specifically for patients affected by **ARS**, genetic testing can give important information to those who are carriers of mutations in *FOXC1* and *PITX2* genes.⁹

Genetic testing has an important role in confirming clinical diagnostic suspicions and can become more important as gene/disease specific treatments will be developed for congenital forms of glaucoma. The advent and improved performance of high-throughput technologies, such as the whole-genome sequencing, and the reduced cost of these technologies, has accelerated the diagnosis. However, the limited study population of patients and families with childhood glaucoma represents a challenge for therapeutic trials.

Findings from rare forms of glaucoma may not only promote diagnosis and treatment to the people affected by them, but may also boost the detection of genes and pathways involved in more common forms of glaucoma, as well as contribute to the development of potential novel therapeutic strategies.

Impact of copy number variants in glaucoma

Copy number variations (CNVs) are a recently discovered new type of genetic variation, defined as a class of structural variants that alter the number of copies of specific genomic regions greater than 1 kb in size. These chromosomal regions can be duplicated or deleted. CNVs have been identified as disease risk in approximately 10% of individuals with neurodevelopmental disorders, and in 8% of pathogenic primary immune deficiency.^{16,17} In neurodevelopmental disorders, application in the clinical setting of the identification of CNVs has contributed to increase the diagnostic rates. However, due to the difficulty in interpretation of the functional consequences, there are still challenges in the use of CNVs in clinical settings. In fact, the evaluation of the potential pathogenicity of CNVs is frequently done by searching for overlaps with disease-associated genes or with previously reported pathogenic CNVs. Nowadays, many bioinformatic tools and approaches for analysis of CNVs and their pathological and clinical consequences have been developed. This extends their application in the clinical setting for many conditions.¹⁸⁻²⁰ In Chapter 4, using SNP-array data, we investigated whether common CNVs can help to identify new glaucoma candidate disease genes, and to detect molecular mechanisms underlying POAG. After performing CNV association analysis, a total of 11 genes (*APC*, *BRCA2*, *COL3A1*, *HLA-DRB1*, *HLA-DRB5*, *HLA-DRB6*, *MFSB8*, *NIPBL*, *SCN1A*, *SDHB*, and *ZDHHC11*) were identified. Further functional annotation and pathway analysis suggested involvement of Wnt signalling, p53, and cadherin component related pathways. The genes identified through our CNV analysis do mostly not overlap with previous POAG GWAS findings of single associated genetic variations; a result that emphasizes the importance of the CNV detection as a complementary approach to GWAS. Despite the limitations (e.g., relatively poor agreement between algorithms in CNV detection), SNP-arrays offer important advantages over other techniques (array-CGH and sequencing) to assess CNV at a genome wide level. This includes the possibility of analysing a large number of samples at a relatively low cost and with a small amount of DNA required.

An important step in the CNV analysis is to distinguish associated genetic variations that influence disease pathogenicity from neutral (benign) ones. The classification and clinical impact of CNVs is done through a uniform system. CNVs are generally classified as: benign, likely benign, variant of uncertain significance (or VUS, a CNV for which there is no clear evidence that supports its clinical significance), likely pathogenic, and pathogenic.²¹ Nowadays, thanks to the new tools for CNV detection, a growing number of CNVs is also classified by their potential clinical impact.

For this purpose, many decision support tools for annotation and classification of CNV have been developed (e.g., CNV-Annotator) to support laboratory diagnosticians, genetic counsellors and clinicians.^{21,22} Indeed, in order to ensure consistent classification, standards for evaluating CNVs are constantly being updated, together with recommendations for their interpretation.

Thus, modern CNV interpretation is based on a point-based scoring system that takes in consideration different criteria like genomic region, dosage sensitivity, predicted functional effect, correlated clinical findings with case reports in the literature, the mode of inheritance patterns, and the *de novo* occurrence.²³ Despite all these efforts, a grey area is still occupied by VUS CNVs

and their missing interpretation. To overcome this issue, research groups world-wide are increasingly collaborating by sharing data that might help the clinical interpretation of VUS. This results in faster translation of this knowledge into improved health care for patients. Over the last years, CNV research has been boosted by the growing availability and lower costs of high-throughput sequencing technology, which is able to produce terabytes of genomic data per run, to explore functional implications. Moreover, due to the improving resolution of CNV detection tools, the number of novel structural variants is increasing. Nonetheless, despite the improvements in both methodology and software, clinical interpretation of CNVs remains a challenge and therefore is still not widely used in clinical (genetic) settings.

In summary, combining our data with those of the literature, CNV analysis has proved to be a robust method for exploring mechanisms and pathogenesis of a disease (including glaucoma) and it can become a powerful tool for both research (i.e., gene dosage discovery) and diagnosis, through the evaluation of pathogenic effects. To fully understand the role of CNVs in glaucoma, continued efforts and collaboration between researchers and clinicians are necessary. This is the way to minimize the risk of misclassification and potentially help to improve the genetic-based outcomes.

Mitochondrial genome: another actor responsible for glaucoma complexity

Two important pathophysiological aspects of POAG are the functional decay of the trabecular meshwork and the increased apoptosis rate of retinal ganglion cells (RGCs). Cells in both tissues are affected by oxidative damage due to mitochondrial dysfunction.^{24,25} In chapter 5, we investigated whether mitochondrial genetic variation is involved in POAG. Focusing on homoplasmic mtDNA variations, we performed an association analysis between POAG and mitochondrial genetic variation (SNPs and haplogroups). We found statistically significant associations between POAG and, respectively, two mtSNPs in the genes *MT-CYB* and *MT-ND4*, and haplogroup K.

Mitochondrial dysfunction has been strongly implicated in glaucomatous neurodegeneration. However, how this dysfunction exactly contributes to glaucoma is still not clear. The mitochondrial electron transport chain is a key system in the production of adenosine triphosphate (ATP), the primary carrier of energy in cells, also involved in oxidation-reduction reactions. In these reactions, each electron is located near to molecular oxygen, which brings a leakage of electrons designated to the production of energy. The consequence of this leakage is a decreased production of ATP and an increase in oxidative overload. Factors that can enhance these leakages are mutations in mitochondrial genomes and malfunctions in mitochondrial biogenesis.²⁶ To complicate matters, mitochondrial impairment can also be a consequence of the glaucomatous neurodegeneration: elevated mechanical stress and insufficient retinal perfusion lead to diminished oxidative substrates, disrupted mitochondrial biogenesis, and disrupted electron transport chain. The mitochondrial impairment results in a reduced energy availability, an increased production of reactive oxygen species, accumulation of damaged mitochondria, and the activation of apoptosis.²⁷

An obstacle for the identification of mutations in the mitochondrial genome is represented by its heteroplasmy, which means the presence of two or more copies of mtDNA in the same cell. Indeed, in mammals each mitochondrion harbours approximately 2-10 copies of mitochondrial DNA, where (potentially) mutant mtDNA copies are mixed with wild-type copies.²⁸ In each individual, mutations in the mitochondrial genome may appear in different rates even from one tissue to another.²⁹ But only when a ‘phenotypic threshold effect’ is reached by the mutated mtDNA copies, there is the manifestation of a mitochondrial pathology.³⁰ Nowadays, mitochondrial heteroplasmy can be assayed using data generated from whole-genome, exome, and RNA sequencing.^{31,32} Therefore, detection of heteroplasmic mutations can contribute to the discovery of individuals at high risk.

Patients with mitochondrial risk alleles that are able to alter mitochondrial functions may have different aetiology and response to treatment if compared to patients with POAG that do not carry such risk alleles. Thus, considering glaucoma as a kind of mitochondrial neurodegenerative disease may boost new therapeutic approaches based on increasing mitochondrial biogenesis and on promoting the expression of normal mitochondrial complementary genes.³³⁻³⁵ One approach to identifying POAG patients with a mitochondrial component to their disease would be a genetic analysis of their mitochondrial genome. In future studies, a cumulative genetic risk score could be developed to investigate the phenotypic features of glaucoma patients with an excess of potentially causative and highly penetrant mitochondrial mutations. Understanding the role of these variants could also have the potential to drive further research such as gene replacement therapy, a technique that replaces faulty genes with a correct form using a viral vector.³⁶ The use of gene replacement therapy in mice has shown success for nuclear-encoded mitochondrial genes, with an evident decrease of retinal ganglion cell degeneration.³⁷

A trial conducted in patients suffering from Leber’s hereditary optic neuropathy (LHON), a condition caused by mutations in the mitochondrially-encoded *MT-ND4* gene, showed promising improvements in recovering visual function.³⁸ More research could define glaucoma patients who would benefit from this kind of therapy.

The era of multi-ancestry studies in glaucoma

In the last two decades, GWASs have shown to be a powerful tool for investigating the genetic architecture of human conditions, including glaucoma.

In Chapter 6, we aimed to determine additional genetic factors for POAG by performing a GWAS meta-analysis across six ancestries from 15 biobanks, which are part of the Global Biobank Meta-analysis Initiative. This led to the discovery of 109 genome-wide significantly associated loci, 18 of which were novel and three ancestry-specific loci. Furthermore, we explored the biological implications of our findings by performing extensive post-GWAS analyses: gene and tissue enrichment analyses, transcriptome-wide association studies (TWAS) and a phenome-wide association study. This research led to the discovery of enriched genes related to vascular-related functions and cell proliferation processes (like Wnt signalling). A fifth of the TWAS-prioritised genes responsible for vascular and proliferation functions are primary ciliary related

genes. Our findings suggest a possible link among POAG, vascular and cell proliferation mechanisms, with a potential role for primary cilia in POAG pathogenesis.

The involvement of vascular mechanisms in the pathogenesis of glaucoma has been previously proposed in the literature and has been annotated as the “vascular theory”.³⁹ The vascular theory is a hypothesis that explains the aetiology of glaucoma on the basis of reduced perfusion pressure, faulty vascular autoregulation, or loss of neurovascular coupling. Indeed, glaucoma patients can suffer from systemic reduced blood flow, changes in conjunctival capillaries, increased prevalence of optic nerve head haemorrhages, venous thrombosis, myocardial ischemia, or ischemic lesions in the brain.⁴⁰ Genetic studies provided further evidence for the vascular theory: GWASs in POAG identified significantly associated SNPs in genes involved in abnormal regulation of vascular cell proliferation.^{41–44} For example, a study aimed to test whether SNPs in genes functionally involved in vascular tone regulation were possibly associated with POAG. Here the authors found that genes coding for endothelial nitric oxide synthase (involved in setting vascular tone) were associated with POAG.⁴⁵

Next to findings related to vascular-related functions, cell proliferation pathways also emerged from our enrichment analyses. In healthy cells, proliferation is highly controlled, but when cells accumulate many mutations in the genes that regulate this process, their destiny is to become cancer cells. In this situation, cell proliferation will increase dramatically.⁴⁶ Interestingly, many genes associated with POAG endophenotypes (vertical cup disc ratio and central corneal thickness) are also involved in different types of cancers.^{47,48} It is worth noting that cancer and neurodegenerative mechanisms might share a number of similar underlying mechanisms. For example, the proteins and post-translational modifications that guide cells toward apoptosis, which implies a decreased risk of cancer but increased risk of neurodegeneration, as it occurs in the optic nerve of glaucoma patients. In contrast, a number of other genetic factors have been identified that promote cell proliferation, increased risk for cancer, and decreased risk for neurodegeneration.⁴⁷ An example of these proteins is those encoded by the gene Ataxia Telangiectasia Mutated (*ATM*), which are directly involved in cell cycle arrest at both G1 and S phases. Indeed, mutations in the *ATM* gene cause inactivation that leads to cerebellar neuron loss, and also to an increased risk of developing cancer, especially breast cancer.^{47,49} A biological link between cancer and glaucoma can be also observed in the crucial role played by stem cells. In primary cancers, a group of cells that derive from stem cells called cancer stem cells have an increased capacity of inducing mutations and chromosomal instability. These characteristics in cancer stem cells allow the phenomenon of cancer proliferation. In the human optic nerve lamina region, which is the primary glaucomatous site, stem cells supply neurotrophic factors to RGCs and repair stress-related damage. It has been observed that during aging, loss of stem cells in the optic nerve lamina region contribute to POAG progression.^{50,51} Our analyses highlighted the involvement of genes related to cell proliferation, an observation that warrants further research through functional studies focused on cell cycle proliferation and neurodegenerative effects on RGCs.

Next to vascular and cellular proliferation factors, our studies also suggested that the function of primary cilia may be a key element in POAG pathophysiology. Primary cilia play a role in different processes, such as vision and mechano-sensation. In the eye, primary cilia are located in the trabecular meshwork and in retinal pigment epithelium. Defects in the primary cilia are defined as ciliopathies, and visual impairment is a common feature in affected patients. For instance, patients with Lowe syndrome, a ciliopathy-associated disease, develop ocular hypertension and glaucoma in the first years of life.⁵² Interestingly, a previous study conducted in an *ex vivo* perfused anterior segment system demonstrated that primary cilia are important mechano-sensors for stretch-induced autophagy in the trabecular meshwork cells.⁵³ The trabecular meshwork has a regulatory role in IOP and these cells are sensible to mechanical deformations. This reflects the relevant role of primary cilia in upstream IOP regulation and the necessity to further investigate their contribution with respect to POAG. Primary cilia are also required for maturation of the retinal pigment epithelium, through the WNT pathway. In fact, defects in primary cilia lead to photoreceptor degeneration.⁵⁴ Therefore, ischemic processes in the photoreceptors may contribute to RCGs death, triggered by elevated levels of glutamate present in the photoreceptors.⁵⁵

Future Perspectives: toward personalised medicine in glaucoma

Advances in the field of genetics that lead up to personalised medicine in glaucoma

Glaucoma research has greatly benefited from the development and integration of many methods of analysis. In genetics, the successful era of genotyping methods currently climaxes into high-throughput molecular and cellular analyses that are increasingly being used to detect genes implicated in glaucoma.⁵⁶⁻⁵⁸ Indeed, to evaluate cells, organs and the human body system in the context of glaucoma pathology in a hypothesis-free way, we need to progressively advance towards high-throughput technologies.⁵⁹ High-throughput whole-genome DNA and RNA/cDNA sequencing technologies, mass spectrometry of proteins, which are omics technologies, and bioinformatics have provided further basic insights in our understanding of genetics driving glaucoma aetiology.⁶⁰

Nowadays, it is well-known that the variations in DNA, RNA and protein contribute only partially to the development of a complex disease. For example, environmental factors, such as cigarette smoke exposure, can influence gene expression levels and contribute to disease burden.⁶¹ Hence, to explore the actual disease-causing process, the aforementioned -omics technologies should be complemented together with other strategies and techniques, such as metabolomics. These latter strategies are currently also applied in glaucoma research (Figure 1).⁶²⁻⁶⁵ Of course, it remains essential to correlate these data to detailed phenotyping. An important challenge that -omics studies need to face is that the regulation of gene expression is specific to tissue and cellular subsets. Thus, it is essential to acquire relevant cells and tissue, even when they are not easily accessible, like the ocular tissue. Even though animal models have been used to better understand how glaucoma progress, these models do not perfectly resemble the mechanisms happening in humans. To overcome this issue, nowadays researchers have been using human pluripotent stem cells to understand the glaucomatous neurodegeneration process. The human pluripotent stem has the advantage of being cultured indefinitely and also induced to differentiate into RGCs. When inducing mutations that affect RGCs, for example those in the *OPTN* gene, the human pluripotent stem provides the extraordinary possibility to study in depth the mechanisms and to discover new therapies.⁶⁶

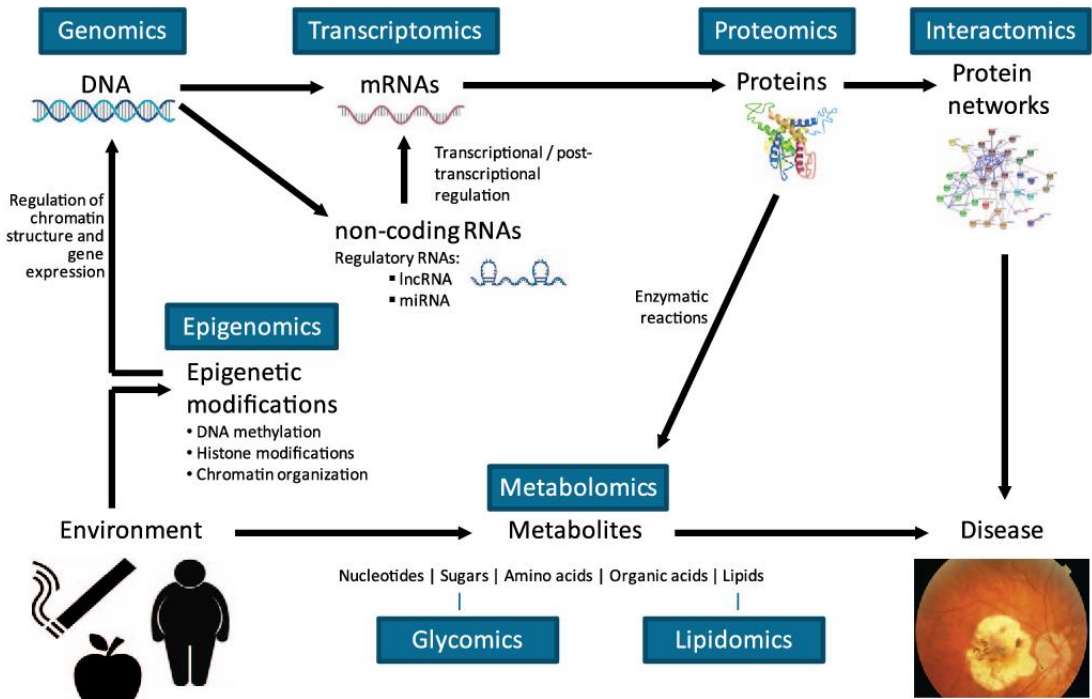


Figure 1. Layers contributing to multifactorial diseases and their relation to each other. The name of the corresponding omics technique is indicated in blue boxes. Source: Investigative Ophthalmology & Visual Science July 2017, Vol.58, BIO88-BIO98. doi: <https://doi.org/10.1167/iov.17-21809>

Another challenge for the -omics area, also related to the limited availability of biological tissues, is the lack of large sample sizes. By comparison, the most recent glaucoma GWAS reached up to a million participants, whereas the sample size of -omics studies typically include only ten to hundreds of biological samples at most.⁶⁷ This issue is further complicated by disease heterogeneity, which makes it difficult to extract meaningful biological information from sample based -omics studies. Considering the complex nature of glaucoma, investigating the whole system might be more informative than focusing on only one specific cell and “one-omic”. A new emerging approach, called multi-omics, describes the phenotypes through the characterization and integration of molecules identified by merging data from different -omics sources.⁶⁸ Such approach is a promising tool, for instance to reveal biomarkers that can be present during asymptomatic stages of disease and that can facilitate prompt detection of patients affected by glaucoma.⁶⁹ Machine learning methods are commonly used to combine the multiple layers of -omics information and biomedical images, helping to advance our knowledge on glaucoma processes.⁶⁹

To conclude, it is reasonable to expect that the field of ophthalmology would benefit from multi-omics technologies in the near future, both in terms of understanding of the disease's pathology and the development of personalised therapies.

What it is still needed to make possible personalised medicine for glaucoma

The recent successful DNA based GWASs have made it possible to predict glaucoma risk using a polygenic risk score (PRS). A PRS provides an estimate of individual disease risk due to genetic makeup, intended as a risk stratifying tool. If GWASs will include more ethnically diverse cohorts, and novel PRS methods will be developed, it will be possible to improve predictive accuracy of the disease, along with the translation processes to a clinical setting.⁷⁰

Although PRS emerges as a the most opportune method to predict health outcome, consistent observations point out that high predictive values are reached only in individuals of recent European ancestry, making it impossible to transfer the use of PRS in other populations.⁷¹ The reason for this is that in the last decades genetic population studies on glaucoma have been strongly imbalanced and predominantly included European participants. This is quite controversial because the African population is the most affected by glaucoma, but, at the same time, it is the least represented in genetic studies. Therefore, this current lack of generalizability of PRS in the clinic is an ethical and scientific challenge.

In Chapter 2, we described the characteristics of POAG presentation among three ethnic groups from Africa and from the Netherlands. We found that African ancestry patients presented POAG at a younger age and in a more advanced disease stage compared to Dutch patients. Therefore, in our study we were able to confirm that POAG patients of African ancestry have more severe symptoms compared to patients from European ancestry. Strategies to identify African ancestry individuals at high risk are needed to manage the burden of POAG. However, as the majority of the studies have been conducted in individuals of European ancestry, the applicability of the PRS in populations different from the European ones is limited. This introduces a major source of inequality and limitations in the application of worldwide precision medicine, especially in populations that need more actions.⁷¹ Duncan and co-workers showed that the predictive accuracy of an European ancestry-derived PRS is lower in Asian and African populations due to different linkage disequilibrium patterns, allele frequencies, and genetic architecture.⁷² Another example that clearly shows the lack of transferability of PRS between populations was reported in a study of Hauser and colleagues. The authors identified a risk locus for POAG in the *APBB2* gene in individuals of African ancestry. When observed in Asian and European ancestries, the variants in this locus were not significant, because of their low frequency (less than 0.1%), and for the different pattern of linkage disequilibrium in Africans.⁷³ Similar results were also observed in the study presented in Chapter 6. Thus, prediction accuracy will be highest in those populations in which most of previous studies were conducted. It is evident that performing multi-ethnicity studies will improve the general prediction accuracy of the PRS, contributing to transferability and health equity. In the longer term, consolidating a more equal representation of all ancestries in GWAS will help to develop better prediction performances, especially in populations with African lineages, who are those at highest risk of glaucoma.⁷¹

To conclude, four actions have been proposed to guarantee that all individuals from different populations can benefit from genomic glaucoma research: (1) increasing the ethnic diversity in

genetic studies, (2) generating more diverse reference genomes, (3) training more scientists in multiple complementary disciplines, and (4) developing better methods for the prediction in numerous ethnic groups and for the separation of genetic and environmental effects.⁷⁴ Glaucoma research and care will certainly benefit from these actions.

Final remarks

In the genetics of complex disease, multi-omics will contribute to fill the picture of the molecular pathology of affected patients. Indeed, this will be true also for glaucoma, thanks to the huge impact that multi-omics will have on the identification of disease-specific biomarkers or of mechanisms linked to drugs response to drugs, just to name a few examples. In spite of that, there are still challenges that need to be overcome: the multi-omics data collected from each patient must be matched, integrated and saved, preferably constantly updated in databases that should be made easily accessible to researchers and clinicians. Yet another challenge is represented by the variability of molecular pathology and phenotype between glaucoma types and patients. But if we get more insights on the glaucoma complexity, then the more we can understand will eventually contribute to predict the disease status in each individual. However, how much all this multi-omics information produced can be utilized in an efficient way in the clinical practice needs more evaluations.

It is clear that personalised medicine is the future of all clinical practice, but it is necessary to further consider the concepts of health and pathology in order to get to know that uniqueness that characterizes each patient. Multi-omics must also become intrinsic to our way of thinking: embrace the biological totality of the patient by linking the smallest molecules and their biological function to the clinical characteristics that make everyone unique.

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