Toward Genetic Screening for Glaucoma

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CHAPTER 8

Summary

Glaucoma is a leading cause of permanent vision loss worldwide, and a stunning 50% of patients from the general population are unaware of their glaucoma status.[1] Population-based studies provide researchers with a global overview of the characteristics within a cohort, and epidemiological research with this data leads to population-based inferences of disease and lifestyle. Additionally, these cohorts afford genome-wide association studies (GWAS), enabling researchers to discover genetic variants in association with complex disorders like glaucoma.[2,3] Glaucoma risk is attributed to both genetic and environmental factors, and traditional screening protocols remain relatively ineffective in Caucasian populations due to its low prevalence.[4] Therefore, in the post-GWAS era, it is imperative to translate both cohort- and GWAS-derived data into useful information for epidemiologists/clinicians to develop effective screening protocols for glaucoma in population-based settings.

The primary aim of this thesis is to explore the feasibility of genetic pre-screening for glaucoma in a population-based setting. The secondary aim is to improve the measurement of self-reported ocular disease status in population-based cohorts by questionnaire data. We exploit GWAS data to create a glaucoma genetic risk score (GRS) to provide an overview of sections of the population who may benefit from timely screening for glaucoma. We also utilize population-based questionnaire data to leverage self-reported status of glaucoma to classify who may be affected and unaware. Furthermore, this "glaucoma proxy" is used as an outcome measure to confirm an association between tinnitus and glaucoma. Finally, we assess the applicability of the above methods for a second ocular disorder, age-related macular degeneration (AMD). In this chapter the main findings and clinical implications, as well as directions for future research, are discussed.

Main findings and clinical implications

What does the literature tell us regarding the heritability of glaucoma?

Heritability of a given trait is a prerequisite for gene-finding studies like GWAS. Heritability in the narrow sense (h^2) is defined as the proportion of phenotypic variance due to additive genetic contributions and is a population parameter (**Chapter 1 and 2**). If a trait has no heritable component, for example spoken language, it is not under genetic control and will not benefit from genetic analysis. With respect to glaucoma, there is increased risk if family members are affected; first-degree relatives are at a 9-fold increased risk, and those with second-degree affected relatives are at a 1.5-fold increased risk to develop glaucoma.[5,6] In the first part of this dissertation (**Chapter 2**), the literature was systematically reviewed regarding the h^2 of glaucoma and related endophenotypes (defined as continuous traits contributing to disease risk but not part of the disease definition).

Due to the lack of reports and inconsistent disease definition, h^2 for glaucoma could not be meta-analysed, although h^2 estimates ranged from moderate to high. With the increases in cohort sizes, attention now needs to be given to h^2 studies where glaucoma can be examined directly, with consistency in case-definition. For glaucoma endophenotype clusters, intraocular pressure (IOP) was reported most frequently. This is expected as it is currently the only modifiable risk factor.[7] Endophenotype cluster h^2 ranged from moderate to high, see **Chapter 2 Figure 4b**.

Secondary analyses revealed that age, ethnicity, and study design were major sources of heterogeneity for endophenotype h^2. Corresponding to the significant age-effect, over one's lifetime a single trait may have different genetic and environmental effects influencing it, such that these variance components essentially become a function of age.[8] For example, variance in birth weight is influenced by the maternal uterine environment, and variance in childhood weight has a decreasing maternal environmental component after weaning and as varying sources of food increase, whereas the variance in adult weight is influenced by environmental rather than maternal factors.[8] Accordingly, as age increases, there is more impact of environmental factors for h^2 of glaucoma endophenotypes. Next, our results indicate ethnicity-based sources of heterogeneity with glaucoma endophenotypes, supporting the well-known association

between ethnicity and glaucoma risk,[9] and confirming the need for ethnicity-based considerations in glaucoma screening. Finally, study type was also a main source of heterogeneity found in the meta-analysis; it is established that twin studies provide higher h^2 estimates than other study types like family-based studies or GWAS.[10,11] Twin-based h^2 studies assume variances explained by the shared environment are identical for mono- and dizygotic twins, with minimal gene-environment correlations/interactions, omitting potentially unmeasured gene by environment interactions to contribute to phenotypic variance.[12,13] These assumptions increase the risk of inflated h^2 estimates. Additionally, twins are always the same age. Different genes may affect a phenotype differently with age, reducing the correlations between family members of different ages, and this is also in line with the reduction of glaucoma endophenotype h^2 with age. With family-based h^2 studies, it is easier to obtain a sufficient sample size, and h^2 is derived by comparing the agreement of family pairs by the expected correlation based on genetic relatedness.[10,11] Recently, advances in genotyping technology has allowed cohorts and consumers to test their DNA at decreasing cost. This has enabled research into genotypic h^2 studies, i.e. common single nucleotide polymorphism (SNP) h^2, by estimating the percentage of phenotypic variance captured by the SNPs on the genotyping platform by way of the genetic relationship matrix.[13,14] This method can use unrelated individuals, allowing for greater flexibility in study design and recruitment. However, individual SNP-effects for complex traits, like glaucoma, may be small and hard to detect, contributing to the missing heritability problem.[10,11]

The collation of h^2 estimates for glaucoma and related endophenotypes highlights the need for further trait- and population-specific h^2 studies. Additionally, as glaucoma (corrected Teikari $h^2 = 0.71$,[15] Charlesworth et al. $h^2 = 0.81$,[16] and sex-specific Cuellar-Partida et al. female $h^2 = 0.52$, male $h^2 = 0.66$[17]) and endophenotypes are highly heritable (Chapter 2), these findings support the potential for further genetic studies. With increasing cohort sizes, meta-GWAS will obtain more genetic variants associated with glaucoma and related endophenotype, with the GWAS results increasing precision for case detection in population-based genetic pre-screening programs.

Are we ready for genetic risk profiling to pre-screen for ocular disorders at a population level?

As discussed in Chapter 2 of this thesis, the heritability of glaucoma and related endophenotypes is moderate to high, indicating the applicability of further genetic studies of glaucoma and related endophenotypes. With the advent of genotyping,

gene-centric studies, and subsequently GWAS of millions of SNPs, we have further elucidated finer details of genetic variants implicated with glaucoma or endophenotypic risk.3 However, most common SNPs (i.e. a minor allele frequency [MAF] of ≥ 5%) found significant in GWAS have relatively small effect sizes, see Figure 1. This can also be said for glaucoma or related endophenotypes; a recent multi ethnic meta-GWAS analysed glaucoma in 34,179 cases and 349,321 controls, finding 44 novel risk loci and confirming 83 previously known loci.18 As expected, the locus with the highest effect is a well-known and rare nonsense mutation (a point mutation that results in a premature stop codon, resulting in a non-functional protein product) in the MYOC gene.[19]

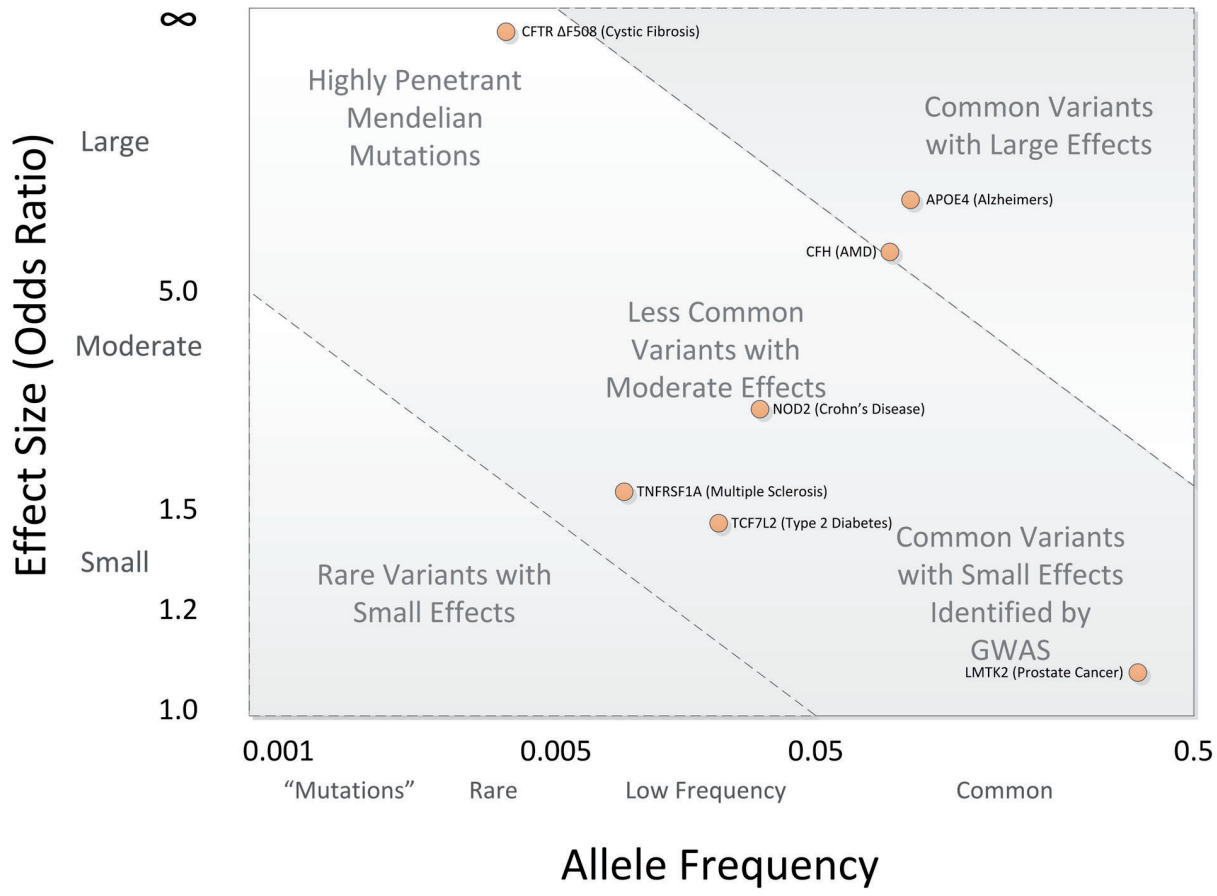

Figure 1. Scope of allelic effects for diseases. The frequency and effect size of disease alleles are often considered together. Alleles for Mendelian disorders are very rare and have large effect sizes (upper left), whereas most GWAS uncover common SNPs with small effect sizes (lower right). Most of the GWAS discoveries lie between the two dashed lines. Source: Bush, W. S. & Moore, J. H. Chapter 11: Genome-wide association studies. PLoS Comput. Biol. 8, e1002822 (2012). [20]

Given that Mendelian mutations causing glaucoma are rare and population-private, we can eliminate the possibility of population-based screening derived from these rare variants.[21] However, common significant variants obtained from GWAS, although having small individual effect sizes, can be combined in a genetic risk score (GRS) that increases the variance explained, especially when weighing the SNP by the effect-size obtained by the GWAS.[11,22,23] As GWAS meta-analysis consortia continue to grow in sample size, so does the ability to find novel SNPs associated with glaucoma and related endophenotypes. The genetic correlation between glaucoma and its endophenotypes indicates value for adding endophenotypes into a glaucoma GRS.[24]

In this thesis, Chapter 3 highlights the novel methods that can be used to pre-

select a population based on genetic risk for glaucoma. The general equation for a genetic risk score (1) is

$$GRS = \Sigma_{i=1}^{N} \omega_i \cdot \chi_i \quad (1)$$

where N is the number of SNPs in the GRS, is the weight for SNP i and is the allelic dosage of the risk variant i.[11] The novel aspect of our glaucoma GRS is utilizing genome-wide significant SNPs from both glaucoma and related endophenotypes, and incorporating the reported effect sizes from a glaucoma meta-GWAS to create a highly curated glaucoma GRS, see Chapter 3 Figure 1. To evaluate the robustness of this GRS, we compared the performance for only glaucoma variants (n=47 SNPs), and including endophenotype variants (n=268 SNPs) in a case-control cohort of 734 well-defined POAG cases from the Groningen Longitudinal Glaucoma Study[25] and 1,418 controls from the Groningen Expert Center for Kids with Obesity cohort.[26] Per standard deviation increase in genetic risk, the OR for glaucoma increased from 1.66, $P=6.5*10^{-25}$ with glaucoma variants only, to 2.11 $P=2.2*10^{-44}$ with endophenotypic variants added, with a doubling in explained variance, from 7.2% with only glaucoma variants to 14.1% with endophenotypic variants.[27] Researchers have also highlighted the importance of endophenotype genetics for glaucoma risk, reporting that glaucoma patients (n=2,154) at the top 20% of genetic risk in a weighted IOP GRS (n=146 SNPs) had an earlier age of diagnosis by 3.7 years ($p<0.001$) compared to those at the bottom 20% of genetic risk.[28]

In the EyeLife study (Chapter 3) we selected participants based only on genetic risk and age, optimizing ocular examinations to phenotype for both structural and functional aspects to create a screening protocol tailored to capture glaucoma. It is important to note that we are blinded to the genetic risk of the participants to minimize the chance of bias. We selected screening exams that are non-contact, non-mydriatic, rapid, robust, easy-to-operate, and proven useful in glaucoma screening research, described in detail in Chapter 3. Our emphasis for classifying glaucoma in a population-based setting is focused on both the structure and function of glaucomatous visual field loss. Specifically, incorporating the well-known International Society of Geographical and Epidemiological Ophthalmology glaucoma classifications, modified to incorporate the retinal nerve fiber layer (RNFL) thickness, as well as the Northern Finland Birth Cohort eye study's intuitive 'two-of-three' method, where glaucoma

is defined by the presence of typical and matching glaucomatous changes in at least two of the following: the RNFL thickness, the optic disc, and the visual field.[29,30] Utilizing the established labels in the Rotterdam Study, we define Definite glaucoma as combined functional and structural loss, probable glaucoma as functional or confirmed structural loss, and possible glaucoma as structural loss, as well as healthy.[31] This has resulted in a comprehensive classification tool for population-based ophthalmology. In addition to glaucoma phenotyping, endophenotypes such as IOP, central corneal thickness (CCT), and anterior angle imaging, as well as other ocular parameters such as tear production are also phenotyped within EyeLife, with the eventual goal of ocularly phenotyping all Lifelines participants at the age of retirement, creating a wealth of ocular data for future research.

The theoretical applicability of genetic pre-screening at the population level has been demonstrated in colorectal cancer (CRC) screening.[32] The Begleitende Evaluierung innovativer Testverfahren zur Darmkrebsfrüherkennung study (n = 5,858 participants) aims to evaluate novel non-invasive CRC screening tests.[33] They reported that participants at the highest quintile of genetic risk for colorectal cancer (n= 39 SNPs) were at a 3.5-fold (95%CI; 2.1-5.6) increase in relative risk of advanced colorectal neoplasms, when compared to the lowest quintile of genetic risk.[32] Additionally, genetic selection has been occurring in production animal populations for decades, selecting for animals with higher meat and milk production in Holstein cattle.[34,35] High milk production is a complex multi-genic adult-onset trait, and selection is through use of marker assisted selection, which is similar to a weighted GRS in human research. Using vast pedigree and extensive GWAS, the accuracy of genomic prediction in dairy cattle exceeds 0.8 for production traits.[36]

The EyeLife study is currently the only study to prospectively select an at-risk population based only on genetic risk and age; if found successful, this approach will be a massive step towards personalized medicine. Additionally, this approach needs to be replicated in other cohorts, utilizing the most up-to-date implicated genetic variants.

Are questionnaire-based proxies appropriate for glaucoma research at a population level?

Population-based cohort studies are continuously increasing in size and performing complex phenotypic assessment for potentially hundreds of thousands of participants is a financial and logistical burden that many cohorts cannot bear all at once. This issue can be especially problematic for disorders with a high level of unawareness.

Due to the insidious nature of early glaucomatous visual field loss, where the brain fills in scotomas with nearby visual input, at the population level, 50% of patients remain unaware of their glaucoma until excessive and irreversible damage has occured.[37,38] As a result, self-reported status may underestimate glaucoma prevalence at a cohort level. Thus, questionnaire-based proxies offer promising interim phenotypes, if they can capture those unaware of their disease. Validated questionnaires exist for subjective vision, including the National Eye Institute Visual Functioning questionnaire-25 (NEI-VFQ-25).[39] In the Lifelines cohort, we improved upon self-reported glaucoma status by incorporating subjective vision loss as well as relevant medical questions to uncover who may be affected with glaucoma but unaware (Chapter 4).

Our glaucoma proxy contains varying levels of certainty; definite, probable, possible, and unaffected glaucoma. We defined 'definite' glaucoma using a very strict criterion to allow for us to quantify glaucoma-induced vision loss, additionally the use of 20 age- and gender-matched controls per case, with no self-reporting of conflicting eye diseases, ensures the visual defects we obtained were a function of glaucoma rather than the general aging process.[40] Using only self-reported glaucoma surgery as the threshold for classification at the population level underestimates actual glaucoma population levels, as surgical intervention is performed usually during the later stage of the disease process. Those who self-reported glaucoma (diagnosis or treatment) and had subjective vision patterns passing the first threshold (Chapter 4, Figure 1) were defined as 'probable' glaucoma, both requirements are used as participants with treated ocular hypertension may incorrectly self-identify as having glaucoma, artificially inflating case numbers. This is a major strength of our study, as cohorts using only self-reported glaucoma status likely includes treated ocular hypertensives. Finally, 'Possible' glaucoma was defined in two ways. First, those who self-identify with glaucoma or glaucoma treatment but with no visual impairment, or those with glaucomatous-like visual impairment above the second threshold (97.5% specificity) with no self-reported glaucoma. The former group likely represents possible treated ocular hypertension, and the latter group is of interest as it potentially represents glaucoma cases that are unaware of their status. Incorporating glaucoma-specific subjective vision improves upon self-reported glaucoma status in questionnaire-based studies because of the insidious nature of glaucomatous visual field loss. The specific yet simplistic nature of the NEI-VFQ-25 questions may capture the 50% of glaucoma patients not yet aware of their glaucoma status. They may struggle with seeing things in their periphery or in dim conditions, but may not know why, or think these glaucoma-specific visual characteristics are merely a function of age.[40] The statistical approach to

finding an interim phenotype is logical and applicable to other cohorts; however, the classification equation needs to be re-derived per cohort, especially cohorts of other ethnicities and cultural backgrounds where different baselines of vision exist.[41] As the participants in this cohort are not currently ocularly phenotyped, these differing levels allows for future researchers to choose the appropriate level of certainty for analyses. The prevalence of self-reported glaucoma (combining definite, probable and possible by self-report), was 5.2%; these results reflect other population-based studies where participants were phenotyped for glaucoma.[42]

The varying levels of certainty of the glaucoma proxy already proved to be beneficial for investigating the association between other systemic disorders and glaucoma. To begin, anecdotally, clinical participants in a glaucoma cohort reported a higher frequency of tinnitus than expected, spurring a cohort- and population-based association study, (Chapter 5). The glaucoma proxy was dichotomized to cases, with definite or probable glaucoma, and controls; possible glaucoma was removed from this analysis to omit those who may be treated ocular hypertensives. After adjustment for socio-demographic features, we found that glaucoma patients were at significantly higher odds of having tinnitus within the clinic- and population-based cohorts, suggesting a common mechanism. Future studies could perform GWAS in large cohorts and further in silico analyses to determine common affected pathways of both disorders. Second, the vascular theory of glaucoma postulates that glaucomatous damage may arise from insufficient blood supply to the optic nerve head (Chapter 6),[43] however, ocular blood flow abnormalities with respect to glaucoma can occur at both high and low blood pressure, yielding a U-shaped relationship.[44] This apparent controversy has led to a new hypothesis, the involvement of autonomic nervous dysfunction. Thus, in a cross-sectional population-based analysis, all levels (definite, probable, and possible) of the glaucoma proxy were utilized as cases to examine the link between glaucoma and heart rate variability in the Lifelines cohort. After adjustment, we found that a decreased heart rate variability (an indication of autonomic dysfunction) was associated with an increased risk for glaucoma. Additionally, a 10-mm Hg increase in pressure-related measurements (systolic blood pressure, mean arterial pressure, and pulse pressure) are all significantly associated with increased risk for glaucoma. Finally, participants classified as hypertensive, using ACE-inhibitors, and those using calcium channel blockers were also at a significantly increased risk of glaucoma. Our findings support the notion that low heart rate variation and high blood pressure may play a role in glaucoma pathophysiology; future studies could examine a causal approach such as Mendelian randomization to elucidate possible causal mechanisms of hyper-

tension and hypertension medication on glaucoma.

Are these approaches applicable to other ocular disorders?

The same approaches were taken with AMD in a population-based setting; aiming to improve upon self-reported AMD status by incorporating subjective vision loss as well as relevant medical questions to uncover who may be affected with AMD but unaware (Chapter 7). To create the discriminatory equation, we utilized 110 patients undergoing treatment for neovascular AMD. As this is a clinical population we are sure of the diagnosis, however, consistent treatment for neovascular AMD improves vision, dampening the coefficients in the discriminatory equation.[45] Furthermore, the clinical population had no cases of dry AMD, yet early AMD is by definition the dry variant. Multivariable analyses revealed AMD cases have significantly poorer performance in distance vision and near vision, this is expected as AMD-related damage is localized in the macula, the place of sharp vision.[45–48] We also found that AMD patients have significantly poorer vision in low luminance settings, this may be due to preferential loss of rods in the photoreceptor cell layer.[49] In the Lifelines testing cohort we categorized self-reported AMD status as definite cases, and again utilized Youden's index as the threshold in obtaining possible AMD. Prevalence estimates were in agreement with a meta-analysis done in the European Eye Epidemiology consortium, as well as a Dutch-specific population cohort study.[50,51] The eye questionnaire given to Lifelines participants did not include self-report for retinal vein occlusion, an age-related vascular ocular disorder that affects central vision. As our proxy is derived from functional vision, the classic AMD epidemiological risk factors (age and smoking) that significantly associated with our proxy may be confounded by this disorder. However, we found that females are at significantly higher odds of either self-reporting or possible AMD, and there is no gender effect found with retinal vein occlusion.[52] Nonetheless, we cannot exclude a certain amount of misclassification of AMD status. With future phenotyping as per EyeLife[53] we will be able to confirm the underlying pathology through structural changes in participants classified as possible AMD.

Additionally, we obtained several GRSs for AMD to test against the proxy. Fritsche et al. performed a comprehensive meta-GWAS for AMD (n = 52 SNPs), including sub-analyses (n= 34 SNPs) for Caucasians, intermediate AMD, advanced AMD, geographic atrophy, and choroidal neovascularization.[54] A more recent GWAS obtained 10 novel SNPs associated with early AMD.[55] We also specifically looked at the independently associated SNPs within the CHF gene because of the well-known associa-

tion with AMD.[54] We tested these GRSs in two genotyped populations within Lifelines. Although we found a single association in one genotyped Lifelines population, all GRS failed to replicate in the second population, and failed to replicate when accounting for familial status. The conclusion must be that a questionnaire-based proxy may currently not be ready for population-based AMD research. Conflicting central ocular pathologies creating case contamination and utilizing treating wet AMD to train a vision algorithm to detect early (i.e., dry) AMD contributes to why an AMD proxy may not be currently suitable for population-based research. However, this observation does open the door to further research examining the specific differences in subjective visual field loss for different central ocular pathologies.

Future Perspectives

As we move to larger cohort sizes and decreasing genotyping costs, we need to be cognizant that currently the majority of GWAS are performed in Caucasian cohorts.[56] Given that glaucoma is more prevalent in those of African and Hispanic ancestry, a shift needs to be made to obtain GWAS for glaucoma and related endophenotypes in these populations that are at a higher general risk for glaucoma.[57] This is especially important for genetic pre-screening for glaucoma, as the predictive performance of a Caucasian-derived GRS is lower in cohorts of different ethnicities.[57,58] As the awareness level of glaucoma in developing countries is even less than 50% we need to prioritize appropriate population-based screening strategies.[38] Therefore, deriving population-private (i.e. ethnicity-specific) GRS for glaucoma will be invaluable for the success of population-based screening and as we move closer to personalized medicine.

Increasing cohort sizes also provides researchers the opportunity to address the lack of heritability reports where glaucoma is the direct outcome. However, there needs to be a uniform classification of glaucoma across population-based cohorts, facilitating collaboration and meta-analyses. While the International society of Geographical and Epidemiological Ophthalmology (ISGEO) classification has been used to classify glaucoma in cohort settings, there is no assessment of the retinal nerve fiber layer thickness via OCT or other techniques, which is a crucial aspect to early glaucoma case detection.[59–61] Systematically reviewing and meta-analysing the literature provides the most reliable source of evidence to guide decision-makers as well as providing directions for future research. As glaucoma categorization in EyeLife is robust in case-classification (Chapter 3), it could be employed in other cohorts to compare the EyeLife glaucoma screening sensitivity, specificity, and positive predictive power between cohorts.

With respect to the glaucoma proxy, the second group of possibles, those with visual complaints similar to glaucoma but no self-reported treatment/diagnosis, is of special interest as these participants may represent, at the population-level, the 50% of glaucoma patients unaware of their status. At the time of publication we are ocularly phenotyping EyeLife participants. Once recruitment is complete, we can determine the accuracy of the proxy in classifying glaucoma based only on glaucoma-specific visual complaints. The glaucoma proxy omits participants with conflicting ocular disorders

due to conflicting visual field anomalies, allowing for improvement upon self-reported status for other ocular disorders. Although we had limited success in creating an AMD proxy, we were able to construct and validate a myopia proxy in Lifelines using data obtained from EyeLife.[62]

Final Remarks

In summary, this thesis lays the foundation and is the first study to prescreen a population for glaucoma based only on genetic risk. Additionally, this thesis provides improvements upon self-reported glaucoma status in questionnaire-based cohort settings. The combination of subtle and non-perceptive functional vision loss in early glaucoma underscores the importance of timely and appropriate screening. Correctly interpreting genetic and questionnaire data at a large scale is challenging due to a myriad of factors causing within-population variation. Yet it is essential to correctly identify those at greater risk for glaucoma, where early detection will preserve eye health and vision for longer.

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