Genome-wide association study success in ophthalmology.

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ABSTRACT:

Purpose of Review

Much progress in our understanding of the genetic profile of many ophthalmic diseases has been made over the last decade. Identification of novel gene associations allows insight into the mechanisms of disease and potentially enables the profiling of individuals at increased risk as well as allowing the development of new treatments. We highlight key recent discoveries using the genome-wide association study (GWAS) design.

Recent Findings

Over the last two years we have seen major international collaborations successfully conduct GWAS to identify genetic pathways associated with eye diseases such as myopia, age-related macular degeneration (AMD) and glaucoma. Similarly other studies have identified and confirmed genes associated with ocular biometry (or disease-specific endophenotypes).

Summary

Our understanding of the genetic architecture of common eye diseases such as myopia, AMD and glaucoma is rapidly expanding. With reducing costs of next-generation sequencing we expect a transition to large-scale interrogation at the whole exome and genome level, which will enable the identification of rare variants which confer a level of sensitivity and specificity to predict risk that will allow us to further understand, predict and intervene in genetic-based eye diseases.

Keywords:

Myopia, POAG, AMD, Keratoconus, Gene-environment interaction

INTRODUCTION

Over the past decade there has been a rapid increase in our understanding of the genetic determinants of human disease. The Human Genome Project (HGP), completed in 2003, was the international scientific research project that identified and mapped the 20,000–25,000 human genes.[1,2] This was followed by the HapMap project, which provided the haplotype map of the human genome describing the common patterns of human genetic variation, a key resource in finding variants affecting health and disease.[3] Single-nucleotide polymorphisms (SNPs) are variations in a single base pair of nucleotide sequence in the genome. At any position in the DNA sequence or genetic code there could be one of four nucleotides: adenine (A), guanine (G), thymine (T) or cytosine (C). Most commonly there will be one particular base at a specific position, termed the 'common' allele. Generally, a common polymorphism is defined as a genetic variant in which the frequency of the minor allele in a given population is greater than 1%. SNPs are rarely mutations that cause disease, are occasionally linked to disease-causing mutations, and most often are of uncertain pathogenic significance. All, however, can be used as genetic markers in association studies. To date the majority of large-scale genetics studies have used somewhere between 500,000 and 2,500,000 SNPs genotyped on high throughput genotyping arrays. The SNPs contained on these arrays are generally distributed across the genome, and as such offer the prospect of capturing or tagging all common variation for a specific population. Intervening SNPs that are not directly assessed can be imputed using population data.

Genome-wide association studies (GWAS) are designed to investigate the associations between SNPs and traits or diseases by comparing the frequency of alleles of a group of people with a particular disease (cases) to that of another group without that disease (controls). Alternatively, rather than a present or absent disease classification, GWAS design can also be applied to biometric or continuous traits. Hundreds of thousands of SNPs are genotyped on microarrays in studies designed to find common ancestral mutations that contribute risk for disease.

The output of a GWAS can be displayed in a figure affectionately, referred to as a Manhattan plot, which charts the p value (or $-\log_{10}$ of that p value, such that the more significant an association the higher the 'skyscraper') for the association of each SNP against its relative position in the genome (x axis). Given the large number of

statistical tests performed, and after adjusting for correlated-SNPs, a multiple testing correction threshold for significance is generally set at $<10^{-8}$ (rather than $p<0.05$). Thus, small studies are typically insufficiently powered to identify a signal with a modest genetic effect. To verify findings, and reduce false positive findings due to the 'winners' curse' replication of top signals is performed in independent cohorts[4] and, more recently, meta-analysis of multiple cohorts has greatly increased the power to identify genetic associations, with the statistical association at some loci reaching p values of $<10^{-540}$. Despite these statistically robust findings, it is important to remember that most of these SNPs are common and many unaffected people carry the risk alleles. Most have small effect sizes, usually not even doubling the risk (OR<2.0). The p value of 10^{-8} just means that we are 99.999999% sure that this is a real finding. In this article we aim to summarise recent insights gained from genetic association studies for ocular disease.

AGE-RELATED MACULAR DEGENERATION

(The might of meta-analysis!)

Age-related macular degeneration (AMD) was the first major success in the GWAS era with the *CFH*[5-7] and *ARMS2-HTRA1*[8,9] loci implicated from relatively small sample sizes, less than 200 cases while the Wellcome Trust Case Control Consortium (WTCCC) was investigated 2,000 cases across seven diseases![10] In 2013, Fritsche and colleagues conducted a meta-analysis for AMD and identified the largest number of loci associated with this disease to date. In total Fritsche *et al.* identified and replicated 19 loci, seven of which were novel.[11] To do this they used >17,100 advanced AMD cases and >60,000 controls of European and Asian ancestry. They confirmed the previously implicated genes: *ARMS2-HTRA1, CFH, C2-CFB, C3, TIMP3, APOE, CETP, VEGFA, TNFRSF10A, LIPC, CF1,* and *COL10A1*, and for the first time, SNPs at several novel loci: *COL8A1-FILIP1L, IER3-DDR1, SLC16A8, TGFBR1, RAD51B, ADAMTS9* and *B3GALTL*. Although one can question the value for disease-risk prediction of adding further genes with smaller effect sizes, this work highlights the fact that although missed in the smaller earlier studies, the larger consortia meta-analyses have identified the vascular endothelial growth factor (VEGF) pathway, that we already know is viable for treatment for AMD. The hope is that further genes will similarly open new opportunities for treatment translation for diseases such as glaucoma. Although we are still not clear which of the two genes

ARMS2 or *HTRA1* causes AMD, we are now 99.99% sure that genetic variants in this region are associated with risk of developing AMD.

<u>(Genotype-phenotype correlations)</u>

In 2013 Holliday *et al.* identified the main genes associated with early AMD: *CFH ARMS2* and potentially *APOE*.[12] This phenotypic subtype approach is important in untangling the full genetic picture of AMD, particularly if we are to flag early risk factors. In this approach researchers identify the SNPs associated with the early disease phenotype. In contrast Ardeljan and colleagues investigated SNPs at the *TIMP3* locus, which is mutated in Sorsby's fundus dystrophy but also has SNPs associated with AMD. They characterised the phenotypic influence of *TIMP3*.[13] In this approach these researchers identified the phenotype that goes with the particular SNPs. Zhang and colleagues investigated the genetics of neovascular AMD and polypoidal choroidal vasculopathy (PCV).[14] A missense variant in *CETP* was significantly associated with PCV but not neovascular AMD. Further genotype-phenotype analyses will provide further insight into the pathological sub-groupings of disease.

<u>(GWAS and pathways: A segue into candidate genes)</u>

GWAS discoveries are also particularly useful for identifying disease associated genetic pathways. The initial implication of *CFH* flagged the complement pathway and thus researchers interrogated specific complement genes identifying other SNPs that a full GWAS was not adequately powered to identify. These are listed in the work by Ratnapriya *et al.* as *ABCA4, CFHR1/CFHR3* and *FBLN5*.[15] Similar, by further interrogation through re-sequencing of genes involved in known *CFH*-related pathways a number of less common variants have also been found to be strongly

associated with AMD.[16,17] These rare SNPs are independent of the previously identified common "GWAS" SNPs.

(GWAS and Gene-Environment interactions "GxE")

Naj[18] and colleagues investigated the gene-environment interactions (GxE) for AMD SNPs and the risk factor smoking. The genetic effects were largely restricted to non-smokers while the *SERPIB8* SNP showed less effect in smokers. The proportion of attributable risk for AMD explained by genetic and environmental factors is now amongst the highest for any complex disease; with an estimate that 75% of the gene and environmental factors are already identified.[19] GxE analysis of GWAS data will be a major ongoing area of work.

MYOPIA

(The might of meta-analysis!)

After years of attempting to identify myopia genes with limited success from linkage[20] and smaller GWAS studies[21,22], two large studies were published concurrently by the Consortium for Refractive Error and Myopia (CREAM)[23] and the Direct to consumer DNA testing company, 23andMe[24]. Remarkably there was considerable overlap in findings between these two studies.[25]

23andMe reported the results of the largest GWAS (n= 45,771) of a refractive phenotype conducted to date. They confirmed the two previously reported loci and discovered 20 novel loci associated with age of onset of myopia in a European-derived population: *BMP3, BMP4, DLG2, DLX1, GJD2, KCNMA1, KCNQ5, LAMA2, LRRC4C, PABPCP2, PDE11A, PRSS56, RASGRF1, RBFOX1, RDH5, RGR, SFRP1, SHISA6, TJP2, TOX, ZBTB38,* and *ZIC2.* CREAM concurrently conducted a meta-analysis of GWAS comprising 27 studies (n=37,382) of adults of European descent and five Asian cohorts (n=8,376). CREAM identified: *BICC1, BMP2, BMP3, CACNA1D, CD55, CHD7, CHRNG, CNDP2, CYP26A1, GJD2, GRIA4,KCNJ2, KCNQ5, LAMA2, MYO1D, PCCA, PRSS56, RASGRF1, RDH5, RORB, SIX6, TOX, ZIC2,* and *ZMAT4.* In total, 16 of the 20 novel loci identified by 23andMe were confirmed by CREAM; 14 of the 22 loci discovered by the CREAM analyses were replicated by 23andMe. Although 23andMe have recently come under direct scrutiny from the regulating body, the US Food and Drug Administration, this research by a private company using participants who self-funded a minimal phenotype

questionnaire and publishing the data on 20 novel loci in an open access journal is a stunning achievement. Meanwhile CREAM, funded by tens of millions of taxpayer and philanthropic dollars, performed a meta-analysis of 32 studies using highly debated phenotype/examination protocols and described their findings of 22 novel loci behind a paywall-controlled journal.

(GWAS and pathways: A segue into candidate genes)

The CREAM study identified neurotransmission (*GRIA4*), ion transport (*KCNQ5*), retinoic acid metabolism (*RDH5*), extracellular matrix remodelling (*LAMA2* and *BMP2*) and eye development (*SIX6* and *PRSS56*). The 23andMe study identified multiple genes in many pathways including: extracellular matrix remodelling (*LAMA2, ANTXR2*), the visual cycle (*RDH5, RGR, KCNQ5*), neuronal development (*KCNMA1, RBFOX1, LRRC4C, NGL-1, DLG2, TJP2*), eye and body growth (*PRSS56, BMP4, ZBTB38, DLX1*) and retinal ganglion cell projections (*ZIC2, SFRP1*). An in-depth analysis of a subset of the CREAM identified the following pathways: cell-cell adhesion, biological adhesion, cell morphogenesis involved in differentiation, cell morphogenesis, synaptic transmission, cellular component morphogenesis, neuron differentiation, ion transport, transmission of nerve impulse, metal ion transport, neuron development, cell-cell signalling, cation transport, cell morphogenesis involved in neuron differentiation, regulation of cell death, regulation of programmed cell death, regulation of system process, regulation of apoptosis, calcium ion transport and axonogenesis.[26] Pathway analyses are an evolving area of research, though it is clear that a systems biology approach will improve our ability to identify potential avenues for treatment.

(GWAS and Gene-Environment interactions "GxE")

Two groups, who are part of CREAM, investigated the interaction of genetic factors with level of education, one of the leading environmental factors associated with myopia. A genetic risk score was calculated based on 26 myopia-associated SNPs from CREAM. Educational level was obtained by questionnaire and categorized into completion of primary, intermediate, and higher education. Individuals with a high genetic risk and who had university-level education showed a remarkably high risk of myopia (OR 51.3; 95 % CI 18.5–142.6), while those at high genetic risk with only primary schooling had a much lower increased risk of myopia (OR 7.2, 95 % CI 3.1–

17.0). The combined effect of genetic predisposition and education on the risk of myopia was far higher than the additive effect and thus provides evidence of a gene-environment interaction in which an individual's genetic risk of myopia is significantly affected by his or her educational level.[23] A similar analysis of five Asian studies from Singapore found three genetic loci *SHISA6-DNAH9, GJD2* and *ZMAT4-SFRP1* exhibited a strong association with myopia in individuals with higher secondary or university education whereas the association at these loci was non-significant or of borderline significance in those with lower secondary education or below. A significant interaction with education was also observed for axial length and myopia.[27]

(Myopia endophenotypes)

Increased axial length is a major determinant of myopia. The CREAM consortium identified nine loci associated with axial length: (*RSPO1, C3orf26, LAMA2, GJD2, ZNRF3, CD55, MIP, ALPPL2,* and *ZC3H11B*.[28] Five of these were also associated with refractive error: *LAMA2, GJD2, CD55, ALPPL2,* and *ZC3H11B.* Analysis of the Avon Longitudinal Study of Parents and Children (ALSPAC) and Singapore Chinese Eye Study (SCES) showed there was shared determination of axial length and corneal curvature with coordinated genetic scaling of the human eye.[29]

Several smaller studies have identified genes associated with corneal curvature. In ALSPAC *PDGFRA* influence corneal curvature and corneal astigmatism. However, rather than affecting corneal curvature in isolation, *PDGFRA* influences the size of the eye while maintaining its scaling.[29] Analysis of astigmatism has proven quite difficult, with limited success to date genes *VAX2[30]*while a more specific study of corneal astigmatism did not reproduce the earlier association with *PDGFRA* in a different ethnic group.[31]

Smaller studies investigating the genetics of myopia, identified variants in genes such as *ZFHX1B* and *SNTB1*[32] as well as previously associated loci *MYP10* and *MYP15*.[33] *ZIC2,* and *RASGRF1* were associated with high myopia in a Japanese study,[34] whilst *VIPR2* (in the *MYP4* locus) and *SNTB1* were associated with high myopia in a Chinese population[35], and *RBFOX* in a Caucasian population.[36] The Blue Mountains Eye Study (BMES) replicated one of the two original myopia GWAS (*GJD2*),[37] while the Age-related Eye Disease Study (AREDS) study was unable to

replicate either.[38] These results indicate that studies generally need to be sufficiently powered before implicated risk associated loci can be dismissed.

CENTRAL CORNEAL THICKNESS AND KERATOCONUS

Central corneal thickness (CCT) has been identified as a contributing factor for both primary open-angle glaucoma (POAG) and keratoconus. Numerous genes in collagen related pathways had been identified in recent years and a major collaboration by the International Glaucoma Genetics Consortium (IGGC) who meta-analysed their CCT GWAS data. This work identified 21 loci associated with CCT, highlighting involvement of the collagen and extracellular matrix pathways[39] with one SNP being found to be nominally associated with POAG. Using independent cohorts of keratoconus, SNPs at two separate CCT-associated loci (*FOXO1* and *FNDC3B*) were found to be associated with keratoconus at the genome-wide level.

An Australian clinic-based cohort of keratoconus replicated two of the identified SNPs at *MPDZ-NF1B* and *ZNF469*,[40] while another Australian keratoconus cohort found a major association with *RAB3GAP1* and less so with three other regions[41] and a US keratoconus cohort found association with *COL5A1*.[42] In addition to confirming some known loci, other smaller GWAS, identified a novel gene region associated with CCT in Latinos,[43] and another American study implicated the *RPN2* locus.[44]

GLAUCOMA

In addition to CCT, researchers have performed GWAS for intra-ocular Pressure (IOP) and optic nerve parameters, which are both risk factors for POAG and Normal Tension Glaucoma (NTG).[19] Several smaller GWAS for IOP identified putative associations genes including the BMES-identified *GLCCI1/ICA1* locus.[10] The Twins UK study identified copy number variants (CNVs) associated with IOP at *RAB9BP1* and *SLC2A14/SLC2A3*, which was replicated in the Australian twins and BMES cohorts.[45] Previous glaucoma GWAS had identified the *LOXL1*[46] associated with pseudoexfoliation and the *CAV1/CAV2*,[47] as well as *TCMO1* and *CDKN2BAS*[48] loci as being associated with POAG. In the coming year we expect further meta-analyses of these studies and others as part of the International Glaucoma Genetics Consortium (IGGC).

The role of *CDKN2B/CDKN2B-AS1* genes located on chromosome 9p21 in POAG have now been extensively investigated across different populations: Australians, Americans, Europeans, Japanese and Afro-Caribbeans.[49] In African-American people a weak association between POAG and the *CDKN2B-AS1* region was found,[50] although this was not confirmed in a Ghanaian population. A separate study from Japan identified *HK2* and *NCK2* as being associated with normal tension POAG.[51]

(POAG endophenotypes)

The Twins Study UK also implicated *FAM125B* through a GWAS of IOP.[52] Analysis of IOP in several American cohorts (NEIGHBOR, GLAUGEN and AREDS) confirmed the association of the *TMCO1* locus and supported association of several other POAG-related genes.[53]

The *ATOH7* gene has been found to be associated with optic disc size as well as cup-to-disc ratio and POAG in some studies. Investigation of the BMES and Twins UK data suggested that *ATOH7* is not associated with cup-to-disc ratio when adjusted for age, sex, IOP and disc size, and as such variants at the *ATOH7* may be more important in optic nerve biometry than POAG risk alone.[54]

(GWAS for Primary Angle Closure Glaucoma)

Recently a GWAS for primary angle closure glaucoma (PACG) with a discovery cohort of just under 2000 cases of Asian ancestry, identified a significant associations at three novel loci (PLEKHA7; COL11A1 chromosome 8q).[55] Interestingly, the association of SNPs at PACG chromosome 8q locus was found to be associated with anterior chamber depth in a Caucasian cohort;[56] however, this association has not been well replicated.[57]

CATARACT, RETINAL AND OTHER OCULAR DISEASES

Over the past twelve months a number of GWAS have investigated a broad range of other ocular diseases. A Chinese study identified three loci of suggestive significance for association with risk of developing diabetic retinopathy,[58] whilst a larger meta-analysis identified loci associated with variation in the retinal microcirculation.[59] Interestingly, a GWAS of retinopathy in individuals without diabetes showed no strong evidence for genetic association.[60] Conversely, a GWAS investigating

rhegmatogenous retinal detachment identified several putative genes associated with cell adhesion or migration, including *SS18*, *TIAM1*, *TSTA3* and *LDB2*, as well as a gene *CERS2*.[61] A GWAS in Chinese people identified *GTF2I* as a new susceptibility locus for primary Sjögren's syndrome and confirmed previously reported associations in Europeans in the regions of *STAT4*, *TNFAIP3* and the major histocompatibility complex.[62] Finally, a GWAS of horizontal phoria suggested association with *ALDH5A1*, which encodes the mitochondrial enzyme succinic semialdehyde dehydrogenase (SSADH).[64]

CONCLUSION

It is clear that a considerable amount of insight into the genetic aetiology of ocular disease and traits has been made recently using the GWAS design (Figure 1). As such, the question must be asked: "Why have GWAS yielded so much information for so many ophthalmic diseases?" We are of the opinion that there are four compelling reasons: First, several of the major GWAS discoveries have uncovered loci of particularly large effect and could have been identified by more traditional linkage-based approaches (e.g for AMD, eye colour, pseudoexfoliation syndrome and Fuch's endothelial dystrophy). In the case of AMD where *CFH* and *ARMS2-HTRA1* were identified with only ~100 cases, we are dealing with common, high effect variants, which could have been identified using a method commonly applied to a 'Mendelian disease', just as *OCA2* had been for iris colour.[65] Indeed the locus for *ARMS2-HTRA1* had been identified five times using linkage analysis prior to the GWAS.[66] The late age of onset and subtle nature of the diseases made it less likely that pedigrees of AMD, pseudoexfoliation syndrome and Fuchs Endothelial Dystrophy would be recognised. Surprisingly, despite inspiring some modelling from geneticists such as Victor McKusick, the genetics of eye colour in the general population had not been extensively studied prior to GWAS. Second, several of the traits and diseases in question have very high heritabilities. For example CCT has a heritably of approximately 95% - one of the highest for any human trait;[67] and although it is important to appreciate that the calculated heritability does not reflect the underlying genetic architecture it does indicate that gene(s) are there to be found. Third, the precision of ophthalmic measurements has greatly improved. For example when we consider CCT assessment for LASIK surgery for myopia, 1 diopter of refractive error corrected requires 12-14 microns of corneal removed. Similarly autorefractors are

very precise and retinal and optic nerve images are of high quality. Reducing the variability in measurement error for quantitative traits increases the power to identify firm associations. Finally, ophthalmic studies lend themselves to severe disease enrichment. Ascertaining, cases or controls at the extreme ends of the phenotypic spectrum increases power and such a design was successfully used by Burdon and colleagues to identify two POAG loci in a GWAS with a relatively small sample size.[48]

Through increasing sample size, and adopting new analytical methods or technology GWAS of common and rare variants will continue to uncover more loci associated with ocular diseases and traits. With reducing costs of next-generation sequencing we expect a transition to large-scale interrogation at the whole exome and genome level, which will enable the identification of rare variants which confer a level of sensitivity and specificity to predict risk that will allow us to further understand, predict and intervene in genetic-based eye diseases.

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Figure Legend:

Figure 1:

The number of GWAS publications is growing exponentially. Data from the NHGRI GWAS Catalogue (accessed 31 March 2014: www.genome.gov/GWAStudies)