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**GWAS in myopia: insights into disease and implications for the clinic**

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## GWAS in myopia: insights into disease and implications for the clinic

### Summary

Myopia is the commonest eye trait worldwide and the prevalence is increasing. It is known to be highly heritable; total genetic variation explains up to 70-80% of variance. In an attempt to better understand the genetic architecture of myopia, with an ultimate view to better predict genetic risk and develop targeted treatments, several genome-wide association studies have been performed in the last 6 years. In this review we focus on what a genome-wide association study involves, what studies have been performed in relation to myopia to date, and what they ultimately tell us about myopia variance and functional pathways leading to pathogenesis. The current limitations of genome-wide association studies are reviewed and potential means to improve our understanding of the genetic factors for myopia are described.

### Keywords

Myopia ; Refractive error ; Genetics ; GWAS ; GxE interactions

## Introduction

Myopia is already the commonest eye condition and its prevalence is increasing across the world (1-4). Although myopia is strongly associated with a number of environmental factors, the most important risk factor in determining whether an individual develops the trait is having a family history of myopia, suggesting a genetic predisposition. The heritability of a trait is an estimate of how much phenotypic variation in a population is due to genetic factors. The heritability of refractive error, using spherical equivalent as a quantitative trait, has been determined in a number of family and, more credibly, twin studies [Figure 1]. These indicate the heritability of myopia is high at around 70% (5-15).

**Figure 1** Heritability estimates for refractive error (Abbreviations: T = twin studies, F = family studies).

Myopia is a complex trait influenced by a complicated interplay of genetic and environmental factors. As with many complex traits there is a distribution of refractive error in the population, meaning the risk of ordinary or “simple” myopia developing is not determined by a classic Mendelian single gene mode of inheritance; there are likely many genes, each contributing a small effect to overall myopia risk. This may not be true for very high, familial or syndrome-associated forms of myopia, where a rare dominantly inherited mutation may be important in an individual family, but not important in the overall population risk. Up until the era of genome-wide association studies (GWAS), identification of disease-associated genes relied on family studies (using linkage analysis) or candidate gene studies. In myopia, these were singularly unsuccessful and prior

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3 to 2009 there were no known myopia-associated genes, other than syndromes  
4 where myopia was a part of the phenotypic spectrum (eg Stickler's, Marfan  
5 syndromes). However, with the advent of GWAS a number of genes for myopia  
6 have been identified, providing new insight into how myopia develops with  
7 implications for future research into how this increasingly common eye trait  
8 might be treated.  
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### 16 17 18 19 **Genome-wide association studies (GWAS)**

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21 Genome-wide association studies (GWAS) are approaches that allow a vast array  
22 of markers scattered across an individual's DNA or genome to be rapidly tested  
23 for association with a disease or trait. These 'markers' are variations in the base  
24 pair of nucleotides at specific points along the genome, commonly known as  
25 SNPs (single-nucleotide polymorphisms), and give an indication of what nearby  
26 genes may be associated with the trait.  
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37 In order for this analysis technique to be possible, all of the base pairs, namely  
38 adenine (A), guanine (G), thymine (T) or cytosine (C), forming the human DNA  
39 code had to be sequenced (ie. read and mapped). The human genome project,  
40 completed in 2003, was a major international scientific collaboration that  
41 identified all of the base pairs and genes that make up the human genome,  
42 approximately 20,500 genes in total (16, 17). This has enabled researchers to  
43 have access to a detailed resource on the structure, function and organization of  
44 the complete set of genes that make up the human species. However, to  
45 investigate the association between the human genome and disease, a 'map' of  
46 common patterns of genetic variation and inheritance was required, a 'haplotype  
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3 map'. This was firstly provided by the HapMap project, completed in 2005 (18);  
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5 this international project compared the genetic sequences of individuals of  
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7 African, Asian and European ancestry. Subsequently, the 1000 Genome Project  
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9 that harnessed the increased speed, greater coverage and reduced cost of next-  
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11 generation sequencing was launched. Released in 2012 this has provided the  
12  
13 most detailed catalogue of human genetic variation to date with sequencing of  
14  
15 over 1000 participants internationally (19). These maps of common inheritance  
16  
17 patterns allow identification of what base pair is commonly at one position in the  
18  
19 genome of a certain ethnic population, the 'common' allele, and what base pair  
20  
21 tends not be at that position, the 'minor' allele. SNPs are generally termed a  
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23 common polymorphism when the frequency of the minor allele, in a specific  
24  
25 population, is greater than 1%.  
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33 GWAS rely upon the assumption that common complex traits are caused by  
34  
35 common genetic variations in the population (the "common disease common  
36  
37 variant" hypothesis). Therefore, in a GWAS the association between a trait and  
38  
39 common genetic variants in the form of SNPs is examined. SNPs are not disease-  
40  
41 causing mutations, as found in classical genetic studies of Mendelian rare  
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43 diseases, and they rarely alter protein structure or function, but may relate to  
44  
45 regulation of genes, or alterations in gene expression. In GWAS SNPs are used as  
46  
47 markers, and indicate genes nearby or biological pathways that may be involved,  
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49 allowing researchers to focus in on specific parts of the genome.  
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55 To perform a GWAS for a disease, an individual must be genotyped or sequenced;  
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57 in large-scale genetic studies this is generally undertaken with the use of high-  
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3 throughput genotyping arrays or chips. These provide an output of somewhere  
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5 between 500,000 and 2,500,000 SNPs for that individual, but obviously do not  
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7 include all the common genetic variants (given there are around 3 billion base  
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9 pairs in the human genome). The missing data is therefore imputed using  
10  
11 reference haplotypes, either the HapMap or 1000 Genome data. Associations  
12  
13 between these genetic variations, following extensive data cleaning (quality  
14  
15 control), and disease status is examined in regression models either as a  
16  
17 quantitative trait (eg. refractive error, as spherical equivalent) or as a categorical  
18  
19 case-control trait (eg. 'myopia' or 'no myopia'). The output from such analyses is  
20  
21 a list of associated SNPs with an indication of the strength of effect on myopia  
22  
23 risk (the beta coefficient) and the confidence of the association (p-value).  
24  
25 Significance thresholds are set at less than  $p \leq 5 \cdot 10^{-8}$  to reduce the possibility of  
26  
27 false positive associations, which may occur as result of correlation between  
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29 SNPs and the high number of statistical tests involved. This means large studies  
30  
31 of many thousands of individuals are required to identify statistically significant  
32  
33 associations. Results are generally portrayed graphically as a Manhattan plot,  
34  
35 which plots all the SNPs by chromosome position as a function of their  
36  
37 association p-value; this plot resembles the Manhattan skyline with different  
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39 SNPs reaching higher than others, like skyscrapers, in accordance with variations  
40  
41 in significance. Results of putative genetic associations for a trait ('discovery  
42  
43 stage') must then be verified through replication of associated variants in  
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45 independent population samples, or through experiments that can examine the  
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47 functional implications of the affected gene.  
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3 The first GWAS was performed in 2005 and since then there has been an  
4 exponential rise in the number of studies [Figure 2], reflecting the large  
5 reduction in time and cost of undertaking these types of analysis.  
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12 **Figure 2** Studies, traits and SNP-trait associations from 2005-2013 reveal the  
13 growth in genome-wide association studies. Adapted from (20), Copyright  
14 obtained.  
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21 GWAS have now been successfully performed on a range of ophthalmic diseases  
22 (21, 22). The earliest and arguably the most 'successful' GWAS to date has been  
23 within the ophthalmic field; the discovery of the association of CFH with age-  
24 related macular degeneration was reported in three independent cohorts in  
25 2005 (23-25), one of which was a GWAS, and has since been replicated in dozens  
26 of studies across the world. Subsequent meta-analysis involving large sample  
27 sizes (>17,100 cases and >60,000 controls) has identified 19 loci for AMD  
28 explaining 10-30% of the variance (26), which has an estimated heritability of  
29 45-70%. These genetic associations explain a relatively high proportion of AMD  
30 variance, which disappointingly has proved to be fairly unusual in subsequent  
31 GWAS for other traits. Although GWAS had identified many variants for many  
32 diseases, relatively small effects on disease risk are conferred for the majority of  
33 variants and only a small proportional of familial clustering or heritability is  
34 explained. This issue of 'missing heritability' is a recurrent issue in GWAS and  
35 has prompted researchers to explore additional approaches to examine the  
36 genetic architecture of common complex diseases (27).  
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### Genome-wide association studies in myopia

Refractive error and myopia have been examined using the full range of genetic methodologies. This initially included genome-wide linkage studies in related individuals, and candidate gene association studies. At least 17 loci have been identified through the former and although there was some success with the latter, results have proved poorly reproducible (28-30). The first GWAS study to examine myopia was performed in 2009 on a cohort with high, pathological myopia; subsequent studies have either been performed on myopia case-control cohorts, largely from East Asia where the prevalence of myopia and high myopia is greater, or refractive error as a quantitative trait. A database detailing all published GWAS for myopia, refractive error and other myopia endophenotypes is available at <http://www.ebi.ac.uk/gwas/home>.

The first published GWAS in myopia examined a Japanese population with 297 cases of pathological myopia (defined as axial length > 26mm) and 977 controls from the general population (31). The strongest association was located at 11q24.1, approximately 44kb upstream of the BLID gene, and conferred odds of higher myopia of 1.37 (95% confidence interval (CI) 1.21 - 1.54). Subsequently a meta-analysis of two ethnic Chinese cohorts, published in 2010, was performed for 287 cases of high myopia (defined as  $\leq -6D$ ) and 911 controls (32). The strongest association was an intronic SNP within the CTNND2 gene on 5p15.2. However neither of these initial associations met the conventional GWAS threshold ( $p \leq 5 \times 10^{-8}$ ) for statistical significance.

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3 Li et al also studied an ethnic Chinese population inclusive of 102 high-grade  
4 myopia cases (defined as  $\leq -8D$  with retinal degeneration) and 335 controls (33).  
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7 The strongest association ( $p = 7.70 \times 10^{-13}$ ) was a high frequency variant located  
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10 in a gene desert within the MYP11 myopia linkage locus on 4q25 (34). In a  
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12 similar ethnic Han Chinese population of 419 high myopia cases ( $\leq -6D$ ) and 669  
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14 controls, Shi et al identified the strongest association ( $p = 1.91 \times 10^{-16}$ ) at an  
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16 intronic, high frequency variant within the MIPEP gene on 13q12 (35). Although  
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18 the aforementioned studies attempted replication in independent cohorts, their  
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20 results, published in 2011, have not been replicated in GWAS comprising of  
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22 individuals of similar ethnic background, phenotypic definition or study design.  
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28 In 2013 two papers in Asian populations reported replicated loci for high  
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30 myopia. Shi et al studied a Han Chinese population of 665 cases with high  
31  
32 myopia ( $\leq -6D$ ) and 960 controls (36). Following two-stage replication in three  
33  
34 independent cohorts the most significantly associated variant ( $p = 8.95 \times 10^{-14}$ )  
35  
36 was in the VIPR2 gene within the MYP4 locus, and three further variants all  
37  
38 reaching genome-wide significance were identified within the same linkage  
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40 disequilibrium block in the SNTB1 gene ( $p = 1.13 \times 10^{-8}$  to  $2.13 \times 10^{-11}$ ). Secondly,  
41  
42 Khor et al reported a meta-analysis of four GWAS of East Asian ethnicity totaling  
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44 1603 cases of severe myopia (based on either refractive error or axial length)  
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46 and 3427 controls (37). After replication analysis, the aforementioned SNTB1  
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48 gene was confirmed and a novel variant within the ZFHX1B gene (also known as  
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50 ZEB2) reached genome-wide significance ( $p = 5.79 \times 10^{-10}$ ).  
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3 In European populations, probably illustrating the lower prevalence of high  
4 myopia, there has only been one case-control GWAS from a French population,  
5 published in 2012. In this study of 192 high myopia cases ( $\leq -6D$ ) and 1064  
6 controls a suggestive association was identified within the MYP10 linkage locus,  
7 3kb downstream of PPP1R3B, however this did not reach genome wide  
8 statistical significance and the study failed to replicate any of the previously  
9 reported loci (38).

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21 Greater success has been achieved by considering refractive error as a  
22 quantitative trait, therefore inclusive of all data on the population studied. In  
23 2010 the first two GWAS for refractive error were published, both in European  
24 populations; a British discovery cohort of 4270 individuals (39) and a Dutch  
25 discovery cohort of 5328 individuals (40), with replication in over 10,000  
26 individuals from the two discovery cohorts and a smaller shared pool of  
27 replication samples. Two loci surpassing the GWAS threshold were identified  
28 near the RASGFR1 gene on 15q25.1 ( $p = 2.70 \times 10^{-09}$ ) and the other near GJD2 on  
29 15q14 ( $p = 2.21 \times 10^{-14}$ ). Subsequently, in 2013, a relatively small meta-analysis  
30 was performed on 7280 individuals from five cohorts with refractive error,  
31 inclusive of various ethnic populations across different continents. Replication  
32 was then undertaken in 26,953 samples (41). A novel variant reaching the GWAS  
33 threshold was identified within the RBFOX1 gene on chromosome 16 was  
34 identified ( $p = 3.9 \times 10^{-9}$ ).

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55 The field made a major breakthrough in 2013 when two major GWAS meta-  
56 analysis studies were published. The Consortium for Refractive Error and  
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3 Myopia (CREAM) is an international collaborative initiative between researchers  
4 studying cohorts of both European and Asian descent. A classic meta-analysis of  
5 the GWAS results for a linear regression between genotype and spherical  
6 equivalent of refractive error was performed for 35 participating centers,  
7 comprising 37,382 individuals of European descent and 12,332 of Southeast  
8 Asian ancestry (42). High statistical power was achieved by this large sample  
9 size, enabling replication of the two loci previously identified and identification  
10 of 22 novel loci [Figure 3]: BICC1, BMP2, BMP3, CACNA1D, CD55, CHD7, CHRNG,  
11 CNDP2, CYP26A1, GJD2, CRIA4, KCNJ2, KCNQ5, LAMA2, MYO1D, PCCA, PRSS56,  
12 RASGRF1, RDH5, RORB, SIX6, TOX, ZIC2 and ZMAT4.  
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28 **Figure 3** Manhattan plot of genetic associations for refractive error in the  
29 CREAM combined GWAS meta-analysis.  $-\log_{10}$ -transformed P values for all SNPs.  
30 The upper horizontal line indicates the  $p < 5.0 \times 10^{-8}$  threshold, the lower  
31 horizontal line indicates a p value  $< 1 \times 10^{-5}$  (adapted from (42)).  
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39 A contemporaneous publication by the direct-to-consumer genomics company  
40 23andMe (Mountain View, CA, USA) on a GWAS survival analysis was performed  
41 on 55,177 individuals of European descent using the phenotype of reported  
42 myopia and reported 'age of spectacle wear' as a proxy for myopia severity (43).  
43 The authors identified 20 novel loci: BMP3, BMP4, DLG2, DLX1, GJD2, KCNMA1,  
44 KCNQ5, LAMA2, LRRC4C, PABPCP2, PDE11A, PRSS56, RASGRF1, RBFOX1, RDH5,  
45 RGR, SFRP1, SHISA6, TJP2, TOX, ZBTB38 and ZIC2. Contrary to many  
46 researchers' expectations, the authors identified highly comparable genetic  
47 associations to those obtained using the carefully and expensively collected  
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3 refractive error data in population-based samples in the CREAM consortium. Of  
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5 the 22 loci discovered by CREAM, 14 were replicated by 23andMe, whilst 16 of  
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7 the 20 loci identified by 23andMe were confirmed by CREAM. Surprisingly the  
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9 same 25 genetic loci were identified in both studies with consistent direction of  
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11 effect despite analysis on different scales, namely dioptres for CREAM (more  
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13 negative on the scale indicative of more myopia) and hazard ratios (higher  
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15 positive hazard ratios indicative of more severe myopia) for 23andMe (44, 45).  
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### 20 21 **Genome-wide association studies and myopia endophenotypes**

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23 The most common form of myopia is axial myopia and as such the axial length of  
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25 the eye is a major determinant of the majority of myopia. A number of  
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27 researchers have therefore used this proxy or 'endophenotype' for use in genetic  
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29 association studies of myopia as a quantitative trait. The first of these, published  
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31 in 2012, examined 4944 individuals of East and South East Asian ancestry (46).  
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33 One locus on 1q41 containing the zinc-finger pseudogene ZC3H11B reached  
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35 genome wide significance ( $p = 4.38 \times 10^{-10}$ ), although replication was not  
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37 performed.  
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44 A much larger GWAS meta-analysis for axial length comprising 12,531  
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46 Europeans and 8,216 Asians was published in 2013 (47). Eight, novel genome-  
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48 wide significant loci were identified (RSPO1, C3orf26, LAMA2, GJD2, ZNRF3,  
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50 CD55, MIP, ALPPL2) and the aforementioned ZC3H11B was confirmed.  
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52 Relevantly, five of these loci had been previously associated with refractive  
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54 error.  
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3 Shared determination of an individual's axial length and corneal curvature was  
4 identified in the Avon Longitudinal Study of Parents and Children (ALSPAC) and  
5 Singapore Chinese Eye Study, suggesting that genetic control of these two eye  
6 dimension parameters is by common genetic variants (48). A number of  
7 relatively small GWAS have been performed for corneal curvature with identified  
8 associations in individuals of varying ancestry, including FRAP1, PDGFRA (also  
9 associated with eye size), CMPK1 and RBP3 (49-52). More recently Miyake et al  
10 published a two-stage GWAS for three myopia-related traits: axial length, corneal  
11 curvature and refractive error (53). The study was performed on 9,804 Japanese  
12 individuals with trans-ethnic replication in Chinese and Caucasian individuals. A  
13 novel gene, WNT7B, was identified for axial length ( $p = 3.9 \times 10^{-13}$ ) and corneal  
14 curvature ( $p = 2.9 \times 10^{-40}$ ), whilst the previously reported association with GJD2  
15 and refractive error was replicated.  
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### 35 **Pathways implicated from genome-wide association studies in myopia**

36 Identifying genes associated with myopia is just the first step in gaining the full  
37 utility from GWAS in improving our understanding of myopia etiology. Certain  
38 biological mechanisms are implicated from associated genes, whilst pathway  
39 analysis can enable a more comprehensive, systems biology approach to  
40 understanding how associated genetic variants can ultimately influence ocular  
41 growth. This analysis is of course reliant on what is already known about the  
42 functionality of certain genes.  
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55 Functional pathways (or ontological classifications) implicated by the large  
56 GWAS on myopia to date have been clear and reproducible (54). Interestingly,  
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3 they provide credible evidence that the genetic architecture is fairly consistent  
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5 between two continental populations (European and Asian). As with many  
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7 GWAS, the variants identified have not necessarily fallen within a gene but likely  
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9 functional implications to proximal, relevant genes have been inferred. Although  
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11 this is reasonable, there are other known factors, such as long-range distance  
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13 equilibrium, which may mean alternate genes or pathways could equally be  
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15 involved. Biological processes indicated from the CREAM meta-GWAS include  
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17 neurotransmission (GRIA4), ion transport (KCNQ5), retinoic acid metabolism  
18  
19 (RDH5), extracellular matrix remodeling (LAMA2, BMP2), and eye development  
20  
21 (SIX6, PRSS56) (42). Whilst the 23andMe meta-GWAS similarly implied  
22  
23 extracellular matrix remodeling (LAMA2, ANTXR2), the visual cycle (RDH5, RGR,  
24  
25 KCNQ5), neuronal development (KCNMA1, RBFOX1, LRRC4C, NGL-1, DLG2,  
26  
27 TJP2), eye and body growth (PRSS56, BMP4, ZBTB38, DLX1), and retinal  
28  
29 ganglion cell projections (ZIC2, SFRP1) (43). Enrichment analysis has enabled  
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31 confirmation that groups of genes implied remain remarkably significant  
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33 between different cohorts. Hysi et al reported that plasma membrane, cell-cell  
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35 adhesion, synaptic transmission, calcium ion binding and cation channel activity  
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37 were significantly over-represented in association with refractive error in two  
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39 British cohorts (54).  
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48 Whilst the biological processes implied by these genes may at first seem  
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50 disparate, the protein products and end functions can be highly correlated. By  
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52 examining known protein-protein interactions researchers have identified that  
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54 in fact many of the genes implicated from the meta-GWAS in myopia are related  
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56 to cell cycle and growth pathways such as the MAPK and TGF-beta/SMAD  
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3 pathways, as shown in Figure 4 (45). This network analysis can provide greater  
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5 insight into how refractive error develops and ultimately allow targeted,  
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7 molecular approaches for intervention to be developed by researchers using this  
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9 information.  
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14 **Figure 4** Network connections of genes associated with myopia. Genes identified  
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16 in GWAS are in round grey nodes, linker elements in square nodes, MAPK & TGF-  
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18 beta/SMAD pathway elements are in orange, solid blue edges identify protein-  
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20 protein interactions and dashed blue edges symbolize corregulation  
21  
22 relationships. Adapted from (45).  
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### 25 26 27 28 **Genome-wide association studies and gene-environment interactions**

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30 Although myopia is a highly heritable trait, it is known that environmental  
31  
32 factors are highly influential in determining myopia risk and must be driving the  
33  
34 recent epidemic rise in prevalence (1). One of the most influential and highly  
35  
36 replicated factors is education (4, 55-58); research suggest that those going onto  
37  
38 higher education have double the myopia prevalence than those who leave  
39  
40 school after primary education (4). Education has therefore been the primary  
41  
42 environmental choice for gene-environment (GxE) interaction analyses in  
43  
44 myopia. GxE studies acknowledge that individuals of a differing genotype may  
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46 respond to environmental variation in differing ways; for example in some  
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48 individuals an environmental exposure may trigger a certain gene to be  
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50 unregulated whilst in others there is no effect. This method of analysis therefore  
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52 has the potential to show how existing significantly associated variants are  
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3 modified by environmental exposure, but may also identify variants that were  
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5 previously only suggestively associated with the disease of interest.  
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10 Two research groups have examined this phenomenon by using the myopia-  
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12 associated variants from the CREAM meta-GWAS analysis. In the first, individuals  
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14 of European descent were firstly categorized as having completed a primary,  
15  
16 intermediate or higher education, and then assigned a polygenic risk score based  
17  
18 on the 26 myopia-associated variants from the CREAM meta-GWAS (59). The  
19  
20 effect of higher education and high genetic predisposition was far higher than  
21  
22 the risk of myopia in those with high genetic risk completing only a primary  
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24 education; the odds ratio for those with high genetic risk completing higher  
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26 education was 51.3 (95% CI 18.5 - 142.6) compared to an odds ratio of 7.2 (95%  
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28 CI 3.1 - 17.0) if only primary education was achieved. The combined effect of the  
29  
30 two risk factors was far greater than the sum of the separate factors (synergy  
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32 index = 4.2, 95% CI 1.9-9.5), providing evidence that an interaction effect  
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34 between an environmental factor and an individual's genotype was occurring. A  
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36 similar analysis was performed on five Singaporean cohorts; this analysis  
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38 identified three genes (DNAH9, GJD2 and ZMAT4-SFRP1) that were strongly  
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40 associated with myopia in individuals achieving higher secondary or university  
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42 education but that were either borderline or not statistically significant in  
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44 individuals achieving lower secondary education or below (60).  
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### 51 52 53 **Implications from genome-wide association studies in myopia**

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55 GWAS have enabled considerable progress in our understanding of what genetic  
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57 variants are associated with myopia; the number of variants identified in the  
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3 recent meta-GWAS far exceeds those identified by linkage and candidate gene  
4 studies. However the high heritability of refractive error and myopia, between  
5 70-80% (5-15), is only nominally explained by the variants so far identified. In a  
6 European cohort the variants identified by the CREAM meta-GWAS explain only  
7 3.4% of the variance of refractive error (42). This means approximately 75% of  
8 the expected heritability is 'missing', a recurrent problem in GWAS studies of  
9 complex diseases (27).  
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21 In an attempt to identify missing variants for complex diseases, sample sizes  
22 need to be maximized. It is well known that small sample sizes reduce power and  
23 accuracy in capturing genetic associations. Since the publication of the major  
24 meta-GWAS in refractive error two studies, of relatively small size (less than  
25 1,900 individuals), have failed to fully replicate results (61, 62). Conversely,  
26 results from high-grade GWAS in refractive error were not replicated by the  
27 meta-analysis of CREAM; this may be due to phenotypic or genetic heterogeneity,  
28 or, more likely, lack of statistical power (63, 64). It must be acknowledged that  
29 underpowered GWAS may produce spurious or false-positive results.  
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44 GWAS have confirmed that myopia is highly polygenic with significant variation  
45 in the allelic spectrum of identified loci; that is to say the minor allele frequency,  
46 indicative of how common the polymorphism is within a population, varied  
47 extensively within both the CREAM and 23andMe GWAS (45). However, the  
48 majority of variants had only a small effect on phenotypic variants with the  
49 highest effect sizes limited to the variants with the lowest minor allele frequency  
50 [Figure 5]. GWAS, in its current form, is limited to assessing associations  
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3 between a phenotype and common genetic variants. This means variants of  
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5 lower allelic frequency (rare variants) but potentially large effect sizes have not  
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7 been investigated.  
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12 **Figure 5** Minor allele frequency against effect size for the significant variants  
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14 identified in the CREAM GWAS (adapted from 45).  
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19 We can therefore infer that GWAS will never fully explain all the expected  
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21 heritability from twin studies. A better means of estimating how much variance  
22  
23 can potentially be explained by common genetic variation is to perform a  
24  
25 genome-wide complex trait analysis or SNP-based heritability (65-67). This  
26  
27 technique allows estimation of how much inter-subject variation of a trait can be  
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29 explained by all the available SNPs. The number of SNPs that have been  
30  
31 genotyped or imputed for that individual limits the method, and therefore the  
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33 SNP-based heritability corresponds to a lower-bound estimate. In a pediatric,  
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35 British cohort SNP-based heritability was found to remain stable over childhood  
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37 and, after adjustment for the lack of cycloplegia on the study participants, the  
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39 SNP heritability, averaged over childhood, was 0.35 (standard error=0.09) (68).  
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41 Whilst this would suggest that common genetic variants could explain 35% of  
42  
43 variance, approximately half of the estimated heritability from twins studies. For  
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45 comparison the authors point out that the variance explained by non-genetic risk  
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47 factors, such as time indoors and time spent reading, explain less than 1% of the  
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49 variance in myopia. It therefore remains possible that more common variants of  
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51 small effect could be found using common SNP-based association techniques and  
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53 that there is good merit in continuing to use the technique with ever larger  
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3 sample sizes in attempt to capture more genetic variants. Rarer variants (in the  
4 order of MAF = 1% to 5%), with potentially greater effect on phenotypic  
5 variation, may be identified with improved accuracy using the greater coverage  
6 conferred with the 1000 genomes haplotype map and larger sample sizes.  
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14 One of the key questions for clinicians is can our current, genetic understanding  
15 of myopia allow prediction of future myopia status for patients. Predicting  
16 disease risk is most commonly performed using receiver-operating  
17 characteristic (ROC) curves (69). This is a plot of the sensitivity of a test against  
18 1-specificity of a test using all possible thresholds of high risk versus low risk.  
19 The area under the curve (AUC) is equal to the probability that a randomly  
20 identified individual with the disease has a higher risk than a randomly selected  
21 healthy individual. An AUC, or C statistic, is given as a fraction with a perfect test  
22 yielding an AUC of 1 and a test with no discriminatory power having an AUC of  
23 <0.5. The predictive accuracy of genetic-risk models varies extensively between  
24 diseases but to date confer little benefit over non-genetic risk prediction models  
25 (70). Age related macular degeneration has been a somewhat exception, with an  
26 AUC of 0.82 for the full combination of associated genetic variants identified  
27 through GWAS (71). The utility of prediction models for age-related macular  
28 degeneration in clinical practice has been further tested by adding in phenotypic  
29 and demographic information, such as age and smoking, which increases the  
30 AUC to 0.87 (72). However, in the majority of disease phenotypes an AUC of 0.5  
31 to 0.7 is more commonly achieved (70), which confers little predictive value, and  
32 this is true for myopia at our current level of understanding of the genetic  
33 architecture.  
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5 To increase the potential for predicting genetic risk entails greater  
6 understanding of the genetic architecture of myopia. As discussed, we estimate  
7 there are more common genetic variants to be identified and given that very low  
8 frequency variants are unlikely to contribute greatly to population variance, we  
9 can be optimistic that most of the phenotypic variation in myopia could be  
10 explained by common genetic variants (66). However, there are other genetic  
11 factors contributing to heritability. Genetic risk is a complex result of common  
12 genetic variation, rare genetic variation, gene-environment interactions, gene-  
13 gene interactions, epigenetics, and a host of other variations in our genetic make-  
14 up. Rare genetic variation requires new analysis techniques and more detailed  
15 sequencing of the genome of study participants. Fortunately next-generation  
16 sequencing has enabled reduced costs of high-throughput, high coverage  
17 genotyping, also enabling whole exome and whole genome examination. Higher-  
18 density SNP chips have also been developed, either for higher coverage of the  
19 genome or exome-specific. This means greater coverage of the genome but also  
20 increased accuracy as the reliance on imputation, typically poor for rare SNPs, is  
21 reduced. As methods for analyzing these vast datasets are refined, this will  
22 dramatically increase the potential for identification of rare variants and has  
23 already proved successful (73, 74). Interactions between our environment and  
24 our genome have already proved informative in myopia, whilst interactions  
25 between genes and other genetic architectural analysis techniques hold promise  
26 for the future.

#### Expert commentary

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3 Genome-wide association studies in myopia have undoubtedly transformed our  
4 understanding of the genetic architecture of this complex trait. This is very  
5 relevant as myopia, already the most common eye condition, is increasing in  
6 prevalence throughout world. In light of the fact that myopia is a highly heritable  
7 trait, deeper understanding of how genetic variation leads to development of  
8 myopia is increasingly necessary.  
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19 The genetic variants identified from the major GWAS in myopia have been clear  
20 and reproducible, providing credible evidence for their association. Biological  
21 processes indicated by the identified associations include neurotransmission, ion  
22 transport, retinoic acid metabolism, extracellular matrix remodeling, eye  
23 development, the visual cycle, neuronal development, eye and body growth, and  
24 retinal ganglion cell projections. Enrichment analysis suggests plasma  
25 membrane, cell-cell adhesion, synaptic transmission, calcium ion binding and  
26 cation channel activity appear to be significantly over-represented in association  
27 error. Whilst these biological processes may seem disconnected, protein  
28 products and end functions do appear correlated in myopia risk with many of the  
29 genetic associations related to cell cycle and growth pathways such as the MAPK  
30 and TGF-beta/SMAD pathways.  
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48 However, only around 3% of myopia variance is explained by the genetic  
49 variants identified to date. SNP-based heritability analysis suggests common  
50 genetic variation accounts for approximately 35% of myopia variance. Therefore,  
51 there is more work to be done in an effort to capture all associated common  
52 genetic variants. This requires larger samples and improved genotyping to  
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3 reduce the burden on imputation, which ultimately can lead to poor ability to  
4 capture associated variants or conversely false-positive results. Alternate  
5 analysis techniques and proxy endophenotypes are being explored in an effort to  
6 further increase our ability to identify these variants. The interplay between  
7 genes, and genes and environment is being examined in relation to myopia with  
8 some success, shedding new light on how genetic variation may be modified and  
9 ultimately lead to myopia development in different individuals. It also important  
10 to acknowledge that twin-based estimates of heritability are much higher, at 70-  
11 80%, and suggest that genetic factors other than common genetic variation may  
12 play a role.  
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28 This paper provides a review of our current understanding into the genetics of  
29 myopia. There is much work still to be done, and this will be required before our  
30 ability to predict future development of myopia becomes a reality. GWAS  
31 provides the first step in our ability to identify novel loci and functional  
32 pathways. This must then be built upon with other genetic association modalities  
33 and the use of both animal models, although notably to date there are few  
34 genetic animal models for myopia, and pharmacological studies. Only then can  
35 researchers begin to target the development of myopia and reduce the burden  
36 from this common, sight-threatening disease.  
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### Five-year view

Despite significant progress in recent years, we still can only explain a very small proportion of myopia variance by genetic factors. In the next five years new approaches to try and capture more of the genetic variance will be employed. Firstly the simple approach of 'bigger is better' should be employed; ever-larger meta-analysis of GWAS studies from across the globe must be utilized in a collaborative format to increase the research community's ability to find genes. This may involve using phenotype data that extends beyond the traditional modality of spherical equivalent into combining GWAS performed on proxy phenotypes and endophenotypes.

Secondly a more detailed interrogation of the genome is required to identity rare genetic variants, and notably these variants may play a more significant role in myopia risk. This can be brought about through a number of existing methods. Using currently genotyped data the improved imputation capacity conferred by haplotype maps such as 1000 genomes should be employed to reduce imputational errors leading to false-negative and false-positive associations; notably both of the major GWAS studies on myopia to date are based on HapMap imputed data. An alternate method is employment of the improved genotyping ability that can be achieved with high-density chips and next-generation sequencing. These modalities achieve greater coverage of the genome, reduced genotyping errors and a reduced reliance on imputation. Although there are many obstacles to overcome such as data storage requirements for these vast files, refinement of analysis techniques, and establishment of how results are



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3 interpreted, they do provide a means to attempt to capture the known missing  
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5 heritability in myopia.  
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10 Finally alternate means of understanding the genetic architecture of myopia  
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12 should be employed - extending beyond simple association methods to explore  
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14 interactions and the effect of other 'omics'. This may include incorporation of  
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16 transcriptomics or metabolomics, for example, with existing association methods  
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18 to allow a more systems biology based approach to understanding how genetic  
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20 variation ultimately leads to myopia development.  
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**Key issues**

1. Myopia is the most common eye condition worldwide and the prevalence is increasing.
2. Myopia has a complex trait with strong environmental risk factors such as education and lack of time spent outdoors, and a high heritability of 70-80%.
3. GWAS studies have enabled rapid association of common genetic variants with disease since 2005 in various diseases, most successfully in age-related macular degeneration.
4. Case-control high myopia GWAS studies have been largely performed in Asian populations with a number of genetic variants identified.
5. The largest identification of variants for myopia was performed in two GWAS, by the CREAM consortium and 23andMe, published in 2013; the 26 genetic loci by CREAM identified explain less than 5% of myopia variance.
6. Functional pathways implicated by the genetic variants identified for myopia include plasma membrane, cell-cell adhesion, synaptic transmission, calcium ion binding and cation channel activity, with many of the genetic associations related to cell cycle and growth pathways.
7. Gene by environment analyses suggest interaction effects do occur between the currently identified genetic variants and higher education, one of the strongest risk factors for myopia.
8. In attempt to capture more of the genetic variants for myopia, with the ultimate of aim of enabling risk prediction and developing targeted

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interventions, larger sample sizes are required with deeper coverage of the genome.

For Peer Review Only

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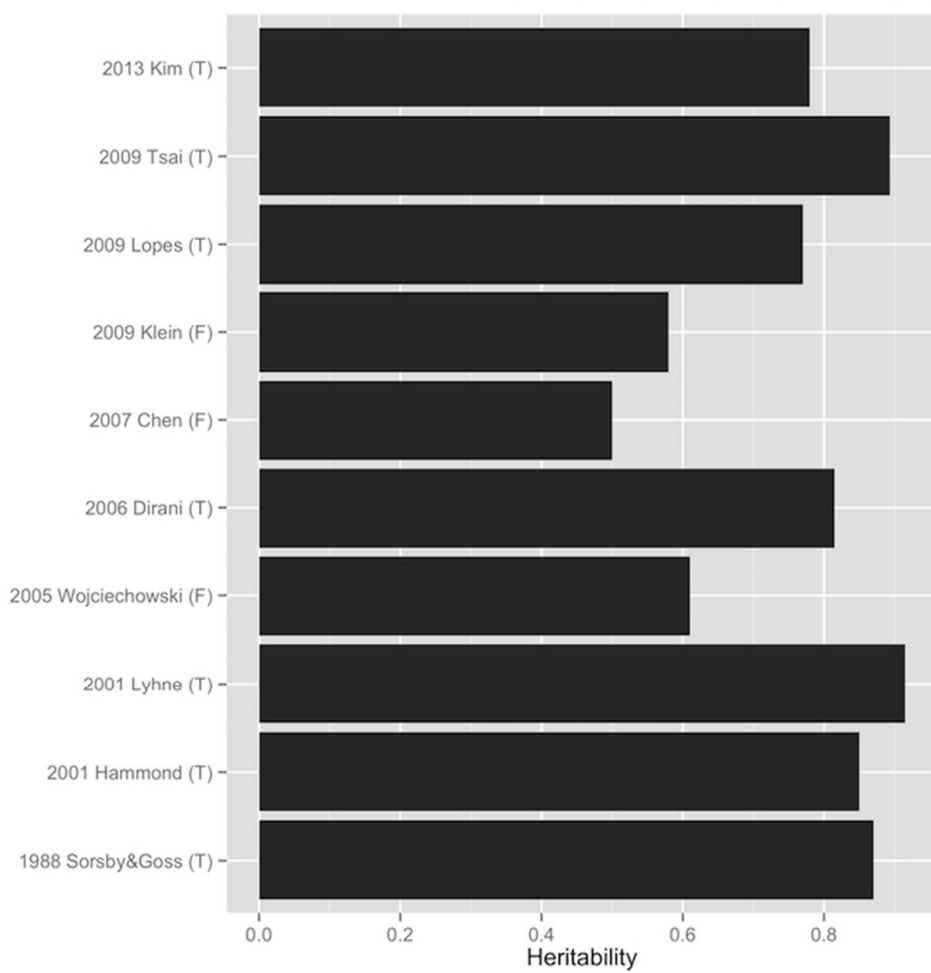


Figure 1  
254x254mm (72 x 72 DPI)

only

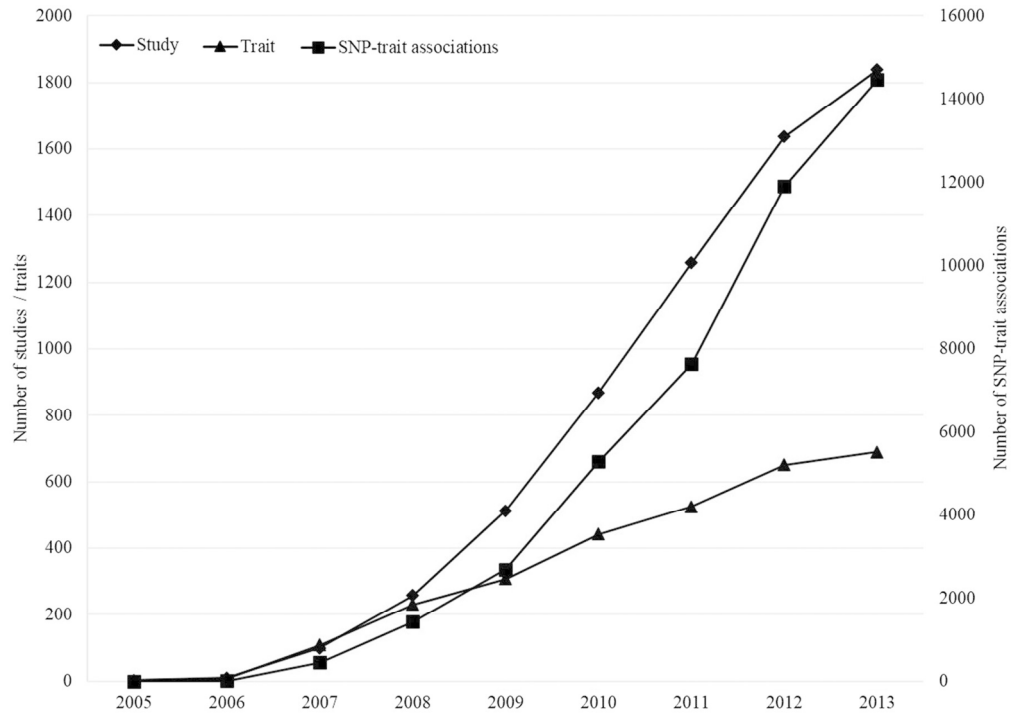


Figure 2  
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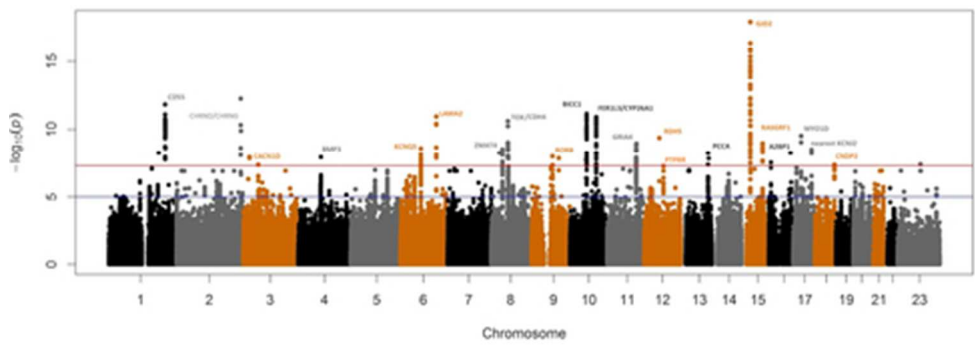


Figure 3  
42x17mm (300 x 300 DPI)

Peer Review Only

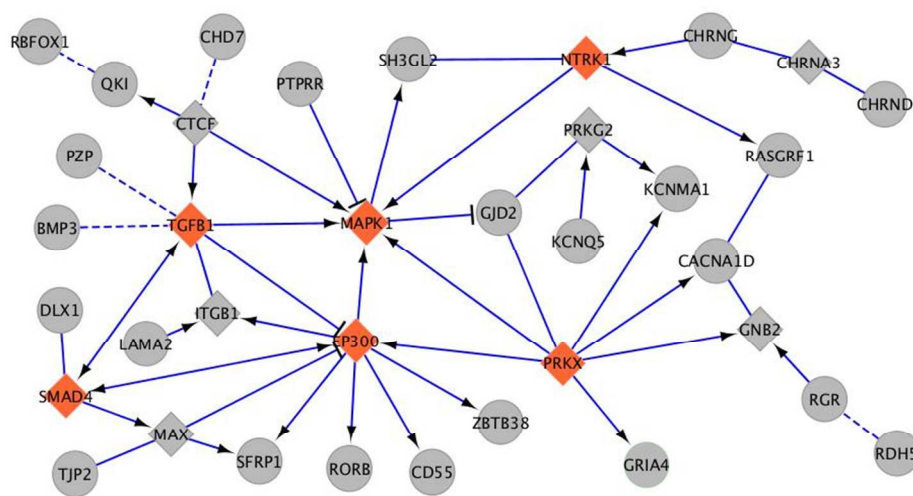


Figure 4  
348x191mm (72 x 72 DPI)

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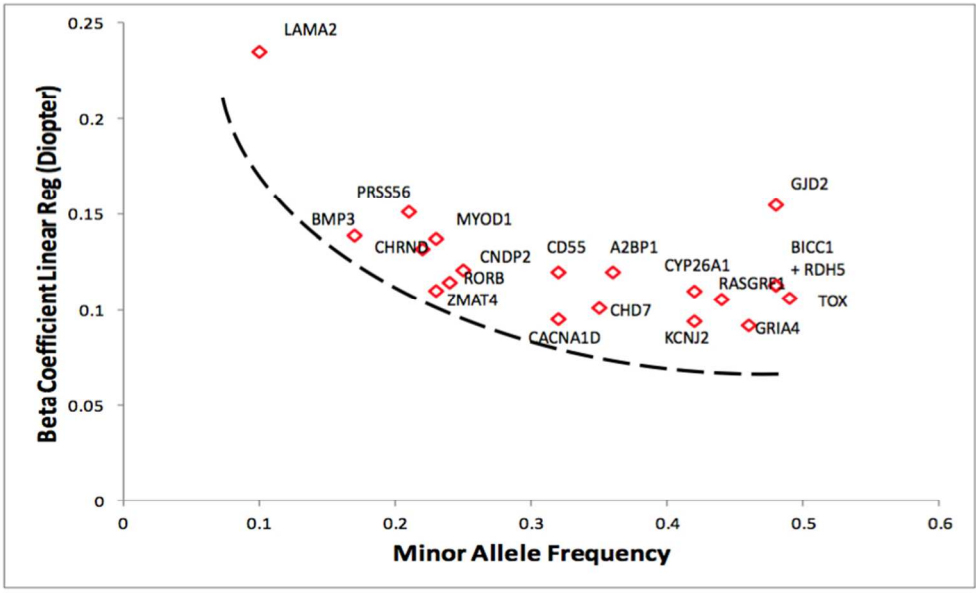


Figure 5  
334x201mm (72 x 72 DPI)

Review Only

## GWAS in myopia: insights into disease and implications for the clinic

### Summary

Myopia is the commonest eye trait worldwide and the prevalence is increasing. It is known to be highly heritable; total genetic variation explains up to 70-80% of variance. In an attempt to better understand the genetic architecture of myopia, with an ultimate view to better predict genetic risk and develop targeted treatments, several genome-wide association studies have been performed in the last 6 years. In this review we focus on what a genome-wide association study involves, what studies have been performed in relation to myopia to date, and what they ultimately tell us about myopia variance and functional pathways leading to pathogenesis. The current limitations of genome-wide association studies are reviewed and potential means to improve our understanding of the genetic factors for myopia are described.

### Keywords

Myopia ; Refractive error ; Genetics ; GWAS ; GxE interactions

## Introduction

Myopia is already the commonest eye condition and its prevalence is increasing across the world (1-4). Refractive error is the term used to describe an error in the accurate focusing of light onto the retinal plane. In myopia, or shortnear-sightedness, there is typically results from axial elongation of the eyeball and this results in an image forming anterior to the retinal plane, whilst in hyperopia the reverse occurs with results when an image forminglies posterior to the retinal plane. Refractive error is the term used to describe an error in the accurate focusing of light onto the retinal plane, encompassing both myopia and hyperopia. Although myopia is strongly associated with a number of environmental factors, the most important risk factor in determining whether an individual develops the trait is having a family history of myopia, suggesting a genetic predisposition. The heritability of a trait is an estimate of how much phenotypic variation in a population is due to genetic factors. The heritability of refractive error, using spherical equivalent as a quantitative trait, has been determined in a number of family and, more credibly, twin studies [Figure 1]. These indicate the heritability of myopia is high at around 70% (5-15).

**Figure 1** Heritability estimates for refractive error (Abbreviations: T = twin studies, F = family studies).

Myopia is a complex trait influenced by a complicated interplay of genetic and environmental factors. As with many complex traits there is a distribution of refractive error in the population, meaning the risk of ordinary or “simple” myopia developing is not determined by a classic Mendelian single gene mode of

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3 inheritance; there are likely many genes, each contributing a small effect to  
4 overall myopia risk. This may not be true for very high, familial or syndrome-  
5 associated forms of myopia - in these cases,~~where~~ a rare dominantly inherited  
6 mutation may be important in an individual family, but not important in the  
7 overall population risk. Up until the era of genome-wide association studies  
8 (GWAS), identification of disease-associated genes relied on family studies (using  
9 linkage analysis) or candidate gene studies. In myopia, these were singularly  
10 unsuccessful and prior to 2009 there were no known myopia-associated genes,  
11 other than syndromes where myopia was a part of the phenotypic spectrum (eg  
12 Stickler's, Marfan syndromes). However, with the advent of GWAS, a number of  
13 genes for myopia have been identified, providing new insight into how myopia  
14 develops with implications for future research into how this increasingly  
15 common eye trait might be treated.  
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### 35 **Genome-wide association studies (GWAS)**

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37 Genome-wide association studies (GWAS) are approaches that allow a vast array  
38 of markers scattered across an individual's DNA or genome to be rapidly tested  
39 for association with a disease or trait. These 'markers' are variations in the base  
40 pair of nucleotides at specific points along the genome, commonly known as  
41 SNPs (single-nucleotide polymorphisms), and give an indication of what nearby  
42 genes may be associated with the trait.  
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53 In order for this analysis technique to be possible, all of the base pairs, namely  
54 adenine (A), guanine (G), thymine (T) or cytosine (C), forming the human DNA  
55 code had to be sequenced (ie. read and mapped). The human genome project,  
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3 completed in 2003, was a major international scientific collaboration that  
4  
5 identified all of the base pairs and genes that make up the human genome,  
6  
7 approximately 20,500 genes in total (16, 17). This has enabled researchers to  
8  
9 have access to a detailed resource on the structure, function and organization of  
10  
11 the complete set of genes that make up the human species. However, to  
12  
13 investigate the association between the human genome and disease, a 'map' of  
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15 common patterns of genetic variation and inheritance was required, [known as](#) a  
16  
17 'haplotype map'. This was firstly provided by the HapMap project, completed in  
18  
19 2005 (18); this international project compared the genetic sequences of  
20  
21 individuals of African, Asian and European ancestry. Subsequently, the 1000  
22  
23 Genome Project, [which](#) ~~that~~ harnessed the increased speed, greater coverage and  
24  
25 reduced cost of next-generation sequencing, was launched. Released in 2012 this  
26  
27 has provided the most detailed catalogue of human genetic variation to date with  
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29 sequencing of over 1000 participants internationally (19). These maps of  
30  
31 common inheritance patterns allow identification of what base pair is commonly  
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33 at one position in the genome of a certain ethnic population, the 'common' allele,  
34  
35 and what base pair tends not be at that position, the 'minor' allele. SNPs are  
36  
37 generally termed a common polymorphism when the frequency of the minor  
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39 allele, in a specific population, is greater than 1%.  
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49 GWAS rely upon the assumption that common complex traits are caused by  
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51 common genetic variations in the population (the "common disease common  
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53 variant" hypothesis). Therefore, in a GWAS the association between a trait and  
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55 common genetic variants in the form of SNPs [are is](#)-examined. SNPs are not  
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57 disease-causing mutations, as found in classical genetic studies of [rare](#) Mendelian  
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3 | diseases, and they rarely alter protein structure or function, but [instead they](#)  
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5 | may relate to regulation of genes or alterations in gene expression. In GWAS  
6 |  
7 | SNPs are used as markers, and indicate [nearby genes](#) ~~nearby~~ or biological  
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9 | pathways that may be involved, allowing researchers to focus in on specific parts  
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11 | of the genome.  
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16 | To perform a GWAS for a disease, an individual must be genotyped or sequenced;  
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18 | in large-scale genetic studies this is generally undertaken with the use of high-  
19 |  
20 | throughput genotyping arrays or chips. These provide an output of somewhere  
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22 | between 500,000 and 2,500,000 SNPs for that individual, but obviously do not  
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24 | include all the common genetic variants (given there are around 3 billion base  
25 |  
26 | pairs in the human genome). The missing data is therefore imputed using  
27 |  
28 | reference haplotypes, either the HapMap or 1000 Genome data. Associations  
29 |  
30 | between these genetic variations, following extensive data cleaning (quality  
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32 | control), and disease status is examined in regression models either as a  
33 |  
34 | quantitative trait (eg. refractive error; [measured by](#) spherical equivalent) or as  
35 |  
36 | a categorical case-control trait (eg. 'myopia' or 'no myopia'). The output from  
37 |  
38 | such analyses is a list of associated SNPs with an indication of the strength of  
39 |  
40 | effect on myopia risk (the beta coefficient) and the confidence of the association  
41 |  
42 | (p-value). Significance thresholds are set at less than  $p \leq 5 \cdot 10^{-8}$  to reduce the  
43 |  
44 | possibility of false positive associations, which may occur as [a](#) result of  
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46 | correlation between SNPs and the high number of statistical tests involved. This  
47 |  
48 | means large studies of many thousands of individuals are required to identify  
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50 | statistically significant associations. Results are generally portrayed graphically  
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52 | as a Manhattan plot, which plots all the SNPs by chromosome position as a  
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3 function of their association p-value; this plot resembles the Manhattan skyline  
4 with different SNPs reaching higher than others, like skyscrapers, in accordance  
5 with variations in significance. Results of putative genetic associations for a trait  
6 ('discovery stage') must then be verified through replication of associated  
7 variants in independent population samples, or through experiments that can  
8 examine the functional implications of the affected gene.  
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19 The first GWAS was performed in 2005 and since then there has been an  
20 exponential rise in the number of studies [Figure 2], reflecting the large  
21 reduction in time and cost of undertaking these types of analysis.  
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28 **Figure 2** Studies, traits and SNP-trait associations from 2005-2013 reveal the  
29 growth in genome-wide association studies. Adapted from (20), Copyright  
30 obtained.  
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37 GWAS have now been successfully performed on a range of ophthalmic diseases  
38 (21, 22). The earliest and arguably the most 'successful' GWAS to date has been  
39 within the ophthalmic field; the discovery of the association of CFH with age-  
40 related macular degeneration was reported in three independent cohorts in  
41 2005 (23-25), one of which was a GWAS, and has since been replicated in dozens  
42 of studies across the world. Subsequent meta-analysis involving large sample  
43 sizes (>17,100 cases and >60,000 controls) has identified 19 loci for AMD  
44 explaining 10-30% of the variance (26), which has an estimated heritability of  
45 45-70%. These genetic associations explain a relatively high proportion of AMD  
46 variance, which disappointingly has proved to be fairly unusual in subsequent  
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3 GWAS for other traits. Although GWAS have identified many variants for many  
4 diseases, relatively small effects on disease risk are conferred for the majority of  
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7 variants and only a small proportion of familial clustering or heritability is  
8  
9 explained. This issue of 'missing heritability' is a recurrent issue in GWAS and  
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11 has prompted researchers to explore additional approaches to examine the  
12  
13 genetic architecture of common complex diseases (27).  
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### 19 **Genome-wide association studies in myopia**

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21 Refractive error and myopia have been examined using the full range of genetic  
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23 methodologies. This initially included genome-wide linkage studies in related  
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25 individuals, which have identified at least 17 loci, and candidate gene association  
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27 studies. ~~At least 17 loci have been identified through the former and although~~  
28  
29 ~~there was some success with the latter, results have proved poorly~~  
30  
31 ~~reproducible which were rarely replicated~~ (28-30). The first GWAS study to  
32  
33 examine myopia was performed in 2009 on a cohort with high, pathological  
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35 myopia; subsequent studies have either been performed on myopia case-control  
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37 cohorts, largely from East Asia where the prevalence of myopia and high myopia  
38  
39 is greater, or on cohorts with refractive error measured as a quantitative trait.  
40  
41 The GWAS catalog A database detailing all published GWAS for myopia,  
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43 refractive error and other myopia endophenotypes was used to identify articles  
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45 for this review (is available at <http://www.ebi.ac.uk/gwas/home>). Articles  
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47 included are summarized in Table 1.  
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55 Table 1 Summary of published GWAS in myopia. † Associations not reaching  
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57 conventional GWAS threshold ( $p \leq 5 \cdot 10^{-8}$ ) for statistical significance  
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### High Myopia GWAS

The first published GWAS in myopia examined a Japanese population with 297 cases of pathological myopia (defined as axial length > 26mm) and 977 controls from the general population (31). The strongest association was located at 11q24.1, approximately 44kb upstream of the BLID gene, and conferred odds of higher myopia of 1.37 (95% confidence interval (CI) 1.21 - 1.54). Subsequently a meta-analysis of two ethnic Chinese cohorts, published in 2010, was performed for 287 cases of high myopia (defined as  $\leq -6D$ ) and 911 controls (32). The strongest association was an intronic SNP within the CTNND2 gene on 5p15.2. However neither of these initial associations met the conventional GWAS threshold ( $p \leq 5 \times 10^{-8}$ ) for statistical significance.

Li et al also studied an ethnic Chinese population inclusive of 102 high-grade myopia cases (defined as  $\leq -8D$  with retinal degeneration) and 335 controls (33). The strongest association ( $p = 7.70 \times 10^{-13}$ ) was a high frequency variant located in a gene desert within the MYP11 myopia linkage locus on 4q25 (34). In a similar ethnic Han Chinese population of 419 high myopia cases ( $\leq -6D$ ) and 669 controls, Shi et al identified the strongest association ( $p = 1.91 \times 10^{-16}$ ) at an intronic, high frequency variant within the MIPEP gene on 13q12 (35). Although ~~these aforementioned~~ studies attempted replication in independent cohorts, their results, published in 2011, have not been replicated in GWAS comprising of individuals of similar ethnic background, phenotypic definition or study design.

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3 In 2013 two papers ~~in Asian populations~~ reported replicated loci for high myopia  
4 in Asian populations. Shi et al studied a Han Chinese population of 665 cases  
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6 with high myopia ( $\leq -6D$ ) and 960 controls (36). Following two-stage replication  
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8 in three independent cohorts the most significantly associated variant ( $p = 8.95 \times$   
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10  $10^{-14}$ ) was in the VIPR2 gene within the MYP4 locus, and three further variants  
11  
12 all reaching genome-wide significance were identified within the same linkage  
13  
14 disequilibrium block in the SNTB1 gene ( $p = 1.13 \times 10^{-8}$  to  $2.13 \times 10^{-11}$ ). ~~Secondly,~~  
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16  
17 Khor et al reported a meta-analysis of four GWAS of East Asian ethnicity  
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19 ~~totaling~~ 1603 cases of “severe” myopia (based on either refractive error  
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21 or axial length) and 3427 controls (37). After replication analysis, the  
22  
23 ~~aforementioned~~ SNTB1 gene was confirmed and a novel variant within the  
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25 ZFHX1B gene (also known as ZEB2) reached genome-wide significance ( $p = 5.79$   
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27  $\times 10^{-10}$ ).  
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35 In European populations, probably illustrating the lower prevalence of high  
36  
37 myopia, there has only been one case-control GWAS from a French population,  
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39 published in 2012. In this study of 192 high myopia cases ( $\leq -6D$ ) and 1064  
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41 controls a suggestive association was identified within the MYP10 linkage locus,  
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43 3kb downstream of PPP1R3B, however this did not reach genome wide  
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45 statistical significance and the study failed to replicate any of the previously  
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47 reported loci (38).  
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### Refractive Error Quantitative GWAS.

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55 Greater success has been achieved by considering refractive error as a  
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57 quantitative treat, ~~therefore including inclusive all subjects in population-based~~  
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3 studies rather than a selected clinic-based sample of all data on the population  
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5 studied of highly affected individuals. In 2010 the first two GWAS for refractive  
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7 error were published, both in European populations; a British discovery cohort  
8  
9 of 4270 individuals (39) and a Dutch discovery cohort of 5328 individuals (40),  
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11 with replication in over 10,000 individuals from the two discovery cohorts and a  
12  
13 smaller shared pool of replication samples. Two loci surpassing the GWAS  
14  
15 threshold were identified near the RASGFR1 gene on 15q25.1 ( $p = 2.70 \times 10^{-09}$ )  
16  
17 and the other near GJD2 on 15q14 ( $p = 2.21 \times 10^{-14}$ ). Subsequently, in 2013, a  
18  
19 relatively small meta-analysis was performed on 7280 individuals from five  
20  
21 cohorts with refractive error, inclusive of various ethnic populations across  
22  
23 different continents. Replication was then undertaken in 26,953 samples (41). A  
24  
25 novel variant reaching the GWAS threshold was identified within the RBFOX1  
26  
27 gene on chromosome 16 was identified ( $p = 3.9 \times 10^{-9}$ ).  
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35 The field made a major breakthrough in 2013 when two major GWAS meta-  
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37 analysis studies were published. The Consortium for Refractive Error and  
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39 Myopia (CREAM) is an international collaborative initiative between researchers  
40  
41 studying cohorts of both European and Asian descent. A classic meta-analysis of  
42  
43 the GWAS results for a linear regression between genotype and spherical  
44  
45 equivalent of refractive error was performed for 35 participating centers,  
46  
47 comprising 37,382 individuals of European descent and 12,332 of Southeast  
48  
49 Asian ancestry (42). High statistical power was achieved by this large sample  
50  
51 size, enabling replication of the two loci previously identified and identification  
52  
53 of 22 novel loci at genome-wide significance [Figure 3]: BICC1, BMP2, BMP3,  
54  
55 CACNA1D, CD55, CHD7, CHRNG, CNDP2, CYP26A1, GJD2, CRIA4, KCNJ2, KCNQ5,  
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3 LAMA2, MYO1D, PCCA, PRSS56, RASGRF1, RDH5, RORB, SIX6, TOX, ZIC2 and  
4  
5 ZMAT4.  
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10 **Figure 3** Manhattan plot of genetic associations for refractive error in the  
11  
12 CREAM combined GWAS meta-analysis.  $-\log_{10}$ -transformed  $p$  values for all  
13  
14 SNPs. The upper horizontal line indicates the  $p < 5.0 \times 10^{-8}$  threshold, the lower  
15  
16 horizontal line indicates a  $p$  value  $< 1 \times 10^{-5}$  (adapted from (42)).  
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21 A contemporaneous ~~publication-GWAS~~ by the direct-to-consumer genomics  
22  
23 company 23andMe (Mountain View, CA, USA) ~~on-using a GWAS~~ survival analysis  
24  
25 was performed on 55,177 individuals of European descent using the phenotype  
26  
27 of reported myopia and reported 'age of spectacle wear' as a proxy for myopia  
28  
29 severity (43). The authors identified 20 novel loci: BMP3, BMP4, DLG2, DLX1,  
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31 GJD2, KCNMA1, KCNQ5, LAMA2, LRRC4C, PABPCP2, PDE11A, PRSS56, RASGRF1,  
32  
33 RBFOX1, RDH5, RGR, SFRP1, SHISA6, TJP2, TOX, ZBTB38 and ZIC2. Contrary to  
34  
35 many researchers' expectations, the authors identified highly comparable  
36  
37 genetic associations to those obtained using the carefully and expensively  
38  
39 collected refractive error data in population-based samples in the CREAM  
40  
41 consortium. Of the 22 loci discovered by CREAM, 14 were replicated by  
42  
43 23andMe, whilst 16 of the 20 loci identified by 23andMe were confirmed by  
44  
45 CREAM. Surprisingly the same 25 genetic loci were identified in both studies  
46  
47 with consistent direction of effect despite analysis on different scales, namely  
48  
49 dioptres for CREAM (more negative on the scale indicative of more myopia) and  
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51 hazard ratios (higher positive hazard ratios indicative of more severe myopia)  
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53 for 23andMe (44, 45).  
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## Genome-wide association studies and myopia endophenotypes

The most common ~~form-type~~ of myopia is axial myopia (~~lens-induced or lenticular myopia is seen in old age due to early nuclear cataract~~) and as such the axial length of the eye is a major determinant of ~~refractive errorthe majority of myopia~~. A number of researchers have therefore used this ~~myopia~~ proxy or 'endophenotype' ~~for use in genetic association studies of myopia as a quantitative trait~~. The first of these, published in 2012, examined 4944 individuals of East and South East Asian ancestry (46). One locus on 1q41 containing the zinc-finger pseudogene ZC3H11B reached genome wide significance ( $p = 4.38 \times 10^{-10}$ ), although replication was not performed.

A much larger GWAS meta-analysis for axial length comprising 12,531 Europeans and 8,216 Asians was published in 2013 (47). Eight, novel genome-wide significant loci were identified (RSP01, C3orf26, LAMA2, GJD2, ZNRF3, CD55, MIP, ALPPL2) and the ~~aforementioned study also replicated the ZC3H11B was confirmed gene~~. Relevantly, five of these loci had been previously associated ~~with in~~ refractive error ~~GWAS~~.

Shared determination of an individual's axial length and corneal curvature was identified in the Avon Longitudinal Study of Parents and Children (ALSPAC) and Singapore Chinese Eye Study, suggesting that ~~shared genetic variants genetic control of these two parameters which contribute to the eye's focuseye dimension parameters is by common genetic variants~~ (48). A number of relatively small GWAS have been performed for corneal curvature ~~in individuals~~

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3 of varying ancestry with identified associations ~~in individuals of varying ancestry~~  
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5 ~~r~~ including FRAP1, PDGFRA (also associated with eye size), CMPK1 and RBP3  
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7 (49-52). More recently Miyake et al published a two-stage GWAS for three  
8  
9 myopia-related traits: axial length, corneal curvature and refractive error (53).  
10  
11 The study was performed on 9,804 Japanese individuals with trans-ethnic  
12  
13 replication in Chinese and Caucasian individuals. A novel gene, WNT7B, was  
14  
15 identified for axial length ( $p = 3.9 \times 10^{-13}$ ) and corneal curvature ( $p = 2.9 \times 10^{-40}$ ),  
16  
17 whilst the previously reported association with GJD2 and refractive error was  
18  
19 replicated.  
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### 26 **Pathways implicated from genome-wide association studies in myopia**

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28 Identifying genes associated with myopia is just the first step in gaining  
29  
30 maximizing information~~the full utility~~ from GWAS ~~in to~~ improving our  
31  
32 understanding of myopia etiology. ~~Certain~~ Some individual biological  
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34 mechanisms ~~are can be~~ implicated from ~~associated~~ genes associated, ~~whilst but~~  
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36 pathway analysis ~~can enables~~ a more comprehensive, systems biology approach  
37  
38 to understanding how associated genetic variants can ultimately influence ocular  
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40 growth. Pathway analysis, however, does rely on previously published work on  
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42 ~~This analysis is of course reliant on what is already known about~~ the  
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44 functionality of certain genes.  
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51 Functional pathways (or ontological classifications) implicated by the large  
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53 GWAS on myopia to date have been clear and reproducible (54). Interestingly,  
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55 they provide credible evidence that the genetic architecture is fairly consistent  
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57 between two continental populations (European and Asian). As with many  
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3 GWAS, the variants identified have not necessarily fallen within a gene but likely  
4 functional implications to proximal, relevant genes have been inferred. Although  
5 this is reasonable, there are other known factors, such as long-range distance  
6 equilibrium, which may mean alternate genes or pathways could equally be  
7 involved. Biological processes indicated from the CREAM meta-GWAS include  
8 neurotransmission (GRIA4), ion transport (KCNQ5), retinoic acid metabolism  
9 (RDH5), extracellular matrix remodeling (LAMA2, BMP2), and eye development  
10 (SIX6, PRSS56) (42). ~~Whilst the 23andMe meta-GWAS similarly implied~~  
11 extracellular matrix remodeling (LAMA2, ANTXR2), the visual cycle (RDH5, RGR,  
12 KCNQ5), neuronal development (KCNMA1, RBFOX1, LRRC4C, NGL-1, DLG2,  
13 TJP2), eye and body growth (PRSS56, BMP4, ZBTB38, DLX1), and retinal  
14 ganglion cell projections (ZIC2, SFRP1) (43). ~~Enrichment analysis has enabled~~  
15 ~~confirmation that groups of genes implied remain remarkably significant~~  
16 ~~between different cohorts.~~ Hysi et al reported that plasma membrane, cell-cell  
17 adhesion, synaptic transmission, calcium ion binding and cation channel activity  
18 were significantly over-represented in association with refractive error in two  
19 British cohorts (54).

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44 Whilst the biological processes implied by these genes may at first seem  
45 disparate, the protein products and end functions can be highly correlated. By  
46 examining known protein-protein interactions researchers have identified that  
47 in fact many of the genes implicated from the meta-GWAS in myopia are related  
48 to cell cycle and growth pathways such as the MAPK and TGF-beta/SMAD  
49 pathways, as shown in Figure 4 (45). This network analysis can provide greater  
50 insight into how refractive error develops, although it must be acknowledged  
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3 [that the risk loci identified from GWAS have not been shown to be causative in](#)  
4 [functional studies and therefore any pathway analysis is speculative, and](#)  
5 [ultimately allow targeted, molecular approaches for intervention to be](#)  
6 [developed by researchers using this information.](#)  
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14 **Figure 4** Network connections of genes associated with myopia. Genes identified  
15 in GWAS are in round grey nodes, linker elements in square nodes, MAPK & TGF-  
16 beta/SMAD pathway elements are in orange, solid blue edges identify protein-  
17 protein interactions and dashed blue edges symbolize coregulation  
18 relationships. Adapted from (45).  
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#### 28 **Genome-wide association studies and gene-environment interactions**

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30 Although myopia is a highly heritable trait, it is known that environmental  
31 factors are highly influential in determining myopia risk and must be driving the  
32 recent epidemic rise in prevalence (1). One of the most influential and highly  
33 replicated factors is education (4, 55-58); research suggest that those going onto  
34 higher education have double the myopia prevalence than those who leave  
35 school after primary education (4). Education has therefore been the primary  
36 environmental choice for gene-environment (GxE) interaction analyses in  
37 myopia. GxE studies acknowledge that individuals of a differing genotype may  
38 respond to environmental variation in differing ways; for example in some  
39 individuals an environmental exposure may trigger a certain gene to be  
40 unregulated whilst in others there is no effect. This method of analysis therefore  
41 has the potential to show how [prior identified existing, significantly associated](#)  
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3 variants are modified by environmental exposure, but may also identify variants  
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5 that were previously only suggestively associated with the disease of interest.  
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10 Two research groups have examined this phenomenon by using the myopia-  
11 associated variants from the CREAM meta-GWAS ~~analysis~~. In the first, individuals  
12 of European descent were ~~firstly~~ categorized as having completed a primary,  
13 intermediate or higher education, and then assigned a polygenic risk score based  
14 on the 26 myopia-associated variants from the CREAM meta-GWAS (59). ~~There~~  
15 ~~appeared to be an interaction between the~~ effect of higher education and ~~having~~  
16 ~~a high genetic predisposition risk score was far higher than the risk of myopia in~~  
17 ~~those with high genetic risk completing only a primary education~~; the odds ratio  
18 ~~for myopia in for~~ those with high genetic risk completing higher education was  
19 51.3 (95% CI 18.5 - 142.6) compared to an odds ratio of 7.2 (95% CI 3.1 - 17.0) if  
20 only primary education was achieved. The combined effect of the two risk factors  
21 was far greater than the sum of the separate factors (synergy index = 4.2, 95% CI  
22 1.9-9.5), providing evidence that an interaction effect between an environmental  
23 factor and an individual's genotype was occurring. A similar analysis was  
24 performed on five Singaporean cohorts; this analysis identified three genes  
25 (DNAH9, GJD2 and ZMAT4-SFRP1) that were strongly associated with myopia in  
26 individuals achieving higher secondary or university education but that were  
27 either borderline or not statistically significant in individuals achieving lower  
28 secondary education or below (60).  
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### 55 **Implications from genome-wide association studies in myopia**

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3 GWAS have enabled considerable progress in our understanding of what genetic  
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5 variants are associated with myopia; the number of variants identified in the  
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7 recent meta-GWAS far exceeds those identified by linkage and candidate gene  
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9 studies. However the high heritability of refractive error and myopia which is  
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11 between 70-80% (5-15), is only ~~nominally partly~~ explained by the variants so far  
12  
13 identified. In a European cohort the variants identified by the CREAM meta-  
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15 GWAS explain only 3.4% of the variance of refractive error (42). This means  
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17 approximately 75% of the expected heritability is 'missing', a recurrent problem  
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19 in GWAS studies of complex diseases (27).  
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27 In an attempt to identify missing variants for complex diseases, sample sizes  
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29 need to be maximized. It is well known that small sample sizes reduce power and  
30  
31 accuracy in capturing genetic associations. Since the publication of the major  
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33 meta-GWAS in refractive error two studies, of relatively small size (less than  
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35 1,900 individuals), have failed to fully replicate results (61, 62). Conversely,  
36  
37 results from high-grade GWAS in refractive error were not replicated by the  
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39 meta-analysis of CREAM; this may be due to phenotypic or genetic heterogeneity,  
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41 or, more likely, lack of statistical power (63, 64). It must be acknowledged that  
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43 underpowered GWAS may produce spurious or false-positive results.  
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49 GWAS have confirmed that myopia is highly polygenic with significant variation  
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51 in the allelic spectrum of identified loci; that is to say the minor allele frequency,  
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53 indicative of how common the polymorphism is within a population, varied  
54  
55 extensively within both the CREAM and 23andMe GWAS (45). However, the  
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57 majority of variants had only a small effect on phenotypic variants with the  
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3 highest effect sizes limited to the variants with the lowest minor allele frequency  
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5 [Figure 5]. GWAS, in its current form, is limited to assessing associations  
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7 between a phenotype and common genetic variants. This means variants of  
8  
9 lower allelic frequency (rare variants) but potentially large effect sizes have not  
10  
11 been investigated.  
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16 **Figure 5** Minor allele frequency against effect size for the significant variants  
17 identified in the CREAM GWAS (adapted from 45).  
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23 We can therefore infer that GWAS will never fully explain all the expected  
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25 heritability from twin studies. A better means of estimating how much variance  
26  
27 can potentially be explained by common genetic variation is to perform a  
28  
29 genome-wide complex trait analysis or SNP-based heritability (65-67). This  
30  
31 technique allows estimation of how much inter-subject variation of a trait can be  
32  
33 explained by all the available SNPs. The number of SNPs that have been  
34  
35 genotyped or imputed for that individual limits the method, and therefore the  
36  
37 SNP-based heritability corresponds to a lower-bound estimate. In a pediatric,  
38  
39 British cohort SNP-based heritability was found to remain stable over childhood  
40  
41 and, after adjustment for the lack of cycloplegia on the study participants, the  
42  
43 SNP heritability, averaged over childhood, was 0.35 (standard error=0.09) (68).  
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48 ~~Whilst this would suggest that common genetic variants could explain 35% of~~  
49 ~~variance, approximately half of the estimated heritability from twin studies. For~~  
50 ~~comparison the authors point out that the variance explained by non-genetic risk~~  
51 ~~factors, such as time indoors and time spent reading, [is explain](#) less than 1% of~~  
52 ~~the variance in myopia.~~ It therefore remains possible that more common  
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3 variants of small effect could be found using common SNP-based association  
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5 techniques and that there is ~~good~~-merit in continuing to use the technique with  
6  
7 ever larger sample sizes in ~~an~~ attempt to capture more genetic variants. Rarer  
8  
9 variants (in the order of MAF = 1% to 5%), with potentially greater effect on  
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11 phenotypic variation, may be identified with improved accuracy using the  
12  
13 greater coverage conferred with the 1000 genomes haplotype map and larger  
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15 sample sizes.  
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21 One of the key questions for clinicians is ~~can-whether~~ our current ~~genetic~~  
22  
23 understanding of myopia ~~genetics~~ allows prediction of future myopia status for  
24  
25 ~~patientschildren~~. Predicting disease risk is most commonly performed using  
26  
27 receiver-operating characteristic (ROC) curves (69). This is a plot of the  
28  
29 sensitivity of a test against 1-specificity of a test using all possible thresholds of  
30  
31 high risk versus low risk. The area under the curve (AUC) is equal to the  
32  
33 probability that a randomly identified individual with the disease has a higher  
34  
35 risk than a randomly selected healthy individual. An AUC, or C statistic, is given  
36  
37 as a fraction with a perfect test yielding an AUC of 1 and a test with no  
38  
39 discriminatory power having an AUC of <0.5. The predictive accuracy of genetic-  
40  
41 risk models varies extensively between diseases but to date confer little benefit  
42  
43 over non-genetic risk prediction models (70). Age related macular degeneration  
44  
45 has been an ~~an-somewhat~~ exception, with an AUC of 0.82 for the full combination of  
46  
47 associated genetic variants identified through GWAS (71). The utility of  
48  
49 prediction models for age-related macular degeneration in clinical practice has  
50  
51 been further tested by adding in phenotypic and demographic information, such  
52  
53 as age and smoking, which increases the AUC to 0.87 (72). However, in the  
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3 majority of disease phenotypes an AUC of 0.5 to 0.7 is more commonly achieved  
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5 (70), which confers little predictive value, and this is true for myopia at our  
6  
7 current level of understanding of the genetic architecture.  
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11 To increase the potential for predicting genetic risk entails greater  
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13 understanding of the genetic architecture of myopia. As discussed, we estimate  
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15 there are more common genetic variants to be identified and given that very low  
16  
17 frequency variants are unlikely to contribute greatly to population variance, we  
18  
19 can be optimistic that most of the phenotypic variation in myopia could be  
20  
21 explained by common genetic variants (66). However, there are other genetic  
22  
23 factors contributing to heritability. Genetic risk is a complex result of common  
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25 genetic variation, rare genetic variation, gene-environment interactions, gene-  
26  
27 gene interactions, epigenetics, and a host of other variations in our genetic make-  
28  
29 up. Rare genetic variation requires new analysis techniques and more detailed  
30  
31 sequencing of the genome of study participants. Fortunately next-generation  
32  
33 sequencing has ~~provided~~ enabled reduced costs of high-throughput, high  
34  
35 coverage genotyping, ~~also~~ enabling whole exome and whole genome  
36  
37 examination. Higher-density SNP chips have also been developed, either for  
38  
39 higher coverage of the genome or exome-specific. This means greater coverage of  
40  
41 the genome but also increased accuracy as the reliance on imputation, typically  
42  
43 poor for rare SNPs, is reduced. As methods for analyzing these vast datasets are  
44  
45 refined, this will dramatically increase the potential for identification of rare  
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47 variants and has already proved successful (73, 74). Interactions between our  
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49 environment and our genome have already proved informative in myopia, whilst  
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3 interactions between genes and other genetic architectural analysis techniques  
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5 hold promise for the future.  
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### 8 9 10 **Expert commentary**

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12 Genome-wide association studies in myopia have undoubtedly transformed our  
13  
14 understanding of the genetic architecture of this complex trait. This is very  
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16 relevant as myopia, already the most common eye condition, is increasing in  
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18 prevalence throughout world. In light of the fact that myopia is a highly heritable  
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20 trait, deeper understanding of how genetic variation leads to development of  
21  
22 myopia is increasingly necessary.  
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28 The genetic variants identified from the major GWAS in myopia have been clear  
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30 and reproducible, providing credible evidence for their association. Biological  
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32 processes indicated by the identified associations include neurotransmission, ion  
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34 transport, retinoic acid metabolism, extracellular matrix remodeling, eye  
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36 development, the visual cycle, neuronal development, eye and body growth, and  
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38 retinal ganglion cell projections. Enrichment analysis suggests plasma  
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40 membrane, cell-cell adhesion, synaptic transmission, calcium ion binding and  
41  
42 cation channel activity appear to be significantly over-represented in ~~association~~  
43  
44 ~~refractive~~ error. ~~Whilst these biological processes may seem disconnected,~~  
45  
46 ~~protein products and end functions do appear correlated in myopia risk, with~~  
47  
48 ~~many~~ Many of the genetic associations are related to cell cycle and growth pathways  
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51 such as the MAPK and TGF-beta/SMAD pathways.  
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3 However, only around 3% of myopia variance is explained by the genetic  
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5 variants identified to date. SNP-based heritability analysis suggests common  
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7 genetic variation accounts for approximately 35% of myopia variance. Therefore,  
8  
9 there is more work to be done in an effort to capture all associated common  
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11 genetic variants. This requires larger samples and improved genotyping to  
12  
13 reduce the burden on imputation, which ultimately can lead to poor ability to  
14  
15 capture associated variants or conversely false-positive results. Alternate  
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17 analysis techniques and proxy endophenotypes are being explored in an effort to  
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19 further increase our ability to identify these variants. The interplay between  
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21 genes, and genes and environment is being examined in relation to myopia with  
22  
23 some success, shedding new light on how genetic variation may be modified and  
24  
25 ultimately lead to myopia development in different individuals. It also important  
26  
27 to acknowledge that twin-based estimates of heritability are much higher, at 70-  
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29 80%, and suggest that genetic factors other than common genetic variation may  
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31 play a role.  
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39 This paper provides a review of our current understanding into the genetics of  
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41 myopia. There is much work still to be done, and this will be required before our  
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43 ability to predict future development of myopia becomes a reality. GWAS  
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45 provides the first step in our ability to identify novel loci and functional  
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47 pathways. This must then be built upon with other genetic association modalities  
48  
49 and the use of both animal models, although notably to date there are few  
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51 genetic animal models for myopia, and pharmacological studies. Only then can  
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53 researchers begin to target [myopia](#) development ~~of myopia~~ and reduce the  
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55 burden from this common, sight-threatening disease.  
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**Five-year view**

Despite significant progress in recent years, we still can only explain a very small proportion of myopia variance by genetic factors. In the next five years new approaches to try and capture more of the genetic variance will be employed. Firstly the simple approach of 'bigger is better' should be employed; ever-larger meta-analysis of GWAS studies from across the globe must be utilized in a collaborative format to increase the research community's ability to find genes. This may involve using phenotype data that extends beyond the traditional modality of spherical equivalent into combining GWAS performed on proxy phenotypes and endophenotypes.

Secondly a more detailed interrogation of the genome is required to identity rare genetic variants, and notably these variants may play a more significant role in myopia risk. This can be brought about through a number of existing methods. Using currently genotyped data the improved imputation capacity conferred by haplotype maps such as 1000 genomes should be employed to reduce imputational errors leading to false-negative and false-positive associations; notably both of the major GWAS studies on myopia to date are based on HapMap imputed data. An alternate method is employment of the improved genotyping ability that can be achieved with high-density chips and next-generation sequencing. These modalities achieve greater coverage of the genome, reduced genotyping errors and a reduced reliance on imputation. Although there are many obstacles to overcome such as data storage requirements for these vast files, refinement of analysis techniques, and establishment of how results are

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3 interpreted, they do provide a means to attempt to capture the known missing  
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5 heritability in myopia.  
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10 Finally alternate means of understanding the genetic architecture of myopia  
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12 should be employed - extending beyond simple association methods to explore  
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14 interactions and the effect of other 'omics'. This may include incorporation of  
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16 transcriptomics or metabolomics, for example, with existing association methods  
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18 to allow a more systems biology based approach to understanding how genetic  
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20 variation ultimately leads to myopia development.  
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### Key issues

1. Myopia is the most common eye condition worldwide and the prevalence is increasing.
2. Myopia has a complex trait with strong environmental risk factors such as education and lack of time spent outdoors, and a high heritability of 70-80%.
3. GWAS studies have enabled rapid association of common genetic variants with disease since 2005 in various [traits/diseases](#), most successfully in age-related macular degeneration.
4. Case-control high myopia GWAS studies have been largely performed in Asian populations with a number of genetic variants identified.
5. The largest identification of variants for myopia was performed in two GWAS, by the CREAM consortium and 23andMe, published in 2013; the 26 genetic loci by CREAM identified explain less than 5% of myopia variance.
6. Functional pathways implicated by the genetic variants identified for myopia include plasma membrane, cell-cell adhesion, synaptic transmission, calcium ion binding and cation channel activity, with many of the genetic associations related to cell cycle and growth pathways.
7. Gene by environment analyses suggest interaction effects do occur between the currently identified genetic variants and higher education, one of the strongest risk factors for myopia.
8. In [an](#) attempt to capture more of the genetic variants for myopia, with the ultimate of aim of enabling risk prediction and developing targeted

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3 interventions, larger sample sizes are required with deeper coverage of  
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5 the genome.  
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Study	Year	Association count	Region of associations	Genes implicated
Nakanishi H et al. A genome-wide association analysis identified a novel susceptible locus for pathological myopia at 11q24.1.	2009	1 †	11q24.1	BLID
Li YJ et al. Genome-wide association studies reveal genetic variants in CTNND2 for high myopia in Singapore Chinese.	2011	1 †	5p15.2	CTNND2
Li Z et al. A genome-wide association study reveals association between common variants in an intergenic region of 4q25 and high-grade myopia in the Chinese Han population.	2011	2	4q25	MYP11 linkage locus
Shi Y et al. Genetic variants at 13q12.12 are associated with high myopia in the Han Chinese population.	2011	1	13q12	MIPEP
Meng W et al. A genome-wide association study provides evidence for association of chromosome 8p23 (MYP10) and 10q21.1 (MYP15) with high myopia in the French Population.	2012	64 †	8p23 10q21.1	MYP10 linkage locus MYP15 linkage locus
Shi Y et al. A genome-wide meta-analysis identifies two novel loci associated with high myopia in the Han Chinese population	2013	5	13q12.12 8q24.12	VIPR2 SNTB1
Khor CC et al. Genome-wide association study identifies ZFHX1B as a susceptibility locus for severe myopia.	2013	2	2q22.3 8q24.12	ZFHX1B SNTB1
Hysi PG et al. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25	2010	1	15q25.1	RASGFR1
Solouki AM et al. A genome-wide association study	2010	1	15q14	GJD2

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identifies a susceptibility locus for refractive errors and myopia at 15q14.				
Stambolian D et al. Meta-analysis of genome-wide association studies in five cohorts reveals common variants in RBFOX1, a regulator of tissue-specific splicing, associated with refractive error.	2013	1	16p13.3	RBFOX1
Verhoeven VJ et al. Genome-wide meta-analyses of multiancestry cohorts identify multiple new susceptibility loci for refractive error and myopia.	2013	26		BICC1, BMP2, BMP3, CACNA1D, CD55, CHD7, CHRNG, CNDP2, CYP26A1, GJD2, CRIA4, KCNJ2, KCNQ5, LAMA2, MYO1D, PCCA, PRSS56, RASGRF1, RDH5, RORB, SIX6, TOX, ZIC2, ZMAT4
Kiefer AK et al. Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia.	2013	22		BMP3, BMP4, DLG2, DLX1, GJD2, KCNMA1, KCNQ5, LAMA2, LRRC4C, PABPCP2, PDE11A, PRSS56, RASGRF1, RBFOX1, RDH5, RGR, SFRP1, SHISA6, TJP2, TOX, ZBTB38, ZIC2