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Meta-analysis of 542,934 subjects of European ancestry identifies 336 novel genes and mechanisms predisposing to refractive error and myopia

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Abstract

Refractive errors, in particular myopia, are a leading cause of morbidity and disability world-wide and their prevalence is rising, largely due to cultural and environmental changes. Genetic investigation is a valuable tool to better understand the molecular mechanisms underlying abnormal eye development and impaired vision. We conducted a meta-analysis of genome-wide association studies involving 542,934 European participants and identified 336 novel genetic loci associated with refractive error that explain an additional 4.6% of spherical equivalent heritability, or an improvement by a third over the previous estimates. Collectively, all associated genetic variants explain 18.4% of heritability and improve the accuracy of myopia prediction (AUC=0.75). Our results suggest that refractive error is genetically heterogeneous, driven by genes participating in the development of every anatomical component of the eye. In addition, our analyses suggest that genetic factors controlling circadian rhythm and pigmentation are also involved in the development of myopia and refractive error. These results may make possible predicting refractive error and the development of personalized myopia prevention strategies in the future.

Results

Association Results.

Analyses were restricted to subjects of European ancestry (Supplementary Figure 1) and combined results from quantitative measures of spherical equivalent and categorical myopia status. Spherical equivalent quantifies RE; a negative spherical equivalent, below a certain threshold defines myopia. We used results obtained from GWAS of directly measured spherical equivalent in 102,117 population-based UK Biobank participants, and 34,998 subjects participating in the GERA Study and combined them with results of analyses of self-reported myopia in 106,086 cases and 85,757 controls from the customer base of 23andMe, Inc. (Mountain View, CA), a personal genomics company. Additionally, we included results from an analysis on the refractive status inferred using demographic and self-reported information on age at first use of prescription glasses among the UK Biobank participants not contributing to the quantitative GWAS (108,956 likely myopes to 70,941 likely non-myopes, see Supplementary Methods). All analyses were adjusted for age, sex and main principal components. To obtain an overall association with RE, we meta-analyzed the results from all studies by using the z-scores.
from the GWAS of the spherical equivalent and the negative values of z-scores from the case-control studies (23andMe and UK Biobank), since myopia is negatively correlated with spherical equivalent. As expected, the large total sample size of the discovery meta-analysis (N=508,855) led to a nominally large genomic inflation factor (λ=1.94). The LD score regression intercept was (1.17), and the \( 1/(\text{mean(chi}^2)-1) \) ratio of 0.097 is fully in line with the expectations of polygenicity. We found associations for 438 discrete genomic regions (Figure 1, Supplementary Table 1), defined by markers contiguously associated at conventional level of GWAS significance \(^{12,13} \) of \( p<5\times10^{-8} \), separated by more than 1 Mbp from other GWAS-associated markers, as recommended elsewhere \(^{14} \). Among them, 308 loci, including 14 on chromosome X, were not described in previous GWAS studies of refractive error \(^7 \). The observed effect sizes were consistent across all the studies (Supplementary Table 1 and Supplementary File 1). The association with RE was statistically strongest for rs12193446 (\( p=9.87\times10^{-328} \)), within LAMA2, a gene previously associated with RE \(^{5,6} \), mutations of which cause muscular dystrophy \(^{15} \). Consistent with these LAMA2 properties, polymorphisms located within the genes coding for both major LAMA2 receptors, DAG1 \(^{16} \) (\( p=1.67\times10^{-38} \) for rs111327216) and ITGA7 \(^{17} \) (\( p=8.57\times10^{-9} \) for rs17117860) which are also known causes of muscular dystrophy \(^{18,19} \), were significantly associated with RE in the discovery meta-analysis.

We compared our discovery meta-analysis findings with GWAS results from 34,079 participants in the CREAM consortium, who were part of a previously reported meta-analysis \(^7 \). To avoid any potential overlap with the UK Biobank participants, only non-UK European CREAM participants were used for replication. Despite the vast power differential, 55 of the SNPs that showed the strongest association in their respective regions in the discovery meta-analysis were significant after Bonferroni correction in the replication sample. A further 142 had a false discovery rate (FDR) < 0.05 and 192 were nominally significant at \( p<0.05 \) (Supplementary Table 2). The effect sizes observed in the discovery and replication samples were strongly correlated (Pearson’s \( r=0.91 \), Supplementary Figure 2). Meta-analysis of all five cohorts (discovery and replication) expanded the number to 449 associated regions of variable length and number of SNPs (Supplementary Figure 3), of which 336 regions were novel (Supplementary Table 3).

Most of the 449 RE-associated regions contained at least one gene linked to severe ocular manifestations in the Online Mendelian Inheritance in Man (OMIM) resource or other genes with interesting link to eye disease (Supplementary Table 4). Although most loci identified through our meta-analyses were novel, several of them hosted genes that harbor mutations leading to myopia or other RE phenotypes. Several genes significantly associated with RE were linked to Mendelian disorders affecting corneal structure, some of which code for transcription factors involved in corneal development \(^{20} \) (Supplementary Table 5). Mutations in these genes cause corneal dystrophies (SLC4A11, \( p=5.81\times10^{-11} \) for rs41281858, TCF4, \( p=4.14\times10^{-8} \), rs41396445; LCAT, \( p=1.26\times10^{-10} \), rs5923; and DCN, \( p=3.67\times10^{-9} \), rs1280632), megalocornea (LTBP2, \( p=1.91\times10^{-24} \), rs73296215) and keratoconus (FNDC3B, \( p=1.89\times10^{-14} \), rs199771582, previously described \(^7 \)). Eleven RE-associated genes were linked to anomalies of the crystalline lens (Supplementary Table 6), including genes linked to autosomal dominant cataracts (PAX6 previously linked to myopia \(^{21} \), \( p=8.31\times10^{-11} \), rs1540320; PITX3, \( p=1.05\times10^{-10} \), rs7923183; MAF, \( p=5.50\times10^{-9} \), rs16951312; CHMP4B, \( p=9.95\times10^{-11} \), rs6087538; TDRD7, \( p=4.79\times10^{-8} \), rs13301794) and lens ectopia (FBN1, \( p=3.30\times10^{-24} \), rs2017765; ADAMTS14, \( p=8.19\times10^{-14} \), rs12131376). Some of the genes affected several eye components. For example, LTBP2 variants are also associated with congenital glaucoma \(^{22} \), and COL4A3 (rs7569375, \( p=1.14\times10^{-8} \)) causes Alport syndrome, which manifests with abnormal lens shape (lenticous) and structural changes in the retina.

Association was also observed within or near 13 genes known to harbor mutations causing microphthalmia (Supplementary Table 7), including TENT1 (\( p=2.48\times10^{-11} \), rs35446926); OTX2
with other pigmentation traits (Supplementary Table 13), although typically association was found for SNPs not strongly associated with this gene forms a conjoint read-through transcript the markers were located within genes involved in systemic pigmentation also previously associated with blindness and retinitis pigmentosa, and age-related macular degeneration (HTRA1/ARMS2). Among genes in novel regions associated with RE, ABCA4 (p=3.20x10^{-10} for rs11165052), and ARMS2/HTRA1 (p=5.72x10^{-13} for rs2142308) are linked to macular disorders and numerous others to retinitis pigmentosa, retinal dystrophy and other retinal diseases, such as FBN2, (p=8.63x10^{-11}, rs6860901), TRAF3IP1 (p=5.71x10^{-16}, rs7596847), CWC27 (p=1.84x10^{-18}, rs1309551). Significant association was found near other genes of interest such as DRD1 (p=4.51x10^{-16}, rs13190379), a dopamine receptor. Together, these results are consistent with previous suggestions of light transmission and transduction in RE^{25}.

Wnt signaling has previously been implicated in experimental myopia^{26}. We found significant association near several Wnt protein-coding genes (WNT7B, a gene previously associated with axial length^{27}, p=1.42x10^{-26} for rs73170583; WNT10A, previously associated with central corneal thickness^{28}, p=1.65x10^{-17} for rs121908120 and WNT3B, p=8.52x10^{-16} for rs70600), suggesting that organogenesis through Wnt signaling is likely to be involved in RE. Significant association were found at genes coding for key canonical (e.g. rs13072632 within the CTNNB1 gene, p=7.30x10^{-27}; AXIN2, rs9895291, p=1.40x10^{-08}) and non-canonical Wnt pathway members (NFATC3, rs147561310, p=1.493x10^{-12}) or at genes coding for both (RHOA, rs7623687, p=1.81x10^{-11} or the previously described^{7} TCF7L2, rs56299331, p=9.38x10^{-46}; Supplementary Table 9).

Similar to previous published analyses^{25}, we found associations for genes involved in sodium, potassium, calcium magnesium and other cation transporters (Supplementary Table 10). The involvement of genes related to glutamatergic synaptic transmission was also notable (Supplementary Table 11). Glutamate is a first synapse transmitter released by photoreceptors towards bipolar cells and is the main excitatory neurotransmitter of the retina, and expression of genes participating in glutamate signaling pathways is significantly altered in myopia models^{29}. These associations support the involvement in RE pathogenesis of neurotransmission and neuronal depolarization and hyperpolarization that was also suggested before^{7}. Associations with POU6F2 gene intronic variants (rs2696187, p=1.11x10^{-11}) also suggests involvement of factors related to development of amacrine and ganglion cells^{30}. Other genes at RE-associated loci were annotated to infantile epilepsy, microcephaly, severe learning difficulty, or other inborn diseases affecting the central nervous system (CNS) in OMIM (Supplementary Table 12).

Polymorphisms in genes linked to oculocutaneous albinism (OCA) were significantly associated with RE (Supplementary Table 13), although typically association was found for SNPs not strongly associated with other pigmentation traits^{31}. Strong association with RE was found near the OCA2 gene causing OCA type 2 (p=1.37x10^{-15}, rs79406658), OCA3 (TYRPI, p=1.18x10^{-11}, rs62538956), OCA5 (SLC39A8, p=4.03x10^{-17}, rs13107325), OCA6 (C10orf11, p=1.73x10^{-16}, rs12256171). In addition, significant association was found near genes linked to ocular albinism (OA) on chromosome X (TBL1X and GPR143, p=2.20x10^{-18}, rs34437079) and Hermansky-Pudlak Syndrome albinism (BLOC1S1, p=2.4610^{-22}, for rs80340147; note that this gene forms a conjoint read-through transcript the BLOC1S1-RDH5 with RDHS). Other associated markers were located within genes involved in systemic pigmentation also previously associated with RE^{2}, such as RALY (p=3.14x10^{-18}, rs2843888), TSPAN10 (p=2.22x^{-50}, rs9747347), as well as melanoma (MCHR2, p=2.37x10^{-15} for rs4839756).
**Functional properties of the associated markers**

 Among the significantly associated markers, 367 unique markers were frameshift or missense variants (Supplementary Table 14). Several are non-synonymous, such as the R141L mutation (rs1048661) within LOXL1, a gene that causes pseudoexfoliation syndrome and glaucoma and A69S (rs10490924) in ARMS2, associated with increased susceptibility to age-related macular degeneration. Other associated variants with predicted deleterious consequences were located in several genes, such as RGR (p=6.89x10^{-6}, rs1042454), a gene previously associated with RE and also retinitis pigmentosa, and within the FBN1 gene, near clusters of mutations that cause Marfan Syndrome and anterior segment dysgenesis.

 Because the functional link between other associated variants and development of RE phenotypes is less obvious, we next performed gene-set enrichment analyses to identify properties that are significantly shared by genes identified by the meta-analysis. An enrichment analysis of Gene Ontology processes (Supplementary Table 15) found enrichment for genes participating in RNA Polymerase II transcription regulation (p=1x10^{-6}) and nucleic acid binding transcription factor activity (p=1.10x10^{-6}), suggesting that many of the genetic associations we identified interfere with gene expression. “Eye development” (p=6.10x10^{-6}) and “Circadian regulation of gene expression” (p=1.10x10^{-4}) were also significantly enriched.

 A transcription factor binding site (TFBS) enrichment analysis identified significant (FDR < 0.05) over-representation of sites targeted by GATA4, EP300, RREB1, for which association was observed in the meta-analyses (Supplementary Table 16). Binding sites of transcription factors involved in eye morphogenesis and development such as MAF (whose mutations cause autosomal cataract), FOXC1 and PITX2 (anterior segment dysgenesis) or CRX (cone-rod dystrophy) were also enriched. CRX and PAX4, binding sites were also significantly enriched; these transcription factors are two of the regulators of circadian rhythm and melatonin synthesis alongside OTX2, for which SNP significant association was observed in our RE meta-analysis (p=6.15x10^{-11} for rs928109). All of these enriched gene-sets are observed for the first time in a GWAS analysis, although the presence of some of the mechanisms that relate them to RE and myopia were hypothesized before.

 Many of the variants associated with RE in our analyses were located within or near genes that are expressed in numerous body tissues (Supplementary Figure 4), and in particular from the nervous system, consistent with our evidence of extraocular, central nervous system involvement in RE. Within the eye, these genes were particularly strongly expressed in eye tissues such as cornea, ciliary body, trabecular meshwork and retina (Supplementary Figure 5, Supplementary Table 17). A stratified LD score regression applied to specifically expressed genes (LDSC-SEG) revealed the results of the GWAS are most strongly correlated with genes expressed in the retina and basal ganglia in the central nervous system but these correlations are not significant after multiple testing correction (Supplementary Figure 6 and Supplementary Table 18). It is possible that the strength of these correlations was constrained by the fact that in most cases, available expression levels were measured in adult samples, while refractive error and myopia are primarily developed in younger ages.

 A Summary data-based Mendelian Randomization (SMR) analysis integrating GWAS with eQTL data from peripheral blood and brain tissues found concomitant association with RE and eQTL transcriptional regulation effects for 159 and 97 genes respectively (Supplementary Tables 19 and 20). A similar analysis integrating GWAS summary data with methylation data from brain tissues found association with both RE and changes in methylation for 134 genes (Supplementary Table 21).
Examine the GWAS Catalog, some of the genetic variants reported here were previously associated with RE, and with other traits, in particular intraocular pressure, intelligence and education; the latter two are known myopia risk factors (Supplementary Table 22). We used LD score regression to assess the correlation of genetic effects between RE and other phenotypes from GWAS summary statistics (Supplementary Table 23). RE genetic risk was significantly correlated with intelligence, both in childhood (rs=-0.27, p=4.76x10^{-9}) and adulthood (fluid intelligence score rs=-0.25, p=1.56x10^{-39}), educational attainment (defined as the number of years spent in formal education, rs=-0.24, p=3.36x10^{-54}), self-reported cataract (rs=-0.31, p=4.70x10^{-10}) and intraocular pressure (IOP, rs=-0.14, p=1.04x10^{-12}). Higher educational attainment appears to cause myopia as demonstrated by Mendelian randomization (MR) studies. A gene by environment interaction GWAS for spherical equivalent and educational attainment (using age at completion of formal full-time education as a proxy) was conducted in 66,242 UK Biobank participants. Despite the relatively well-powered sample, only one locus yielded evidence of statistically significant interaction (rs536015141 within TRPM1, p=2.35x10^{-09}, Supplementary Table 24), suggesting that the true relationship between RE and education is compounded by several factors and may not be linear in nature, as suggested recently. TRPM1 is localized in rod ON bipolar cell dendrites, and rare mutations cause congenital stationary night blindness, often associated with high myopia.

To further explore the nature of the relationship between RE and IOP, we built MR models using genetic effects previously reported for IOP. On average, every 1 mmHg increase in IOP predicts a 0.05-0.09 diopters decrease in spherical equivalent (Supplementary Table 25, Supplementary Figure 7). We also built a MR model to assess the relationship between intelligence and spherical equivalent, but statistical evidence in this case points towards genetic pleiotropy rather than causation (Supplementary Table 26). This suggests that both myopia and intelligence are often influenced by the same factors, but without direct causal path linking one to the other. We found no significant genetic correlations between RE and the glaucoma endophenotype vertical cup to disc ratio (rs=-0.01, p=0.45), or hair pigmentation (rs=-0.03, p=0.35). Therefore, RE and pigmentation may have different allelic profiles with limited sharing of genetic risk.

We subsequently carried out a conditional analysis on the meta-analysis summary results and found a total of 904 independent SNPs significantly associated with RE. 890 of these markers were available in the EPIC-Norfolk Study, an independent cohort that did not participate in the RE meta-analysis (Supplementary Figure 8). These markers alone explained 12.1% of the overall spherical equivalent phenotypic variance in a regression model or 18.4% (SE=0.04) of the spherical equivalent heritability. Newly associated markers found in our meta-analysis, but not in the previous large GWAS, explain 4.6% (SE=0.01) of the spherical equivalent phenotypic variance in EPIC-Norfolk Study, which is an improvement of one third compared to heritability explained by previously associated markers.

Predictive models, based on the above-mentioned 890 SNPs, along with age and sex, were predictive of myopia (versus all non-myopia controls) with areas under the receiving operating characteristic curve (AUC) of 0.67, 0.74 and 0.75 (Figure 2), depending on the severity cutoff for myopia (< -0.75D, < -3.00D and < -5.00D respectively). The performance of the predictions appears not to improve for myopia definitions of -3.00D or worse, suggesting that the information extracted from our meta-analysis is more representative of the genetic risk for common myopia seen in the general population, than for more severe forms of myopia, which may have a distinctive genetic architecture.
Analysis of the distribution of effects and number of associated variants needed to explain all RE heritability

Using information from over half a million population-based participants SNPs identified in these analyses still only explain 18.4% of the spherical equivalent heritability. We next assessed how many common SNPs are likely to explain the entire heritable component of RE, and what sample sizes are likely to be needed in the future to identify them, using the likelihood-based approach described elsewhere. We estimate that approximately 13,808 (SE=969) polymorphic variants are likely to be behind the full RE heritability. Similar to other quantitative phenotypic traits that are previously published, our analyses estimate that 10.3% (SE=1.0%) of the phenotypic variance is likely explained by a batch of approximately 543 (SE=81) common genetic variants of relatively large effect size and a further 20.8% (SE=0.9%) of the entire phenotypic variance explained by the remainder. With increased sample sizes, we project that the proportion of variance explained will continue to improve fast but will start plateauing for sample sizes above one million, after which further increases in sample size will likely yield ever diminishing additional phenotypic variance (Supplementary Figure 9).

Discussion

Our results provide evidence for at least two major sets of mechanisms in the pathogenesis of RE. The first affect intraocular pressure, eye structure, ocular development and physiology, and the second are CNS-related, including circadian rhythm control. Contributors to RE include all anatomical factors that alter refractive power relative to eye size, light transmittance, photoconductance and higher cerebral functions.

The findings implicate almost every single anatomical components of the eye, which along with the central nervous system participate in the development of RE. The healthy cornea contributes to 70% of the optical refractive power of the eyes and genes involved in corneal structure, topography and function may directly contribute to RE through direct changes in the corneal refraction. Our results show that several genes involved in lens development also contribute to RE in the general population. It is unclear if their contribution is mediated through alterations in biomechanical properties that affect eyes’ ability to accommodate, changes to the lens refractive index, or alterations in light transmission properties that impair the ability to focus images on the retina.

Many retinal genes are implicated in the development of refractive error, reflecting the role of light in mediating eye growth and the importance of the retina’s role in light transduction and processing. Associations with RE at genes coding for gated ion channels and glutamate receptors point to the photoreceptor-bipolar cell interface as a potentially key factor in RE. Rare mutations in several of our associated genes cause night blindness, implicating the rod system in the pathophysiology of RE, but many also affect cone pathways. The TRPM1 gene, important for rod ON bipolar cell polarity, is also implicated in the gene-education interaction analysis. Associations observed for the VSX1 and VSX2, its negative regulator, genes implicate the cone bipolar cells.

The association with genes involved in pigmentation, including most of the OCA-causing genes, raises questions about the relationship between melanin, pigmentation and eye growth and development. These associations are unlikely to be influenced by any cryptic population structure in our samples, which our analyses were designed to control. None of the major pigmentation-associated SNPs was directly associated with RE and there was no significant correlation of genetic effects between RE and pigmentation.
The mechanisms linking pigmentation with RE are unclear. Foveal hypoplasia and optic disc dysplasias are common in all forms of albinism. Although melanin synthesis is disrupted in albinism, both melanin and dopamine are synthesized through shared metabolic pathways. Disc and chiasmal lesions in albinism are often attributed to dopamine, but we found limited evidence supporting an association with RE for genetic variants involved in dopamine signaling. The scarcity of association with RE for genes involved in dopamine-only pathways contrasts with the abundance of association for genes involved in pigmentation and melanin synthesis. This may suggest that melanin metabolism is connected to RE through other mechanisms that are independent from the metabolic pathways it shares with dopamine production. Melanin reaches the highest concentrations in the retinal pigment epithelium at the outmost layer of the retina, and anteriorly, in the iris and variations in pigmentation may affect the intensity of the light reaching the retina. Light exposure is a major protective factor for development of myopia. It is possible that pigmentation plays a role in light signal transmission and transduction.

Animal model experiments suggest that in addition to local ocular mechanisms, emmetropization (the process by which the eye develops to minimize refractive error) is strongly influenced by the CNS. The strong correlation of genetic risks between RE and intelligence and association found for genes linked to severe learning disability support an involvement of the CNS in emmetropization and RE pathogenesis.

Results from gene-set enrichment analysis demonstrate an interesting evolution with increasing sample sizes. While smaller previous studies were sufficiently powered to discover enrichment of low, cell-level properties, such as cation channel activity and participation in the synaptic space structures, significantly more powered recent studies have found additional evidence for enrichment and involvement of more integrated physiological functions, such as light signal processing in retinal cells and others. Beyond the identification of a much larger number of genes and explaining significantly higher proportions of heritability, our results, based on a considerably more statistically powered sample, uphold the previous findings and support the involvement of the same molecular and physiological mechanisms that were previously described.

In line with expectations from a higher power of association to discover genes and gene sets individually responsible for even smaller proportions of the refractive error variance, we find evidence for even higher regulatory mechanisms, that act more holistically over the eye development or integrate eye growth and homeostasis with other processes of extraocular nature. For example, we found evidence that binding sites of transcription factors involved in the control of circadian rhythm are significantly enriched among genes associated with refractive error. Circadian rhythm is important in emmetropization and its disruption leads to myopia in animal knock-out models, potentially through dopamine-mediated mechanisms, or changes in IOP and diurnal variations.

Most of the loci identified through our meta-analysis are not subject to particularly strong and systematic evolutionary pressures (Supplementary Figure 10). The variability in minor allele frequencies observed across loci associated with RE may therefore be the result of genetic drift. However, given the variety of the different visual components whose disruptions can result in RE, this variability may also be the result of overall balancing forces which encourage high allelic diversity of genes involved in RE, providing additional buffering capacity to absorb environmental pressures or genetic disruptions on any of the individual components of the visual system.

Our results cast light on potential mechanisms that contribute to RE in the general population and have identified the genetic factors that explain a considerable proportion of the heritability and phenotypic variability of RE. This allows us to improve significantly our ability to make predictions of myopia risk and generate novel hypotheses on how multiple aspects of visual processing affect emmetropization, which may pave the way to personalized risk management and treatment of RE in the population in the future.
Online Methods provided separately
Figure 1. All GWAS-associated regions from the main meta-analysis. Each band is a true scale of genomic regions associated with refractive error listed in Supplementary Table 1 (+250kbp on each side to make smaller regions more visible). The different color codes represent the significance (p-value) for the genetic variant within that region that displays the strongest evidence for association.
Figure 2. Receiver Operating Characteristic (ROC) curves for myopia predictions, using information from 890 SNP markers identified in the meta-analysis. The three different colors represent three different curves for each of the different definition of myopia: red – all myopia (< -0.75D), blue – moderate myopia (< -3.00 D) and green - severe myopia (defined as < -5.00 D).
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