Genetics of Myopia in a Participant Driven, Web-Based Cohort

Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia

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Introduction

Myopia, or nearsightedness, is the most common eye disorder worldwide. In the United States, an estimated 30-40% of the adult population has clinically relevant myopia (more severe than -1 diopter), and the prevalence has increased markedly in the last 30 years. Myopia is a refractive error that results primarily from increased axial length of the eye. The increase in eye length leads to improper focusing of images anterior to the retinal plane.

Genetics plays a substantial role in myopia. Heritability estimates from twin and sibling studies range from 50-90%.[1] To date, there have been seven genome-wide association studies (GWAS) on myopia or related phenotypes (pathological myopia, refractive index, and ocular axial length): two in Europeans[2,3] and five in Asian populations. Each of these publications has identified a different single association with myopia. In addition there have been several linkage studies and an exome sequencing study of severe myopia. Using our web-based research platform, we were able to cheaply and efficiently collect information on myopia from over 40,000 people, making this the largest study to date.

Methods

We conducted a genome-wide association study on age of onset of nearsightedness. All participants were drawn from the customer base of 23andMe, Inc., a personal genetics company. All participants were of primarily European ancestry, and no pair was more closely related than at the level of first cousins.

Phenotypic Data
Our discovery cohort consisted of 43,360 participants who reported via a web-based questionnaire whether they had been diagnosed with nearsightedness, and if so, at what age (Table 1).

A separate, non-overlapping set of 4,277 participants who answered a standalone question about whether they used corrective eyewear for nearsightedness before the age of 10 were used as a replication cohort (Table 1).

Genotyping
Participants were genotyped for 586,916 to 1,008,949 SNPs on one of three Illumina-based BeadChips. An additional 7,356,559 imputed SNPs were included in the analysis.

Table 1. Summary of the cohorts used in the analysis

	Number	% female	Age (SE)	Age of onset (SE)
Discovery, myopic	26038	46.1	48.6 (15.7)	16.4 (11.0)
Discovery, not myopic	17322	39.6	49.1 (17.1)	—
Replication, myopic at 10	800	45.1	47.7 (14.9)	≤ 10
Replication, not myopic at 10	3477	45.2	50.0 (16.6)	—

Sex, current age, and age of onset for discovery and replication cohorts.

Statistical Analyses
We performed a genome-wide survival analysis using a Cox proportional hazards model on the discovery cohort. This model assumes that there is an (unknown) baseline probability of developing myopia as a function of age. The model then tests whether each single nucleotide polymorphism (SNP) is associated with a significantly higher or lower probability of developing myopia compared to baseline. The Cox model can be thought of as a generalization of an analysis of myopia age of onset that allows for the inclusion of non-myopic controls, resulting in considerably increased statistical power. Analyses controlled for sex and five principal components of genetic ancestry.

Results and Discussion

A total of 19 SNPs crossed our threshold for genome-wide significance ($p < 5\times10^{-8}$) in the discovery cohort (Figure 1). These 19 SNPs include the two SNPs previously associated with refractive error in Europeans near *GJD2/ACTC1,* and near *RASGRF1*. Of these 19, 9 were significant in our replication cohort. Given the small size of the replication cohort and the less precise assessment of age of onset, it is unsurprising that not all the SNPs replicated (see Table 2; replicated SNPs are highlighted).

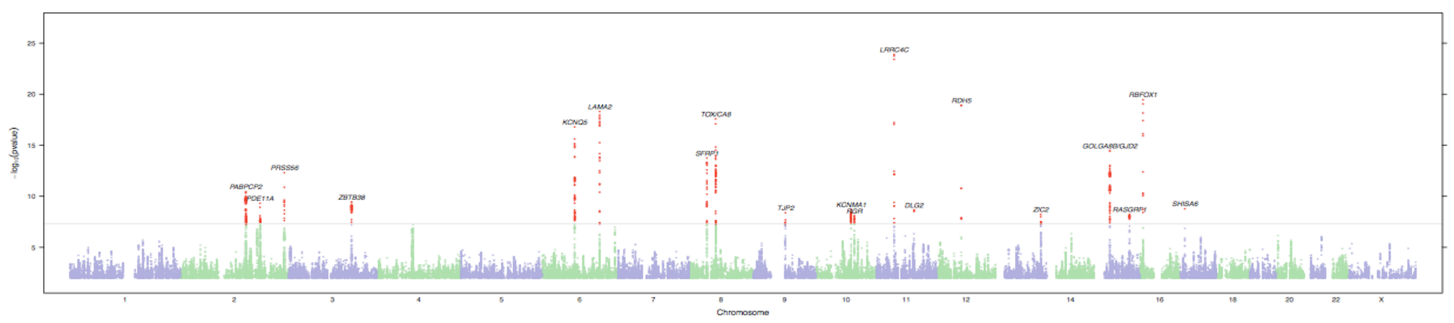

Figure 1. Manhattan Plot of Associations with Myopia in the Discovery Cohort

Several of the 17 novel associations identified lie in or near genes with direct links to processes implicated in myopia: extracellular matrix (ECM) remodeling, the visual cycle, retinal neuron development, eye and body growth, and neuronal development/signaling.

Table 2. Index SNPs for regions significant in the discovery cohort.

rsid	Genes	p-value	p_{repl}
rs12193446	*LAMA2*	$1 \cdot 10^{-42}$	$4.9 \cdot 10^{-4}$
rs11602008	*LRRC4C*	$1.3 \cdot 10^{-24}$	0.012
rs17648524	*RBFOX1*	$3.5 \cdot 10^{-20}$	0.27
rs3138142	*RDH5*	$1.2 \cdot 10^{-19}$	0.0074
chr8:60178580	*TOX/CA8*	$2.6 \cdot 10^{-18}$	0.26
rs7744813	*KCNQ5*	$1.7 \cdot 10^{-17}$	0.0016
rs524952	*GOLGA8B/GJD2*	$3.3 \cdot 10^{-15}$	0.0019
rs2137277	*SFRP1*	$1.8 \cdot 10^{-14}$	0.52
rs1550094	*PRSS56*	$4.9 \cdot 10^{-13}$	0.019
rs11681122	*PABPCP2*	$3.6 \cdot 10^{-11}$	0.085
rs7624084	*ZBTB38*	$3.8 \cdot 10^{-10}$	0.19
rs1898585	*PDE11A*	$4.9 \cdot 10^{-10}$	0.011
rs2908972	*SHISA6*	$1.7 \cdot 10^{-9}$	0.053
rs6480859	*KCNMA1*	$2.0 \cdot 10^{-9}$	0.82
rs10736767	*DLG2*	$2.2 \cdot 10^{-9}$	0.53
rs11145746	*TJP2*	$4.2 \cdot 10^{-9}$	0.77
rs4291789	*ZIC2*	$6 \cdot 10^{-9}$	$2.2 \cdot 10^{-4}$
rs4778882	*RASGRF1*	$6.1 \cdot 10^{-9}$	0.017
rs745480	*RGR*	$8 \cdot 10^{-9}$	0.32

Extracellular Matrix Remodeling
LAMA2 encodes the laminin alpha 2 subunit. Laminins are extracellular structural proteins that are integral parts of the ECM, and play a role in the development and maintenance of different eye structures.

The Visual Cycle

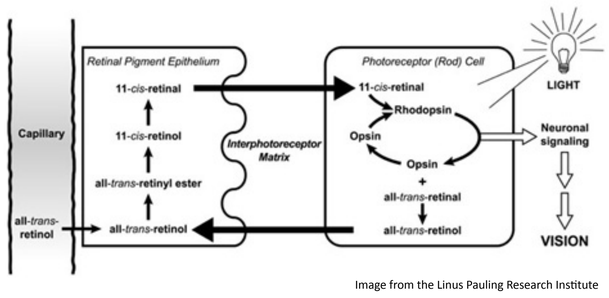

Image from the Linus Pauling Research Institute

RDH5 and *RGR* play critical roles in the regeneration of 11-cis-retinal in the retinal pigment epithelium (RPE), the light sensitive component of photoreceptors. Mutations in *RDH5* have been linked with fundus albipunctatus, a rare form of congenital stationary night blindness; mutations in *RGR* have been linked with retinitis pigmentosa. *KCNQ5* encodes potassium channels thought to contribute to ion flow across the RPE.

Retinal Ganglion Cell Projections

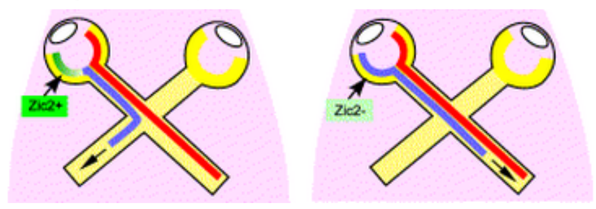

Image from Aruga, J (2004) The role of *ZIC genes in neural development*

*Z*IC2 regulates ipsilateral retinal ganglion cell development.[4] *ZMAT4* has no known link to vision, but rs2137277 also lies 385 kb downstream of *SFRP1* (secreted frizzled-related protein 1), which is involved in differentiation of the optic cup from the neural retina, retinal neurogenesis, development and function of photoreceptor cells, and growth of retinal ganglion cells.

Eye and Body Growth
We find an association with a missense mutation in *PRSS56.* Other mutations in *PRSS56* have been linked with strikingly small eyes and severe decreases in eye length. Two of our SNPs, near *TOX/CA8* and near *ZBTB38*, are in linkage disequilibrium with SNPs previously associated with height.

Neuronal Development and Signaling
Five associations are in genes involved in neuronal development and signaling, but without a known role in vision:

- *KCNMA1* encodes the pore-forming alpha subunit of a MaxiK channel, a family of large conductance, voltage and calcium-sensitive potassium channels involved in the control of smooth muscle and neuronal excitation.
- *RBFOX1* belongs to a family of RNA binding proteins that regulates the alternative splicing of several neuronal transcripts implicated in neuronal development and maturation.
- *LRRC4C* encodes a binding partner for netrin G1 and promotes the outgrowth of thalamocortical axons.
- *DLG2* plays a critical role in the formation and regulation of protein scaffolding at postsynaptic sites.
- *TJP2*; The duplication and subsequent overexpression of *TJP2* is found in adult-onset progressive nonsyndromic hearing loss.

Conclusions

- Our identification of 17 novel genetic associations suggests several different genetic pathways in the development of human myopia
- Some of the associations are consistent with the current view, based largely on animal models, that a visually-triggered signaling cascade from the retina ultimately guides the scleral remodeling that leads to eye growth, and that the RPE plays a key role in this process.
- A number of the novel associations also point to the potential importance of early neuronal development in the eventual development of myopia, particularly the growth and topographical organization of retinal ganglion cells, a pathway not previously implicated in myopia.

Acknowledgments

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References

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