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A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14

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Refractive errors are the most common ocular disorders worldwide and may lead to blindness. Although this trait is highly heritable, identification of susceptibility genes has been challenging. We conducted a genome-wide association study for refractive error in 5,328 individuals from a Dutch population-based study with replication in four independent cohorts (combined 10,280 individuals in the replication stage). We identified a significant association at chromosome 15q14 (rs634990, $P = 2.21 \times 10^{-14}$). The odds ratio of myopia compared to hyperopia for the minor allele (minor allele frequency = 0.47) was 1.41 (95% CI 1.16–1.70) for individuals heterozygous for the allele and 1.83 (95% CI 1.42–2.36) for individuals homozygous for the allele. The associated locus is near two genes that are expressed in the retina, *GJD2* and *ACTC1*, and appears to harbor regulatory elements which may influence transcription of these genes. Our data suggest that common variants at 15q14 influence susceptibility for refractive errors in the general population.

Refractive errors are by far the most common cause of visual impairment in humans[1–5]. They result from aberrant coordinated effects of the ocular biometric components, most notably of axial length. Elongation of the eye axis leads to myopia (nearsightedness), whereas a shortened axis causes hyperopia (farsightedness). Refractive errors often cause alterations in the anatomical structure of the eye, increasing the risk of clinical complications[6]. Myopia may lead to ocular morbidity such as glaucoma and retinal detachment, and high myopia in particular can cause posterior staphyloma and macular degeneration[7–11]. Treatment options for myopia are limited, and myopia is the fifth most common cause of impaired vision and the seventh most common cause of legal blindness worldwide[10,11].

The etiology of refractive errors and myopia is complex and largely uncharacterized. The current notion is that eye growth is triggered by a visually evoked signaling cascade that begins in the retina, traverses the choroid and subsequently mediates scleral remodeling. Established risk factors for myopia are education, reading, outdoor exposure and familial predisposition[11–14]. Familial aggregation studies have quantified a strong genetic basis for myopia; the estimated recurrence risk for siblings of individuals with myopia (λ_s) varied between 1.5 and 3.0 for low myopia and between 4.9 and 19.8 for high myopia, and heritability estimates of myopia (h^2) ranged from 0.60 to 0.90 (ref. 15). Segregation analyses suggested the involvement of multiple genes rather than a single major gene effect[11,13,15]. In an attempt to identify causal genes, previous linkage mapping studies mainly focused on highly myopic probands with multiple affected relatives and thereby identified at least 20 putative genetic

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Table 1 Genome-wide association and replication for refractive error at locus 15q14

		Discovery cohort				Replication									
		RS-I (n = 5,328)				RS-II (n = 2,008)		RS-III (n = 1,970)		ERF (n = 2,032)		TwinsUK (n = 4,270)		Meta-analysis (n = 15,608)	
SNP	Position	MA	MAF	β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P	β (s.e.m.)	P
rs688220	32,786,167	A	0.45	−0.27 (0.05)	1.76×10^{-8}	−0.28 (0.08)	3.80×10^{-4}	−0.22 (0.08)	9.27×10^{-3}	−0.03 (0.07)	6.24×10^{-1}	−0.15 (0.07)	2.60×10^{-2}	−0.20 (0.0009)	2.79×10^{-11}
rs580839	32,786,121	A	0.44	−0.27 (0.05)	1.89×10^{-8}	−0.27 (0.08)	4.96×10^{-4}	−0.22 (0.08)	7.95×10^{-3}	−0.03 (0.07)	6.34×10^{-1}	−0.16 (0.07)	1.92×10^{-2}	−0.20 (0.0009)	2.53×10^{-11}
rs619788	32,782,398	A	0.44	−0.27 (0.05)	1.92×10^{-8}	−0.27 (0.08)	4.94×10^{-4}	−0.22 (0.08)	7.72×10^{-3}	−0.03 (0.07)	6.27×10^{-1}	−0.16 (0.07)	1.85×10^{-2}	−0.20 (0.0009)	2.53×10^{-11}
rs4924134	32,781,857	G	0.44	−0.27 (0.05)	2.04×10^{-8}	−0.27 (0.08)	4.76×10^{-4}	−0.27 (0.08)	6.58×10^{-3}	−0.06 (0.07)	4.10×10^{-1}	−0.16 (0.07)	1.85×10^{-2}	−0.21 (0.0009)	1.36×10^{-12}
rs560766	32,788,234	A	0.44	−0.26 (0.05)	4.27×10^{-8}	−0.28 (0.08)	4.54×10^{-4}	−0.21 (0.08)	1.29×10^{-2}	−0.03 (0.07)	6.65×10^{-1}	−0.18 (0.07)	7.68×10^{-3}	−0.20 (0.0009)	2.49×10^{-11}
rs7176510	32,786,771	T	0.45	−0.26 (0.05)	5.16×10^{-8}	−0.28 (0.08)	5.10×10^{-4}	−0.22 (0.08)	9.62×10^{-3}	−0.02 (0.07)	7.51×10^{-1}	−0.16 (0.07)	1.76×10^{-2}	−0.20 (0.0009)	6.25×10^{-11}
rs7163001	32,777,866	A	0.44	−0.26 (0.05)	5.23×10^{-8}	−0.28 (0.08)	4.08×10^{-4}	−0.23 (0.08)	5.89×10^{-3}	−0.07 (0.07)	3.01×10^{-1}	−0.16 (0.07)	1.87×10^{-2}	−0.21 (0.0009)	5.61×10^{-12}
rs11073060	32,777,143	A	0.44	−0.26 (0.05)	5.76×10^{-8}	−0.28 (0.08)	4.05×10^{-4}	−0.23 (0.08)	5.82×10^{-3}	−0.08 (0.07)	2.72×10^{-1}	−0.16 (0.07)	1.91×10^{-2}	−0.21 (0.0009)	3.65×10^{-12}
rs8032019	32,778,782	G	0.40	−0.26 (0.05)	6.09×10^{-8}	−0.28 (0.08)	5.57×10^{-4}	−0.13 (0.09)	1.30×10^{-1}	−0.05 (0.07)	5.12×10^{-1}	−0.16 (0.07)	1.96×10^{-2}	−0.19 (0.0009)	3.71×10^{-10}
rs685352	32,795,627	G	0.44	−0.25 (0.05)	8.80×10^{-8}	−0.25 (0.08)	1.28×10^{-3}	−0.19 (0.08)	1.98×10^{-2}	−0.07 (0.07)	3.06×10^{-1}	−0.24 (0.07)	4.43×10^{-4}	−0.21 (0.0009)	4.19×10^{-12}
rs524952	32,793,178	A	0.47	−0.25 (0.05)	1.03×10^{-7}	−0.30 (0.08)	2.09×10^{-4}	−0.19 (0.08)	2.56×10^{-2}	−0.06 (0.07)	4.13×10^{-1}	−0.32 (0.07)	4.15×10^{-6}	−0.23 (0.0009)	3.18×10^{-14}
rs634990	32,793,365	C	0.47	−0.25 (0.05)	1.03×10^{-7}	−0.30 (0.08)	2.15×10^{-4}	−0.20 (0.08)	2.03×10^{-2}	−0.05 (0.07)	5.11×10^{-1}	−0.33 (0.07)	2.93×10^{-6}	−0.23 (0.0009)	2.21×10^{-14}
rs11073059	32,776,966	A	0.44	−0.25 (0.05)	1.20×10^{-7}	−0.28 (0.08)	3.96×10^{-4}	−0.23 (0.08)	5.83×10^{-3}	−0.08 (0.07)	2.72×10^{-1}	−0.16 (0.07)	1.91×10^{-2}	−0.20 (0.0009)	8.45×10^{-12}
rs11073058	32,776,918	T	0.44	−0.25 (0.05)	1.30×10^{-7}	−0.28 (0.08)	3.93×10^{-4}	−0.23 (0.08)	5.84×10^{-3}	−0.08 (0.07)	2.71×10^{-1}	−0.16 (0.07)	1.90×10^{-2}	−0.20 (0.0009)	8.45×10^{-12}

RS-I, Rotterdam Study I; RS-II, Rotterdam Study II; RS-III, Rotterdam Study III; ERF, Erasmus Rucphen Family Study; TwinsUK, the Twin Cohort recruited in London; MA, minor allele; MAF, minor allele frequency; β, effect size on spherical equivalent in diopters.

loci[11]. Replication of these results has been limited, and these candidate genes have been shown to have little to no effect in unselected populations. To our knowledge, no genome-wide association studies (GWAS) for refractive error in the general population have previously been reported.

We performed a GWAS in the population-based Rotterdam Study (RS-I, n = 5,328) for refractive error measured as a quantitative trait. Study design and baseline characteristics are provided in the Online Methods and **Supplementary Table 1**. The mean spherical equivalent in this older population (> 55 years of age) of European descent was +0.86 (s.d. = 2.45) diopters. Refractive errors occurred in 52% (n = 2,790) of the participants, with measurements ranging from −19 to +10 diopters.

We genotyped the entire cohort using the Illumina HumanHap 550k and 610Q arrays (Online Methods). Genotypes for more than 2.5 million autosomal SNPs were imputed with reference to the HapMap Phase 2 CEU population build 36. Comparison of the observed and expected distributions (for the quantile-quantile plot, see **Supplementary Fig. 1**) showed modest inflation of the test statistics (genomic control inflation factor (λ_{GC}) = 1.054 for RS-I). Using an additive model, we identified a significant association on chromosome 15q14 (rs688220, $P = 1.76 \times 10^{-8}$; **Table 1** and **Fig. 1**). We took forward 31 SNPs spread across four loci on chromosomes 15q14, 14q24, 1q41 and 10p12.3 reaching $P < 10^{-6}$ (**Supplementary Table 2**) for further investigation in four independent replication cohorts: RS-II (n = 2,008, λ_{GC} = 1.012), RS-III (n = 1,970, λ_{GC} = 1.012) and the Erasmus Rucphen Family Study (ERF, n = 2,032, λ_{GC} = 1.037) from The Netherlands, and a twin study from the United Kingdom (TwinsUK, n = 4,270, λ_{GC} = 1.04). The designs of RS-II and RS-III were population based, whereas those of ERF and TwinsUK were

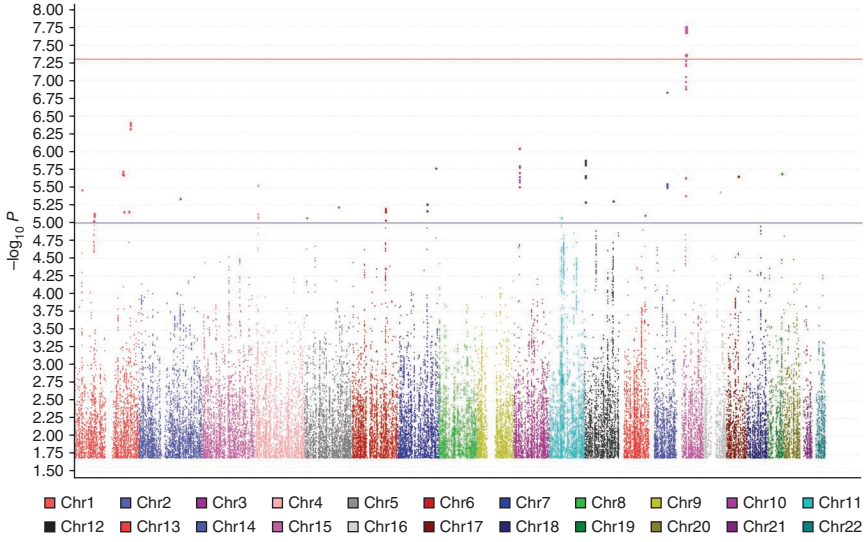

Figure 1 Genome-wide signal intensity (Manhattan) plot of the discovery cohort Rotterdam Study-I. The statistical significance values across the 22 autosomes of each SNP's association with refractive error (measured as spherical equivalent) are plotted as $-\log_{10} P$ values. SNPs with minor allele frequency ≥ 0.01 were included. The blue horizontal line indicates $P = 10^{-5}$ and the red line indicates $P = 5 \times 10^{-8}$.

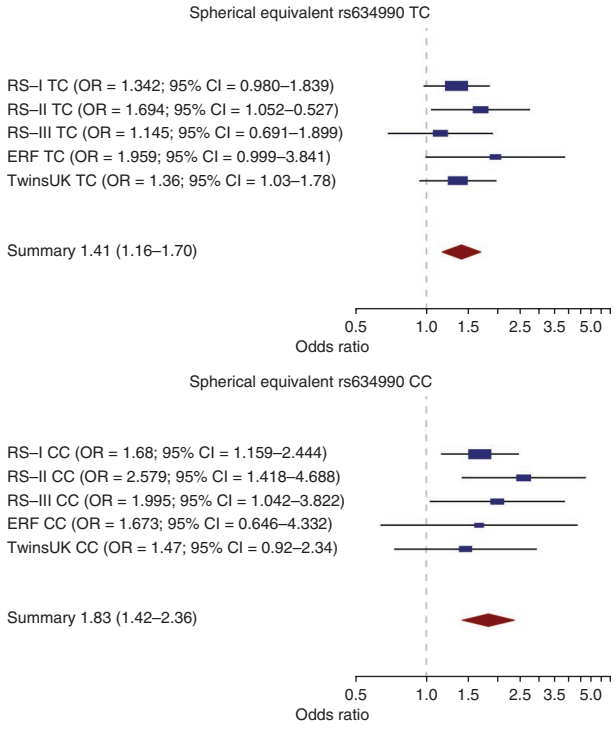

Figure 2 Forest plot of associations for myopia (spherical equivalent ≤ –3 diopters) versus hyperopia (spherical equivalent ≥ +3 diopters). Forest plot of the estimated per-genotype odds ratio for top SNP rs634990 for the five studies separately and for the meta-analysis of all studies. RS-I, Rotterdam Study I; RS-II, Rotterdam Study II; RS-III, Rotterdam Study III; ERF, Erasmus Rucphen Family Study; TwinsUK, the Twin Cohort recruited in London; OR, odds ratio.

family based. Cohorts were not selected on the basis of a disease phenotype. All studies consisted predominantly of individuals of European ancestry and all used similar protocols to evaluate refractive error (Online Methods and **Supplementary Table 2**).

Meta-analysis of the combined discovery and replication cohorts showed a significant association between refractive errors and the locus on 15q14 (the most significant association in meta-analysis was at rs634990 with a combined $P = 2.21 \times 10^{-14}$; **Table 1**). Frequencies of the risk alleles at this region were similar across the studies. The *P* values were nominally significant ($P < 0.05$) for the 14 top SNPs in RS-II, RS-III and TwinsUK, and the direction of the effect (regression coefficient β) of the minor alleles was consistent (**Table 1**). rs634990 accounted for 0.5% of the variance in spherical equivalent.

To determine the effect of this locus on the risk of clinically relevant outcomes, we compared subjects with myopia to those with hyperopia in a logistic regression analysis. We found strong evidence that the C allele of rs634990 conferred a higher risk of myopia than the T allele (**Fig. 2**). The odds ratio (OR) of low to moderate or high myopia versus low to moderate or high hyperopia was 1.41 (95% CI 1.16–1.70) for heterozygotes and 1.83 (95% CI 1.42–2.36) for homozygotes.

The locus on 15q14 (**Fig. 3**) is within an intergenic region in the vicinity of the genes *GJD2* (39 kb from rs634990 at its 3′ end), *ACTC1* (74 kb from rs634990 at its 3′ end) and *GOLGA8B* (180 kb from rs634990 at its 5′ end). We investigated a potential function for these genes in eye growth development by examining gene expression levels in the retina of postmortem human eyes (**Supplementary Table 3**) and observed moderate to high expression of *GJD2* and *ACTC1* and much lower expression of *GOLGA8B*. *GOLGA8B* encodes the 67-kDa protein Golgi autoantigen golgin-67, which belongs to a family of Golgi auto-antigens and is localized at the cytoplasmic surface of the Golgi complex[16]. A specific function of *GOLGA8B* in the retina has not been reported. *ACTC1* encodes the 42-kDa smooth muscle actin cardiac muscle alpha actin 1. The functional role of *ACTC1* in the eye is currently unclear, but actins that are similar to it, such as α-SMA, have been shown to be increased in developing myopic eyes[17]. α-SMA influences the number of contractile myofibroblasts in the sclera and contributes to extracellular matrix remodeling. As these are key factors occurring in eye enlargement, it is intriguing to know whether *ACTC1* has these characteristics as well.

The function of *GJD2* makes this gene an interesting candidate gene for refractive error. *GJD2* encodes the 36-kDa protein Connexin36 (also known as CX36 and gap junction protein delta 2), which is a neuron-specific protein belonging to a multigene family of integral membrane proteins[18]. CX36 forms gap junction channels between adjacent membranes of neuronal cells, is present in photoreceptors, amacrine and bipolar cells, and plays a critical role in the transmission process of the retinal electric circuitry by enabling intercellular transport of small molecules and ions[18–21]. Further exploration of *GJD2* using Ingenuity analysis (see URLs, Online Methods and **Supplementary Fig. 2**) alluded to a role for the gene in eye growth regulation as well as in lens fiber maturation in knockdown animals[22,23]. To identify possible causal variants in this gene, we performed direct sequencing of all exons and intron-exon boundaries of *GJD2* in 47 individuals with either high myopia, high hypermetropia or emmetropia. We found neither new mutations nor frequency differences of any variants

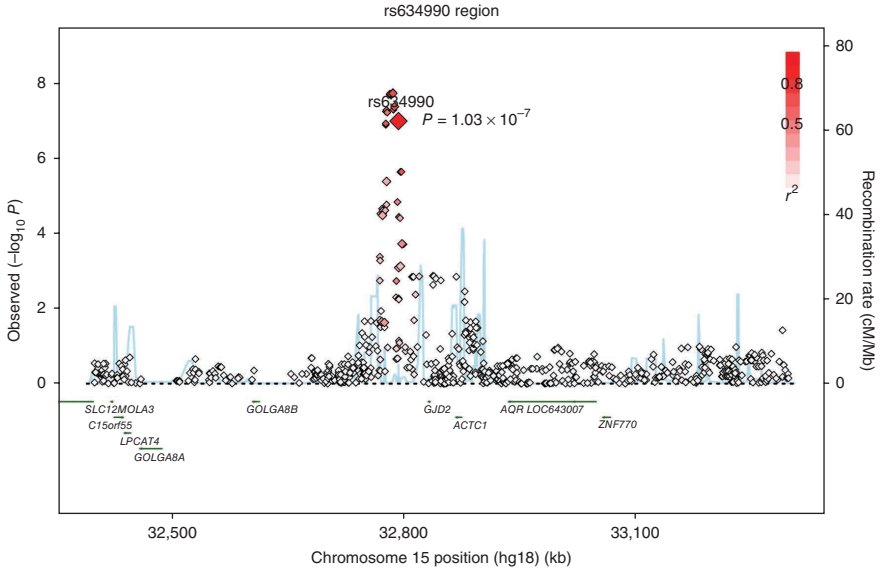

Figure 3 Regional plot at chromosome 15q14. Log_{10} *P* values from the discovery cohort RS-I as a function of genomic position (HapMap release 22 build 36). The *P* value for the top SNP is denoted by the large diamond and *P* values for other genotyped and imputed SNPs are shown as smaller diamonds. *P* values for SNPs of unknown type are presented as squares. Superimposed on the plot are gene locations (green) and recombination rates (blue).

between groups (**Supplementary Table 4**), and we conclude that linkage disequilibrium with common functional variants in *GJD2* is unlikely to explain the observed association.

The next step was to assess whether associated variants within the intergenic region itself may have functional consequences. We evaluated the expression of SNPs within the associated 15q14 locus in lymphoblastoid cell lines. At least two of the top associated SNPs from the meta-analysis significantly altered expression, suggesting that these may regulate gene expression (rs560766, $P = 1.0 \times 10^{-5}$; rs580839, $P = 9.5 \times 10^{-6}$; **Supplementary Table 5**). Subsequently, we searched for regulatory elements[24,25] in the entire 53-kb locus of highly significantly correlated SNPs using the UCSC Genome Browser and found the predicted presence of seven DNase I hypersensitive sites, six enhancers based on experimentally validated H3 chromatin signatures in Hela and K562 cells[24,25], 20 peaks of sequence conservation in alignments of multiple species of placental mammals and 1 insulator site (**Supplementary Fig. 3** and ref. 25). Enhancers are known to facilitate transcription of distal genes, and their range of activity is confined by insulators[25]. Notably, the greatest peak of our association coincided with an insulator site. Precedents of genomic alterations of insulators causing hereditary disease have been previously reported[26,27]. We speculate that variants or mutations in regulatory elements at 15q14 may lead to illegitimate transcription of genes in the area, for example, of *ACTC1* and *GJD2*.

In GWAS in general, sources of heterogeneity may cause spurious findings. To address this issue and minimize potential biases, we applied genomic control to the cohort-level test statistics in the population cohorts, and we corrected the statistics using the identity-by-descent structure in the family-based cohorts. Three studies, RS-II, RS-III and TwinsUK significantly replicated our initial findings. The fourth study, ERF, showed the same direction of association as the other three studies, albeit a nonsignificant association, and revealed similar risks of myopia for carriers of the risk allele (**Fig. 2**). Thus, the observed effects of the genetic variants at 15q14 are relatively homogeneous among the five studies, enhancing the credibility of the findings.

In a companion paper in this issue, Christopher Hammond and colleagues report a GWAS for refractive errors in the TwinsUK study[28]. Researchers in that study found a genome-wide significant association (most significant combined $P = 1.85 \times 10^{-9}$ for rs939658 and $P = 2.07 \times 10^{-9}$ for rs8027411) at a locus on chromosome 15q25, explaining 0.81% of the variance in spherical equivalent measurements. This locus includes the promoter of *RASGRF1*. This gene is known to be functionally involved in eye development[29] and, similar to *GJD2*, is involved in synaptic transmission of photoreceptor responses[30]. TwinsUK and RS-I are two of the largest existing refractive error cohorts with GWAS data. Our studies each identified one different genome-wide significant locus, and we both estimated the variation in refractive error explained by these SNPs to be small. The findings of our studies suggest that the genetic variance in refractive error is mostly determined by multiple variants with a low to moderate penetrance, which is similar to the pattern found for traits such as height[31].

The mutual replication of the direction and the β coefficient of the effect of variants at 15q14 and 15q25 supports the association of these genomic loci to refractive error and myopia. To unravel the mechanism behind myopia, the next steps should include comprehensive resequencing of the entire associated regions and the flanking genes, validation in cohorts of other ethnicities, functional assays and study of risk modulation by environmental factors. This may help to uncover new pathogenic pathways for refractive errors and may eventually lead to new strategies to reduce the sight-threatening consequences of myopia.

URLs. Ingenuity, http://www.ingenuity.com; MACH, http://www.sph.umich.edu/csg/abecasis/MACH; R, http://www.r-project.org; METAL, http://www.sph.umich.edu/csg/abecasis/metal/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Accession codes. The relevant expression data are deposited in the GEO database under accession number GSE20191.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

A.M.S., V.J.M.V. and C.C.W.K. performed analyses and drafted the manuscript. C.M.v.D., B.A.O., F.R., A.G.U., A.H., P.T.V.M.d.J., J.R.V. and C.C.W.K. designed the study and obtained funding. D.D.G.D., L.M.v.K., L.H., W.D.R., M.C., R.K., J.J.M.W.-A., T.G.M.F.G., F.C.C.R. and S.M.A.S. helped in data collection. A.J.M.H.V., M.K.I., N.A., M.S., Y.S.A., A.A.B.B., A.A.L.J.v.O. and A.I. participated in the genetic analyses. P.G.H., T.L.Y., D.A.M., T.D.S. and C.J.H. were responsible for data from the TwinsUK study. M.K.I., R.W.A.M.K., G.v.R., P.G.H., C.J.H., C.M.v.D., A.J.M.H.V., B.A.O., J.R.V. and A.A.B.B. critically reviewed the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Bourne, R.R., Dineen, B.P., Ali, S.M., Noorul Huq, D.M. & Johnson, G.J. Prevalence of refractive error in Bangladeshi adults: results of the National Blindness and Low Vision Survey of Bangladesh. *Ophthalmology* **111**, 1150–1160 (2004).
2. Dandona, R. *et al.* Population-based assessment of refractive error in India: the Andhra Pradesh eye disease study. *Clin. Experiment. Ophthalmol.* **30**, 84–93 (2002).
3. Kempen, J.H. *et al.* The prevalence of refractive errors among adults in the United States, Western Europe, and Australia. *Arch. Ophthalmol.* **122**, 495–505 (2004).
4. Sawada, A. *et al.* Refractive errors in an elderly Japanese population: the Tajimi study. *Ophthalmology* **115**, 363–370 (2008).

5. Vitale, S., Ellwein, L., Cotch, M.F., Ferris, F.L. III & Sperduto, R. Prevalence of refractive error in the United States, 1999–2004. *Arch. Ophthalmol.* **126**, 1111–1119 (2008).
6. McBrien, N.A. & Gentle, A. Role of the sclera in the development and pathological complications of myopia. *Prog. Retin. Eye Res.* **22**, 307–338 (2003).
7. Saw, S.M. *et al.* How blinding is pathological myopia? *Br. J. Ophthalmol.* **90**, 525–526 (2006).
8. Curtin, B.J. & Karlin, D.B. Axial length measurements and fundus changes of the myopic eye. *Am. J. Ophthalmol.* **1**, 42–53 (1971).
9. Saw, S.M., Gazzard, G., Shih-Yen, E.C. & Chua, W.H. Myopia and associated pathological complications. *Ophthalmic Physiol. Opt.* **25**, 381–391 (2005).
10. Tano, Y. *et al.* Pathologic myopia: where are we now? *Am. J. Ophthalmol.* **134**, 645–660 (2002).
11. Young, T.L. *et al.* Molecular genetics of human myopia: an update. *Optom. Vis. Sci.* **86**, E8–E22 (2009).
12. Dirani, M. *et al.* Outdoor activity and myopia in Singapore teenage children. *Br. J. Ophthalmol.* **93**, 997–1000 (2009).
13. McBrien, N.A. *et al.* Myopia: recent advances in molecular studies; prevalence, progression and risk factors; emmetropization; therapies; optical links; peripheral refraction; sclera and ocular growth; signalling cascades; and animal models. *Optom. Vis. Sci.* published online, doi:10.1097/01.opx.0000344146.84135.68 (19 December 2008).
14. Saw, S.M., Hong, C.Y., Chia, K.S., Stone, R.A. & Tan, D. Nearwork and myopia in young children. *Lancet* **357**, 390 (2001).
15. Young, T.L., Metlapally, R. & Shay, A.E. Complex trait genetics of refractive error. *Arch. Ophthalmol.* **125**, 38–48 (2007).
16. Eystathioy, T., Jakymiw, A., Fujita, D.J., Fritzler, M.J. & Chan, E.K. Human autoantibodies to a novel Golgi protein golgin-67: high similarity with golgin-95/gm 130 autoantigen. *J. Autoimmun.* **14**, 179–187 (2000).
17. Jobling, A.I., Gentle, A., Metlapally, R., McGowan, B.J. & McBrien, N.A. Regulation of scleral cell contraction by transforming growth factor-beta and stress: competing roles in myopic eye growth. *J. Biol. Chem.* **284**, 2072–2079 (2009).
18. Kihara, A.H. *et al.* Connexin36, an essential element in the rod pathway, is highly expressed in the essentially rodless retina of Gallus gallus. *J. Comp. Neurol.* **512**, 651–663 (2009).
19. Deans, M.R., Volgyi, B., Goodenough, D.A., Bloomfield, S.A. & Paul, D.L. Connexin36 is essential for transmission of rod-mediated visual signals in the mammalian retina. *Neuron* **36**, 703–712 (2002).
20. Striedinger, K. *et al.* Loss of connexin36 increases retinal cell vulnerability to secondary cell loss. *Eur. J. Neurosci.* **22**, 605–616 (2005).
21. Güldenagel, M. *et al.* Visual transmission deficits in mice with targeted disruption of the gap junction gene connexin36. *J. Neurosci.* **21**, 6036–6044 (2001).
22. Rong, P. *et al.* Disruption of Gja8 (a8 connexin) in mice leads to microphthalmia associated with retardation of lens growth and lens fiber maturation. *Development* **129**, 167–174 (2002).
23. White, T.W. Targeted ablation of Connexin50 in mice results in microphthalmia and zonular pulverulent cataracts. *J. Cell Biol.* **143**, 815–825 (1998).
24. Heintzman, N.D. *et al.* Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* **459**, 108–112 (2009).
25. Heintzman, N.D. & Ren, B. Finding distal regulatory elements in the human genome. *Curr. Opin. Genet. Dev.* **19**, 541–549 (2009).
26. Delaloy, C. *et al.* Deletion of WNK1 first intron results in misregulation of both isoforms in renal and extrarenal tissues. *Hypertension* **52**, 1149–1154 (2008).
27. Mihaly, J. *et al.* Dissecting the regulatory landscape of the *Abd-B* gene of the bithorax complex. *Development* **133**, 2983–2993 (2006).
28. Hysi, P.G., Young, T.L. *et al.* A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25 which contains the *RASGRF1* gene. *Nat. Genet.* advance online publication, doi:10.1038/ng.664 (12 September 2010).
29. Jones, C. & Moses, K. Cell-cycle regulation and cell-type specification in the developing *Drosophila* compound eye. *Semin. Cell Dev. Biol.* **15**, 75–81 (2004).
30. Fernández-Medarde, A. *et al.* RasGRF1 disruption causes retinal photoreception defects and associated transcriptomic alterations. *J. Neurochem.* **110**, 641–652 (2009).
31. Lettre, G. *et al.* Identification of ten loci associated with height highlights new biological pathways in human growth. *Nat. Genet.* **40**, 584–591 (2008).

ONLINE METHODS

Participants. *Discovery cohort.* The Rotterdam Study (RS-I) was a prospective population-based cohort study of 7,983 residents aged 55 years and older living in Ommoord, a suburb of Rotterdam, The Netherlands[32]. The baseline examination for the ophthalmic portion of the study took place between 1991 and 1993 and included 6,775 persons. Individuals were excluded from the study if they had undergone bilateral cataract surgery, laser refractive procedures or other intra-ocular procedures which might alter refraction. Complete data on refractive error and genome-wide SNPs were available on 5,328 persons, 99% of whom were of European ancestry.

Replication cohorts. The first three replication studies originated from The Netherlands. The first cohort was RS-II, an independent cohort which included 2,157 new participants aged 55 and older living in Ommoord since 2000 (ref. 32) who had good quality genotyping data. Baseline examinations for this cohort took place between 2000 and 2002, and follow-up examination took place from 2004 to 2005. The second replication cohort was RS-III, a study which included 2,082 new participants aged 45 and older living in Ommoord since 2006 who had good quality genotyping data. Baseline examination for this cohort took place between 2006 and 2009. The third replication study was the Erasmus Rucphen Family (ERF) Study, a family-based study in a genetically isolated population in the southwest of The Netherlands. This study included 2,032 living descendants of Erasmus Rucphen aged 18 years and older originating from 22 families who had at least six children baptized in the community church between 1880 and 1900 and who had good quality genotyping data. The fourth replication cohort was derived from the United Kingdom (TwinsUK). This study was an adult twin registry of over 10,000 healthy volunteer twins based at St. Thomas' Hospital in London. Participants were recruited and phenotyped between 1998 and 2008. A total of 4,270 participants of European descent had complete data on ocular phenotype and genotype[33].

As in the discovery cohort, participants in the four replication cohorts were excluded if they had undergone bilateral surgery which inhibited evaluation of the original refractive error.

Measurements of refractive error. All studies used a similar protocol for phenotyping. Participants underwent an ophthalmologic examination which included non-dilated automated measurement of refractive error (RS I–III) and ERF used the Topcon RM-A2000 autorefractor; the TwinsUK cohort used the Humphrey-670 (Humphrey Instruments) from 1998 to 2002 and then the ARM-10 (Takagi Seiko), best-corrected visual acuity and keratometry. Each spherical equivalent was calculated from the standard formula:

$$\text{spherical equivalent} = \text{sphere} + (\text{cylinder}/2)$$

In addition to investigating spherical equivalent as a quantitative trait, we stratified spherical equivalent measurements into categories of refractive error to evaluate findings from a clinical viewpoint. Myopia was categorized into low (spherical equivalent from −1.5 to −3 diopters), moderate (spherical equivalent from −3 to −6 diopters) and high (spherical equivalent of −6 diopters or lower). For hyperopia, the categories used were low (spherical equivalent from +1.5 to +3 diopters), moderate (spherical equivalent from +3 to +6 diopters) and high (spherical equivalent of +6 diopters or higher), respectively. We considered spherical equivalent from −1.5 to +1.5 diopters as emmetropia.

Ethics. All measurements in RS-I–III and ERF were conducted after the Medical Ethics Committee of the Erasmus University had approved the study protocols and all participants had given a written informed consent in accordance with the Declaration of Helsinki. In the TwinsUK study, all twins gave fully informed consent under a protocol reviewed by the St. Thomas' Hospital Local Research Ethics Committee.

Genotyping. *Discovery cohort.* All persons attending the baseline examination from 1990–1993 consented to genotyping and had DNA extracted from blood leucocytes. Genotyping of autosomal SNPs was performed in persons with high quality extracted DNA (n = 6,449) using the Illumina Infinium II HumanHap550 chip v3.0 array according to the manufacturer's protocols. Samples with low call rate (<97.5%, n = 209), with excess autosomal heterozygosity (>0.336, n = 21) and with sex-mismatch (n = 36) were excluded, as were outliers identified by the identity-by-state clustering analysis (outliers were defined as being >3 s.d. from population mean, n = 102 or having identity-by-state probabilities >97%, n = 129). 5,974 individuals had good quality genotyping data.

Replication cohorts. In RS-II, the majority of the 2,516 DNA samples were genotyped using the HumanHap550 Duo Arrays; 133 samples (5%) were genotyped using the Human610-Quad Arrays (Illumina). In the RS-III cohort, all DNA samples were genotyped using the Illumina Infinium II HumanHap550 chip v3.0 array. In ERF, DNA was genotyped on one of four different platforms (Illumina 6k, Illumina 318K, Illumina 370K and Affymetrix 250K). Genotyping for the TwinsUK cohort took place in stages; in the first stage, 1,810 individuals were genotyped using Illumina's HumanHap 300k duo chip and at a later stage, 2,578 persons were genotyped using Illumina's HumanHap610-Quad chip.

Imputation. The set of genotyped input SNPs used for imputation in each study was selected based on highest quality GWAS data. The call rate was set at >98% in RS-I–III, the minor allele frequency was set at >0.01, and the Hardy-Weinberg P value was set at $>10^{-6}$. We used the Markov Chain Haplotyping (MACH) package version 1.0.15 software (Rotterdam, The Netherlands; imputed to plus strand of NCBI build 36, HapMap release #22; see URLs) for the analyses. For each imputed SNP, a reliability of imputation was estimated as the ratio of the empirically observed dosage variance to the expected binomial dosage variance (O/E ratio).

Statistical analysis. *Discovery cohort.* Refractive error measured at baseline as a continuous variable was used as the outcome in the analysis. We calculated the mean spherical equivalent measurements for individuals with measurements available for both eyes and included the spherical equivalent of only one eye if data from the other eye were missing. Linear regression models with a 1 degree of freedom trend test were used to examine the associations between SNPs and spherical equivalent, adjusted for age and gender. Using these linear regression models, we calculated regression coefficients with corresponding 95% CIs. ORs of myopia and hyperopia were calculated with logistic regression analysis, adjusting for age and gender. GWAS analyses were performed using GRIMP[34].

We used genomic control to obtain optimal and unbiased results and applied the inverse variance method of each effect size estimated for both autosomal SNPs that were genotyped and imputed in both cohorts. $P < 5 \times 10^{-8}$ was considered genome-wide significant.

Replication analyses. The top SNPs with $P < 1 \times 10^{-6}$ from the discovery analysis were examined in the replication cohorts RS-II, RS-III, ERF and TwinsUK using SPSS version 15.0.0 for Windows (SPSS Inc.) and R statistical package version 2.8.1 for Linux. A meta-analysis was performed on all five studies using METAL for Linux.

GRIMP[34] was used for the analysis of the population-based replication cohorts. To adjust for family relationships, the GenABEL package[35] was used in the ERF study, and Merlin was used in the TwinsUK Study[36]. SNPs which deviated significantly from Hardy-Weinberg equilibrium (using the threshold of $P < 10^{-6}$) and SNPs which had minor allele frequency < 0.01 were excluded.

Gene expression data in human eye tissue. Human gene expression data were obtained essentially as described[37]. In short, postmortem eye bulbs (retinal pigment epithelium was obtained from six donor eyes, choroid was obtained from three donor eyes and photoreceptors were obtained from three donor eyes), provided by the Corneabank Amsterdam, were rapidly frozen using liquid nitrogen. Donors were between 63 and 78 years old and had no known history of eye pathology.

Cryosections were cut from the macula, and we used histology to confirm a normal histological appearance. Retinal pigment epithelium, photoreceptor and choroidal cells were isolated from macular sections using a Laser Microdissection System (PALM). Total RNA was isolated and the mRNA component was amplified, labeled and hybridized to a 44K microarray (Agilent Technologies)[38]. At least three to six microarrays were performed per tissue. Sample isolation, procedures and expression microarray analysis were carried out according to MIAMI guidelines.

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As a measure of the level of expression, we sorted all the genes represented on the 44K microarray by increasing their expression, and we calculated the corresponding percentiles (**Supplementary Table 3**).

Ingenuity database search. We explored the Ingenuity knowledge database using the keyword 'eye development' for all genes involved in 'function or diseases'. This search provided approximately 100 genes, which formed a new network for eye development. We subsequently added *GJD2* to the network, and we used the Path Explorer tool to search for possible functional relationships between *GDJ2* and these eye development genes in human, mouse, rat and *in vitro* models (**Supplementary Fig. 2a**). We continued the search using the keyword 'eye growth' for all genes involved in 'function or diseases' and investigated functional links between molecules using the connect tool and upstream-downstream analysis (**Supplementary Fig. 2b**).

32. Hofman, A. *et al.* The Rotterdam Study: 2010 objectives and design update. *Eur. J. Epidemiol.* **24**, 553–572 (2009).
33. Spector, T.D. & MacGregor, A.J. The St. Thomas' UK Adult Twin Registry. *Twin Res.* **5**, 440–443 (2002).
34. Estrada, K. *et al.* GRIMP: A web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. *Bioinformatics* **25**, 2750–2752 (2009).
35. Aulchenko, Y.S., Ripke, S., Isaacs, A. & van Duijn, C.M. GenABEL: an R library for genome-wide association analysis. *Bioinformatics* **23**, 1294–1296 (2007).
36. Abecasis, G.R., Cherny, S.S., Cookson, W.O. & Cardon, L.R. Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. *Nat. Genet.* **30**, 97–101 (2002).
37. Booij, J.C. *et al.* Functional annotation of the human retinal pigment epithelium transcriptome. *BMC Genomics* **20**, 10–164 (2009).
38. Van Soest, S.S. *et al.* Comparison of human retinal pigment epithelium gene expression in macula and periphery highlights potential topographic differences in Bruch's membrane. *Mol. Vis.* **10**, 1608–1617 (2007).