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1 **Meta-analysis of 542,934 subjects of European ancestry identifies 336** 2 **novel genes and mechanisms predisposing to refractive error and** 3 **myopia**

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46 **Abstract**

47 **Refractive errors, in particular myopia, are a leading cause of morbidity and disability world-wide and**
48 **their prevalence is rising, largely due to cultural and environmental changes. Genetic investigation is a**
49 **valuable tool to better understand the molecular mechanisms underlying abnormal eye development**
50 **and impaired vision. We conducted a meta-analysis of genome-wide association studies involving**
51 **542,934 European participants and identified 336 novel genetic loci associated with refractive error**
52 **that explain an additional 4.6% of spherical equivalent heritability, or an improvement by a third over**
53 **the previous estimates. Collectively, all associated genetic variants explain 18.4% of heritability and**
54 **improve the accuracy of myopia prediction (AUC=0.75). Our results suggest that refractive error is**
55 **genetically heterogeneous, driven by genes participating in the development of every anatomical**
56 **component of the eye. In addition, our analyses suggest that genetic factors controlling circadian**
57 **rhythm and pigmentation are also involved in the development of myopia and refractive error. These**
58 **results may make possible predicting refractive error and the development of personalized myopia**
59 **prevention strategies in the future.**

60

61 Refractive errors (RE) occur when converging light rays from an image do not clearly focus on the retina.
62 They are the seventh most prevalent clinical condition¹ and the second leading cause of disability in the
63 world². The prevalence of RE is rapidly increasing, mostly driven by a dramatic rise in the prevalence of
64 one of its forms, myopia (near-sightedness). Although the causes of such a rise over a short time are
65 likely due to environmental and cultural changes from the mid-20th century³, RE are highly heritable⁴.
66 Several studies^{5,6} have previously sought to identify genes controlling molecular mechanisms leading to
67 RE and myopia. However, the variance and heritability that can be attributed to known genetic factors is
68 modest⁷ and our knowledge of pathogenic mechanisms remains partial. Here, we conduct a meta-
69 analysis combining data from quantitative spherical equivalent and myopia status from large and
70 previously unpublished genome-wide association studies (GWAS) of more than half a million subjects
71 from the UK Biobank, 23andMe and the Genetic Epidemiology Research on Adult Health and Aging
72 (GERA) cohorts, with subsequent replication and meta-analysis with data previously reported from the
73 Consortium for Refractive Error and Myopia (CREAM).

74

75 **Results**

76 ***Association Results.***

77 Analyses were restricted to subjects of European ancestry (Supplementary Figure 1) and combined
78 results from quantitative measures of spherical equivalent and categorical myopia status. Spherical
79 equivalent quantifies RE; a negative spherical equivalent, below a certain threshold defines myopia. We
80 used results obtained from GWAS of directly measured spherical equivalent in 102,117 population-
81 based UK Biobank participants⁸, and 34,998 subjects participating in the GERA Study⁹ and combined
82 them with results of analyses of self-reported myopia in 106,086 cases and 85,757 controls from the
83 customer base of 23andMe, Inc. (Mountain View, CA), a personal genomics company¹⁰. Additionally, we
84 included results from an analysis on the refractive status inferred using demographic and self-reported
85 information on age at first use of prescription glasses among the UK Biobank participants not
86 contributing to the quantitative GWAS (108,956 likely myopes to 70,941 likely non-myopes, see
87 Supplementary Methods). All analyses were adjusted for age, sex and main principal components. To
88 obtain an overall association with RE, we meta-analyzed the results from all studies by using the z-scores

89 from the GWAS of the spherical equivalent and the negative values of z-scores from the case-control
90 studies (23andMe and UK Biobank), since myopia is negatively correlated with spherical equivalent. As
91 expected, the large total sample size of the discovery meta-analysis (N=508,855) led to a nominally large
92 genomic inflation factor ($\lambda=1.94$). The LD score regression intercept was (1.17), and the (intercept-
93 1)/(mean(χ^2)-1) ratio of 0.097 is fully in line with the expectations of polygenicity¹¹.

94 We found associations for 438 discrete genomic regions (Figure 1, Supplementary Table 1), defined by
95 markers contiguously associated at conventional level of GWAS significance^{12,13} of $p < 5 \times 10^{-08}$, separated
96 by more than 1 Mbp from other GWAS-associated markers, as recommended elsewhere¹⁴. Among them,
97 308 loci, including 14 on chromosome X, were not described in previous GWAS studies of refractive
98 error⁷. The observed effect sizes were consistent across all the studies (Supplementary Table 1 and
99 Supplementary File 1). The association with RE was statistically strongest for rs12193446 ($p=9.87 \times 10^{-328}$),
100 within *LAMA2*, a gene previously associated with RE^{5,6}, mutations of which cause muscular
101 dystrophy¹⁵. Consistent with these *LAMA2* properties, polymorphisms located within the genes coding
102 for both major *LAMA2* receptors, *DAG1*¹⁶ ($p=1.67 \times 10^{-08}$ for rs111327216) and *ITGA7*¹⁷ ($p=8.57 \times 10^{-09}$ for
103 rs17117860) which are also known causes of muscular dystrophy^{18,19}, were significantly associated with
104 RE in the discovery meta-analysis.

105 We compared our discovery meta-analysis findings with GWAS results from 34,079 participants in the
106 CREAM consortium, who were part of a previously reported meta-analysis⁷. To avoid any potential
107 overlap with the UK Biobank participants, only non-UK European CREAM participants were used for
108 replication. Despite the vast power differential, 55 of the SNPs that showed the strongest association in
109 their respective regions in the discovery meta-analysis were significant after Bonferroni correction in the
110 replication sample. A further 142 had a false discovery rate (FDR) < 0.05 and 192 were nominally
111 significant at $P < 0.05$ (Supplementary Table 2). The effect sizes observed in the discovery and replication
112 samples were strongly correlated (Pearson's $r=0.91$, Supplementary Figure 2). Meta-analysis of all five
113 cohorts (discovery and replication) expanded the number to 449 associated of regions of variable length
114 and number of SNPs (Supplementary Figure 3), of which 336 regions were novel (Supplementary Table
115 3).

116 Most of the 449 RE-associated regions contained at least one gene linked to severe ocular
117 manifestations in the Online Mendelian Inheritance In Man (OMIM) resource or other genes with
118 interesting link to eye disease (Supplementary Table 4). Although most loci identified through our meta-
119 analyses were novel, several of them hosted genes that harbor mutations leading to myopia or other RE
120 phenotypes. Several genes significantly associated with RE were linked to Mendelian disorders affecting
121 corneal structure, some of which code for transcription factors involved in corneal development²⁰
122 (Supplementary Table 5). Mutations in these genes cause corneal dystrophies (*SLC4A11*, $p=5.81 \times 10^{-11}$ for
123 rs41281858, *TCF4*, $p=4.14 \times 10^{-08}$, rs41396445; *LCAT*, $p=1.26 \times 10^{-10}$, rs5923; and *DCN*, $p=3.67 \times 10^{-09}$,
124 rs1280632), megalocornea (*LTBP2*, $p=1.91 \times 10^{-24}$, rs73296215) and keratoconus (*FNDC3B*, $p=1.89 \times 10^{-14}$,
125 rs199771582, previously described⁷). Eleven RE-associated genes were linked to anomalies of the
126 crystalline lens (Supplementary Table 6), including genes linked to autosomal dominant cataracts (*PAX6*
127 previously linked to myopia²¹, $p=8.31 \times 10^{-11}$, rs1540320; *PITX3*, $p=1.05 \times 10^{-10}$, rs7923183; *MAF*,
128 $p=5.50 \times 10^{-09}$, rs16951312; *CHMP4B*, $p=9.95 \times 10^{-11}$, rs6087538; *TDRD7*, $p=4.79 \times 10^{-08}$, rs13301794) and
129 lens ectopia (*FBN1*, $p=3.30 \times 10^{-24}$, rs2017765; *ADAMTSL4*, $p=8.19 \times 10^{-14}$, rs12131376). Some of the genes
130 affected several eye components. For example, *LTBP2* variants are also associated with congenital
131 glaucoma²², and *COL4A3* (rs7569375, $p=1.14 \times 10^{-08}$) causes Alport syndrome, which manifests with
132 abnormal lens shape (lenticonus) and structural changes in the retina.

133 Association was also observed within or near 13 genes known to harbor mutations causing
134 microphthalmia (Supplementary Table 7), including *TENM3* ($p=2.48 \times 10^{-11}$, rs35446926); *OTX2*

135 ($p=6.15 \times 10^{-11}$, rs928109); *VSX2*, ($p=4.60 \times 10^{-10}$, rs35797567); *MFRP*, ($p=2.85 \times 10^{-16}$, rs10892353) and the
136 previously identified⁶ *TMEM98*, ($p=3.49 \times 10^{-43}$, rs62067167). Association was also found for *VSX1*
137 ($p=4.59 \times 10^{-08}$ for rs6050351), a gene that is closely regulated by *VSX2*²³ and believed to play important
138 roles in eye development²⁴. Many of the genes nearest associated SNPs have been linked to inherited
139 retinal disease (Supplementary Table 8), including 32 genes linked to cone-rod dystrophies, night
140 blindness and retinitis pigmentosa, and age-related macular degeneration (*HTRA1/ARMS2*). Among
141 genes in novel regions associated with RE, *ABCA4* ($p=3.20 \times 10^{-10}$ for rs11165052), and *ARMS2/HTRA1*
142 ($p=5.72 \times 10^{-23}$ for rs2142308) are linked to macular disorders and numerous others to retinitis
143 pigmentosa, retinal dystrophy and other retinal diseases, such as *FBN2*, ($p=8.63 \times 10^{-11}$, rs6860901),
144 *TRAF3IP1* ($p=5.71 \times 10^{-16}$, rs7596847), *CWC27* ($p=1.84 \times 10^{-18}$, rs1309551). Significant association was
145 found near other genes of interest such as *DRD1* ($p=4.51 \times 10^{-16}$, rs13190379), a dopamine receptor.
146 Together, these results are consistent with previous suggestions of light transmission and transduction
147 in RE^{7,25}.

148 *Wnt* signaling has previously been implicated in experimental myopia²⁶. We found significant association
149 near several *Wnt* protein-coding genes (*WNT7B*, a gene previously associated with axial length²⁷,
150 $p=1.42 \times 10^{-26}$ for rs73175083; *WNT10A*, previously associated with central corneal thickness²⁸,
151 $p=1.65 \times 10^{-17}$ for rs121908120 and *WNT3B*, $p=8.52 \times 10^{-16}$ for rs70600), suggesting that organogenesis
152 through *Wnt* signaling is likely to be involved in RE. Significant association were found at genes coding
153 for key canonical (e.g. rs13072632 within the *CTNNB1* gene, $p=7.30 \times 10^{-27}$; *AXIN2*, rs9895291, $p=1.40 \times 10^{-08}$)
154 and non-canonical *Wnt* pathway members (*NFATC3*, rs147561310, $p=1.493 \times 10^{-12}$) or at genes coding
155 for both (*RHOA*, rs7623687, $p=1.81 \times 10^{-11}$ or the previously described⁷ *TCF7L2*, rs56299331, $p=9.38 \times 10^{-46}$;
156 Supplementary Table 9).

157 Similar to previous published analyses²⁵, we found associations for genes involved in sodium, potassium,
158 calcium magnesium and other cation transporters (Supplementary Table 10). The involvement of genes
159 related to glutamatergic synaptic transmission was also notable (Supplementary Table 11). Glutamate is
160 a first synapse transmitter released by photoreceptors towards bipolar cells and is the main excitatory
161 neurotransmitter of the retina, and expression of genes participating in glutamate signaling pathways is
162 significantly altered in myopia models²⁹. These associations support the involvement in RE pathogenesis
163 of neurotransmission and neuronal depolarization and hyperpolarization that was also suggested
164 before⁷. Associations with *POU6F2* gene intronic variants (rs2696187, $p=1.11 \times 10^{-11}$) also suggests
165 involvement of factors related to development of amacrine and ganglion cells³⁰. Other genes at RE-
166 associated loci were annotated to infantile epilepsy, microcephaly, severe learning difficulty, or other
167 inborn diseases affecting the central nervous system (CNS) in OMIM (Supplementary Table 12).

168 Polymorphisms in genes linked to oculocutaneous albinism (OCA) were significantly associated with RE
169 (Supplementary Table 13), although typically association was found for SNPs not strongly associated
170 with other pigmentation traits³¹. Strong association with RE was found near the *OCA2* gene causing OCA
171 type 2 ($p=1.37 \times 10^{-15}$, rs79406658), *OCA3* (*TYRP1*, $p=1.18 \times 10^{-11}$, rs62538956), *OCA5* (*SLC39A8*, $p=4.03 \times 10^{-17}$,
172 rs13107325), *OCA6* (*C10orf11*, $p=1.73 \times 10^{-16}$, rs12256171). In addition, significant association was
173 found near genes linked to ocular albinism (OA) on chromosome X (*TBL1X* and *GPR143*³², $p=2.20 \times 10^{-18}$,
174 rs34437079) and Hermansky-Pudlak Syndrome albinism (*BLOC1S1*, $p=2.4610^{-22}$, for rs80340147; note
175 that this gene forms a conjoint read-through transcript the *BLOC1S1-RDH5* with *RDH5*). Other associated
176 markers were located within genes involved in systemic pigmentation also previously associated with
177 RE⁷, such as *RALY* ($p=3.14 \times 10^{-18}$, rs2284388), *TSPAN10* ($p=2.22 \times 10^{-50}$, rs9747347), as well as melanoma
178 (*MCHR2*, $p=2.37 \times 10^{-15}$ for rs4839756).

179

180

181 **Functional properties of the associated markers**

182 Among the significantly associated markers, 367 unique markers were frameshift or missense variants
183 (Supplementary Table 14). Several are non-synonymous, such as the R141L mutation (rs1048661) within
184 *LOXL1*, a gene that causes pseudoexfoliation syndrome and glaucoma³³ and A69S (rs10490924) in
185 *ARMS2*, associated with increased susceptibility to age-related macular degeneration³⁴. Other
186 associated variants with predicted deleterious consequences were located in several genes, such as *RGR*
187 ($p=6.89 \times 10^{-68}$, rs1042454), a gene previously associated with RE^{7,10} and also retinitis pigmentosa³⁵, and
188 within the *FBN1* gene, near clusters of mutations that cause Marfan Syndrome and anterior segment
189 dysgenesis³⁶.

190 Because the functional link between other associated variants and development of RE phenotypes is less
191 obvious, we next performed gene-set enrichment analyses to identify properties that are significantly
192 shared by genes identified by the meta-analysis. An enrichment analysis of Gene Ontology processes
193 (Supplementary Table 15) found enrichment for genes participating in RNA Polymerase II transcription
194 regulation ($p=1 \times 10^{-06}$) and nucleic acid binding transcription factor activity ($p=1.1 \times 10^{-06}$), suggesting
195 that many of the genetic associations we identified interfere with gene expression. “Eye development”
196 ($p=6.1 \times 10^{-06}$) and “Circadian regulation of gene expression” ($p=1.1 \times 10^{-04}$) were also significantly
197 enriched.

198 A transcription factor binding site (TFBS) enrichment analysis identified significant (FDR < 0.05) over-
199 representation of sites targeted by *GATA4*, *EP300*, *RREB1*, for which association was observed in the
200 meta-analyses (Supplementary Table 16). Binding sites of transcription factors involved in eye
201 morphogenesis and development such as *MAF* (whose mutations cause autosomal cataract), *FOXC1* and
202 *PITX2* (anterior segment dysgenesis) or *CRX* (cone-rod dystrophy) were also enriched. *CRX* and *PAX4*,
203 binding sites were also significantly enriched; these transcription factors are two of the regulators of
204 circadian rhythm and melatonin synthesis³⁷ alongside *OTX2*, for which SNP significant association was
205 observed in our RE meta-analysis ($p=6.15 \times 10^{-11}$ for rs928109). All of these enriched gene-sets are
206 observed for the first time in a GWAS analysis, although the presence of some of the mechanisms that
207 relate them to RE and myopia were hypothesized before³⁸.

208 Many of the variants associated with RE in our analyses were located within or near genes that are
209 expressed in numerous body tissues (Supplementary Figure 4), and in particular from the nervous
210 system, consistent with our evidence of extraocular, central nervous system involvement in RE. Within
211 the eye, these genes were particularly strongly expressed in eye tissues such as cornea, ciliary body,
212 trabecular meshwork³⁹ and retina⁴⁰ (Supplementary Figure 5, Supplementary Table 17). A stratified LD
213 score regression applied to specifically expressed genes (LDSC-SEG)⁴¹ revealed the results of the GWAS
214 are most strongly correlated with genes expressed in the retina and basal ganglia in the central nervous
215 system but these correlations are not significant after multiple testing correction (Supplementary Figure
216 6 and Supplementary Table 18). It is possible that the strength of these correlations was constrained by
217 the fact that in most cases, available expression levels were measured in adult samples, while refractive
218 error and myopia are primarily developed in younger ages.

219 A Summary data-based Mendelian Randomization (SMR) analysis⁴² integrating GWAS with eQTL data
220 from peripheral blood⁴³ and brain tissues⁴⁴ found concomitant association with RE and eQTL
221 transcriptional regulation effects for 159 and 97 genes respectively (Supplementary Tables 19 and 20). A
222 similar analysis integrating GWAS summary data with methylation data from brain tissues found
223 association with both RE and changes in methylation for 134 genes (Supplementary Table 21).

224

225

226 ***Pleiotropy and genetic effects shared between RE and other conditions***

227 Examining the GWAS Catalog⁴⁵, some of the genetic variants reported here were previously associated
228 with RE, and with other traits, in particular intraocular pressure, intelligence and education; the latter
229 two are known myopia risk factors (Supplementary Table 22). We used LD score regression to assess the
230 correlation of genetic effects between RE and other phenotypes from GWAS summary statistics
231 (Supplementary Table 23). RE genetic risk was significantly correlated with intelligence, both in
232 childhood⁴⁶ ($r_g = -0.27$, $p = 4.76 \times 10^{-09}$) and adulthood (fluid intelligence score $r_g = -0.25$, $p = 1.56 \times 10^{-39}$),
233 educational attainment (defined as the number of years spent in formal education, $r_g = -0.24$, $p = 3.36 \times 10^{-54}$),
234 self-reported cataract ($r_g = -0.31$, $p = 4.70 \times 10^{-10}$) and intraocular pressure (IOP, $r_g = -0.14$, $p = 1.04 \times 10^{-12}$).

235 Higher educational attainment appears to cause myopia as demonstrated by Mendelian randomization
236 (MR) studies⁴⁷. A gene by environment interaction GWAS for spherical equivalent and educational
237 attainment (using age at completion of formal full-time education as a proxy) was conducted in 66,242
238 UK Biobank participants. Despite the relatively well-powered sample, only one locus yielded evidence of
239 statistically significant interaction (rs536015141 within *TRPM1*, $p = 2.35 \times 10^{-09}$, Supplementary Table 24),
240 suggesting that the true relationship between RE and education is compounded by several factors and
241 may not be linear in nature, as suggested recently⁴⁸. *TRPM1* is localized in rod ON bipolar cell dendrites,
242 and rare mutations cause congenital stationary night blindness⁴⁹, often associated with high myopia.

243 To further explore the nature of the relationship between RE and IOP, we built MR models using genetic
244 effects previously reported for IOP⁵⁰. On average, every 1 mmHg increase in IOP predicts a 0.05-0.09
245 diopters decrease in spherical equivalent (Supplementary Table 25, Supplementary Figure 7). We also
246 built a MR model to assess the relationship between intelligence and spherical equivalent, but statistical
247 evidence in this case points towards genetic pleiotropy rather than causation (Supplementary Table 26).
248 This suggests that both myopia and intelligence are often influenced by the same factors, but without
249 direct causal path linking one to the other. We found no significant genetic correlations between RE and
250 the glaucoma endophenotype vertical cup to disc ratio ($r_g = -0.01$, $p = 0.45$), or hair pigmentation ($r_g = -0.03$,
251 $p = 0.35$). Therefore, RE and pigmentation may have different allelic profiles with limited sharing of
252 genetic risk.

253

254 ***Conditional analysis and risk prediction***

255 We subsequently carried out a conditional analysis⁵¹ on the meta-analysis summary results and found a
256 total of 904 independent SNPs significantly associated with RE. 890 of these markers were available in
257 the EPIC-Norfolk Study, an independent cohort that did not participate in the RE meta-analysis
258 (Supplementary Figure 8). These markers alone explained 12.1% of the overall spherical equivalent
259 phenotypic variance in a regression model or 18.4% (SE=0.04) of the spherical equivalent heritability.
260 Newly associated markers found in our meta-analysis, but not in the previous large GWAS⁷, explain 4.6%
261 (SE=0.01) of the spherical equivalent phenotypic variance in EPIC-Norfolk Study, which is an
262 improvement of one third compared to heritability explained by previously associated markers⁷.

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

270

271 ***Analysis of the distribution of effects and number of associated variants needed to explain all RE***
272 ***heritability***

273 Using information from over half a million population-based participants SNPs identified in these
274 analyses still only explain 18.4% of the spherical equivalent heritability. We next assessed how many
275 common SNPs are likely to explain the entire heritable component of RE, and what sample sizes are
276 likely to be needed in the future to identify them, using the likelihood-based approach described
277 elsewhere⁵². We estimate that approximately 13,808 (SE=969) polymorphic variants are likely to be
278 behind the full RE heritability. Similar to other quantitative phenotypic traits that are previously
279 published⁵², our analyses estimate that 10.3% (SE=1.0%) of the phenotypic variance is likely explained by
280 a batch of approximately 543 (SE=81) common genetic variants of relatively large effect size and a
281 further 20.8% (SE=0.9%) of the entire phenotypic variance explained by the remainder. With increased
282 sample sizes, we project that the proportion of variance explained will continue to improve fast but will
283 start plateauing for sample sizes above one million, after which further increases in sample size will
284 likely yield ever diminishing additional phenotypic variance (Supplementary Figure 9).

285

286 ***Discussion***

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

292 The findings implicate almost every single anatomical components of the eye, which along with the
293 central nervous system participate in the development of RE. The healthy cornea contributes to 70% of
294 the optical refractive power of the eyes⁵³ and genes involved in corneal structure, topography and
295 function may directly contribute to RE through direct changes in the corneal refraction. Our results show
296 that several genes involved in lens development also contribute to RE in the general population. It is
297 unclear if their contribution is mediated through alterations in biomechanical properties that affect
298 eyes' ability to accommodate, changes to the lens refractive index, or alterations in light transmission
299 properties that impair the ability to focus images on the retina.

300 Many retinal genes are implicated in the development of refractive error, reflecting the role of light in
301 mediating eye growth and the importance of the retina's role in light transduction and processing⁷.
302 Associations with RE at genes coding for gated ion channels and glutamate receptors point to the
303 photoreceptor-bipolar cell interface as a potentially key factor in RE. Rare mutations in several of our
304 associated genes cause night blindness, implicating the rod system in the pathophysiology of RE, but
305 many also affect cone pathways. The *TRPM1* gene, important for rod ON bipolar cell polarity⁵⁴, is also
306 implicated in the gene-education interaction analysis. Associations observed for the *VSX1* and *VSX2*, its
307 negative regulator, genes implicate the cone bipolar cells⁵⁵.

308 The association with genes involved in pigmentation, including most of the OCA-causing genes, raises
309 questions about the relationship between melanin, pigmentation and eye growth and development.
310 These associations are unlikely to be influenced by any cryptic population structure in our samples,
311 which our analyses were designed to control. None of the major pigmentation-associated SNPs³¹ was
312 directly associated with RE and there was no significant correlation of genetic effects between RE and
313 pigmentation.

314 The mechanisms linking pigmentation with RE are unclear. Foveal hypoplasia⁵⁶ and optic disc⁵⁷
315 dysplasias are common in all forms of albinism⁵⁸. Although melanin synthesis is disrupted in albinism,
316 both melanin and dopamine are synthesized through shared metabolic pathways. Disc and chiasmal
317 lesions in albinism are often attributed to dopamine⁵⁹, but we found limited evidence supporting an
318 association with RE for genetic variants involved in dopamine signaling. The scarcity of association with
319 RE for genes involved in dopamine-only pathways contrasts with the abundance of association for genes
320 involved in pigmentation and melanin synthesis. This may suggest that melanin metabolism is connected
321 to RE through other mechanisms that are independent from the metabolic pathways it shares with
322 dopamine production. Melanin reaches the highest concentrations in the retinal pigment epithelium at
323 the outmost layer of the retina, and anteriorly, in the iris and variations in pigmentation may affect the
324 intensity of the light reaching the retina. Light exposure is a major protective factor for development of
325 myopia^{60,61} It is possible that pigmentation plays a role in light signal transmission and transduction.

326 Animal model experiments suggest that in addition to local ocular mechanisms, emmetropization (the
327 process by which the eye develops to minimize refractive error) is strongly influenced by the CNS⁶². The
328 strong correlation of genetic risks between RE and intelligence and association found for genes linked to
329 severe learning disability support an involvement of the CNS in emmetropization and RE pathogenesis.

330 Results from gene-set enrichment analysis demonstrate an interesting evolution with increasing sample
331 sizes. While smaller previous studies were sufficiently powered to discover enrichment of low, cell-level
332 properties, such as cation channel activity and participation in the synaptic space structures²⁵,
333 significantly more powered recent studies have found additional evidence for enrichment and
334 involvement of more integrated physiological functions, such as light signal processing in retinal cells
335 and others⁷. Beyond the identification of a much larger number of genes and explaining significantly
336 higher proportions of heritability, our results, based in a considerably more statistically powered sample,
337 uphold the previous findings and support the involvement of the same molecular and physiological
338 mechanisms that were previously described.

339 In line with expectations from a higher power of association to discover genes and gene sets individually
340 responsible for even smaller proportions of the refractive error variance⁶³, we find evidence for even
341 higher regulatory mechanisms, that act more holistically over the eye development or integrate eye
342 growth and homeostasis with other processes of extraocular nature. For example, we found evidence
343 that binding sites of transcription factors involved in the control of circadian rhythm are significantly
344 enriched among genes associated with refractive error. Circadian rhythm is important in
345 emmetropization and its disruption leads to myopia in animal knock-out models³⁸, potentially through
346 dopamine-mediated mechanisms, or changes in IOP and diurnal variations.

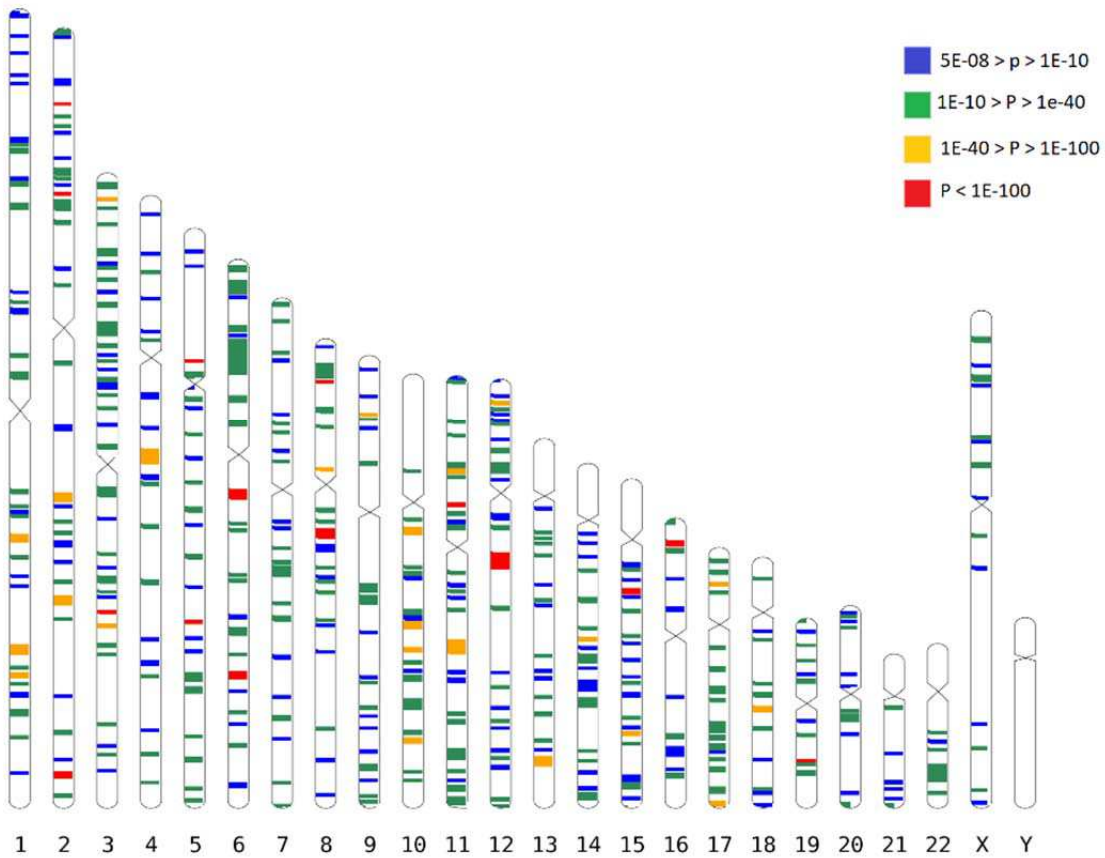
347 Most of the loci identified through our meta-analysis are not subject to particularly strong and
348 systematic evolutionary pressures (Supplementary Figure 10). The variability in minor allele frequencies
349 observed across loci associated with RE may therefore be the result of genetic drift. However, given the
350 variety of the different visual components whose disruptions can result in RE, this variability may also be
351 the result of overall balancing forces which encourage high allelic diversity of genes involved in RE,
352 providing additional buffering capacity to absorb environmental pressures⁴⁸ or genetic disruptions on
353 any of the individual components of the visual system.

354 Our results cast light on potential mechanisms that contribute to RE in the general population and have
355 identified the genetic factors that explain a considerable proportion of the heritability and phenotypic
356 variability of RE. This allows us to improve significantly our ability to make predictions of myopia risk and
357 generate novel hypotheses on how multiple aspects of visual processing affect emmetropization, which
358 may pave the way to personalized risk management and treatment of RE in the population in the future.

359

360 **Online Methods provided separately**

361 **Figure 1.** All GWAS-associated regions from the main meta-analysis. Each band is a true scale of genomic
362 regions associated with refractive error listed in Supplementary Table 1 (+250kbp on each side to make
363 smaller regions more visible). The different color codes represent the significance (p-value) for the
364 genetic variant within that region that displays the strongest evidence for association.

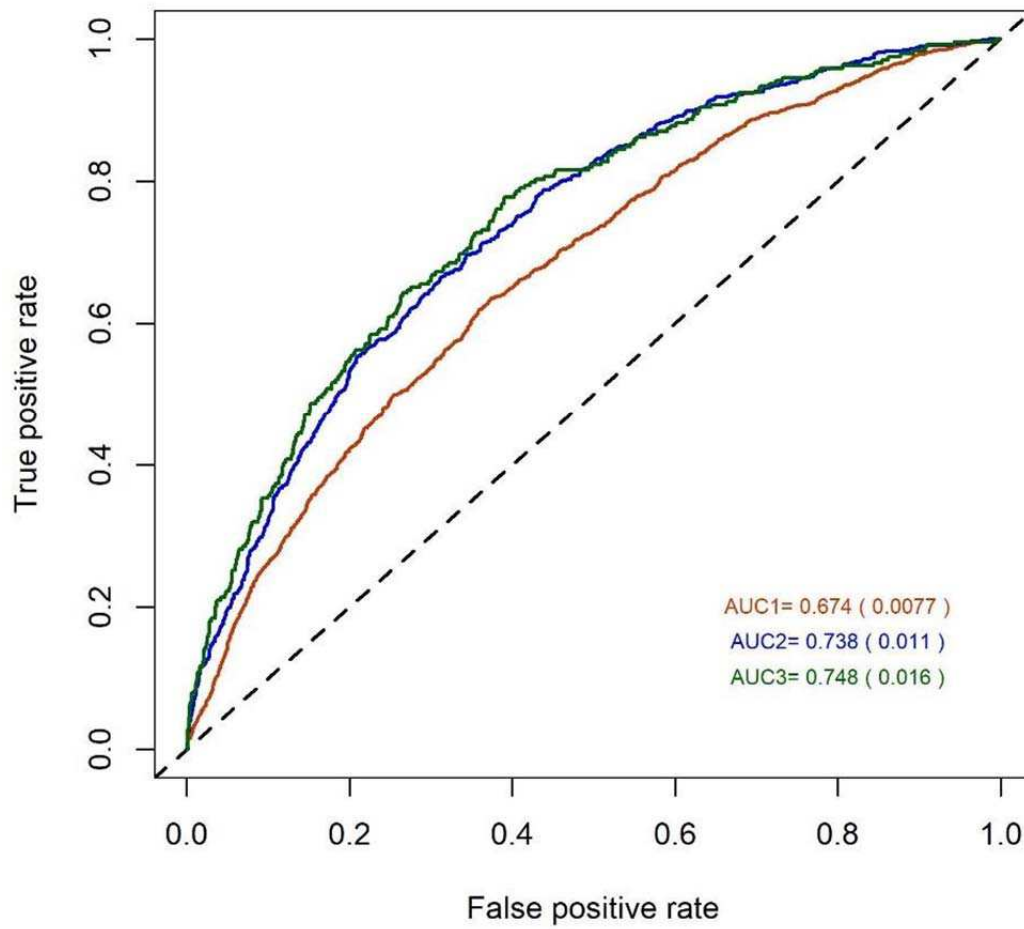


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368 **Figure 2.** Receiver Operating Characteristic (ROC) curves for myopia predictions, using information from
369 890 SNP markers identified in the meta-analysis. The three different colors represent three different
370 curves for each of the different definition of myopia: red – all myopia (< -0.75D), blue – moderate
371 myopia (< -3.00 D) and green - severe myopia (defined as < -5.00 D).



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375 References:

- 376 1. Vos, T. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability
377 for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global
378 Burden of Disease Study 2016. *The Lancet* **390**, 1211-1259 (2017).
- 379 2. WHO. The Global Burden of Disease. 2004 Update ISBN-13: 9789241563710. ISBN-10
380 **651629118**(2008).
- 381 3. Williams, K.M. *et al.* Increasing Prevalence of Myopia in Europe and the Impact of Education.
382 *Ophthalmology* **122**, 1489-97 (2015).
- 383 4. Sanfilippo, P.G., Hewitt, A.W., Hammond, C.J. & Mackey, D.A. The heritability of ocular traits.
384 *Surv Ophthalmol* **55**, 561-83 (2010).
- 385 5. Kiefer, A.K. *et al.* Genome-wide analysis points to roles for extracellular matrix remodeling, the
386 visual cycle, and neuronal development in myopia. *PLoS Genet* **9**, e1003299 (2013).
- 387 6. Verhoeven, V.J. *et al.* Genome-wide meta-analyses of multiancestry cohorts identify multiple
388 new susceptibility loci for refractive error and myopia. *Nat Genet* **45**, 314-8 (2013).
- 389 7. Tedja, M.S. *et al.* Genome-wide association meta-analysis highlights light-induced signaling as a
390 driver for refractive error. *Nat Genet* **50**, 834-848 (2018).
- 391 8. Cumberland, P.M. *et al.* Frequency and Distribution of Refractive Error in Adult Life:
392 Methodology and Findings of the UK Biobank Study. *PLoS One* **10**, e0139780 (2015).
- 393 9. Kvale, M.N. *et al.* Genotyping Informatics and Quality Control for 100,000 Subjects in the
394 Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. *Genetics* **200**, 1051-
395 60 (2015).
- 396 10. Pickrell, J.K. *et al.* Detection and interpretation of shared genetic influences on 42 human traits.
397 *Nat Genet* **48**, 709-17 (2016).
- 398 11. Bulik-Sullivan, B.K. *et al.* LD Score regression distinguishes confounding from polygenicity in
399 genome-wide association studies. *Nat Genet* **47**, 291-5 (2015).
- 400 12. Dudbridge, F. & Gusnanto, A. Estimation of significance thresholds for genomewide association
401 scans. *Genet Epidemiol* **32**, 227-34 (2008).
- 402 13. Pe'er, I., Yelensky, R., Altshuler, D. & Daly, M.J. Estimation of the multiple testing burden for
403 genomewide association studies of nearly all common variants. *Genet Epidemiol* **32**, 381-5
404 (2008).
- 405 14. Wood, A.R. *et al.* Defining the role of common variation in the genomic and biological
406 architecture of adult human height. *Nat Genet* **46**, 1173-86 (2014).
- 407 15. Oliveira, J. *et al.* LAMA2 gene analysis in a cohort of 26 congenital muscular dystrophy patients.
408 *Clin Genet* **74**, 502-12 (2008).
- 409 16. Colognato, H. *et al.* Identification of dystroglycan as a second laminin receptor in
410 oligodendrocytes, with a role in myelination. *Development* **134**, 1723-36 (2007).
- 411 17. Burkin, D.J. & Kaufman, S.J. The alpha7beta1 integrin in muscle development and disease. *Cell*
412 *Tissue Res* **296**, 183-90 (1999).
- 413 18. Ervasti, J.M. & Campbell, K.P. Dystrophin-associated glycoproteins: their possible roles in the
414 pathogenesis of Duchenne muscular dystrophy. *Mol Cell Biol Hum Dis Ser* **3**, 139-66 (1993).
- 415 19. Mayer, U. *et al.* Absence of integrin alpha 7 causes a novel form of muscular dystrophy. *Nat*
416 *Genet* **17**, 318-23 (1997).
- 417 20. Jean, D., Ewan, K. & Gruss, P. Molecular regulators involved in vertebrate eye development.
418 *Mech Dev* **76**, 3-18 (1998).

- 419 21. Hammond, C.J., Andrew, T., Mak, Y.T. & Spector, T.D. A susceptibility locus for myopia in the
420 normal population is linked to the PAX6 gene region on chromosome 11: a genomewide scan of
421 dizygotic twins. *Am J Hum Genet* **75**, 294-304 (2004).
- 422 22. Ali, M. *et al.* Null mutations in LTBP2 cause primary congenital glaucoma. *Am J Hum Genet* **84**,
423 664-71 (2009).
- 424 23. Clark, A.M. *et al.* Negative regulation of Vsx1 by its paralog Chx10/Vsx2 is conserved in the
425 vertebrate retina. *Brain Res* **1192**, 99-113 (2008).
- 426 24. Heon, E. *et al.* VSX1: a gene for posterior polymorphous dystrophy and keratoconus. *Hum Mol*
427 *Genet* **11**, 1029-36 (2002).
- 428 25. Hysi, P.G. *et al.* Common mechanisms underlying refractive error identified in functional analysis
429 of gene lists from genome-wide association study results in 2 European British cohorts. *JAMA*
430 *Ophthalmol* **132**, 50-6 (2014).
- 431 26. Ma, M. *et al.* Wnt signaling in form deprivation myopia of the mice retina. *PLoS One* **9**, e91086
432 (2014).
- 433 27. Miyake, M. *et al.* Identification of myopia-associated WNT7B polymorphisms provides insights
434 into the mechanism underlying the development of myopia. *Nat Commun* **6**, 6689 (2015).
- 435 28. Cuellar-Partida, G. *et al.* WNT10A exonic variant increases the risk of keratoconus by decreasing
436 corneal thickness. *Hum Mol Genet* **24**, 5060-8 (2015).
- 437 29. Stone, R.A. *et al.* Image defocus and altered retinal gene expression in chick: clues to the
438 pathogenesis of ametropia. *Invest Ophthalmol Vis Sci* **52**, 5765-77 (2011).
- 439 30. Zhou, H., Yoshioka, T. & Nathans, J. Retina-derived POU-domain factor-1: a complex POU-
440 domain gene implicated in the development of retinal ganglion and amacrine cells. *J Neurosci*
441 **16**, 2261-74 (1996).
- 442 31. Hysi, P.G. *et al.* Genome-wide association meta-analysis of individuals of European ancestry
443 identifies new loci explaining a substantial fraction of hair color variation and heritability. *Nat*
444 *Genet* **50**, 652-656 (2018).
- 445 32. Fabian-Jessing, B.K. *et al.* Ocular albinism with infertility and late-onset sensorineural hearing
446 loss. *Am J Med Genet A* **176**, 1587-1593 (2018).
- 447 33. Thorleifsson, G. *et al.* Common sequence variants in the LOXL1 gene confer susceptibility to
448 exfoliation glaucoma. *Science* **317**, 1397-400 (2007).
- 449 34. Rivera, A. *et al.* Hypothetical LOC387715 is a second major susceptibility gene for age-related
450 macular degeneration, contributing independently of complement factor H to disease risk. *Hum*
451 *Mol Genet* **14**, 3227-36 (2005).
- 452 35. Morimura, H., Saindelle-Ribeau, F., Berson, E.L. & Dryja, T.P. Mutations in RGR, encoding a
453 light-sensitive opsin homologue, in patients with retinitis pigmentosa. *Nat Genet* **23**, 393-4
454 (1999).
- 455 36. Robinson, P.N. *et al.* Mutations of FBN1 and genotype-phenotype correlations in Marfan
456 syndrome and related fibrillinopathies. *Hum Mutat* **20**, 153-61 (2002).
- 457 37. Rohde, K., Moller, M. & Rath, M.F. Homeobox genes and melatonin synthesis: regulatory roles
458 of the cone-rod homeobox transcription factor in the rodent pineal gland. *Biomed Res Int* **2014**,
459 946075 (2014).
- 460 38. Chakraborty, R. *et al.* Circadian rhythms, refractive development, and myopia. *Ophthalmic*
461 *Physiol Opt* **38**, 217-245 (2018).
- 462 39. Carnes, M.U., Allingham, R.R., Ashley-Koch, A. & Hauser, M.A. Transcriptome analysis of adult
463 and fetal trabecular meshwork, cornea, and ciliary body tissues by RNA sequencing. *Exp Eye Res*
464 **167**, 91-99 (2018).
- 465 40. Ratnapriya, R. *et al.* Retinal transcriptome and eQTL analyses identify genes associated with age-
466 related macular degeneration. *Nat Genet* **51**, 606-610 (2019).

- 467 41. Finucane, H.K. *et al.* Heritability enrichment of specifically expressed genes identifies disease-
468 relevant tissues and cell types. *Nat Genet* **50**, 621-629 (2018).
- 469 42. Zhu, Z. *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait
470 gene targets. *Nat Genet* **48**, 481-7 (2016).
- 471 43. Westra, H.J. *et al.* Systematic identification of trans eQTLs as putative drivers of known disease
472 associations. *Nat Genet* **45**, 1238-1243 (2013).
- 473 44. Qi, T. *et al.* Identifying gene targets for brain-related traits using transcriptomic and methylomic
474 data from blood. *Nat Commun* **9**, 2282 (2018).
- 475 45. Buniello, A. *et al.* The NHGRI-EBI GWAS Catalog of published genome-wide association studies,
476 targeted arrays and summary statistics 2019. *Nucleic Acids Res* **47**, D1005-D1012 (2019).
- 477 46. Benyamin, B. *et al.* Childhood intelligence is heritable, highly polygenic and associated with
478 FBNP1L. *Mol Psychiatry* **19**, 253-8 (2014).
- 479 47. Mountjoy, E. *et al.* Education and myopia: assessing the direction of causality by mendelian
480 randomisation. *BMJ* **361**, k2022 (2018).
- 481 48. Pozarickij, A. *et al.* Quantile regression analysis reveals widespread evidence for gene-
482 environment or gene-gene interactions in myopia development. *Commun Biol* **2**, 167 (2019).
- 483 49. Audo, I. *et al.* TRPM1 is mutated in patients with autosomal-recessive complete congenital
484 stationary night blindness. *Am J Hum Genet* **85**, 720-9 (2009).
- 485 50. Khawaja, A.P. *et al.* Genome-wide analyses identify 68 new loci associated with intraocular
486 pressure and improve risk prediction for primary open-angle glaucoma. *Nat Genet* **50**, 778-782
487 (2018).
- 488 51. Yang, J. *et al.* Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies
489 additional variants influencing complex traits. *Nat Genet* **44**, 369-75, S1-3 (2012).
- 490 52. Zhang, Y., Qi, G., Park, J.H. & Chatterjee, N. Estimation of complex effect-size distributions using
491 summary-level statistics from genome-wide association studies across 32 complex traits. *Nat*
492 *Genet* **50**, 1318-1326 (2018).
- 493 53. Zadnik, K. *et al.* Normal eye growth in emmetropic schoolchildren. *Optom Vis Sci* **81**, 819-28
494 (2004).
- 495 54. Li, Z. *et al.* Recessive mutations of the gene TRPM1 abrogate ON bipolar cell function and cause
496 complete congenital stationary night blindness in humans. *Am J Hum Genet* **85**, 711-9 (2009).
- 497 55. Chow, R.L. *et al.* Vsx1, a rapidly evolving paired-like homeobox gene expressed in cone bipolar
498 cells. *Mech Dev* **109**, 315-22 (2001).
- 499 56. Struck, M.C. Albinism: Update on ocular features. *Current Ophthalmology Reports* **3**, 232-237
500 (2015).
- 501 57. Mohammad, S. *et al.* Characterization of Abnormal Optic Nerve Head Morphology in Albinism
502 Using Optical Coherence Tomography. *Invest Ophthalmol Vis Sci* **56**, 4611-8 (2015).
- 503 58. Yahalom, C. *et al.* Refractive profile in oculocutaneous albinism and its correlation with final
504 visual outcome. *Br J Ophthalmol* **96**, 537-9 (2012).
- 505 59. Lopez, V.M., Decatur, C.L., Stamer, W.D., Lynch, R.M. & McKay, B.S. L-DOPA is an endogenous
506 ligand for OA1. *PLoS Biol* **6**, e236 (2008).
- 507 60. Karouta, C. & Ashby, R.S. Correlation between light levels and the development of deprivation
508 myopia. *Invest Ophthalmol Vis Sci* **56**, 299-309 (2014).
- 509 61. Wu, P.C., Tsai, C.L., Wu, H.L., Yang, Y.H. & Kuo, H.K. Outdoor activity during class recess reduces
510 myopia onset and progression in school children. *Ophthalmology* **120**, 1080-5 (2013).
- 511 62. Troilo, D., Gottlieb, M.D. & Wallman, J. Visual deprivation causes myopia in chicks with optic
512 nerve section. *Curr Eye Res* **6**, 993-9 (1987).
- 513 63. de Leeuw, C.A., Neale, B.M., Heskes, T. & Posthuma, D. The statistical properties of gene-set
514 analysis. *Nat Rev Genet* **17**, 353-64 (2016).

