

# 1 **Towards the identification of causal genes for age-related macular degeneration**

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17

## 18 **Abstract**

19 Age-related macular degeneration (AMD) is a leading cause of visual impairment in ageing  
20 populations and has no radical treatment or prevention. Although genome-wide association studies  
21 (GWAS) have identified many susceptibility loci for AMD, the underlying causal genes remain  
22 elusive. Here, we prioritized nine putative causal genes by integrating expression quantitative trait  
23 locus (eQTL) data from blood ( $n = 2,765$ ) with AMD GWAS data (16,144 cases vs. 17,832 controls)  
24 and replicated six of them using retina eQTL data ( $n = 523$ ). Of the six genes, altering expression of  
25 *cnn2*, *sarm1* and *bloc1s1* led to ocular phenotype, impaired vision and retinal pigment epithelium  
26 (RPE) loss in zebrafish. Essential photoreceptor and RPE genes were downregulated in *cnn2*- and  
27 *sarm1*-knockdown zebrafishes. Through integration of GWAS and eQTL data followed by functional  
28 validation, our study reveals potential roles of *CNN2*, *SARM1* and *BLOC1S1* in AMD pathogenesis  
29 and demonstrates an efficient platform to prioritise causal genes for human complex diseases.

## 30 Introduction

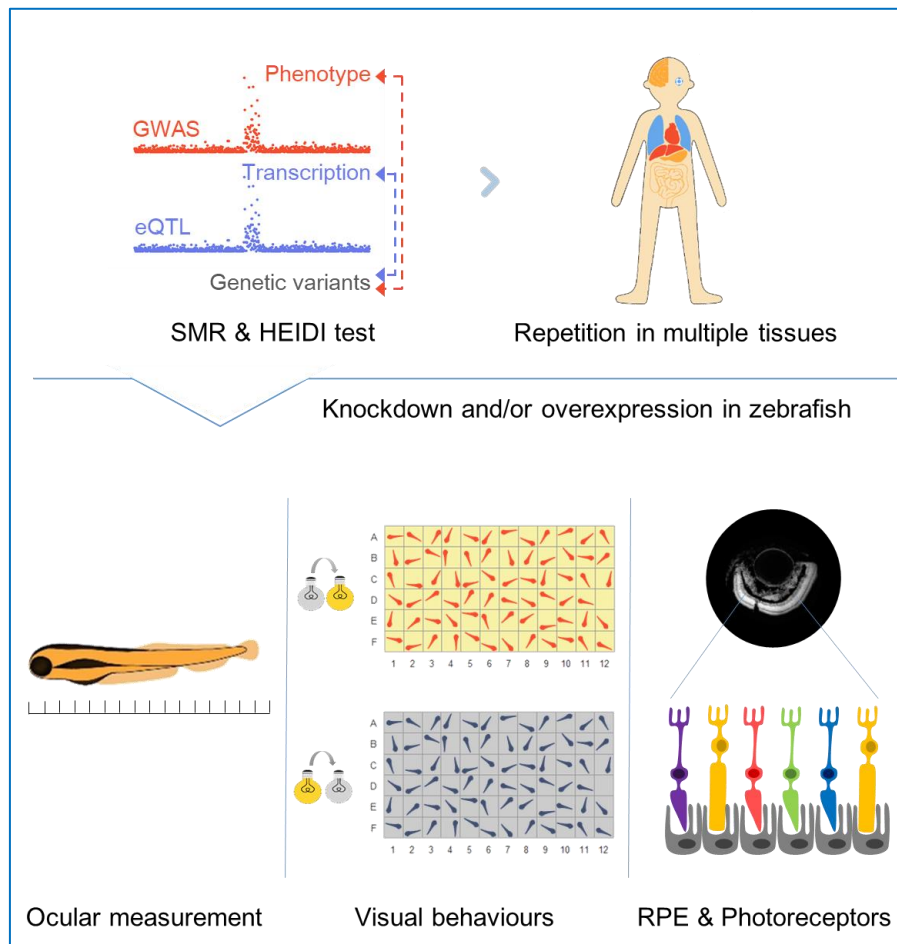
31 Age-related macular degeneration (AMD) is an incurable blinding disorder caused by dysfunction of the  
32 retinal pigment epithelium (RPE) and progressive loss of photoreceptors in the macula<sup>1</sup>. It results in visual  
33 impairment of central vision and disability of daily life activities, such as reading, walking and face  
34 recognition. The prevalence of AMD is 8.69% in the age range of 45-85 years globally, and it is projected  
35 to affect 196 million people worldwide in 2020<sup>2</sup>. As such, AMD is highly endorsed as a major health and  
36 social problem for both individuals and communities, especially in elderly populations<sup>3</sup>.

37  
38 AMD is one of the most genetically well-defined complex diseases. Genome-wide association studies  
39 (GWAS) with increasing sample sizes have identified 52 susceptibility loci which together explain more  
40 than 50% of the heritability of liability<sup>4-6</sup>. These findings provide important clues for understanding the  
41 genetic architecture of the disease, but the causal genes at those susceptibility loci and underlying  
42 mechanisms remain largely unclear. For example, a nonsynonymous variant, *CFH* p. Arg1210Cys (allele  
43 frequency = 0.00017 in ExAC), increases AMD risk by >20-fold<sup>7</sup>, but there has been no evidence showing  
44 its functional impact on the regulation of gene expression, structural and functional integrity of the protein-  
45 coding region, or interplay with the genes nearby<sup>8</sup>. This is partly because of linkage disequilibrium (LD)  
46 between single-nucleotide polymorphisms (SNPs) and causative variants that GWAS mapping resolution<sup>9</sup>.  
47 This could also be the reason that the trait-associated variants, especially those residing in non-coding  
48 regions, exert an impact on gene expression through distal regulation<sup>10</sup>.

49  
50 With the availability of data from large-scale GWAS<sup>5</sup>, expression quantitative trait locus (QTL) studies<sup>11</sup>  
51 and advanced integrative statistical methods<sup>12,13</sup>, we sought to test the hypothesis that genetic variants at  
52 some of the susceptibility loci affect the risk of AMD through genetic regulation of transcriptional levels.  
53 We used the summary-data-based Mendelian randomization (SMR) here<sup>12</sup>, which features the statistical  
54 power because of the flexibility to utilize GWAS and eQTL data from two independent studies. We  
55 subsequently used heterogeneity in dependent instruments (HEIDI) approaches to distinguish  
56 causality/pleiotropy (i.e., the same causal variant(s) affecting AMD susceptibility and the expression level  
57 of a gene) from linkage (i.e., two distinct causal variants in LD, one affecting AMD susceptibility and the  
58 other affecting certain gene expression). This analytical framework has been successfully used in various  
59 common diseases, such as diabetes, autoimmune diseases, and psychiatric disorders<sup>14,15</sup>, and is for the first  
60 time used in ocular diseases in this study.

61  
62 We then established an experimental scheme that utilized morpholino oligonucleotide (MO)-induced  
63 knockdown and/or mRNA overexpression zebrafish as an animal model to assay the putative causal genes  
64 identified by the above integrative analysis (**Figure 1**). Valid procedures were designed to provide  
65 morphological and functional assessments of the zebrafish ocular phenotypes, thereby demonstrating the  
66 functional relevance to AMD pathogenesis for these prioritised genes. Moreover, the research workflow  
67 that combines integrative analysis of large-scale data in humans and functional validation in zebrafish is

68 general and can be used as a new paradigm to efficiently and effectively highlight susceptible genes for  
69 human complex diseases and then provide insights for additional prospective therapeutic applications.



70

71 **Fig. 1 Schematics of study design.** First, blood eQTL and AMD GWAS data were integrated through  
72 SMR and HEIDI to identify expression–phenotype associations before replications in retina or other  
73 48 human tissues in GTEx. Then, functional experiments were conducted in zebrafish to measure  
74 ocular sizes, light-induced behavioural patterns, qPCR of photoreceptors and RPE genes and retinal  
75 immunostaining to validate the prioritised genes.

76

## 77 Results

### 78 Associating gene expression with AMD risk by an integrative analysis

79 To prioritize genes whose expression levels are associated with AMD risk, we used SMR<sup>12</sup> to test if a  
80 variant has a joint association with AMD risk and the expression level of a gene, using GWAS and eQTL  
81 summary data. The GWAS summary data were derived from a study of the International AMD Genomics  
82 Consortium with 16,144 AMD cases vs. 17,832 controls<sup>5</sup>. The eQTL summary data were generated by the  
83 Consortium for the Architecture of Gene Expression (CAGE) from a study of 36,778 gene expression  
84 probes in peripheral blood of 2,765 individuals<sup>11</sup>. All the individuals were predominantly of European  
85 ancestry, and both datasets are publicly available (URLs). And the test was performed for each of the genes  
86 with at least an eQTL at  $P_{\text{eQTL}} < 5e-8$  (Methods).

87

88 In total, we identified 16 genes (tagged by 21 probes) at a genome-wide significance level

89 ( $P_{smr} = 5.9 \times 10^{-6}$ , correcting for 8459 tests, i.e., 8459 probes with at least an eQTL at  $P_{eQTL} < 5e-8$ )

90 (**Supplementary Table 1**). We then employed the HEIDI method<sup>12</sup> to reject SMR associations due to

91 linkage (removing probes with  $P_{HEIDI} < 0.05$ ) (**Methods**). And the LD information, required for the HEIDI

92 test, was computed from genotype data of randomly selected 20,000 individuals from European ancestry in

93 the UK Biobank<sup>16</sup>. Consequently, 9 genes (tagged by 12 probes) were retained (**Table 1**), and some of

94 which, including *BLOC1S1*, *PILRB*, and *TMEM199*, have been reported in a recent study that used a

95 different strategy to integrate AMD GWAS with retinal eQTL data<sup>17</sup>. We found that 58.3% of the identified

96 probes were not tagging the closest genes to the top associated GWAS signals, consistent with the

97 observations from previous studies<sup>10,12</sup>. The eQTL variants of all the prioritised genes were common with

98 minor allele frequencies (MAF) ranging from 0.11 to 0.49. It is of note that the association of rs7212349

99 (i.e., the top associated variant for gene *SARM1* with  $P_{eQTL} = 1.0e-22$ ) with AMD ( $P_{GWAS} = 1.8e-7$ ) did not

100 reach the conventional genome-wide significance threshold, suggesting a gain of power in gene discovery

101 by leveraging eQTL data, in line with previous work<sup>12</sup>.

**Table 1 Putative causal genes for AMD identified from SMR & HEIDI analysis using the blood or retina eQTL data.**

Probe ID	Chr	Gene	topSNP	A1	A2	Freq	CAGE-Blood				Retina
							$P_{\text{GWAS}}$	$P_{\text{eQTL}}$	$P_{\text{SMR}}$	$P_{\text{HEIDI}}$	$P_{\text{SMR}}$
1685534	7	<i>PILRB</i>	rs7792525	G	A	0.19	1.09e-08	1.72e-23	7.01e-07	0.121	3.88e-08
1807712	7	<i>PILRB</i>	rs1964242	A	G	0.19	7.07e-09	4.41e-76	3.31e-08	0.317	2.68e-08
1723984	7	<i>PILRB</i>	rs73401450	C	G	0.19	1.07e-08	3.28e-205	1.89e-08	0.183	3.83e-08
1768754	7	<i>PILRB</i>	rs61735533	A	G	0.19	1.60e-08	7.93e-255	2.48e-08	0.257	5.37e-08
1773395	12	<i>BLOC1S1-RDH5</i>	rs56108400	T	G	0.24	2.36e-08	8.3e-82	8.31e-08	0.407	2.65e-06
1773395	12	<i>BLOC1S1-RDH5</i>	rs56108400	T	G	0.24	2.36e-08	8.3e-82	8.31e-08	0.407	7.64e-01
1748481	17	<i>TMEM199</i>	rs708100	G	A	0.49	2.50e-08	1.86e-18	2.56e-06	0.351	1.94e-04
1746265	17	<i>SARM1</i>	rs7212349	T	C	0.45	1.81e-07	1.00e-22	4.09e-06	0.128	4.39e-03
2043615	17	<i>C17orf90</i>	rs11150803	A	C	0.47	4.36e-09	6.77e-81	2.03e-08	0.012	6.74e-01
1805131	17	<i>C17orf90</i>	rs9910935	T	C	0.47	1.71e-09	1.13e-17	8.40e-07	0.139	8.27e-01
1708486	19	<i>CNN2</i>	rs3087680	C	A	0.11	4.57e-08	3.26e-56	2.38e-07	0.760	7.80e-05
1743205	19	<i>ABCA7</i>	rs3087680	C	A	0.11	4.57e-08	4.44e-136	9.33e-08	0.737	7.51e-02
1796316	20	<i>MMP9</i>	rs3918261	G	A	0.14	1.02e-09	9.78e-34	4.98e-08	0.196	3.06e-02

102 Chr represents chromosome; A1 is the effect allele; Freq is frequency of the effect allele in the reference sample.

103 Note: *BLOC1S1-RDH5* is an integrated probe in CAGE, but separated probes in EyeGex-Retina.

104 **Replication of the SMR associations in retina and other tissues**

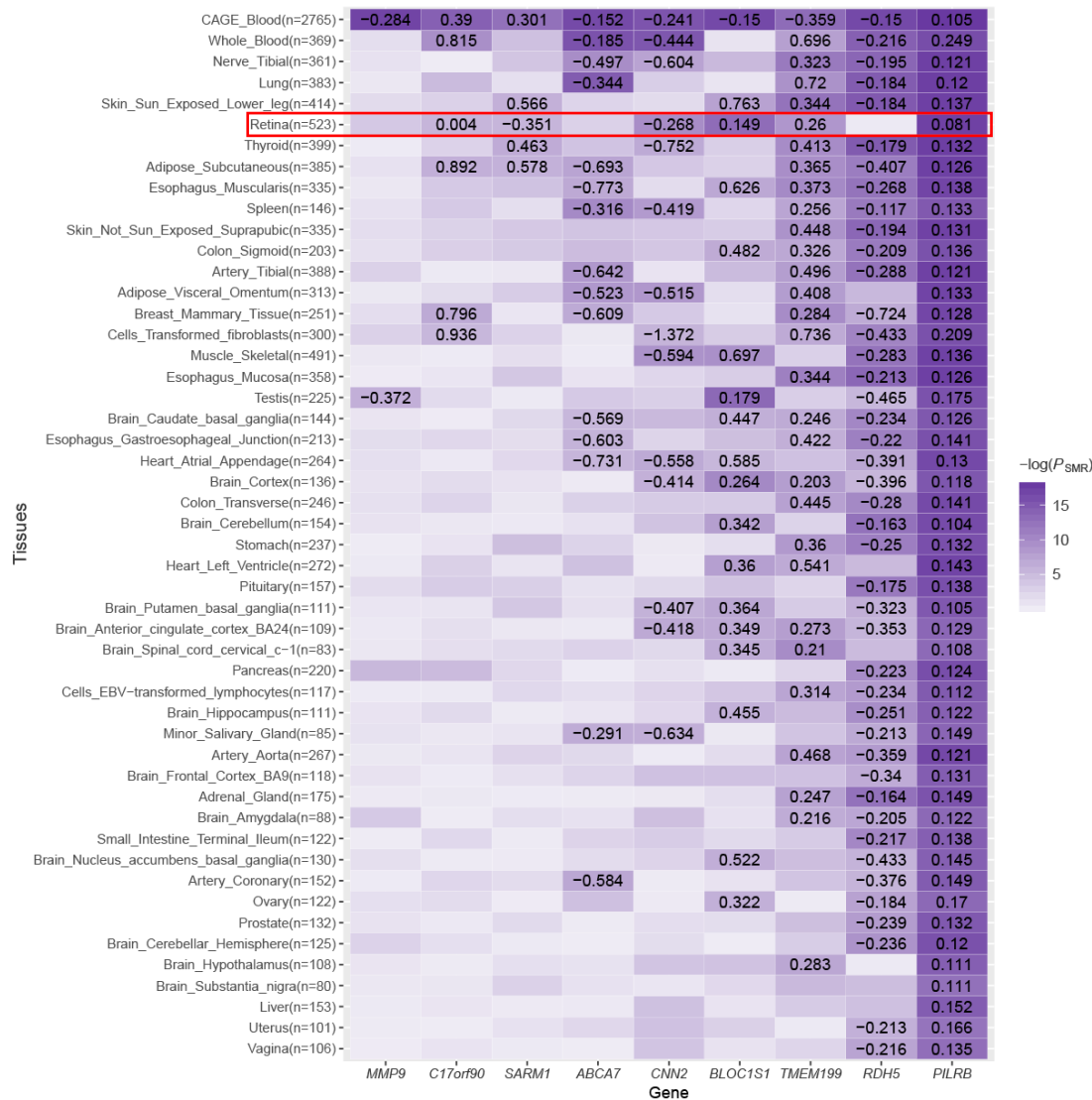
105 Given possible tissue-specific genetic effects, we replicated SMR associations of the nine significant genes  
106 in retina. The retinal eQTL summary data were retained from 523 postmortem subjects<sup>17</sup>, available in the  
107 Eye Genotype Expression (EyeGex) database (**URLs**). Six of the nine genes were replicated at  
108  $P_{smr} = 5.6 \times 10^{-3}$  (i.e., 0.05/9), a relatively high replication rate given the small sample size of the EyeGex  
109 data (**Table 1** and **Supplementary Figure 1**). However, for some of the replicated genes, the eQTL effects  
110 were significantly different, even in opposite directions, between the retina and CAGE-blood. For instance,  
111 the estimated effect of *SARM1* on AMD risk was 0.301 (standard error (SE) = 0.065 and  $P_{eQTL} = 4.1e-06$ )  
112 in CAGE-blood, consistent in several other tissues (see **Supplementary Figure 1**), but was -0.351 (SE =  
113 0.123 and  $P_{eQTL} = 4.4e-03$ ) in retina, suggesting that a strong tissue-specific effect and the importance to  
114 replicate and interpret discovery results in disease-relevant tissue(s).

115

116 It is unclear whether AMD is a localized disease (occurring only in affected retinas) or an ocular  
117 manifestation of a systemic process, since risk factors such as cigarette smoking, nutrition, and  
118 cardiovascular disease have a significant impact on disease progression<sup>19-23</sup>. Thus, we conducted SMR  
119 analysis for the nine genes in a wider range of tissues available in the GTEx project (**Supplementary**  
120 **Figure 1**). Intriguingly, the results showed that *PILRB*, a key activator in immune function, is significant  
121 across all 48 human tissues with effect sizes ranging from 0.081 (in retina) to 0.249 (in whole blood),  
122 implying that systemic immune pathways may be involved in AMD pathogenesis. In fact, *PILRB* is not  
123 an exception; all the nine genes were significant in at least two tissues and did not display obvious tissue-  
124 specific effects except for *SARM1* shown above, consistent with the results from previous studies that cis-  
125 eQTL effects are largely consistent across tissues<sup>24</sup>. Our results also suggest that the across-tissue  
126 replication rate depends heavily on the size of replication sample, supporting that using blood eQTL data  
127 from large samples gains power for gene discovery.

128

129 **Supplementary Figure 1: Heatmap of SMR results of the nine prioritised genes in multiple**  
 130 **tissues.** Each row represents a prioritised gene, and each column represents a tissue.  $-\log(P\text{-value}_{\text{SMR}})$   
 131 is plotted in white-purple scale. The purple color indicates more significant and the white means less  
 132 significant. Each tile with a number available indicates it reaches the significant threshold  $5.6E-3$   
 133 (correcting for 9 tests), with the number being the estimated SMR effect. Note that the overall mean  
 134 SMR p-value is decreasing towards top and right. Replication in retina is highlighted by a red  
 135 rectangle.



136  
137

### 138 **Knockdown of *cnn2* or *sarm1* led to ocular abnormalities in zebrafish**

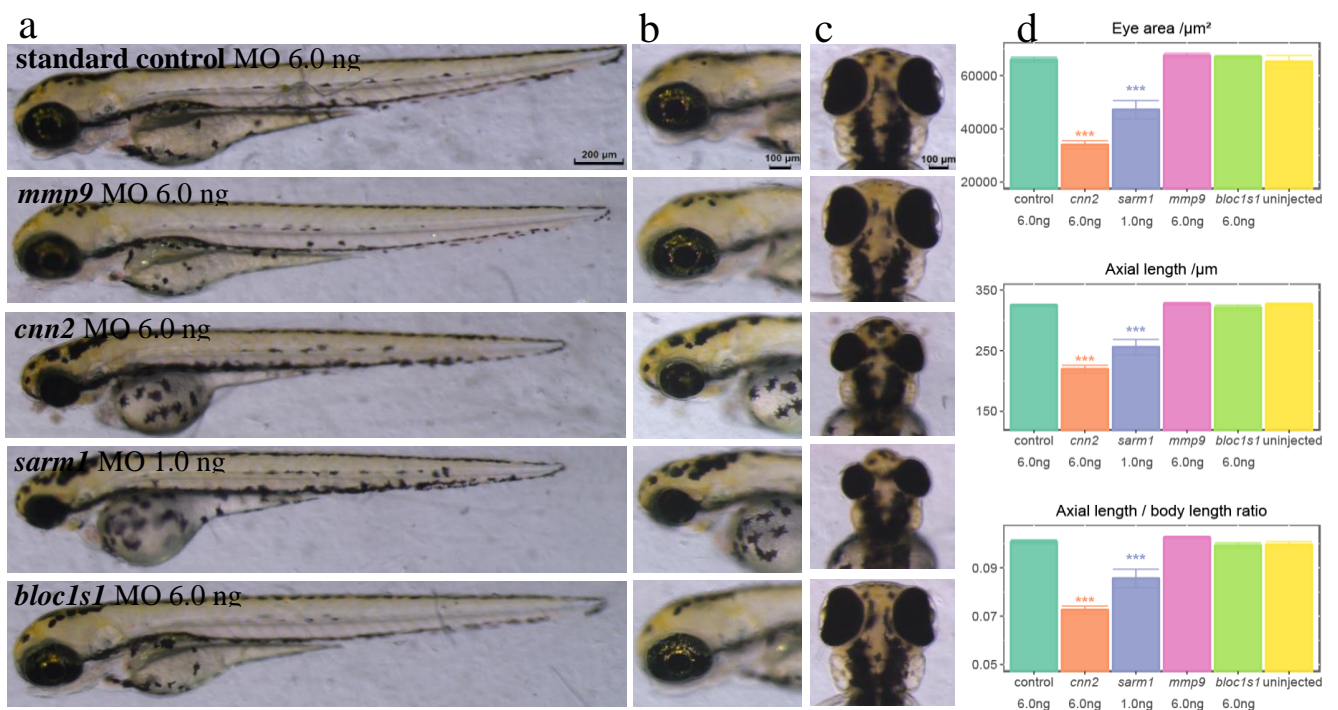
139 To validate the functional relevance of the prioritised genes to AMD pathogenesis, we sought an animal  
 140 model that is amenable to manipulating gene expression and that can reliably evaluate ocular phenotypes.  
 141 We chose zebrafish also because it has been extensively used to model ocular and other disorders<sup>25-27</sup>.  
 142 Accounting for sequence homology between human and zebrafish, we obtained 4 of the 9 prioritised genes  
 143 (*CNN2*, *SARM1*, *BLOC1S1* replicated at  $P_{\text{SMR}} < 5.6e-3$  and *MMP9* at  $P_{\text{SMR}} < 0.05$  using the retina eQTL  
 144 data; **Table 1**) with orthologue similarity above 60%, reported by either Ensembl or GeneCards, for  
 145 functional follow-up (**URLs, Supplementary Table 2**). Then, MO technology, blocking the translation  
 146 process of a certain mRNA, was used to knock down the corresponding gene. We observed that

147 downregulation of *cnn2* and *sarm1* at doses of 6 ng MO and 1.0 ng, respectively, led to obvious decreases  
 148 in axis length (by 32.5% and 21.3%, respectively) and eye area (by 48.5% and 28.5%, respectively),  
 149 whereas suppression of *mmp9* and *bloc1s1* showed no significant difference at 3 days post fertilisation  
 150 (dpf) (**Figure 2** and **Supplementary Figure 2**).

151

152 To further confirm the results, we conducted dose-dependent and rescue experiments. We found that in  
 153 comparison to the control, eye sizes of *cnn2*-MO morphants were smaller and the degree was inversely  
 154 proportional to the MO dose (from 4.0 to 6.0 ng) without higher mortality (**Supplementary Figure 3**). For  
 155 the *sarm1*-MO morphants, abnormal ocular phenotypes occurred when the MO dose was extremely low  
 156 (0.50 ng), and the impact accumulated when the dose increased (from 0.50 to 4.0 ng), indicating that  
 157 ocular development might be sensitive to the *sarm1* expression level (**Supplementary Figure 4**).

158 Importantly, rescue of the smaller eyes was achieved in the mRNA and MO co-injected larvae for both  
 159 genes (**Figure 3a,d**). Axial length was recovered by 95.8% for *sarm1*-MO and 91.0% for *cnn2*-MO  
 160 (**Supplementary Note 1**). This study provides evidence for potential roles of *CNN2* and *SARM1* in ocular  
 161 development and disease-causing mechanisms.

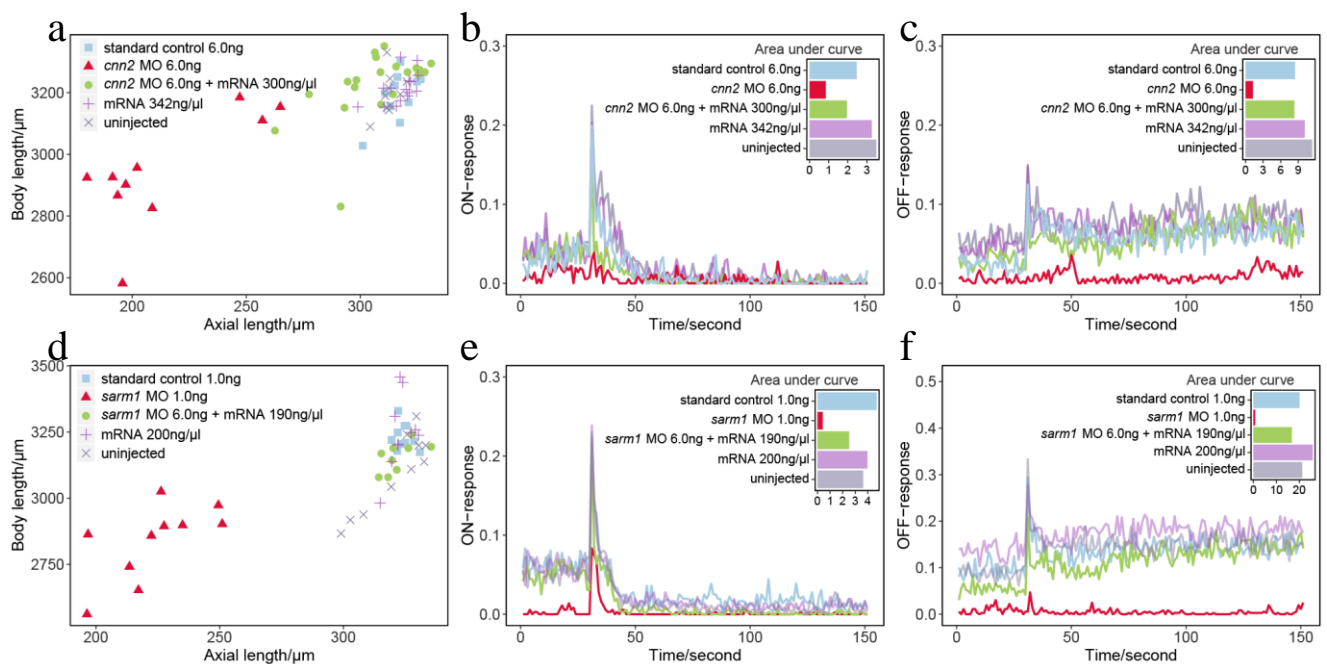


162  
 163 **Fig. 2 Phenotypes of *cnn2*-, *sarm1*-, *mmp9*-, *bloc1s1*-deficient zebrafish.** (a) Lateral view of whole  
 164 bodies. (b) Magnified lateral view of zebrafish eyeballs exhibits apparent decreases in eye area for  
 165 *cnn2*- and *sarm1*-deficient fishes. (c) Vertical view of eyeballs showing shorter axial length in *cnn2*  
 166 and *sarm1* knockdown larvae. (d) Quantification of eye area, axial length and ratio of axial length and  
 167 body length, respectively. Bar plots are shown as the mean  $\pm$  s.e.m. T-test was performed between each  
 168 group and the standard control. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . N=10 for each group.



### 169 **Knockdown of *cnn2* or *sarm1* led to functional impairment in zebrafish**

170 We then used a visual motor response (VMR) assay to evaluate the visual condition at the behavioural  
 171 level 5 dpf. According to a standard protocol<sup>28</sup>, zebrafish were placed in a 96-well plate and the locomotor  
 172 response to light alteration was monitored. We found that, for *CNN2*, three groups, including the uninjected  
 173 control, standard MO control and *cnn2* mRNA, had a brief spike at approximately 0.20-0.22 of motor  
 174 activity for ON response and 0.12-0.15 for OFF response (**Figure 3b, 3c**). In comparison, the response of  
 175 the *cnn2* MO-injected group was weakened and delayed, with the peak dramatically decreasing by 61.9%  
 176 for lights-ON and by 78.6% at 20 s later when lights-OFF. Of note, augmentation of *cnn2* mRNA with MO  
 177 morphants could save a visual response to 0.16 for lights-ON (recovering by 61.5%); when lights-OFF,  
 178 their motor activities were intensified to 0.08 (recovering by 45.5%), and the baseline was notably  
 179 improved. Actually, functional recovery relied on a sufficient dose of injected mRNA. Our pre-experiment  
 180 suggested that only partial rescue of visual function could be realized when injecting less *cnn2* mRNA  
 181 (**Supplementary Figure 5**). In addition, the *SARM1* group showed similar results but with more severe  
 182 visual impairment (**Figure 3e,f**). Notably, simple injection of *sarm1* mRNA seemed to slightly promote the  
 183 OFF response, implying an underlying therapeutic target. Taken together, the results of the visual function  
 184 assays further elucidate the reduction in visual motor activities specifically caused by forced  
 185 downregulation of *cnn2* or *sarm1*, indicating their essential roles in maintaining normal visual function.



186

### 187 **Fig. 3 Morphological and functional effects of knockdown and rescue of *cnn2* and *sarm1* in**

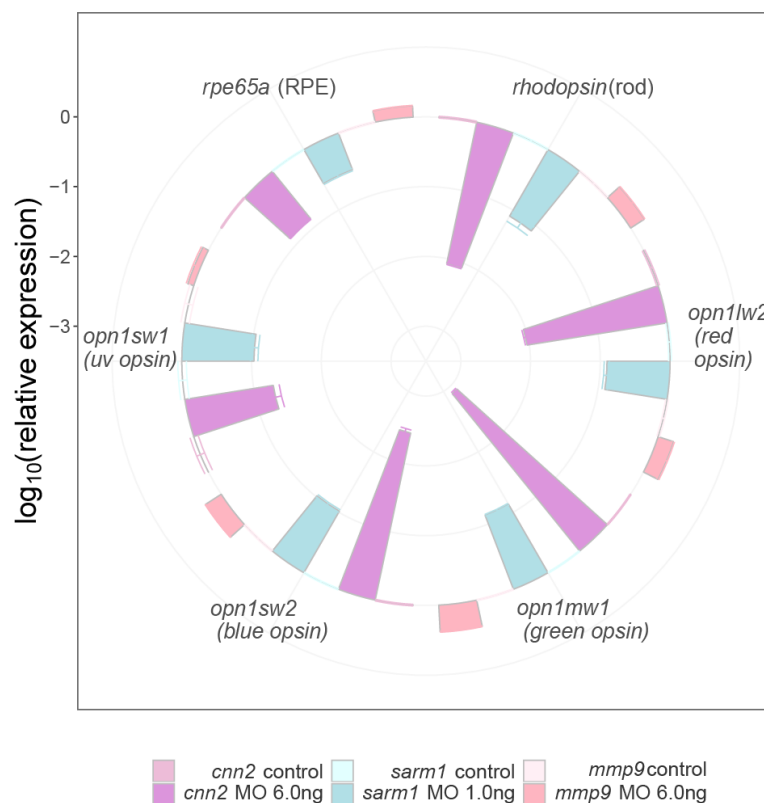
188 **zebrafish larvae. (a, d)** The scatter plots of body length and eyeball axial length. The *cnn2* or *sarm1*  
 189 MO group (labelled by red triangle) was clearly separate from the other groups, showing a reduction  
 190 in eye size. In addition, injecting corresponding mRNA (labelled by green dot) could rescue the  
 191 reduced ocular size. **(b, c, e, f)** VMR testing for ON and OFF responses. Lights-ON or lights-OFF  
 192 stimuli occurred at 30 s. Real-time motor activities of zebrafish were recorded by lines. The area  
 193 under the curve reflected the sum of motor activities during the 150 s. The responses of the *cnn2* or

194 *sarm1* MO group (labelled by red line) were dramatically weakened and could also be saved by  
195 injecting mRNA (labelled by green line).

196

### 197 **Knockdown of *cnn2* or *sarm1* downregulated the expression of photoreceptor and RPE signature** 198 **genes**

199 Considering the potential role of *CNN2* and *SARM1* in AMD pathophysiology, we sought to test the  
200 hypothesis whether visual impairment could be attributed to defects of vital photoreceptor or RPE genes.  
201 Thus, we conducted reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis to  
202 measure the expression levels of retinal signature genes: *rhodopsin*, four kinds of cone opsins (*red*, *green*,  
203 *blue*, and *uv*) and RPE-specific gene *rpe65a*<sup>29,30</sup>. For the *cnn2* 6.0 ng MO group and the *sarm1* 1.0 ng MO  
204 group, all these genes showed dramatic decreases compared to the standard MO control; *rhodopsin*  
205 decreased by 117.1-fold and 12.6-fold respectively, so as to cone opsin (*green opsin* by 797.5-fold and  
206 13.6-fold, *blue opsin* by 279.8-fold and 10.4-fold, *red opsin* by 107.4-fold and 8.0-fold, and *uv opsin* by  
207 19.2-fold and 10.8-fold), and *rpe65a* (by 7.5-fold and 3.6-fold) (**Figure 4**). However, regarding the *mmp9*  
208 6.0 ng MO group, the expression levels of all six genes slightly increased by 1.2-2.4 folds. Combined with  
209 the results above, the transcriptional reduction of signature genes in RPE and photoreceptors, caused by  
210 downregulation of *cnn2* or *sarm1*, is in line with ocular abnormality and visual behaviour impairments.  
211 Since opsin gene expression is a determinant factor for photoreceptor degeneration<sup>31-33</sup>, we hypothesize  
212 that downregulation of *CNN2* and *SARM1* are likely involved in photoreceptor degeneration during the  
213 pathogenic process of AMD.



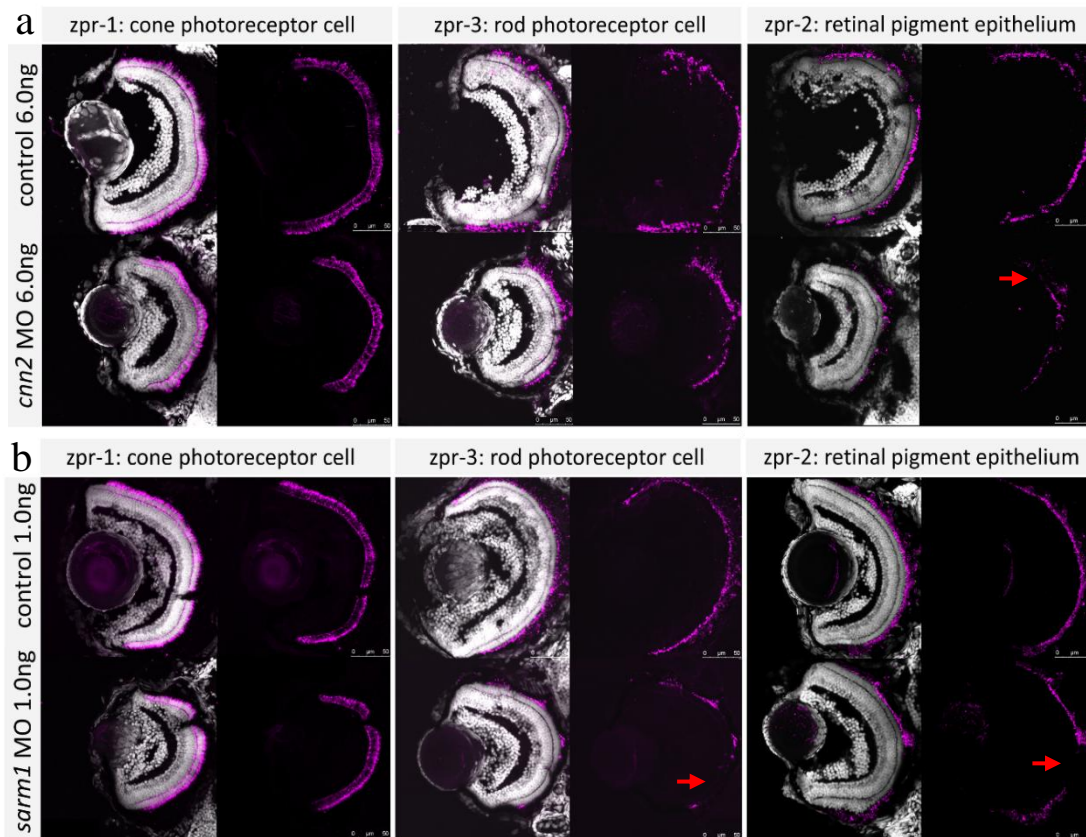
214

215 **Fig. 4 Real-time qPCR of photoreceptor and RPE genes in the MO zebrafish oculus. The x axis**

216 represents photoreceptor and RPE signature genes, and the y axis represents the  
217  $\log_{10}(\text{relative expression})$ . Centripetal bars indicate downregulation, and centrifugal bars indicate  
218 upregulation. The *cnn2*- and *sarm1*- MO groups revealed relatively low expression for all gene  
219 markers, whereas the *mmp9* group showed a slight increase. The bar plot is shown as the mean $\pm$ s.e.m.  
220

### 221 **Knockdown of *cnn2* or *sarm1* disrupted RPE in zebrafish**

222 In addition to the alteration of photoreceptors and RPE at transcriptional level, we investigated the retinal  
223 morphological consequences of the knockdown by cryosection and immunostaining to further verify the  
224 functional relevance of *CNN2* and *SARM1* with AMD pathogenesis. Using DAPI for nuclear staining, zpr-  
225 1 for cones, zpr-2 for RPE and zpr-3 for rods, we found that laminations were basically intact in both the  
226 *cnn2* 6.0 ng MO group and the *sarm1* 1.0 ng MO group (**Figure 5**). For the *cnn2* 6.0 ng MO group,  
227 staining of cones and rods showed no apparent difference from the standard control, but RPE displayed  
228 obvious disorganization and cell loss. For the *sarm1* 1.0 ng MO group, staining of RPE also demonstrated  
229 significant deficiency, with most rods disrupted (**Supplementary Figure 6**), whereas cones remained  
230 complete. In fact, a prior experiment of the *sarm1* 6.0 ng MO group observed severe lamination disruption,  
231 indicating that extreme insufficiency of *sarm1* has a severely adverse impact on ocular development  
232 (**Supplementary Figure 7**). Altogether, for both *cnn2* and *sarm1*, RPE were morphologically injured,  
233 which is in accordance with a well-accepted understanding that degeneration of RPE is a fundamental  
234 trigger of the cascade of events resulting in AMD pathology<sup>34</sup>. However, photoreceptors seemed not  
235 significantly affected. A likely hypothesis is that, in a certain condition, functional changes might precede  
236 morphological changes. That is, in the developmental stage, a small number of healthy RPE tissues could  
237 burden delivering oxygen and metabolites, where photoreceptors maintain morphological normality but are  
238 gradually functionally injured due to downregulation of *cnn2* or *sarm1* and photoreceptor core genes, then  
239 cumulative damage tips the balance and consequently leads to degeneration<sup>34</sup>.



240

241 **Fig. 5 Retinal architecture of *cnn2*- and *sarm1*-deficient morphants.** Nuclear layers were stained

242 by DAPI in grey, and each group showed intact lamination. zpr-1, zpr-2 and zpr-3 were stained in

243 purple for each column. (a) Signals of zpr-1 and zpr-3 in the *cnn2* MO 6.0 ng group were detected at

244 relatively normal levels, but the signal of zpr-2 was much lower compared with the standard control.

245 (b) Signals of zpr-3 and zpr-2 were distributed significantly less in the out layer of retina for the

246 *sarm1* MO 1.0 ng group, and zpr-1 remained intact.

247

### 248 **Overexpression of *bloc1s1* resulted in mild impaired ocular phenotypes in zebrafish**

249 Given the significant SMR associations of *BLOC1S1* in both discovery and replication analyses where the

250 effect size was positive in retina and negative in blood, we assumed that GWAS loci in *BLOC1S1* affect the

251 risk of AMD by upregulating its expression. Therefore, we injected an additional zebrafish group with

252 *bloc1s1* mRNA. Compared to controls, smaller eyes were observed in *bloc1s1*-overexpressing fishes

253 (Figure 6a) with axial length decreased by 7%. For the visual behaviour, the ON response was not

254 apparently affected, but impairment of the OFF response was observed in the *bloc1s1* mRNA-injected

255 group with the peak of OFF response declined by 59.1% (Figure 6b,c). Immunostaining results showed

256 that disruption of cones, rods and RPE in *bloc1s1*-overexpression group occurred (Figure 6d) but more

257 than one half of staining repetition had no notable difference in comparison to the standard control

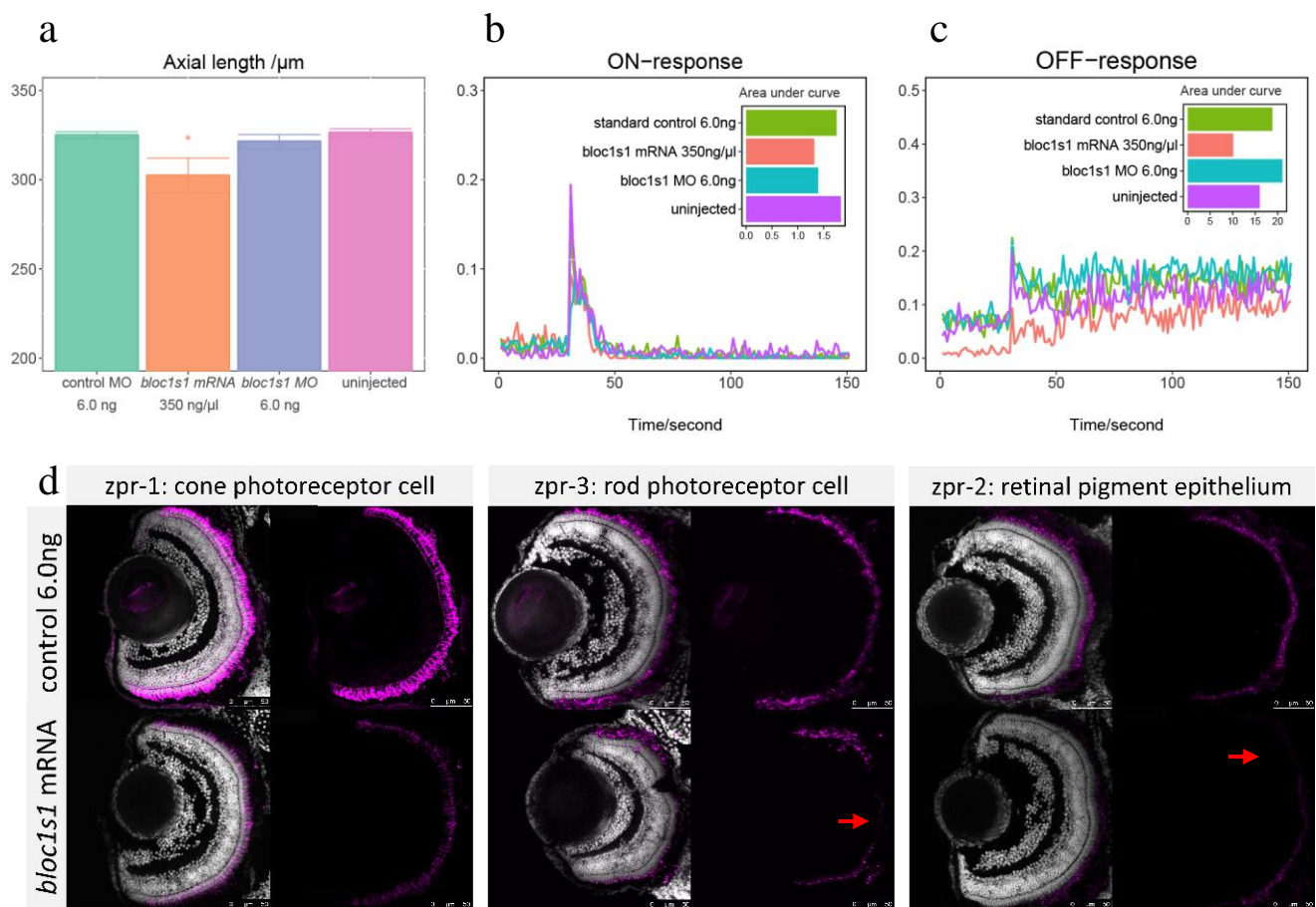
258 (Supplementary Figure 8). In terms of qPCR, however, rod gene was down-regulated by 1.4-fold ( $P =$

259 5.4e-4) and uv cone gene was up-regulated significantly by 1.3-fold ( $P = 0.025$ ) but other retinal genes

260 remained same (**Supplementary Figure 9**), likely in the early stage of degeneration since a previous study  
 261 showed that aggregation of S-opsin (short-wavelength cone opsin, including uv opsin) is accompanied by  
 262 the onset of cone degeneration through activating endoplasmic reticulum (ER) stress in a murine model<sup>31</sup>.  
 263 Overall, impaired ocular phenotypes were caused by *bloc1s1*-overexpressing, but the impairment was  
 264 milder than that in the knockdown groups above.

265

266 Despite that the detrimental effects of overexpression of *bloc1s1* were moderate, the phenotypic results in  
 267 zebrafish were in line with the estimated SMR effect in retina rather than that in blood. Here, the positive  
 268 SMR effect means that increased expression level of a gene is associated with increased phenotype (or  
 269 disease risk) and vice versa. The estimated SMR effect of *BLOC1S1* was in positive and those of *CNN2*  
 270 and *SARM1* were negative, predicting that increased expression level of *BLOC1S1* and decreased  
 271 expression levels of *CNN2* and *SARM1* are associated with increased AMD risk. In a word, the prediction  
 272 from SMR and HEIDI analysis was validated in zebrafish that knockdown of *cnn2* and *sarm1* and  
 273 overexpression of *bloc1s1* caused ocular abnormalities.



274

275 **Fig. 6 Phenotypes of *bloc1s1*-overexpression zebrafish larvae.** (a) The bar plot of axial length. The  
 276 *bloc1s1*-overexpression group showed a slight but significant reduction in ocular sizes. (b, c) The  
 277 VMR testing for ON and OFF responses. The ON response was not apparently affected, but  
 278 impairment of the OFF response was observed in the *bloc1s1* mRNA-injected group. (d)

279 Immunostaining of *bloc1s1*-mRNA zebrafish retinae showed apparently low expression for zpr-1, zpr-

280 2 and zpr-3.

281

## 282 Discussion

283 In this study, we aimed to shed light on the biological knowledge expected from integrated analysis of  
284 AMD GWAS and eQTL summary data. Initially, we identified 9 putative causal genes for AMD using the  
285 SMR and HEIDI methods based on a blood eQTL dataset (**Table 1**), and subsequently replicated 6 of the 9  
286 genes using a retina eQTL dataset (**Table 1** and **Supplementary Figure 1**). We also tested the 9 genes in  
287 48 other tissues using the GTEx eQTL data and showed that the across-tissue replication rate depended  
288 heavily on the size of replication sample (**Supplementary Figure 1**). We carried 4 of the 9 genes forward  
289 for functional assay in zebrafish and for the first time demonstrated the functional relevance of *CNN2*,  
290 *SARM1* and *BLOC1S1* to AMD. Injection of respective *cnn2* MO, *sarm1* MO and *bloc1s1* mRNA in  
291 zebrafish larvae exhibited different degrees of ocular defects (**Figures 2** and **6**), vision loss (**Figures 3** and  
292 **6**), relatively low transcription of essential photoreceptor and/or RPE genes (**Figure 4**) and RPE  
293 degeneration (**Figures 5** and **6**). Importantly, knockdown-induced phenotypes could be rescued by  
294 augmentation of corresponding mRNA (**Figure 3**), indicating the potential as targets to design new  
295 treatments. Finally, the phenotypic effects of alternating transcription of the prioritised genes in zebrafish  
296 were in line with the directions of estimated SMR effects in human retinas. All these results provide strong  
297 evidence supporting the potential role of *CNN2*, *SARM1* and *BLOC1S1* in AMD pathogenesis.

298

299 There is no denying that the study has some limitations. First, we used a large blood eQTL data set ( $n =$   
300 2,765) for discovery and a relatively small retina eQTL data set ( $n = 523$ ) for replication, following the  
301 suggestion from a recent study<sup>14</sup>. This strategy would have missed genes with a cis-eQTL effect in retina  
302 but not in blood. However, we considered that if cis-eQTL effects are similar across tissues<sup>18,24</sup>, using a  
303 blood eQTL data set of large sample size could increase the power of gene discovery in comparison to  
304 using a retina eQTL data set of small sample size. In addition, blood is much more accessible than retina so  
305 that the growth of eQTL data from blood is expected to be faster than that from retina, suggesting that the  
306 power of our analysis strategy could be substantially improved in the future by leveraging blood eQTL  
307 data sets with sample sizes of orders of magnitude larger than that used in this study<sup>35</sup>.

308

309 Second, zebrafish is an ideal but not perfect animal model. AMD is an age-related disease. However,  
310 zebrafish larvae were studied instead of elder zebrafish because the most efficient duration of MO-  
311 mediated knockdown effects is the first two or three days of development, and efficiencies decrease later<sup>36</sup>,  
312 rendering it difficult to observe age-related morphological and functional changes. Generating a primate  
313 AMD model is the most scientifically valid since humans or primates are the only mammals with a macula  
314 and foveal centralis, where AMD manifests, but it is extremely challenging in particular with respect to  
315 costs and time scale of the experiment. Instead, an important role of the zebrafish models is to serve as an  
316 efficient screening test to narrow down the most plausible causal genes through our comprehensive  
317 evaluation approaches. And the robust ocular changes of the prioritised genes, even in larvae time, should

318 not be underestimated. It is acknowledged that individual SNPs generally confer small effects on gene  
319 expression<sup>11</sup>, but MO interventions dramatically eliminate transcriptional levels, resulting in more severe  
320 and noticeable phenotypes. Moreover, accumulating evidence indicates that the origins of age-related  
321 disorders occur during foetal life<sup>37,38</sup>, and AMD should not be an exception.

322

323 Third, the genetic mechanisms of gene regulation and the relationship of gene regulation to AMD  
324 manifestation remain a mystery. Hypotheses of non-coding SNPs that influence gene expression include  
325 transcriptional, posttranscriptional, or posttranslational process<sup>9</sup>, such as non-coding RNA function or  
326 histone modification, allowing for more specific regulatory mechanistic studies. Of note, it is more  
327 important to identify the causal gene than the causal variant because the ultimate goal is to identify the  
328 causal gene, which can correspond to multiple causal variants. A mass of downstream research is required  
329 to understand the underlying molecular mechanisms. Intriguing clues include compelling biology such as  
330 inflammatory response and lipid metabolism and underlying overlapping pathophysiology with other age-  
331 related diseases for genes such as *CNN2*, *PILRB* and *ABCA7* that are also located at risk loci for late onset  
332 Alzheimer's disease (AD) (**Supplementary Table 3** for the description of each prioritised gene).

333

334 In summary, we performed an integrative data analysis that efficiently pinpointed novel susceptibility  
335 genes for AMD, and demonstrated the functional relevance of *CNN2*, *SARM1* and *BLOC1S1* to the disease  
336 using zebrafish models. The gene discovery procedure, combining statistical analysis of large-scale data  
337 and experimental validation, can be applied to other complex disorders to fill the knowledge gaps between  
338 genetic variants and phenotypes.

339

## 340 **Online Methods**

### 341 **Data used for the integrative analysis**

342 The GWAS summary data used in this study were derived from the latest and largest AMD GWAS meta-  
343 analysis<sup>5</sup> (see URLs section), consisting of 16,144 advanced AMD patients and 17,832 controls of  
344 predominantly European ancestry. The total number of SNPs was up to 12 million. The SNP effects were  
345 expressed as log odds ratios. Because the SNP allele frequency was not available, we estimated the allele  
346 frequencies using the UK Biobank data<sup>16</sup>.

347

348 The eQTL summary-level statistics were obtained from the CAGE data<sup>11</sup>, consisting of 36,778  
349 transcription phenotypes and ~8 million SNPs on 2,765 peripheral blood samples (of predominantly  
350 European ancestry). Transcription levels were measured using Illumina gene expression arrays. For  
351 replication in the retina, the summary cis-eQTL data were obtained from 523 postmortem retinas with ~ 9  
352 million SNPs and 15,124 gene expression traits<sup>17</sup>, available in the Eye Genotype Expression (EyeGex)  
353 database (URLs). For replication in other multiple tissues, we used the GTEx v7 data, containing a set of  
354 cis-eQTL summary data across 48 human tissues (URLs). Transcription levels in both EyeGex and GTEx  
355 were measured by RNA-seq. The eQTL effects in all the three data sets were expressed in standard

356 deviation (SD) units of transcription levels.

357

### 358 **SMR and HEIDI test for pleiotropic association**

359 SMR and HEIDI analyses were developed to identify genes whose expression levels were associated with  
360 a complex trait because of pleiotropy/causality (i.e., the trait and gene expression are associated due to the  
361 same set of causal variants at a locus)<sup>12</sup>. First, the SMR test takes the top associated cis-eQTL of the gene  
362 as an instrumental variable to test for association of a transcript (as an exposure) with AMD (as an  
363 outcome). An SMR estimate of the effect of gene expression on AMD is the ratio of the estimated effect of  
364 the instrument on a transcript (eQTL effect) and that on the disease (GWAS effect). The standard error  
365 (SE) of the SMR effect is computed using the Delta method, and the significance of the effect is assessed  
366 by the Wald test. To exclude the SMR associations due to linkage (i.e., the trait and gene expression are  
367 associated due to distinct set of causal variants in LD), the HEIDI analysis uses multiple SNPs in a cis-  
368 eQTL region to test against the null hypothesis that the trait is associated with gene expression because of  
369 the same set of underlying causal variants (pleiotropy/causality). Under the null, the SMR effects estimated  
370 using different eQTL SNPs in the cis region are expected to be the same. Significant heterogeneity in SMR  
371 effects detected at different SNPs in LD with the top associated cis-eQTL would be considered as linkage  
372 and rejected from the analysis.

373

### 374 **Morpholino, mRNA rescue and overexpression experiments**

375 For the *cnm2* mRNA rescue experiment, we generated zebrafish cDNA from total RNA using RT-PCR on a  
376 full-length *cnm2* fragment. For the *sarm1* mRNA rescue and *bloc1s1* overexpression study, we attained  
377 specific cDNA from the pUC57 vector cloned into template cDNA sequences of *sarm1* or *bloc1s1*, which  
378 were obtained through oligoribonucleotide synthesis (Sangon Biotech, Shanghai, China). The  
379 amplification primers of *cnm2* included the forward (5'-  
380 TAATACGACTCACTATAGGGGCCACCATGTCTTCGCAG-3') and the reverse (5'-  
381 TTAGTAATCTTGGCCGTCGTCCTGATAGC-3'); *sarm1* included the forward (5'-  
382 TAATACGACTCACTATAGGGGCCACCATGTTTTTGTCCCTCG-3') and the reverse (5'-  
383 CTACTTCTTTTGTGGCTCTTTTTTGTCCG-3'); *bloc1s1*, included the forward (5'-  
384 TAATACGACTCACTATAGGGGCCACCATGCTCTCGCGG-3') and the reverse (5'-  
385 TCATGTGGATGCCGGCTGGAC-3'); they all flank the T7 promoter sequence (5'-  
386 TAATACGACTCACTATAGGG-3') and enhancing sequence (Kozak). PCR template DNA was purified  
387 using the QIAquick PCR Purification Kit (Qiagen, Germany). Capped and tailed full-length mRNA was  
388 then synthesized using an mMACHINE™ T7 ULTRA Transcription Kit (Invitrogen,  
389 Carlsbad, CA) before purification using the RNeasy Mini Kit (Qiagen, Germany) following the  
390 manufacturer's protocols. Rescue mRNAs were co-injected with MO into the one-cell stage embryos. For  
391 overexpression, corresponding mRNAs were injected into one- to two-cell stage embryos.

392

### 393 **Measurement of eye parameters and body length**



394 The eye parameter and body length measurements of 3 days post-fertilisation (dpf) embryos were assessed  
395 using stereomicroscopy (SZX116, OLYMPUS, Japan). Pictures of the vertical and lateral view of each  
396 larva were recorded by a microscopic camera. Axial length, eye area, and body length were quantified by  
397 built-in software (OLYMPUS cellsens standard, version 1.14). For data collection, 10-15 larvae were  
398 included in each group; experiments were replicated three times. Student's t-test was performed between  
399 controls and treatment conditions for each phenotype. Statistical differences were calculated and visualized  
400 by R software (version 3.5.3) and the ggplot2 package (version 3.1.0).

401

#### 402 **Visual behaviour experiments**

403 Visual motor response (VMR) of 5 dpf was measured using a Zebrabox (VMR machine ViewPoint 2.0,  
404 France) to evaluate lights-ON and lights-OFF responses. All larvae with different treatments were  
405 separately placed in a 96-well plate with adequate water to ensure free activities (12 larvae for each  
406 treatment). The Zebrabox protocol was set to apply: 1) dark adaption for 3 hrs; 2) ON light for 30 mins; 3)  
407 OFF light for 30 mins; and 4) repeat step 2 and step 3 three times. Motor activities were recorded every  
408 second. The duration of 150 s (30 s before and 120 s after light switching) was used to evaluate the visual  
409 motor activities of larvae. Aggregate data of three repetitions were compiled and visualized in the figures.  
410 The area under the curve, calculated by R package MESS (Version 1.0), was used to quantify the overall  
411 motor response during 150 s of light alterations. Twelve injected larvae for each group were randomly  
412 selected for experiments routinely conducted between 11:00 and 17:00.

413

#### 414 **Real-time quantitative PCR**

415 Zebrafish oculus was isolated from the control and experimental groups (n=30 pairs for each) at 3 dpf.  
416 Total RNA was then extracted with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA).  
417 RNA concentrations were determined using a NanoDrop instrument (NanoDrop Technologies, Thermo,  
418 US). Following the manufacturer's instructions, purified RNA (500 ng) was used to generate cDNA using  
419 PrimeScript reverse transcriptase (TaKaRa, Dalian, China). Real-time quantitative PCR was performed  
420 through FastStart Universal SYBR Green Master (RocheApplied Science, Mannheim, Germany). Specific  
421 primers are provided in Supplementary Table 4. The relative expression levels were detected by the  
422 StepOne Plus™ Real-time PCR System (Life Technologies, Carlsbad, CA, USA) and calculated by the 2-  
423  $\Delta\Delta$  CT method. The fold change was compared to the standard MO control. All experiments were  
424 performed in triplicate.

425

#### 426 **Immunohistochemistry**

427 For immunostaining purposes, morphants and control larvae at 3 dpf were fixed in 4% paraformaldehyde  
428 and were rinsed with 15% and 30% sucrose in PBS for dehydration. Zebrafish samples were then directly  
429 frozen in Richard-Allan Scientific™ Neg-50™. Cryosections of zebrafish eyes that were 18- $\mu$ m were  
430 stained with zpr-1, zpr-2 and zpr-3 zebrafish-specific antibodies (1:400; provided by ZFIN) overnight at  
431 4 °C. Alexa Fluor 594 anti-mouse secondary antibodies (1:400, provided by ZFIN) were used for

432 incubation in blocking solution for 2 hrs at room temperature. DAPI (4,6-diamidino-2-phenylindole) was  
433 counterstained for the cell nucleus. Coverslips were mounted, and a confocal microscope (TCS SP8, Leica,  
434 Germany) was used to analyse gene expression and retinal architecture.

435

#### 436 URLs

437 AMD summary results: <http://amdgenetics.org/>

438 eQTL summary results: <http://cnsgenomics.com/software/smr/#DataResource>

439 SMR software: <http://cnsgenomics.com/software/smr>

440 EyeGex: <https://gtexportal.org/home/datasets>

441 Ensembl Orthologs: <http://asia.ensembl.org/index.html>

442 GeneCards Orthologs: <https://www.genecards.org/>

443

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451

#### 452 References

- 453 1 DeAngelis, M. M. *et al.* Genetics of age-related macular degeneration (AMD). *Human molecular genetics*  
454 **26**, R45-r50, doi:10.1093/hmg/ddx228 (2017).
- 455 2 Wong, W. L. *et al.* Global prevalence of age-related macular degeneration and disease burden projection  
456 for 2020 and 2040: a systematic review and meta-analysis. *The Lancet. Global health* **2**, e106-116,  
457 doi:10.1016/s2214-109x(13)70145-1 (2014).
- 458 3 Velez-Montoya, R. *et al.* Current knowledge and trends in age-related macular degeneration: genetics,  
459 epidemiology, and prevention. *Retina (Philadelphia, Pa.)* **34**, 423-441,  
460 doi:10.1097/iae.000000000000036 (2014).
- 461 4 Klein, R. J. *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science (New*  
462 *York, N.Y.)* **308**, 385-389 (2005).
- 463 5 Fritsche, L. G. *et al.* A large genome-wide association study of age-related macular degeneration  
464 highlights contributions of rare and common variants. *Nature genetics* **48**, 134-143,  
465 doi:10.1038/ng.3448 (2016).
- 466 6 Chen, W. *et al.* Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence  
467 susceptibility to age-related macular degeneration. *Proceedings of the National Academy of Sciences of*  
468 *the United States of America* **107**, 7401-7406, doi:10.1073/pnas.0912702107 (2010).
- 469 7 Zhan, X. *et al.* Identification of a rare coding variant in complement 3 associated with age-related  
470 macular degeneration. *Nature genetics* **45**, 1375-1379, doi:10.1038/ng.2758 (2013).
- 471 8 Fritsche, L. G. *et al.* Age-related macular degeneration: genetics and biology coming together. *Annual*  
472 *review of genomics and human genetics* **15**, 151-171, doi:10.1146/annurev-genom-090413-025610  
473 (2014).
- 474 9 Edwards, Stacey L., Beesley, J., French, Juliet D. & Dunning, Alison M. Beyond GWASs: Illuminating the  
475 Dark Road from Association to Function. *The American Journal of Human Genetics* **93**, 779-797,  
476 doi:10.1016/j.ajhg.2013.10.012 (2013).
- 477 10 Wu, Y. *et al.* Integrative analysis of omics summary data reveals putative mechanisms underlying  
478 complex traits. *Nature communications* **9**, 918, doi:10.1038/s41467-018-03371-0 (2018).

- 479 11 Lloyd-Jones, L. R. *et al.* The Genetic Architecture of Gene Expression in Peripheral Blood. *American*  
480 *journal of human genetics* **100**, 228-237, doi:10.1016/j.ajhg.2016.12.008 (2017).
- 481 12 Zhu, Z. *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait gene  
482 targets. *Nature genetics* **48**, 481-487, doi:10.1038/ng.3538 (2016).
- 483 13 Gusev, A. *et al.* Integrative approaches for large-scale transcriptome-wide association studies. *Nature*  
484 *genetics* **48**, 245-252, doi:10.1038/ng.3506 (2016).
- 485 14 Xue, A. *et al.* Genome-wide association analyses identify 143 risk variants and putative regulatory  
486 mechanisms for type 2 diabetes. *Nature communications* **9**, 2941, doi:10.1038/s41467-018-04951-w  
487 (2018).
- 488 15 Hauberg, M. E. *et al.* Large-Scale Identification of Common Trait and Disease Variants Affecting Gene  
489 Expression. *The American Journal of Human Genetics* **100**, 885-894, doi:10.1016/j.ajhg.2017.04.016  
490 (2017).
- 491 16 Bycroft, C. *et al.* The UK Biobank resource with deep phenotyping and genomic data. *Nature* **562**, 203-  
492 209, doi:10.1038/s41586-018-0579-z (2018).
- 493 17 Ratnapriya, R. *et al.* Retinal transcriptome and eQTL analyses identify genes associated with age-related  
494 macular degeneration. *Nature genetics*, doi:10.1038/s41588-019-0351-9 (2019).
- 495 18 Qi, T. *et al.* Identifying gene targets for brain-related traits using transcriptomic and methylomic data  
496 from blood. *Nature communications* **9**, 2282, doi:10.1038/s41467-018-04558-1 (2018).
- 497 19 Lim, L. S., Mitchell, P., Seddon, J. M., Holz, F. G. & Wong, T. Y. Age-related macular degeneration. *Lancet*  
498 *(London, England)* **379**, 1728-1738, doi:10.1016/s0140-6736(12)60282-7 (2012).
- 499 20 Myers, C. E. *et al.* Cigarette smoking and the natural history of age-related macular degeneration: the  
500 Beaver Dam Eye Study. *Ophthalmology* **121**, 1949-1955, doi:10.1016/j.ophtha.2014.04.040 (2014).
- 501 21 Wu, J., Cho, E., Willett, W. C., Sastry, S. M. & Schaumberg, D. A. Intakes of Lutein, Zeaxanthin, and Other  
502 Carotenoids and Age-Related Macular Degeneration During 2 Decades of Prospective Follow-up. *JAMA*  
503 *ophthalmology* **133**, 1415-1424, doi:10.1001/jamaophthalmol.2015.3590 (2015).
- 504 22 Seddon, J. M. *et al.* Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular  
505 degeneration. Eye Disease Case-Control Study Group. *Jama* **272**, 1413-1420 (1994).
- 506 23 Cheung, C. M. & Wong, T. Y. Is age-related macular degeneration a manifestation of systemic disease?  
507 New prospects for early intervention and treatment. *Journal of internal medicine* **276**, 140-153,  
508 doi:10.1111/joim.12227 (2014).
- 509 24 Battle, A., Brown, C. D., Engelhardt, B. E. & Montgomery, S. B. Genetic effects on gene expression across  
510 human tissues. *Nature* **550**, 204-213, doi:10.1038/nature24277 (2017).
- 511 25 Roosing, S. *et al.* Disruption of the basal body protein POC1B results in autosomal-recessive cone-rod  
512 dystrophy. *American journal of human genetics* **95**, 131-142, doi:10.1016/j.ajhg.2014.06.012 (2014).
- 513 26 Huang, X. F. *et al.* Mutation of IPO13 causes recessive ocular coloboma, microphthalmia, and cataract.  
514 *Experimental & molecular medicine* **50**, 53, doi:10.1038/s12276-018-0079-0 (2018).
- 515 27 Zheng, S. S., Han, R. Y., Xiang, L., Zhuang, Y. Y. & Jin, Z. B. Versatile Genome Engineering Techniques  
516 Advance Human Ocular Disease Researches in Zebrafish. *Frontiers in cell and developmental biology* **6**,  
517 75, doi:10.3389/fcell.2018.00075 (2018).
- 518 28 Emran, F., Rihel, J. & Dowling, J. E. A behavioral assay to measure responsiveness of zebrafish to changes  
519 in light intensities. *Journal of visualized experiments : JoVE*, doi:10.3791/923 (2008).
- 520 29 Tzima, E. *et al.* Transcriptional and Behavioral Responses of Zebrafish Larvae to Microcystin-LR Exposure.  
521 *International journal of molecular sciences* **18**, doi:10.3390/ijms18020365 (2017).
- 522 30 Zhang, L. *et al.* Expression profiling of the retina of pde6c, a zebrafish model of retinal degeneration.  
523 *Scientific data* **4**, 170182, doi:10.1038/sdata.2017.182 (2017).
- 524 31 Zhang, T., Zhang, N., Baehr, W. & Fu, Y. Cone opsin determines the time course of cone photoreceptor  
525 degeneration in Leber congenital amaurosis. *Proceedings of the National Academy of Sciences of the*  
526 *United States of America* **108**, 8879-8884, doi:10.1073/pnas.1017127108 (2011).
- 527 32 Nir, I., Agarwal, N. & Papermaster, D. S. Opsin gene expression during early and late phases of retinal  
528 degeneration in rds mice. *Experimental eye research* **51**, 257-267, doi:10.1016/0014-4835(90)90022-m  
529 (1990).
- 530 33 Cunea, A., Powner, M. B. & Jeffery, G. Death by color: differential cone loss in the aging mouse retina.  
531 *Neurobiology of aging* **35**, 2584-2591, doi:10.1016/j.neurobiolaging.2014.05.012 (2014).
- 532 34 Ferrington, D. A., Sinha, D. & Kaarniranta, K. Defects in retinal pigment epithelial cell proteolysis and the  
533 pathology associated with age-related macular degeneration. *Progress in retinal and eye research* **51**,  
534 69-89, doi:10.1016/j.preteyeres.2015.09.002 (2016).
- 535 35 Vösa, U. *et al.* Unraveling the polygenic architecture of complex traits using blood eQTL metaanalysis.

- 536 *bioRxiv*, 447367, doi:10.1101/447367 (2018).
- 537 36 Sumanas, S. & Larson, J. D. Morpholino phosphorodiamidate oligonucleotides in zebrafish: a recipe for  
538 functional genomics? *Briefings in functional genomics & proteomics* **1**, 239-256,  
539 doi:10.1093/bfgp/1.3.239 (2002).
- 540 37 Wei, K. *et al.* Developmental origin of age-related coronary artery disease. *Cardiovascular research* **107**,  
541 287-294, doi:10.1093/cvr/cvv167 (2015).
- 542 38 Briana, D. D. & Malamitsi-Puchner, A. Developmental origins of adult health and disease: The metabolic  
543 role of BDNF from early life to adulthood. *Metabolism: clinical and experimental* **81**, 45-51,  
544 doi:10.1016/j.metabol.2017.11.019 (2018).