Primary open-angle glaucoma (POAG) is a leading cause of blindness worldwide. To identify new susceptibility loci, we performed meta-analysis on genome-wide association study (GWAS) results from eight independent studies from the United States (3,853 cases and 33,480 controls) and investigated the most significantly associated SNPs in two Australian studies (1,252 cases and 2,592 controls), three European studies (875 cases and 4,107 controls) and a Singaporean Chinese study (1,037 cases and 2,543 controls). A meta-analysis of the top SNPs identified three new associated loci: rs35934224[T] in TXNRD2 (odds ratio (OR) = 0.78, \( P = 4.05 \times 10^{-11} \)) encoding a mitochondrial protein required for redox homeostasis; rs7137828[T] in ATXN2 (OR = 1.17, \( P = 8.73 \times 10^{-10} \)); and rs2745572[A] upstream of FOXC1 (OR = 1.17, \( P = 1.76 \times 10^{-10} \)).

Using RT-PCR and immunohistochemistry, we show TXNRD2 and ATXN2 expression in retinal ganglion cells and the optic nerve head. These results identify new pathways underlying POAG susceptibility and suggest new targets for preventative therapies.

Glaucoma is a clinically and genetically complex disease that is the leading cause of irreversible blindness worldwide\(^1,2\). POAG, the most common form of the disease in most populations\(^3\), is characterized by retinal ganglion cell apoptosis and progressive optic nerve damage\(^4\). Recent GWAS have identified interesting risk loci for POAG\(^5–9\), but these account for only a fraction of disease heritability. To identify new POAG susceptibility loci, we have completed a meta-analysis of GWAS summary findings from individuals of European descent from the United States with replication in an Australian study (ANZRAG, Australian and New Zealand Registry of Advanced Glaucoma) and further evaluation in a second Australian study (BMES, Blue Mountains Eye Study), three European studies and a Singaporean Chinese data set.

For stage 1 (discovery), we performed meta-analysis on summary data from eight independent data sets (3,853 cases and 33,480 controls; Supplementary Table 1) with European ancestry from the United States with replication in an Australian study (ANZRAG, Australian and New Zealand Registry of Advanced Glaucoma) and further evaluation in a second Australian study (BMES, Blue Mountains Eye Study), three European studies and a Singaporean Chinese data set.

A full list of affiliations appears at the end of the paper.
sets except OHTS, controls had an intraocular pressure (IOP) of <21 mm Hg (Supplementary Table 2). For each data set, site-specific quality control (sample and genotype call rates ≥95%), principal-components analysis (EIGENSTRAT\(^1\)) and imputation (IMPUTE2 (ref. 12) or MACH\(^13,14\)) were completed using the 1000 Genomes Project reference panel (March 2012) (Supplementary Table 3 and Supplementary Note). Imputed variants with minor allele frequencies <5% or imputation quality scores \(\hat{r}^2\) <0.7 were removed before analysis. Dosage data, in the form of estimated genotypic probabilities, were analyzed in ProbABEL\(^15\) for each data set using logistic regression models, adjusting for age, sex, any significant eigenvectors \(P < 0.05\) and study-specific covariates. The genomic inflation \(\lambda\) was less than 1.05 for each individual data set (Supplementary Fig. 1). Estimated genotypic probabilities for 6,425,680 variants underwent meta-analysis in Meta\(^16\) using the inverse variance–weighted method.

To confirm that the results were not skewed by a particular data set, we completed a sensitivity analysis by selectively removing each data set in turn and carrying out meta-analysis on the remaining seven data sets. The odds ratios from each grouping of seven data sets were highly correlated with the results obtained from all eight data sets (Supplementary Fig. 2).

The stage 1 genome-wide association results are shown in Supplementary Figure 3, and the association results for all SNPs with \(P < 1 \times 10^{-5}\) are shown in Supplementary Table 4. The association for one SNP (rs2745572[A]) located in a region on 6p 50 kb 5′ to FOXC1 reached genome-wide significance \((OR = 1.25, P = 2.36 \times 10^{-9})\) in stage 1 (Table 1). Additionally, 873 SNPs, including SNPs located in regions not previously associated with POAG on 1p, 2p, 2q, 5p, 6p, 9q, 10q, 12q, 20p and 22p, had association \(P < 1 \times 10^{-5}\) (Supplementary Table 4).

Table 1 Association and meta-analyses of the NEIGHBORHOOD and ANZAG cohorts for the top ranked loci

| Chr. | SNP | Position | A1 | A2 | Gene | OR (NEIGHBORHOOD, discovery, stage 1) | OR (ANZAG, replication, stage 2) | Meta-analysis NEIGHBORHOOD + ANZAG |
|------|-----|----------|----|----|------|--------------------------------------|---------------------------------|-----------------------------------|
| 1    | rs7518099 | 165,736,880 | T | C | TMCO1 | 0.70 \(1.32 \times 10^{-13}\) | 0.71 \(8.02 \times 10^{-6}\) | 0.70 \(6.35 \times 10^{-18}\) |
| 4    | rs1173201 | 7,924,690 | T | C | AFAP1 | 0.85 \(3.93 \times 10^{-6}\) | 0.87 \(6.77 \times 10^{-6}\) | 0.83 \(1.98 \times 10^{-10}\) |
| 6    | rs2745572 | 1,548,369 | A | G | FOXC1 | 1.25 \(2.36 \times 10^{-9}\) | 1.18 \(6.46 \times 10^{-3}\) | 1.23 \(6.50 \times 10^{-11}\) |
| 9    | rs7866783 | 22,056,359 | A | G | CDKN2B-AS1 | 0.70 | 1.04 \(10^{-23}\) | 0.67 | 2.92 \(10^{-12}\) | 0.69 | 1.22 \(10^{-34}\) |
| 9    | rs2472493 | 107,695,848 | A | G | ABCA1 | 0.83 | 1.24 \(10^{-7}\) | 0.70 | 2.08 \(10^{-10}\) | 0.79 | 2.44 \(10^{-15}\) |
| 12   | rs7137828 | 111,932,800 | T | C | ATXN2 | 1.17 \(6.53 \times 10^{-6}\) | 1.22 \(4.36 \times 10^{-4}\) | 1.18 | 9.20 \(10^{-9}\) |
| 13   | rs33912345 | 60,976,537 | A | C | SIX6 | 0.76 | 8.94 \(10^{-15}\) | 0.78 | 6.21 \(10^{-10}\) | 0.76 | 1.71 \(10^{-19}\) |
| 17   | rs9897123 | 10,020,501 | T | C | GAS7 | 0.85 | 6.86 \(10^{-6}\) | 0.79 | 1.45 \(10^{-5}\) | 0.83 | 5.85 \(10^{-10}\) |
| 22   | rs35934224 | 19,872,645 | T | C | TXNRD2 | 0.79 | 1.39 \(10^{-6}\) | 0.74 | 2.01 \(10^{-4}\) | 0.77 | 1.08 \(10^{-9}\) |

Association results for the SNPs reaching genome-wide significance in the discovery cohort (rs2745572) as well as other top ranked loci showing replication. Genomic position is based on Build 37 of the reference genome. A1 is the effect allele for both cohorts. NEIGHBORHOOD, National Eye Institute Glaucoma Human Genetics Collaboration Heritable Overall Operational Database; ANZAG, Australian and New Zealand Registry of Advanced Glaucoma; chr, chromosome; OR, odds ratio; Het, heterogeneity \(\hat{r}^2\) index; Het \(P\) value for heterogeneity. Loci not previously reported are denoted in bold text.

Figure 1 Association results for the regions reaching genome-wide significance after stage 2. These plots show the regional association and recombination rates for the top SNPs in the discovery cohort (NEIGHBORHOOD; 3,853 cases and 33,480 controls) after meta-analysis with data for these SNPs from ANZAG (1,155 cases and 1,992 controls). In each plot, the solid diamond represents the top ranked SNP in the region, identified on the basis of two-sided \(P\) values. Pairwise correlation \(\hat{r}^2\) between the top SNP and the other SNPs in the region is indicated by color. Blue spikes show the estimated recombination rates. The box underneath each plot shows the gene annotations in the region. Each plot was created using LocusZoom for the top ranked SNP in each region with a 400-kb region surrounding it. (a) The top SNP for this plot is rs2745572 on chromosome 6 upstream of FOXC1 with association \(P = 6.50 \times 10^{-11}\). (b) The top SNP for this plot is rs7137828 on chromosome 12 within ATXN2 with association \(P = 9.20 \times 10^{-9}\). (c) The top SNP for this plot is rs35934224 on chromosome 22 within TXNRD2 with association \(P = 1.08 \times 10^{-9}\).
Next, we investigated the associations of the most significant stage 1 SNPs (\(P < 1 \times 10^{-5}\)) in a replication data set of individuals of European ancestry from Australia (ANZRAG; 1,155 cases and 1,992 controls) (Supplementary Note) and performed a meta-analysis of these SNPs in the NEIGHBORHOOD and ANZRAG data sets using effect sizes and their standard errors (stage 2). In the meta-analysis, SNPs in new regions 50 kb\(^5\) to \(FOX C1\) (top SNP rs2745572[A], \(OR = 1.23, P = 6.5 \times 10^{-11}\)), within intron 14 of \(ATXN2\) (top SNP rs7137828[T], \(OR = 1.18, P = 9.2 \times 10^{-9}\)) and within intron 11 of \(TXNRD2\) (top SNP rs35934224[T], \(OR = 0.77, P = 1.8 \times 10^{-9}\)) reached genome-wide significance (Table 1 and Supplementary Table 5). The regional association results for these SNPs are shown in Figure 1.

For each of the three new regions reaching genome-wide significance after stage 2, we further examined their association with POAG in a second Australian data set (BMES; 107 cases and 600 controls), three European data sets (875 cases and 4,107 controls in total) and a study of Singaporean Chinese (1,037 cases and 2,543 controls) (stage 3).

The association for all three top SNPs exceeded genome-wide significance in meta-analysis of all data sets (Fig. 2 and Supplementary Fig. 4): \(TXNRD2\) rs35934224[T], combined \(P = 4.05 \times 10^{-11}\), \(OR = 0.78\); \(ATXN2\) rs7137828[T], combined \(P = 4.40 \times 10^{-10}\), \(OR = 1.17\); and \(FOX C1\) rs2745572[A], combined \(P = 1.76 \times 10^{-10}\), \(OR = 1.17\) (Supplementary Tables 6 and 7). The top SNP within \(ATXN2\) (rs7137828) is very rare in the Singaporean Chinese population and thus could not be evaluated.

SNPs in the \(G A S 7\) region, previously associated with IOP, a quantitative trait that when elevated is a risk factor for glaucoma\(^7\)–\(^9\), were significantly associated with POAG after stage 2 (top SNP rs9897123[T], \(OR = 0.83, P = 5.85 \times 10^{-10}\)) (Table 1). Other POAG-associated loci identified in recent studies\(^5\)–\(^9\) were also confirmed, including \(T M C O 1, C D K N 2 B-A S 1, S I X 6, A B C A 1\) and \(A F A P 1\) (Table 1 and Supplementary Table 8). SNPs in \(P M M 2\) recently identified in Chinese individuals with POAG\(^9\) were nominally associated with POAG (top SNP rs12444233[T], \(OR = 1.13, P = 0.0016\)).

POAG, like many complex human diseases, displays clinical subphenotypes\(^20,21\). In particular, optic nerve degeneration in POAG can occur without elevation of IOP, a clinical subtype defined as normal-tension glaucoma (NTG)\(^22\). The NEIGHBORHOOD POAG data set included 725 NTG cases (maximum IOP \(\leq 21\) mm Hg) and 1,868 high-tension glaucoma (HTG) cases (maximum IOP \(>21\) mm Hg) (pretreatment IOP data were not available for 1,260 cases).

Meta-analysis of NTG cases (using all the controls from the data sets with NTG cases) identified one new associated locus on chromosome 12q (rs2041895[C], \(OR = 1.48, P = 2.41 \times 10^{-9}\)) in stage 1 (Supplementary Fig. 5 and Supplementary Table 9). The direction of effect was consistent (\(OR = 1.13\)) in the ANZRAG NTG data set but did not reach significance (\(P = 0.11\)) and the combined association result (NEIGHBORHOOD + ANZRAG) fell just short of genome-wide significance (\(P = 6.5 \times 10^{-11}\)), within intron 14 of \(ATXN2\) (top SNP rs7137828[T], \(OR = 1.76 \times 10^{-10}\), \(P = 1.9 \times 10^{-1}\)) and 8q22 (ref. 7) (top SNP rs284491[T], \(OR = 1.35 \times 10^{-12}\) and \(8.9 \times 10^{-12}\)) and, in the HTG subgroup (1,868 cases), \(12q\) (rs2041895[C], \(OR = 1.48, P = 2.41 \times 10^{-9}\)) in stage 1.

Meta-analysis of NTG cases (using all the controls from the data sets with NTG cases) identified one new associated locus on chromosome 12q (rs2041895[C], \(OR = 1.48, P = 2.41 \times 10^{-9}\)) in stage 1 (Supplementary Fig. 5 and Supplementary Table 9). The direction of effect was consistent (\(OR = 1.13\)) in the ANZRAG NTG data set but did not reach significance (\(P = 0.11\)) and the combined association result (NEIGHBORHOOD + ANZRAG) fell just short of genome-wide significance (\(P = 6.5 \times 10^{-11}\)), possibly owing to a smaller number of NTG cases in the ANZRAG data set (\(n = 363\)). In the NEIGHBORHOOD discovery data set, we confirmed previous NTG associations on 9p (ref. 7) (CDKN2B-AS1 top SNP rs1333037[T], \(OR = 1.67, P = 1.35 \times 10^{-12}\)) and 8q22 (ref. 7) (top SNP rs284491[T], \(OR = 0.66, P = 2.30 \times 10^{-8}\)) and, in the HTG subgroup (1,868 cases), confirmed associations with \(T M C O 1\) (refs. 6,17) and \(S I X 6\) (refs. 7,23) (Supplementary Fig. 6 and Supplementary Table 10). The SNPs in the \(FOX C1\) region associated with POAG overall were also significant in the NEIGHBORHOOD HTG subgroup (most significant SNP rs2317961, \(OR = 0.76, P = 2.58 \times 10^{-8}\)).

To assess the possible functional effects of SNPs at the three newly identified POAG-associated loci, we accessed and applied data from...
Figure 3. ATXN2 and TXNRD2 are expressed in mouse retina and optic nerve head. (a) Representative images of immunofluorescence using an antibody to ATXN2 show the presence of ATXN2 (green) in cells in the ganglion cell layer (arrows; upper panels) as well as punctate staining in the inner plexiform layer (arrowhead; right-most upper panel). Only a low level of punctate staining was observed in the optic nerve head (arrowheads; lower panels). (b) Representative images of immunofluorescence using an antibody to TXNRD2 show the presence of TXNRD2 (green) in cells in the ganglion cell layer (arrows; upper panels) as well as substantial punctate staining in the inner plexiform layer (arrowheads; right-most upper panel). Staining was also observed in cells in the optic nerve head (lower panels), indicative of astrocytes that form pial columns (arrows; right-most lower panel). Punctate staining was also observed in the optic nerve head (arrowheads; lower panels). For each antibody, at least three sections from six eyes were assessed. No staining (not even punctate staining) was observed in control tissue with no primary antibody (data not shown). Blue, DAPI.

In all rows, the right-most panels show magnified views of the boxed regions in the center panels. Scale bars: upper left and center panels in a and b, 20 μm; lower left and center panels in a, 15 μm; lower left and center panels in b, 25 μm; right-most panels in a and b, 5 μm.

The most significant SNP (rs35934224) is located in a binding site. Both SP1 and ESR2 are expressed in human and rat retinal cells33,34 and could influence expression of ATXN2 (ref. 35).

The TXNRD2 region that includes 22 SNPs associated at the genome-wide significance level after stage 2 (Supplementary Table 5) is significantly enriched for enhancers in lymphoid cells (P = 0.01, RegulomeDB v2). The most significant SNP (rs7137828) is located in an SP1 transcription factor binding site, and another associated SNP (rs653178), which is in linkage disequilibrium (LD) with rs7137828 (r² > 0.8, European ancestry), is located in an ESR2 (estrogen receptor 2) binding site. Both SP1 and ESR2 are expressed in human and rat retinal cells33,34 and could influence expression of ATXN2 (ref. 35).

FOXC1 is a member of the forkhead family of transcription factors, and rare coding-sequence mutations (missense, nonsense and copy number variation (CNV)) are known to cause anterior segment dysgenesis and early-onset glaucoma with dominant inheritance39,40. FOXC1 has not been previously implicated in common adult-onset forms of glaucoma, including POAG or HTG. Interestingly, association over GMDS, located 3′ to FOXC1, has been identified in a study using some of the same samples analyzed here8. In our study, we found genome-wide significant association adjacent to FOXC1 in the 5′ regulatory region and less significant association in GMDS (top SNP rs9378638[C], OR = 0.83, P = 7.50 × 10⁻⁶). The top SNPs in the two regions are approximately 400 kb apart and are not in LD. Conditional analysis confirmed that the odds ratio and P value for the significantly associated SNPs 5′ to FOXC1 are unchanged by conditioning on the GMDS peak SNP, suggesting that these are independent associations (Supplementary Fig. 10). The 5′ regulatory SNPs associated with POAG and HTG identified by this study could be involved in regulation of FOXC1 expression.

The ATXN2 and TXNRD2 genomic regions have not been previously associated with POAG or with any glaucoma-related quantitative traits such as optic nerve parameters or IOP. Expansions of a CAG repeat in ATXN2 can cause spinocerebellar ataxia 2 with optic atrophy, and intermediate-length expansions can contribute to the development of amyotrophic lateral sclerosis (ALS)41. Very recently, two other genes known to be responsible for Mendelian forms of NTG have also been shown to contribute to ALS42,43. The ATXN2-SH2B3 region has been associated with retinal venular caliber in individuals with European ancestry44. We analyzed the expression of ATXN2 mRNA in normal human ocular tissues by RT-PCR and found expression in the cornea, trabecular meshwork, ciliary body, retina and optic nerve.
(Supplementary Fig. 11). Immunolabeling of sections of a normal mouse eye showed evidence of TXNRD2 in the retinal ganglion cells and optic nerve (Fig. 3).

TXNRD2 encodes thioredoxin reductase 2, a mitochondrial protein necessary for reducing the levels of damaging reactive oxygen species generated by oxidative phosphorylation and other mitochondrial functions. Cellular oxidative stress has been hypothesized as a cause of retinal ganglion cell dysfunction in glaucoma and over-expression of thioredoxin 2, the substrate of thioredoxin reductase 2 (encoded by TXNRD2), increased retinal ganglion cell survival in an experimental glaucoma model. We confirmed by RT-PCR that TXNRD2 is expressed in normal human ocular tissue (Supplementary Fig. 11), including the retina and optic nerve. Immunolabeling in mice showed strong staining in retinal ganglion cells as well as in the optic nerve (Fig. 3). These data suggest that the reduction of reactive oxygen species levels by TXNRD2 could prevent mitochondrial dysfunction and retinal ganglion cell apoptosis in glaucoma. To our knowledge, TXNRD2 is the first mitochondrial protein associated with glaucoma risk.

In this study, we identified common variants near FOXC1, ATXN2 and TXNRD2 as new risk loci for POAG. These genes suggest new pathways that may contribute to glaucoma development, including abnormal ocular development (FOXC1), neurodegeneration (ATXN2) and mitochondrial dysfunction secondary to accumulating reactive oxygen species (TXNRD2). Targeting these pathways could lead to effective and potentially preventative glaucoma therapies.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Summary data for the NEIGHBORHOOD POAG meta-analysis are available on the NEIGHBORHOOD website (under 'Publications'; see URLs).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.N.C.B., L.R.P., J.H.K., J.L.H. and J.L.W. were involved in designing the study. R.R.A., C.C.K., M.B., D.L.B., H.C., W.G.C., G.G., I.D.V., J.H.F., P.J.F., C.E., D.G., T.G., A.W.H., E.H., D.J.H., R.K.L., Z.L., P.R.L., D.A.M., P. McGuffin, P. Mitchell, S.E.M., S.A.P., Q.Q., T.R., J.E.R., P.M.R., E.R., R.R., J.S.S., W.K.S., K. Singh, A.J.S., R.M.T., F.T., A.C.V., D.Y., G.W., T.Y.W., B.L.Y., D.J., K.Z., N.W., B.W., R.N.W., M.A.P.-V., T.A., E.N.V., S.M., J.E.C., M.A.H., L.R.P., J.L.H. and J.L.W. were involved in participant recruitment, sample collection or genotyping. Analysis was performed by J.N.C.B., S.J.L., J.H.K., P.G., C.C.K., K.P.B., A.A.B., A.B., H.A., D.I.C., R.P.L., P.G.H., C.A.G., A.A.-K., C.-Y., A.P.C., M.R., K. Small, Y.E.S., S.S.V., J.J.W., C.J.H., K.P., L.R.P., S.M., J.L.H. and J.L.W. The laboratory experiments were designed and conducted by K.W.P., Y.L., G.H. and J.L.W. Clinician assessment was performed by R.R.A., D.L.B., W.G.C., J.H.F., D.G., A.W.H., R.K.L., P.R.L., D.A.M., S.E.M., T.R., R.R., J.S.S., K. Singh, A.I.S., F.T., A.C.V., G.W., T.Y.W., D.J.Z., K.Z., J.E.C., L.R.P. and J.L.W. The initial draft of the manuscript was written by J.N.C.B., L.R.P., J.H.K., J.L.H. and J.L.W.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Study design. Imputed genotypes (1000 Genomes Project panel, March 2012) for 3,853 cases and 33,480 controls from eight independent data sets were used as the discovery cohort for this GWAS for POAG (stage 1). The association results for the top SNPs from the discovery cohort were replicated in 1,155 cases and 1,992 controls from an Australian POAG study of individuals of European ancestry (stage 2), followed by further replication (stage 3) in a second Australian study (BMES) and three European studies (EPIC, GER and UK) (982 cases and 4,707 controls in total) and in a Singaporean Chinese data set of 1,037 cases and 2,543 controls. The details for all data sets, including genotyping platforms, quality control, imputation methods and diagnostic criteria, are listed in the Supplementary Note. All patients and controls were enrolled into the study following written informed consent and approval from the relevant national and regional institutional review boards for each sample collection.

Meta-analysis (discovery, stage 1). Quality control was performed for each data set as described in the Supplementary Note. Overall sample and genotype call rates were 99.5% for each site. Samples with log R ratio (LRR) and B allele frequency (BAF) values suggestive of CNVs were removed before analysis. Principal components (eigenvectors) were computed for all participants using EIGENSTRAT.51 For each data set, logistic regression was performed in ProbABEL15 for all analyses (POAG overall, HTG and NTG), controlling for age, sex and study-specific covariates, including study-specific ancestry (NEIGHBORHOOD; Trait Analysis)52. Conditional analyses were performed using GCTA (Genome Complex Loci of interest in the discovery cohort Replication (stages 2 and 3). Meta-analysis (discovery, stage 1). Meta-analysis of the results was performed in Metal16, implementing the inverse variance–weighted method and applying genomic control correction.

Replication (stages 2 and 3). Loci of interest in the discovery cohort (NEIGHBORHOOD; P < 1 × 10−7) were evaluated in the first replication cohort (ANZRAG) and meta-analysis was performed with the NEIGHBORHOOD results (stage 2). The top SNPs for the three newly identified regions were evaluated in five additional data sets, one Australian (BMES) data set, three European (EPIC, GER and UK) data sets and a Singaporean Chinese data set.

Power calculations. Power calculations were performed as described48. For the stage 1 discovery analysis, power calculations using a disease prevalence of 2% (ref. 2) indicated that there was 96% power of detecting loci at P < 1.0 × 10−5 (the threshold for carryover to stage 2) at minor allele frequencies as low as 30% with per-allele odds ratios of 1.17. The entire sample set (stages 1, 2 and 3) had 99% power to detect loci at P < 5.0 × 10−8 at minor allele frequencies as low as 30% with per-allele odds ratios as low as 1.17.

Candidate genes and functional effects. Genes of interest in the associated region were identified using Ensembl49, UCSC Genome Bioinformatics50 and Genecards51. To predict the functional effects of the top POAG-associated SNPs, we used ENCODE Project data24, HaploReg (v2)25 and RegulomeDB29. We used SCAN25, Genevar26 and GTEx27 as well as a study of UK twins applying RNA-seq and 1000 Genomes Project imputation27 to investigate eQTLs within genomic regions of interest.

Statistical analyses. Conditional analyses were performed using the top SNPs in the FOXC1, ATXN2 and TXNRD2 regions as well as the top SNP in the previously reported GMD5 region5, conditioning on the risk allele in the region of interest. Conditional analyses were performed using GCTA (Genome Complex Trait Analysis)32. Forest plots to visualize the effect sizes of top SNPs in each region by data set were created using the rmeta package in R. The odds ratios and 95% confidence intervals for each displayed SNP were plotted, and P values are listed for each analysis (Fig. 2) and each NEIGHBORHOOD data set (Supplementary Fig. 4).

Sensitivity analysis using the leave-one-out method was performed by excluding each NEIGHBORHOOD data set from a meta-analysis of the other seven data sets. We compared the odds ratios from these analyses by calculating the Pearson's product-moment correlation coefficient between each leave-one-out analysis and the overall meta-analysis of eight NEIGHBORHOOD data sets (stage 1) (Supplementary Fig. 2). Correlations were calculated in R using the corplot package and ellipse option.

Expression analysis of genes at associated loci in ocular tissues. Total RNA was extracted from dissected tissues from normal human donor eyes as previously described53,54 using an RNA isolation kit from Life Technologies. Reverse-transcription reactions were completed using Superscript III reverse transcriptase from Life Technologies. Primer sequences were designed to specifically amplify TXNRD2 and ATXN2. PCR reactions were performed using the recommended conditions with Platinum Taq DNA polymerase (Life Technologies) and applying a touchdown program. Amplified PCR products were visualized by gel electrophoresis on 2% agarose gels.

Immunohistochemistry. CS7BL/6J mice (males and females) were maintained on a 12-h light/12-h dark cycle. All experiments were approved by the Animal Care and Use Committee at The Jackson Laboratory. Eyes from 2- to 4-month-old CS7BL/6J mice were enucleated and fixed in 4% paraformaldehyde for 2 h, rinsed in 0.1 M phosphate buffer, immersed in 30% sucrose overnight and frozen in OCT. Sections (15 μm) were placed on Fisherbrand Superfrost Plus slides and stored at −70 °C until required. Sections were incubated overnight at 4 °C in the following primary antibodies: rabbit antibody to TXNRD2 (1:50 dilution; Acris) and rabbit antibody to ATXN2 (1:50 dilution; Acris). All antibodies were diluted in PB1 (1% Triton X-100 in 1× PBS). Sections were blocked in 2.5% chicken serum (in PBT) for 1 h and then incubated overnight at 4 °C. After incubation with primary antibody, sections were washed three times in PBT and incubated with secondary antibody (goat anti-rabbit IgG) for 4 h at 4 °C. All sections were then counterstained with DAPI and mounted with Aqua PolyMount. Images were acquired on a Leica SP5 confocal microscope. For each antibody, at least three sections from six eyes were assessed. Antibodies were obtained from Acris: atxin 2 (21776-1 AP); immunogen, Ag16470; GenBank ID (clone info), BC114546; purification method, Antigen affinity purification; Txnrd2 (16360-1-AP): immunogen, Ag8367; GenBank ID (clone info), BC007489; purification method, Antigen affinity purification. All images in Figure 3 were acquired on a Leica SP5 confocal microscope. Images in the left and center panels were acquired with a 20× glycerol objective; the images in the right panels were acquired with a 63× glycerol objective. Excitation was performed using a 405-nm diode laser (DAPI) and an argon laser (ATXN2 or TXNRD2). Image acquisition was performed using sequential scanning: scan 1, PMT 1 (gain 966) for DAPI; scan 2, PMT 2 (gain 1013) for ATXN2 or TXNRD2.

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