Supplementary Information

Genome-wide association study identifies susceptibility loci for open angle glaucoma at *TMCO1* and *CDKN2B-AS1*

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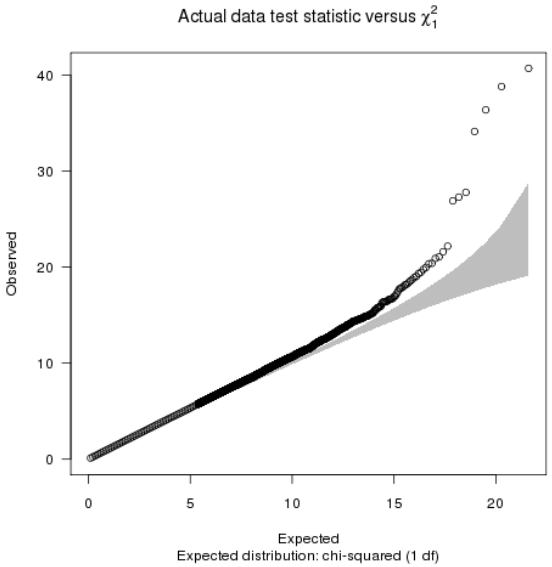
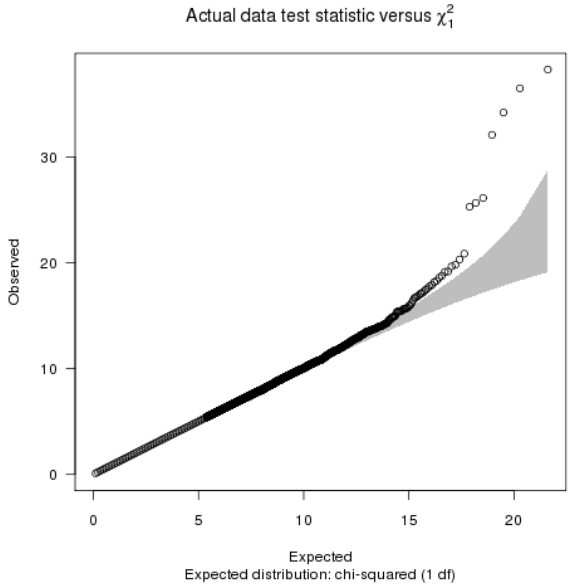
Supplementary Figures

Supplementary Figure 1: Q-Q plots. A) Genotyped SNPs. B) genotyped SNPs, corrected for genomic control parameter of 1.06. C) Advanced glaucoma discovery cohort cases compared with WTCCC 58BC controls after removal of outlier samples and adjustment for 10 principal components of ancestry.

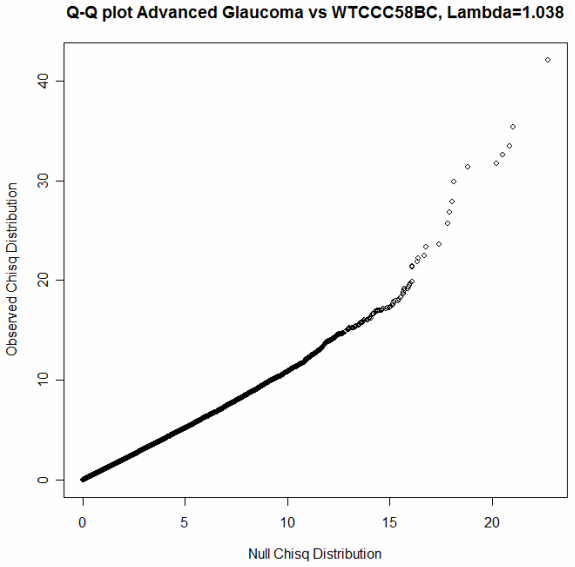
A



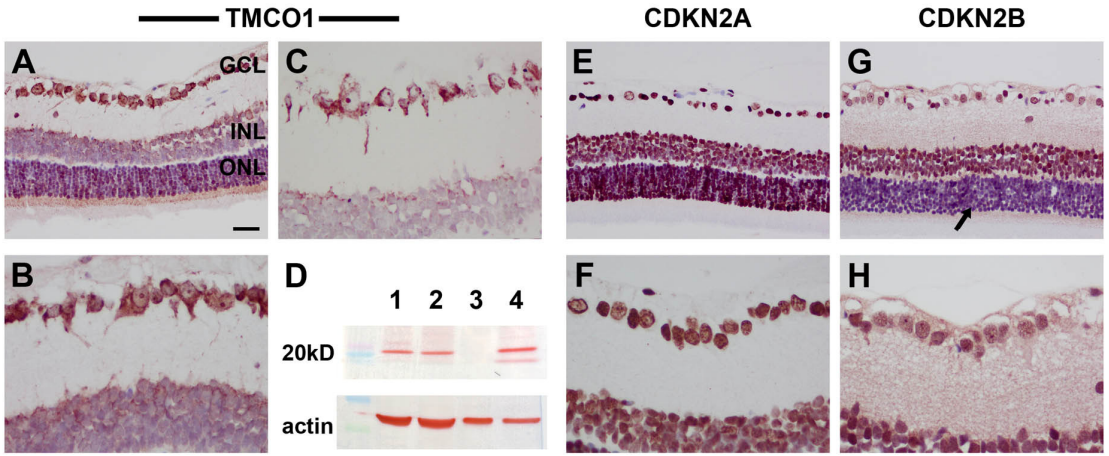
B



C



Supplementary Figure 2: Expression of TMCO1 (A-D), CDKN2A (E-F) and CDKN2B (G-H) in rat retina as determined by immunohistochemistry and Western blotting. Nuclei are counterstained with hematoxylin. TMCO1 immunolabelling was observed throughout the retina in a predominantly cytoplasmic subcellular localization (A, B), with strongest staining evident in the GCL, such that decreasing the concentration of the primary antibody by only 2-fold resulted in retention of robust somato-dendritic labelling in the GCL, but not elsewhere (C). Western blot analysis using the TMCO1 antibody revealed a band at approximately 20 kD in rat liver, brain and retina (D). Lanes: 1=liver, 2=brain, 3=optic nerve, 4=retina. CDKN2A and CDKN2B both displayed nuclear patterns of localization. CDKN2A (E-F) was robustly and ubiquitously expressed in cells of the retina and was often associated with the nuclear membrane. CDKN2B (G-H) immunolabelling was less intense than CDKN2A and was almost undetectable in the photoreceptor layer (arrow). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bar: A, E and G, 30 µm; B, C, F and H, 15µm.



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Supplementary Tables

Supplementary Table 1: Replication results under the allelic model. A) Association results for each cohort by severity of glaucoma. B) Association results in the combined replication cohorts. Also shown are p-values following adjustment for age and sex in a logistic regression under an additive model. This was done with and without the WTCCC 58BC cohort, as this entire cohort is the same age (i.e. 52 years in 2010). C) Allele frequency in each case cohort and the total control cohort for genome-wide significant SNPs. Odds Ratio (OR) are given for comparison of each case cohort with the total control cohort (n= 8600). The "Less Severe Rep Cases" includes the 93 BMES cases as well as the 465 other cases. A1=Allele 1, A2=Allele 2, Adv=advanced OAG, Rep=replication. * Included in replication study due to previously reported associations with other diseases. ** Adjusted for age and sex. NA=Not available in the WTCCC 58BC control data from Illumina 550 array.

A) Replication cohorts by severity of glaucoma					Advanced Replication cohort				Less Severe Replication cohort			
SNP	Chr	Build 36 position	Gene	Allele	Case freq	Control freq	P-value	OR(95%CI)	Case freq	Control freq	P-value	OR(95%CI)
rs4656461	1	163953829	*TMCO1*	G	0.17	0.12	0.010	1.47(1.09-1.97)	0.15	0.12	0.026	1.28(1.03-1.89)
rs7411708	1	163992902	*TMCO1*	C	0.42	0.41	0.591	1.06(0.86-1.31)	0.43	0.39	0.022	1.19(1.03-0.39)
rs10918276	1	163993294	*TMCO1*	C	0.42	0.40	0.434	1.09(0.88-1.34)	0.43	0.39	0.024	1.19(1.03-1.39)
rs7518099	1	164003504	*TMCO1*	C	0.16	0.12	0.032	1.38(1.03-1.86)	0.15	0.12	0.022	1.29(1.04-1.61)
rs7041637	9	21951866	*CDKN2A*	C	0.71	0.69	0.493	1.08(0.86-1.35)	0.70	0.74	0.009	0.80(0.68-0.95)
rs3731239	9	21964218	*CDKN2A*	T	0.69	0.65	0.078	1.22(0.98-1.52)	0.66	0.61	0.010	1.23(1.05-1.44)
rs3217992	9	21993223	*CDKN2B*	A	0.43	0.42	0.817	1.03(0.83-1.26)	0.40	0.35	0.028	1.19(1.02-1.39)
rs1063192	9	21993367	*CDKN2B*	T	0.66	0.59	0.006	1.35(1.09-1.68)	0.61	0.53	$1.57x10^{-4}$	1.34(1.15-1.57)
rs7049105	9	22018801	*CDKN2B-AS1*	G	0.55	0.52	0.170	1.16(0.94-1.42)	0.51	0.44	$6.64x10^{-4}$	1.30(1.12-1.51)
rs1412829*	9	22033926	*CDKN2B-AS1*	T	0.68	0.62	0.015	1.31(1.06-1.63)	0.63	0.55	$7.74x10^{-5}$	1.37(1.14-1.60)
rs10120688	9	22046499	*CDKN2B-AS1*	A	0.56	0.52	0.153	1.16(0.95-1.43)	0.51	0.46	0.013	1.21(1.04-1.41)
rs4977756	9	22058652	*CDKN2B-AS1*	A	0.69	0.63	0.042	1.25(1.01-1.56)	0.64	0.58	0.003	1.27(1.08-1.48)
rs4977574	9	22088574	*CDKN2B-AS1*	G	0.54	0.51	0.374	1.10(0.89-1.35)	0.53	0.49	0.031	1.23)1.16-1.37)
rs2383207	9	22105959	*CDKN2B-AS1*	G	0.58	0.52	0.030	1.26(1.02-1.55)	0.55	0.51	0.028	1.18(1.02-1.38)
rs10757278*	9	22114477	*CDKN2B-AS1*	G	0.52	0.50	0.389	1.10(0.89-1.35)	0.51	NA	NA	NA
rs1333049*	9	22115503	*CDKN2B-AS1*	C	0.53	0.50	0.220	1.14(0.92-1.41)	0.53	NA	NA	NA
rs7020996*	9	22119579	*CDKN2B-AS1*	T	0.15	0.13	0.318	1.16(0.86-1.57)	0.12	NA	NA	NA

B) Combined replication cohorts

SNP	Chr	Build 36 position	Gene	Allele	Case freq	Control freq	P-value	OR (95% CI)	Adjusted P-value **	Adjusted P-value** excluding WTCCC
rs4656461	1	163953829	*TMCO1*	G	0.16	0.12	$7.56x10^{-6}$	1.39(1.20-1.61)	$9.74x10^{-8}$	$5.71x10^{-7}$
rs7411708	1	163992902	*TMCO1*	C	0.43	0.39	0.018	1.15(1.02-1.29)	0.017	0.233
rs10918276	1	163993294	*TMCO1*	C	0.43	0.40	0.013	1.15(1.03-1.29)	0.011	0.178
rs7518099	1	164003504	*TMCO1*	C	0.16	0.12	$2.14x10^{-5}$	1.37(1.18-1.59)	$3.30x10^{-7}$	$1.44x10^{-6}$
rs7041637	9	21951866	*CDKN2A*	C	0.70	0.73	0.014	0.86(0.76-0.99)	0.137	0.894
rs3731239	9	21964218	*CDKN2A*	T	0.68	0.62	$2.26x10^{-6}$	1.33(0.18-1.50)	$5.87x10^{-6}$	0.012
rs3217992	9	21993223	*CDKN2B*	A	0.41	0.36	$5.04x10^{-4}$	1.22(1.09-1.37)	0.004	0.221
rs1063192	9	21993367	*CDKN2B*	T	0.63	0.55	$7.46x10^{-10}$	1.44(1.28-1.61)	$3.43x10^{-8}$	0.003
rs7049105	9	22018801	*CDKN2B-AS1*	G	0.53	0.46	$4.84x10^{-7}$	1.33(1.19-1.49)	$4.92x10^{-6}$	0.034
rs1412829*	9	22033926	*CDKN2B-AS1*	T	0.65	0.56	$2.93x10^{-10}$	1.45(1.29-1.63)	$7.98x10^{-9}$	0.0017
rs10120688	9	22046499	*CDKN2B-AS1*	A	0.53	0.48	$3.98x10^{-5}$	1.24(1.12-1.38)	$1.28x10^{-4}$	0.001
rs4977756	9	22058652	*CDKN2B-AS1*	A	0.66	0.59	$4.19x10^{-7}$	1.33(1.19-1.48)	$1.27x10^{-6}$	$1.13x10^{-5}$
rs4977574	9	22088574	*CDKN2B-AS1*	G	0.53	0.48	$5.67x10^{-4}$	1.22(1.09-1.36)	$9.72x10^{-4}$	0.116
rs2383207	9	22105959	*CDKN2B-AS1*	G	0.56	0.50	$8.56x10^{-5}$	1.25(1.12-1.40)	$1.12x10^{-4}$	0.029
rs10757278*	9	22114477	*CDKN2B-AS1*	G	0.52	0.47	0.005	1.21(1.06-1.38)	0.084	0.084
rs1333049*	9	22115503	*CDKN2B-AS1*	C	0.53	0.48	0.002	1.23(1.08-1.42)	0.113	0.113
rs7020996*	9	22119579	*CDKN2B-AS1*	T	0.14	0.87	0.663	0.96(1.79-1.17)	0.402	0.402

C) Allele frequency and odds ratios for each case cohort vs all controls

		All controls	Adv Discovery Cases		Adv Rep Cases		All Adv Cases		Less Severe Rep Cases	
		n=8600	n=590		n=334		n=924		n=558	
SNP	**A1**	**A1 Freq**	**A1 Freq**	**OR(95%CI)**	**A1 Freq**	**OR(95%CI)**	**A1 Freq**	**OR(95%CI)**	**A1 Freq**	**OR(95%CI)**
rs4656461	G	0.12	0.19	1.70(1.45-1.99)	0.17	1.49(1.20-1.85)	0.18	1.62(1.42-1.86)	0.15	1.29(1.07-1.54)
rs7518099	C	0.12	0.18	1.67(1.42-1.96)	0.16	1.41(1.13-1.77)	0.18	1.58(1.38-1.81)	0.15	1.35(1.12-1.61)
rs10120688	A	0.49	0.58	1.43(1.27-1.62)	0.56	1.35(1.15(1.59)	0.57	1.40(1.27-1.55)	0.52	1.13(0.99-1.28)
rs4977756	A	0.60	0.69	1.49(1.31-1.70)	0.69	1.47(1.23-1.74)	0.69	1.48(1.33-1.65)	0.64	1.20(1.05-1.38)

Supplementary Table 2: Haplotype association in the combined discovery and replication cohorts. Odds ratios (OR) and p-values are calculated for each haplotype compared to all others combined at that locus. Chromosome 1 - SNPs included in the haplotype are rs4656461; rs7411708; rs10918276; rs7518099. The risk haplotype GCCC is the only haplotype to contain the risk allele at every position while the protective haplotype ATAT contains the non-risk allele at every position. Chromosome 9 - SNPs included in the haplotype are rs3217992; rs1063192; rs7049105; rs1412829; rs10120688; rs4977756. These six SNPs form a block of linkage disequilibrium within the associated locus and contain the most associated SNPs under single SNP analysis. The risk haplotype TAGTAA is the only haplotype to contain the risk allele at every position while the protective haplotype CGACGG contains the non-risk allele at every position.

Locus	Haplotype	Freq Cases (n=1482)	Freq Controls (n=4892)	P-value	OR (95% CI)
Chromosome 1[1]	GCCC	0.165	0.117	**2.36×10^{-11}**	1.49 (1.32-1.67)
	ACCT	0.274	0.285	0.285	0.94 (0.86-1.03)
	ATAT	0.561	0.598	**3.7×10^{-4}**	0.85 (0.78-0.92)
Chromosome 9[2]	CGACGG	0.316	0.390	**2.37×10^{-12}**	0.72 (0.66-0.79)
	CGATGG	0.012	0.013	0.525	0.56 (0.23-1.35)
	TAGTAA	0.431	0.368	**3.88×10^{-9}**	1.28 (1.18-1.40)
	CAGTAA	0.103	0.098	0.397	1.06 (0.92-1.22)
	CAATAA	0.012	0.014	0.497	0.87 (0.59-1.28)
	CGACGA	0.026	0.030	0.352	0.88 (0.67-1.14)
	CAATGA	0.100	0.087	0.043	1.16 (1.00-0.33)

[1] Chromosome 1 locus overall likelihood ratio test for haplotype association gives p-value 6.56×10^{-12}.
[2] Chromosome 9 locus overall likelihood ratio test for haplotype association gives p-value 2.59×10^{-9}.

Supplementary Table 3: Sequence analysis of coding regions of *TMCO1* in 12 glaucoma cases (24 chromosomes) homozygous for the risk allele at top ranked SNP rs4656461. SNPs rs4656461 and rs7518099 were assessed in the genome-wide scan and are included here for comparison with exonic SNPs identified by sequencing. All SNPs identified were in the 3'UTR of the *TMCO1* gene. Three of the homozygous cases were heterozygous at the second SNP rs7518099 and one was homozygous for the wild-type allele. One novel variant was identified in the 3'UTR in the single case that was homozygous at rs4656461 and wild-type at rs7518099. Allele frequencies for each variant reported in the CEU HapMap sample are also shown. "NR"= No result

SNP ID	**rs4656461**	**rs7518099**	**rs14223**	**novel variant**	**rs7524755**	**rs6426937**	**rs16849835**	**rs1913845**	**rs1913846**	**rs6660601**
bp position on chr1	**163953829**	**164003504**	**165693863**	**165694878**	**165694897**	**165694918**	**165695203**	**165695579**	**165695726**	**165695855**
Alleles 1/2	A/G	T/C	G/T	T/A	C/T	C/G	A/C	C/T	C/T	C/T
HapMap CEU freq, allele 1	0.84	0.84	0.36	1.00	0.85	0.64	1.00	0.68	0.66	0.16
sample 1	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 2	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 3	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 4	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 5	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 6	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 7	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 8	GG	CC	GG	TT	TT	CC	AA	CC	TT	CC
sample 9	GG	CT	GT	TT	TC	CG	AA	CT	TC	CT
sample 10	GG	CT	GT	TT	TC	NR	AA	CC	TT	CT
sample 11	GG	CT	NR	TT	TC	CG	AA	CC	TC	CT
sample 12	GG	TT	GG	TA	CC	CC	AC	CC	TT	TT

Supplementary Table 4: Comparison of control cohort with WTCCC 58BC controls. A) Minor allele frequencies at genome-wide significant SNPs in each historic control cohort. B) Genome-wide significant association results for discovery cohort cases (n=551) and WTCCC 58BC controls (n=1423). Samples were removed as outliers if they differed from the mean on any of the first 10 principal components by >6SD. Results are adjusted for 10 principal components of ancestry as calculated in EIGENSTRAT following removal of SNPs in regions of extended linkage disequilibrium. In this analysis, λ=1.036, thus no further adjustment for genomic control was made.

A	SNP	Chr	Gene	Twin controls	Endometriosis controls	WTCCC 58BC controls
	rs4656461	1	*TMCO1*	0.118	0.121	0.119
	rs4977756	9	*CDKN2B-AS1*	0.397	0.404	0.421

B	SNP	Chr	Gene	Build 36 position	Allele	Freq cases	Freq WTCCC 58BC	P-value
	rs504022*	21	Gene Desert	43555566	A	0.05	0.01	8.8×10^{-11}
	rs10120688	9	*CDKN2B-AS1*	22046499	A	0.58	0.46	2.8×10^{-9}
	rs4977756	9	*CDKN2B-AS1*	22058652	G	0.31	0.42	7.3×10^{-9}
	rs7049105	9	*CDKN2B-AS1*	22018801	G	0.56	0.44	1.2×10^{-8}
	rs1063192	9	*CDKN2B*	21993367	G	0.36	0.47	1.8×10^{-8}
	rs7034696**	9	*ADAMTSL1*	18794130	A	0.39	0.30	2.2×10^{-8}
	rs4656461	1	*TMCO1*	163953829	G	0.19	0.12	4.5×10^{-8}

*rs504022 was included in the replication study. No evidence for replication was observed (p=0.20 in the advanced replication cohort, p=0.33 in the less-severe cohort). No other SNPs in this region were ranked in the top 1000 SNPs in this analysis.
**rs7034696 was not included in the replication results as the genotyping assay was unsuccessful. Further work is required to determined the contribution (if any) of this SNP to glaucoma. No other SNPs in this region were ranked in the top 1000 SNPs in this analysis.

Supplementary Table 5: Primer sequences. F=Forward primer, R=Reverse primer. A) Primers used for PCR amplification and direct sequencing of exons of the *TMCO1* gene. Exon 7 (containing the 3'UTR) was sequenced in 7 overlapping fragments designated 7-1 to 7-8. A region of 430 basepairs in exon 7 containing a complex microsatellite was not sequenced. B) Primers for RT-PCR analysis of genes located in the glaucoma associated loci in human ocular tissues. C) Primer sequences used for RT-PCR analysis of *CDKN2B-AS1* splice variants in human retina. D) Primers used for real-time RT-PCR analysis of *Tmco1*, *Cdkn2a* and *Cdkn2b* genesin retinae of rat model of glaucoma.

A	**Exon**	**Primer Sequences 5' > 3'**	**Product Size (bp)**	**Annealing Temperature**
***TMCO1* sequencing**	1	F: CCCTTCAGCTCCAGTGAGTT R: AAGGCTCGCGATCTTTCC	399	59°C
	2	F: TTTGGCTGAAGAGTCAGTTGT R: TCTAGTTGGTATTACACATTTTGCAT	400	59°C
	3	F: TCAGTAGCTATGAGAGTGGACCAG R: TCCTGTACACCTCACAAAATGG	400	59°C
	4	F: TGCTCTGCTGCATTTGAATC R: ACTTCCATTTGGTCCAGGAA	379	59°C
	5	F: CCTGGGAGACAGAGCAAGAC R: TGAAGCAAAACATTAACAACTGTG	373	59°C
	6	F: GCATGTCACCCTCTCTTTGTT R: TGAGCAACTGAAAGAACTCAG	398	59°C
	7-1	F: TTGCCTGAGAGCTACATAAAACA R: TTGCTACAAAACAGTTGCCAGT	249	59°C
	7-2	F: CCCAATCCTTACTGTGCTTTC R: CAGCTAAAAATTTGGCCTCTT	600	57°C
	7-3	F: GCAAGTTGGCTGTCTATGAGC R: CATGGCAGAAAATGAAGGCTA	575	57°C
	7-4	F: TCTTGTGGGGGCACTCTTAG R: ACCTTGTAGGGCTGTCTTGG	618	57°C
	7-5	F: ATCTGCCCAAGGTGAAGGTA R: GGGCATCATTTTCTTCATTATCA	600	57°C
	7-6	F: TGTCTGATAATGGATGGTAATGACT R: CTGGTGATGCTGTGGACCTA	590	57°C
	7-7	F: CAAGGAGTTATTAAGGGCTACTGC R: CTTTTACTTAAAGGGCAGTATTGAAA	595	57°C
	7-8	F: CATTTTTACACCTAAGAAAATGGACA R: GCTCATTATTTTGAAGACCAAGG	632	57°C

B	Gene	Primer Sequences 5' > 3'	Product Size (bp)	Annealing Temperature	Elongation time	Number of cycles
RT-PCR	*TMCO1*	F: TCAAGCTGAATTCAAGCACTATGTTCGCGGACACTCTC R: GAGGCCCAAGTAGTAAGGCTACCT	681	62°C	45 sec	32
	CDKN2A	F: TTACGGTCGGAGGCCGATCCA R: GAGGGACCTTCCGCGGCATC	327	56°C	30 sec	37
	ARF	F: AGCAGCCGCTTCCTAGAAGACCA R: AGGGACCTTCCGCGGCATCT	328	56°C	30 sec	37
	CDKN2B	F: TTTCGGGAGGCGCGCGATC R: GGTGCTCTGCAGCGTCGTGA	993	56°C	1 min	32
	CDKN2B-AS1	F: TGCCTGCCCTGTCGAGGAACA R: AAGCAGTACTGACTCGGGAAAGGA	232	58°C	30 sec	39
	MTAP	F: TTCTTGTGCCAGAGGAGTGTGCCA R: CTTCAGGTTATGGAGGGTTTCTGAC	427	56°C	30 sec	32
	GAPDH	F: ACCACCATGGAGAAGGCTGG R: CTCAGTGTAGCCCAGGATGC	527	56°C	30 sec	25

C	Exon	Primer sequence (5' to 3')	Exon	Nucleotide position	Accession number
CDKN2B-AS1 **Splice variants**	1	F:CTCGTCGAAAGTCTTCCATTCT	1	112-133	DQ485453, NR_003529.3
	19	R: GGCAAATCACTTTTCATCTTTCTGTAT	19	3258-3284	DQ485453, NR_003529.3
	12-3'	R: CCCAACAAGATAGAGAAGCAGGTA	12	2591-2614	DQ485454
	13	R: GCTCCGTAATCATCTCCAGTGT	13	736-757	GQ495924

D	Gene	Primer sequences 5' > 3'	Product size (bp)	Annealing temperature	Accession number
Real-time RT-PCR	*Gapdh*	F: TGCACCACCAACTGCTTAGC R: GGCATGGACTGTGGTCATGAG	87	63°C	NM_017008
	Tmco1	F: TGAAGGCGGAAGTGGAAAAA R: AACAAAAGCCAATCGCAAACA	177	59°C	NM_001009631
	Cdkn2a	F: CGTGCGGTATTTGCGGTATCT R: GCCAGAAGTGAAGCCAAGGA	171	59°C	NM_031550
	Cdkn2b	F: AGATCCCAACGCCGTCAAC R: CAGCACCATTAGCGTGTCCAG	184	61°C	NM_130812

Supplementary Note

Participant Recruitment:

Discovery cohort cases

Participants were drawn from the Australian & New Zealand Registry of Advanced Glaucoma (ANZRAG) and the Glaucoma Inheritance Study in Tasmania (GIST). Both studies include clinic-based recruitment of glaucoma patients. The GIST aimed to recruit all cases of glaucoma in Tasmania (an island state of Australia)[1,2], and ANZRAG aims to recruit cases of advanced glaucoma Australia-wide through ophthalmologist referral. Enrolment in the advanced OAG category of ANZRAG was defined by severe visual loss resulting from OAG. This includes best-corrected visual acuity worse than 6/60 due to OAG, or a reliable 24-2 Visual Field with a mean deviation of worse than -22db or at least 2 out of 4 central fixation squares affected with a Pattern Standard Deviation of < 0.5%. The field loss must be due to OAG, and the less severely affected eye was also required to have signs of glaucomatous disc damage. Clinical exclusion criteria for this study included: i) pseudoexfoliation or pigmentary glaucoma, ii) angle closure or mixed mechanism glaucoma, iii) secondary glaucoma due to aphakia, rubella, rubeosis or inflammation, iv) infantile glaucoma, v) glaucoma in the presence of a known associated syndrome, vi) mutation in the *MYOC* gene (by direct sequencing of exon 3). Identical criteria were applied to GIST participants to select equivalent advanced glaucoma cases. All participants provided written informed consent. Approval was obtained from the Human Research Ethics Committees (HRECs) of Southern Adelaide Health Service/Flinders University, University of Tasmania and The Royal Victorian Eye and Ear Hospital.

Discovery cohort controls

Two controls sets were used. Firstly, 1949 Australian parents (80% of the sample) and siblings of adolescent twins recruited as part of the Brisbane Adolescent Twin Study described in detail elsewhere[3,4]. Secondly, 2318 genotyped endometriosis patients were used as additional controls[5]. Endometriosis patients were recruited by QIMR between 1995 and 2002. Approval for both control arms was obtained from the Queensland Institute of Medical Research (QIMR) HREC.

These controls were not screened for glaucoma and hence will include some present (or future) glaucoma cases. Although this slightly reduces power to test for association relative to screened controls, given the low prevalence of advanced glaucoma (~ 3/1000), the reduction in power is small and can be overcome through the use of large control sets[6].

To verify that the use of a disease based control set did not alter the results, parallel analyses were conducted using the genotypes from the 1958 British Birth Cohort (n = 1,438) provided by the Sanger Institute as part of the Wellcome Trust Case-Control Consortium.

Replication cohorts

Approval for these cohorts was obtained from the HRECs of Southern Adelaide Health Service/Flinders University, University of Tasmania, Royal Victorian Eye and Ear Hospital and Western Sydney Area.

Replication 1 - Advanced OAG: The cohort consisted of a group of 334 ANZRAG and GIST participants meeting the criteria for advanced glaucoma but not included in the discovery cohort. These cases were selected using identical entry criteria to the Discovery cohort but were recruited after the discovery phase genotyping was performed. Controls consisted of 434 examined normal participants ascertained from retirement home facilities in South Australia and Tasmania.

Replication 2 - Less Severe OAG: The less severe cohort comprised individuals with a definite diagnosis of OAG, but with less severe visual field loss than that required to meet entry criteria for the advanced category of ANZRAG. These 465 Australian participants were drawn from GIST and the South Australian population (through the eye clinic at Flinders Medical Centre, Adelaide, Australia). OAG was defined by concordant findings of typical glaucomatous visual field defects on

the Humphrey 24-2 test, with corresponding optic disc rim thinning, including an enlarged cup-disc ratio (≥ 0.7), or cup-disc ratio asymmetry (≥0.2) between the two eyes. Clinical exclusion criteria were as for the discovery cohort. Controls were drawn from the publically available Wellcome Trust Case Control Consortium 1958 Birth Cohort.

Replication 3 - Blue Mountains Eye Study: The Blue Mountains Eye Study is a population based study of individuals aged over 50 years living in the Blue Mountains, near Sydney, Australia [7]. OAG was defined as for the Replication 2 cohort except the Humphrey 30-2 test was used. All participants were examined for OAG. Within the cohort 93 participants were diagnosed with glaucoma and the remaining 2761 samples were designated as controls.

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