Genetic Risk for Primary Open-Angle Glaucoma Determined by *LMX1B* Haplotypes

Soo Park,[1] *Yalda Jamshidi,*[2] *Daniela Vaideanu,*[3] *Maria Bitner-Glindzicz,*[4] *Scott Fraser,*[3] *and Jane C. Sowden*[1]

PURPOSE. Primary open-angle glaucoma (POAG) is a common disease requiring early diagnosis and treatment to avoid asymptomatic visual field loss and eventual blindness. *LMX1B* mutations cause dominantly-inherited Nail-Patella syndrome in which approximately 33% of patients develop glaucoma. This study investigated the wider role of *LMX1B* in POAG.

METHODS. The contribution of variation at the *LMXIB* locus to risk of glaucoma was investigated in a case-control genetic association study in 272 patients with high-tension glaucoma (HTG), 37 patients with normal-tension glaucoma (NTG), 58 patients with ocular hypertension (OHT), and 276 controls.

RESULTS. Significant SNP associations were found for each patient group: rs7859156 was associated with HTG ($P = 0.0015$; odds ratio [OR], 0.64) and OHT ($P = 0.0482$; OR, 0.59); rs7854658 was associated with NTG ($P = 0.0041$; OR, 0.30). A protective ATG haplotype (including rs7859156) was less prevalent in patients with raised intraocular pressure (22.7% in combined HTG+OHT group vs. 31.7% in controls; $P = 0.0005$), and in patients with glaucoma (22.9% in combined HTG+NTG group vs. 31.7% in controls; $P = 0.0008$). ATG carriers in these combined groups had a decreased risk of developing glaucoma (OR, 0.72 and OR, 0.73, respectively). A GCAGAC haplotype (including rs7854658) was also less prevalent in glaucoma patients (16.5% vs. 24.7%; $P = 0.0005$) and carriers had a decreased risk of developing glaucoma (OR, 0.70).

CONCLUSIONS. *LMX1B* haplotypes influence susceptibility to glaucoma in the general population, suggesting altered *LMX1B* function predisposes to glaucomatous damage and that this role may be independent of raised intraocular pressure. (*Invest Ophthalmol Vis Sci.* 2009;50:1522–1530) DOI:10.1167/iovs.08-2483

Glaucoma is the second leading cause of blindness worldwide, affecting approximately 67 million people.[1] Primary open-angle glaucoma (POAG) is the most common form, with an estimated prevalence of 1.2% for the age group 40 to 89 years in the white UK population.[2] High-tension glaucoma (HTG) with raised intraocular pressure (IOP > 21 mm Hg) is the most common form of POAG, whereas the less common normal-tension glaucoma (NTG) shows IOP within the normal range. POAG is characterized by progressive retinal ganglion cell death, resulting in visual field loss and a typical excavation of the optic nerve head. Substantial disease progression frequently occurs before diagnosis. However, sight can often be preserved if early diagnosis is made.[3]

Raised IOP is recognized as one of the strongest known risk factors for glaucoma.[4–7] Individuals with ocular hypertension (OHT) have raised IOP without clinical signs of a glaucomatous optic neuropathy; however, 10% will convert to POAG over a 10-year period.[8] Other risk factors include age,[9,10] black ethnicity,[11] reduced central corneal thickness (CCT),[12] enlarged cup/disc ratio (CDR),[13–15] and family history of the disease.[16]

Population-based studies showed that the prevalence of glaucoma in siblings of patients was 10.4%, with the lifetime risk of glaucoma being 9.2 times higher in siblings and offspring of glaucoma patients compared to siblings and offspring of controls.[16] Moreover, twin studies found a higher degree of concordance among monozygotic twins.[17,18] Genetic linkage studies in rare pedigrees showing Mendelian patterns of adult-onset POAG inheritance have identified 14 genetic loci (GLC1A-N).[19–29] Notably however, few of these genes have been robustly associated with POAG in the general population. Among the three identified genes *MYOC* (GLC1A), *OPTN* (GLC1E), and *WDR36* (GLC1G), only *MYOC* is established as directly causative, while due to conflicting results, the exact roles of *OPTN*[30,31] and *WDR36*[32] in POAG remain uncertain. In addition, mutations in *MYOC* account for only a small—approximately 5%—of POAG.[33–37] The emerging view is that POAG is a complex, multifactorial disease resulting from interactions between several genetic and environmental factors.[38] While linkage studies have been the predominant method used to identify POAG loci, with advances in genome technology and bioinformatics it has become feasible to undertake studies to unravel the complex genetics of glaucoma. Association studies using single nucleotide polymorphisms (SNPs) offer a proficient method for assessing candidate genes with small effects[39] and are increasingly being applied to elucidate genetic mechanisms in complex disease.[40] Even small cohorts have proved to be remarkably powerful.[41]

Genes that cause developmental glaucoma,[42] with the exception of the *CYP1B1* gene, have yet to be assessed as genetic susceptibility factors for POAG. *CYP1B1* causes primary congenital glaucoma. It is also involved in cases of juvenile open-angle glaucoma (JOAG)[43,44] and a recent study identified a *CYP1B1* polymorphism as a susceptibility factor for POAG.[45,46]

Developmental glaucoma refers to glaucomas that are associated with developmental malformations of the anterior segment of the eye.[47] Anterior segment dysgenesis (ASD) may lead to an incomplete development, or dysfunction, of the structures that form the aqueous drainage pathway, and can result in IOP elevation secondary to aqueous outflow obstruction, predisposing to glaucoma.[42] Between 30% and 75% of ASD patients develop glaucoma, and a number of gene mutations have been identified underlying these conditions.[48–50] We hypothesize that subclinical mutations/polymorphisms in genes that are important for anterior segment development may produce subtle and undetected abnormalities in anterior segment structure and/or function which predispose to glaucoma. Therefore, the presence of specific alleles of these genes may be a significant susceptibility factor for the development of POAG.

The murine *Lmx1b* gene is expressed in the developing anterior segment, in the trabecular meshwork and ciliary body, which are the sites of aqueous drainage and production, respectively.[51] *Lmx1b* encodes a LIM homeobox transcription factor that regulates programs of gene expression essential for normal morphogenesis and cell differentiation of the anterior segment.[51] Targeted homozygous mutation resulted in ASD phenotypes, including iris and ciliary body hypoplasia, and corneal stromal defects.[51] Significantly, mutations in the human homologue *LMX1B* on chromosome 9q34.1 cause a rare autosomal dominant condition, Nail-Patella Syndrome (NPS; Mendelian Inheritance in Man [MIM] 161200) in which approximately 33% of patients over aged 40 years develop open-angle glaucoma.[52] As well as causing dysplasia of the nails and absent or hypoplastic patellae, *LMX1B* mutations cause a spectrum of ocular phenotypes that vary in severity, including rare anterior segment anomalies of iris processes, ptosis, hypertelorism, epicanthal folds, bilateral sclerocornea, microcornea, keratoconus, congenital cataracts, and microphakia.[53–55] However, developmental abnormalities of the anterior segment are not clinically detectable in patients carrying *LMX1B* mutation in the majority of cases,[52,56] a feature which is more consistent with POAG.

In this study, we investigate whether variant alleles of *LMX1B* play a role in POAG in the general population. We perform a case-control genetic association study to compare the prevalence of *LMX1B* SNPs in four groups—HTG, NTG, OHT, and a normal control group. *LMX1B* haplotypes were identified and their prevalence assessed in patients with glaucomatous optic neuropathy (HTG and NTG patients), and in patients with raised IOP (HTG and OHT patients). *LMX1B* has not been analyzed in any association studies to date, and here we identify protective *LMX1B* haplotype associations for both glaucoma and raised IOP.

METHODS

Recruitment of Patients

All the participating subjects were recruited from glaucoma outpatient clinics at the Sunderland Eye Infirmary (Sunderland, UK), a secondary ophthalmology referral center. The subjects were treated in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants after the nature and possible consequences of the study were explained. The study had Local Research Ethics Committee approval. A cohort of HTG cases ($n = 272$), and unrelated controls ($n = 276$) matched for ethnicity, age, and sex were recruited to the study. Cases with NTG ($n = 37$) and OHT ($n = 58$) were also collected. All cases ($n = 367$) and controls were of white British descent.

Control participants, either accompanying spouses or friends of individuals with glaucoma, were recruited randomly. All controls underwent a complete ophthalmic examination to exclude individuals with glaucoma from the control group, and were confirmed to have no visual complaints and IOP of less than 22 mm Hg with a normal disc appearance. Individuals with a family history of glaucoma were excluded.

All case subjects underwent a complete ophthalmic examination, including best visual acuity and visual field testing (Humphrey SITA standard 24-2 perimetry; Carl Zeiss Meditec AG, Jena, Germany), slit-lamp examination of the anterior segment (including gonioscopy), measurement of IOP by Goldmann applanation tonometer, posterior segment examination of the retina and optic disc after pupil dilation, and measurement of the CDR. Adult individuals with a diagnosis of HTG, NTG, or OHT older than aged 40 years were enrolled based on the following clinical criteria:

Presence of glaucomatous optic neuropathy (defined by loss of neuroretinal rim) with compatible and reproducible visual field loss for HTG and NTG, and absence of detectable glaucomatous damage or field loss for OHT;

Open drainage angles on gonioscopy;

IOP consistently 22 mmHg or greater on diurnal testing for HTG and OHT, and 21 mm Hg or less for NTG;

Absence of a secondary cause for glaucomatous optic neuropathy;

Absence of nonglaucomatous field losses and disc changes (i.e., high myopia).

Selection and Analysis of Single Nucleotide Polymorphisms

We used a tagging single nucleotide polymorphism (tSNP) approach to screen the *LMX1B* gene including 10 kb of upstream and downstream flanking region in patient and control groups, using tSNPs selected from the HapMap database (HapMap Data Release #22/Phase II Apr 2007; http://www.hapmap.org). Genotyping was performed by high-throughput SNP genotyping (Genomics Laboratory, Wellcome Trust Centre for Human Genetics, Cambridge, UK; MassARRAY iPLEX Assay; Sequenom Inc, San Diego, CA). (Details of primer sequences and SNP genotyping provided on request.) Allele frequencies for each SNP were tested for agreement with Hardy-Weinberg expectations ($P > 0.05$) using a χ^2 goodness-of-fit test.

LD and Haplotype Structure of the *LMX1B* Genomic Region

Haplotypes were inferred using haplotype analysis software (Haploview v.4.0; www.broad.mit.edu /mpg/haploview), and associations between tSNP or haplotype and glaucoma were investigated. The method of Gabriel et al., as implemented in Haploview,[57] was used to construct LD blocks from tSNPs with minor allele frequencies (MAF) of 5% or more. LD between tSNPs was measured by the pairwise D' statistic and the LD structure was examined using the 80% confidence bounds of D' to define sites of historical recombination between tSNPs.

Haplotypes were constructed from genotype data in the full-size case-control panel within blocks by using an accelerated expectation-maximization algorithm method.[58] In each haplotype block, common haplotypes with frequencies of 1% or more were inferred that accounted for >98% of the chromosomes. Differences in genotype and haplotype frequencies between cases and controls were determined using a χ^2 distribution with 2° of freedom. Permutation testing was performed to calculate corrected P values for multiple testing with 1000 simulations.

SNP and haplotype effects were investigated with and without adjustment for the covariates of age and sex using analysis software (THESIAS v.3.1; www.genecanvas.org). Thesias is based on the maximum likelihood model described in Tregouet et al.[59] Odds ratios (ORs) were calculated (THESIAS v.3.1 with 95% confidence intervals (CIs) for each genotype, with the respective wild-type as the reference), and

TABLE 1. Comparison of Age, Sex, Mean CDR, and Mean IOP between HTG, OHT, NTG, HTG+OHT Group (with Raised IOP), HTG+NTG Group (with Glaucomatous Optic Neuropathy) and Control Group

	NTG	OHT	HTG	Controls	Cases Combined (OHT+HTG)	P*	Cases Combined (NTG+HTG)	P†
n	37	58	272	276	330		309	
Age	77.51	65.19‡	71.17	70.76	70.12	0.437	71.93	0.158
(SD)	(8.849)	(11.494)	(10.448)	(9.313)	(10.863)		(10.462)	
% Male	27.0‡	41.4	54.4‡	42.8	52.1	0.022	51.1	0.047
Mean CDR	0.7419‡	0.3629	0.7175‡	0.2120	0.6552	<0.0001	0.7204	<0.0001
(SD)	(0.138)	(0.138)	(0.182)	(0.231)	(0.221)		(0.177)	
Mean IOP	17.64	27.16‡	29.13‡	15.45	28.79	<0.0001	27.75	<0.0001
(SD)	(2.090)	(4.021)	(5.506)	(2.352)	(5.326)		(6.415)	

P values were calculated using independent samples t-test, except a χ^2 test was used for sex, using analysis software (SPSS version 15; SPSS Inc., Chicago, IL).

* P and † P indicate significant difference between controls and the HTG + OHT group or the HTG + NTG group, respectively.

‡ Significant difference $(P > 0.05)$ between controls and the separate case groups (HTG, NTG, OHT).

covariate-adjusted haplotype-phenotype by comparison to the most frequent haplotype.

RESULTS

Among the cases, 272 (74.3%) were classified as HTG, 37 (10.1%) as NTG, and 58 (15.6%) as OHT (Table 1). The mean IOP was significantly higher in the HTG and OHT groups compared with the control group, whereas CDR was significantly higher in the HTG and NTG groups compared to controls (Table 1).

A total of 23 tagging SNPs (with MAF > 0.10 at a minimum r^2 of 0.8) spanning a region of 102,314 bp, including the 82,314 bp *LMX1B* gene, were selected from the HapMap database and genotyped in all individuals (Fig. 1). The genotype distributions of these 23 SNPs in the controls did not differ from those expected from Hardy-Weinberg equilibrium. The pairwise LD matrix revealed six main haplotype blocks (Fig. 2).

Associations between Individual SNPs and Glaucoma

The allele frequencies of the 23 tSNPs among the three separate case groups (HTG, NTG, OHT) and controls were assessed; tSNPs showing significant effects are shown in Table 2.

In the HTG group, four tSNPs (all situated in intron 2) had allele frequencies that differed significantly between patients and controls. Protective allele frequencies of rs7859156, rs6478750, and rs7854658 were higher in controls than in HTG patients ($P = 0.0015$, 0.0258, and 0.0057, respectively), with ORs of 0.64, 0.72, and 0.65, respectively, after adjustment for sex. By contrast, the risk allele frequency of rs10987385 was higher in the HTG patients than in controls ($P = 0.0180$), with an OR of 1.43 after adjustment for sex.

In the OHT group, two of these tSNPs (both situated in intron 2) had allele frequencies that differed significantly between patients and controls. Protective allele frequencies of rs7859156 and rs6478750 were higher in controls than in OHT patients ($P = 0.0482$ and $P = 0.0309$, respectively), both with ORs of 0.59 after adjustment for age.

In the NTG group, five tSNPs had allele frequencies that differed significantly between NTG patients and controls, but only one of these alleles (rs7854658) was in common with the significant HTG tSNPs. The protective allele frequency of rs7854658 was higher in the controls than in NTG patients ($P = 0.0041$) with an OR of 0.30. In addition, risk allele frequencies of rs944103, rs16929236, rs10733682, and rs867559 were higher in NTG patients than in controls ($P = 0.0488$, 0.0064, 0.0489, and 0.0295, respectively), with ORs of 1.71, 2.30, 1.75, or 1.82, respectively, after adjustment for sex. rs7854658 and rs944103 are situated in intron 2, whereas rs16929236, rs10733682, and rs867559 are situated in the 3' region of *LMX1B*. After permutation testing, all P values for
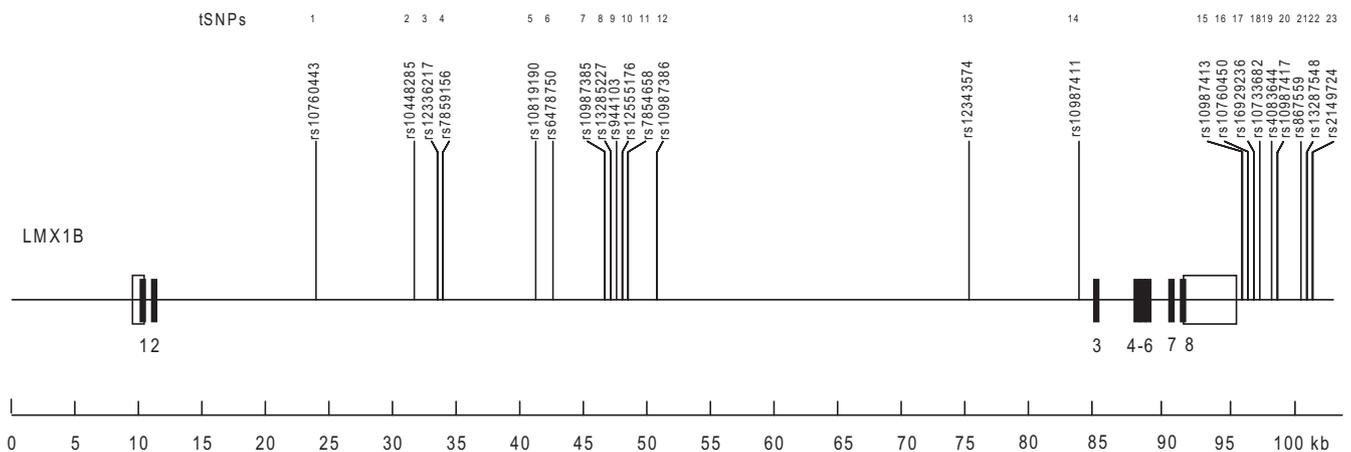

FIGURE 1. *LMX1B* gene structure. Eight coding exons are indicated as *solid boxes* and numbered accordingly. Untranslated exons are shown as *open boxes*. The relative position of the 23 tSNPs (labeled above with the respective haplotype) are also shown.
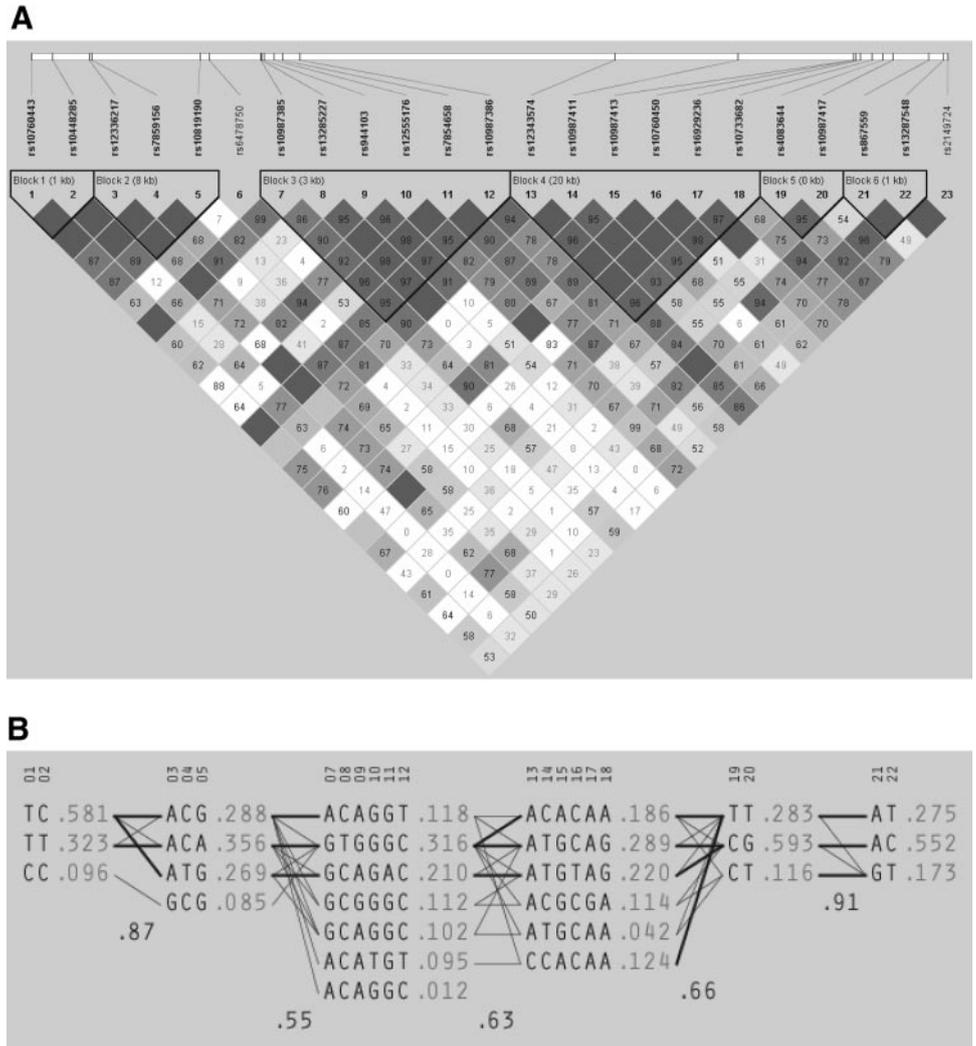
FIGURE 2. (A) Diagram of block structure of *LMX1B* generated using haplotype analysis software (Haploview v.4.0; www.broad.mit.edu /mpg/ haploview). LD plots were identified by strong LD. Shades of gray indicate the computed pairwise D' value; darker end of the gray scale indicates a higher D' value. (B) The selected tSNPs and estimated haplotype frequencies in the six major haplotype blocks (1 to 6) are shown. Marker numbers and arrows above the haplotypes indicate tSNPs. The frequency of each haplotype within a block is given to the right of the haplotype. The thickness of the lines connecting the haplotypes across blocks represents the relative frequency (i.e., high [*thick*] vs. low [*thin*]) with which a given haplotype is associated with the haplotype in the neighboring block.

TABLE 2. Distribution of tSNPs between HTG, OHT, NTG, HTG+OHT, and HTG+NTG, Compared to the Wild-Type Control Group

Phenotype	tSNP	SNP ID	Allele	Case Counts (%)	Control Counts (%)	*P*	Permutation *P*	OR (95% CI)
HTG	**4**	**rs7859156**	**T**	**121 (23.1)**	**171 (31.8)**	**0.0015**	**0.0376**	**0.64 (0.49–0.85)**
	6	rs6478750	C	193 (36.3)	236 (42.9)	0.0258	NS	0.72 (0.55–0.93)
	7	rs10987385	A	147 (27.7)	118 (21.5)	0.0180	NS	1.43 (1.07–1.91)
	11	rs7854658	A	95 (18.0)	137 (24.9)	0.0057	NS	0.65 (0.49–0.89)
OHT	4	rs7859156	T	24 (22.2)	171 (31.8)	0.0482	NS	0.59 (0.34–1.01)
	6	rs6478750	C	35 (31.8)	236 (42.9)	0.0309	NS	0.59 (0.35–0.97)
NTG	9	rs944103	G	41 (55.4)	238 (43.3)	0.0488	NS	1.71 (1.06–2.77)
	11	rs7854658	A	7 (9.7)	137 (24.9)	0.0041	NS	0.30 (0.14–0.64)
	17	rs16929236	G	17 (23.0)	64 (11.6)	0.0064	NS	2.30 (1.30–4.05)
	18	rs10733682	A	44 (59.5)	260 (47.3)	0.0489	NS	1.75 (1.05–2.90)
	21	rs867559	G	22 (29.7)	104 (18.9)	0.0295	NS	1.82 (1.09–3.05)
HTG + OHT	**4**	**rs7859156**	**T**	**145 (22.9)**	**171 (31.8)**	**7.0E-4**	**0.0120**	**0.63 (0.48–0.82)**
	6	rs6478750	C	228 (35.5)	236 (42.9)	0.0090	NS	0.69 (0.54–0.89)
	7	rs10987385	A	175 (27.3)	118 (21.5)	0.0205	NS	1.39 (1.05–1.84)
	11	rs7854658	A	116 (18.2)	137 (24.9)	0.0047	NS	0.66 (0.50–0.88)
HTG + NTG	**4**	**rs7859156**	**T**	**138 (23.2)**	**171 (31.8)**	**0.0013**	**0.0360**	**0.64 (0.49–0.84)**
	6	rs6478750	C	220 (36.3)	236 (42.9)	0.0217	NS	0.73 (0.57–0.94)
	7	rs10987385	A	162 (26.8)	118 (21.5)	0.0366	NS	1.35 (1.02–1.79)
	11	**rs7854658**	**A**	**102 (17.0)**	**137 (24.9)**	**0.0010**	**0.0270**	**0.62 (0.46–0.83)**

tSNPs significantly distributed after 1000 permutation tests are highlighted in bold.

TABLE 3. Distribution of Haplotypes between HTG+OHT Cases and Controls

	Haplotype Frequency	All Subjects (%)	Case (%)	Control (%)	*P*	Permutation *P*	OR (95% CI) *P* Value after Adjustment for Sex
Block 2	ACA	35.6	35.8	35.2	0.8269	1.0000	
(tSNP 3 to 5)	ACG	28.8	31.7	25.4	0.0156	0.3020	1.33 (0.99–1.78) *P* = 0.0591
	ATG	**26.9**	**22.7**	**31.7**	**5.0E-4**	**0.0100**	**0.72 (0.53–0.97) *P* = 0.0330**
	GCG	8.5	9.4	7.4	0.2110	0.9987	
Block 3	GTGGGC	31.6	31.4	31.8	0.8865	1.0000	
(tSNP 7 to 12)	GCAGAC	21.0	17.8	24.7	0.0035	0.0710	0.75 (0.54–1.03) *P* = 0.0770
	ACAGGT	11.8	14.0	9.4	0.0143	0.2770	1.61 (.08–2.41) *P* = 0.0190
	GCGGGC	11.2	12.4	9.8	0.1574	0.9875	
	GCAGGC	10.2	9.8	10.6	0.6668	0.9875	
	ACATGT	9.5	9.8	9.2	0.7580	1.0000	
	ACAGGC	1.2	1.2	1.3	0.7729	1.0000	

Haplotypes significantly distributed after 1000 permutation tests are highlighted in bold.
P values after adjustment for sex are provided only for haplotypes that are significantly distributed.

these differences between patients in each group and controls increased beyond the significant level of 0.05, except for rs7859156, which had a permutated *P* value of 0.0376 after 1000 tests in the HTG group.

Next, we tested the allele frequencies of the 23 tSNPs in the combined groups of all patients with raised IOP (HTG and OHT), or all patients with glaucomatous optic neuropathy—that is, all the POAG patients (HTG and NTG), compared with the control group. In both groups, HTG+OHT and HTG+NTG, the same four tSNPs had an allele frequency that differed significantly between patients and controls (Table 2). Protective allele frequencies of rs7859156, rs6478750, and rs7854658 were higher in controls than cases, and risk allele frequencies of rs10987385 were higher in patients than in controls. In both combined groups, tSNP rs7859156, remained significantly different after 1000 permutation tests (*P* < 0.05). In addition, in the combined POAG case group (HTG+NTG), a second tSNP rs7854658 remained significantly different after permutation testing.

Associations between Haplotypes and Raised IOP (OHT+HTG)

We examined the difference in frequency distribution of all common haplotypes between the combined raised IOP patient group (HTG+OHT) and the controls (Table 3) and found a significant haplotype effect of block 2 and block 3. These effects replicated those found in the separate HTG group (Table 4). Within block 2, two haplotypes (ATG and ACG defined by tSNPs rs12336217, rs7859156, and rs10819190) were differentially distributed in patients and controls (Table 3). Of these, haplotype ATG was most significant, and was less prevalent among patients compared with controls (22.7% vs. 31.7%; *P* = 0.0005) and remained significant after permutation testing.

Haplotype ATG carriers were at a decreased risk of developing raised IOP compared with noncarriers (OR, 0.72) and this remained significant after adjustment for sex (*P* = 0.0330). The LD observed between SNPs and different functional effects of the haplotypes are potential sources of bias when studying multiple SNPs. Therefore, haplotype background analysis was performed in Thesias and although rs12336217, rs7859156, and rs10819190 are in LD, the rs7859156 polymorphism exerted an independent decreased risk to glaucoma (OR, 0.54; 95% CI, 0.39–0.75; *P* = 0.0003).

Within block 3, we observed two haplotypes (GCAGAC, ACAGGT; defined by rs10987385, rs13285227, rs944103, rs12555176, rs7854658, and 10987386) that were differentially distributed in patients and controls, although these associations did not withstand permutation testing (Tables 3, 4).

These data suggest that *LMX1B* haplotypes, or perhaps an unknown nearby functional variant in LD, affect IOP and consequently predispose to glaucoma.

TABLE 4. Distribution of Haplotypes between HTG Cases and Controls

	Haplotype	All Subjects (%)	Case (%)	Control (%)	*P*	Permutation *P*	OR (95% CI) *P* Value after Adjustment for Sex
Block 2	ACA	35.0	34.8	35.2	0.8804	1.0000	
(tSNP 3 to 5)	ACG	28.7	32.2	25.4	0.0129	0.2720	1.39 (1.02–1.90) *P* = 0.0377
	ATG	**27.4**	**22.8**	**31.7**	**0.0010**	**0.0300**	**0.75 (0.54–1.03) *P* = 0.0750**
	GCG	8.6	9.9	7.4	0.1515	0.9770	
Block 3	GTGGGC	31.8	31.8	31.8	0.9653	1.0000	
(tSNP 7 to 12)	GCAGAC	21.1	17.5	24.7	0.0043	0.1040	0.73 (0.52–1.02) *P* = 0.0648
	ACAGGT	11.7	14.2	9.4	0.0128	0.2720	1.64 (1.09–2.48) *P* = 0.0189
	GCGGGC	11.0	12.3	9.8	0.1818	0.9890	
	GCAGGC	10.1	9.5	10.6	0.4851	1.0000	
	ACATGT	9.2	9.2	9.2	0.9892	1.0000	
	ACAGGC	1.4	1.4	1.3	0.9327	1.0000	

Haplotypes significantly distributed after 1000 permutation tests are highlighted in bold.
P values after adjustment for sex are provided only for haplotypes that are significantly distributed.

TABLE 5. Distribution of Haplotypes between HTG+NTG Cases and Controls

	Haplotype	All Subjects (%)	Case (%)	Control (%)	P	Permutation P	OR (95% CI) P Value after Adjustment for Sex
Block 2	ACA	35.3	35.3	35.2	0.9718	1.0000	
(tSNP 3 to 5)	ACG	28.9	32.2	25.4	0.0110	0.2350	1.36 (1.01-1.83) P = 0.0437
	ATG	**27.1**	**22.9**	**31.7**	**8.0E-4**	**0.0220**	**0.73 (0.54–0.99) P = 0.0450**
	GCG	8.4	9.3	7.4	0.2452	1.0000	
Block 3	GTGGGC	31.6	31.4	31.9	0.8768	1.0000	
(tSNP 7 to 12)	**GCAGAC**	**20.4**	**16.5**	**24.7**	**5.0E-4**	**0.0150**	**0.70 (0.51–0.98) P = 0.0360**
	GCGGGC	11.9	13.9	9.8	0.0350	0.5760	1.51 (1.003-2.28) P = 0.0480
	ACAGGT	11.7	13.8	9.4	0.0202	0.3980	1.6 (1.09-2.46) P = 0.0170
	GCAGGC	10.5	10.3	10.8	0.7689	1.0000	
	ACATGT	9.1	9.1	9.3	0.9033	1.0000	
	ACAGGC	1.3	1.2	1.3	0.8697	1.0000	

Haplotypes significantly distributed after 1000 permutation tests are highlighted in bold.
P values after adjustment for sex are provided only for haplotypes that are significantly distributed.

Associations between Haplotypes and Glaucoma (HTG+NTG)

Examination of the difference in frequency distribution of all common haplotypes between the combined POAG patient group (HTG+NTG) and the controls (Table 5) found a significant effect of the same block 2 and block 3 haplotypes on glaucoma. Within block 2, the ACG and ATG haplotypes were differentially distributed in patients and controls, with haplotype ATG showing the strongest effect. ATG was significantly less prevalent among patients compared with controls (22.9% vs. 31.7%; *P* = 0.0008) and remained significant after permutation testing. ATG carriers were at a decreased risk of developing glaucoma compared with noncarriers (OR, 0.73), and this remained significant after adjustment for sex (*P* = 0.0450). Haplotype background analysis was performed to determine whether any of the tSNPs within the haplotype block 2 had an independent effect on glaucoma risk. Although rs12336217, rs7859156, and rs10819190 are in LD, rs7859156 polymorphism exerted an independent increased risk (OR, 0.54; 95% CI, 0.39–0.75; *P* = 0.0002).

Within block 3, we observed three haplotypes (GCAGAC, GCGGGC, and ACAGGT, defined by rs10987385, rs13285227, rs944103, rs12555176, rs7854658, and 10987386) that were differentially distributed in patients and controls; one of these, GCAGAC, was significant after permutation testing. Haplotype GCAGAC was significantly less prevalent among patients compared with controls (16.5% vs. 24.7%; *P* = 0.0005, and turned into *P* = 0.0150 after 1000 permutation tests). GCAGAC carriers were at a decreased risk of developing glaucoma compared with noncarriers (OR, 0.70; *P* = 0.0360) after adjustment for sex in multivariate logistic regression analysis.

Crucially, the relation between haplotypes in block 2 and 3 and glaucoma risk did not persist after adjusting for CDR in Thesias. CDR is significantly increased in both NTG and HTG compared with controls (Table 1). Thus, the influence of these *LMX1B* haplotypes, or a nearby functional variant in LD, on glaucoma risk may be partially explained by their relation with CDR.

DISCUSSION

Approximately 1% to 2% of individuals 40 years of age or older in the general population develop POAG, compared with 33% of patients with the rare condition NPS (MIM 161200), which is caused by dominant mutations in the *LMX1B* gene. In this study, we investigated *LMX1B* as a candidate genetic risk factor for the most common form of glaucoma and identified significant associations between *LMX1B* alleles and haplotypes and POAG.

In the large HTG cohort, we found that the frequency of the protective tSNP rs7859156 in intron 2 was significantly reduced compared with the controls, and this effect withstood stringent permutation testing. Interestingly, a number of weak associations (with tSNPs rs6478750, rs10987385, and rs7854658) in the HTG group were replicated in the small separate OHT and NTG groups.

The HTG group is characterized by glaucomatous damage and raised IOP. The other two patient groups each have only one of these characteristics, either glaucomatous damage without raised IOP (NTG), or raised IOP without glaucomatous damage (OHT). To test the relationship between the variant alleles of *LMX1B* and these two characteristics, we grouped the patients into those with raised IOP (HTG and OHT), or those with glaucomatous damage irrespective of IOP (HTG and NTG). Notably, in both of the combined groups the significant association with the protective tSNP rs7859156 in intron 2 was replicated and enhanced. Moreover, tSNP rs7854658, identified in the separate NTG and HTG groups, was highly significant and withstood permutation testing in the combined NTG+HTG group. This was an interesting result and was confirmed by haplotype analysis, suggesting that the effect of *LMX1B* on glaucoma susceptibility may involve functions that are broader than effects on IOP. As the HTG cases are highly represented in the combined groups, in future studies it will be important to establish whether a larger group of NTG cases show a strong association. Recent studies have shown that 26.7% of individuals with NPS older than aged 50 years develop NTG,[54] whereas 11.9% of individuals older than aged 40 years develop OHT.[56] These data are also consistent with the proposal that the role of *LMX1B* in glaucoma pathology may be complex.

Several studies have shown that increased CCT may occur in certain types of dominantly inherited developmental glaucoma, including iris hypoplasia (*FOXC1* duplication),[60] aniridia (*PAX6/Pax6*),[61,62] and dysgenic lens (*Foxe3*).[60] Conversely, a reduced corneal thickness was associated with Axenfeld-Rieger malformation of the anterior segment and *PITX2* mutation.[63] A thinner CCT was also found to be a risk factor for developing POAG in individuals with OHT.[64] CCT data was not collected for the cases and controls in the present study. However, analysis of the relationship between *LMX1B* haplotypes and

CCT will be important in future work, as like these other developmental glaucoma genes, LMX1B may affect CCT.

CCT variation is known to affect the accuracy of IOP measurement on applanation tonometry, with thick corneas giving falsely high IOP readings and thin corneas giving falsely low readings.[65,66] In the present study, IOP measurements were checked by a Tono-Pen which is less affected by CCT,[67] in addition to performing applanation tonometry. Although normagrams based on varying CCT exist for adjusting IOP readings,[65,68,69] there is as yet no generally accepted correction formula.[70] However, two recent studies that adjusted IOP for CCT found that the correction did not alter the diagnosis of HTG or NTG,[71] and did not affect the relationship between the prevalence of POAG and IOP, respectively.[72]

We identified six main haplotype blocks across the LMX1B gene. Haplotypes within two of these blocks containing the significantly varying tSNPs were found to show significantly altered distributions in the two combined patient groups, POAG (HTG+NTG) and raised IOP with, or without HTG (HTG+OHT). The block 2 ATG haplotype is associated with both groups and with HTG alone, whereas the block 6 GCA-GAC haplotype shows the strongest association with the HTG+NTG group. The block 2 haplotype (spanning a region of ~6700 bp) and the block 3 haplotype (spanning a region of ~28,000 bp) are both located within the large intron 2 of LMX1B. This region shows stretches of greater than 85% conservation between the sequences of humans and mouse, and a functional role (e.g., gene expression regulatory regions) has been suggested.[73] Significantly, the block 2 haplotype is in complete LD with the promoter and 5′ upstream regions.[73] Therefore, despite being an intronic sequence, this haplotype may tag important conserved regions involved in gene regulation.

Our proposal that levels of LMX1B expression are relevant to risk of elevated IOP and glaucoma is in line with the well-documented sensitivity of ocular development to gene dosage. For example, variations in the copy number of FOXC1[74] and PAX6,[75] which are presumed to alter levels of gene expression, or mutations in PITX2 that increase or decrease levels of transcription factor function,[76] cause anterior segment anomalies and glaucoma. These developmental glaucoma genes are obvious candidates for further association studies for POAG, and it is possible that the combined inheritance of multiple loci may give a greater association with HTG and/or OHT, and may serve as important prognostic indicators in the general population.

Despite the progress in identifying genes that are involved in POAG, understanding of the underlying pathogenic mechanism is still limited. This is partly because if each causal gene only makes a small contribution to the overall phenotype, then unraveling the role each plays in the biochemical and cellular pathways involved in IOP control and retinal ganglion cell function is complex. New insight into a possible Lmx1b pathway relevant to glaucoma pathology was provided by a report showing mutations in the collagen Col4a1 gene resulted in ASD with optic nerve hypoplasia, and raised as well as low IOP.[77] Lmx1b is known to directly regulate expression of Col4a3 and Col4a4 in the kidney.[78,79] Lmx1b$^{-/-}$ mice showed defective collagen fibrillogenesis in the eye as well as ciliary body hypoplasia,[51] though there is not yet evidence that Lmx1b regulates Col4a1. Considered together, our data and other studies suggest that altered LMX1B activity levels cause abnormalities and dysfunction in the IOP-regulating anterior segment structures that are age-related and are clinically undetectable, resulting in POAG. While JOAG can be a consequence of LMX1B mutation in NPS,[52,56] the mean age at which open-angle glaucoma or OHT has been detected in NPS is older than 40 years, at 63.4 (range, 55-72)[54] and 47.9 years (range, 23 to 78)[56] respectively, a feature consistent with POAG.

This is the first study to show an association between variants of LMX1B and POAG. These data indicate altered LMX1B function may be associated with the common forms of POAG in the general population, and provides a platform for further investigation of LMX1B as a genetic risk factor for adult-onset glaucoma.

References

1. Quigley HA. Number of people with glaucoma worldwide. *Br J Ophthalmol.* 1996;80:389–393.
2. Tuck MW, Crick RP. The age distribution of primary open angle glaucoma. *Ophthalmic Epidemiol.* 1998;5:173–183.
3. Heijl A, Leske MC, Bengtsson B, Hyman L, Bengtsson B, Hussein M. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. *Arch Ophthalmol.* 2002;120:1268–1279.
4. Wilson R, Martone J. Epidemiology of chronic open angle glaucoma. In: Ritch R, Shields M, Krupin T, eds. *The Glaucomas.* 2nd ed. St. Louis: Mosby; 1996:725–753.
5. Le A, Mukesh BN, McCarty CA, Taylor HR. Risk factors associated with the incidence of open-angle glaucoma: the visual impairment project. *Invest Ophthalmol Vis Sci.* 2003;44:3783–3789.
6. Leske MC, Wu SY, Nemesure B, Hennis A. Incident open-angle glaucoma and blood pressure. *Arch Ophthalmol.* 2002;120:954–959.
7. The AGIS Investigators. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol.* 2000;130:429–440.
8. Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol.* 2002;120:701–713.
9. Gillespie BW, Musch DC, Guire KE, et al. The collaborative initial glaucoma treatment study: baseline visual field and test-retest variability. *Invest Ophthalmol Vis Sci.* 2003;44:2613–2620.
10. Leske MC, Heijl A, Hussein M, Bengtsson B, Hyman L, Komaroff E. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. *Arch Ophthalmol.* 2003;121:48–56.
11. Tielsch JM, Sommer A, Katz J, Royall RM, Quigley HA, Javitt J. Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. *JAMA.* 1991;266:369–374.
12. Leske MC, Wu SY, Hennis A, Honkanen R, Nemesure B. Risk factors for incident open-angle glaucoma: the Barbados Eye Studies. *Ophthalmology.* 2008;115:85–93.
13. Armaly MF, Krueger DE, Maunder L, et al. Biostatistical analysis of the collaborative glaucoma study. I. Summary report of the risk factors for glaucomatous visual-field defects. *Arch Ophthalmol.* 1980;98:2163–2171.
14. Hart WM Jr, Yablonski M, Kass MA, Becker B. Multivariate analysis of the risk of glaucomatous visual field loss. *Arch Ophthalmol.* 1979;97:1455–1458.
15. Yablonski ME, Zimmerman TJ, Kass MA, Becker B. Prognostic significance of optic disk cupping in ocular hypertensive patients. *Am J Ophthalmol.* 1980;89:585–592.
16. Wolfs RC, Klaver CC, Ramrattan RS, van Duijn CM, Hofman A, de Jong PT. Genetic risk of primary open-angle glaucoma. Population-based familial aggregation study. *Arch Ophthalmol.* 1998;116:1640–1645.

17. Gottfredsdottir MS, Sverrisson T, Musch DC, Stefansson E. Chronic open-angle glaucoma and associated ophthalmic findings in monozygotic twins and their spouses in Iceland. *J Glaucoma.* 1999;8:134–139.

18. Teikari JM. Genetic factors in open-angle (simple and capsular) glaucoma. A population-based twin study. *Acta Ophthalmol (Copenh).* 1987;65:715–720.

19. Monemi S, Spaeth G, DaSilva A, et al. Identification of a novel adult-onset primary open-angle glaucoma (POAG) gene on 5q22.1. *Hum Mol Genet.* 2005;14:725–733.

20. Sarfarazi M, Child A, Stoilova D, et al. Localization of the fourth locus (GLC1E) for adult-onset primary open-angle glaucoma to the 10p15–p14 region. *Am J Hum Genet.* 1998;62:641–652.

21. Sheffield VC, Stone EM, Alward WL, et al. Genetic linkage of familial open angle glaucoma to chromosome 1q21–q31. *Nat Genet.* 1993;4:47–50.

22. Stoilova D, Child A, Trifan OC, Crick RP, Coakes RL, Sarfarazi M. Localization of a locus (GLC1B) for adult-onset primary open angle glaucoma to the 2cen-q13 region. *Genomics.* 1996;36:142–150.

23. Trifan OC, Traboulsi EI, Stoilova D, et al. A third locus (GLC1D) for adult-onset primary open-angle glaucoma maps to the 8q23 region. *Am J Ophthalmol.* 1998;126:17–28.

24. Wirtz MK, Samples JR, Kramer PL, et al. Mapping a gene for adult-onset primary open-angle glaucoma to chromosome 3q. *Am J Hum Genet.* 1997;60:296–304.

25. Wirtz MK, Samples JR, Rust K, et al. GLC1F, a new primary open-angle glaucoma locus, maps to 7q35–q36. *Arch Ophthalmol.* 1999;117:237–241.

26. Baird PN, Foote SJ, Mackey DA, Craig J, Speed TP, Bureau A. Evidence for a novel glaucoma locus at chromosome 3p21–22. *Hum Genet.* 2005;117:249–257.

27. Pang CP, Fan BJ, Canlas O, et al. A genome-wide scan maps a novel juvenile-onset primary open angle glaucoma locus to chromosome 5q. *Mol Vis.* 2006;12:85–92.:85–92.

28. Wang DY, Fan BJ, Chua JK, et al. A genome-wide scan maps a novel juvenile-onset primary open-angle glaucoma locus to 15q. *Invest Ophthalmol Vis Sci.* 2006;47:5315–5321.

29. Wiggs JL, Lynch S, Ynagi G, et al. A genomewide scan identifies novel early-onset primary open-angle glaucoma loci on 9q22 and 20p12. *Am J Hum Genet.* 2004;74:1314–1320.

30. Alward WL, Kwon YH, Kawase K, et al. Evaluation of optineurin sequence variations in 1,048 patients with open-angle glaucoma. *Am J Ophthalmol.* 2003;136:904–910.

31. Wiggs JL, Auguste J, Allingham RR, et al. Lack of association of mutations in optineurin with disease in patients with adult-onset primary open-angle glaucoma. *Arch Ophthalmol.* 2003;121:1181–1183.

32. Fingert JH, Alward WL, Kwon YH, et al. No association between variations in the WDR36 gene and primary open-angle glaucoma. *Arch Ophthalmol.* 2007;125:434–436.

33. Suzuki Y, Shirato S, Taniguchi F, Ohara K, Nishimaki K, Ohta S. Mutations in the TIGR gene in familial primary open-angle glaucoma in Japan. *Am J Hum Genet.* 1997;61:1202–1204.

34. Alward WL, Fingert JH, Coote MA, et al. Clinical features associated with mutations in the chromosome 1 open-angle glaucoma gene (GLC1A). *N Engl J Med.* 1998;338:1022–1027.

35. Alward WL, Kwon YH, Khanna CL, et al. Variations in the myocilin gene in patients with open-angle glaucoma. *Arch Ophthalmol.* 2002;120:1189–1197.

36. Fingert JH, Heon E, Liebmann JM, et al. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. *Hum Mol Genet.* 1999;8:899–905.

37. Stone EM, Fingert JH, Alward WL, et al. Identification of a gene that causes primary open angle glaucoma. *Science.* 1997;275:668–670.

38. Fan BJ, Wang DY, Lam DS, Pang CP. Gene mapping for primary open angle glaucoma. *Clin Biochem.* 2006;39:249–258.

39. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet.* 2005;6:95–108.

40. Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005;308:421–424.

41. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005;308:385–389.

42. Gould DB, Smith RS, John SW. Anterior segment development relevant to glaucoma. *Int J Dev Biol.* 2004;48:1015–1029.

43. Vincent AL, Billingsley G, Buys Y, et al. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. *Am J Hum Genet.* 2002;70:448–460.

44. Acharya M, Mookherjee S, Bhattacharjee A, et al. Primary role of CYP1B1 in Indian juvenile-onset POAG patients. *Mol Vis.* 2006;12:399–404.

45. Bhattacharjee A, Banerjee D, Mookherjee S, et al. Leu432Val polymorphism in CYP1B1 as a susceptible factor towards predisposition to primary open-angle glaucoma. *Mol Vis.* 2008;14:841–850.

46. Melki R, Lefort N, Brezin AP, Garchon HJ. Association of a common coding polymorphism (N453S) of the cytochrome P450 1B1 (CYP1B1) gene with optic disc cupping and visual field alteration in French patients with primary open-angle glaucoma. *Mol Vis.* 2005;11:1012–1017.

47. Gould DB, John SW. Anterior segment dysgenesis and the developmental glaucomas are complex traits. *Hum Mol Genet.* 2002; 11:1185–1193.

48. Shields M, Ritch R, Krupin T. Classification of glaucomas. In: Ritch R, Shields M, Krupin T, eds. *The Glaucomas.* 2nd ed. St. Louis: Mosby; 1996:717–725.

49. Strungaru MH, Dinu I, Walter MA. Genotype-phenotype correlations in Axenfeld-Rieger malformation and glaucoma patients with FOXC1 and PITX2 mutations. *Invest Ophthalmol Vis Sci.* 2007;48:228–237.

50. Idrees F, Vaideanu D, Fraser SG, Sowden JC, Khaw PT. A review of anterior segment dysgeneses. *Surv Ophthalmol.* 2006;51:213–231.

51. Pressman CL, Chen H, Johnson RL. LMX1B, a LIM homeodomain class transcription factor, is necessary for normal development of multiple tissues in the anterior segment of the murine eye. *Genesis.* 2000;26:15–25.

52. Mimiwati Z, Mackey DA, Craig JE, et al. Nail-patella syndrome and its association with glaucoma: a review of eight families. *Br J Ophthalmol.* 2006;90:1505–1509.

53. Spitalny LA, Fenske HD. Hereditary osteo-onychodysplasia. *Am J Ophthalmol.* 1970;70:604–608.

54. Bongers EM, Huysmans FT, Levtchenko E, et al. Genotype-phenotype studies in nail-patella syndrome show that LMX1B mutation location is involved in the risk of developing nephropathy. *Eur J Hum Genet.* 2005;13:935–946.

55. Lichter PR, Richards JE, Downs CA, Stringham HM, Boehnke M, Farley FA. Cosegregation of open-angle glaucoma and the nail-patella syndrome. *Am J Ophthalmol.* 1997;124:506–515.

56. Sweeney E, Fryer A, Mountford R, Green A, McIntosh I. Nail patella syndrome: a review of the phenotype aided by developmental biology. *J Med Genet.* 2003;40:153–162.

57. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science.* 2002;296:2225–2229.

58. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263–265.

59. Tregouet DA, Barbaux S, Escolano S, et al. Specific haplotypes of the P-selectin gene are associated with myocardial infarction. *Hum Mol Genet.* 2002;11:2015–2023.

60. Lehmann OJ, Tuft S, Brice G, et al. Novel anterior segment phenotypes resulting from forkhead gene alterations: evidence for cross-species conservation of function. *Invest Ophthalmol Vis Sci.* 2003;44:2627–2633.

61. Ramaesh T, Collinson JM, Ramaesh K, Kaufman MH, West JD, Dhillon B. Corneal abnormalities in Pax6+/- small eye mice mimic human aniridia-related keratopathy. *Invest Ophthalmol Vis Sci.* 2003;44:1871–1878.

62. Brandt JD, Casuso LA, Budenz DL. Markedly increased central corneal thickness: an unrecognized finding in congenital aniridia. *Am J Ophthalmol.* 2004;137:348–350.

63. Asai-Coakwell M, Backhouse C, Casey RJ, Gage PJ, Lehmann OJ. Reduced human and murine corneal thickness in an Axenfeld-Rieger syndrome subtype. *Invest Ophthalmol Vis Sci.* 2006;47: 4905–4909.

64. Gordon MO, Beiser JA, Brandt JD, et al. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol.* 2002;120:714–720.

65. Whitacre MM, Stein RA, Hassanein K. The effect of corneal thickness on applanation tonometry. *Am J Ophthalmol.* 1993;115:592–596.

66. Feltgen N, Leifert D, Funk J. Correlation between central corneal thickness, applanation tonometry, and direct intracameral IOP readings. *Br J Ophthalmol.* 2001;85:85–87.

67. Bhan A, Browning AC, Shah S, Hamilton R, Dave D, Dua HS. Effect of corneal thickness on intraocular pressure measurements with the pneumotonometer, Goldmann applanation tonometer, and Tono-Pen. *Invest Ophthalmol Vis Sci.* 2002;43:1389–1392.

68. Ehlers N, Bramsen T, Sperling S. Applanation tonometry and central corneal thickness. *Acta Ophthalmol (Copenh).* 1975;53:34–43.

69. Stodtmeister R. Applanation tonometry and correction according to corneal thickness. *Acta Ophthalmol Scand.* 1998;76:319–324.

70. Brandt JD. Corneal thickness in glaucoma screening, diagnosis, and management. *Curr Opin Ophthalmol.* 2004;15:85–89.

71. Miyazawa A, Fuse N, Mengkegale M, et al. Association between primary open-angle glaucoma and WDR36 DNA sequence variants in Japanese. *Mol Vis.* 2007;13:1912–1919.

72. Francis BA, Varma R, Chopra V, Lai MY, Shtir C, Azen SP. Intraocular pressure, central corneal thickness, and prevalence of open-angle glaucoma: The Los Angeles Latino Eye Study. *Am J Ophthalmol.* July 29 2008; [Epub ahead of print].

73. Dunston JA, Hamlington JD, Zaveri J, et al. The human LMX1B gene: transcription unit, promoter, and pathogenic mutations. *Genomics.* 2004;84:565–576.

74. Lehmann OJ, Ebenezer ND, Ekong R, et al. Ocular developmental abnormalities and glaucoma associated with interstitial 6p25 duplications and deletions. *Invest Ophthalmol Vis Sci.* 2002;43: 1843–1849.

75. Dora N, Ou J, Kucerova R, Parisi I, West JD, Collinson JM. PAX6 dosage effects on corneal development, growth, and wound healing. *Dev Dyn.* 2008;237:1295–1306.

76. Kozlowski K, Walter MA. Variation in residual PITX2 activity underlies the phenotypic spectrum of anterior segment developmental disorders. *Hum Mol Genet.* 2000;9:2131–2139.

77. Gould DB, Marchant JK, Savinova OV, Smith RS, John SW. Col4a1 mutation causes endoplasmic reticulum stress and genetically modifiable ocular dysgenesis. *Hum Mol Genet.* 2007;16:798–807.

78. Rohr C, Prestel J, Heidet L, et al. The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes. *J Clin Invest.* 2002;109:1073–1082.

79. Morello R, Zhou G, Dreyer SD, et al. Regulation of glomerular basement membrane collagen expression by LMX1B contributes to renal disease in nail patella syndrome. *Nat Genet.* 2001;27:205–208.

E R R A T U M

Erratum in: "XIAP Effects on Retinal Detachment–Induced Photoreceptor Apoptosis" by Zadro-Lamoureux et al. (*Invest Ophthalmol Vis Sci.* 2009;50:1448–1453.)

The title of the article was printed incorrectly. The correct title is shown above.