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

Soo Park,1 Yalda Jamsbidi,2 Daniela Vaideanu,3 Maria Bitner-Glindzicz,4 Scott Fraser,5 and Jane C. Sowden1

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 LMX1B 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, 1.09); rs7854658 was associated with NTG (P = 0.0041; OR, 0.50). 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 GCAGGC 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.08-2485

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.2–7 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.16,17-20 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 OPTN30,31 and WDR3632 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 effects39 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)10,44 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.\textsuperscript{51} 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.\textsuperscript{52,55} Between 30% and 75% of ASD patients develop glaucoma, and a number of gene mutations have been identified underlying these conditions.\textsuperscript{56–58} 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 \textit{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.\textsuperscript{51} \textit{Lmx1b} encodes a LIM homeobox transcription factor that regulates programs of gene expression essential for normal morphogenesis and cell differentiation of the anterior segment.\textsuperscript{51} Targeted homozygous mutation resulted in ASD phenotypes, including iris and ciliary body hypoplasia, and corneal stromal defects.\textsuperscript{51} Significantly, mutations in the human homologue \textit{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 aged over 40 years develop open-angle glaucoma.\textsuperscript{55} As well as causing dysplasia of the nails and absent or hypoplastic patellae, \textit{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 microphthalmia.\textsuperscript{55–58} However, developmental abnormalities of the anterior segment are not clinically detectable in patients carrying \textit{LMX1B} mutation in the majority of cases,\textsuperscript{52,56} a feature which is more consistent with POAG.

In this study, we investigate whether variant alleles of \textit{LMX1B} play a role in POAG in the general population. We perform a case-control genetic association study to compare the prevalence of \textit{LMX1B} SNPs in four groups—HTG, NTG, OHT, and a normal control group. \textit{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). \textit{LMX1B} has not been analyzed in any association studies to date, and here we identify protective \textit{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 (\(n = 567\)) 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 perimeter; 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 \textit{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 April 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 \(\chi^2\) goodness-of-fit test.

**LD and Haplotype Structure of the \textit{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 Haplovlp,\textsuperscript{77} 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 \(D^\prime\) statistic and the LD structure was examined using the 80% confidence bounds of \(D^\prime\) 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.\textsuperscript{56} 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 \(\chi^2\) distribution with \(2^\Omega\) 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.\textsuperscript{79} 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

<table>
<thead>
<tr>
<th></th>
<th>NTG</th>
<th>OHT</th>
<th>HTG</th>
<th>Controls</th>
<th>Cases Combined (OHT+HTG)</th>
<th>Cases Combined (NTG+HTG)</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>58</td>
<td>272</td>
<td>276</td>
<td>330</td>
<td>309</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age (SD)</td>
<td>77.5 ± 8.4</td>
<td>65.1 ± 11.4</td>
<td>71.7 ± 10.4</td>
<td>70.7 ± 9.315</td>
<td>70.12 ± (10.8653)</td>
<td>71.93 ± (10.4623)</td>
<td>0.437</td>
<td>0.158</td>
</tr>
<tr>
<td>% Male</td>
<td>27 ± 0.5</td>
<td>41.4 ± 4.4</td>
<td>54.4 ± 4.4</td>
<td>42.8 ± 4.4</td>
<td>52.1 ± 4.4</td>
<td>51.1 ± 4.4</td>
<td>0.022</td>
<td>0.047</td>
</tr>
<tr>
<td>Mean CDR (SD)</td>
<td>0.74 ± 0.09</td>
<td>0.36 ± 0.29</td>
<td>0.717 ± 0.182</td>
<td>0.212 ± 0.251</td>
<td>0.655 ± 0.221</td>
<td>0.720 ± &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean IOP (SD)</td>
<td>17.6 ± 2.09</td>
<td>27.16 ± 4.021</td>
<td>29.13 ± 5.506</td>
<td>15.45 ± 2.352</td>
<td>28.79 ± 5.326</td>
<td>27.5 ± (6.4153)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

P values were calculated using independent samples t-test, except a χ² 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 r² 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 rs941103, 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.](https://iovs.arvojournals.org/)
**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

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>tSNP</th>
<th>SNP ID</th>
<th>Allele</th>
<th>Case Counts (%)</th>
<th>Control Counts (%)</th>
<th>$P$</th>
<th>Permutation $P$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTG</td>
<td>4</td>
<td>rs7859156</td>
<td>T</td>
<td>121 (23.1)</td>
<td>171 (31.8)</td>
<td>0.0015</td>
<td>0.0376</td>
<td>0.64 (0.49–0.85)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>rs6478750</td>
<td>C</td>
<td>193 (36.3)</td>
<td>236 (42.9)</td>
<td>0.0258</td>
<td>NS</td>
<td>0.72 (0.55–0.93)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>rs10987385</td>
<td>A</td>
<td>147 (27.7)</td>
<td>118 (21.5)</td>
<td>0.0180</td>
<td>NS</td>
<td>1.43 (1.07–1.91)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>rs7854658</td>
<td>A</td>
<td>95 (18.0)</td>
<td>137 (24.9)</td>
<td>0.0057</td>
<td>NS</td>
<td>0.65 (0.49–0.89)</td>
</tr>
<tr>
<td>OHT</td>
<td>4</td>
<td>rs7859156</td>
<td>T</td>
<td>24 (22.2)</td>
<td>171 (31.8)</td>
<td>0.0482</td>
<td>NS</td>
<td>0.59 (0.34–1.01)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>rs6478750</td>
<td>C</td>
<td>35 (31.8)</td>
<td>236 (42.9)</td>
<td>0.0309</td>
<td>NS</td>
<td>0.59 (0.35–0.97)</td>
</tr>
<tr>
<td>NTG</td>
<td>9</td>
<td>rs944103</td>
<td>G</td>
<td>41 (55.4)</td>
<td>238 (43.3)</td>
<td>0.0488</td>
<td>NS</td>
<td>1.71 (1.06–2.77)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>rs7854658</td>
<td>A</td>
<td>7 (9.7)</td>
<td>137 (24.9)</td>
<td>0.0041</td>
<td>NS</td>
<td>0.30 (0.14–0.64)</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>rs16929236</td>
<td>G</td>
<td>17 (23.0)</td>
<td>116 (21.1)</td>
<td>0.0064</td>
<td>NS</td>
<td>2.30 (1.30–4.05)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>rs10733682</td>
<td>A</td>
<td>44 (59.5)</td>
<td>260 (47.3)</td>
<td>0.0489</td>
<td>NS</td>
<td>1.75 (1.05–2.90)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>rs867559</td>
<td>G</td>
<td>22 (29.7)</td>
<td>104 (18.9)</td>
<td>0.0295</td>
<td>NS</td>
<td>1.82 (1.09–3.05)</td>
</tr>
<tr>
<td>HTG/OHT</td>
<td>4</td>
<td>rs7859156</td>
<td>T</td>
<td>145 (22.9)</td>
<td>171 (31.8)</td>
<td>7.0E-4</td>
<td>0.0120</td>
<td>0.63 (0.48–0.82)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>rs6478750</td>
<td>C</td>
<td>228 (35.5)</td>
<td>236 (42.9)</td>
<td>0.0090</td>
<td>NS</td>
<td>0.69 (0.54–0.89)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>rs10987385</td>
<td>A</td>
<td>175 (27.3)</td>
<td>118 (21.5)</td>
<td>0.0205</td>
<td>NS</td>
<td>1.39 (1.05–1.84)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>rs7854658</td>
<td>A</td>
<td>116 (18.2)</td>
<td>137 (24.9)</td>
<td>0.0047</td>
<td>NS</td>
<td>0.66 (0.50–0.88)</td>
</tr>
<tr>
<td>HTG/NTG</td>
<td>4</td>
<td>rs7859156</td>
<td>T</td>
<td>138 (23.2)</td>
<td>171 (31.8)</td>
<td>0.0013</td>
<td>0.0360</td>
<td>0.64 (0.49–0.84)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>rs6478750</td>
<td>C</td>
<td>220 (36.3)</td>
<td>236 (42.9)</td>
<td>0.0217</td>
<td>NS</td>
<td>0.73 (0.57–0.94)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>rs10987385</td>
<td>A</td>
<td>162 (26.8)</td>
<td>118 (21.5)</td>
<td>0.0366</td>
<td>NS</td>
<td>1.35 (1.02–1.79)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>rs7854658</td>
<td>A</td>
<td>102 (17.0)</td>
<td>137 (24.9)</td>
<td>0.0010</td>
<td>0.0270</td>
<td>0.62 (0.46–0.85)</td>
</tr>
</tbody>
</table>

tSNPs significantly distributed after 1000 permutation tests are highlighted in bold.
TABLE 4. Distribution of Haplotypes between HTG+OHT Cases and Controls

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>All Subjects (%)</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>P</th>
<th>Permutation P</th>
<th>OR (95% CI)</th>
<th>P Value after Adjustment for Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(tSNP 3 to 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>35.6</td>
<td>35.8</td>
<td>35.2</td>
<td>0.8269</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACG</td>
<td>28.8</td>
<td>31.7</td>
<td>25.4</td>
<td>0.0156</td>
<td>0.3020</td>
<td>1.33 (0.99–1.78) P = 0.0591</td>
<td></td>
</tr>
<tr>
<td>ATG</td>
<td>26.9</td>
<td>22.7</td>
<td>31.7</td>
<td>5.0E-4</td>
<td>0.0100</td>
<td>0.72 (0.53–0.97) P = 0.0330</td>
<td></td>
</tr>
<tr>
<td>GGC</td>
<td>8.5</td>
<td>9.4</td>
<td>7.4</td>
<td>0.2110</td>
<td>0.9987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(tSNP 7 to 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTGGGC</td>
<td>31.6</td>
<td>31.4</td>
<td>31.8</td>
<td>0.8865</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCAGAC</td>
<td>21.0</td>
<td>17.8</td>
<td>24.7</td>
<td>0.0035</td>
<td>0.0710</td>
<td>0.75 (0.54–1.03) P = 0.0770</td>
<td></td>
</tr>
<tr>
<td>ACAGGT</td>
<td>11.8</td>
<td>14.0</td>
<td>9.4</td>
<td>0.0143</td>
<td>0.2770</td>
<td>1.61 (.08–2.41) P = 0.1990</td>
<td></td>
</tr>
<tr>
<td>GCAGGCC</td>
<td>11.2</td>
<td>12.4</td>
<td>9.8</td>
<td>0.1574</td>
<td>0.9875</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCAGGC</td>
<td>10.2</td>
<td>9.8</td>
<td>10.6</td>
<td>0.6668</td>
<td>0.9875</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CATGTC</td>
<td>9.5</td>
<td>9.8</td>
<td>9.2</td>
<td>0.7580</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGC</td>
<td>1.2</td>
<td>1.2</td>
<td>1.3</td>
<td>0.7729</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

effects replicated those found in the separate HTG group (Table 4). Within block 2, two haplotypes (ATG and ACG defined by tSNPs rs12356217, 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.

Haplotypes 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 rs12356217, 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 = 0.0005).

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

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>All Subjects (%)</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>P</th>
<th>Permutation P</th>
<th>OR (95% CI)</th>
<th>P Value after Adjustment for Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(tSNP 3 to 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACA</td>
<td>35.0</td>
<td>34.8</td>
<td>35.2</td>
<td>0.8804</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACG</td>
<td>28.7</td>
<td>32.2</td>
<td>25.4</td>
<td>0.0129</td>
<td>0.2720</td>
<td>1.39 (1.02–1.90) P = 0.0377</td>
<td></td>
</tr>
<tr>
<td>ATG</td>
<td>27.4</td>
<td>22.8</td>
<td>31.7</td>
<td>0.0010</td>
<td>0.0300</td>
<td>0.75 (0.54–1.03) P = 0.0750</td>
<td></td>
</tr>
<tr>
<td>GGC</td>
<td>8.6</td>
<td>9.9</td>
<td>7.4</td>
<td>0.1515</td>
<td>0.9770</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(tSNP 7 to 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTGGGC</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>0.9653</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCAGAC</td>
<td>21.1</td>
<td>17.5</td>
<td>24.7</td>
<td>0.0045</td>
<td>0.1040</td>
<td>0.73 (0.52–1.02) P = 0.0648</td>
<td></td>
</tr>
<tr>
<td>ACAGGT</td>
<td>11.7</td>
<td>14.2</td>
<td>9.4</td>
<td>0.0128</td>
<td>0.2730</td>
<td>1.64 (1.09–2.48) P = 0.0189</td>
<td></td>
</tr>
<tr>
<td>GCAGGC</td>
<td>10.1</td>
<td>9.5</td>
<td>10.6</td>
<td>0.4851</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACATGTC</td>
<td>9.2</td>
<td>9.2</td>
<td>9.2</td>
<td>0.9892</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGC</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
<td>0.9327</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Block 2 (tSNP 3 to 5)</th>
<th>Haplotype</th>
<th>All Subjects (%)</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>P</th>
<th>Permutation P</th>
<th>OR (95% CI)</th>
<th>Value after Adjustment for Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>35.3</td>
<td>35.3</td>
<td>35.2</td>
<td>0.9718</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACG</td>
<td>28.9</td>
<td>32.2</td>
<td>25.4</td>
<td>0.0110</td>
<td>0.2350</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATG</td>
<td>27.1</td>
<td>22.9</td>
<td>31.7</td>
<td>8.0E-4</td>
<td>0.0220</td>
<td>1.73 (0.54–0.99)</td>
<td>0.0450</td>
<td></td>
</tr>
<tr>
<td>GCC</td>
<td>8.4</td>
<td>9.3</td>
<td>7.4</td>
<td>0.2452</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GTGGGC</td>
<td>31.6</td>
<td>31.4</td>
<td>31.9</td>
<td>0.8768</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCAGAC</td>
<td>20.4</td>
<td>16.5</td>
<td>24.7</td>
<td>5.0E-4</td>
<td>0.0150</td>
<td>0.70 (0.51–0.98)</td>
<td>0.0360</td>
<td></td>
</tr>
<tr>
<td>GCGGGC</td>
<td>11.9</td>
<td>13.9</td>
<td>9.8</td>
<td>0.0350</td>
<td>0.5760</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGT</td>
<td>11.7</td>
<td>13.8</td>
<td>9.4</td>
<td>0.0202</td>
<td>0.3980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCAGGC</td>
<td>10.5</td>
<td>10.3</td>
<td>10.8</td>
<td>0.7689</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACATGT</td>
<td>9.1</td>
<td>9.1</td>
<td>9.3</td>
<td>0.9033</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGC</td>
<td>1.3</td>
<td>1.2</td>
<td>1.3</td>
<td>0.8697</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Block 3 (tSNP 7 to 12)</th>
<th>Haplotype</th>
<th>Subjects (%)</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>P</th>
<th>Permutation P</th>
<th>OR (95% CI)</th>
<th>Value after Adjustment for Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACAGG</td>
<td>11.7</td>
<td>13.8</td>
<td>9.4</td>
<td>0.0202</td>
<td>0.3980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGG</td>
<td>11.8</td>
<td>13.9</td>
<td>9.8</td>
<td>0.0350</td>
<td>0.5760</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGT</td>
<td>11.7</td>
<td>13.8</td>
<td>9.4</td>
<td>0.0202</td>
<td>0.3980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGG</td>
<td>10.5</td>
<td>10.3</td>
<td>10.8</td>
<td>0.7689</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACATGT</td>
<td>9.1</td>
<td>9.1</td>
<td>9.3</td>
<td>0.9033</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAGGC</td>
<td>1.3</td>
<td>1.2</td>
<td>1.3</td>
<td>0.8697</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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; 95% CI, 0.54–0.99). However, within block 3, we observed three haplotypes (GCAGAC, GCGGGC, and ACAGGT) that were differentially distributed in patients and controls, with haplotype GCAGAC being significantly less prevalent among patients compared with controls (16.5% vs. 24.7%; P = 0.0002). 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, rs9414103, 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, whereas 11.9% of individuals older than aged 40 years develop OHT. 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), aniridia (*PAX6/Pax6*), and dysgenic lens (*Foxe3*). Conversely, a reduced corneal thickness was associated with Azenfeld-Rieger malformation of the anterior segment and *PITX2* mutation. A thinner CTT was also found to be a risk factor for developing POAG in individuals with OHT. 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. In the present study, IOP measurements were checked by a Tono-Pen which is less affected by CCT, in addition to performing applanation tonometry. Although normal eyes on varying CCT exist for adjusting IOP readings, there is as yet no generally accepted correction formula. However, two recent studies that adjusted IOP for CCT found that the correction did not alter the diagnosis of HTG or NTG, and did not affect the relationship between the prevalence of POAG and IOP, respectively.

We identified six main haplotype blocks across the LMX1B gene. Haplotype 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 high 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. Significantly, the block 2 haplotype is in complete LD with the promoter and 5’ upstream regions. Therefore, despite being an intrinsic 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 and PAX6, which are presumed to alter levels of gene expression, or mutations in PITX2 that increase or decrease levels of transcription factor function, 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. Lmx1b is known to directly regulate expression of Col4a1 in the kidney, mice showed defective collagen fibrillogenesis in the eye as well as ciliary body hypoplasia, 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, 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) and 47.9 years (range, 23 to 78) 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.

Acknowledgments

The authors thank the subjects for their participation in this study: Charles Mein, Paul Rutland, and David Garway-Heath for advice during this study; and Lyn-Louise Buckingham at the Genomics Laboratory, Wellcome Trust Centre for Human Genetics.

References


**ERRATUM**


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