Tag SNPs detect association of the CYP1B1 gene with primary open angle glaucoma

Kathryn P. Burdon,1 Alex W. Hewitt,1,2 David A. Mackey,2 Paul Mitchell,3 Jamie E. Craig1

1Department of Ophthalmology, Flinders University, Adelaide, Australia; 2Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia; 3Centre for Vision Research, Department of Ophthalmology and Westmead Millennium Institute, University of Sydney, Westmead, Australia

Purpose: The cytochrome p450 family 1 subfamily B (CYP1B1) gene is a well known cause of autosomal recessive primary congenital glaucoma. It also acts as an autosomal modifier of disease severity in primary open angle glaucoma (POAG), particularly in juvenile onset families. However, the role of common variation in the gene in relation to POAG has not been thoroughly explored.

Methods: Seven tag single nucleotide polymorphisms (SNPs), including two coding variants (L432V and N543S), were genotyped in 860 POAG cases and 898 examined normal controls. Each SNP and haplotype was assessed for association with disease. In addition, a subset of 396 severe cases and 452 elderly controls were analyzed separately.

Results: There was no association of any individual SNP in the full data set. Two SNPs (rs162562 and rs10916) were nominally associated under a dominant model in the severe cases (p<0.05). A common haplotype (AGCAGCC) was also found to be nominally associated in both the full data set (p=0.048, OR [95%CI]=0.83 [0.69–0.90]) and more significantly in the severe cases (p=0.004, OR [95%CI]=0.68 [0.52–0.89]) which survives correction for multiple testing.

Conclusions: Although no major effect of common variation at the CYP1B1 locus on POAG was found, there could be an effect of SNPs tagged by rs162562 and represented on the AGCAGCC haplotype.

The cytochrome p450 family 1 subfamily B (CYP1B1) is a member of the CYP450 superfamily. While its exact function and effect on cells is not clear, the gene is inducible by dioxins and has several endogenous substrates including 17β-estradiol, retinoic acid, and melatonin as well as many exogenous substrates [1]. It was first recognized as a cause of primary congenital glaucoma (PCG) following linkage mapping [2] and candidate gene screening [3] in a panel of 17 Turkish families with recessive PCG. This finding has since been replicated in many ethnic groups with over 80 mutations now reported from many different populations [1,4,5]. In many cases, compound heterozygosity is observed as the cause of recessive disease. The proportion of PCG cases accounted for by CYP1B1 mutations varies significantly between ethnic groups, from around 20% in Australia and Japan to nearly 100% in Saudi Arabia and Slovakian Gypsies [4].

Primary Open Angle Glaucoma (POAG) is the most prevalent form of glaucoma and leads to significant levels of irreversible blindness worldwide. The genetics of this complex trait are not well understood, although many loci and several genes have been reported [6]. The most common known genetic cause of POAG is the myocilin gene (MYOC). Mutations in this gene account for 2%–4% of POAG in Caucasians [7] and up to 36% in juvenile onset (JOAG) families [8]. CYP1B1 mutations have also been identified in JOAG and POAG patients. Melki et al. [9] reported compound heterozygotes in three French families containing patients with PCG as well as JOAG. Acharya et al. [10] reported nine individuals from India (4 JOAG and 5 POAG) with single heterozygous mutations in CYP1B1 and Kumar et al. [11] reported four mutations in 27 Indian POAG patients, including two who were compound heterozygotes. Lopez-Garrido et al. [12] presented heterozygous mutations in 10 Spanish POAG patients. CYP1B1 has also been suggested as a modifier of POAG in carriers of MYOC mutations [13]. A common polymorphism was associated with cupping of the optic disc, which may be relevant to POAG [14] although other studies found no association of CYP1B1 mutations with disc changes in POAG [15].

Although several studies have reported rare variants in the CYP1B1 gene in glaucoma patients that were not detected in controls [10–15], no large scale re-sequencing of normal population has been performed to determine the spectrum of rare variants in this gene. There are 75 reported coding variants in dbSNP, of which 45 are non-synonymous, 11 are insertions or deletions, and 4 are truncating mutations. While the majority have not yet been thoroughly validated as common polymorphisms, the number of reported frameshift and non-synonymous variants suggests that CYP1B1 activity is not compromised by most mutational events, at least in the heterozygous state. Thus, the presence of rare variants in the sequenced glaucoma patients is not surprising.
between \textit{CYP1B1} and POAG is therefore currently circumstantial. Chakrabarti et al. [15] assessed six common polymorphisms in a small cohort of POAG and primary angle closure glaucoma (PACG) patients as well as controls and found no association of any haplotypes with glaucoma status. This study aims to evaluate the contribution of common polymorphisms in \textit{CYP1B1} to POAG.

\section*{METHODS}

\textit{Patients}: Participants were drawn from the Glaucoma Inheritance Study in Tasmania (GIST), the Australian \& New Zealand Registry of Advanced Glaucoma (ANZRAG) and the Blue Mountains Eye Study (BMES). The GIST and ANZRAG includes a clinic-based recruitment of glaucoma patients. The GIST aimed to capture all cases of glaucoma in Tasmania (an island state of Australia) and ANZRAG aims to capture cases of advanced glaucoma Australia-wide through ophthalmologist referral [16,17]. In both cases, normal elderly controls were ascertained from nursing home facilities in Launceston, Tasmania (for GIST) and Adelaide, South Australia (for ANZRAG). The BMES is a population based study of individuals aged over 50 years living in the Blue Mountains, west of Sydney, Australia [18]. All participants, including normal controls in all three studies were examined. Glaucoma was defined by concordant findings of typical glaucomatous visual field defects on the Humphrey 24–2 (for BMES) or 30–2 (for GIST and ANZRAG) test, together with corresponding optic disc rim thinning, including an enlarged cup-disc ratio ($\geq 0.7$) or cup-disc ratio asymmetry ($\geq 0.2$) between the two eyes. Intraocular pressure was not considered in the diagnostic criteria. Advanced POAG was defined by a vertical cup:disc ratio $>0.95$, a best-corrected visual acuity worse than 6/60 due to POAG, or on a reliable Humphrey Visual Field (Carl Zeiss Pty. Ltd., Sydney, Australia) a mean deviation of $\leq -22$ db or at least 10 out of 16 central squares involved with a Pattern Standard Deviation of $<0.5\%$. The field loss had to be due to POAG, and the less severely affected eye was required to have signs of glaucomatous disc damage and a glaucomatous field defect. Clinical exclusion criteria included: i) pseudoxfoliative glaucoma, ii) pigmentary glaucoma, iii) angle closure or mixed mechanism glaucoma; iv) secondary glaucoma due to aphakia, rubella, rubeosis or inflammation; v) congenital or infantile glaucoma, juvenile glaucoma with age of onset less than 20 years; or vi) glaucoma in the presence of a known syndrome.

All control subjects were required to have no known family history of POAG, as well as a normal intraocular pressure, optic disc and visual field. The population-based BMES control cohort comprised the eldest subgroup of people meeting control inclusion criteria.

\textit{SNP selection and genotyping}: Using the tagger program implemented in Haploview 4.0 [19] tag single nucleotide polymorphisms (SNPs) across the \textit{CYP1B1} gene were selected on the basis of linkage disequilibrium patterns observed in the Caucasian (CEU) samples genotyped as part of the International HapMap Project [20]. Only SNPs with minor allele frequency greater than 5\% in HapMap were considered. Two coding SNPs (rs1056836 and rs1800440) coding L432V and N453S respectively) and four 3′UTR SNPs (rs162549, rs2855358, rs10916, and rs162562) for the \textit{CYP1B1} 3′UTR were force included to capture as much coding variation as possible. In addition, intronic SNPs rs10175368 and rs1625562 were selected. These eight tag SNPs captured all alleles with an $r^2$ of at least 0.8 (mean $r^2=0.96$) and were genotyped in all individuals using iPLEX GOLD chemisty (Sequenom Inc., San Diego, CA) on an Autoflex Mass Spectrometer (Sequenom Inc.) at the Australian Genome Research Facility, Brisbane, Australia. The 3′UTR SNP rs2855658 failed genotyping and was removed from the analysis. This SNP did not tag any other HapMap SNPs.

\textit{Statistical analysis}: All analyses were conducted using the statistical genetics software packages Plink [21] and Haploview [19]. Hardy Wienberg equilibrium was assessed in all samples and in controls separately. Association was tested under the five genetic models implemented in Plink. These models are the allelic test (allele1 versus allele2), genotypic (11 versus 12 versus 22), dominant (11 and 12 versus 22), recessive (11 versus 12 and 22) and the Cochran-Armitage Trend test. Association of common haplotypes (>1\% frequency) was also assessed in Plink using the conditional haplotype test. All analyses were conducted in the full data set as well as a sub-set of cases with severe disease (from GIST and ANZRAG) compared to elderly (>81 years old).

\begin{table}
\centering
\begin{tabular}{lcccclccc}
\hline
\textbf{Cohort} & \textbf{N} & \textbf{Sex} & \textbf{Age} \\
& & \textbf{Cases} & \textbf{Controls} & \textbf{Cases} & \textbf{Controls} & \textbf{p-value} & \textbf{Cases} & \textbf{Controls} & \textbf{p-value} \\
\hline
Full Sample & 860 & 897 & 0.52 & 0.53 & 0.82 & 74.8 & 80.5 & <0.001 \\
Severe Sample & 396 & 452 & 0.53 & 0.53 & 0.98 & 77.0 & 85.3 & <0.001 \\
ANZRAG & 230 & 285 & 0.47 & 0.55 & 0.05 & 82.4 & 76.0 & <0.001 \\
GIST & 476 & 101 & 0.60 & 0.68 & 0.16 & 73.3 & 86.3 & <0.001 \\
BMES & 154 & 511 & 0.36 & 0.48 & 0.01 & 77.7 & 81.1 & <0.001 \\
\hline
\end{tabular}
\caption{Demographics by recruitment center.}
\end{table}
| SNP          | Position (bp) | Allele | Minor allele frequency | Genotype frequency |
|--------------|---------------|--------|------------------------|--------------------|
|              |               |        | Cases | Controls | Cases | Controls | Cases | Controls | Cases | Controls | Cases | Controls |
| rs162549     | 38148960      | T      | 0.24  | (384)     | 0.23  | (3946)   | 0.07  | (53)     | 0.34  | (278)    | 0.59  | (478)    |
|              | rs10916       | G      | 0.21  | (347)     | 0.23  | (401)    | 0.05  | (38)      | 0.32  | (271)    | 0.63  | (530)    |
| rs162562     | 38151019      | C      | 0.20  | (346)     | 0.23  | (404)    | 0.04  | (37)      | 0.32  | (272)    | 0.63  | (556)    |
|              | rs162556      | G      | 0.19  | (313)     | 0.18  | (318)    | 0.04  | (34)      | 0.29  | (245)    | 0.67  | (566)    |
| rs1800440    | 38151643      | G      | 0.19  | (313)     | 0.18  | (318)    | 0.04  | (34)      | 0.29  | (245)    | 0.67  | (566)    |
|              | rs1056836     | G      | 0.45  | (739)     | 0.45  | (788)    | 0.21  | (168)     | 0.50  | (403)    | 0.30  | (243)    |
|              | rs10175368    | C      | 0.44  | (732)     | 0.47  | (829)    | 0.17  | (147)     | 0.52  | (438)    | 0.30  | (256)    |
|              | rs1900440     | T      | 0.28  | (471)     | 0.27  | (478)    | 0.08  | (65)      | 0.41  | (341)    | 0.51  | (431)    |

Allele 1 is the minor allele in each case. Note that SNPs are presented in genomic order and the gene is transcribed from the reverse strand.
of age) examined controls. Power calculations [22] revealed that, assuming a prevalence of 3% in this population, for a sample of this size (860 cases versus 898 controls) under an additive model we had 99% power to detect a genotype relative risk of 1.1 for an allele frequency of 0.4 at α=0.007 (allowing for multiple testing of 7 SNPs). For an allele frequency of 0.2, the power is 71% for a relative risk of 1.1, 92% for 1.2, and 98% for 1.3.

RESULTS

In total, 860 cases and 897 examined, normal, unrelated controls were available. Sex and age distribution for the full cohort and each sub-cohort are given in Table 1. Overall, the age of cases is significantly less than the controls, although in the ANZRAG cohort the cases are slightly older. There were no differences in the proportion of each cohort that is female.

All seven SNPs were in Hardy–Weinberg equilibrium. Allele and genotype frequencies by glaucoma status are shown in Table 2. Linkage disequilibrium across the region is high with all seven SNPs falling into a single block, although the correlation between SNPs rs162556 and rs10175368 is low (Figure 1), consistent with values observed in the HapMap data set. Thus using the block definition of Gabriel et al. [23] there are two haplotype blocks as shown in Figure 1.

Single SNP association analysis was conducted for five genetic models in Plink. No SNP was associated under the allelic test, nor in any of the other genetic models (Table 3). When the analysis was restricted to severe cases and elderly controls (≥81 years) only, SNPs rs162562 and rs10916 were nominally associated (p<0.05); however, these results do not survive correction for the number of SNPs assessed. The associations were also nominally significant under the recessive model and trend test. In addition, a logistic regression adjusted for age and sex was conducted. Nominally significant results at the same two SNPs were observed in the severe cohort, but do not survive multiple testing correction (Table 3).

Haplotype analysis was conducted in Plink. No overall association between the CYP1B1 locus and POAG was detected in either the full sample (p=0.140) nor the severe cases and elderly controls (p=0.189); however, one specific haplotype of the seven SNPs (AGCAGCC) was nominally
associated in both data sets (Table 4). This is a relatively common haplotype that is slightly under-represented in POAG cases, particularly severe cases. The association does survive correction for multiple testing of the seven common haplotypes in the severe cases (corrected p-value=0.028). The associated haplotype is the most common haplotype to carry a C at SNP rs162562, which was nominally significant in the single SNP analysis of the severe cases. This allele is observed in only one other haplotype (AGCAGTG) which only differs from the associated haplotype at the 6th SNP (rs162556), but is rarer and is not associated with POAG.

**DISCUSSION**

The association of the CYP1B1 gene with PCG is well understood in populations world-wide, although the mechanism of disease is not. The gene is also associated with JOAG and may interact with the MYOC gene to cause the early onset observed in JOAG families. However, the role of CYP1B1 in later onset POAG is unclear. The majority of studies to date have sequenced the coding region of the gene in a small cohort of POAG patients and most have identified missense mutations not observed in a control cohort. This approach has identified many apparent mutations that may contribute to the risk of POAG in rare cases, but does not provide evidence for a contribution of this locus to most (or even a significant proportion) of POAG cases. In addition, there are many reported missense, frameshift and truncating variants of this gene in non-POAG individuals, many of which have not been reported in the POAG cohorts, making interpretation of the published data in relation to POAG susceptibility difficult.

The present CYP1B1 study is the largest cohort of POAG patients examined to date (n=860) and we were well powered to identify common genetic variants at the level of relative risk of 1.1 or 1.2. In addition, all controls (n=898) are at least 50 years of age and have been thoroughly examined for glaucoma phenotypes. We have taken a tag SNP approach to assess the role of common variation throughout the CYP1B1 locus for an association with POAG in both a general POAG cohort as well as a cohort of severe (typically slightly younger onset) cases compared to elderly (>81 years) examined normal controls. These data do not provide evidence for a substantial role of this locus in POAG, although one haplotype may be protective for severe glaucoma. The odds ratio for the nominally associated haplotype is 0.68 when compared to all other common haplotypes. Power calculations [22] revealed that for a sample of this size (860 cases versus 898 controls) under an additive model we had 88% power to detect a genotype relative risk of 0.68 (or 1.47) for an allele frequency of 0.16 at α=0.007 (allowing for multiple testing of 7
| Haplotype     | Full sample (over-all p-value=0.156) | Restricted sample (over-all p-value=0.140) |
|--------------|--------------------------------------|------------------------------------------|
|              | f cases    | f controls | p-value  | OR (95%CI)    | f cases    | f controls | p-value  | OR (95%CI)    |
| ATAGCCC      | 0.19       | 0.18       | 0.536    | 1.06 (0.89–1.25) | 0.20       | 0.18       | 0.305    | 1.13 (0.89–1.44) |
| ATAACCTT     | 0.28       | 0.27       | 0.452    | 1.06 (0.91–1.23) | 0.28       | 0.26       | 0.304    | 1.12 (0.90–1.39) |
| TTAAGTC      | 0.23       | 0.22       | 0.303    | 1.00 (0.93–1.28) | 0.23       | 0.23       | 0.733    | 1.04 (0.83–1.30) |
| AGCAGCC      | 0.16       | 0.18       | 0.037    | 0.83 (0.69–0.90) | 0.13       | 0.19       | 0.004*   | 0.68 (0.52–0.89) |
| TTAAGCC      | 0.02       | 0.01       | 0.256    | 1.09 (0.93–1.28) | 0.01       | 0.01       | 0.617    | 1.26 (0.50–3.17) |
| AGCAGTC      | 0.05       | 0.04       | 0.441    | 1.14 (0.82–1.57) | 0.05       | 0.04       | 0.351    | 1.23 (0.90–1.91) |
| ATTACCC      | 0.08       | 0.09       | 0.115    | 0.82 (0.64–1.05) | 0.08       | 0.09       | 0.811    | 0.96 (0.68–1.36) |

Results are presented for the full sample and the sample restricted to severe cases and elderly controls. All p-values and odds ratios (OR) are shown for the specific haplotype versus all other haplotypes. Nominally significant values are in bold. The asterisk indicates survives Bonferroni correction for 7 observed haplotypes tested.
Molecular Vision 2010; 16:2286-2293 <http://www.molvis.org/molvis/v16/a245>

Vasiliou V, Gonzalez FJ. Role of CYP1B1 in glaucoma. Annu Rev Pharmacol Toxicol 2008; 48:333-58. [PMID: 17914928]

Sarfarazi M, Akarsu AN, Hassain A, Turacil ME, Aktan SG, Barsoum-Homsy M, Chevrette L, Sayli BS. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. Genomics 1995; 30:171-7. [PMID: 8568416]

Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P450B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. Hum Mol Genet 1997; 6:641-7. [PMID: 9097971]

Dimasi DP, Hewitt AW, Straga T, Pater J, MacKinnon JR, Elder JE, Casey T, Mackey DA, Craig JE. Prevalence of CYP1B1 mutations in Australian patients with primary congenital glaucoma. Clin Genet 2007; 72:255-60. [PMID: 17718864]

Yang M, Guo X, Liu X, Shen H, Jia X, Xiao X, Li S, Fang S, Zhang Q. Investigation of CYP1B1 mutations in Chinese patients with primary congenital glaucoma. Mol Vis 2009; 15:432-7. [PMID: 19247456]

Fan BJ, Wang DY, Lam DS, Pang CP. Gene mapping for primary open angle glaucoma. Clin Biochem 2006; 39:249-58. [PMID: 16332362]

Fingert JH, Heon E, Liebmann JM, Yamamoto T, Craig JE, Rait J, Kawase K, Hoh ST, Buys YM, Dickinson J, Hockey RR, Williams-Lyn D, Trope G, Kitazawa Y, Ritch R, Mackey DA, Alward WL, Sheffield VC, Stone EM. Analysis of myocilin mutations in 1703 glaucoma patients from five different populations. Hum Mol Genet 1999; 8:899-905. [PMID: 10196380]

Shimizu S, Lichter PR, Johnson AT, Zhou Z, Higashi M, Gottfredsdottir M, Othman M, Moroi SE, Rzoska FW, Schertzer RM, Clarke MS, Schwartz AL, Downs CA, Vollrath D, Richards JE. Age-dependent prevalence of mutations at the GLC1A locus in primary open-angle glaucoma. Am J Ophthalmol 2000; 130:165-77. [PMID: 11004290]

Melki R, Colomb E, Lefort N, Brezin AP, Garchon HJ. CYP1B1 mutations in French patients with early-onset primary open-angle glaucoma. J Med Genet 2004; 41:647-51. [PMID: 15342693]

Acharya M, Mookherjee S, Bhattacharjee A, Bandyopadhyay AK, Daulat Thakur SK, Bhaduri G, Sen A, Ray K. Primary role of CYP1B1 in Indian juvenile-onset POAG patients. Mol Vis 2006; 12:399-404. [PMID: 16688110]

Kumar A, Basavaraj MG, Gupta SK, Qamar I, Ali AM, Bajaj V, Ramesh TK, Prakash DR, Shetty JS, Dorairaj SK. Role of CYP1B1, MYOC, OPTN, and OPTC genes in adult-onset primary open-angle glaucoma: predominance of CYP1B1 mutations in Indian patients. Mol Vis 2007; 13:667-76. [PMID: 17563717]

López-Garrido MP, Sánchez-Sánchez F, López-Martinez F, Aroca-Aguilar JD, Blanco-Marchite C, Coca-Prados M, Escrivan J. Heterozygous CYP1B1 gene mutations in Spanish patients with primary open-angle glaucoma. Mol Vis 2006; 12:748-55. [PMID: 16862072]

Vincent AL, Billingsley G, Buys Y, Levin AV, Priston M, Trope G, Williams-Lyn D, Héon E. Digenic inheritance of early-onset glaucoma: CYP1B1, a potential modifier gene. Am J Hum Genet 2002; 70:448-60. [PMID: 11774072]

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; 11:1012-7. [PMID: 16319821]

Chakraborti S, Devi KR, Komatireddy S, Kaur K, Parikh RS, Mandal AK, Chandrasekhar G, Thomas R. Glaucoma-associated CYP1B1 mutations share similar haplotype backgrounds in POAG and PACG phenotypes. Invest Ophthalmol Vis Sci 2007; 48:5439-44. [PMID: 18055790]

Green CM, Kearns LS, Wu J, Barbour JM, Wilkinson RM, Ring MA, Craig JE, Wong TL, Hewitt AW, Mackey DA. How significant is a family history of glaucoma? Experience from the Glaucoma Inheritance Study in Tasmania. Clin Experiment Ophthalmol 2007; 35:793-9. [PMID: 18173405]

Hewitt AW, Chappell AJ, Straga T, Landers J, Mills RA, Craig JE. Sensitivity of confocal laser tomography versus optical coherence tomography in detecting advanced glaucoma. Clin Experiment Ophthalmol 2009; 37:836-41. [PMID: 20092591]

Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia. The Blue Mountains Eye Study. Ophthalmology 1996; 103:1661-9. [PMID: 8874440]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263-5. [PMID: 15755982]

International HapMap Consortium. The International HapMap Project. Nature 2003; 426:789-96. [PMID: 14685227]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559-75. [PMID: 17561642]

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart
M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. Science 2002; 296:2225-9. [PMID: 12029063]