Association of Polymorphisms of Tumor Necrosis Factor and Tumor Protein p53 with Primary Open-Angle Glaucoma

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PURPOSE. To evaluate the variants of 10 genes for association with primary open-angle glaucoma (POAG) in a Chinese population.

METHODS. A total of 405 unrelated patients with POAG (255 high-tension glaucoma [HTG], 100 normal-tension glaucoma [NTG], and 50 juvenile-onset open-angle glaucoma [JOAG]) and 201 control subjects were recruited. Seventeen variants in 10 genes with reported association with POAG were genotyped for analysis of allele and haplotype frequencies between cases and control subjects. These genes included CDH1 (cadherin 1, type 1, E-cadherin), CDKN1A (cyclin-dependent kinase inhibitor 1A), CYP1B1 (cytochrome P450, family 1, subfamily B, polypeptide 1), GSTM1 (glutathione S-transferase mu 1), GSTT1 (glutathione S-transferase theta 1), MTHR (5,10-methylene tetrahydrofolate reductase), NOS3 (nitric oxide synthase 3), OPAL (optic atrophy 1), TNF (tumor necrosis factor), and TP53 (tumor protein p53).

RESULTS. One SNP (−308G>A; rs1800629) in TNF demonstrated a significant association with HTG (P = 0.012). The allele G frequency was higher in HTG patients than in control subjects (94.6% vs. 90.3%; OR = 1.89). One haplotype consisting of rs1799724 and rs1800629 was significantly associated with HTG (P = 0.015, corrected P = 0.045). One SNP (R72P; rs1042522) in TP53 was significantly associated with NTG (P = 0.018). The allele G frequency was higher in NTG patients than in control subjects (56.1% vs. 45.8%; OR = 1.52). The significance of these associations survived the Bonferroni correction (corrected P < 0.024). Other gene variants were not significantly associated with HTG (P > 0.065) or NTG (P > 0.13). None of the studied variants was significantly associated with JOAG (P > 0.17).

CONCLUSIONS. The findings suggest that variants in TNF and TP53 are risk factors for POAG, whereas variants in other studied genes are not major risk factors for POAG, at least in the Chinese population. (Invest Ophthalmol Vis Sci. 2010;51: 4110–4116) DOI:10.1167/iovs.09-4974

Glaucoma is a heterogeneous group of diseases characterized by apoptosis of the retinal ganglion cells and progressive degeneration of the optic nerve. It is the second leading cause of blindness worldwide, estimated to affect more than 60 million people by 2010.1 Primary open-angle glaucoma (POAG) and exfoliation glaucoma (XFG) are common forms of glaucoma in most populations. Family segregation studies have shown that genetic factors make major contributions to the etiology of these diseases.2 Lysyl oxidase-like 1 (LOXL1) was recently associated with XFG as a major gene, accounting for more than 90% of XFG cases in most populations.3–6 However, the genetics of POAG appears to be more complex, and no major associated genes have been identified thus far.7

At least 20 genetic loci have been linked to POAG, 14 of which are designated GLC1A to GLC1N.8 Only three causative genes have been identified from these loci: myocilin (MYOC),9,10 optineurin (OPTN),11,12 and WD repeat domain 36 (WDR36).13 Mutations in these genes account for only approximately 10% of patients with POAG.7,12,13 More than 20 other genes have been associated with POAG, but most of them have not been reported in single studies; only a few of them have been investigated in multiple studies.7,14 However, papers on these associations, such as those for optic atrophy 1 (OPA1), are often conflicting in the different studies, probably due to small samples or ethnic differences.15,16 Thus, further evaluation of these genes is warranted in larger samples and different populations.

Retinal ganglion cell death in glaucoma has been characterized—especially that involving the apoptotic pathway.17 Growing evidence supports the possibility that oxidative stress and mitochondrial dysfunction contribute to retinal ganglion cell death by apoptosis and lead to glaucoma.18,19 In addition, elevated intraocular pressure (IOP) is an important risk factor for glaucoma. Thus, an understanding of the genes that are involved in apoptosis, oxidative stress, mitochondrial dysfunction, and regulating IOP should assist in elucidation of the mechanisms of glaucoma. In the present study, we investigated 10 genes for their association with POAG in a Chinese case-control cohort containing 405 patients with POAG and 201 control subjects. Five of these genes are involved in apoptosis: CDH1 (cadherin 1, type 1, E-cadherin),20 CDKN1A (cyclin-dependent kinase inhibitor 1A),21 MTHR (5,10-methylene tetrahydrofolate reductase),22 TNF (tumor necrosis factor),23 and TP53 (tumor protein p53).24 Two are involved in oxidative stress: GSTM1 (glutathione S-transferase mu 1)25 and GSTT1 (glutathione S-transferase theta 1).26 One is associated with mitochondrial dysfunction: OPA1.15 Two are involved in regulating IOP: CYP1B1 (cytochrome P450, family 1, subfamily B, polypeptide 1)27 and NOS3 (nitric oxide synthase 3).28 Seventeen variants in

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Individuals with homozygous deletions were deemed to have a null genotype and those with one or two copies of the relevant genes had a positive genotype. Single-nucleotide polymorphisms (SNPs) in other genes (CDH1, CDKNIA, CYP1B1, MTHFR, NOS1, OPA1, TNF, and TP53) were genotyped either by SNP genotyping assay (TaqMan; Applied Biosystems [ABI], Foster City, CA) or by direct sequencing. Oligonucleotide primers were obtained from ABI (assay by demand) and the assays were performed according to the manufacturer's instructions. For direct sequencing, products from PCR amplification were purified and sequenced using dye-termination chemistry (BigDye; ABI) and an automated genetic analyzer (model 3130; ABI).

Statistical Analysis
Statistical analyses for the SNPs were performed using PLINK (ver. 1.06), which is a free statistical analysis toolset, designed to perform a range of basic and large-scale analyses for genome-wide association studies in a computationally efficient manner.30 Hardy-Weinberg equilibrium was assessed by using the $\chi^2$ test. The minor allele frequencies of each SNP between patients with HTG, NTG, or JOAG and control subjects were compared by using Fisher's exact test. Odds ratio (OR) and 95% confidence interval (CI) were calculated by using the logistic regression method. Haplotype frequencies were estimated with the standard E-M algorithm and tested with the $\chi^2$ test. The omnibus $P$-value was obtained from the omnibus test. Specific $P$-values were obtained from the haplotype-specific test. OR and 95% CI were calculated for each haplotype compared with all the other haplotypes.

Statistical analyses for the deletions in GSTM1 and GSTTI were performed (SAS ver. 9.1; SAS Institute, Cary, NC). Genotype frequencies of each gene between patients with HTG, NTG, or JOAG and control subjects were compared by Fisher's exact test. Multiple comparisons were corrected by using the Bonferroni method. Gene-wide correction was performed with all studied variants within each gene.

RESULTS
Characteristics and genotype counts of the 17 variants were summarized in Table 2. All SNPs followed Hardy-Weinberg equilibrium in the control group. One promoter SNP (rs1800629 > G; rs1800629) in TNF demonstrated a significant association with HTG ($P = 0.012$; Table 3). The allele G frequency of rs1800629 was higher in the HTG patients than in the control subjects (94.6% vs 90.3%; OR = 1.89; 95% CI, 1.14–3.13). One haplotype consisting of rs1799724 and rs18006629 in TNF was significantly associated with HTG ($P = 0.015$, Bonferroni-corrected $P = 0.045$; Table 4). One nonsynonymous SNP (R72P; rs10425222) in TP53 was significantly associated with NTG ($P = 0.018$; Table 5). The allele G frequency of rs10425222 was higher in the NTG patients than in the control subjects (56.1% vs 45.8%; OR = 1.52; 95% CI, 1.08–2.13). These associations survived the gene-wide Bonferroni correction (corrected $P < 0.024$). Other gene variants were not significantly associated with HTG ($P > 0.063$) or NTG.

Table 1. Demographic and Clinical Features of the Study Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Gender (M/F)</th>
<th>Age at Diagnosis* (y)</th>
<th>Highest IOP (mm Hg)</th>
<th>Vertical Cup–Disc Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>HTG</td>
<td>255</td>
<td>167/88</td>
<td>35–90</td>
<td>62.8 ± 12.4</td>
<td>22–67</td>
</tr>
<tr>
<td>NTG</td>
<td>100</td>
<td>54/46</td>
<td>35–88</td>
<td>63.2 ± 11.5</td>
<td>10–21</td>
</tr>
<tr>
<td>JOAG</td>
<td>50</td>
<td>33/17</td>
<td>4–34</td>
<td>21.3 ± 9.2</td>
<td>25–69</td>
</tr>
<tr>
<td>Control</td>
<td>201</td>
<td>120/81</td>
<td>50–90</td>
<td>69.8 ± 8.7</td>
<td>6–21</td>
</tr>
</tbody>
</table>

* For the control subjects, age at diagnosis refers to age at inclusion.
Except for one SNP rs3176352 in CDKN1A for association with POAG in a Chinese case–control sample. In the present study, we investigated 17 variants in 10 genes associated with JOAG (\(P > 0.17\); Table 3). Replication of the association of these gene variants with POAG in an independent sample is thus warranted. In the present study, only one SNP (rs1800629) in TNF and one SNP (rs1042522) in TP53 were found to be significantly associated with HTG and NTG, respectively. All the other 15 variants were not significantly associated with HTG or NTG. In addition, none of the studied variants was significantly associated with JOAG, suggesting that these genes are not major risk factors for JOAG. Our results suggested that TNF and TP53 have significant roles in the development of POAG. Intriguingly, both TNF and TP53 are important components involved in the apoptosis pathway. In experimental models, an increased TNF expression

### Table 3. Single-Variant Association of the Studied Genes with HTG, NTG, and JOAG

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Minor Allele*</th>
<th>HTG Minor Allele Frequency*</th>
<th>NTG Minor Allele Frequency*</th>
<th>JOAG Minor Allele Frequency*</th>
<th>(P)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH1</td>
<td>rs1801026</td>
<td>T</td>
<td>0.177</td>
<td>0.157</td>
<td>0.163</td>
<td>0.174</td>
</tr>
<tr>
<td>CDKN1A</td>
<td>rs3176352</td>
<td>C</td>
<td>0.415</td>
<td>0.424</td>
<td>0.478</td>
<td>0.401</td>
</tr>
<tr>
<td>CDKN1A</td>
<td>rs1801270</td>
<td>A</td>
<td>0.482</td>
<td>0.450</td>
<td>0.435</td>
<td>0.490</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>rs1800444</td>
<td>G</td>
<td>0.002</td>
<td>0.005</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>rs1056836</td>
<td>G</td>
<td>0.081</td>
<td>0.086</td>
<td>0.098</td>
<td>0.092</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>rs100112</td>
<td>G</td>
<td>0.212</td>
<td>0.207</td>
<td>0.174</td>
<td>0.224</td>
</tr>
<tr>
<td>MTHFR</td>
<td>rs1801133</td>
<td>T</td>
<td>0.216</td>
<td>0.202</td>
<td>0.217</td>
<td>0.179</td>
</tr>
<tr>
<td>NOS3</td>
<td>rs207074</td>
<td>C</td>
<td>0.129</td>
<td>0.101</td>
<td>0.130</td>
<td>0.119</td>
</tr>
<tr>
<td>NOS3</td>
<td>rs3918226</td>
<td>T</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NOS3</td>
<td>rs1799983</td>
<td>T</td>
<td>0.101</td>
<td>0.091</td>
<td>0.163</td>
<td>0.112</td>
</tr>
<tr>
<td>OPA1</td>
<td>rs166850</td>
<td>T</td>
<td>0.069</td>
<td>0.056</td>
<td>0.043</td>
<td>0.072</td>
</tr>
<tr>
<td>OPA1</td>
<td>rs1051941</td>
<td>C</td>
<td>0.351</td>
<td>0.369</td>
<td>0.424</td>
<td>0.373</td>
</tr>
<tr>
<td>TNF</td>
<td>rs1799724</td>
<td>T</td>
<td>0.135</td>
<td>0.096</td>
<td>0.120</td>
<td>0.112</td>
</tr>
<tr>
<td>TNF</td>
<td>rs1800629†</td>
<td>A</td>
<td>0.054</td>
<td>0.061</td>
<td>0.098</td>
<td>0.097</td>
</tr>
<tr>
<td>TP53</td>
<td>rs1042522†</td>
<td>C</td>
<td>0.480</td>
<td>0.439</td>
<td>0.511</td>
<td>0.542</td>
</tr>
<tr>
<td>GSTM1</td>
<td>Gene deletion</td>
<td>Null</td>
<td>0.480</td>
<td>0.414</td>
<td>0.413</td>
<td>0.405</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Gene deletion</td>
<td>Positive</td>
<td>0.488</td>
<td>0.424</td>
<td>0.435</td>
<td>0.470</td>
</tr>
</tbody>
</table>

Significant SNPs and \(P\) values are shown in bold type.

* For GSTM1 and GSTT1, minor allele refers to minor genotype.
† Obtained from Fisher’s exact test versus control subjects. NA, not available.
‡ The allele G frequency of rs1800629 was higher in the HTG patients (94.6%) than in control subjects (90.3%; \(P = 0.012\); OR = 1.89, 95% CI = 1.14–3.13).
§ The allele G frequency of rs1042522 was higher in the NTG patients (56.1%) than in control subjects (45.8%; \(P = 0.018\); OR = 1.52, 95% CI = 1.08–2.15).
level has been demonstrated in glaucomatous eyes,\textsuperscript{31,32} and TNF has been reported to participate directly in the apoptosis of retinal ganglion cells.\textsuperscript{33} \(p53\) is involved in the apoptosis of retinal ganglion cells through acting as a transcription factor and upregulating the expression of the proapoptotic gene \textit{BAX} and downregulating the expression of the antiapoptotic gene \textit{BCL2}.\textsuperscript{34} In zebrafish mutants, loss of \textit{Wdr36} function leads to an activation of the \(p53\) stress-response pathway, suggesting that defects in \(p53\) pathway genes may influence the impact of \textit{WDR36} variants on POAG.\textsuperscript{35}

\textit{TNF} was initially reported to associate with POAG in a Chinese population (Fig. 1). The allele A frequency of the promoter SNP rs1800629 (\(-308G>A\)) was higher in patients with POAG than in control subjects (42.5\% vs. 21.4\%; \(OR = 2.72; 95\% CI, 1.66–4.45\)).\textsuperscript{23} This association was replicated in an Iranian population, with a higher allele A frequency of rs1800629 in patients with POAG than in control subjects (9.3\% vs. 2.5\%; \(OR = 3.99; 95\% CI, 1.71–9.33\)).\textsuperscript{30} However, the frequencies of allele A in both cases and control subjects in the Iranian study were much lower than those reported in the Chinese study (21.4\%).\textsuperscript{23} A study in a Japanese population revealed no significant association between three promoter SNPs (\(-308G>A\), \(-857C>T\) and \(-863C>A\)) of \textit{TNF} and POAG, despite a possible interaction between \textit{TNF} and \textit{OPTN}.\textsuperscript{37} Similarly, a study in a Caucasian population from southern Austria also did not find a significant association between two promoter SNPs (\(-308G>A\) and \(-238G>A\)) of \textit{TNF} and POAG.\textsuperscript{38} In the present study, we identified a significant association between \textit{TNF} and HTG. In contrast to previous studies in which the allele A of rs1800629 was more frequent in POAG patients than in control subjects,\textsuperscript{23,30} we found that the allele G frequency was higher in the HTG patients than in the control subjects (94.6\% vs. 90.3\%; \(OR = 1.89; 95\% CI, 1.14–3.13\); Table 3). Our haplotype analysis further confirmed this association (Table 4). The allele A frequency in our control subjects (9.7\%) was similar to that in the HapMap data from Chinese (3.3\%) but was much lower than that reported in the initial Chinese study (21.4\%).\textsuperscript{23} In a study of lipopolysaccharide-stimulated whole-blood-cell cultures, the allele A of rs1800629 was associated with increased TNF production.\textsuperscript{39} However, investigators in another study were unable to replicate this effect of the allele A on TNF production.\textsuperscript{40} In the present study, the allele A of rs1800629 was found to be protective against POAG, whereas the allele G was a risk factor. A similar protective effect of the allele A has been indicated in other diseases in specific populations, such as XFG in Turks and ischemic stroke in Asians.\textsuperscript{34,41} It remains unclear how the G allele works as a risk factor to influence the development of POAG.

\textit{TP53} was originally shown to be associated with POAG in a Chinese population (Fig. 1). The allele C frequency of the nonsynonymous SNP rs1042522 (\(R72P\)) was higher in POAG patients than in control subjects (57\% vs. 36\%; \(OR = 2.39; 95\% CI, 1.14–5.01\)).\textsuperscript{24} Two studies in Caucasian populations replicated this association and showed that specific haplotypes consisting of rs1042522 and rs17878362 (a 16-bp ins/del polymorphism in intron 3) were significantly associated with POAG.\textsuperscript{43,44} However, no significant association was identified between rs1042522 and POAG in several studies in Indian, Australian, Japanese, Turkish, and Brazilian populations.\textsuperscript{45–49} In addition, no significant association was found between rs17878362 and POAG in Indian and Australian populations.\textsuperscript{45–46} In contrast to the original study in the Chinese, in which the allele C of rs1042522 was more frequent in POAG patients than in control subjects,\textsuperscript{24} we found that the allele G frequency was higher in our NTG patients than in the control subjects.\textsuperscript{24} Our results are consistent with those in a study by Daugherty et al.,\textsuperscript{44} who reported a higher frequency of the

\begin{table}[h]
\centering
\caption{Haplotype Analysis of TNF in Patients with HTG and Control Subjects}
\begin{tabular}{llllll}
\hline
Haplotype & Haplotype Frequency & & & & \\
\hline
 & HTG & Control & \(P\) & OR (95\% CI) & \\
CG & 0.815 & 0.802 & 0.58 & 1.10 (0.79–1.53) & \\
TG & 0.133 & 0.106 & 0.23 & 1.28 (0.86–1.93) & \\
CA* & 0.052 & 0.092 & 0.015 & 0.53 (0.32–0.89) & \\
Total & 1.000 & 1.000 & 0.04† & & \\
\hline
\end{tabular}
\footnote{Haplotype CA shown in bold type was significantly associated with HTG (\(P = 0.015\); Bonferroni-corrected \(P = 0.045\)).}
\footnote{This \(P\) value was obtained from the omnibus test, whereas the other \(P\) values were obtained from the haplotype-specific test. OR was calculated for each haplotype compared with all the other haplotypes.}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{The gene structure of \textit{TNF} and \textit{TP53} and the polymorphic sites for association with POAG published to date. Solid squares: translated coding exons; horizontal lines: introns. The polymorphisms shown inside the squares were investigated in the present study.}
\end{figure}
allele G in POAG patients than in control subjects (80% vs.
71%) and even higher in NTG (84%) patients than in HTG (78%) patients. Note that the allele G frequency in our control
subjects (45.8%) was similar to that in the HapMap data from
Chinese (51.1%) subjects but was much lower than that in the
original Chinese study (64%).24 SNP rs1042522 is a common
sequence polymorphism, located in the proline-rich domain of
p53, which is essential for the protein to fully induce apop-
tosis. In cell lines containing inducible alleles and in cells with
endogenous p53, the allele T frequency was similar in POAG patients, in
an independent and larger Chinese sample. For rs1801026,
endogenous p53, the G allele of rs1042522 was shown to have
a 15-fold increased apoptotic ability compared with the C
allele.50 Thus, it is possible that concurrence of the G allele
with higher risk of POAG is due to increased susceptibility of
retinal ganglion cells to apoptosis. On the basis of p53 involve-
ment in the development of POAG, apoptosis would more
likely have a greater role in NTG than in HTG.16 This hypo-
thesis is further supported by findings of a significant associ-
ation of TP53 with NTG other than HTG in our present study and
the study by Daugherty et al.44

In the present study, no significant association was identi-
fied for CDH1, CDKN1A, CYP1B1, GSTM1, GSTT1, MTHFR,
NOS3, and OPA1, suggesting that genetic variants in these
genes may not be major risk factors for the development of
POAG in Chinese patients.

Association with POAG of a 3’UTR SNP (rs1801026) in the
CDH1 gene and a SNP in exon 2 (rs1801270) of CDKN1A had
been reported in the Chinese.20-21 We did not reveal any
significant association of either SNP with POAG in this study on
an independent and larger Chinese sample. For rs1801026,
whereas the allele T frequency was similar in POAG patients, in
control subjects, it was much lower in our study (17.4%) than
in the original study (52.4%).20 However, our results in control
subjects are very close to statistics in the dbSNP database for Asians (16.7%). As for rs1801270, association with POAG had
not been replicated in two studies of Caucasians.18,51

Rare heterozygous mutations in the CYP1B1 gene have
been consistently reported in POAG, especially in JOAG.27-52
One study of an Indian population has suggested an associ-
ation between a common SNP (rs1056836) in exon 3 of CYP1B1 and
POAG.53 However, our study, like many other reported stud-
ies, did not support the notion that common polymorphisms of
CYP1B1 are associated with the development of POAG.24-27 It is
noteworthy that unlike in Caucasians, the allele G of
rs18001440 was very rare in our Chinese sample, at 0.5% in
patients with POAG, and is completely absent in control sub-
jects, consistent with the HapMap database (1.1% in Chinese).
We think it is more likely that CYP1B1 contributes to only a
small proportion of POAG with Mendelian inheritance. How-
ever, complex forms of POAG are unlikely to be due to com-
mon polymorphisms of CYP1B1.

The GSTM1-positive genotype was first shown to be associ-
ated with POAG in an Estonian population.25 Only one study in a
Turkish population support this finding,26 whereas several
other studies did not find any association.58-59 There were even
reports of a reversed association in which the null genotype
was more common in POAG patients.60-62 The GSTT1 null
genotype was associated with POAG in studies of a Turkish
population20 and of an Arabian population,63 but not in other
Turkish or European studies.25-27-61 In the present study in the
Chinese, we did not find any association of GSTM1 and GSTT1
with POAG.

One SNP in exon 5 (rs18011335) of the MTHFR gene was
associated with POAG in a German population.22 However,
this association with POAG has not yet been replicated in other
studies, including three studies of Caucasians63-65 and two of
Asians,66,67 although one study of Koreans suggested a possible
association with younger NTG.68 In the present study, we did
not find any association between this SNP and POAG in the
Chinese.

A promoter SNP (rs3918226) in the NOS3 gene associated
with POAG was first reported in an Australian population.28
However, another promoter SNP (rs2070744) of NOS3 was not
associated with POAG in two other studies.59,70 In addition,
the 27-bp repeat alleles in intron 4 of NOS3 were associated
with POAG in British and Pakistani populations10,71 but not in
a Chinese population.29 In the present study, we did not find
any association between two NOS3 SNPs (rs2070744 and
rs1799983) and POAG. Of note, the minor allele T of
rs3918226 in NOS3 was not found in our Chinese sample (Table 3).
One recent study in Caucasians demonstrated that three
SNPs of NOS3 (rs3918188, rs2070744, and rs1800779)
were significantly associated with HTG among women but not
among men.72 The other two SNPs (rs1799983 and rs7830) did
not show this sex-dependent association. In the present study,
stratified analysis did not identify a significant difference be-
tween the men and the women in association of two NOS3
SNPs (rs2070744 and rs1799983) with HTG (P heterogeneity
by sex > 0.14; data not shown).

Two SNPs (rs166850 and rs10451941) in intron 8 of the
OPAI gene were initially associated with NTG in a British
population.15 To date, this association has been replicated in
two studies of Caucasians7-6,7 and in one Japanese study,75 but
not in two other studies of Caucasians.16,67 two studies of
Africans,16,76 and one study of Koreans.77 In the present study,
we did not find any association of OPA1 with POAG in our
Chinese sample.

The pathogenesis of POAG is genetically heterogeneous and
complex. Despite several genetic loci and genes that have been
associated with POAG, the major genes that confer significant
susceptibility remain unknown.7,14 In particular, the complex
forms of POAG are most likely caused by interactions of mul-
tiple genes and environmental factors. To date, most associa-
tion studies for POAG have investigated only single genes or
single gene variants without accounting for contributions from
gene-gene and gene-environment interactions. This deficit in
studies of POAG-associated genes may be a main reason for the
conflicting results in different association studies, in addition to
too many differences. An ideal replication study could be per-
fomed in the same population with exactly the same sampling
criteria as in the original study. Unfortunately, almost none of the
association studies published have been replicated thusly.
It is also noted that original studies that identified significant
associations were usually performed in small samples and thus
lack sufficient representation of the study populations, leading
to false-positive associations. Another problem caused by small
samples is lack of sufficient power to detect true associations
in replication studies, leading to false-negative associations.
In this regard, associations obtained from the studies using large
samples are more reliable. Data from the HapMap project have shown obviously dif-
ferent allele frequencies of many genes between populations.
It is therefore crucial to avoid population stratification in ge-
netic association studies. One of the strengths of the present
study is the well-matched cases and control subjects in ethnic-
ity, and thus the observed association in our study is unlikely to
be affected by population stratification. Another major strength
of our study is the larger sample size in both the disease and
control groups that gave our study sufficient power to detect
a true association. In addition, we classified patients with POAG
into three subgroups and analyzed association for HTG, NTG,
and JOAG separately, which also improved the study’s power
by reducing the phenotype heterogeneity.

One limitation of our study is that we did not screen the
whole gene region but investigated significant variants in each
gene as reported by other groups. As a result, we could not rule
out the possibility of the existence of other variants in these genes that might be associated with POAG in our samples. Further comprehensive study of whole genes would be helpful to examine this issue. In addition, the associations we observed in the present study were nominally significant after the gene-wide Bonferroni correction. In genome-wide association studies, up to 500-K SNPs are tested for association and thus the Bonferroni-corrected significance level is usually set as $1 \times 10^{-7}$ (0.05/500 K). However, in the present study, our null hypothesis was that the allele frequencies of all variants tested in a specific gene were not significantly different between cases and control subjects, the gene-wide Bonferroni-corrected significance level was thus set as a function of the number of variants tested in a specific gene. In addition, one critical assumption of the Bonferroni correction is thus considered to be overly conservative, especially for replication studies that have tended to obtain less significant association than the initial studies.

In summary, our findings suggest that variants in $TNF$ and $TP53$ are significant risk factors for POAG. However, variants in other studied genes are not major risk factors for the development of this complex disorder, at least in the Chinese population.

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