Assessment of *TGIF* as a Candidate Gene for Myopia

Kelly K. Pertile,1,2 Maria Schäche,1,2 F. M. Amirul Islam,1,2 Christine Y. Chen,1,2 Mohamed Dirani,1,2 Paul Mitchell,2,3 and Paul N. Baird1,2

**Purpose.** Transforming growth β-induced factor (*TGIF*) has been identified as a candidate gene for high myopia through genetic linkage studies and through its role in ocular growth in animal studies. However, the association of single nucleotide polymorphisms (SNPs), based solely on myopia refraction, has so far been inconclusive. This is the first study conducted to investigate the association of *TGIF* with refraction and ocular biometric measurements.

**Methods.** Twelve tag SNPs (tSNPs) encompassing the *TGIF* gene and 2 kb upstream of its promoter region were used to evaluate the association between *TGIF* variants with both ocular biometric measures and refraction. A total of 257 cases of myopia (spherical equivalent [SE] worse than −0.50 D) and 294 control subjects (no myopia) were genotyped. Genotype frequencies were analyzed by χ² test and one-way ANOVA.

**Results.** Two tSNPs showed significant association with biometric measures, with the SNP rs8082866 being associated with both axial length (P = 0.013) and corneal curvature (P = 0.007) and the SNP rs2020436 being associated with corneal curvature (P = 0.022). However, these associations became nonsignificant after multiple testing (Bonferroni correction).

**Conclusions.** Findings of this study suggest that the *TGIF* gene is unlikely to play a major role in either ocular biometric measures or refraction in a Caucasian population. Further studies should focus on other genes in the MYP2 linkage region or other linked regions to identify myopia-causing genes. (Invest Ophthal Vis Sci. 2008;49:49–54) DOI:10.1167/iovs.07-0896

Myopia is one of the leading causes of visual impairment and blindness in the world, affecting approximately 25% of individuals in Western and European countries.1–4 Ocular components, such as corneal curvature, anterior chamber depth, lens power, and ocular axial length are all seen to be important determinants in the development of myopia.5

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Myopia is a complex disease with both genetic and environmental factors implicated in its pathogenesis. Family studies have shown an increased risk of myopia in children with myopic parents, compared with those with no myopic parents,5–7 as well as a four-fold increased sibling risk.8 In addition, parental myopia has been shown to influence an offspring’s ocular components. Children with two myopic parents have longer eyes than do children with only one myopic parent or no myopic parents.9 A genetic component for ocular biometric measures and refraction has also been demonstrated in population, twin, and family-based studies.10–17

Genetic (genome-wide linkage) studies of myopia have so far identified 15 chromosomal regions harboring a disease gene (MYP1–15). Of these candidate loci, nine have been identified for high myopia (spherical equivalent [SE] −4.25 D or worse; MYP1–5, -11, -12, -13, and -15)18–26 and six for low/moderate myopia (≤ −1.00 D; MYP6–10 and MYP14).12,27,28 Six of these regions (MYP2, -3, -6, -10, and -13) have been replicated in independent linkage studies.29–35 The MYP2 region has been replicated twice in high-myopia families and therefore provides a likely location for a candidate gene in high myopia.19,31,32

The transforming growth β-induced factor (*TGIF*) gene (National Center for Biotechnology Information [NCBI] Entrez Gene ID: 7050), is a good candidate gene for myopia because of its physical location within the MYP2 region and its functional role in ocular development. *TGIF* is expressed in the sclera, retina, cornea, and optic nerve and competitively inhibits its binding to the retinoic acid receptor to a retinoid-responsive promoter.19,36–38 Animal studies using form-deprivation myopia have demonstrated that transforming growth factor (TGIF)-β, which is induced by TGIF, mediates retinal control of ocular growth.39,40

Genetic evidence supporting a role for *TGIF* in myopia has come from analysis of a Chinese cohort where six single-nucleotide polymorphisms (SNPs) were significantly associated with high myopia (≤ −6.00 D).41 However, a significant association with this gene could not be replicated in a second Chinese case–control study of high myopia individuals.42 A Japanese case–control study of high myopia individuals also analyzed this gene by using 13 SNPs across the *TGIF* gene and failed to identify significant association.43 In the only Caucasian study to date, coding regions, and intron–exon boundaries of *TGIF* were sequenced in 10 cases (≤ −6.00 D) from European high-myopia families and 10 unrelated emmetropic control individuals (0.00 D). No significant sequence variants were detected in the high-myopia individuals compared to control subjects.57

Currently published studies of the *TGIF* gene have concentrated on the myopia phenotype (refraction) as the trait of interest. Given that the *TGIF* gene has a biological role in eye growth, it may be more prudent to examine whether association of this gene exists at the individual trait level. As a consequence, we undertook a tag SNP (tSNP) approach to examine association of the *TGIF* gene with not only refraction but also the individual and continuous ocular biometric traits of axial length, corneal curvature, and anterior chamber depth, which would provide an alternative approach to studying myopia candidate genes.
TABLE 1. Refractive Status and Ocular Biometric Measures of Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>High Myopia n = 117</th>
<th>Low/Moderate Myopia n = 140</th>
<th>Emmetropia n = 148</th>
<th>Hypermetropia n = 146</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refraction (SE), D (± 6.00 D)</td>
<td>−6.00 to −19.25 (−8.57) (2.39)</td>
<td>−5.88 to −2.00 (−3.77) (0.96)</td>
<td>−0.50 to 0.75 (0.11) (0.28)</td>
<td>2.12 to 16.63 (4.22) (1.96)</td>
</tr>
<tr>
<td>Axial length, mm (n = 551)</td>
<td>24.42 to 31.41 (26.74) (1.31)</td>
<td>23.27 to 27.53 (24.92) (0.87)</td>
<td>22.08 to 25.53 (23.30) (0.67)</td>
<td>16.11 to 23.44 (22.15) (0.89)</td>
</tr>
<tr>
<td>Anterior chamber depth, mm (n = 416)</td>
<td>2.34 to 4.90 (3.59) (0.46)</td>
<td>2.07 to 4.79 (3.54) (0.39)</td>
<td>2.61 to 5.14 (3.39) (0.39)</td>
<td>2.21 to 5.06 (3.10) (0.44)</td>
</tr>
<tr>
<td>Corneal curvature, D (n = 418)</td>
<td>41.15 to 49.66 (44.13) (1.52)</td>
<td>41.24 to 47.06 (44.27) (1.31)</td>
<td>40.33 to 47.98 (44.06) (1.46)</td>
<td>41.06 to 47.88 (43.98) (1.31)</td>
</tr>
</tbody>
</table>

Data are the range (mean) (SD).

Materials and Methods

Subjects

Individuals used in this study were recruited through the Genes in Myopia (GEM) Study, the GEM Twin Study, the Melbourne Visual Impairment Project (VIP), and the Blue Mountains Eye Study (BMES). The methodologies for each study have been published elsewhere. Individuals from this study were categorized into high myopia (≥ 6.00 D), low/m moderate myopia (≥ −5.99 D and < −0.50 D), emmetropia (−0.50 D and ≤ +0.75 D), and hypermetropia (≥ +0.76 D). Individuals with a history of ocular diseases, such as age-related macular degeneration or keratoconus or eye insufficiency, were excluded from the analysis of this study. The ocular biometric measurements of axial length, corneal curvature (average of K1 and K2), and anterior chamber depth were also obtained. There was no significant difference (P < 0.05) for all eye measurements between the right and left eye; therefore, only the right eye measures were used in the final analysis.

Written informed consent was obtained from all individuals before any testing, and ethics approval was provided by the Human Research Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH), Melbourne. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

SNP Genotyping

Twelve SNPs were identified for the TGIF gene using an SNP tagging approach. This approach began with the identification of all SNPs within the TGIF region (Table 2, Fig. 1). All known SNP markers were tagged by the Tagger program incorporated in the Haploview program, ver. 3.32 to identify the final subset of 12 tSNPs. The tagging criteria included common SNPs that had a minor allele frequency (MAF) of >0.1 and an r² threshold of >0.8 in the CEPH (CEU) population. The CEU population consists of Utah residents with ancestry from northern and western Europe.

Genomic DNA was isolated from peripheral blood lymphocytes of all participants using standard techniques. Genotyping was performed by the Australian Genome Research Facility (AGRFB, Brisbane, Australia) using the MassArray platform and MALDI-TOF analysis (Sequenom, San Diego, CA).

Hardy-Weinberg Equilibrium Test

Genotyping data from the AGRF was assessed with the χ² test for deviations from Hardy-Weinberg equilibrium (HWE). This analysis was performed with the software program JLIN: a Java-based linkage disequilibrium plotter. Any SNPs not passing this test were excluded from further analysis.

Qualitative Genetic Analysis

A series of χ² tests were performed with commercial software (SPSS; ver. 14.0; SPSS Inc, Chicago, IL) to compare the allele and genotype frequencies between affected (individuals with high and low/moderate myopia) individuals and unrelated control subjects (emmetropes and hypermetropes). Seven comparisons were undertaken including (1) any myopia versus no myopia, (2) high myopia versus no myopia, (3) low/moderate myopia versus no myopia, (4) high myopia versus emmetropia, (5) high myopia versus hypermetropia, (6) low/moderate myopia versus emmetropia, and (7) low/moderate myopia versus hypermetropia.

Quantitative Genetic Analysis

Ocular biometric measures of axial length, corneal curvature, and anterior chamber depth were analyzed by using quantitative analysis, performed by comparing the mean value for each trait in a one-way ANOVA test. Sex has been found to be a significant covariate for ocular biometric components; therefore, separate analyses were conducted on the men and the women.

Results

Clinical Data

A total of 551 unrelated subjects (358 women; 193 men; mean age ± SD, 55.41 ± 12.65 years) were included in the study. The cohort consisted of 257 cases and 294 controls, for the number of individuals in each refractive category, refer to Table 1. The mean age of individuals with high myopia was 50.62 ± 13.33 years; low/moderate myopia, 52.09 ± 12.65 years; and emmetropia, 55.12 ± 9.51 years and was 62.70 ± 11.70 years in individuals with hypermetropia. Refraction and ocular biometric measures (axial length, corneal curvature, and anterior chamber depth) for the right eye of participants are described in Table 1.

Tag SNPs Identified and HWE Test

In total, 12 tSNPs were genotyped for TGIF, including 9 in intronic regions, 1 in an exon—intron boundary, 1 in an exon, and 1 upstream of the 5’ region (Table 2, Fig. 1). All known common variants (MAF > 0.1) of TGIF were tagged by the selected panel of tSNPs with r² > 0.8. However, no tSNPs could be selected to cover SNPs at the end of the TGIF gene because there were none that met our criteria. SNPs within this region were rare and had MAF’s less than 0.1 (most had an MAF = 0.0, according to the HapMap data). Therefore, these SNPs were not tagged by our set of 12 tSNPs.
TABLE 2. Quantitative Analysis of tSNPs with Ocular Biometric Measures

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Position (dbSNP 16)</th>
<th>Sequence Variation</th>
<th>Corneal Curvature</th>
<th>Axial Length</th>
<th>Anterior Chamber Depth</th>
<th>Cornus Curvature</th>
<th>N</th>
<th>Female</th>
<th>Male</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11081045</td>
<td>3,420,242</td>
<td>A/T</td>
<td>rs11081045</td>
<td>3,420,242</td>
<td>A/T</td>
<td>rs11081045</td>
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</tr>
</tbody>
</table>

SNP IDs are reference SNP numbers from the public dbSNP database. SNPs with genotype frequencies significantly different within the ocular biometric measures are in bold (P < 0.05); however, after the Bonferroni adjustment, none of these tSNPs are significant (P > 0.05). Based on one-way ANOVA.

Qualitative Genetic Analysis

Using a bivariate approach for refraction (SE), we analyzed the genotype frequencies for each tSNP comparing the groups of "any myopia" versus "no myopia," "high myopia" versus "no myopia," "low/moderate myopia" versus "no myopia," "high myopia" versus "emmetropia," "high myopia" versus "hypermetropia," "low/moderate myopia" versus "emmetropia," and "low/moderate myopia" versus "hypermetropia." None of the 12 tSNPs showed a significant difference of P < 0.05 in genotype frequencies between the groups (Supplementary Table S1, online at http://www.iovs.org/cgi/content/full/49/1/49/DC1).

Quantitative Genetic Analysis

We undertook a quantitative analysis using ocular biometric traits to examine the associations of these tSNPs with continuous data. Measurements of the participant’s axial length, anterior chamber depth, and corneal curvature were all normally distributed. Significant associations were observed for the tSNP rs8082866 with axial length (P = 0.013) and corneal curvature (P = 0.007), as well as for the tSNP rs2020436 with corneal curvature (P = 0.022; Table 2). Since multiple tests were undertaken in this analysis, we used the Bonferroni correction to identify tSNPs that showed significance at P < 0.004. After this correction, none of the previously identified tSNP remained significant.

When the men and women were analyzed separately we found that the tSNPs rs8082866 and rs2020436 were no longer significant. SNP rs238135 previously showed no significant associations when looking at the men and women combined; there was a significant association in the men for corneal curvature (P = 0.054). However, after a Bonferroni correction of P < 0.004, this tSNP did not remain significant.

Discussion

This is the first case-control study to undertake a tSNP approach in a myopia candidate gene to examine associations with refraction and ocular biometric measures.

Previous studies of the TGIF gene have involved either SNP analysis or direct sequencing of coding regions and intron-exon boundaries. Only one study, in a Chinese population, has suggested significant association of six SNPs using "1 high-myopia and 105 unrelated control individuals." All the significant SNPs were located in the equivalent of the current exon 10 of this gene (NCBI Build 36). This region was not covered in our study using tSNPs as all SNPs in this region of the gene had an MAF of <0.1. In addition, the Lam study did not adjust for multiple testing, which is important in identifying false-positive associations. In the previous Japanese study high-myopia cases were defined according to an unconventional control definition of <−9.25 D and >−4.00 D. This definition may have underrepresented high myopia in the range between −9.25 and −4.00 D. Selection of SNPs was through the NCBI dbSNP database based on their population frequency validation, multiple submitters, and high-profile submitters using the public dbSNP database. A more comprehensive way to examine the TGIF gene is through a tSNP approach that efficiently encompasses all the known common
variants and most of the unknown common variants in the gene. This approach also does not require a causative variant to be directly tested, but can highlight regions (haplotypes) that harbor disease-associated variants. Therefore, association studies that incorporate linkage disequilibrium information may offer more power than individual SNP analysis to identify causal genetic variants underlying complex disease.51

Our tag SNP approach identified 12 tSNPs that efficiently tagged common variants with a MAF > 0.1 in the TGIF gene. Using these 12 tSNPs, we undertook association studies using the qualitative measure of refractive error, as previously used in other studies as well as a quantitative analysis based on individual ocular biometric measures. The advantage of this approach is that myopia most likely represents a phenotype based on a varied etiological spectrum of environmental and genetic effects. Thus, the examination of individual quantitative traits may be more useful in identifying specific genetic drivers that underlie this condition. We were able to confirm that the TGIF gene was not associated with high, moderate, or low myopia in our population. However, our association study with biometric measures indicated a significant association (P < 0.05) of the tSNP rs8082866 with both axial length and corneal curvature, whereas the tSNP rs2020436 was associated only with corneal curvature. Through breaking the sample group down into male and female components and analyzing these separately, we hoped that the association would be strengthened if the underlying genetic variant was different in both sexes, but the association was not strengthened. However, dividing the group into men and women evidently reduced the sample size and this reduction may caused a positive result to be undetectable.

One of the main issues in association studies is how to evaluate the significance of multiple testing of SNPs. The Bonferroni correction is commonly applied, but it is usually too conservative, whereas an alternative approach would be to use replication of a nominal probability in a second data set. This method is less stringent; however, we did not have access to a second dataset, and so we applied the Bonferroni correction. Significant associations for this study would therefore require an adjusted P < 0.004. None of the previously significant tSNPs were significant at this level. We realize that this correction is a limitation and may lead to loss of significant findings, but in light of not having verification, this approach appeared to be the most efficacious. A larger case–control study of the two tSNPs significant before Bonferroni correction in a separate population would be a more definitive way to determine whether our findings are real or false.

In this study, tSNPs with an MAF > 0.1 were analyzed, which excluded the 3’ end of TGIF. This meant that we were unable to test those SNPs initially identified by Lam et al.41 in what is now identified as exon 10 (exon 3 in the Lam study). Although our selection parameter would tag common variants of the TGIF gene (MAF > 0.1), we cannot exclude the possibility that other rarer variants in this gene, not in linkage disequilibrium with our tSNPs, might be associated with myopia. It is plausible that SNPs with a minor allele frequency of <10% could still have a major effect on a common trait, such as myopia. There are currently two views on allelic frequencies and common diseases: the common disease/common allele hypothesis and the common disease/multiple rare allele hypothesis. We have assessed the first hypothesis to check whether common variants contribute to myopia susceptibility;
however, we cannot rule out the second hypothesis of alleles with low population frequencies being responsible for susceptibility to myopia.

Assessment of ocular biometric measures as quantitative traits is a novel approach to assess association of SNPs from the TGIF gene. Phenotypic definitions of myopia based on refraction vary greatly between studies, and defining myopia as “high,” “moderate,” and “low” tends to limit statistical power by defining myopia as a series of categories rather than as a continuum. Furthermore, the underlying biology of refraction suggests that it is probably influenced by both genes and environmental factors, of which several traits including the ocular biometric components of axial length, anterior chamber depth, and corneal curvature are implicated. Although these underlying components have been shown to be influenced by a genetic component,10,11,15,16,52 the exact genes underlying each of these traits has so far not been identified.

In conclusion, this is the first case–control association study to evaluate all ranges of refraction as well as ocular biometric measures in a Caucasian population. The lack of significant association with TGIF tSNPs suggests that TGIF is an unlikely candidate gene for myopia and its underlying ocular biometric determinants. We have also shown that sex is not a significant covariate for ocular biometric traits which is in contrast to previously published data.11,16 Recent studies, however, have implicated the hepatocyte growth factor gene (HGFG in high myopia53 as well as two collagen genes associated with myopia54,55. The HGF gene was analyzed in a high-myopia family-based association study of Han Chinese, the collagen type I alpha 1 (COL1A1) gene with high myopia in a Japanese cohort44 and the collagen type II alpha 1 (COL2A1) gene with common forms of myopia in a predominantly Caucasian population.55 These genes are implicated in eye growth and may provide alternative candidate genes for further exploration in the analysis of biometric traits and myopia. Future investigations in identifying myopia candidate genes should therefore focus on genes located in the MYP2 region and other myopia-linked regions as well as genes involved with eye growth, as we have now shown that the likelihood that TGIF is a good candidate gene for myopia is low.

References