Replication Study Supports CTNND2 as a Susceptibility Gene for High Myopia

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PURPOSE. The CTNND2 gene is located in the linkage interval of high myopia locus MYP16 and two single-nucleotide polymorphisms (SNPs; rs6885224 and rs12716080) in CTNND2 were recently shown to associate with high myopia. This study evaluated such associations in an independent case-control series.

METHODS. A total of 2773 unrelated individuals were enrolled in this study, including 1203 subjects with high myopia (spherical refraction at each meridian ≤ −6.00 D), 615 subjects with moderate myopia (−6.00 D < spherical refraction ≤ −4.00 D), and 955 controls (−0.50 D to +1.00 D, spherical equivalent). Genomic DNA was prepared from venous leukocytes. SNPs rs6885224 and rs12716080 in CTNND2 were determined by Sanger sequencing. Allele and genotype frequencies of the SNPs were compared between cases and controls by χ2 test (α = 0.05).

RESULTS. One SNP, rs6885224, in CTNND2 showed significant differences in genotype and allele frequencies between high myopia and controls (genotype P = 2.17E−5, allele P = 5.29E−6; odds ratio [OR] = 0.69, 95% confidence interval [CI] = 0.591–0.812), as well as between moderate myopia and controls (genotype P = 0.009, allele P = 0.005, OR = 0.765, 95% CI = 0.633–0.924). rs12716080 showed no statistical difference between myopias and controls.

CONCLUSIONS. These results confirmed the strong association between CTNND2 polymorphism and myopia. The minor allele C of rs6885224 was protective against myopia in this study but was a risk allele in a previous study. (Invest Ophthalmol Vis Sci. 2011;52:8258–8261) DOI:10.1167/iovs.11-7914

Myopia is the most common cause of visual impairment, with an average prevalence of 30% worldwide.1–3 It affects approximately 50% to 70% of populations in some urban areas in East Asia.4–9 High myopia, defined as refractive error equal to or greater than −6 D, is a leading cause of blindness due to its associated myopic retinopathy and other complications.9,10 Both environmental factors and genetic factors contribute to myopia development,11–15 whereas the molecular mechanism of myopia is still undetermined.

Molecular genetic study provides a unique tool to investigate the molecular basis of myopia. Linkage studies have mapped at least 17 myopia susceptibility loci, such as MYP1–3 and MYP5–18.16–29 Genome-wide association studies (GWAS) have identified single-nucleotide polymorphisms (SNPs) in different chromosomal regions significantly associated with myopia.30–32 However, the exact responsible genes in these loci await further studies.

Of those myopia susceptible loci, one, MYP16 (MIM 612554), was mapped to chromosome 5p15.33–p15.2 based on linkage evidence of three Chinese families with autosomal dominant high myopia.27 Recently, a GWAS study identified a strong association between high myopia and genetic variations in CTNND2,33 which is located inside the linkage interval of MYP16. It would be of interest to know whether this association can be replicated by independent studies. In this study, association of the two reported SNPs (rs6885224 and rs12716080 in CTNND2) with myopia was evaluated in an independent case-control series.

Materials and Methods

Subjects

Written informed consent, conforming to the tenets of the Declaration of Helsinki, was obtained from the participating individuals or their guardians before the collection of clinical data and genomic samples in this study. The Institutional Review Board of Zhongshan Ophthalmic Center approved this study.

A total of 2773 unrelated Chinese subjects were collected at the Ophthalmic Genetic and Molecular Biology Laboratory of Zhongshan Ophthalmic Center, including 1203 subjects with high myopia (spherical refraction at each meridian ≤ −6.00 D), 615 subjects with moderate myopia (−6.00 D < spherical refraction ≤ −4.00 D), and 955 controls (−0.50 D to +1.00 D, spherical equivalent).

The criteria for 1203 subjects with high myopia were as follows: spherical refraction ≤ −6.00 D and exclusion of other known ocular or systemic diseases. The criteria for 615 subjects with moderate myopia were: −6.00 D < spherical refraction ≤ −4.00 D; best corrected visual acuity of ≥0.8; myopia occurred during school age without family history of high myopia; college students with at least 12 years education in schools; and exclusion of other known eye or systemic diseases. The control individuals met the following criteria: received at least 12 years education in schools; bilateral refraction between −0.50 D and +1.00 D (spherical equivalent); best unaided visual acuity of ≥1.0; without family history of high myopia; and without other known eye or systemic diseases. A cutoff of −4.00 D for moderate myopia is based on reported refractive errors development in Hong Kong children.34–36 According to the reports, the average annual change in spherical equivalent refraction (SER) for children with myopia (SER ≤ −0.50 D) was −0.63 D, compared with −0.29 D
for those who were not myopic at the beginning of the study. Then those children who were not myopic at the beginning of study would develop a refractive of −3.6 D (−0.29 D times 12 school years) until university age. Myopia subjects with genetic inclination in addition to the environmental factors should carry a refractive error lower than −3.60 D (more myopic) at university age. So a cut-off of −4.00 D for moderate myopia was chosen, given that this study aimed to reveal genetic basis for myopia susceptibility.4

The results of ophthalmologic examinations were recorded, including vision acuity (unaided, near, and/or best), color vision, slit-lamp, and direct ophthalmoscope examination. Refractive errors were measured with an auto refractometer (Topcon KR-8000, Paramus, NJ) after cycloplegia (Mydrin-P, Santen Pharmaceutical Co. Ltd., Osaka, Japan). Ocular biometric axial length was measured by an optical biometer (IOL Master V5.0; Carl Zeiss Meditec AG, Jena, Germany). Additional examinations, including an electroretinogram and fundus photograph, were taken in selected individuals. The refractions had been compared between right eye and left eye in the subjects, and no statistical difference existed, so we selected the data of right eye for analysis.

Genotyping

Genomic DNA was prepared from venous leukocytes of each individual using the standard phenol/chloroform method. The SNPs rs6885224 and rs12716080 were genotyped by Sanger sequencing. Primers used for amplification and sequencing are listed in Table 1. Sequencing was performed with a cycle sequencing kit (ABI BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA), using a genetic analyzer (ABI 3100 Genetic Analyzer; Applied Biosystems). Sequencing results from all the subjects were compared with CTNND2 consensus sequence (National Center for Biotechnology Information, Build37.2 NG_023544.1) using DNA and protein sequence analysis software (SeqMan II program of the Lasergene package; DNAStar Inc., Madison, WI).

Statistical Analysis

Commercial analytical software (SPSS ver. 13; SPSS Science, Chicago, IL) was applied for computing all the data. Hardy–Weinberg equilibrium (HWE) was initially evaluated for each SNP distribution in each group. The respective allele and genotype frequencies of each SNP were compared between myopia and controls by χ² test (α = 0.05), and P < 0.05 was considered as statistically significant between myopias and controls. The minor allele frequency, minor allele odds ratio (OR), and its 95% confidence interval (95% CI) were calculated to estimate the effect size of the minor allele on myopia. Software (Haploview 4.2) was used to calculate r², which indicates the extent of linkage disequilibrium of these two SNPs.

Results

Basic information of the subjects is listed in Table 2. Genotyping of the two SNPs (rs12716080 and rs6885224) in CTNND2 was successful for all 2773 subjects. These two SNPs in each group were in HWE. One SNP, rs6885224, showed significant differences in genotype and allele frequencies between high myopia and controls (genotype P = 2.17E×10⁻⁵, allele P = 5.29E×10⁻⁶, OR = 0.69, 95% CI = 0.591–0.812), as well as between moderate myopia and controls (genotype P = 0.009, allele P = 0.005, OR = 0.765, 95% CI = 0.635–0.924). The other SNP, rs12716080, showed no significant differences either between high myopia and controls or between moderate myopia and controls (Table 3). Based on the genotype data of 2773 samples, r² = 0.517 (<0.80) was figured out, which indicates that these two SNPs are not in linkage disequilibrium. The minor allele C of rs6885224 was protective against myopia in this study but was a risk allele in the previous study.35

Discussion

Previously, a genome-wide linkage scan mapped a myopia locus (MYP16, MIM 612554) on chromosome 5p15.33-p15.2, based on analysis of three Chinese families with autosomal dominant high myopia living in Hong Kong.27 This locus was further supported by an additional genome-wide linkage scan of Asian families.37 Recently, a GWAS study on Singapore Chinese revealed that genetic variations in the noncoding region of CTNND2, the SNPs rs6885224 and rs12716080, were associated with high myopia.35 The CTNND2 gene is located inside the linkage interval of MYP16. In this study, the SNPs rs6885224 and rs12716080 in CTNND2 were analyzed in 1818 myopia subjects (1203 with high myopia and 615 with moderate myopia) and 955 normal controls. Our results not only confirmed the association between rs6885224 in CTNND2 and high myopia, but also suggested that this SNP may associate with moderate myopia in the Chinese population. These lines of evidence suggested that MYP16 might be a common locus responsible for high myopia in Chinese as well as in Japanese. Whether the genetic variants in CTNND2 are associated with high myopia in other populations requires further studies.

CTNND2 encodes an adhesive junction associated protein of the armadillo/β-catenina super family, which interacts with several transcriptional factors such as Pax6, E2F1, and Hes136–39 and is involved in brain and eye development and cancer formation.41,42 Heterozygous deletion of CTNND2 was

Table 1. Primers Used for Amplification and Sequencing

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer Sequence (5’→3’)</th>
<th>Amplicon Length (bp)</th>
<th>Annealing Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12716080-F</td>
<td>ACGCCCTGCGACACTACAAGA</td>
<td>383</td>
<td>62</td>
</tr>
<tr>
<td>rs12716080-R</td>
<td>ACCGCTGGGACTCTACAAAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs6885224-F</td>
<td>ATGGCCCTGGGTTTCTTTTT</td>
<td>222</td>
<td>58</td>
</tr>
<tr>
<td>rs6885224-R</td>
<td>TCTGCCACCATATCGTCATCA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Basic Information of the 2773 Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>High Myopia</th>
<th>Moderate Myopia</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>1203</td>
<td>615</td>
<td>955</td>
</tr>
<tr>
<td>Refraction</td>
<td>S ≤ -6.00 D</td>
<td>-6.00 D ≤ S ≤ -4.00 D</td>
<td>0.50 D ≤ S ≤ 1.00 D</td>
</tr>
<tr>
<td>Age, y</td>
<td>18.53 ± 6.64</td>
<td>20.70 ± 1.59</td>
<td>24.49 ± 2.82</td>
</tr>
<tr>
<td>Sex, male</td>
<td>49.33%</td>
<td>58.47%</td>
<td>50.30%</td>
</tr>
<tr>
<td>Mean refraction</td>
<td>-8.56 ± 1.66D</td>
<td>-5.06 ± 0.52 D</td>
<td>0.17 ± 0.46 D</td>
</tr>
</tbody>
</table>
found in Cri-du-Chat syndrome,\textsuperscript{43} but increased expression of \textit{CTNND2} was associated with prostate tumors and breast tumors.\textsuperscript{44–45} Whether expression of \textit{CTNND2} was altered in myopia individuals is worthy of further study.

The SNP rs6885224 is situated in the noncoding region of \textit{CTNND2}. A 20-kilobase genomic DNA region encompassing rs6885224 in \textit{CTNND2} is speculated to regulate mRNA transcription (http://genome.ucsc.edu/; University of California, Santa Cruz, Santa Cruz, CA) and, therefore, may affect the expression of gene \textit{CTNND2}. Similarly, the noncoding region was also suggested to play a possible regulatory role in two other GWAS studies for myopia.\textsuperscript{31–32} where significant associations were found between genomic regions at 15q14 and 15q25 and myopia but there were no coding sequence variations.

The association was found only for rs6885224 but not for rs12716080 in this study. A previous study suggested that these two SNPs are not in linkage disequilibrium, with $r^2 = 0.517$. This may explain that only one of the two SNPs is associated with myopia in our study.

As observed in this study, the effect direction of the minor allele at rs6885224 was in the opposite direction compared with the original study.\textsuperscript{35} Several previous association studies on other diseases have also reported that the minor allele was observed in the opposite direction for a certain disease in different studies.\textsuperscript{46–52} Lin et al.\textsuperscript{35} demonstrated that multilocus effects and variation in interlocus correlations contributed to this phenomenon. One possible explanation is that the ancestry variations predisposed to myopia may occur independently on different alleles in different study populations. Elucidating the functional variations predisposing to myopia at this locus in further studies may lead to the understanding of this mystery.

In conclusion, our study supports the association between SNP rs6885224 in \textit{CTNND2} and high myopia. This result together with previous linkage and GWAS studies imply that the genomic region around rs6885224 in \textit{CTNND2} may be an important locus predisposing myopia in Chinese population. Additional studies are expected to reveal how the SNPs in \textit{CTNND2} could be associated with high myopia.

### Acknowledgments

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