Clinical Ophthalmology

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ORIGINAL RESEARCH

Associations between *CRYBA4* gene variants and high myopia in a Japanese population

This article was published in the following Dove Press journal:
Clinical Ophthalmology

Clinical Ophthalmology downloaded from https://www.dovepress.com/ by 3.234.214.113 on 14-Dec-2019
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Purpose: The crystallin beta A4 (*CRYBA4*) gene variant, rs2009066, was previously reported to be associated with high myopia in a southern Chinese population. In the present study, we investigated whether *CRYBA4* variants were associated with high myopia in a Japanese population.

Methods: We recruited 1,063 Japanese patients with high myopia (spherical equivalent [SE] ≤−9.00 D in both eyes) and 1,009 healthy Japanese subjects (SE >−1.00 D). We genotyped rs2009066 and three tagging single-nucleotide polymorphisms (SNPs), rs16982456, rs2071861, and rs4276, in the *CRYBA4* region.

Results: We did not find any significant association between these four SNPs and high myopia in an allele analysis. However, rs2009066 and rs2071861, which were in strong linkage disequilibrium (LD; r^2=0.86), showed a marginal association with high myopia in the recessive genotype model of risk alleles (rs2009066 G allele: P=0.032, odds ratio [OR] =1.31; rs2071861 A allele: P=0.037, OR =1.31). Nevertheless, this association became insignificant after correcting for multiple testing (Pc >0.05).

Conclusion: This study showed no significant association between *CRYBA4* variants and high myopia in a Japanese population. Our findings did not correspond with a previous study. Further genetic studies with other populations are needed to elucidate a potential contribution of the *CRYBA4* region in the development of high myopia.

Keywords: high myopia, *CRYBA4*, association study, polymorphism

Introduction

Myopia is the most frequent ocular disorder worldwide in the modern world.[1] High myopia is distinguished from common myopia, based on a spherical equivalent (SE) refractive error of <−6 D or an axial length (AL) of >26 mm. It is well known that high myopia is associated with an increased risk of various ocular diseases, including retinal detachment, glaucoma, and cataract.[2] The prevalence of high myopia in East Asian and Southeast Asian populations is higher than in Caucasian populations.[3–7]

Although the pathogenesis of high myopia remains uncertain, previous studies have indicated that genetic and environmental factors, such as education, are involved in the progression of high myopia.[8–13] Familial linkage studies and twin studies have identified 25 myopia loci (MYP1 to MYP25) (OMIM; http://omim.org/),[14] and many genome-wide association studies have dealt with several candidate loci/genes for myopia, refractive error, and/or elongation of the AL in several ethnic populations.[15–20] Most of the identified genes were not confirmed in follow-up studies on either common or high myopia, and it is not yet apparent whether the same genetic background is associated with both myopias.

Family-based linkage analyses have identified a large number of chromosomal regions, for example, in the MYP loci (*MYP1–3*, *MYP5–6*, *MYP15–17*, *MYP18–19*,

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Dovepress
http://dx.doi.org/10.2147/OPTH.S146038

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MYP21–22, *MYP24–25*), associated with high myopia (OMIM; http://omim.org/).[21] Among these, we focused on the *MYP6* locus on chromosome 22q13.33 containing the SCO2 cytochrome c oxidase assembly protein (*SCO2*) gene, which encodes a copper chaperone required for cytochrome c oxidase assembly.[22] Tran-Viet et al showed that heterozygous mutations in the *SCO2* gene cause high-grade myopia in patients in the USA and that *SCO2* expression was significantly downregulated in myopic mouse retinae.[23] Another study also identified other heterozygous mutations in *SCO2* causing high myopia in the People's Republic of China.[24] These findings suggest *SCO2* mutations are an important risk factor for the development of high myopia. However, Wakazono et al suggested that *SCO2* mutations may play a limited role in extreme myopia among Japanese patients.[25] Furthermore, the *SCO2* E140K mutation identified by Tran-Viet et al was not associated with high myopia in a Poland population.[26] Thus, in the *MYP6* locus, the contribution of *SCO2* to myopia development is still unclear.

Recently, Ho et al performed a case–control association study with 26 potential candidate genes in a southern Chinese population to identify myopia susceptibility genes in the *MYP6* locus. That study identified a novel gene, crystallin beta A4 (*CRYBA4*), which conferred susceptibility to high myopia.[27] *CRYBA4* is part of a four-gene cluster of β-crystallin spanning approximately 1.4 Mbp on chromosome 22. Previous studies[28,29] have shown that missense mutations in the *CRYBA4* gene are involved in cataractogenesis and microphthalmia and indicated that CRYBA4 plays an important role in eye development. These evidences suggest that *CRYBA4* variants may be a risk factor for the development of high myopia.

To date, no replication study for the findings on *CRYBA4* has been performed in other ethnic populations. The aim of the current study was to investigate whether *CRYBA4* variants were associated with high myopia in a Japanese population. Here, we assessed multiple single-nucleotide polymorphisms (SNPs) in the *CRYBA4* gene to determine their association with the risk of disease in Japanese patients with high myopia.

Methods

Subjects

We recruited 1,063 unrelated Japanese individuals with high myopia (SE ≤−9.00 D in both eyes) and 1,009 unrelated healthy Japanese control subjects (SE >−1.00 D in both eyes) at Yokohama City University, Okada Eye Clinic, and Aoto Eye Clinic in Japan. All participants were unrelated to each other, but had similar social backgrounds and resided in the same urban area. They were diagnosed with comprehensive ophthalmologic tests, which included an AL measurement, a fundus examination, and determinations of spherical power and corneal curvature (Autorefractor; NIDEK ARK-730A, ARK-700A TOPCON KP-8100P, BIO & PACHY Meter AL-2000; Tomey Corporation). The subjects with high myopia had no known genetic diseases associated with myopia and/or high myopia, including glaucoma, keratoconus, or Marfan syndrome. We excluded individuals younger than 20 years old from the control cohort to exclude individuals with potential myopia. Written informed consent was obtained from all participants. The study methodology adhered to the tenets of the Declaration of Helsinki, and the study was approved by the relevant Ethics Committees at Yokohama City University, Okada Eye Clinic, and Aoto Eye Clinic.

SNP genotyping of the *CRYBA4* gene region

Genomic DNA was extracted from peripheral blood samples with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Procedures were performed under standardized conditions to prevent variation in DNA quality.

We evaluated rs2009066, which is located 3 kb downstream of the *CRYBA4* gene. rs2009066 was previously reported to be the gene most significantly associated with high myopia.[25] We also selected three tagging SNPs (rs16982456, rs2071861, and rs4276) that covered the entire *CRYBA4* gene, including 10 kb upstream and downstream (minor allele frequency ≥5%, pairwise r^2≥0.8), with the LD tag SNP selection tool in the SNPinfo web server (https://snpinfo.niehs.nih.gov/). Genotyping was performed with the polymerase chain reaction (PCR) method; we used the TaqMan 5′ exonuclease assay with validated TaqMan primer-probe sets supplied by Applied Biosystems (Foster City, CA, USA). The PCR mixture (total volume, 10 μL) contained 1× TaqMan Universal PCR Master Mix (Applied Biosystems), 24 nM of each primer-probe set, and 3 ng genomic DNA. The PCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. Probe fluorescence was detected with the StepOne Plus Real-Time PCR System (Applied Biosystems).

Statistical analysis

Allele frequencies, genotype frequencies, Hardy–Weinberg equilibrium (HWE), and LD were assessed with SNP & Variation Suite 8.4.0 software (Golden Helix, Inc., Bozeman,

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MT, USA; http://www.goldenhelix.com), SNPStats software (Catalan Institute of Oncology, Barcelona, Spain; http://bioinfo.iconcologia.net/SNPstats),[30] and Haploview 4.1 software.[31] The obtained *P*-values were corrected for multiple hypothesis testing with Bonferroni's method. A corrected *P* (*Pc*) value <0.05 was considered significant. Multiple inheritance models were used to analyze genotype data in assessing each risk allele. These inheritance models were additive (R/R vs R/nR vs nR/nR), dominant (R/R + R/nR vs nR/nR), and recessive (R/R vs R/nR + nR/nR), where R was the risk allele and nR was the non-risk allele. *P*-values and odds ratios (ORs) in genotype models were adjusted for age and sex (P_{adj} and OR_{adj}, respectively).

Results

The clinical characteristics of the study populations are shown in Table 1. Patient age ranged from 13 to 72 years (mean 38.7±11.8 years), and 43.1% of patients were male. The average SEs of the patients were −11.21±1.92 D in the right eye and −11.13±1.95 D in the left eye. The average ALs of the patients were 27.64±1.18 mm (range 23.92–33.85 mm) for the right eye and 27.60±1.16 mm (range 23.99–33.17 mm) for the left eye. The ages of controls ranged from 20 to 87 years (mean 58.3±12.2 years), and 44.3% were male. The average SEs were 0.49±0.64 D (range −0.75 to 3.50 D) in the right eye and 0.49±0.63 D (range −0.75 to 3.00 D) in the left eye. The average ALs were 23.22±0.80 mm (range 18.76–25.96 mm) and 23.20±0.79 mm (range 18.99–26.05 mm) for the right and left eyes, respectively.

Table 1 Clinical characteristics of the study populations

Parameters	High myopia[a] (n=1,063)	Controls[b] (n=1,009)
Male (%)	43.1	44.3
Mean age[c] (years)	38.7±11.8	58.3±12.2
Age range (years)	13–72	20–87
Mean SE[c] (D)		
Right eyes	−11.21±1.92	0.49±0.64
Left eyes	−11.13±1.95	0.49±0.63
Range of SE (D)		
Right eyes	−9.00 to −22.75	−0.75 to 3.50
Left eyes	−9.00 to −24.50	−0.75 to 3.00
Mean AL[c] (mm)		
Right eyes	27.64±1.18	23.22±0.80
Left eyes	27.60±1.16	23.20±0.79
Range of AL (mm)		
Right eyes	23.92–33.85	18.76–25.96
Left eyes	23.99–33.17	18.99–26.05

Notes: [a]High myopia cases defined as SE ≤−9.0 D in both eyes. [b]Controls are defined as SE >−1.0 D in both eyes. [c]Data presented are mean ± standard deviation unless otherwise indicated.
Abbreviations: SE, spherical equivalent; AL, axial length.

Among the controls, the genotype frequencies of all four SNPs analyzed were in HWE (*P*>0.05). Figure 1 shows the overall LD patterns for the four SNPs. These four SNPs were located in one haplotype block, and three SNPs (rs2071861, rs4276, and rs2009066) were in strong LD with each other ($r^2 \geq 0.41$).

Table 2 shows the allelic association results for the four SNPs. None of the four SNPs showed any significant association with high myopia. Table 3 shows the genotype association results of the four SNPs, calculated for each of the three inheritance models (additive, dominant, and recessive models). No significant association was found for any of the four SNPs in the additive or dominant models. On the other hand, rs2009066 and rs2071861, which were in almost complete LD (r^2=0.86), showed significant differences between cases and controls in the recessive model of risk alleles (*P*=0.032, OR =1.31 and *P*=0.037, OR =1.31, respectively). However, the differences did not remain significant after applying the Bonferroni correction for multiple testing (*Pc* >0.05). We did not find any significant

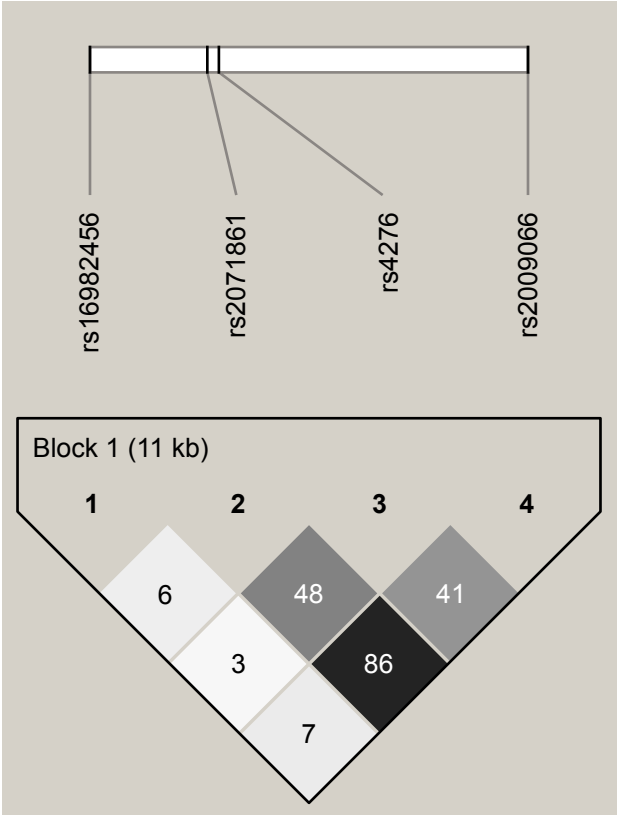

Figure 1 Linkage disequilibrium plot of four *CRYBA4* SNPs for 2,072 study participants.
Notes: The r^2 value corresponding to each SNP pair is expressed as a percentage, and it is shown within the respective diamond. The darker shades of black represent greater r^2 values.
Abbreviation: SNP, single-nucleotide polymorphism.

Table 2 Allelic association results for *CRYBA4* SNPs

SNP	Position[a]	Allele	Risk allele	Risk allele frequency (%)		*P*-value	OR (95% CI)
				Cases	Controls		
rs16982456	27018049	C/T	T	94.2	94.0	0.723	1.05 (0.81–1.36)
rs2071861	27021189	A/G	A	52.6	50.6	0.211	1.08 (0.96–1.22)
rs4276	27021425	A/G	A	34.7	33.7	0.533	1.04 (0.92–1.18)
rs2009066	27029545	A/G	G	55.1	53.6	0.345	1.06 (0.94–1.20)

Note: [a]Position on chromosome 22 according to the human assembly GRCh37.
Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

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association between the other two SNPs and high myopia in the recessive model.

Discussion

High myopia is characterized by AL elongation and thinning of the sclera,[32–34] but there are several distinct clinical phenotypes.[35–37] High myopia is thought to be caused by multiple genes interacting with the environment. Although many candidate gene studies[4–9] and genome-wide association studies[10–16] have been conducted, the genetic basis for susceptibility to high myopia has not been fully elucidated.

Ho et al recently identified *CRYBA4* as a new gene associated with susceptibility to high myopia. They performed a case–control association study using southern Chinese subjects in Hong Kong.[27] They reported that rs2009066 was the SNP most significantly associated with high myopia and its risk allele G had a dominant inheritance effect and a high OR of 1.74.[27] In the current study, we found a lack of association between *CRYBA4* variants and high myopia in our Japanese population, although the rs2009066 allele G had a possible effect (OR =1.31, but not significant) on disease risk in the recessive inheritance model which is inconsistent with observations from the previous study. This discrepancy may result from complex differences between the two studies. Indeed, these two studies focused on different ethnic groups and had different sample sizes; moreover, there may have been differences in environmental factors, and possibly, genotyping errors.

One of the possible limitations in the present and previous studies is the medium sample size (1,063 patients and 1,009 controls from Japan, 658 patients and 655 controls from Hong Kong). Limited sample sizes can sometimes lead to false-positive or false-negative results in a case–control association study. Various complex diseases often involve the integration of multiple genetic and environmental factors, and risk alleles for such diseases confer small effect sizes (OR <1.5).[38] Therefore, it is suggested that the sample sizes in these two studies may have not enough statistical power to accurately detect the association between *CRYBA4* variants and high myopia (a common disease with small effect sizes).

The structural stability of crystallins is important for maintaining transparency and refractive error in the lens.[39] In the human lens, there are three major crystallin classes, α-crystallin, β-crystallin, and γ-crystallin, which contain 40%, 35%, and 25% of total crystallin proteins, respectively.[28] β-crystallins are the major constituents of human lens.[40] CRYBA4 is a 196-amino-acid protein and constitutes 5% of the total crystallins in the young human lens.[40] The expression of CRYBA4 is limited to the lens fiber cells; it is not found in the retina, cornea, iris, choroid, sclera, etc.[41] *CRYBA4* mutations have been reported to be involved in cataractogenesis and microphthalmia,[28,29] and it has been suggested that *CRYBA4* variants may affect eye development. Considering the functional role of CRYBA4, it is not surprising that it has been associated with ocular abnormalities, including myopia. However, only one study has reported that

Table 3 Genotype association results for *CRYBA4* SNPs

SNP	Allele	Risk allele	Genotype ((R/R)/(R/nR)/(nR/nR)) frequency (%)		Genetic models[a]						
			Cases	Controls	Additive model		Dominant model		Recessive model		
					P-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	*Pc*	OR (95% CI)
rs16982456	C/T	T	88.7/11.0/0.3	88.4/11.1/0.5	0.899	1.02 (0.73–1.43)	0.999	1.00 (0.18–5.48)	0.894		1.02 (0.72–1.46)
rs2071861	A/G	A	28.1/48.9/23.0	24.3/52.7/23.0	0.437	1.07 (0.91–1.25)	0.370	0.88 (0.68–1.16)	0.037	0.150	1.31 (1.02–1.69)
rs4276	A/G	A	12.0/45.2/42.7	10.7/46.1/43.2	0.526	1.06 (0.89–1.25)	0.773	1.03 (0.82–1.30)	0.380		1.17 (0.82–1.66)
rs2009066	A/G	G	30.6/49.0/20.4	27.2/52.9/19.9	0.256	1.10 (0.93–1.29)	0.638	0.93 (0.70–1.24)	0.032	0.128	1.31 (1.02–1.68)

Notes: [a]To assess each risk allele, genotype data were analyzed with multiple inheritance models, as follows: additive (R/R vs R/nR vs nR/nR), dominant (R/R + R/nR vs nR/nR), and recessive (R/R vs R/nR + nR/nR). *P*-values and ORs were adjusted for age and sex.
Abbreviations: SNP, single-nucleotide polymorphism; R, risk allele; nR, non-risk allele; OR, odds ratio; CI, confidence interval; *Pc*, corrected *P*-value.

rs2009066, which is located near the *CRYBA4* gene, was a marker for susceptibility to high myopia.[27] In contrast, the present study showed no association between rs2009066 and high myopia in the Japanese population. Although our results do not support those of previous study, we could not still exclude the possibility that *CRYBA4* variants may affect the risk of high myopia. To clarify the role of *CRYBA4* gene variants in the risk of high myopia, further genetic analyses should be performed in different populations.

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Acknowledgments

The authors thank all the subjects for their participation in this study. They also thank Dr Yasuhito Iijima and the medical staff at the Aoto Eye Clinic for their assistance with sample collection and diagnosis.

Disclosure

The authors report no conflicts of interest in this work.

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