Association study of fibroblast growth factor 10 (FGF10) polymorphisms with susceptibility to extreme myopia in a Japanese population

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Purpose: The fibroblast growth factor 10 (FGF10) gene polymorphism rs339501 was previously reported to be associated with high myopia in a Chinese population. In the present study, we investigated whether FGF10 polymorphisms are associated with extreme myopia in a Japanese population as well.

Methods: A total of 433 Japanese patients with extreme myopia (≤ −10.00 diopters) and 542 Japanese healthy controls (+1.50 to −1.50 diopters) were recruited. We genotyped seven tagging single-nucleotide polymorphisms (SNPs), including rs339501, in FGF10. We also performed an imputation analysis to evaluate the potential association of ungenotyped FGF10 SNPs, and 34 SNPs were imputed.

Results: It was found that rs339501 and rs12517396 exhibited the strongest association with extreme myopia (p=3.9 × 10⁻⁴, corrected p [Pc]=0.0039). A significant association was also observed for rs10462070 (p=6.5 × 10⁻⁴, Pc=0.0059). These three SNPs were in strong linkage disequilibrium (D' ≥0.99, r² ≥0.96). However, the frequency of the A allele of rs339501 was increased in cases compared to controls, which differs from the increased frequency of the G allele in cases in the previous Chinese population.

Conclusions: Three FGF10 SNPs in complete linkage disequilibrium—rs339501, rs12517396, and rs10462070—were associated with extreme myopia in the Japanese population, and the risk allele of rs339501 differed from the previous Chinese population. Therefore, these three SNPs may not be an important risk factor for susceptibility to extreme myopia. Further studies are needed to elucidate the possible contribution of the FGF10 region in the development of extreme myopia.

Myopia is a very common refractive error that has a significant impact on public health and economics around the world. High myopia, which is a refractive error ≤ −6 diopters (D), is a major cause of blindness associated with an increased risk of various ocular and systemic diseases, including retinal detachment, glaucoma, and cataracts [1]. The prevalence of high myopia has been reported to range from 1.0% to 9.6% in the general population, but it exhibits variable incidence in different countries, with a preponderance in Asia [2-5].

Although the cause of high myopia is unclear, family correlation studies and twin studies have shown that genetic factors play a significant role in its development [6-11], with a relationship between the genetic basis of eye growth and the development of myopia. Twin studies revealed a correlation between axial length and refractive error that was much higher in monozygotic twins compared to dizygotic twins [12,13]. The pattern of inheritance in high myopia appears to be heterogeneous, with an autosomal dominant to autosomal recessive pattern [9]. Therefore, risk factors that contribute to the development of high myopia include genetic heterogeneity and axial length [14,15]. Familial linkage studies have attempted to identify candidate genes that might contribute to myopia, and significant linkages have been reported at 18 loci, specifically MYP1 to MYP18 [16]. Many recent genome-wide association studies (GWASs) have been conducted to identify genes involved in myopia or high myopia, and many candidate loci/genes have been reported [17-27].

The fibroblast growth factor (FGF) family of proteins plays important roles in the proliferation and differentiation of a wide variety of cells and tissues. A defect in FGF10 leads to the development and differentiation of several ocular tissues [28-30]. Sclera remodeling, which is one of the important mechanisms in the development of myopia, involves alterations in both the degradation and synthesis of extracellular matrix components [31], and FGF10 can modulate extracellular matrix–associated genes [32-35]. Recently, His et al. [36]
reported that the sclera of myopic mouse eyes have higher levels of FGF10 mRNA. The G allele of FGF10 polymorphism rs339501 was also found to be associated with higher FGF10 expression and the risk of extreme myopia (<-10 D) in a Chinese population residing in Taiwan. Therefore, higher expression of FGF10 caused by the G allele of rs339501 could represent a risk for myopia. The aim of the present study was to investigate whether genetic polymorphisms in FGF10 are associated with extreme myopia in Japanese patients.

METHODS

Subjects: We recruited 433 unrelated Japanese individuals with extreme myopia (refractive error ≤-10.00 D in at least one eye) and 542 unrelated healthy Japanese controls (+1.50 to −1.50 D in both eyes) at Yokohama City University and Okada Eye Clinic in Japan. All participants were diagnosed by comprehensive ophthalmologic tests, including axial length, fundus examination, spherical power, and corneal curvature (Autorefractor; NIDEK [Gamagori, Japan] ARK-730A, ARK-700A TOPCON [Tokyo, Japan] KP-8100P, BIO and PACHY Meter AL-2000; Tomey Corporation, Nagoya, Japan). The individuals with extreme myopia had no known genetic diseases associated with myopia and/or high myopia, including glaucoma, keratoconus, or Marfan syndrome. Patient age ranged from 12 to 76 years (mean 40.6±12.0 years), and 44.4% of patients were male. The average spherical refractive errors were −11.9±2.20 D (range −6.75 to −22.75 D) in the right eye (OD) and −11.9±2.29 D (range −8.50 to −23.0 D) in the left eye (OS). The average axial length was 28.0±1.18 mm (range 26.0 to 33.1 mm) for OD and 28.0±1.23 mm (range 26.0 to 34.7 mm) for OS. The average corneal refraction was 43.8±1.46 D (range 39.5 to 47.8 D) for OD and 43.8±1.52 D (range 39.8 to 53.0 D) for OS. Control individuals were healthy volunteers and not related to each other or the patients. The controls were sex-matched (47.2% male) to the patients with an age range of 24 to 75 years (mean 40.6±12.0 years). All participants had similar social backgrounds and resided in the same urban area. Informed consent was obtained from all participants. The study methodology adhered to the tenets of the Declaration of Helsinki and was approved by the relevant ethics committees in each participating institute.

Single-nucleotide polymorphism genotyping of the FGF10 gene region: Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Procedures were performed under standardized conditions to prevent variation in DNA quality. Seven tagging single nucleotide polymorphisms (SNPs) covering the FGF10 region including 10 kb upstream and downstream from the gene were selected from HapMap Japanese data (minor allele frequency ≥5%, pairwise r² ≥0.8; Table 1; NCBI). Genotyping was performed using the TaqMan 5’ exonuclease assay with validated TaqMan primer-probe sets supplied by Applied Biosystems (Foster City, CA). PCR was performed using a reaction mixture with a total volume of 10 μl containing 1X 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 ºC for 10 min, followed by 40 cycles of denaturation at 92 ºC for 15 s and annealing/ extension at 60 ºC for 1 min. The probe’s fluorescence signal was detected using the StepOnePlus Real-Time PCR System (Applied Biosystems).

Imputation analysis of the FGF10 gene region: We performed an imputation analysis to evaluate the potential association of ungenotyped SNPs in the FGF10 region, including 10 kb upstream and downstream from the gene. The genotypes of 433 cases and 542 controls were imputed using MACH v1.0 [37,38]. For the reference panel, we used Japanese data from HapMap phase 3. For quality control, we excluded SNPs from the reference panel if they had a call rate <95%, leaving 34 SNPs for the imputation. As none of the SNPs had a squared correlation between imputed and true genotypes <0.3, the 34 imputed SNPs were included in the association analysis (Table 1).

Statistical analysis: Allele frequencies, Hardy–Weinberg equilibrium, and linkage disequilibrium (LD) were assessed using Haploview 4.1 software [39]. Differences in allele haplotype frequencies between cases and controls were assessed by χ². The obtained p values were corrected for multiple testing using a permutation test (10,000 iterations) in Haploview. A corrected p (Pc) value <0.05 was considered significant. Conditional logistic regression analysis was performed to assess the effect of each SNP on disease susceptibility using PLINK [40].

RESULTS

The genotype frequencies of all seven tagging and 34 imputed SNPs were in Hardy–Weinberg equilibrium among both cases and controls. Figure 1 and Table 2 show the results of the association analysis of 41 SNPs in FGF10. Of the seven tagging SNPs, rs339501 exhibited a strong association with extreme myopia (p=3.9 × 10⁻⁴, Pc=0.0030), and the frequency of the A allele of rs339501 was increased in cases compared to controls (90.0% versus 84.5%, odds ratio [OR]=1.64), which is the opposite of that reported in the previous Chinese population. In other tagging SNPs, the frequencies of the A allele of rs2330545 and A allele of rs1384449 were also increased in cases compared to controls (p=0.047 and
# Table 1. The 41 FGF10 SNPs in the present study.

| SNP     | Position on chromosome five (Build 37.1) | Gene location |
|---------|------------------------------------------|---------------|
| rs1448044 | 44,296,986                               | 3′-UTR        |
| rs10072476 | 44,296,400                               | 3′-UTR        |
| rs9292903  | 44,299,998                               | 3′-UTR        |
| rs1979079  | 44,300,650                               | 3′-UTR        |
| rs1374961  | 44,303,760                               | 3′-UTR        |
| rs6451758  | 44,305,515                               | Introns 2     |
| rs10462070 | 44,305,749                               | Introns 2     |
| rs10473352 | 44,308,252                               | Introns 2     |
| rs1374962  | 44,311,070                               | Introns 1     |
| rs16873956 | 44,312,489                               | Introns 1     |
| rs10060796 | 44,313,151                               | Introns 1     |
| rs1839090  | 44,313,282                               | Introns 1     |
| rs980510   | 44,318,532                               | Introns 1     |
| rs13436788 | 44,318,624                               | Introns 1     |
| rs10057630 | 44,327,864                               | Introns 1     |
| rs4866891  | 44,328,270                               | Introns 1     |
| rs987642   | 44,331,905                               | Introns 1     |
| rs1011814  | 44,335,820                               | Introns 1     |
| rs10512844 | 44,338,759                               | Introns 1     |
| rs2330544  | 44,339,764                               | Introns 1     |
| rs2330545  | 44,339,810                               | Introns 1     |
| rs7708529  | 44,347,131                               | Introns 1     |
| rs1482689  | 44,359,428                               | Introns 1     |
| rs12517396 | 44,359,526                               | Introns 1     |
| rs339509   | 44,360,892                               | Introns 1     |
| rs17234079 | 44,362,204                               | Introns 1     |
| rs1482672  | 44,362,769                               | Introns 1     |
| rs339502   | 44,364,007                               | Introns 1     |
| rs2121875  | 44,365,545                               | Introns 1     |
| rs339501   | 44,365,633                               | Introns 1     |
| rs1750845  | 44,373,060                               | Introns 1     |
| rs1384449  | 44,377,060                               | Introns 1     |
| rs16901816 | 44,381,698                               | Introns 1     |
| rs2973644  | 44,384,183                               | Introns 1     |
| rs1482679  | 44,385,415                               | Introns 1     |
| rs2973646  | 44,387,537                               | Introns 1     |
| rs2973649  | 44,391,161                               | 5′-UTR        |
| rs1482680  | 44,392,142                               | 5′-UTR        |
| rs723166   | 44,396,015                               | 5′-UTR        |
| rs10473354 | 44,396,353                               | 5′-UTR        |
| rs10941665 | 44,398,696                               | 5′-UTR        |

Genotyped SNPs are indicated in bold.
p=0.019, respectively), although this increase did not reach significance after correcting for multiple testing (Pc >0.05).

Of 34 imputed SNPs, rs12517396 showed the strongest significance, equivalent to rs339501, and the C allele was associated with a risk of extreme myopia (p=3.9 × 10^{-4}, Pc=0.0030, OR=1.64). The A allele of rs10462070 was also strongly associated with a risk of extreme myopia (p=6.5 × 10^{-4}, Pc=0.0059, OR=1.62). Another 20 imputed SNPs showed moderate association (p<0.05) with the disease but this did not reach significance after correction (Pc >0.05).

Figure 1 shows the overall LD patterns for the 41 SNPs in 975 individuals. Strong LD was observed throughout
| SNP      | Position on chromosome five (Build 37.1) | Gene location | Alleles | Risk allele | Risk allele frequency | Cases (n=433) | Controls (n=542) | P     | Pc     | OR (95%CI) |
|----------|----------------------------------------|----------------|---------|-------------|-----------------------|---------------|------------------|-------|--------|------------|
| rs10462070 | 44,305,749 | Intron 2 | A/G | A          | 0.901 | 0.849 | 6.5×10⁻⁴ | 0.0059 | 1.62 (1.22–2.13) |
| rs12517396 | 44,359,526 | Intron 1 | A/C | C          | 0.900 | 0.845 | 3.9×10⁻⁴ | 0.0030 | 1.64 (1.25–2.16) |
| rs339501   | 44,365,633 | Intron 1 | A/G | A          | 0.900 | 0.845 | 3.9×10⁻⁴ | 0.0030 | 1.64 (1.25–2.16) |
the FGF10 gene region and 40 SNPs from rs1448044 to rs10473354 were located in one haplotype block (Block 1). The three SNPs with the strongest signal, rs339501—rs12517396, and rs10462070—were in complete LD in Block 1 (D^2 \geq 0.99, r^2 \geq 0.96). Twenty-two SNPs with moderate association were also in Block 1. To elucidate the effect of rs339501, rs12517396, and rs10462070 on disease susceptibility, we performed conditional logistic regression analysis. However, we could not determine which variant was the causal SNP for the observed associations in this study because of the complete LD among the three SNPs.

**DISCUSSION**

Myopia is a complex disease that involves both environmental factors and multiple interacting genetic factors. In particular, determination of the role of genetic factors in high myopia has been influenced by its high prevalence, genetic heterogeneity, and potentially modulating environmental factors. In the past few years, previous GWASs have reported many genomic loci/genes that confer susceptibility to myopia [17-27]. Although Hsi et al. recently reported that FGF10 rs339501 is associated with extreme myopia (refractive error \leq -10.00 D) but not high myopia (\leq -6.00 D) in a Chinese population using a candidate gene approach [36], the GWASs have not identified FGF10 as a myopia susceptibility gene. At least two possible explanations exist for this difference. First, the GWAS platforms may not have included the significant SNP rs339501 and other SNPs in strong LD with rs339501 that would lead to the detection of an association between the FGF10 region and myopia. Second, none of the GWASs focused on extreme myopia; they used high myopia, pathological myopia (axial length \geq 28 mm), axial length, or refractive error.

The A allele frequency of rs339501 was found to have a role in the risk of extreme myopia in our Japanese population. This finding differs from the previous study of a Chinese population [36] in which extreme myopia cases had a significantly higher frequency of the G allele compared to controls. We also found that two other SNPs, rs12517396 and rs10462070, in complete LD with rs339501 were strongly associated with extreme myopia, but these SNPs were not included in the previous study. These three SNPs are intronic variants that can significantly affect gene expression levels and contribute to the development of human diseases [41-43]. Hsi et al. reported that the G allele of rs339501 significantly increases the expression of FGF10, suggesting that the increased FGF10 expression caused by the G allele increases the risk for myopia. However, because our results showed that the A allele of rs339501 is associated with a risk of extreme myopia in our Japanese population, it suggests that the G allele is not a risk factor for the susceptibility of extreme myopia in all populations.

Drastic differences in the allelic distribution of disease risk–associated SNPs among different ethnic populations have been reported in exfoliation syndrome (XFS). XFS is strongly associated with certain SNPs, including rs1048661, rs2165241, and rs3825942 of the lysyl oxidase-like 1 (LOXL1) gene, in many different ethnic groups [44-46], suggesting that LOXL1 is the major susceptibility gene for the development of XFS. However, the allelic distributions of rs1048661 and rs2165241 were different between East Asian populations, including Japanese, Chinese, and Korean, and other ethnic populations such as Caucasian, Middle Eastern, and black South African; the risk alleles of rs1048661 and rs2165241 for XFS in East Asians were the opposite of those reported for other ethnic populations [44-46]. On the other hand, the risk allele of rs3825942 for XFS was different between black South Africans and all other reported ethnicities, including East Asians and Caucasians [44-46]. Although the reasons for discrepancies in the allelic distributions of the LOXL1 SNPs among XFS patients with different ethnicities are unclear, it has been suggested that these SNPs are not the true causal variants of XFS, and that unidentified genetic variants in strong LD with these SNPs may play important roles in the development of XFS.

In this study, we found that the risk allele of FGF10 rs339501 for extreme myopia in the Japanese population is different from that reported in the Chinese population residing in Taiwan. The disparity between our results and those of the original report can be explained based on the association between XFS and LOXL1 SNPs; another FGF10 variant may be the true genetic factor and the associations observed in the present and previous studies may have resulted from strong LD with the true FGF10 variant. Variable LD patterns among different ethnic groups could explain the conflicting results; the true risk-associated allele in FGF10 may be linked to the G allele of rs339501 in the Chinese population and the A allele of rs339501 in the Japanese population. This explanation does not seem to be unreasonable because a close similarity exists in the genetic backgrounds of the Japanese and Chinese populations [47]. In addition, our study and the original study used limited sample sizes of extreme myopia (433 from Japan, 125 from Taiwan). Limited sample sizes can sometimes lead to false positive or negative results in an association study. Therefore, further association studies of FGF10 variants with larger sample sizes of Japanese, Chinese, and other ethnic populations are needed. We also need to consider the disparity in gender between the present and the original study. Men comprised 44.4% of patients with extreme myopia.
in the present study, whereas 65.4% of patients were men in the original study. In recent genetic studies of extreme myopia, 30–40% of the patients were men [48-50], suggesting that extreme myopia is more common in women, although the association of gender with extreme myopia still needs to be elucidated. Therefore, sampling bias may have existed in the original study.

In conclusion, we found that the FGF10 variants, including rs339501 reported in the previous study, are associated with extreme myopia in our Japanese population, whereas the disease risk–associated allele differed between the present and the previous study. Our findings suggest that the FGF10 variants studied in the present study are not an important risk factor for susceptibility to extreme myopia. However, because FGF10 variants may still affect the risk of extreme myopia, further genetic studies are needed to clarify the contribution of the FGF10 region in the development of extreme myopia.

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