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Human Molecular Genetics, 2013, Vol. 22, No. 25 **5288–5294**
doi:10.1093/hmg/ddt385
Advance Access published on August 9, 2013

Genome-wide association study identifies *ZFHX1B* as a susceptibility locus for severe myopia

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Received April 20, 2013; Revised July 17, 2013; Accepted August 5, 2013

Severe myopia (defined as spherical equivalent < -6.0 D) is a predominant problem in Asian countries, resulting in substantial morbidity. We performed a meta-analysis of four genome-wide association studies (GWAS), all of East Asian descent totaling 1603 cases and 3427 controls. Two single nucleotide polymorphisms (SNPs) (rs13382811 from *ZFHX1B* [encoding for ZEB2] and rs6469937 from *SNTB1*) showed highly suggestive evidence of association with disease ($P < 1 \times 10^{-7}$) and were brought forward for replication analysis in a further 1241 severe myopia cases and 3559 controls from a further three independent sample collections. Significant evidence of replication was observed, and both SNP markers surpassed the formal threshold for genome-wide significance upon meta-analysis of both discovery and replication stages ($P = 5.79 \times 10^{-10}$, per-allele odds ratio (OR) = 1.26 for rs13382811 and $P = 2.01 \times 10^{-9}$, per-allele OR = 0.79 for rs6469937). The observation at *SNTB1* is confirmatory of a very recent GWAS on severe myopia. Both genes were expressed in the human retina, sclera, as well as the retinal pigmented epithelium. In an experimental mouse model for myopia, we observed significant alterations to gene and protein expression in the retina and sclera of the unilateral induced myopic eyes for *Zfhx1b* and *Sntb1*. These new data advance our understanding of the molecular pathogenesis of severe myopia.

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INTRODUCTION

Myopia is a world-wide eye disability, suspected to be due to both genetic and environmental influences. The hereditable elements responsible for predisposition to myopia are difficult to identify and dissect due to substantial confounding by environmental factors. This is particularly complicated in studies of common myopia, typically defined as spherical equivalent (SE) of < -0.5 D, in which the robust identification of genetic determinants requires the deployment of very large sample sizes (1–3).

Natural variation in ocular biometric quantitative traits has been shown to be significantly influenced by a large number of genetic sequence variants, each conferring modest effect sizes (4–7). Thus far, three genome-wide association studies (GWAS) on SE quantitative trait have been performed on ever larger sample sizes, revealing very strong evidence of association between multiple single nucleotide polymorphism (SNP) markers and inter-individual SE variation (8–11). Notably, the effect sizes for all of these identified loci are modest, suggesting a multi-factorial etiology and possible presence of unmeasured environmental confounding, in keeping with other common diseases (12). To a more limited extent, GWAS on severe myopia (defined as SE < -6.0 D in either eye) has also been performed (13–16), and robustly associating genetic loci surpassing the formal threshold for genome-wide significance ($P < 5 \times 10^{-8}$) are now beginning to emerge (15,16).

Severe myopia is more common in East Asians than whites, affecting up to 10% of the population aged 40 years and older, and can be associated with vision loss due to myopic macular degeneration, glaucoma and retinal detachment. To date, successful GWAS of severe myopia have been conducted in individuals of Chinese descent from China (15,16), the most recent of which involved 665 cases and 960 controls in the discovery stage (15). To identify further genetic determinants for severe myopia, we conducted a meta-analysis of four genome-wide association studies across four independent Asian collections enrolled from mainland China, Hong Kong, Japan and Singapore (Table 1). The collections from China and Hong Kong have been described in prior publications. Altogether, these total 1603 severe myopia cases and 3427 emmetropic controls. Replication experiments were conducted in a further 1241 severe myopia cases and 3559 controls from Hong Kong, Japan and Singapore.

RESULTS

After stringent quality control filters on samples and SNPs (see Materials and Methods), a total of 494 215 SNPs (see Supplementary Material for summary statistics of the complete GWAS dataset) were observed to be successfully genotyped in two or more sample collections, and 250 531 SNPs were found common across all four study collections. Statistical associations between each SNP genotype and severe myopia were measured using logistic regression, modeling for a trend per-copy effect of the minor allele. A quantile–quantile (QQ) plot of the P-values obtained from the four-collection meta-analysis showed low evidence of genomic inflation of the association test statistics ($\lambda_{gc} = 1.057$), giving minimal evidence of cryptic population stratification or differential genotyping success rates between cases and controls which could confound the association results. Observed against this background was several point P-values showing extreme deviation at the tail end of the QQ distribution. These data points could represent true positive associations with severe myopia, and reflected two distinct genetic loci ($P < 1 \times 10^{-7}$) (Supplementary Material, Figs S1 and S2). The first locus is rs13382811 mapping within *ZFHX1B* (also known as *ZEB2*; per-allele odds ratio (OR) = 1.33, $P = 7.44 \times 10^{-9}$) (Supplementary Material, Fig. S3). The second marker is rs6469937 mapping within *SNTB1* (per-allele OR = 0.75, $P = 6.08 \times 10^{-8}$)) (Supplementary Material, Fig. S4), which is located within the same linkage disequilibrium region as the three SNP markers recently described as strongly associated with severe myopia (15).

A power calculation based on a per-allele OR of between 1.20 and 1.30 (due to the winner's curse phenomenon), minor allele frequency between 0.20 and 0.30, and a replication sample size of 1232 severe myopia cases and 3559 emmetropic controls showed that only SNPs surpassing $P = 1 \times 10^{-7}$ in the GWAS discovery stage would be sufficiently powered to achieve genome-wide significance upon meta-analysis should the effect be a true positive (Supplementary Material, Table S1). Based on these estimations, we proceeded to conduct replication experiments of both SNPs in a further 1232 severe myopia cases and 3559 emmetropic controls enrolled across three countries (Table 1). Both SNP markers showed significant evidence of replication (Fig. 1A and B, Supplementary Material, Table S2a and b), with minimal to no heterogeneity across the three study collections ($0\% \leq I^2$ index $< 25\%$). Meta-analyzing data from all seven collections, we note genome-wide significant association with severe myopia ($P = 5.79 \times 10^{-10}$, per-allele OR = 1.26 for rs13382811 and $P = 2.01 \times 10^{-9}$, per-allele OR = 0.79 for rs6469937) for both SNP markers, again with no evidence of inter-collection heterogeneity.

Differential gene expressions for *ZEB2* and *SNTB1* from the tissues of myopic (with SE < -5.0 D) and fellow non-occluded eyes of the experimental mice were compared with age-matched control tissues (Fig. 2). The mRNA levels of *ZEB2* and *SNTB1* were significantly downregulated in myopic retina/RPE compared with naive control retina/RPE and this was upregulated in the myopic sclera. Fold change for ZEB2 in retina/RPE/sclera −3.1/−7.8/2; $P = 0.00004$; $P = 0.0002$ and $P = 0.0003$, respectively. Fold change for SNTB1 in retina/RPE/sclera −5.6/−22/17.4; $P = 0.0001$; $P = 0.0008$ and $P = 0.00006$, respectively.

We performed additional analysis assessing for association within the current severe myopia dataset for previously reported severe myopia loci (Supplementary Material, Table S3), as well as previously reported loci influencing SE (Supplementary Material, Table S4) identified via the GWAS approach. All SNP markers showing evidence of association surpassing $P < 1 \times 10^{-4}$ in the GWAS meta-analysis discovery stage are shown in Supplementary Material, Table S5.

DISCUSSION

Severe myopia is much more extreme phenotype compared with common myopia (defined as SE < -0.5 D) and the study of individuals at the more severe end of the quantitative phenotype spectrum has documented usefulness (17,18). Furthermore, severe myopia can cause significant visual impairment via myopic macular degeneration, retinal detachment and glaucoma. Severe myopia is more common in East Asians, affecting 5–10% of persons 40 years and older. In an attempt to minimize

Table 1. Sample collections used in both stages of the study.

Collection	Cases	Age (mean: [range])	Controls	Age (mean: [range])	Genotyping platform
GWAS stage					
China (Sichuan)	419	34.3 [12–76]	669	32.7 [22–55]	Illumina 610K
Hong Kong	232	50.6 [12–87]	244	69.0 [39–94]	Illumina 370K
Japan	500	57.8 [14–91]	1194	50.0 [20–79]	Illumina 550K
Singapore	452	40.9 [10–75]	1320	43.5 [10–85]	Illumina 610K
Total GWAS	1603		3427		
Replication stage					
Hong Kong	106	56.2 [21–85]	178	75.0 [55–99]	
Japan	728	56.6 [11–86]	3248	52.2 [30–75]	
Singapore	407	19.5 [16–37]	133	18.7 [16–25]	
Total replication	1241		3559		
Total samples	2844		6986		

spurious associations due to population stratification or environmental effects (as >70% Asians are myopic to some degree), we utilized emmetropic adults (-0.5 D < SE < $+1.0$ D) as far as possible, adjusted for genetic stratification, and included multiple populations in the study design. Also, we only considered markers showing low inter-cohort heterogeneity, in order to avoid genotyping artifacts driven by specific sample collections. This current study in 2844 severe myopia cases and 6986 controls identified *ZFHX1B* as a new susceptibility locus for severe myopia, and confirmed a recent observation at *SNTB1* where the minor allele of several SNP markers were significantly associated with decreased susceptibility towards severe myopia.

Many different experimental animal models (chick, rabbit, tree shrew, macaque and tree shrew) (19,20) had been used for the studies of emmetropization and myopia generation. These animal models were used to characterize the optical parameters of and study the mechanisms of induced myopia (21). Studies of the chick eye have formed the basis for several hypotheses of myopic development, but the chick does not possess a fovea or retinal blood supply. It is thus unclear whether these differences alter the pathways of emmetropization. Even closely related primate species can exhibit different responses to form deprivation conditions, suggesting differing mechanisms of eye growth control. Monocular occlusion of the rhesus macaque, for instance, results in myopia when the ciliary muscle is paralyzed or the optic nerve cut, but does not in the stump tailed macaque, suggesting a role of excessive accommodation in the development of myopia in the stump tail but not the rhesus.

Given such variability in the models, a persisting element of continued myopia research must be an evaluation of the relevance of any given model to the human condition. In this regard, the study of changing patterns of gene expression within and among species during emmetropization and myopic progression may offer a productive avenue for future research. Experimental myopia has been induced in the mouse by us and others (22–24). The mouse myopia model was developed and assayed because of the availability of the whole-genome sequence, comprehensive protein database and more importantly, the availability of molecular tools like whole-genome gene chip. With our mouse models and noninvasive methods for measuring and monitoring axial length, we were able to monitor the progress of myopia in the same animal without the need to sacrifice it.

ZFHX1B encodes for Zinc finger E-box-binding homeobox 2, also known as SMAD-interacting protein-1 (SMADIP1). It functions as a transcriptional co-repressor involved in the transforming growth factor-β (TGF-β) signaling pathway, and has also been implicated in Mowat–Wilson syndrome (25,26). Members of this TGF-β signaling pathway, such as *BMP2* and *BMP3*, have only recently been implicated in a large multi-ethnic GWAS ($N > 45\,000$) as quantitative trait loci for SE (27). Notably, multiple genes encoding for zinc finger proteins (including *ZIC2* (27), *ZC3H11B* (28) and *ZNF644* (29)) have been shown to associate with refractive error in recent GWAS and exome sequencing studies. Mowat–Wilson syndrome is a genetic condition that could affect multiple distinct parts of the body. Common manifestations of this disorder frequently include characteristic facial features, intellectual disability, delayed development, Hirschsprung disease and other associated birth defects. This syndrome is often associated with an unusually small head (microcephaly), structural brain abnormalities and intellectual disability ranging from moderate to severe. Although ocular disorders are not particularly pronounced in patients with Mowat–Wilson syndrome apart from wide-set eyes, DNA sequencing experiments performed on a patient presenting with Down syndrome, Hirschsprung disease, high myopia and ocular coloboma revealed a non-synonymous amino acid substitution (953Arg→Gly) which was not present in 200 matched normal chromosomes (30). This significant association observed between natural genetic variation within *ZFHX1B* and severe myopia is thus in keeping with current knowledge on the biological pathways for refractive errors. The second locus, *SNTB1*, has only been recently described as a susceptibility locus for severe myopia [15]. It encodes for Beta-1 syntrophin. The Syntrophin family of proteins associate with ion channel and signaling proteins of the dystrophin-associated protein complex. Syntrophins have a diverse role of acting as molecular adaptors for many cellular signaling pathways (31,32). Beta-1 syntrophin, one of the homologous isoforms, has been implicated in the regulation of ABCA1 protein levels in human fibroblast cell lines (33).

We were able to show nominal evidence of association ($0.001 < P < 0.05$) at some of the previously reported GWAS loci for high myopia, namely *CTNND2*, *MIPEP-C1QTNF9B* on Chromosome 13q12 and *VIPR2* (Supplementary Material, Table S1). For the previously reported SE loci, we could observe this level of association at directly genotyped SNPs found at Chromosome 15q14 and 15q25, which were the first SE loci identified via GWAS. The allele associated with

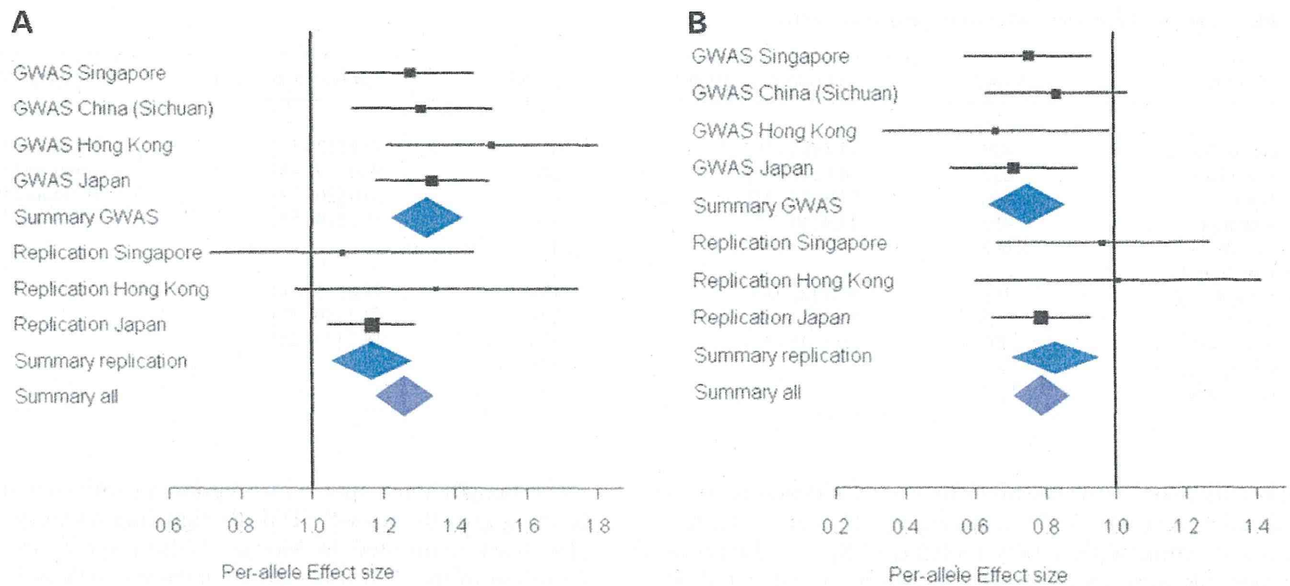

Figure 1. (**A**) Forest plot of the meta-analysis for all sample collections at *ZFHX1B* rs13382811. (**B**) Forest plot of the meta-analysis for all sample collections at *S[illegible]* rs6469937.

increased negativity of SE in quantitative trait analysis (8,9) was also found to be associated with the increased risk of severe myopia in this current study.

In summary, our GWAS on seven independent sample collections of East Asians (five Chinese and two Japanese) identify *ZFHX1B* as a new susceptibility locus for severe myopia. It offers new information into the pathogenesis of severe myopia in Chinese and Japanese, and further implicates the TGF-β biological pathway as an important determinant of severe extreme of refractive error disorders.

MATERIALS AND METHODS

Samples for GWAS stage

Details of the GWAS from Sichuan (419 severe myopia cases and 669 emmetropic controls) and Hong Kong (232 severe myopia cases and 244 emmetropic controls) have been described elsewhere (15,16). The 452 severe myopia cases from Singapore are of Chinese descent, and were enrolled from the SCORM, SP2 and SCES collection. The emmetropic controls were enrolled from the same studies. For Japan, a total of 500 severe myopia cases with axial length ≥28 mm in both eyes and 1194 controls were enrolled for this study.

Sample for replication stage

We enrolled a further 1232 severe myopia cases and 3559 controls from Hong Kong, Japan and Singapore.

Hong Kong

A total of 338 unrelated individuals with severe myopia (refractive error in at least one eye ≤ −6 D and axial length in at least one eye ≥26 mm) and 422 unrelated emmetropic control individuals were recruited from the CUHK Eye Centre (Chinese University of Hong Kong), Hong Kong Eye Hospital and the eye clinics of the Prince of Wales Hospital, Hong Kong. As 232 severe myopia cases and 244 emmetropic controls have undergone GWAS genotyping and were included in the discovery stage, there remained 106 severe myopia cases and 178 emmetropic controls for the replication stage.

Japan

The 728 individuals with severe myopia (axial length ≥26 mm in both eyes) were recruited from Kyoto University Hospital, and Tokyo Medical and Dental University Hospital. For the controls, we used 3248 unrelated individuals recruited from the Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience (The Nagahama Study).

Singapore

A total of 407 individuals with severe myopia and 133 emmetrophic controls were volunteers recruited by DSO National Laboratories from military personnel. The severe myopia [illegible] spherically equivalent refractive error of at least −6 D [illegible] either eye. All subjects were of self-declared Chinese ancestry (all four grandparents), with an age range of between 16 and 25 years.

Genotyping and quality checks

Genome-wide genotyping for the Sichuan (China) and Hong Kong sample collections were performed as previously described (15). The Japanese severe myopia cases were genotyped using the Illumina 550K and 660 W Bead chips, whilst the Japanese controls were genotyped using the Illumina 610K Bead chip. The Singaporean severe myopia collections were genotyped using the Illumina 610K platform according to manufacturer's instructions. Post-genotyping quality checks were conducted on a per-SNP and per-sample basis. SNP markers showing any one of these characteristics were removed from further analysis:

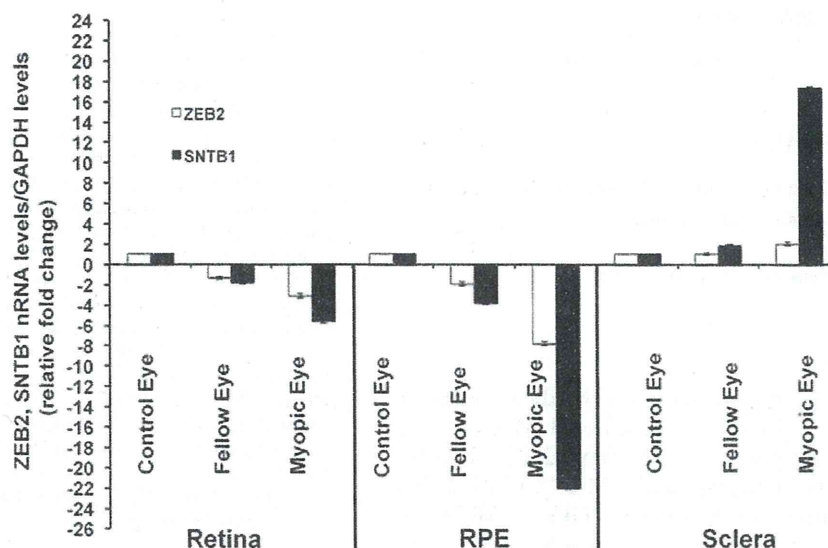

Figure 2. Transcription quantification of *ZEB2* and *SNTB1* in mouse retina. RPE and sclera in induced myopic eyes, fellow eyes and independent control eyes. (transcription quantification of *ZEB2* and *SNTB1* in mouse retina, RPE and sclera in induced myopic eyes, fellow eyes and independent control eyes. Myopia was induced using −15 D negative lenses in the right eye of mice for 6 weeks. Uncovered left eyes were served as fellow eyes and age-matched naive mice eyes were controls. Quantification of mRNA expression in mice retina, RPE and sclera is via qRT-PCR. The bar represents the fold changes of mRNA for *ZEB2* and *SNTB1* genes after normalization using *GAPDH* as reference. The mRNA levels of *ZEB2* and *SNTB1* in myopic and fellow retina, RPE and sclera are compared with independent controls).

(i) call rate $< 95\%$,
(ii) minor allele frequency $< 1\%$ and
(iii) significant deviation from Hardy–Weinberg equilibrium ($P < 10^{-6}$).

Samples showing any of the following characteristics were removed from further analysis:

(i) call rate $< 95\%$,
(ii) extremes of heterozygosity,
(iii) significant ancestral outlier on principal component analysis (PCA) and
(iv) first degree relatives in which case the sample with the lower call rate within the pair would be excluded.

Replication genotyping was performed using the Taqman allelic discrimination assay (Applied Biosystems) according to maufacturer's instructions in all three sample collections.

Statistical analysis

Statistical analyses were conducted using PLINK version 1.07 (34) and the R statistical software package (http://www.r-project.org/). SNP association analysis with severe myopia was performed using logistic regression testing for a trend per-copy of the minor allele, within a multiplicative model for the OR. Additional adjustments compensating for the significant axes of genetic stratification were also performed, when data are available (e.g. in the GWAS stage). PCA and quantile–quantile distributions were plotted using the R statistical software package. PCA assessing for genetic stratification was performed using EIGENSTRAT, and has been described previously for the Chinese (Sichuan) and Hong Kong collections (15,16). The plots contrasting principal component scores between severe myopia cases and controls for each collection are presented in Supplementary Material, Figures S5–S8. Good genetic matching between cases and controls was observed along the top five axes of variation of genetic ancestry.

Ethical approval for animal study

Animal study approval was obtained from the Sing Health IACUC (AAALAC accredited). All procedures performed in this study complied with the Association of Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmology and Vision Research.

Differential gene expression in a mouse model of myopia

Experimental myopia was induced in B6 wild-type mice ($n = 36$) by applying a −15.00 D spectacle lens on the right eye (experimental eye) for 6 weeks since post-natal Day 10. The left eyes were uncovered and served as contra-lateral fellow eyes. Age-matched naive mice eyes were used as independent control eyes ($n = 36$). Eye biometry, refraction and data analysis were followed as described previously (22,35).

We used quantitative real-time PCR (qRT-PCR) to validate the gene expression. Tissue collection, RNA extraction, qRT-PCR methods and analysis were followed as described previously (28). qRT-PCR primers were designed using Probe Finder 2.45 (Roche Applied Science, Indianapolis, IN, USA) and this was performed using a Lightcycler 480 Probe Master (Roche Applied Science). The primer sequences for ZEB2 and SNTB1 were forward: 5′-ccagaggaaacaaggatttcag-3′ and reverse: 5′-aggcctgacatgtagtcttgtg-3′ (NM_015753.3) and forward: 5′-tttggaggcaaagaaggaga-3′ and reverse: 5′-aggagtggatgatgaaaacga-3′ (NM_016667.3), respectively.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

The authors are grateful to the many individuals (both patients and controls) who participated in this study.

Conflict of Interest statement. None declared.

FUNDING

This study is supported by the Singapore Bio-Medical Research Council (BMRC 06/1/21/19/466), the National Medical Research Council of Singapore (NMRC 0796/2003, NMRC 1176/2008, NMRC/IRG/1117/2008 and NMRC/CG/T1/2010) and the Genome Institute of Singapore (GIS/12-AR2105). K.O.-M. acknowledges funding from the Japan Society for the Promotion of Science (JSPS 22390322 and 23659808). The animal model experiments were supported by the National Medical Research Council of Singapore (NMRC/IRG/1117/2008 and NMRC/CG/T1/2010 to V.A.B.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Molecular Vision 2013; 19:1074-1081 <http://www.molvis.org/molvis/v19/1074>
Received 22 June 2012 | Accepted 19 May 2013 | Published 21 May 2013

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Insulin-like growth factor 1 is not associated with high myopia in a large Japanese cohort

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Purpose: To investigate whether genetic variations in the insulin-like growth factor 1 (*IGF-1*) gene are associated with high myopia in Japanese.

Methods: A total of 1,339 unrelated Japanese patients with high myopia (axial length ≥26 mm in both eyes) and two independent control groups were evaluated (334 cataract patients without high myopia and 1,194 healthy Japanese individuals). The mean axial length (mm±SD) in the case group was 29.18±1.85 mm, and the mean spherical equivalent (D±SD) of the phakic eyes was −12.69±4.54 D. We genotyped five tagging single nucleotide polymorphisms (SNPs) in *IGF-1*: rs6214, rs978458, rs5742632, rs12423791, and rs2162679. Chi-square tests for trend, multivariable logistic regression, and haplotype regression analysis were conducted.

Results: We found no significant association between the *IGF-1* SNPs and high or extreme myopia (axial length ≥28 mm in both eyes, 837 subjects) in the additive model, even when compared with the cataract and general population controls, with or without adjustments for age and sex. The evaluation using dominant and recessive models also did not reveal any significant associations. The haplotype analysis with a variable-sized sliding-window strategy also showed a lack of association of *IGF-1* SNPs with high or extreme myopia.

Conclusions: The results of the present study using a Japanese subset do not support the proposal that the *IGF-1* gene determines susceptibility to high or extreme myopia in Caucasians and Chinese. Further studies are needed to confirm our reports in other populations and to identify the underlying genetic determinants of these ocular pathological conditions.

Myopia is a common visual disorder found worldwide and poses major public health concerns, especially in East Asian populations. Myopic eyes with long axial lengths (≥26 mm) or a high degree of myopic refractive error (≤−6 D) are classified as high myopia [1]. High myopia is associated with various ocular complications [2], and these pathological conditions are one of the leading causes of legal blindness in developed countries [3-5]. Therefore, elucidating the pathological mechanisms underlying high myopia and discovering methods for preventing or delaying its onset are important.

Myopia is a complex disease caused by environmental and genetic factors. To date, although many studies have evaluated various candidate genes and susceptible loci of high myopia [6-11], no single gene has been consistently responsible for the condition. In addition to candidate gene studies, a genome-wide approach has also been performed by several groups. Our group previously determined a susceptibility locus for pathological myopia in 2009, using a genome-wide association study [12]. In addition, recent genome-wide association studies have revealed myopia susceptibility loci on chromosome 15 [13,14], and we confirmed that these susceptibility loci are also present in high myopia [15]. However, susceptibility genes for myopia have not yet been determined.

Insulin-like growth factor 1 (*IGF-1*) is similar to insulin in function and structure and is a member of a protein family involved in mediating growth and development. Recently, a single nucleotide polymorphism (SNP) in *IGF-1* was reported to be associated with several types of myopia, including high myopia, in Caucasians [16]. However, these associations were not confirmed by a Polish family study that used single-marker association analysis, a family-based association test, a pedigree disequilibrium test, and haplotype analysis [17]. Nevertheless, subsequent Chinese studies reported a significant association of *IGF-1* polymorphisms with high or extreme myopia; Mak et al. [18] reported an association with high myopia according to haplotype analysis but not single-marker analysis, and Zhuang et al. [19] reported an association with extreme myopia but not with high myopia according to the single-marker and haplotype analyses. The *IGF-1*

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gene is located at a well replicated myopia susceptibility locus, MYP3 [20-23]. Since several previous animal studies indicated that insulin and *IGF-1* were involved in myopia development [24-26], resolving these conflicting results and clarifying whether *IGF-1* polymorphisms are indeed associated with high myopia are essential. In the present study, we conducted a systematic case-control study to validate the association between polymorphisms of the *IGF-1* gene and high and extreme myopia, using a large cohort of 2,867 unrelated Japanese individuals.

METHODS

Subjects: A total of 1,339 unrelated Japanese patients with high myopia who had agreed to participate in genomic study were recruited from the Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Fukushima Medical University Hospital, Kobe City Medical Center General Hospital, and Ozaki Eye Hospital. All the patients underwent a comprehensive ophthalmic examination, including dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, and measurements of the axial length by applanation A-scan ultrasonography or partial coherence interferometry (IOLMaster, Carl Zeiss Meditec, Dublin, CA). To be classified as having high myopia, the subjects had to have an axial length ≥26 mm in both eyes. Of the 1,339 patients with high myopia, 837 had extreme myopia, which is defined as an axial length ≥28 mm in both eyes.

As control subjects, two cohorts were included. One cohort was composed of selected controls, comprising 334 cataract patients with axial lengths <25.0 mm in both eyes (control 1). These patients were recruited from the Department of Ophthalmology at Kyoto University Hospital, Ozaki Eye Hospital, Japanese Red Cross Otsu Hospital, and Nagahama City Hospital. The axial length was measured with applanation A-scan ultrasonography or partial coherence interferometry before cataract surgery, and dilated fundus examination was performed after surgery. If the fundus examination results revealed myopic changes such as lacquer cracks/peripapillary atrophy, staphyloma, or choroidal neovascularization, the subject was eliminated from control 1. The other cohort was composed of general population controls, comprising 1,194 healthy Japanese individuals recruited from the Aichi Cancer Center Research Institute, who had agreed to participate in genomic study (control 2).

All the procedures adhered to the tenets of the Declaration of Helsinki. The institutional review board and ethics committee of each participating institute approved the protocols. All the patients were fully informed of the purpose and procedures of the study, and written consent was obtained from each patient.

DNA extraction: Total genomic DNAs were prepared from 14 ml of venous blood. DNA was purified using a DNA extraction kit (QuickGene-610L, Fujifilm, Minato, Tokyo, Japan).

Single nucleotide polymorphism selection and genotyping: We selected tag SNPs in *IGF-1* based on HapMap Phase II (Build 36) genotype data [27] using the Haploview software (ver. 4.2). Although previous studies tagged relatively minor SNPs (minor allele frequencies [MAFs] ≥0.05 or 0.10 were applied), all such minor SNPs showed no association with high or extreme myopia. In addition, rs6214 and rs12413791, which were reported to be associated with high and extreme myopia, respectively, showed a MAF of 43% and 31% in HapMap JPT (Japanese in Tokyo, Japan). Thus, we tagged all the major (MAF ≥30%) SNPs that showed a Hardy–Weinberg p≥0.05. Using a tagger pairwise program provided in Hapmap project (R2 cutoff of 0.90), we selected five SNPs: rs6214, rs978458, rs5742632, rs12423791, and rs2162679. These tags provided 100% coverage of the major HapMap SNPs within an 84.65-kilobase region spanning the *IGF-1* gene.

The samples of the high myopia cases and cataract controls were genotyped using a commercially available assay (TaqMan SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA). The individuals recruited from the Aichi Cancer Center Research Institute were genotyped using Illumina HumanHap 610 chips (Illumina Inc., San Diego, CA). Because rs12413791 was not included in this chip, the genotype for rs12423791 was imputed using the MACH software, based on the HapMap Phase II JPT genotype data.

Statistical analyses: Deviations in genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed for each group with the chi-square test. The chi-square test for trend or its exact counterpart was used to compare the genotype distributions of the two groups. To adjust for age and sex, we performed a multivariable logistic regression analysis. In addition, dominant and recessive models were also calculated using the chi-square test. These statistical analyses and power calculation were performed with R software (R Foundation R 2.13.0 for Statistical Computing, Vienna, Austria) and PLINK software (ver. 1.07). We also conducted a haplotype analysis by using a variable-sized sliding-window strategy [28] using the PLINK software. p≤0.05 was considered statistically significant. The Bonferroni correction was used for multiple comparisons.