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A Novel Genetic Variant of *BMP2K* Contributes to High Myopia

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Loss of eye growth regulation may cause myopia, because modulation of optic globe size is essential for the generation of normal optic power. Evidence has implied variations of *BMP2* gene expression mediate ocular development and retinal tissue remodeling. Given BMP2 as a potential regulator involved in myopia development, we investigate whether gene BMP2-inducible kinase (BMP2K, BIKe), whose expression is up-regulated during BMP2-induced osteoblast differentiation, contributes to susceptibility of high myopia. Participants grouped into high myopia had a spherical equivalent greater than −6.00 D, compared with a control group of spherical equivalent less than −0.5 D. Genotyping of polymorphisms 1379 G/A (rs2288255) and 3171 C/G (rs12507099), corresponding with 405 Gly/Ser and 1002 Thr/Ser variation in the *BMP2K* gene were determined by PCR-restriction fragment length polymorphism and associative study performed by comparing high myopic subjects and healthy controls. The frequency of A allele in the *BMP2K* gene 1379 G/A polymorphism showed a significant difference between cases and controls ($P < 0.001$, OR = 2.99, 95% CI = 1.62–5.54) and subjects with either AA or AG genotype show higher risk than GG genotype ($P < 0.001$, OR = 3.07, 95% CI = 1.59–5.92), while 3171 C/G polymorphism was not significant from this survey. These data suggest that *BMP2K* gene 1379 G/A variant is strongly correlated with high myopia and may contribute to a genetic risk factor for high degrees of myopic pathogenesis. J. Clin. Lab. Anal. 23:362–367, 2009. © 2009 Wiley-Liss, Inc.

Key words: high myopia; bone morphogenetic protein; single nucleotide polymorphism

INTRODUCTION

Despite occurrence of myopia differs from either country or ethnic groups, myopia is the most common optic disorder in Asian populations with high prevalence up to 70%, based on epidemiological survey (1–3). High predisposition of myopia not only causes aberrant eyesight but also greater risk of blindness and visual impairment, including cataract, retinal detachment, glaucoma, and macular degeneration (4–7), and thus may induce profound impact on public health and social problems. Many animals, as well as human, have ability to modulate axial eye length and focus optical images on the retina to generate normal optical power, known as emmetropisation (8), but patients with myopic pathology show refractive errors and/or loss of adjustment, leading to concentrate their visual image prior to the retina. Occurrences of high myopia may be complicated and multiple factors. Adjusting the level of hard study or stressful life in Asia, life pressure has been considered an environmental factor in high

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prevalence of myopia (9–11). Additional evidence reveals that familial inheritance and genetic basis are also highly responsible for susceptibility to myopic developmental processes (12,13).

Progress of myopia remains unclear. In biological terms, regulation of eye size is likely a key influence on pathological myopia: elongation of ocular globe to result in increased axial length is a feature obtained in patients with myopia and refractive defect (14). Studies on some biological factors in the regulation of this process or on ocular globe remodeling may provide possible clues to identify candidate genes of eye size control and understand more about the causes of pathological forms and high-risk incidence of myopia. Sclera is a specific connective tissue composed of extracellular matrix elements and collagen fibrils lying at the exterior of ocular globe to serve as framework for eye structure and shape maintenance (15,16). During the myopia progression, sclera undergoes dramatic structural change in response to environmental pressure or genetic inheritance. Compared with persons showing normal optic power, high myopic patients tend to have thinner scleral layer and longer-than-average ocular globe, extending axial dimension of the eye and result in refractive error (17–19).

Retina is another fascinating tissue to mediate axial length of eye size (20). Exploration of retinal development uncovered bone morphogenetic proteins (BMPs) and their signaling molecule cascades having an essential influence on the co-operation of eye development through BMP elements and receptors (21–24), despite its original discovery was required for osteogenesis and bone morphogenesis (25,26). Study of chick model of myopia to search for biological factors of eye size control has revealed that *BMP2* gene expression is down regulated at an early form-deprivation stage (27), and in the presence of BMP receptors and secreted BMP2 molecule in chicken embryonic retina have profound effects on eye development, stimulating lens fiber cell differentiation and avoiding cell death in lens epithelium (28), indicating the variation of *BMP2* gene expression as potentially involved in the disturbance of ocular globe development and contribution to myopia development.

In search for regulated factors accounting for variation of eye size, BMP2 is a crucial candidate for retinal development and patterning (29,30). Our study evaluates whether a novel protein BMP2-inducible kinase (BMP2K, BIKe), whose expression is up-regulated during BMP2 induction (31), is correlated with high myopia. To achieve this aim, during performing PCR-restriction fragment length polymorphism (PCR-RFLP) to map the *BMP2K* gene polymorphisms at 1379 G/A and 3171 C/G, we observed that *BMP2K* gene variant may contribute to genetic background of high myopia pathogenesis in Taiwanese population.

MATERIALS AND METHODS

Subjects

All participants enrolled in this study were based on the tenets of the Declaration of Helsinki for research and recruited at China Medical University Hospital in Taiwan. Institutional ethics committee approved this project, with informed consent obtained from all subjects. Their ages ranged from 16 to 25 years, with male-to-female ratio of 1.8:1.0, and all were medical school students. Subjects also met the following criteria: Taiwanese population, no kinship, visual acuity with distance correct of 0.2 log MAR (20/32) or better. Patients with historical diagnosis of any known ocular diseases, intraocular surgery, orthokeratology, retinopathy, prematurity, neonatal problems, and genetic disease and/or connective tissue disorders associated with myopia, such as Stickler or Marfan syndromes, were excluded. The cohort composed of 201 high myopia cases and 86 normal controls collected from February to November 2004. All were diagnosed by measuring refractive error in diopter (D) plus mean spherical equivalent (SE) of the two eyes after one drop of cycloplegic drug (1% mydricyle, Alcon, Berlin) application. Those with myopia greater than or equal to −6.00 D in both eyes were included in the case group; subjects had no or mild myopia, with less than or equal to −0.5 D collected as the control group.

Determination of *BMP2K* Gene Variant by PCR-RFLP

Genomic DNA was extracted from peripheral blood samples by a standard protocol including proteinase K digestion and phenol–chloroform precipitation. Two nonsynonymous single nucleotide polymorphisms (SNP), 1379 G/A (rs2288255) and 3171 C/G (rs12507099), across exonic *BMP2K* locus were selected through public dbSNP database. These polymorphisms of 1379 G/A and 3171 C/G cause codon change at 405 Gly/Ser and 1002 Thr/Ser, respectively. To identify allele preference for the SNPs, PCR-RFLP was designed for genotyping. In brief, PCR reactions were carried out in a total volume of 25 µl, containing 5 ng genomic DNA, and specific primers (1379 G/A: forward 5′-CCC AAC CTC AAA CCC TAT TAG TTG-3′ and reverse 5′-GGA GAA AGA TGA GGA CTA ATG CG-3′; 3171 C/G: forward 5′- GTA GAT GTA TTT GGC TCC AC-3′ and reverse 5′-TTG GAG TCT GAG ATG GTT AA-3′). PCR amplification protocol was set as 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 50°C for

30 sec, 72°C for 1 min, and last elongation step at 72°C for 7 min. A 593 bp size of PCR product from 1379 G/A polymorphism was generated and genotyping was preformed by restriction enzyme *Bst*EII (New England Biolabs, Mississauga, Ontario, Canada) in a total volume of 20 µl at 37°C overnight. Genotyping 3171 C/G polymorphism was determined by a 341 bp size PCR products using restriction enzyme *Bfa*I. The digestion fragments were ascertained by agarose gel electrophoresis, and stained with ethidium bromide.

Statistical Analysis

Distributions of allele and genotype frequencies were performed from each genotyping data by either χ^2 test or Fisher's extract test (one cell has an expected count smaller than 1) evaluation. Statistical analysis of the odds ratios (OR) and 95% confidence interval (CI) were carried out with SPSS version 10.0 software (Chicago, IL) based on the presence of reference allele and genotype frequencies. Statistical analysis considered only P values below 0.05 as significant. Adherence to Hardy–Weinberg equilibrium constant was tested using the χ^2 test with one degree of freedom.

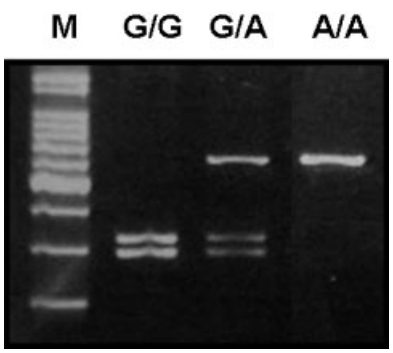

Fig. 1. Genotyping of *BMP2K* gene 1379 G/A polymorphism using PCR followed by restriction enzyme *Bst*EII digestion to generate two fragments with 310 and 283 bp length. Lane 1: DNA marker; lanes 2 and 4: homozygous GG and AA genotypes, respectively; lane 3: heterozygous GA genotype.

RESULTS

Genotypes for *BMP2K* Gene Polymorphisms

BMP2K gene 1379 G/A polymorphism, causing codon change 405 Gly/Ser, was assayed by PCR-RFLP. Genomic DNA samples from high myopia cases and healthy controls were prepared for PCR reaction and obtained a fragment with 593-bp length. Theoretically, two smaller fragments of 310 and 283 bp were obtained in the presence of restriction enzyme *Bst*EII treatment. Figure 1 reveals PCR products as digestible, indigestible homozygous "GG" and "AA" genotypes, as well as heterozygous "GA" genotype for *BMP2K* gene. Genotyping 3171 C/G *BMP2K* gene variant was determined by restriction enzyme *Bfa*I application. The C allele sequence was separated into two fragments, 120 and 221 bp by *Bfa*I, whereas the G allele remained intact (341 bp).

Different Frequencies of *BMP2K* Gene Polymorphisms in High Myopia Cases and Controls

Table 1 shows distributions of allele and genotype frequencies for *BMP2K* gene 1379 G/A polymorphism in high myopia cases and controls. There was statistical difference in allele frequency for genotyping distribution between high myopia cases and controls ($P = 0.00029$): allele frequency of G:A 80.3:19.7% in high myopia group vs. 92.4:7.6% in control group. Odds ratio of "A" allele presented higher risk of 2.99 folds (95% CI = 1.62–5.54) than "G" allele. Also, marked difference appeared among the distributions of AA, AG, and GG genotype frequencies ($P < 0.001$). Combining AA and AG genotypes, odds ratio in high myopic susceptibility was 3.07 higher risk (95% CI = 1.59–5.92) than GG homozygote, and AA homozygous genotype heightened probability of high myopia. Thus, subjects with "A" allele in the *BMP2K* gene 1379 G/A polymorphism tend to have a higher incidence of pathogenic high myopia.

TABLE 1. Comparison of the Allele and Genotype Frequencies of *BMP2K* Gene 1379 G/A Polymorphism Between Cases with High Myopia and Healthy Controls

		Cases		Controls				
Polymorphism		Number	(%)	Number	(%)	*P*-value	Odds ratio (95% CI)	Remarks
1379 G/A	G	323	80.3	159	92.4	0.00029[a]	1	A vs. G
	A	79	19.7	13	7.6		2.99 (1.62–5.54)	
	GG	130	64.7	73	84.9	0.00098[b]	1	AA+GA vs. GG
	GA	63	31.3	13	15.1		3.07 (1.59–5.92)	
	AA	8	4.0	0	0.0			

CI, confidence interval. Hardy–Weinberg equilibrium test: $\chi^2 = 0.0113$ and 0.5749 for cases and controls, respectively ($P > 0.5$).
[a]*P*-values were calculated by χ^2 test.
[b]Fisher's exact test (2×3).

TABLE 2. Comparison of the Alleles and Genotypes 3171C/G Polymorphism in *BMP2K* Gene Between High Myopia Patients and Healthy Controls

		Cases		Controls				
Polymorphism		Number	(%)	Number	(%)	*P*-value[a]	Odds ratio (95% CI)	Remarks
3171 C/G	C	148	72.5	124	75.6	0.5063	0.85 (0.53–1.37)	C vs. G
	G	56	27.5	40	24.4		1	
	CC	54	52.9	45	54.9	0.4916	0.45 (0.11–1.80)	CC vs. GG
	CG	40	39.2	34	41.5		0.44 (0.11–1.80)	CG vs. GG
	GG	8	7.8	3	3.6		1	

CI, confidence interval. Hardy–Weinberg equilibrium test: $\chi^2 = 0.0243$ and 1.2648 for cases and controls, respectively ($P > 0.5$).
[a]*P*-values were calculated by χ^2 test.

With 3171 C/G *BMP2K* gene polymorphism, distribution of allele frequency for C:G was 72.5:27.5% in high myopia group vs. 75.6:24.4% in control group (Table 2). Neither C/G allele distribution nor CC/CG/GG genotype frequency showed significant association between high myopia and control groups ($P > 0.05$). In both 1379 G/A and 3171 C/G genotype distributions, they were in accordance with the Hardy–Weinberg equilibrium.

DISCUSSION

We tested a hypothesis of whether polymorphism within *BMP2K* gene influences susceptibility to high myopia in Taiwanese population. All subjects were young medical school students engaged in intense professional studies, making risk factors of education or near-viewing pervasive from our survey. While genotyping in *BMP2K* gene 1379 G/A (rs2288255), which causes protein codon Gly to Ser alteration at 405 position, we found that frequency of either "AA" or "AG" genotypes revealing higher distribution in high myopia cases than "GG" genotype, as tabulated in statistical analysis. Moreover, subjects with "A" allele had higher risk for high myopia than "G" allele carriers, since there was significant difference in our cohort of case–control participants. Therefore, survey of genetic factor associated with high myopia reveals that polymorphism in BMP2K codon 405 is a probable candidate responsible for this disease progression.

Incidence of myopia is rising according to epidemiological evidence, especially in Asia, motivating us to uncover genetic factors involved in pathological myopia for the purposes of decreasing social health costs and preventing this disease. Development of myopia predominantly results from dysfunction of eye growth cooperating by visual signals and outer coat of ocular globe membranes, sclera, choroids, and retina (20,30,32). In particular, it increases axial elongation and reduces tolerance for the intraocular pressure of the eye (33,34), which may cause irreversible injury to these membranes, increasing risk of high degrees of myopia. Other pathologic diseases such as glaucoma, cataract, and retinal detachment also reveal strong correlation between mechanical stresses and excessive increase in eye size (7).

Exploring cellular factors essential for the control of eye size may help us understand myopic pathology. Changes of TGFβ expression and activities of TGFβ-associated signal transduction have been linked with scleral changes and remodeling. Sclera is a connective tissue consisting mainly of collagen (35). In a previous study of a tree shrew model of high myopia, collagen production fell 28% (36). This drop in TGFβ expression seems to mediate collagen level down-synthesis in sclera (37), and these disturbances may alter scleral extracellular matrix organization. In the longer term, they may lead to enlargement of eye size and thinness of the sclera, a crucial process for the pathogenesis of high myopia (38).

Besides profound influences of TGFβ on scleral remodeling and myopia, expression of BMPs, including BMP2 molecule and BMPs associated components, has also been responsible for ocular globe patterning and remodeling (29,30). From RT-PCR and Northern blot analysis, BMP2 appeared in mouse retina neurons, retinal pigmented epithelium, and human optic nerve head (22,39). To culture retinal ganglion cells with BMP2 shows BMP2 increasing cell survival, promoting neurite development (40) and BMPs released by retina, suggesting a possible role in the survival and differentiation of lens fiber cells (28). Other research also discovered that adult human cornea can express BMP2 protein, hinting that BMP2 might involve in the proliferation and modulation of keratocyte chemotaxis of human corneal fibroblasts (41,42). BMPs molecules are members of TGFβ superfamily and originally identified to induce cartilage and bone development (25,26,43). BMPs functions include control of a broad range of biological processes: e.g., regulation of cell

growth, differentiation, and promoting the synthesis of extracellular matrix proteins via a set of serine/threonine kinase receptors (44). After stimulation, activated BMPs transduce their signal to a series of family proteins known as Smads, similar downstream molecular cascades in TGFβ activation (28,45). Smad4 is probably an important target after BMP stimulation, and activated Smad4 proteins might translocate to nuclei so as to regulate transcription activity of some specific proteins (46,47). Regulation of BMPs signaling cascades may be significant for ocular development, so we try to test correlation between *BMP2K* gene polymorphism and high myopia, a progressive disease linked with eye structure patterning and remodeling.

According to prior data, BMP2K is a serine/threonine kinase protein and contains nuclear localization signal to direct protein to nuclei and may affect transcription activities of target genes (31). Study on the effect of shock wave-medicated changes to human osteoblast highlights stimulated cell proliferation and differentiation, along with induced up-regulation of *BMP2K* gene expression (48). Likewise, protein level of BMP2K increased during BMP2-induced osteoblast differentiation, and its function acts mostly as a regulator of cell differentiation process, suggesting that maintenance of skeletal homeostasis is important for BMP2K protein (31). With BMP2 highly responsible for eye size control and *BMP2* gene down expression at form-deprivation myopia model (27), BMP2-induced protein kinase, BMP2K, may be significant for myopia, but not about BMP2K's association with this progression. We assayed two nonsynonymous SNPs, 1379 G/A and 3171 C/G, in *BMP2K* gene for genotyping in cases vs. control study. Both variants obeyed Hardy–Weinberg equilibrium ($P > 0.05$). Lack of association was found between 3171 C/G polymorphism and high myopia, but the variant at 1379 G/A in *BMP2K* gene showed significant difference between patients with high myopia and healthy controls ($P = 0.00029$). Even though after the Bonferroni correction at 0.05 α level, P value below 0.025 (0.05/2) is still considered as significant. In our results, allele "A" in 1379 G/A polymorphism is a likely risk factor for myopia due to a higher prevalence in high myopic groups than healthy controls. This polymorphism in 1379 G/A, corresponding with 405 Gly/Ser variation, may cause changes of BMP2K protein activity participating in ocular globe development and leading to myopic progression.

Although this traditional uninformative 1379 G/A polymorphism has been shown to carry important information about the responsiveness of high degrees of myopia, the possible action of BMP2K-mediated eye development still needs to be determined. Present data supply important insight into BMP family as potential signaling molecules in high myopic pathogenesis.

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