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A new locus for autosomal dominant high myopia maps to 4q22-q27 between D4S1578 and D4S1612

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Purpose: Myopia is the most common visual problem in the world. High myopia, the extreme form of myopia that can be complicated by retinal detachment and macular degeneration, affects 1%-2% of the general population. The genes responsible for nonsyndromic high myopia have not been identified although several chromosome loci have been suggested. Additional loci for the majority of high myopia, especially in Asian populations, await discovery. A large Chinese family with autosomal dominant high myopia was collected in order to map the genetic locus as an initial step towards identifying the genetic cause of high myopia in this family.

Methods: A Chinese family with 12 individuals affected with high myopia was ascertained from a small village in central China. Phenotypic information and DNA samples were collected from 18 individuals, including 11 affected and 7 unaffected individuals. A genome-wide scan was performed using markers spaced at about 10 cM intervals for genotyping and two-point linkage analysis was carried out. Candidate genes were sequenced.

Results: High myopia, ranging from -5.00 D to -20.00 D with typical fundus changes, is transmitted as an autosomal dominant trait in this family. High myopia in this family shows linkage to markers in a 20.4 cM region between D4S1578 and D4S1612, with maximum lod scores of 3.11 and 3.61 at θ=0 by D4S1564 and by the D4S2986-D4S1572-D4S1564-D4S406-D4S1580-D4S402 haplotype, respectively. Sequence analysis of the retinal pigment epithelium-derived rhodopsin homolog (RRH; OMIM 605224) gene inside the linked region did not identify any causative mutations.

Conclusions: A novel locus (MYP11) for autosomal dominant high myopia in a Chinese family maps to 4q22-q27 but is not associated with mutations in RRH.

Myopia, affecting an average of about 30% (3%-84%) of people throughout the world, is a leading cause of visual impairment [1-3], and Chinese populations residing in industrialized or urban/suburban areas have a significantly higher incidence of myopia than other populations [1-5]. Both genetic and environment factors have been implicated in the etiology of myopia, but the precise molecular mechanisms responsible for myopia are as yet unknown [1,2,6]. High myopia, its extreme form, is associated with vision threatening retinal detachment and macular degeneration and is the fourth most common cause of blindness [7]. Identifying genetic markers for myopia would be a useful first step towards identifying and understanding the molecular defects that lead to the pathophysiology of myopia.

High myopia, occurring in 1-2% of the general population [8-10], is a common hereditary variation. It may be inherited as an autosomal dominant, autosomal recessive, or as X-linked recessive trait. The possibility of complex genetic traits should also be considered for some high myopia. Five loci for high myopia have been reported, including 4 autosomal dominant loci on 18p11.31, 12q21-q23, 7q36, and 17q21-q22, and one X-linked recessive on Xq28 [7,9-12]. However, it is estimated that these loci are responsible for only a small portion of high myopia [13]. Additional loci remain to be identified, as do the genes in the known loci causing high myopia. Several loci contributing to common myopia were also suggested by a twin study and linkage analysis of a group of Ashkenazi Jewish families [14,15].

In this study we describe a Chinese family with high myopia and map the disease to a novel locus on chromosome 4q22-q27 between D4S1578 and D4S1612. This region contains no known loci for common or high myopia.

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METHODS

Family and clinical data: A family with autosomal dominant high myopia was identified in a Chinese family of Han ethnicity living in a small village in central China. This family contains 12 affected individuals in four generations. Eighteen individuals, including 11 affected and 7 unaffected, participated in this study. Informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (863-Plan) by the Ministry of Public Health of China was obtained from the participating individuals prior to the study. Medical and ophthalmic histories were obtained, and ophthalmological examination (by XG and QZ) included visual acuity, slit-lamp, and funduscopic examinations. Refractive error was measured by retinoscopy. A subject was considered to have high myopia if he or she met the following criteria: (1) myopia was noted before school age; (2a) cycloplegic refraction

was -5.00 D or lower (spherical equivalent) in individuals less than 30 years of age or (2b) manifest refraction was -5.00 D or lower (spherical equivalent) in individuals more than 30 years of age as defined previously [7]; and (3) exclusion of other known ocular or systemic diseases. Electroretinogram (ERG) responses were recorded in the proband consistent with ISCEV standards [16]. Genomic DNA was prepared from venous blood as described previously [17].

Genotyping, linkage analysis, and candidate gene screening: Genotyping for all participating family members was performed using 5'-fluorescently labeled microsatellite markers. After initial analysis of high myopia candidate loci, a genome-wide scan was carried out using panels 1 to 27 of the ABI PRISM linkage Mapping Set Version 2, which includes 382 markers spaced at intervals of about 10 cM. PCR was conducted at 94 °C for 8 min; followed by 10 cycles of amplification at 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s; 20 cycles at 89 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; and 72 °C for 10 min. After mixing with GENESCAN™ 400 HD (ROX™) standard (ABI) and deionized formamide, PCR products were denatured at 95 °C for 5 min and then immediately placed on ice for 5 min. The amplicons were separated on Long Ranger sequencing gels (Cambrex Bio Science, Rockland, ME) on an ABI 377 DNA sequencer. Genotyping data were collected by using GeneScan Analysis 3.0 and analyzed by Genotyper 2.5 of the software package from ABI. Two-point linkage analysis was performed using the MLINK program of the FASTLINK implementation of the LINKAGE program package [18,19]. The myopia in the family was analyzed as an autosomal dominant trait with full penetrance and

Table 1. Clinical information of individuals in the family

ID	Gender	Age (yrs)	Refractive error (uncorrected visual acuity) OD	Refractive error (uncorrected visual acuity) OS	Myopia phenotype	Onset age (yrs)
3	F	62	+1.00	+1.00	No	-
4	F	57	-15.75	-20.00	Yes	childhood
6	F	35	-8.00	-8.00	Yes	childhood
7	M	37	0	0	No	-
8	M	32	-10.00	-10.00	Yes	4
9	F	34	0	0	No	-
10	M	30	-11.50	*	Yes	4
11	F	26	-1.00	0	No	-
12	M	28	-8.50	-7.50	Yes	childhood
13	F	22	0	0	No	-
14	M	23	-5.25	-5.00	Yes	4
16	M	12	-10.75	-13.00	Yes	childhood
17	F	2.5	-4.50	-5.50	Yes	2.5
18	M	12	+1.50	+1.50	No	-
19	F	5	-6.50	-5.50	Yes	5
20	F	4	-5.50	-6.50	Yes	4
21	M	2.5	+2.00	+2.00	No	-
22	F	3	-9.00	-10.75	Yes	3

All affected individuals developed myopia before school age. The refractive error for a pthisical eye is listed as an asterisk (*).

with a disease-gene allele frequency of 0.0133 as previously suggested [9]. Haplotypes were generated using the Cyrillic 2.1 program and confirmed by inspection. Marker allele frequencies were arbitrarily set as equal for the genome wide scan and were calculated from 90 unrelated unaffected individuals for haplotype linkage analysis. Primers situated in the flanking region of the RRH gene (NM_006582) were designed to amplify each exon including exon-intron boundaries (primer sequences available on request). The amplicon was sequenced with the ABI BigDye Terminator cycle sequencing kit v3.1, according to the manufacturer's recommendations, using an ABI 3100 Genetic Analyzer. Sequencing results from both

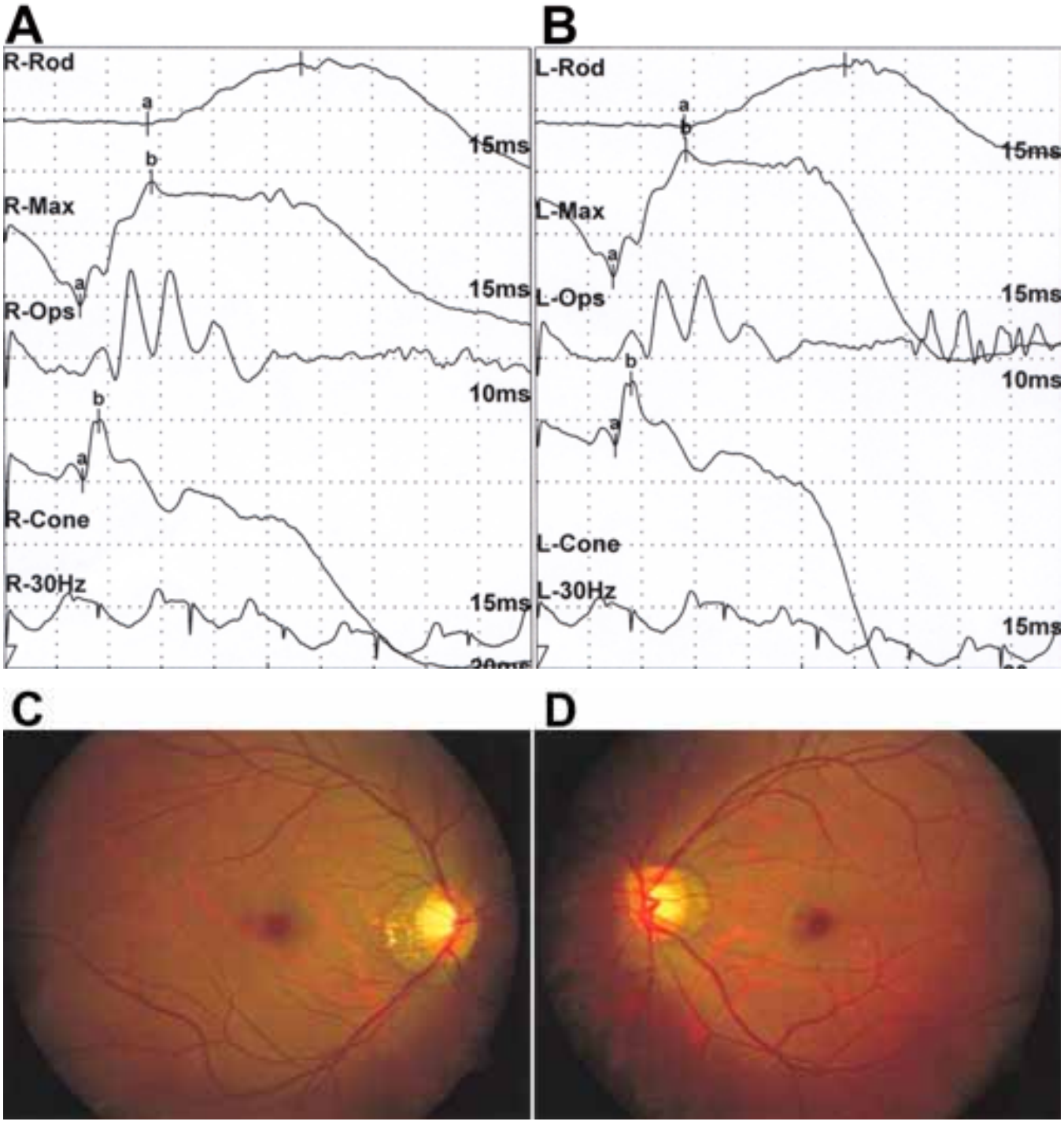

Figure 1. ERG recording and fundus photographs of the proband. **A**,**B**: ERG recorded under scotopic and photopic conditions according to ISCEV standards of individual 12 in Figure 2. Panel **A** shows the right eye and panel **B** shows the left eye. **C**,**D**: Photographs showing typical changes of high myopia, including optic nerve head crescents and "tigroid" appearance of the posterior retina. Panel **C** shows the right fundus and panel **D** shows the left fundus of individual 12.

affected and unaffected individuals and consensus sequences from the NCBI human genome database were imported into SeqMan (DNASTAR) and then aligned to identify the variations.

RESULTS

The family described in this study lives a typical rural lifestyle in a small village in central China, about 100 km away from the nearest city. The myopia in the family ranged from -5.00 D to -20.00 D, except individual 17 who, at the age of 2 years and 6 months had myopia of -4.5 D (right eye) and -5.5 D (left eye). This child was treated as affected in the linkage study based on the age of onset, degree of myopia, and the criteria previously suggested [7,15]. All 11 affected individuals developed myopia before school age, and four of these were found to have developed high myopia before the age of 5 years by on site examination. None of the individuals who married into the family had myopia greater than -1.00 D. All unaffected siblings or offspring were either emmetropes or hyperopes (Table 1). No other families living in the same village at the time this family was collected had high myopia. Ophthalmological examination excluded known ocular diseases associated with myopia, including keratoconus, spherophakia, ectopia lentis, retinal dystrophy, and optic atrophy. Fundus examination revealed changes typical of high myopia in persons of Chinese ethnicity as shown in the proband in Figure 1. Males and females in this family were affected with equal severity. The ERG recorded in the proband was

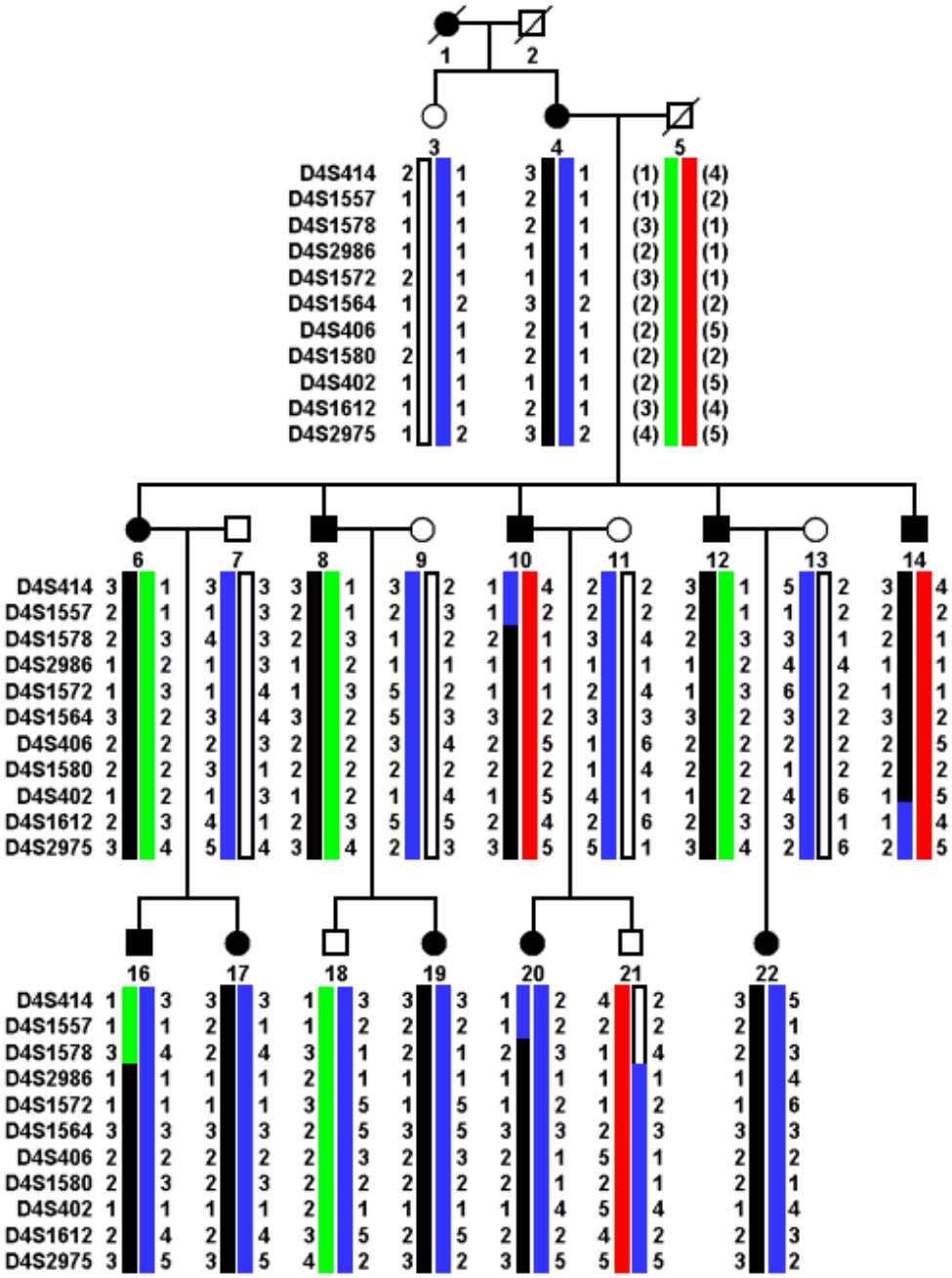

Figure 2. Pedigree and haplotype diagram of the family with high myopia. Black bars represent the disease allele. The white, green, blue, and red bars are all normal alleles and indicate how the different alleles are transmitted in the family. Squares or circles filled with black indicate individuals affected with high myopia.

comparatively normal except for a mild reduction of cone amplitude (Figure 1). An ocular A-scan in the proband recorded an axial length of 28.13 mm (OD) and 28.00 mm (OS). Keratometric measurement of the proband was 44.12/41.00 D (OD) and 44.12/41.00 D (OS). No systemic abnormalities were noted in any affected individual.

Markers around the 4 known loci for autosomal dominant high myopia showed no statistically significant or suggestive evidence of linkage in this family (not shown). Upon completion of a genome-wide scan of chromosomes 1 through 22, a two-point linkage analysis yielded lod scores greater than 1.0 with markers D1S2878, D4S1572, D4S406, D4S402, D5S647, D5S641, D7S640, and D10S1693, corresponding to 5 possible loci, on chromosomes 1, 4, 5, 7, and 10. The loci on chromosome 1, 5, 7, and 10 were excluded by lod scores of minus infinity for closely flanking markers and by nonsegregation of the supposed disease alleles (haplotypes) in affected family members (not shown). In contrast, fine mapping and haplotype analysis confirms the locus on chromosome 4q22-q27 (Figure 2, Table 2). This locus maps to a 20.4 cM (25.24 Mb) region between D4S1578 and D4S1612. All 6 microsatellite markers examined inside this region show positive lod scores, with D4S1564 showing the highest lod score, 3.11 at θ=0.

Haplotypes of family members in this region support the linkage results (Figure 2). Recombination at D4S1578 in affected individual 16 with further recombination at D4S1557 in affected individual 10 confirmed in individual 20 sets the proximal boundary. Recombination at D4S1612 in individual 14 sets the distal boundary for the linked region. Linkage analysis based on the haplotypes generated by 6 linked markers (D4S2986, D4S1572, D4S1564, D4S406, D4S1580, and D4S402) gives a lod score of 3.61 at θ=0 (Table 2), close to the theoretical maximum lod score which could be generated in this type of family. Individual 7 shared part of the haplotye observed in affected family members, did not have myopia. This may reflect an ethnic relation in a distant progenitor.

RRH (retinal pigment epithelium-derived rhodopsin homolog) is located inside the linked region [20]. Sequence analysis of its seven exons and exon-intron boundaries revealed no potentially causative mutations.

DISCUSSION

In this study, autosomal dominant high myopia in a Chinese family is assigned to a new locus on chromosome 4q22-q27 between D4S1578 and D4S1612. Exclusion of known loci for autosomal dominant high myopia, positive two point lod scores of all 6 markers inside the linked region, haplotype observation, and haplotypes of the six linked markers giving a lod score of 3.61 at θ=0, all support this new locus for autosomal dominant high myopia in the family.

It is important (but can be difficult) to differentiate a hereditary high myopia from acquired high myopia in a linkage analysis. Isolation of the environmental influences on familial myopia is potentially more important than simply setting a phenotypic boundary to separate high myopia from common myopia. As children living in industrialized societies or cities such as Hong Kong begin attending school, annual myopic changes increase about -0.63 D in children with myopia and -0.29 D without myopia [21]. Some preschool children with moderate myopia living in such places would be possibly expected to have high myopia in a few years as they initiate increased “near work”. In this case, age of onset may be critical but environment impact is still a problem. Genetic factors, as opposed to environmental factors, might be expected to dominate the development of myopia for people living in remote areas [1,2]. Compared to the vast progress in identifying genes for retinal degeneration, so far no gene causing

Table 2. Two-point linkage results for markers in the high myopia region at 4q22-4q27

	Position		Lod score at θ								
Markers	cM	Mb	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Zmax	θmax
D4S414*	99.20	91.33	-∞	-0.74	0.44	0.77	0.78	0.53	0.23	0.83	0.15
D4S1557	99.20	95.32	-∞	-2.33	-1.06	-0.61	-0.25	-0.07	-0.01	0.00	0.55
D4S1578	103.10	97.44	-∞	1.25	1.72	1.72	1.38	0.87	0.35	1.75	0.07
D4S2986	103.60	100.01	0.76	0.81	0.92	0.95	0.87	0.67	0.38	0.95	0.10
D4S1572*	106.30	104.13	1.54	1.52	1.42	1.30	1.03	0.74	0.40	1.54	0.00
D4S1564	111.10	108.73	3.11	3.05	2.80	2.47	1.78	1.04	0.33	3.11	0.00
D4S406*	115.80	112.08	2.11	2.07	1.91	1.70	1.26	0.79	0.32	2.11	0.00
D4S1580	120.80	116.49	0.50	0.54	0.64	0.66	0.57	0.38	0.13	0.66	0.10
D4S402*	123.50	120.51	1.37	1.41	1.48	1.46	1.28	0.96	0.54	1.48	0.07
D4S1612	123.50	122.68	-∞	1.56	2.02	2.02	1.68	1.17	0.60	2.05	0.07
D4S2975	125.10	125.80	-∞	1.56	2.02	2.02	1.68	1.17	0.60	2.05	0.07
Haplotype			3.61	3.55	3.30	2.98	2.28	1.52	0.72	3.61	0.00

Two-point linkage results for markers in the high myopia region at 4q22-4q27 support location of the disease locus between D4S1578 and D4S1612. Asterisks indicate markers included in the ABI PRISM MD10 linkage Mapping Set Version 2 which were used in the initial genome-wide scan. The haplotype referenced in the “Markers” column is the haplotype with markers D4S2986-D4S1572-D4S1564-D4S406-D4S1580-D4S402.

nonsyndromic myopia (common or high) when mutated, has been identified. The most likely explanation of this difficulty lies in the gene-environment interplay affecting even Mendelian myopia [1,2,6]. A portion of high myopia may actually be a complex disorder like common myopia. In addition, since even high myopia is common, its occurrence in individuals married into the family can also make linkage analysis problematic. Thus, families showing a minimum environment impact on myopia development provide an optimal setting for identification of the causative genes.

The family reported here meets the above criteria for a linkage study of high myopia. They all maintain a rural lifestyle in a small village. All affected individuals developed high myopia before school age. In this family, individuals with high myopia are clearly distinguishable from unaffected individuals, especially since no individuals marrying into the family have myopia greater than -1.00 D. All affected individuals have typical fundus changes of high myopia generally observed in Chinese. Except for high myopia, no other ocular and systemic abnormalities were documented.

Five loci for high myopia have been reported so far. The first locus MYP1 (OMIM 310460) on Xq28 was reported in 1990 based on linkage analysis of Bornholm eye disease with F8C [11]. The second locus MYP2 (OMIM 160700) on chromosome 18p11.31 was identified based on genome-wide scan of 8 families [9]. The myopia in 7 of the 8 families, including one Chinese family living in Hawaii, was mapped to 18p11.31. The third locus MYP3 (OMIM 603221) on chromosome 12q21-q23 and fifth locus MYP5 (OMIM 608474) on chromosome 17q21-q22 were identified based on a study of a large Italian/German family and a large English/Canadian family, respectively [7,12]. The fourth locus MYP4 (OMIM 608367) on chromosome 7q36 was suggested based on a maximum multipoint lod score of 2.81 generated from study of 21 French families and 2 Algerian families [10]. These loci were reported to be responsible for a small portion of high myopia based on a study of 51 UK families with high myopia [13]. Linkage of common myopia to these loci has not been found [14,15]. However, several possible loci for common myopia have been suggested [14,15], including MYP6 (OMIM 608908) on chromosome 22q12, MYP7 (OMIM 609256) on chromosome 11p13, MYP8 (OMIM 609257) on chromosome 3q26, MYP9 (OMIM 609258) on chromosome 4q12, and MYP10 (OMIM 609259) on chromosome 8p23. All these loci were identified in a study of families living in industrialized societies. The locus reported here is neither in the region of the 5 known loci for high myopia nor in the region of the 5 loci suggestied for common myopia. This is the first family living in the rural area of a developing country in which a high myopia locus has been mapped.

In summary, a new locus for autosomal dominant high myopia in a Chinese family was mapped to chromosome 4q22-q27 with the highest lod score of 3.61 at θ=0. This is the sixth locus for high myopia. Currently, other candidate genes inside the linked region are being screened for mutations. Recruitment of new families for analysis (which is also being pursued) would possibly narrow down the linked region and therefore facilitate the cloning of the causative gene. Identification of the mutant gene potentially would provide significant insight into the molecular mechanisms underlying the most common cause of visual impairment.

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