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A Second Locus for Familial High Myopia Maps to Chromosome 12q

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Summary

Myopia, or nearsightedness, is the most common eye disorder worldwide. "Pathologic" high myopia, or myopia of $\leqslant -6.00$ diopters, predisposes individuals to retinal detachment, macular degeneration, cataract, or glaucoma. A locus for autosomal dominant pathologic high myopia has been mapped to 18p11.31. We now report significant linkage of high myopia to a second locus at the 12q21-23 region in a large German/Italian family. The family had no clinical evidence of connective-tissue abnormalities or glaucoma. The average age at diagnosis of myopia was 5.9 years. The average spherical-component refractive error for the affected individuals was −9.47 diopters. Markers flanking or intragenic to the genes for the 18p locus, Stickler syndromes type I and II (12q13.1-q13.3 and 6p21.3), Marfan syndrome (15q21.1), and juvenile glaucoma (chromosome 1q21-q31) showed no linkage to the myopia in this family. The maximum LOD score with two-point linkage analysis in this pedigree was 3.85 at a recombination fraction of .0010, for markers D12S1706 and D12S327. Recombination events identified markers D12S1684 and D12S1605 as flanking markers that define a 30.1-cM interval on chromosome 12q21-23, for the second myopia gene. These results confirm genetic heterogeneity of myopia. The identification of this gene may provide insight into the pathophysiology of myopia and eye development.

Introduction

Myopia in the United States is common (25% of adults) (Sperduto et al. 1983) and has a high societal cost with respect to associated eye pathology, eye examinations, and dollars spent each year on refractive aids (National Advisory Council, Strabismus, Amblyopia and Visual Procesing Panel 1993). It has been estimated that 5.6% of blindness among schoolchildren in the United States is attributable to myopia (National Advisory Council, Strabismus, Amblyopia and Visual Procesing Panel 1993). Severe myopia of $\leqslant -6.00$ diopters (D), described as "pathologic" myopia, is associated with glaucoma, macular degeneration, cataracts, and retinal detachment and contributes significantly to loss of vision in adults (Curtin 1985). Pathologic myopia occurs primarily because of increased axial length of the eye, rather than corneal or lenticular conical changes. The cause of myopia is unknown, and both genetic and environmental factors have been implicated. Analysis of selected pedigrees suggests that high myopia is inherited as a dominant gene (Flach 1942; Franceschetti 1953; Francois 1961).

We have performed a genome screen for myopia-susceptibility loci in several medium to large multigenerational families with autosomal dominant high myopia of $\leqslant -6.00$ and have found significant linkage at 18p11.31 (Young et al. 1998). This analysis was performed on the assumption that there may be several loci associated with myopia, because it is a common disorder. In our original analysis, significant linkage to chromosome 18p11.31 was found in seven of eight families (Young et al. 1998). One of eight families studied in the original analysis did not show linkage either to that 18p locus or to the 12q locus that we herein describe. We now report significant linkage to a 12q locus in a single large family.

Subjects And Methods

Subjects

A large Italian/German family (family MYO-10) consented to participate in the study. Criteria for selection included a history of onset of myopia at <12 years of age, in all affected subjects (parents and offspring); myopia of $\leqslant -6.00$ D; and two or more generations affected. Children <12 years of age were classified as unknown if they had a myopic cyloplegic refractive error.

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The diagnosis of myopia was determined on the basis of the refractive error. No participants had known ocular disease or insult that could predispose to myopia; nor did any of them have a known genetic disease associated with myopia, such as Stickler syndrome or Marfan syndrome.

Ophthalmology examination and blood collection were performed by one of the authors of us (T.L.Y.), as described elsewhere (Young et al. 1998). In most instances, participants declined axial-length measurements of their eyes and keratometry measurements of their corneas. Details of the ophthalmic examination are summarized in table 1. This study was approved by the University of Minnesota Hospital and Clinics Institutional Review Board.

DNA Analysis/Marker Typing

DNA analysis was performed as described elsewhere, with multiplexed primer pairs and fluorescent detection techniques by means of an LI-COR DNA 4000 infrared sequencer (LI-COR) (Young et al. 1998). For fine mapping, additional markers were selected from genetic maps of 12q (Gyapay et al. 1994; Dib et al. 1996).

Linkage Analysis

Linkage analysis was performed in the manner that has been described elsewhere (Young et al. 1998) for the chromosome 18p locus. Standard marker databases were used for intermarker recombination frequencies and order (as taken from The Genome Data Base [Fasman et al. 1994], Généthon [Dib et al. 1996], The Cooperative Human Linkage Center, and the Marshfield Medical Research Foundation). Genetic distances between isolated 12q markers were additionally determined by means of the CEPH panel of reference families and the analysis program CRIMAP (Lander and Green 1987), as well as by radiation-hybrid analysis of chromosome 12 markers by use of the automated services, for radiation-hybrid mapping, of the Stanford Institute for Genome Research (Stewart et al. 1997).

Analysis of the genotype data was performed by a nonparametric multipoint method, GENEHUNTER (Kruglyak et al. 1996). This analysis did not contribute any additional mapping data.

Results

One large multigeneration family with autosomal dominant high myopia was characterized. Figure 1 shows the pedigree structure of this family (family MYO-10). The average age at diagnosis of myopia in affected individuals was 5.9 years (range 4–8 years). The average spherical-component refractive error for the affected individuals was −9.47 D (range −6.25 to −15.00 D). Glaucoma, keratoconus, lenticonus, and dislocated lens were not present in study participants. The representative average ± SD axial length of 30.06 ± 0.54

Table 1

Refractive-Error Characteristics in Family MYO-10

Person (Gender)	Refractive Error	Age at Onset (years)	Age at Exam (years)	OD Axial Length/ OS Axial Length (mm)	OD Keratometry/ OS Keratometry (D)
3 (M)	−7.50 + 1.50 × 28, − 6.50 + 1.00 × 148	7	>70		
4 (F)	+8.00 sphere, +9.25 sphere		70		
5 (M)	−14.00 + 0.75 × 35, − 13.25 sphere	5	61	29.68/30.44	44.25/43.50, 44.00/43.00
6 (F)	−3.50 + 0.50 × 65, − 4.00 + 1.00 × 65		59		
7 (M)	−13.00 sphere, −14.00 sphere	6	72		
9 (M)	−1.75 + 1.25 × 15, − 2.00 + 0.75 × 170		64		
10 (F)	−6.25 + 1.75 × 110, − 3.50 + 1.25 × 85	7	63		
11 (F)	−8.25 + 0.75 × 105, − 9.00 + 0.50 × 73	5	34		
12 (F)	−9.00 + 1.50 × 100, − 8.25 + 1.00 × 90	5	35		
13 (M)	−4.50 + 0.50 × 90, − 5.25 + 0.50 × 82		37		44.25/45.00, 44.25/45.25
14 (F)	−10.50 + 1.00 × 47, − 10.25 + 1.25 × 145	4	37		45.75/46.00
15 (M)	−12.00 sphere, −13.00 sphere	5	32		
16 (F)	−4.50 sphere, −4.50 sphere		29		
17 (F)	−9.00 + 0.75 × 80, − 8.50 + 0.75 × 84	7	26		
18 (M)	−7.75 + 2.25 × 90, − 8.00 + 2.25 × 90	6	46		46.00/44.75, 46.00/45.00
19 (M)	−7.75 + 2.25 × 90, − 8.00 + 2.25 × 90	5	40		
20 (M)	−13.00 sphere, −15.00 sphere	5	38		
21 (M)	−7.75 sphere, −7.75 sphere	7	35		
22 (F)	−6.75 + 0.50 × 178, − 7.75 + 0.50 × 177	7	33		
23 (M)	−3.50 + 0.25 × 100, − 4.00 + 0.50 × 80	8	8		
24 (F)	+0.25 sphere, plano + 0.25 × 180	No glasses	4		
25 (F)	Plano, plano	No glasses	7		

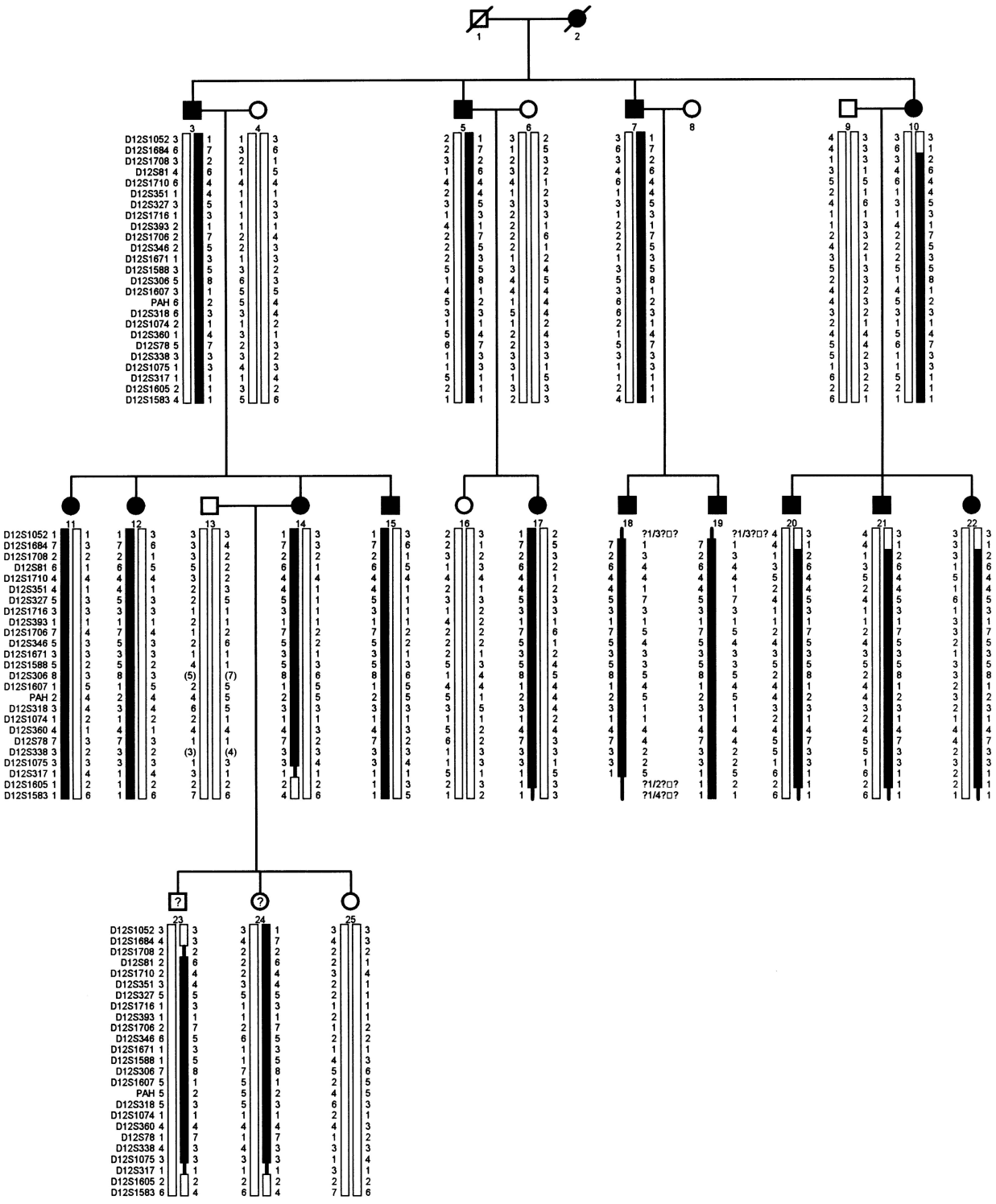

Figure 1 Family MYO-010, with familial high myopia. Circles and squares denote females and males, respectively; blackened symbols denote affected individuals; a diagonal line through a symbol denotes that the individual is deceased; a questions mark within a symbol denotes that the affection status is unknown. The alleles for all polymorphic markers are shown for each studied individual. Haplotypes were constructed on the basis of the minimum number of recombinations between these markers. The chromosome assumed to carry the inherited disease allele is depicted as a blackened bar, and unblackened bars represent the normal haplotypes. Only essential matings are shown; nonparticipating family members are not shown. Allele determination for markers D12S306 and D12S338 for individual 13 was not possible, since numerous attempts at PCR amplification failed; therefore, the allele assignments (in parentheses) were inferred on the basis of those of the offspring. For individuals 18 and 19, only one set of parental-allele information was available; therefore, genotype information was indeterminate (denoted by question marks) for markers D12S1052, D12S1605, and D12S1583. A thinner black line extending from a blackened bar denotes identical parental marker-allele assignment. Note that individuals 14, 23, and 24 are recombinant for the telomeric marker D12S1605. Individuals 10 and 20–23 are recombinant for the centromeric marker D12S1684.

Table 2

Two-Point Linkage Analysis, of High Myopia and Markers on Chromosome 12q

Marker[a]	Total LOD Score at θ =							Maximum Recombination Fraction	Maximum LOD Score
	.0	.01	.05	.1	.2	.3	.4		
D12S1052	.10	.31	.58	.63	.48	.26	.07	.0900	.63
D12S1684	2.28	2.46	2.64	2.55	2.08	1.43	.65	.0520	2.64
D12S1708	2.93	2.88	2.65	2.35	1.74	1.10	.44	.0010	2.93
D12S81	3.45	3.39	3.12	2.78	2.07	1.31	.53	.0010	3.45
D12S1710	2.84	2.79	2.57	2.29	1.69	1.05	.40	.0010	2.84
D12S351	3.32	3.26	3.00	2.67	1.99	1.26	.52	.0010	3.32
D12S327	3.85	3.78	3.51	3.16	2.40	1.57	.68	.0010	3.85
D12S1716	3.02	2.96	2.72	2.42	1.78	1.11	.43	.0010	3.02
D12S393	3.39	3.33	3.09	2.77	2.09	1.36	.56	.0010	3.39
D12S1706	3.85	3.79	3.51	3.16	2.40	1.57	.68	.0010	3.85
D12S346	3.82	3.75	3.48	3.13	2.37	1.56	.67	.0010	3.82
D12S1671	3.01	2.95	2.71	2.40	1.77	1.10	.42	.0010	3.01
D12S1588	1.89	1.85	1.68	1.47	1.03	.59	.20	.0010	1.89
D12S306	3.53	3.46	3.19	2.84	2.11	1.33	.54	.0010	3.53
D12S1607	3.76	3.69	3.42	3.07	2.33	1.52	.65	.0010	3.76
PAH	3.80	3.73	3.46	3.11	2.36	1.54	.66	.0010	3.80
D12S318	1.99	1.95	1.79	1.58	1.14	.67	.22	.0010	1.99
D12S1074	1.07	1.05	.95	.82	.55	.30	.08	.0010	1.07
D12S360	3.76	3.69	3.42	3.07	2.33	1.52	.65	.0010	3.76
D12S78	3.84	3.77	3.50	3.14	2.38	1.56	.66	.0010	3.84
D12S338	1.66	1.63	1.48	1.30	.91	.52	.16	.0010	1.66
D12S1075	2.50	2.46	2.27	2.02	1.49	.94	.37	.0010	2.50
D12S317	1.11	1.08	.97	.84	.55	.28	.07	.0010	1.11
D12S1605	$-\infty$	.88	1.37	1.40	1.14	.73	.28	.0810	1.41
D12S1583	$-\infty$	-.59	.06	.26	.32	.21	.09	.1700	.33

[a] Order shown is from centromere to telomere

mm in individual 5 was significantly longer ($P < .001$ [Student's *t*-test]) than the published adult normal value of 24.2 ± 0.85 mm (Sorsby et al. 1962). The average keratometry reading of affected individuals 5, 14, and 18, 44.83 ± 1.11 D, was not significantly higher ($P = .005$ [Student's *t*-test]) than the published adult normal value of 43.1 ± 1.62 D (Sorsby et al. 1962).

Exclusion of Candidate Genes/Regions

The genes responsible for several autosomal dominant disorders that present with high myopia were evaluated as candidate genes, including those for juvenile-onset glaucoma, mild Stickler syndrome, and Marfan syndrome. Selected candidate-gene markers (Young et al. 1998) for these disorders were tested for possible linkage in family MYO-10. The results revealed significant nonlinkage (LOD score −2.00) with regard to these candidate disorders (data not shown).

Evaluation of significantly linked 18p markers showed significant nonlinkage in the MYO-10 family. Markers D18S476, D18S481, and D18S63 resulted in LOD scores of −3.10, −6.58, and −4.72, respectively, at a recombination fraction of .0100.

Genome Screen

Two-point linkage analysis, of an intragenic marker for the gene phenylalanine hydroxylase (PAH) and myopia, demonstrated a cumulative LOD score of 3.80 at a recombination fraction of .0010 in family MYO-10. Twenty additional markers were analyzed in this region. The LOD-score results for two-point analysis, for each marker and myopia, are shown in table 2. The maximum LOD scores for family MYO-10 were 3.85 at a recombination fraction of .0010, for markers D12S1706 and D12S327.

Haplotype Analysis

Haplotype analysis of affected individuals revealed recombination events that narrow the region containing the gene, as shown in figure 1. By haplotype analysis, it was discovered that individuals 23 and 24 had inherited the allele pattern of their affected relatives, although at the time of examination they were not diagnosed as affected. Individual 24 was 4 years of age at the time of her initial eye examination and blood draw. Her refractive error was essentially plano at that time. Most children in this age group have, on average, a moderately hyperopic refractive error of +1.00 to +2.50 D, and

therefore it would appear that individual 24 was pre-myopic at the time of her examination. The most current refractive error for individual 23 at 9.5 years of age was $-4.00 + 0.75 \times 110$ for the right eye (OD) and $-5.25 + 1.00 \times 73$ for the left eye (OS). The most current refractive error for individual 24, who now, at 5.5 years of age, wears spectacles, is -2.75 sphere OD and -3.00 sphere OS. It is anticipated that individuals 23 and 24 will become highly myopic ($\leqslant -6.00$ D) at <12 years of age. For linkage analysis, however, individuals 23 and 24 were classified as unknown, since they did not meet the criteria of $\leqslant -6.00$ D- myopia.

The critical region was found to be between markers D12S1684 and D12S1605. A centromeric recombinant event was inferred to be present between markers D12S1708 and D12S1684 in individual 10 and to have been inherited by her offspring. A telomeric recombination event occurs between markers D12S1605 and D12S1075 in individual 14 and has been inherited in persons 23 and 24. Individual 14 is uninformative for D12S317. This potentially narrows the region to a 30.1-cM interval between these two markers (fig. 2).

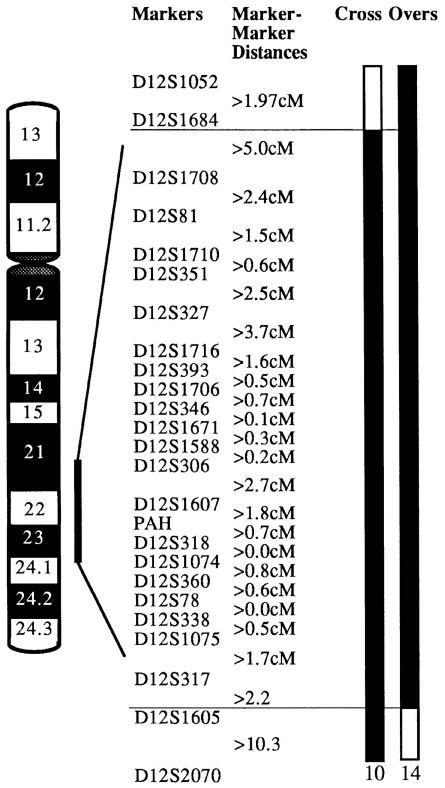

Figure 2 Ideogram of chromosome 12q: placement of markers in band 12q21-23 and interval containing the MYP3 locus, in family MYO-10. Genetic distances are in centimorgans. The areas of informative crossovers seen in affected individuals 10 and 14 are indicated by the blackened portions of their haplotypes

Discussion

To our knowledge, this is the second genetic locus for high familial myopia. LOD-score analysis places a gene for myopia on chromosome 12q21-23, within a 30.1-cM interval. This study provides evidence for genetic heterogeneity of autosomal dominant high myopia, already anticipated clinically. Since the chromosome 18p locus has been designated "MYP2" by the Human Gene Nomenclature Committee, we take the liberty of naming this second identified locus for nonsyndromal autosomal dominant high myopia on chromosome 12q "MYP3." The first locus, MYP1, has been appointed for a sex-linked high myopia at Xq28 (Schwartz et al. 1990).

Exclusion of linkage to the candidate-gene regions for juvenile glaucoma, Stickler syndrome, and Marfan syndrome was essential, to ensure that none of the families that we studied exhibited a mild phenotypic expression or phenocopy of high myopia for any of these autosomal dominant, early-onset disorders.

A search for genes and/or expressed sequence tags physically mapped between the two markers D12S1684 and D12S317 reveals 119 unidentified transcripts, 12 mRNAs, and 30 sequences for regulatory or structural genes (National Center for Biotechnology Information database). Selected genes among these are those for PAH, lumican, decorin, and dermatan sulfate proteoglycan (DSPG3). Although an intragenic polymorphic marker for PAH shows significant linkage to the myopia in this pedigree, it is unclear how mutations in PAH may be associated with high myopia. Of 328 different mutations by state that have been collected by the PAH Mutation Analysis Consortium Database, the majority are rare mutations causing hyperphenlyalaninemia, and the remainder are polymorphic variants without apparent effect on phenotype (Nowacki et al. 1998).

Of even greater interest are candidate genes relevant to ocular structures that map to this region. Decorin and lumican are members of the small interstitial proteoglycan family of proteins that are expressed in the extracellular matrix of various tissues. Both interact with collagen and limit the growth of fibril diameter (Hedbom and Heinegard 1989; Bianco et al. 1990; Rada et al. 1993; Hedlund et al. 1994). Decorin and lumican are present in corneal stroma and in the interstitial matrices of the heart, aorta, skeletal muscle, skin, and intervertebral disks (Chakravarti et al. 1995). DSPG3, another small interstitial proteoglycan, is expressed in cartilage, as well as in ligament and placental tissues (Deere et al. 1996). Whereas the presence of lumican or DSPG3 has not been demonstrated in sclera, other members of the proteoglycan family, such as decorin, biglycan, and aggrecan, have been found to be present in this tissue (Rada et al. 1997). Fibrillogenesis of the sclera may be affected

by mutations in these candidate proteins, as has been demonstrated in other connective-tissue disorders that manifest with myopia, such as Sticker syndrome and Marfan syndrome.

In summary, we have mapped a second genetic locus for high myopia. We continue in our efforts to reduce the 30.1-cM critical region for high myopia, through recruitment and analysis of new families prior to conducting gene-isolation experiments to identify and clone the gene responsible for this myopia phenotype. We continue our work, as well, to identify other possible chromosomal loci for myopia. Mutational characterization of the genes for high myopia will, it is hoped, provide insight into the molecular mechanisms underlying eye growth regulation.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, The, http://www.chlc.org/ChlcMaps.html
Généthon, http://www.genethon.fr
Genome Database, The http://gdbwww.gdb.org
Human Gene Nomenclature Committee, http://www.gene.ucl.ac.uk/nomenclature
Marshfield Medical Research Foundation, http://www.marshmed.org/genetics
National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/msrch2
Stanford Institute for Genome Research, http://www.shgc.stanford.edu

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