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Evidence That a Locus for Familial High Myopia Maps to Chromosome 18p

Terri L. Young,[1] Shawn M. Ronan,[1] Leslie A. Drahozal,[1] Scott C. Wildenberg,[2,3] Alison B. Alvear,[1] William S. Oetting,[2,3] Larry D. Atwood,[3,4] Douglas J. Wilkin,[5] and Richard A. King[2,3]

Departments of [1]Ophthalmology and [2]Medicine, [3]Institute of Human Genetics, and [4]Division of Epidemiology, University of Minnesota, Minneapolis; and [5]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda

Summary

Myopia, or nearsightedness, is the most common human eye disorder. A genomewide screen was conducted to map the gene(s) associated with high, early-onset, autosomal dominant myopia. Eight families that each included two or more individuals with $\geqslant -6.00$ diopters (D) myopia, in two or more successive generations, were identified. Myopic individuals had no clinical evidence of connective-tissue abnormalities, and the average age at diagnosis of myopia was 6.8 years. The average spherical component refractive error for the affected individuals was −9.48 D. The families contained 82 individuals; of these, DNA was available for 71 (37 affected). Markers flanking or intragenic to the genes for Stickler syndrome types 1 and 2 (chromosomes 12q13.1-q13.3 and 6p21.3, respectively), Marfan syndrome (chromosome 15q21.1), and juvenile glaucoma (chromosome 1q21-q31) were also analyzed. No evidence of linkage was found for markers for the Stickler syndrome types 1 and 2, the Marfan syndrome, or the juvenile glaucoma loci. After a genomewide search, evidence of significant linkage was found on chromosome 18p. The maximum LOD score was 9.59, with marker D18S481, at a recombination fraction of .0010. Haplotype analysis further refined this myopia locus to a 7.6-cM interval between markers D18S59 and D18S1138 on 18p11.31.

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Address for correspondence and reprints: Dr. Terri L. Young, University of Minnesota, Department of Ophthalmology, Box 493, 420 Delaware Street, Minneapolis, MN 55455. E-mail: young061@gold.tc.umn.edu

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Introduction

Myopia is common (25% of adults) in the United States (Sperduto et al. 1983), and its cost to society is high (National Advisory Council, Strabismus, Amblyopia and Visual Processing Panel 1993). Refractive eye examinations are estimated to cost $1 billion annually, and $1.5 billion is spent each year on eyeglasses (National Advisory Council, Strabismus, Amblyopia and Visual Processing Panel 1993). It has been estimated that 5.6% of blindness among U.S. school children is attributable to myopia (National Advisory Council, Strabismus, Amblyopia and Visual Processing Panel 1993). Severe myopia of $\geqslant -6.00$ diopters (D), described as "pathologic" myopia, is associated with glaucoma, macular degeneration, cataracts, and retinal detachment, and it contributes significantly to loss of vision in adults (Curtin 1985). The frequency of myopia at or above this level in myopic populations has been reported to range from 27% to 33.2%, which corresponds to rates of 1.7%–2.1% for the general population (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; both genetic and environmental factors have been implicated. Evidence for a genetic component to myopia is provided by population and family studies. The incidence of myopia among school children in Taiwan approaches 70% (Lin et al. 1988), which suggests that this population is genetically susceptible to myopia. Children of myopic parents are more likely to have myopia than are children of nonmyopic parents (Goldschmidt 1981; Ashton 1985; Gwiazda et al. 1993; Minkovitz et al. 1993). The ocular components (axial length, anterior chamber depth, and corneal curvature) and refractive errors of MZ twins are more closely aligned than are those of DZ twins (Francois 1961; Sorsby et al. 1962*b*; Teikari et al. 1991).

Autosomal recessive and autosomal dominant modes of inheritance of myopia have been suggested. Recessive

Table 1

Summary of Ophthalmologic Biometric Parameters Contributing to Refractive Error for the Participants in Eight Pedigrees

Subject (Sex)	Refractive Error (OD; OS)	Age at Onset (years)	Age at Exam (years)	Axial Length (OD; OS [mm])	Keratometry (OD; OS [D])
Pedigree 1:					
3 (F)	−6.00+2.00 × 15; −6.25+1.75 × 105	7	76		
5 (F)	−1.75+1.50 × 5; −2.00+2.00 × 163	15	76		
8 (M)	−3.50+1.50 × 10; −2.00+.75 × 135	14	84		
10 (M)	−9.00+1.25 × 100; −7.75+.50 × 150	8	39		
11 (F)	−5.00+2.00 × 80; −5.25+2.25 × 86	8	35		
12 (M)	−8.00+1.50 × 97; −7.75+2.25 × 86	6	34		
13 (M)	−6.00+.50 × 105; −6.50+1.50 × 60	7	…		
14 (M)	−7.50+3.25 × 95; −6.50+2.50 × 90	6	46		
15 (M)	−10.50+2.50 × 82; −11.50+2.75 × 97	4	48		
16 (F)	−11.00+.75 × 60; −12.25+1.00 × 70	4	54	27.59; 28.00	41.00/43.50; 41.00/43.50
17 (M)	−3.00+.50 × 95; −4.50+1.50 × 60	7	45		
18 (M)	−7.50+1.50 × 40; −8.50+1.75 × 135	6	52		
19 (M)	−6.00+1.50 × 50; −6.50+.75 × 80	9	53		
20 (M)	−.50 sph; −2.00+1.00 × 180	17	42		
21 (F)	Plano	…	59		
22 (F)	Plano	…	50		
Pedigree 2:					
2 (F)	−6.00+2.50 × 5; −6.75+2.50 × 178	12	83		
3 (F)	−6.00 sph; −6.50 sph	9	60		
4 (M)	−4.00+2.00 × 180; −4.50+1.50 × 180	7	67		
5 (F)	−14.75+.50 × 107; −14.50+.50 × 105	10	30	28.96; 29.37	Not obtained
6 (M)	−16.00 sph OU	5	38		
7 (M)	−5.50+1.25 × 63; −5.50 sph	9	36		
8 (F)	−6.75 sph OU	11	33		
9 (F)	−12.25+.75 × 120; −12.50+1.00 × 180	6	36		
10 (M)	Plano OU	…	6		
11 (M)	−.25 sph OU	…	8		
Pedigree 3:					
1 (M)	+1.25+.50 × 147; +.25+1.00 × 90	…	68		
2 (F)	−7.50+2.00 × 60; −7.00+1.00 × 15	13	63		
3 (F)	Plano; −.50 sph	…	33		
4 (F)	−17.00+.50 × 137; −18.50+.37 × 25	4	38	28.69; Pt declined OS	46.50/46.50; 47.25/47.50
5 (M)	−5.00 sph; −6.50+.75 × 8	12	37		
6 (M)	−19.25+2.25 × 135; −21.00+3.00 × 60	2	4		
7 (M)	−8.50+2.00 × 106; −7.00+.25 × 87	.5	2		
Pedigree 4:					
1 (M)	−10.25+.50 × 170; −10.75+1.25 × 175	7	66	25.10; 25.00	42.00/42.50; 42.75/43.00
2 (F)	+1.00 sph; +.25 sph	…	62		
4 (F)	−7.25+.50 × 18; −7.25+.50 × 60	11	33		
5 (F)	−6.25+.75 × 115; −5.25+.75 × 72	8	22		
6 (M)	−4.75+1.00 × 175; −4.50+.75 × 160	13	35		
7 (F)	−6.25 sph; −6.00 sph	5	12		
8 (M)	Plano OU	…	10		
Pedigree 5:					
1 (M)	−.50+.75 × 178; −.50+1.00 × 50	24	75		
2 (F)	−7.00 sph; −7.25 sph	7	67		
3 (M)	−3.00+.50 × 69; −2.75+.25 × 99	13	37		
4 (M)	−3.25+1.50 × 110; −1.75+.75 × 80	12	38		
5 (F)	−2.75+.25 × 180; −2.75+.50 × 105	12	36		
6 (M)	−10.00+1.50 × 46; −8.25+1.75 × 105	6	35		
7 (F)	−8.00+.25 × 90; −8.00+.25 × 90	6	33		
8 (M)	−2.75+.25 × 76; −2.75+.25 × 91	15	31		

(*continued*)

Table 1 (continued)

Subject (Sex)	Refractive Error (OD; OS)	Age at Onset (years)	Age at Exam (years)	Axial Length (OD; OS [mm])	Keratometry (OD; OS [D])
9 (M)	+.25+.50 × 170; +.25+.25 × 170	...	29		
10 (F)	−6.25+.75 × 50; −8.00+1.00 × 120	7	25		
11 (M)	−8.00+1.00 × 50; −8.00+.75 × 79	5	23	28.0; 28.1	
12 (M)	+1.75 sph; +1.00	...	7		41.50/42.875; 41.875/42.75
13 (F)	+.75 sph; +.50 sph	...	6		
Pedigree 6:					
1 (M)	−8.75+4.00 × 160; OS not available	7	80	25.25; 25.10	
2 (F)	−5.50+4.00 × 18; Plano+2.00 × 80	8	72		45.87/44.00; 46.50/44.25
3 (F)	−12.00+2.00 × 100; −10.25 sph	4	48		
4 (F)	−7.25+1.25 × 180; −7.25+1.25 × 20	5	47		
5 (M)	−1.25+.25 × 12; −1.50+1.00 × 163	16	49		
6 (M)	Plano OU	...	19		
Pedigree 7:					
1 (M)	−7.25+1.00 × 145; −8.25+1.25 × 170	16	...	26.05; 24.70	
2 (F)	−.25 sph; −.50 sph	...	...		43.12/43.75; 43.50 /43.87
3 (F)	−5.00 sph; −5.75 sph	7	...		
4 (M)	−.75+.75 × 140; −2.00 sph	18	29		
5 (M)	−10.75+2.00 × 10; −10.50+2.50 × 70	6	23		
Pedigree 8:					
3 (M)	−11.00 sph OU	11	50		
5 (M)	−7.25 sph OU	13	48		
6 (F)	−4.00 sph OU	14	47		
7 (M)	−14.00 sph; −12.00 sph	6	34	29.10; 28.64	
8 (M)	−10.00 sph OU	9	33		
9 (F)	−.75 sph; −.75 sph	17	18		44.75/45.37; 45.00/45.25
10 (M)	Plano OU	...	20		

NOTE.—M = male; F = female; OD = right eye; OS = left eye; sph = sphere; Pt = patient; Plano = neutral refractive error; and OU = both eyes.

inheritance has been the recent paradigm and may represent the most common mode of inheritance (Macklin 1927; Karlsson 1975; Edwards et al. 1991). Analysis of selected pedigrees, however, suggests that myopia may also be inherited as a dominant gene with variable penetrance. One of the earliest reports of autosomal dominant myopia is that by Flach (1942), who reported a three-generation family. Four-generation families with severe myopia have been described by Francois (1961) and by Franceschetti (1953). DelBono et al. (1995) recently described 52 two- and three-generation families with two or more individuals affected by juvenile-onset myopia ($\geqslant -0.75$ D by age 15 years).

We conducted a genome screen to search for myopia-susceptibility loci in eight medium-to-large multigenerational families with an autosomal dominant pattern of myopia of $\geqslant -6.00$ D. Candidate loci for the Stickler syndromes, Marfan syndrome, and juvenile glaucoma were also analyzed to exclude linkage to the myopia in these families.

Subjects and Methods

Patients and Families

Eight families consented to participate in the study. Criteria for selection included a history of onset of myopia at age <12 years in all affected subjects (parents and offspring), myopia of $\geqslant -6.00$ D, and two or more generations affected. The diagnosis of myopia was determined by the refractive error. Anisometropic individuals, with a refractive error of <-6.00 D for one eye and >-6.00 D for the other eye, with at least a 2-D difference between the two eyes, were considered unaffected. Individuals were excluded if there was known ocular disease or insult that could predispose to myopia, such as retinopathy of prematurity or early-age media opacification, or if they had a known genetic disease associated with myopia, such as Stickler or Marfan syndrome.

Affection status is difficult to determine for a common

Table 2

Linkage Analysis between High Myopia and the Loci for Juvenile Glaucoma, Marfan Syndrome, and Stickler Syndrome Types 1 and 2

Disorder Excluded	Gene	Locus	Marker	Cumulative LOD Score at $\theta = .00$
Juvenile open-angle glaucoma	Trabecular meshwork-induced glucocorticoid-response protein	1q21-q31	D1S196	−8.36
			D1S215	−7.33
			D1S218	−8.83
			D1S433	−4.18
Marfan syndrome	Fibrillin	15q15-q21.1	D15S117	−8.20
			D15S648	−12.43
Stickler syndrome type 2	Collagen 11A2	6p21.3-p22.3	D6S276	−8.70
			D6S299	−10.09
Stickler syndrome type 1	Collagen 2A1	12q13.1-q13.3	Polymorphic VNTR located 3′ to COL2A1	–10.31

NOTE.—Linkage analysis was performed with a myopia gene frequency of .0133, at 100% penetrance of the gene. Familial high myopia was assumed to have autosomal dominant inheritance, and the MLINK program was used to calculate two-point LOD scores between myopia and each marker.

complex quantitative trait such as myopia. Despite the fact that refraction depends on corneal curvature, lens power, and axial length (measurements difficult to obtain in most of our participants), historically in the literature pathologic myopia has been defined as a refractive error of $\geqslant -6.00$ D. The degree and progression of myopia has environmental influences as well. An affection status of $\geqslant -6.00$ D degrees of myopia was chosen to select for the more severe phenotypic form of this disorder, under the assumption that this would disproportionately represent a genetic etiology. This is further supported by the low frequency of pathologic myopia represented in the general population (Curtin 1985).

Anisometropic individuals were classified as "unaffected" to insure that the phenotype/genotype was as specific as possible. Anisometropia may be secondary to a nongenetic event, such as inadvertent occlusion of one eye or unilateral ptosis (Hoyt et al. 1981) or retinal hemorrhage during early childhood (Miller-Meeks et al. 1990). There may not be residual structural changes observable during a routine screening of an adult.

An ophthalmology examination was performed by one of the authors (T.L.Y.) at the time of sample collection, for most participants, and for at least one member of each family. For family members who were not locally available, examination records were obtained from the individual's ophthalmologist. The ophthalmologic evaluation included retinoscopy, a slit-lamp evaluation of the anterior segment, measurement of intraocular pressure, and a fundus examination, with special notation on the health and degree of cupping of the optic nerve head. Cycloplegic retinoscopy was performed in children age $\leqslant 10$ years. In most instances, at least one affected adult from each family also underwent axial-length measurements of their eyes and keratometry measurements of their corneas. Most participants declined to have these measurements taken. Details of the ophthalmic examinations are summarized in table 1. Venous blood was collected after informed consent was obtained. This study was approved by the University of Minnesota Hospital and Clinics Institutional Review Board.

DNA Analysis/Marker Typing

DNA was isolated from peripheral blood lymphocytes by use of standard techniques. The genome screen used polymorphic microsatellite markers from the Weber 4a and 8a sets (Research Genetics; Weber and May 1989; Dubovsky et al. 1995). For fine mapping, additional markers were selected from genetic maps of 18p (Gyapay et al. 1994; Dib et al. 1996). The 5′ marker of each primer set was modified with a special M13 sequence that allowed fluorescent detection (Oetting et al. 1995). Three to four primer pairs were multiplexed in the amplification reaction.

PCR reactions were prepared in 96-well plates with 2.0 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 μM each dNTP, 1.0 pmol each marker primer, 0.16 pmol M13 primer, 0.8 U *Taq* DNA polymerase (Fisher Biotech Scientific), and 20 ng DNA. After 26 amplification cycles, aliquots were mixed with a formamide sample buffer and electrophoresed through preheated 6% polyacrylamide, 7 M urea denaturing gels in an automated Li-Cor DNA 4000 infrared sequencer. Alleles were visualized as an autoradiogram-like image on an IBM 486 computer, and size was determined by use of RFLP Scan software (Scanalytics). Allele sizes were directly imported to a database (Fox Pro), which was then used for LOD score determination.

Linkage Analysis

Linkage analysis was performed using the C version of the LINKAGE package FASTLINK (Cottingham et

Table 3

Two-Point Linkage Analysis between High Myopia and Markers on Chromosome 18p11.31

	Total LOD Score at θ =							− Pedigree 8		+ Pedigree 8	
	.0	.01	.05	.1	.2	.3	.4	θ_{max}	Z_{max}	θ_{max}	Z_{max}
Telomere											
D18S1140	−∞	3.26	3.57	3.37	2.62	1.68	.69	.0440	3.58	.1160	2.50
D18S59	5.96	5.84	5.37	4.75	3.47	2.15	.87	.0010	5.96	.0660	3.96
D18S476	8.76	8.62	8.04	7.26	5.53	3.62	1.59	.0010	8.76	.0290	7.40
D18S1146	1.40	1.37	1.27	1.13	.85	.56	.27	.0010	1.40	.0010	1.40
D18S481	9.59	9.42	8.74	7.86	5.96	3.87	1.67	.0010	9.59	.0250	8.16
D18S63	8.30	8.16	7.55	6.77	5.09	3.28	1.38	.0010	8.30	.0010	8.30
D18S1138	−∞	6.57	6.66	6.18	4.79	3.13	1.34	.0290	6.75	.0550	5.95
D18S52	−∞	6.95	6.40	5.70	4.21	2.65	1.10	.0320	7.09	.0320	7.09
D18S62	−∞	4.01	4.86	4.75	3.85	2.56	1.09	.0620	4.88	.0840	4.50
D18S1150	−∞	−1.05	1.34	2.02	2.09	1.55	.72	.1520	2.17	.1780	1.91
D18S1116	−∞	−.41	1.82	2.35	2.16	1.42	.53	.1250	2.40	.1160	2.83
Centromere											

Note.—LOD scores were generated using a dominant mode of inheritance and a penetrance of 100%.

al. 1993; Schaffer et al. 1994) and the utility programs Makeped, Linkage control program, and Linkage report program from LINKAGE 5.1 (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986). Marker-allele–frequency estimates were based on the frequencies of alleles in married-in unrelated individuals in the families. Two-point LOD scores were also calculated with all alleles set at equal frequencies, to control for allele-frequency effects. Standard marker databases were used for intermarker recombination frequencies and order, and genetic distances between isolated 18p markers were determined by use of the CEPH panel of reference families and the analysis program CRIMAP (Lander and Green 1987).

Severe myopia occurs in the general population at a frequency of 1.7%–2.1% (Curtin 1985). Linkage analysis was performed with use of a myopia-gene frequency of .0133, at 100% penetrance of the gene. For initial data analysis, familial high myopia was assumed to have autosomal dominant inheritance, and the MLINK program was used to calculate two-point LOD scores between myopia and each marker. Recombination frequencies were assumed to be equal between men and women. All affected individuals and informative spouses were included in the linkage analysis. The LODSCORE program was used to calculate maximum LOD scores at the lowest recombination frequency. Tests for heterogeneity were determined by the HOMOG program (Ott 1983).

Table 4

Two-Point Linkage Analysis between High Myopia and Markers on Chromosome 18p11.31 for Pedigree 1

	Total LOD Score at θ =								
	.0	.01	.05	.1	.2	.3	.4	θ_{max}	Z_{max}
Telomere									
D18S1140	−∞	1.54	1.99	1.97	1.59	1.03	.39	.067	2.01
D18S59	2.70	2.65	2.45	2.20	1.64	1.01	.36	.001	2.70
D18S476	2.79	2.76	2.62	2.41	1.90	1.28	.56	.001	2.79
D18S1146	.50	.48	.43	.37	.24	.12	.03	.001	.50
D18S481	3.91	3.84	3.57	3.22	2.45	1.60	.66	.001	3.91
D18S63	3.84	3.77	3.51	3.16	2.39	1.55	.63	.001	3.84
D18S1138	3.90	3.84	3.57	3.22	2.45	1.60	.66	.001	3.90
D18S52	3.83	3.76	3.50	3.15	2.40	1.55	.64	.001	3.83
D18S62	3.81	3.74	3.48	3.13	2.38	1.54	.63	.001	3.81
D18S1150	−∞	.25	.83	.97	.90	.63	.26	.122	.98
D18S1116	−∞	−.38	.76	1.07	1.06	.74	.29	.142	1.13
Centromere									

Exclusion of Candidate Genes/Regions

The genes responsible for several disorders that present with high myopia and autosomal dominant mode of inheritance were evaluated as candidate genes. These include the genes for juvenile-onset glaucoma, mild Stickler syndrome phenotype, and Marfan syndrome. Juvenile glaucoma is a rare but aggressive form of glaucoma that usually segregates in an autosomal dominant fashion with high penetrance, with a usual age at onset of 5–45 years (Johnson et al. 1993). One locus for juvenile-onset glaucoma maps to 1q21-31 (Sheffield et al. 1993; Wiggs et al. 1994), with a recent identification of mutations of the gene encoding for trabecular meshwork–induced glucocorticoid-response protein in this region (Stone et al. 1997). Four closely linked markers (D1S196, D1S215, D1S218, and D1S433) were used. Markers intragenic to the collagen 11A2 gene for Stickler syndrome type 2 at 6p21.3-6p22.3 (D6S276 and D6S299) (Brunner et al. 1994) and to the fibrillin gene for the Marfan syndrome at 15q15-q21.1, (D15S117 and D15S648) (Dietz et al. 1991; Lee et al. 1991) were used. For Stickler syndrome type 1, a polymorphic VNTR located just 3′ to the collagen 2 A1 gene was used

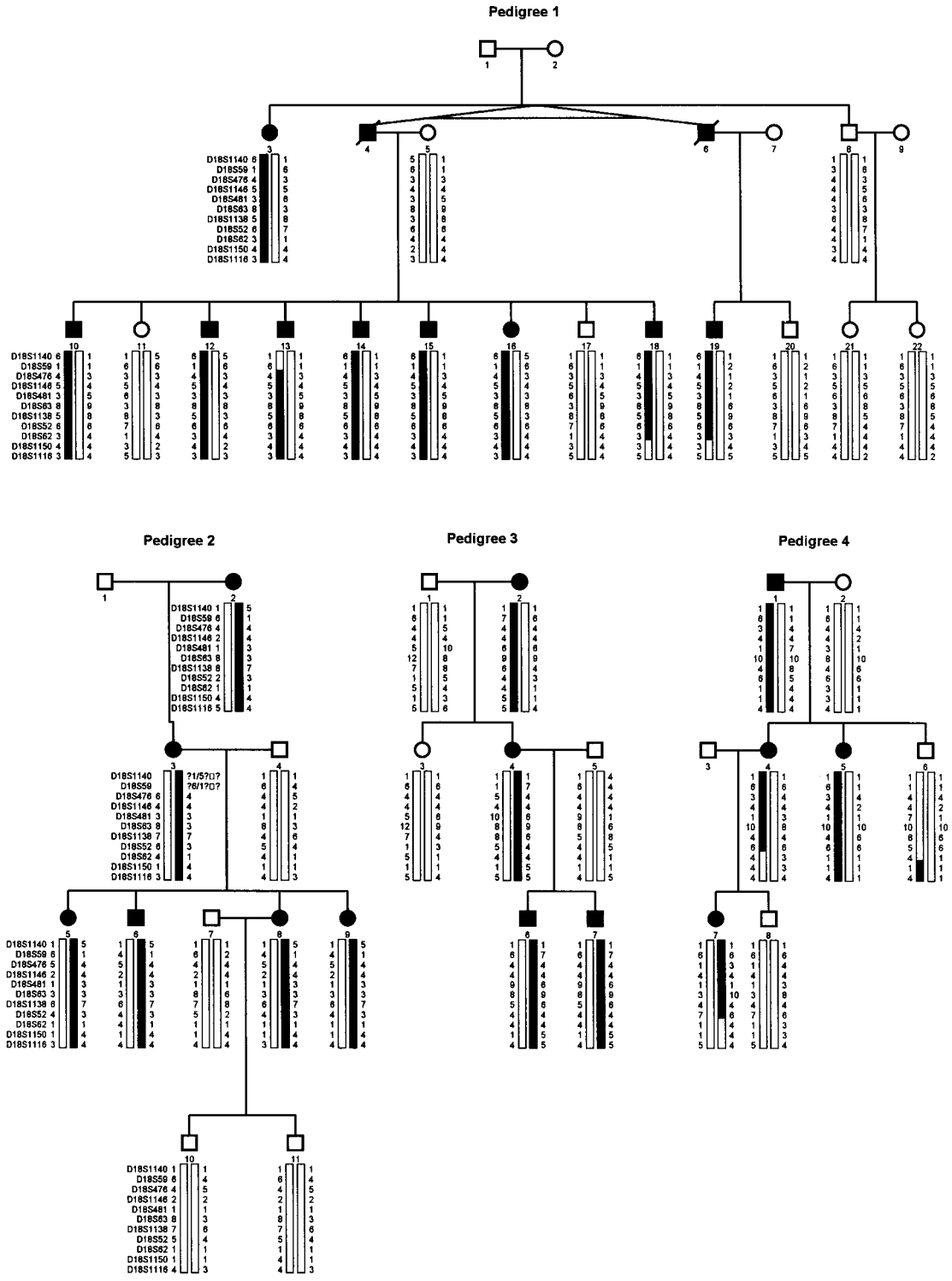
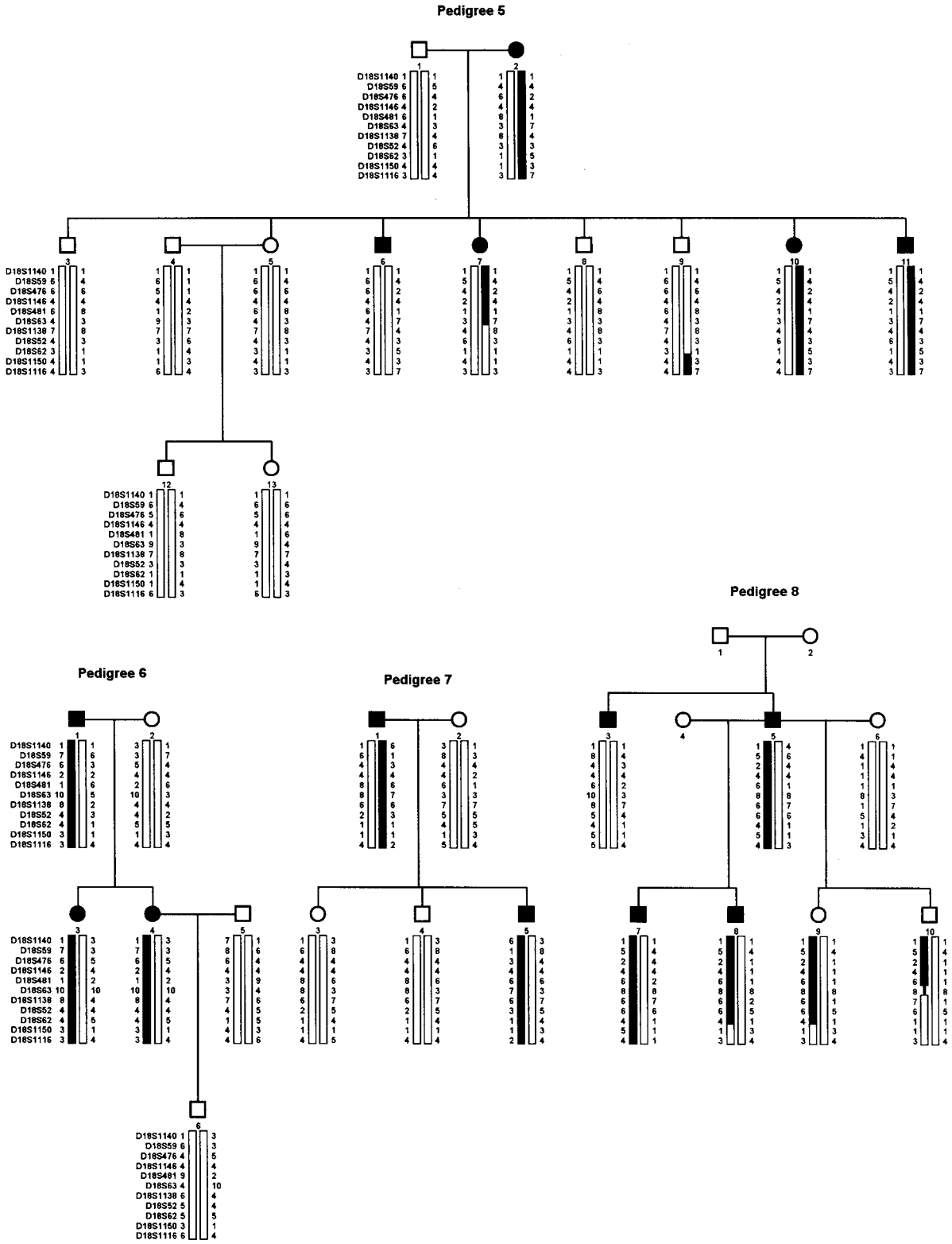


Figure 1 Eight families with familial high myopia. Circles and squares denote females and males, respectively; blackened symbols denote affected individuals; and symbols with slashes denote deceased individuals. The alleles for D18S1140, D18S59, D18S476, D18S1146, D18S481, D18S63, D18S1138, D18S52, D18S62, D18S1150, and D18S1116 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 disease allele is blackened. Only essential matings are shown; nonparticipating family members are not shown.


Pedigree 5
Pedigree 6
Pedigree 7
Pedigree 8

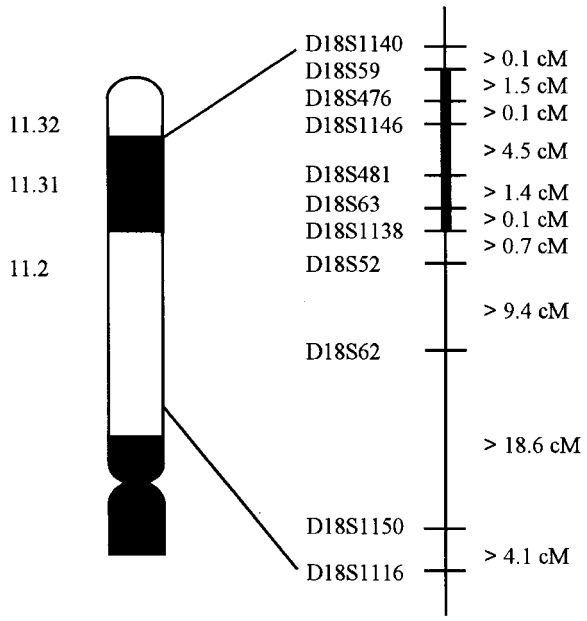


Figure 2 Schematic representation of a partial linkage map of 11 microsatellite markers located at 18p. The mapping order and genetic distances (in centimorgans) were obtained from the Généthon comprehensive genetic map of the human genome. The bold segment denotes the linked interval determined by haplotype analysis.

(Francamano et al. 1987; Berg and Olaisen 1993; Wilkin et al. 1993).

Results

Clinical Characteristics

Eight multigeneration families with autosomal dominant high myopia were identified. The average age at diagnosis of myopia was 6.8 years (range 1.5–9.5 years). The average spherical component for the affected individuals was (−9.48 ± 3.53 D, range −6.00 to −21.00 D). Cataract, glaucoma, keratoconus, lenticonus, or dislocated lens were not present in study participants. The average axial length (27.18 ± 1.75 mm) of the affected participants was significantly longer ($P = .0003$, Student's *t*-test) than the published normal value (24.2 ± 0.85 mm; Sorsby et al. 1962*a*). The average keratometry reading (43.97 ± 1.75 D) of affected members was not significantly steeper ($P = .1135$, Student's *t*-test) than the published normal value (43.1 ± 1.62 D; Sorsby et al. 1962*a*). Table 1 is a summary of the ophthalmic examination results for the participants. Individual 7, pedigree 4, had a cycloplegic refraction, at age 10 years, of −5.50 sphere, right eye (OD), and −5.00 sphere, left eye (OS). This indicates that, despite a noncycloplegic refraction at age 12 years, at the time of her participation in the study, she clearly had high myopia not associated with accommodative variation, which would be minimized with a cycloplegic refraction.

Genome Screen

The eight families showed exclusion to the candidate-gene regions discussed above (table 2). The initial genome screen was carried out with four families. Sixty-two microsatellite markers were analyzed in the genome screen before two-point linkage analysis between marker D18S59 and myopia gave a cumulative LOD score of 3.81, at a recombination fraction (θ) of .00, in these families. To determine whether this might be an area of significant linkage, we typed the families for 10 additional markers in this region. Four additional families that had become available were added to the analysis. The eight families gave a combined maximum LOD score of 8.30, at $\theta = .0010$, for marker D18S63. The LOD score results of two-point analysis for each marker for the combined eight pedigrees are shown in table 3. Family 1 alone showed significant linkage to the chromosome 18 markers. The maximum LOD score for pedigree 1 was 3.91, at $\theta = .0010$, for marker D18S481. Table 4 shows the results of two-point analysis for pedigree 1 alone. The maximum LOD score for pedigree 5 was 2.11, at $\theta = .0010$, for marker D18S481. Affected-meioses analysis was also performed, with 100% penetrance, and there was no difference in LOD scores.

Haplotype Analysis

Haplotype analysis of affected individuals revealed critical recombination events that narrow the region of the gene. The haplotypes for the eight pedigrees are given in figure 1. Pedigree 8 is problematic and appears not to be linked to this region. This family could not be phenotypically excluded, however, and therefore was included in the initial linkage analysis. Haplotype analysis indicated that individuals 3 and 5 appear not to be related. Individual 3 also appears to be a phenocopy for the disease locus. Individuals 9 and 10 appear to have incomplete penetrance. Repeat DNA sampling was performed on this family. We have elected to submit analysis data both with and without pedigree 8 (table 3). The maximum LOD score for the seven families, excluding pedigree 8, was 9.59, at $\theta = .0010$, for marker D18S481.

From this analysis, the critical region of interest appears to be between markers D18S59, telomerically, and D18S1138, centromerically, because of recombination events observed in affected individuals 13, 18, and 19 in pedigree 1, individuals 4 and 7 in pedigree 4, and individual 7 in pedigree 5. Although two-point linkage analysis does not show a recombination event for D18S59 for pedigree 1 alone, haplotype analysis shows that individual 13 must have inherited allele 6 from his deceased affected father and not from his mother. To

assume otherwise would presume a double-crossover event. This potentially narrows the region to a 7.6-cM interval between these two markers (fig. 2).

Genetic Heterogeneity

On the basis of haplotype analysis, pedigree 8 did not show linkage to this region and was excluded from tests of heterogeneity. The null hypothesis of homogeneity across the seven families was tested with markers D18S63, D18S481, and D18S476. The proportion α of families segregating with the linked gene had a maximum-likelihood estimate of 1.00. Under the assumption of linkage, the null hypothesis was not rejected ($\chi^2 = 0.000$, $P = 1.00$). Therefore, there was no evidence of genetic heterogeneity in these families.

Discussion

To our knowledge, this is the first genetic locus mapped for high familial myopia. LOD score analysis places a gene for myopia on chromosome 18p11.31, within a 7.6-cM interval. To date, there appear to be no additional D18 markers to use to further refine this interval. Four markers in this region—AFMB082, D18S54, D18S1154, and D18S53—did not provide scorable alleles despite numerous modifications of PCR conditions.

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

Pedigree 1 is a family of Chinese descent, living in Hawaii, and the only family of Asian ethnicity in our studies. It is well known that the incidence of myopia in many Asian populations is significantly higher than the incidence in European and African populations (Rasmussen 1936; Lin et al. 1988; Rajan et al. 1995). The pedigree 1 haplotype may reflect a common one for this ethnic group. Further studies with more families clustered by ethnic group may aid in identifying distinct haplotypes on this basis.

A search for genes and/or expressed sequence tags physically mapped between markers D18S63 and D18S59 revealed 49 unidentified transcripts, 2 mRNAs for an open reading frame (KIAA0249 and KIAA0211), and 13 mRNAs and 25 sequences for regulatory or structural genes (National Center for Biotechnology Information database). Among these are adenylate cyclase–activating polypeptide, thymidylate synthase, protein tyrosine phosphatase receptor, the α subunit of guamine nucleotide-binding protein, protein tyrosine phosphatase, Niemann-Pick C disease protein, and the α subunit of laminin (LAMA). Of these, LAMA is a biologically relevant candidate gene for this newly identified myopia locus, since it is a component of a structural glycoprotein found in the ocular scleral wall.

Laminin is present in the eye as a constituent of the elastic system in the trabecular meshwork (Marshall et al. 1990) and zonular (oxytalan) fibers of the lens (Marshall et al. 1992). It has also been identified in the astrocytic and vascular endothelial-cell basement membranes of the laminar-beam margins of the rodent lamina cribosa (Morrison et al. 1995). More recently, Marshall (1995) has localized laminin to the oxytalan and elaunin microfibrils of human sclera by immunoelectron microscopy. These microfibrils comprise two of the three components of the elastic-fiber system that make elastic tissue more stretchable than collagen, a fibrous protein that provides tensile strength (Ross 1971; Cleary 1983). Marshall suggests that laminin may bind these microfibrils to collagen fibrils, since laminin has been shown to have binding sites for several extracellular matrix components, including collagen (Ayad et al. 1994). These properties of laminin and its localization in the sclera make LAMA an attractive candidate gene.

In summary, we have mapped a genetic locus for high myopia. Studies are currently underway in our laboratory to reduce the 7.6-cM critical region for high myopia, prior to performance of gene-isolation experiments to identify and clone the gene responsible for myopia phenotypes. We also continue our work to identify other possible loci for myopia. The future cloning and mutational characterization of the gene(s) for high myopia will, it is hoped, elucidate the molecular mechanisms underlying exaggerated eye growth and lead to a better understanding of the clinical consequence of mutations of these loci.

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Electronic-Database Information

URLs for data in this article are as follows:

Généthon comprehensive genetic map of the human genome, http://www.genethon.fr/genethon_en.html (for mapping order and genetic distances of 11 microsatellite markers used)

National Center for Biotechnology Information database, http://www.ncbi.nlm.nih.gov/ (for genes and/or expressed se-

quence tags physically mapped between markers D18S63 and D18S59)

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