Exclusion of Lumican and Fibromodulin as Candidate Genes in MYP3-linked High-Grade Myopia

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Abstract

Purpose: The proteoglycans lumican and fibromodulin regulate collagen fibril assembly and show expression in ocular tissues. A recent mouse knockout study implicates lumican and fibromodulin as functional candidate genes for high myopia.[1] Lumican maps within the chromosome 12q21-q23 autosomal dominant high-grade myopia-3 (MYP3) interval, and fibromodulin maps to chromosome 1q32. We screened individuals for lumican and fibromodulin sequence alterations from the original MYP3 family, and from a second high-grade myopia pedigree that showed suggestive linkage to both the MYP3 interval and to chromosome 1q32.

Methods: A total of 10 affected (average spherical refractive error = -16.13 diopters) and 5 unaffected individuals from the 2 families were screened by direct DNA sequencing. Six primer pairs spanning intron-exon boundaries and coding regions were designed for the 3-exon 1804bp lumican gene. Two primer pairs for the 2-exon 2863bp fibromodulin gene were designed. Polymerase chain reaction (PCR) products were sequenced and analyzed using standard fluorescent methods. Sequences were quality scored and aligned for polymorphic analysis.

Results: Direct DNA sequencing of lumican amplicons yielded the expected sequence with no evidence of polymorphism or pathologic mutation. Sequencing of fibromodulin amplicons revealed 6 polymorphisms, 1 of which was novel. One polymorphism was a silent mutation, and five were in the 3' un-translated (UTR) region. No polymorphism segregated with high myopia.

Conclusions: Although null and double-null *Lum* and *Fmod* mouse models have been developed for high myopia, our human cohort did not show affected status association with these genes. Sequencing of the human lumican and fibromodulin genes has excluded them as candidate genes for MYP3- associated high-grade myopia

Introduction

Previously, we reported significant linkage of autosomal dominant high myopia to a locus at chromosome 12q21-23 in a large German/Italian family (Figure 1).[2] This locus was named the high-grade myopia MYP3 locus by the Human Gene Nomenclature Committee.

A recent mouse knockout study, implicated lumican (LUM) and fibromodulin (FMOD) as functional candidate genes for high myopia.[1] LUM maps within the chromosome 12q21-q23 MYP3 interval, and FMOD maps to chromosome 1q32.

Both proteins are members of the small interstitial proteoglycan family and are believed to participate in the assembly of the extracellular matrix interacting with type I and type II collagen fibrils. Fibrillogenesis of the scleral wall with consequent axial globe expansion may be affected by mutations in these candidate genes. This has been demonstrated in connective-tissue disorders with myopia as a consistent phenotypic feature, such as Sticker or Marfan syndromes.

We sought to determine if the LUM and FMOD genes are causally related to MYP3-associated high myopia by direct DNA sequencing. We screened subjects from the original MYP3 family, and from a second family (Pedigree-2) that showed suggestive linkage to the MYP3 interval and chromosome 1q32. While the effect of a single gene may be determined independently, it is now important to examine any proposed susceptibilty allele in relation to other known genes.

Methods

Patients: Representatives of the MYP3 pedigree (6 subjects, 4 affected), and Pedigree-2 (9 subjects, 5 affected) were studied (Figure 1). Each affected subject presented with high myopia and elongated axial lengths. Controls were obtained from family marry-ins, non-myopic related family members, and unrelated subjects. Table 1 displays the refractive error of all subjects studied. Total genomic DNA was extracted from 10-15ml of venous blood from all participants after informed consent was obtained.

Genotyping Family-2: The average refractive error for affected adults ranged from -5.00 to -32.00 diopters. Syndromic myopia linkage was excluded by using intragenic or flanking markers for Stickler syndrome types 1, 2, and 2B; Marfan syndrome; Ehlers-Danlos syndrome type 4; and juvenile glaucoma. DNA analysis was performed as previously described, using multiplexed microsatellite primer pairs and fluorescence detection techniques. For fine-point mapping, additional markers were selected from the ABI HD-5 microsatellite marker set. Analysis of the genotype data was performed using parametric and non-parametric methods (GENEHUNTER 2.1).[3] Suggestive linkage was detected on chromosome 1q32, and within the MYP3 interval with a lod-score of 1.41 for markers D1S484, and markers D12S1583 and D12S79, respectively (Table 2).

Figure 1.

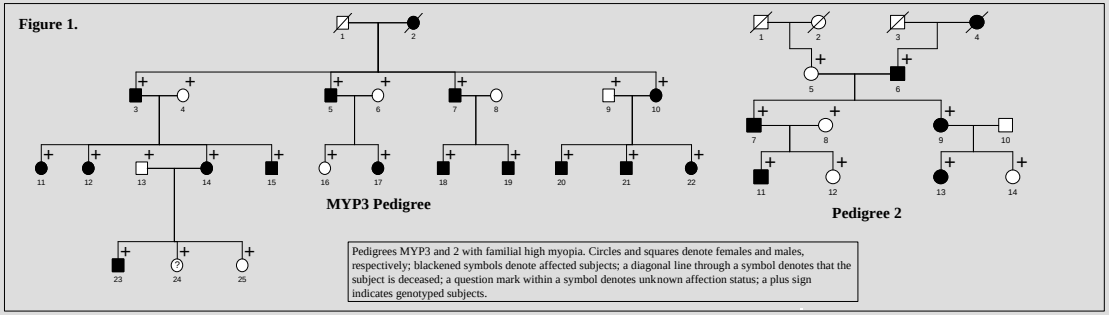


Table 1. Spherical Refractive Errors for Pedigrees MYP3 and 2.

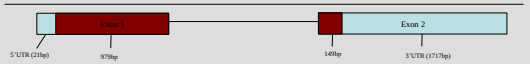
MYP3 Pedigree Subjects	OD/OS	Pedigree 2 Subjects	OD/OS
6	-3.50/-4.00	12	+1.25/+0.50
4	+8.00/+9.00	14	Plano OU
25	Plano OU	11	-23.75/-20.00
5	-14.00/-13.50	7	-32.00/-32.00
3	-7.50/-6.50	5	-16.50/-16.25
23	-3.50/-4.00(8yo)	9	-28.00/-25.00
20	-13.00/-15.00		
15	-12.00/-13.00	Neg. Control	Plano OU
7	-13.00/-14.00		

Table 2. Linkage Analysis of Pedigree 2 for Chromosomes 12q22-24 and 1q23-32 Microsatellite Markers.

Marker	LOD score	NPL score	p-value	information
D12S346	0.336437	1.976623	0.062500	0.574837
D12S78	0.406907	2.038583	0.062500	0.740833
D12S1613	0.030702	0.855281	0.062500	0.462905
D12S1583	1.406333	2.038583	0.062500	0.745644
D12S1646	-0.001336	0.151861	0.281250	0.218872
D12S79	1.406333	2.038583	0.062500	0.734998
D12S1718	-0.000996	0.000000	0.281250	0.108170
D12S86	1.405871	1.914664	0.062500	0.800000
D12S324	1.405871	1.914664	0.062500	0.800000
D12S1659	0.002513	0.000000	0.281250	0.200000
D12S1723	1.115712	0.801826	0.062500	0.527807
D1S2726	-0.267785	-0.300887	0.500000	0.574837
D1S252	0.445379	0.238118	0.250000	0.350000
D1S484	1.406333	2.038583	0.062500	0.727807
D1S2878	1.328281	1.731621	0.062500	0.674837
D1S196	0.288437	0.595295	0.125000	0.318872
D1S218	-1.470127	0.196812	0.250000	0.800000
D1S238	-2.449207	-0.325590	0.562500	0.700000

Figure 2.

Exonic Structure of Fibrmodulin Gene on NT_004671.15 Chromosome 1q32



Exonic Structure of Lumican Gene on NT_019546.15 - Chromosome 12q21.33

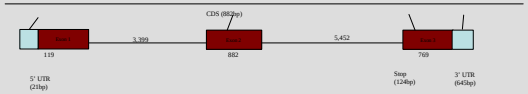


Table 3. Polymorphisms Observed in Fibromodulin (FMOD) Gene.

Subject-Pedigree	14672146 wt=G/G rs#7543148	14666200 wt= G/G rs#3738022	14665831 wt= G/G Novel	14664950 wt= C/C rs#4605	14664864 wt= C/C rs#7208	14664736 wt= A/A rs#2886220
12-2		A/A	G/G	C/C	C/C	A/A
14-2		G/A	G/G	G/C	C/T	A/G
11-2	A/A	A/A	G/G	C/C	C/C	A/A
7-2		A/A	G/G	C/C	C/C	A/A
5-2		A/A	G/G	C/C	C/C	A/A
9-2		G/A	G/G	G/C	C/T	A/G
6-MYP3		G/A	G/G	G/C	C/T	A/G
4-MYP3	A/A	G/A	G/G	G/G	C/C	A/A
25-MYP3	A/G		G/G	G/G	C/C	A/A
5-MYP3	A/A	G/A	G/G	G/C	C/T	A/G
3-MYP3		G/A		G/C	C/T	A/G
23-MYP3	A/G	G/A	G/G	G/G	C/T	A/G
20-MYP3			G/G	G/C	C/T	A/G
15-MYP3		A/A	G/G	G/C	C/C	A/A
7-MYP3		G/G	G/G	G/G	T/T	G/G
Control	A/G	A/A	G/A	G/C	C/C	A/A

Each column represents a separate sequence variant with base-pair position on the scaffold sequence. Wt is wild-type sequence published on scaffold NT_04671.15. Rs# is reference cluster SNP identification. Yellow highlighting denotes affected subjects.

Methods II

DNA Amplification and Mutation Screening: The genomic structures of LUM and FMOD, as reported in MapViewer (build 34) of the reference human genome sequence is outlined in Figure 2. The genomic structures of LUM and FMOD comprise 3 exons spanning 8.1kb and 2 exons spanning 7.7kb, respectively. The mature 1804bp LUM mRNA encodes a protein of 339 residues. The 2863bp FMOD mRNA encodes 337 residues.

Six oligonucleotide primer pairs were designed to amplify the exonic sequences with 50-200 base pairs extensions beyond the intron-exon boundary for LUM, and 2 were designed to completely sequence FMOD. Amplicons were sequenced using BigDye™ Terminator v3.1 on an ABI 3700® Genetic Analyzer (Applied Biosystems, Foster City, CA). Chromatograms were trimmed for quality and aligned using Sequencher™ (Gene Codes, Ann Arbor, MI). Resulting contigs were compared between normal and affected individual DNA samples. Novel SNPs were submitted to dbSNP databases.

Results

No polymorphisms where observed in the LUM gene. Six exonic polymorphisms were found by direct sequencing of the FMOD gene (Table 3). Of these, 5 were in UTR, and 1 at position 14666200 was a synonomous substitution in the FMOD protein sequence. Five polymorphisms corresponded with previously reported SNPs in public databases. One polymorphism at position 14665831 was novel, and has been submitted to the dbSNP database. None of the sequence variants co-segregated with the affected phenotype.

Discussion

Mutation analysis of the encoded LUM and FMOD genes did not identify sequence alterations associated with the disease phenotype in two high myopia pedigrees which mapped to either or both gene loci. Further studies of MYP3 candidate genes are needed to determine the gene causative for this potentially blinding disorder.

References

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