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RESEARCH ARTICLE

Novel Mutations in the Small Leucine-Rich Repeat Protein/Proteoglycan (SLRP) Genes in High Myopia

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The importance of the genetic component in high myopia has been well established in population and family studies, but only a few candidate genes have been explored to date. The extracellular matrix small leucine-rich repeat proteins/proteoglycans (SLRPs) regulate collagen fibril diameter and spacing. Given their role in extracellular matrix assembly and expression in the eye, they are likely to regulate its shape and size. Analysis of 85 English and 40 Finnish subjects with high myopia (refractive error of −6 diopters [D] or greater) resulted in 23 sequence variations in four SLRP genes, *LUM, FMOD, PRELP,* and *OPTC*. We observed higher number of variations in *OPTC* in English patients than in controls (p = 0.042), and a possibly protective variation in *LUM* (c.893−105G>A) with p-value of 0.0043. Two intronic variations, six nonsynonymous and one synonymous amino acid changes, were not found in any of the nonmyopic controls. Five changes were detected in opticin, Thr177Arg, Arg229His, Arg325Trp, Gly329Ser, and Arg330His, and all but one (Arg229His) were shown to cosegregate with high myopia in families with incomplete penetrance. A homology model for opticin revealed that Arg229His and Arg325Trp are likely to disrupt the protein structure, and PolyPhen analysis suggested that Thr177Arg, Arg325Trp, and Gly329Ser changes may be damaging. A Leu199Pro change in lumican and Gly147Asp and Arg324Thr variations in fibromodulin are located in the highly conserved leucine-rich repeat (LRR) domains. This study provides new insight into the genetics of high myopia, suggesting that sequence variations in the SLRP genes expressed in the eye may be among the genetic risk factors underlying the pathogenesis of high myopia. Hum Mutat 28(4), 336–344, 2007. **© 2006 Wiley-Liss, Inc.**

KEY WORDS: high myopia; *FMOD*; *LUM*; *OPTC*; *PRELP*; SLRP; sequence variations

INTRODUCTION

Myopia is the most common eye condition, with a wide variability in prevalence between ethnic groups, from 10 to 20% in Africa and 30 to 40% in Europe and the United States to figures as high as 70 to 90% in Asia [Chow et al., 1990; Saw et al., 1996; Zadnik, 1997; Wong et al., 2000]. High myopia, variably defined as a refractive error of at least −6 or −8 diopters (D), has been estimated to affect 1 to 3% of the population [Fuchs, 1960; Sperduto et al., 1983; Fredrick, 2002]. It is a common cause of blindness due to complications such as rhegmatogenous retinal detachment and choroidal neovascularization, and several syndromic diseases, including Stickler syndrome types 1 and 2, Ehlers-Danlos syndrome VI, and Marfan syndrome, have high myopia and mutations in extracellular matrix (ECM) genes as common features [Kaplan et al., 1986; Naiglin et al., 1999]. Genetic heterogeneity is also seen in nonsyndromic high myopia, since 10 chromosomal loci, MYP1–MYP10, at Xq28, 18p11.31, 12q23–12q24, 7q36, 17q21–17q22, 22q12, 4q12, 3q26, 8p23, and 11p13. respectively, have been detected so far, although no mutations have been implicated at these loci to date [Young et al., 1998; Naiglin et al., 2002; Paluru et al., 2003; Stambolian et al., 2004; Hammond et al., 2004].

The refractive state of the eye is determined by its axial length and the focusing power of the cornea and crystalline lens. Since the ECM of the cornea and sclera has a key role in determining the shape of these tissues, mutations in ECM genes are likely to be important in high myopia [Gentle et al., 2003]. Furthermore, ECM components of the vitreous may be important, as Stickler syndrome is caused by mutations in genes that encode vitreous humor collagens [Bishop, 2000].

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The ECM small leucine-rich repeat protein/proteoglycans (SLRP) are a family of ECM molecules to which a number of functions have been ascribed, including regulation of the diameter and spacing of collagen fibrils, inhibition of transforming growth factor-β, and control of cell proliferation [Iozzo, 1999; Ameye and Young, 2002]. Thus ocular SLRPs could play a role in regulating eye shape and axial length through a variety of mechanisms.

The SLRPs contain tandem leucine-rich repeats (LRRs) of approximately 24 amino acids with the consensus sequence $LX_2LXLX_2NX(L/I)$ [Scott et al., 2004]. These LRRs form curved solenoid structures with a beta sheet on their inner surface and variable topology on their outer surfaces [Scott et al., 2004]. There are disulfide-bonded capping motifs at either end of the LRR domains. At least two members of the SLRP family, decorin and opticin, form stable dimers through interactions between the LRR domains [Scott et al., 2003, 2004; Le Goff et al., 2003]. Opticin (OPTC; MIM 605127), fibromodulin (FMOD; MIM 600245), lumican (LUM; MIM 600616), and proline arginine-rich end LRR protein (PRELP; MIM 601914) are members of the SLRP family [Iozzo, 1999; Reardon et al., 2000] that are expressed in the eye. The *OPTC*, *PRELP*, and *FMOD* genes form a cluster on chromosome 1q31–1q32 [Sztrolovics et al., 1994; Grover et al., 1996; Hobby et al., 2000] and the *LUM* gene is located on chromosome 12q22–12q23 [Grover et al., 1995; Chakravarti et al., 1995].

One immunolocalization study localized opticin specifically to the vitreous humor [Ramesh et al., 2004], but others found a wider distribution in the eye [Friedman et al., 2002]. In situ hybridization studies have demonstrated opticin expression in the nonpigmented ciliary epithelium of the eye, and in specific regions of the developing chick brain [Takanosu et al., 2001; Bishop et al., 2002; Frolova et al., 2004]. In addition to the sclera and cornea, PRELP is expressed in several nonocular tissues, such as the kidney, aorta, liver, skeletal muscle, skin, and tendon [Grover et al., 1996; Bengtsson et al., 2002], and it has been thought to function as an anchoring molecule connecting basement membranes to the underlying connective tissue [Bengtsson et al., 2002]. However, in adult eye, PRELP mRNA was detected in the nonpigmented epithelium of the ciliary body in pattern of expression that resembled that of OPTC mRNA [Takanosu et al., 2001]. Lumican and fibromodulin are both expressed in sclera, tendon, and cartilage, and lumican is also expressed in the cornea and skin [Blochberger et al., 1992; Chakravarti et al., 1998; Dunlevy and Summers Rada, 2004; Säämänen et al., 2001]. They have both been shown to participate in the regulation of collagen fibril assembly [Chakravarti et al., 1998; Ezura et al., 2000]. *Fmod/Lum* double-null mice showed features of high myopia, such as increased axial length, thin sclera, and retinal detachment [Chakravarti et al., 2003]. The ocular expression of opticin, lumican, fibromodulin, and PRELP together with their proposed functions suggests that mutations in these genes may contribute to myopia in humans. We set out here to investigate the role of *OPTC*, *LUM*, *FMOD*, and *PRELP* in high myopia by searching for such gene mutations in 125 unrelated individuals with high myopia.

MATERIALS AND METHODS

Subjects

A total of 85 English and 40 Finnish unrelated individuals with high myopia, defined as a refractive error of at least −6 D in both eyes, and a positive family history (i.e., at least one member of their immediate family being affected with refractive error of at least −6 D in both eyes) were included in the study. DNA samples from family members were available for three of the Finnish and one of the English patients. Altogether 12 samples from relatives were analyzed to study the cosegregation of the sequence variations with high myopia. The first control set consisted of 65 English and 37 Finnish individuals, which were extended to 208 English and 100 Finnish individuals with a refractive error of within 2 D of emmetropia. All Finnish subjects and controls were Caucasian, and the respective portions for the English were 98% and 98.5%. The study was approved by the Ethics Committee of the Northern Ostrobothnia Hospital District and the Central Manchester Local Research Ethics Committee. Blood samples were collected from all subjects for genomic DNA isolation after obtaining written consent.

DNA Analysis

Genomic DNA extracted from EDTA anticoagulated blood samples was used for mutation screening by conformation-sensitive gel electrophoresis (CSGE) [Körkkö et al., 1998]. The sequences corresponding to the coding region and exon-flanking sequences of *OPTC*, *FMOD*, *LUM*, and *PRELP* were amplified by PCR. The primer sequences used in the PCR analysis are shown in Table 1. The amplifications were carried out in a volume of 23 μL containing 20 to 40 ng of genomic DNA, 5 to 10 pmol of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.6 U Ampli*Taq* Gold DNA polymerase (Applied Biosystems Roche, Brenchburg, NJ). The conditions, after initial denaturation at 95°C for 10 min, were 35 cycles of 30 s at 95°C, 30 s at 55 to 63°C, and 30 s at 72°C, followed by final extension at 72°C for 8 min. The PCR products were denatured at 95°C for 5 min, followed by annealing at 68°C for 30 min to generate heteroduplexes for CSGE. A 3-μL aliquot of the reaction mixture was analyzed on a 1.2% agarose gel to check the quantity and quality of the PCR product. Approximately 20 ng of the product was used for heteroduplex analysis by CSGE as previously described [Körkkö et al., 1998], with the exception that the gels were stained with SYBR Gold nucleic acid gel stain (Molecular Probes, Eugene, OR). Products containing heteroduplexes were sequenced using a DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Buckinghamshire, England) and an ABI PRISM™ 377 sequencer to define the underlying sequence variations.

DNA mutation numbering is based on the nomenclature proposed by den Dunnen and Antonarakis [2000] and by den Dunnen and Paalman [2003]. cDNA sequences were used for numbering (GenBank accession numbers *OPTC*: NM_014359.3; *LUM*: NM_002345.3; *FMOD*: NM_002023.3; and *PRELP*: NM_002725.3), where +1 corresponds to the A of the ATG translation initiation codon in the reference sequence and *1 to the nucleotide after translation stop codon. The initiation codon is referred as codon 1.

PolyPhen, Ensembl, and Statistical Analysis

PolyPhen (which stands for polymorphism phenotyping), an automatic tool for predicting the impact of an amino acid substitution on protein structure and function, was used to analyze the consequences of the observed missense mutations. PolyPhen analyses are based on straightforward empirical rules that are applied to the sequence, phylogenetic, and structural information characterizing the substitution (http://tux.embl-heidelberg.de/ramensky). The Ensembl system was used to analyze the conservativeness of the locations of the opticin, lumican and fibromodulin variations within species (www.ensembl.org). Statistical analyses were performed using Fisher's exact test with

TABLE 1. Primer Sequences, Sizes of PCR Products, and Annealing Temperatures Used in the Analysis

Gene	Exon	Primer sequences (5′-3′)	Size (bp)	Temp °C
OPTC	1	F: AGCTGGACATCAGAAGGAATG R: CCAAACACTTTGCCCTCATT	232	58
	2	F: GGAGAGCCTGTCCCTCAGAT R: TCTGACTCCCAGTGTCATGC	373	55
	3	F: CACTGAGGTTCCCAGAGTCC R: CAACCACGTTCCACTTTGGT	312	58
	4	F: GCCCCAGAGGCTAAAGAGAT R: CTGCCCCCACTCCTCTTT	246	58
	5	F: CCAACCTGGACAAGGAAAGA R: GGAGGTGAGTGGTGGAGGTA	361	58
	6	F: GGCCACCTGTCTCTGGTCT R: CCTCTCTCTGCCACGACCT	269	62
	7	F: GGGACAGCTGATGTGAGCC R: GGACCCGATTTCTAGTGGC	364	55
	8	F: CCTCTCCCCCACACTAGACA R: GGAAGTACACAAGCCCATCC	352	62
LUM	2A	F: GACGCTGTTAGATGCACTAAAAATAGC R: GATCAGTTACATTCTCAAAGGCC	432	60
	2B	F: AATGGTGCCTCCTGGAATCAAG R: ATTGTTGTCTAAGTAGAGAGTTAG	460	58
	2C	F: GAAAGAGGATGCTGTTTCAGCTG R: TTACTTACTCTCAAGTTGATTGACCTCC	358	60
	e3	F: GGTTGCAAATTCAAACATATACTATA R: ATGGATACTATGAAAACTGACACACAG	367	60
FMOD	1A	F: GCCTACTCTGCCTCACTGAATC R: CCGTGGAGAGCAATCCAGAGCAG	493	63
	1B	F: AATCGCAACCTCAAGTACCTG R: TTGTGCTCCATGTACAGCTGC	482	55
	1C	F: GAAGTGGGCAGTTCCATGAGG R: CTCAGAATAGTGTCTTTCAAGTGA	405	58
	2	F: GCTAATCTCACTGTGGCTTCAG R: GTCCATCCTGGACCTTCCAG	316	58
PRELP	2A	F: ACTAGTTACTGAGGGCCCAAGGAT R: CTGGTCTATCTTGCGGATTCGGTTG	497	58
	2B	F: GCATCCATTACCTCTATCTCCAG R: GTAGAGCTGGTGAATGGCGGT	437	60
	2C	F: CGACGGCGTCTTCAAGCCCGA R: GTGGAAGTGCTACACAAGCCAA	412	60
	3	F: GTCTACAGTCCTTGCTCTCGG R: TGCCTGGGCCCCAGCCTTCA	331	60

F, forward; R, reverse.

GraphPad software (GraphPad Software, Inc., San Diego, CA; www.graphpad.com).

Homology Model for the Opticin LRR Domain

Examination of the sequences of human opticin and bovine decorin, for which a crystal structure at a resolution of 2.15 Å is available [Scott et al., 2004], suggested that a model of the LRR domain of opticin could be constructed using the software package Modeller 8v0 [Marti-Renom et al., 2000]. As opticin has fewer LRRs than decorin, the first step was to generate a truncated form of the decorin template structure by removal of the whole LRRs 5, 8, 9, and 10, in order to minimize the differences in the lengths and sequences of the target and template LRRs. This template reconstruction was done using XFIT [McRee, 1999], with sequence alignments generated with ClustalW [Higgins et al., 1994]. The alignment used for modeling the opticin LRR domain required only four single amino acid deletions and one single amino acid insertion in the decorin sequence.

RESULTS

DNA Analysis

Screening of the *OPTC*, *LUM*, *FMOD*, and *PRELP* genes from the 125 patients with high myopia resulted in the identification of 23 sequence variations. Six nonsynonymous changes and one synonymous change, in addition to two intronic variations observed in the highly myopic patients, were not present in any of the 308 controls analyzed for these genes (Table 2). None of the patients were homozygous or compound heterozygous for mutations in one gene nor had any patient mutations in several genes.

Four of the sequence variations that were unique to the highly myopic subjects were located in the *OPTC* gene. A c.530C>G converted a codon ACA for Thr177 to AGA for Arg, and a c.989G>A variation changed a codon CGC for Arg330 to CAC for His in two Finnish patients. A c.686G>A change altered a codon CGC for Arg229 to CAC for His in one English patient, and a c.985G>A change a codon GGC for Gly329 to AGC for Ser in two English patients. In addition, a c.973C>T change altered a codon CGG for Arg325 to TGG for Trp in two high myopes, but this alteration was also observed in two control samples.

The cosegregation of these variations with high myopia was studied in the families of four patients. The opticin Thr177Arg variation was observed in one myopic family member (Fig. 1A, III:3), but was absent from two nonmyopic relatives, the patient's father (II:1) and uncle (II:4). The Arg325Trp variation in OPTC was found in the patient's mother, who had a refraction of −5.25 D in both eyes (Fig. 1B, II:2), and also in one brother with moderate

TABLE 2. Sequence Variations Found in the *OPTC*, *LUM*, *FMOD*, and *PRELP* Genes

Gene	Sequence variation[e]	English patients (n = 85)	English controls (n = 208)	Finnish patients (n = 40)	Finnish controls (n = 100)	PolyPhen result
OPTC[a]	c.402C>T/p.L134L[f]	1	0	0	1	n.a.
	c.530C>G/p.T177R[f]	0	0	1	0	Possibly damaging
	c.686G>A/p.R229H[f]	1	0	0	0	Benign
	c.803T>C/p.L268P[f]	9	21	1	6	Benign
	c.810G>A/p.L270L[f]	4	2	0	0	n.a.
	c.973C>T/p.R325W[f]	1	1	1	1	Probably damaging
	c.985G>A/p.G329S[f]	2	0	0	0	Possibly damaging
	c.989G>A/p.R330H[f]	0	0	1	0	Benign
LUM[b]	**c.1-57delT[f]**	1	1	0	0	n.a.
	c.1-98T>C[f]	1	1	0	0	n.a.
	c.507T>C/p.N169N[f]	6	5	1	5	n.a.
	c.596 T>C/p.L199P[f]	1	0	0	0	Possibly damaging
	c.865-69A>C[f]	1	0	0	0	n.a.
	c.865-105G>A[f]	4	35	1	6	n.a.
FMOD[c]	**c.137C>A/p.P46L[f]**	2	1	0	2	Benign
	c.237G>A/p.E79E	4	5	3	2	n.a.
	c.440G>A/p.G147D[f]	0	1	1	1	Benign
	c.971G>A/p.R324T[f]	1	0	0	0	Benign
PRELP[d]	**c.97G>A/p.G33R[f]**	2	2	0	0	n.a.
	c.408C>T/p.N149N	2	0	0	0	n.a.
	c.469A>G/p.M157V	1	3	1	3	Possibly damaging
	c.1042A>C/p.N348H	4	3	1	3	Benign
	c.1150*27T>C	0	0	1	0	n.a.

*GenBank accession numbers for cDNA sequences:
[a]NM_014359.3,
[b]NM_002345.3,
[c]NM_002023.3,
[d]NM_002725.3,
[e]Mutation numbering is based on the nomenclature proposed by den Dunnen and Antonarakis [2000], and den Dunnen and Paalman [2003],
[f]Analyzed for total amount of control samples available (n = 308). Novel mutations are presented in bold.
n.a., not analyzed.

myopia (−3.5/−3.75 D). The father, who was emmetropic, and a sister with low myopia, did not carry the variation. Both parents and one sister of the patient with an opticin Arg330His change were analyzed (Fig. 1C, II:3 and I:1, respectively), and the mother proved to have neither myopia nor the variation, the sister had high myopia and carried the same variation, but so did the father, who was emmetropic. The parents of the English patient with a Gly329Ser change were also analyzed, and the mother, who was emmetropic, carried the variation, but father, with low myopia, did not.

In the case of the *LUM* gene, a c.596T>C change, converting a codon CTG for Leu199 to CCG for Pro, was found in one English subject and one unique intronic variation was observed (c.893−69A>C). One unique sequence variation was detected in the *FMOD* gene only in subjects with high myopia: a c.971G>A change that converted a codon AGG for Arg324 to AAG for Thr. One intronic variation was found in the flanking sequence of exon 3 of the *PRELP* gene (c.1188+27T>C) and one nonsynonymous variation at position c.408C>T coding for Asp149. These were not observed in control individuals. The family members of the subjects with unique sequence variations in *LUM*, *FMOD*, and *PRELP* did not consent to examination. These variations were not found in any of the 100 Finnish and 208 English control samples. Statistical analysis of the total number of variations observed in these four SLRP genes showed no significant difference between the patients and controls. However, more variations were observed in the English patients in the *OPTC* gene ($p = 0.0422$). In addition, the c.893−105G>A variation in the *LUM* gene was more common in the English controls (16.8%) than it was in the English patients (4.7%) with p-value of 0.0043.

PolyPhen and Ensembl Analysis

The results of the PolyPhen prediction program, which was used to analyze the impact of each observed missense mutation on the protein structure and function, indicated five probably or possibly damaging changes: Thr177Arg, Arg325Trp, and Gly329Ser in opticin; Leu199Pro in lumican; and Met157Val in PRELP (Table 2). Analyses of the conservativeness of the locations of the opticin, lumican, and fibromodulin variations within species using the Ensembl system revealed that the following variations were located in highly conserved positions: Thr177Arg, Arg229His, Arg325Trp, and Gly329Ser in opticin; Leu199Pro in lumican; and Pro46Leu, Glu147Asp, and Arg324Thr in fibromodulin (Fig. 2).

Homology Model for the Opticin LRR Domain

In order to gain an insight into possible consequences of the detected mutations for the structure of opticin, we generated a model of its LRR domain based on the recently published crystal structure of decorin [Scott et al., 2004]. Circular dichroism spectroscopy has shown that opticin and decorin have very similar secondary structures, dominated by a β-sheet and β-turn, with a little α-helix. Moreover, both proteins exist in solution as stable dimers [Scott et al., 2003; Le Goff et al., 2003]. A striking feature of the structure of decorin, which is reflected in the model of the opticin monomer (Fig. 3), is the presence of a series of salt bridges formed by acidic and basic residues brought into proximity on adjacent LRRs by the unique fold in this domain. The integrity of these salt bridges is probably critical for the stability of decorin and opticin under physiological conditions.

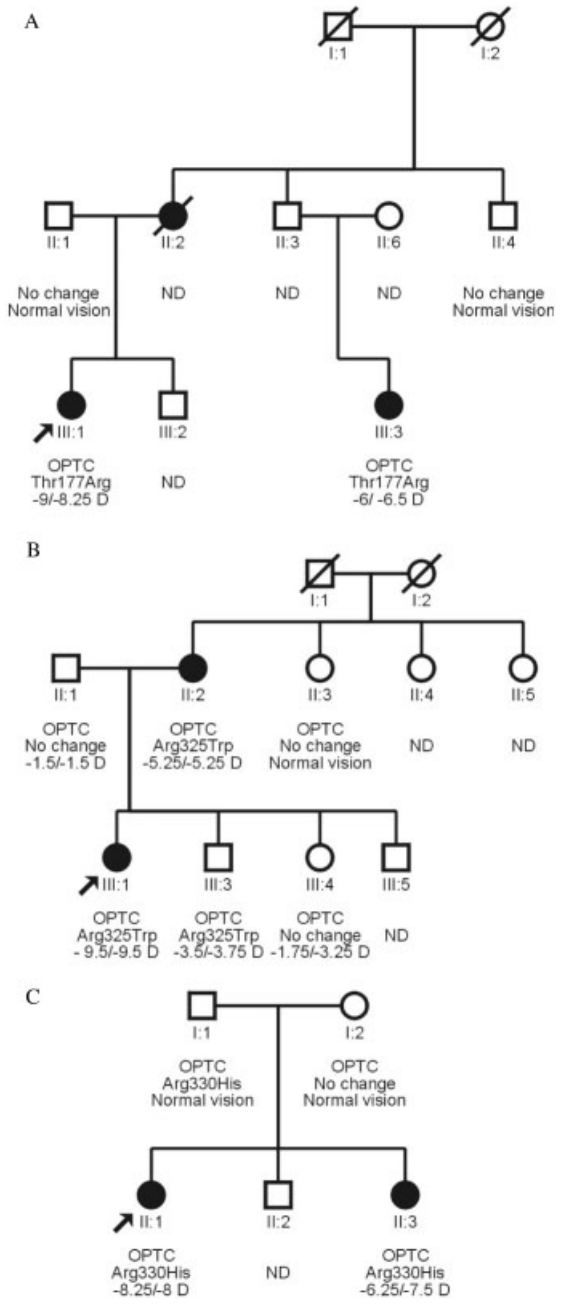

FIGURE 1. **Cosegregation of the observed missense mutations in *OPTC* in high myopic families. Pedigrees: Thr177Arg (A), Arg325Trp (B), and Arg330His (C).**

The summary of all analyses conducted on the sequence variations (cosegregation in high myopia families, PolyPhen and Ensembl analyses, structural modeling) are presented in Table 3.

DISCUSSION

The importance of the genetic component in intermediate and high myopia has been well established in population and family studies [Goss et al., 1988; Young et al., 1998]. A total of 10 loci have been identified to date, but the genes responsible have not yet been discovered. SLRPs expressed in the eye, especially in the sclera and vitreous, are typically associated with collagen fibrils of the ECM, and alterations in these components are likely to cause changes in scleral shape and in the vitreous collagen fibrillar network. Additional support for their role in the pathogenesis of high myopia has been obtained from experiments with *Fmod/Lum* null mice [Chakravarti et al., 2003].

In this work we investigated the genes encoding four SLRP proteins, opticin, PRELP, lumican, and fibromodulin, in 125 patients and found 13 sequence variations that altered an amino acid, six of which were not present in any of the 308 controls analyzed. Furthermore, eight nonsynonymous changes were highly conserved between species, implying an important role in the gene structure.

We analyzed the results further to determine whether we could obtain more evidence that these variations are genetic risk factors for the development of high myopia by studying cosegregation and whether the observed amino acid substitutions could be predicted to affect protein structure and function. Although all the patients had a family history of high myopia, the relatives of only four consented to take part in the study. The opticin changes Thr177Arg, Arg325Trp, Gly329Ser, and Arg330His were found in all the highly myopic relatives analyzed, but there were three variations, Arg325Trp, Gly329Ser, and Arg330His, that also occurred in family members with only low myopia or emmetropia. So while these data are supportive to a degree, they do not in their own right provide compelling evidence. However, perhaps this is not surprising as it is recognized that high myopia is a "complex disease" resulting from the influence of either alleles of reduced penetrance (susceptibility genes), environmental factors, or both [Farbrother et al., 2004; Zlotogora, 2003].

The effect upon protein structure and function was analyzed using PolyPhen analysis, sequence conservation across species, and, in the case of opticin, by introducing the mutations into a structural model of the LRR domains. The Polyphen analysis identified the Thr177Arg, Arg325Trp, and Gly329Ser mutations of opticin as possibly damaging. Some of the affected amino acids were highly conserved between species, suggesting that the amino acid substitutions may perturb structure and function, including Arg229His, Arg325Trp, and Gly329Ser. However, in the case of Arg330His, a His residue is present at this position in other species, suggesting that the amino acid substitution is benign. The structural model was only informative about three of the amino acid substitutions Thr177Arg, Arg229His, and Arg325Trp, but it did suggest that all three could perturb the protein structure. An Arg229 to His change, yielding a side-chain that is shorter and less likely to be protonated at physiological pH, or alteration of Arg325 to the uncharged hydrophobic residue Trp, would in each case eliminate a positively-charged residue that could be predicted to be involved in salt bridges of the kind observed here. The introduction of an additional positive charge by the Thr177Arg change in a region of opticin already dominated by positively-charged amino acids is also expected to be highly destabilizing. Misfolding of the LRR domain could impair the secretion and/or function of opticin, either directly or through aggregation driven by exposure of the hydrophobic core. The low degree of sequence similarity between the C-termini of opticin and decorin precluded any inferences about the possible structural consequences of the Gly329Ser or Arg330His mutations.

Although our results suggest that the opticin Arg325Trp change may be a risk allele for high myopia and disrupt the protein structure, it has been reported by Friedman et al. [2002] in a patient suffering from sporadic primary open-angle glaucoma or normal-tension glaucoma, and was also detected in a control individuals in that and our study. The myopic statuses of the

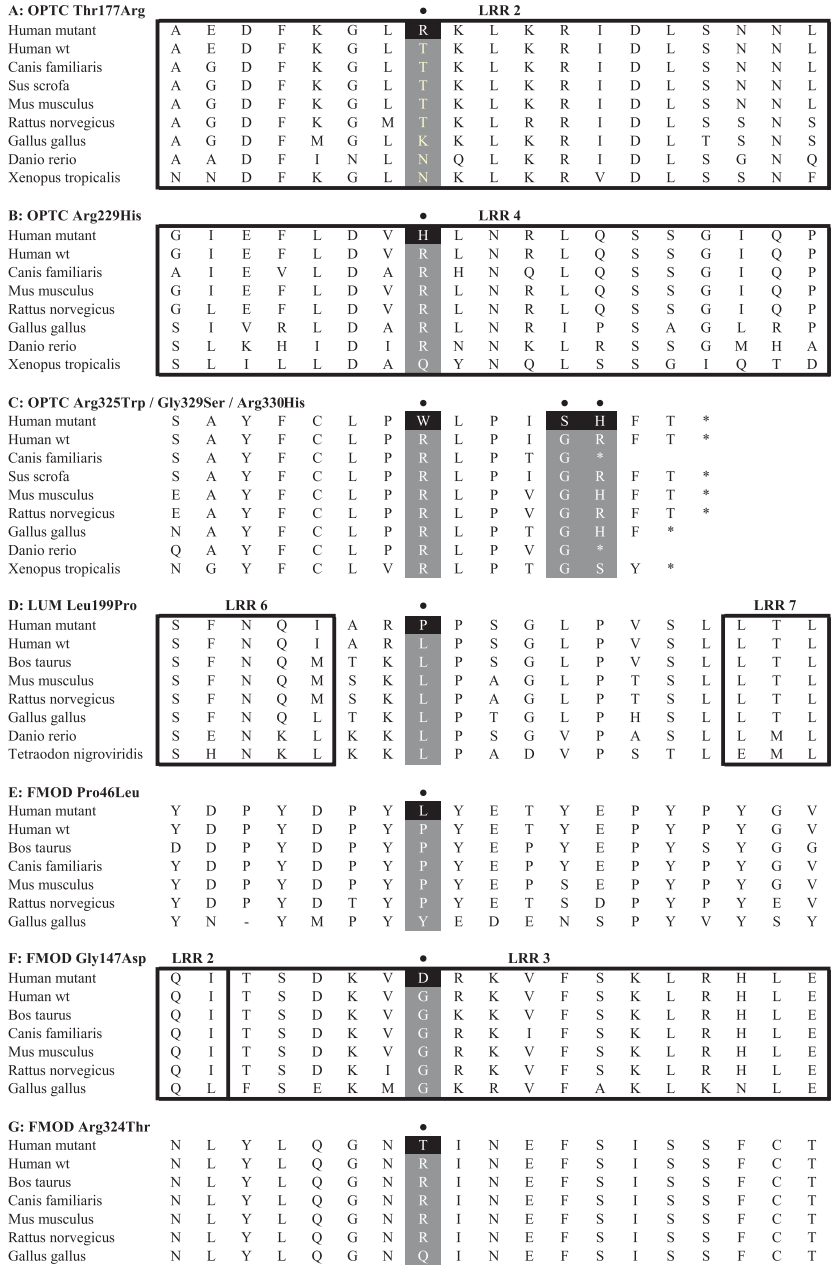

FIGURE 2. **Conservativeness of the altered amino acids (highlighted), studied by Ensembl ortholog prediction. Sequences belonging to the LRR domains are indicated with boxes and marked according to the domain number. A–C: Changes in opticin (Thr177Arg, Arg229His, Arg325Trp, Gly329Ser, and Arg330His). D: Changes in lumican (Leu199Pro). E–G: Changes in FMOD (Pro46Leu, Gly147Asp, Arg324Thr). Each mutation is indicated by a dot.**

patient and control in the Friedman's study are not known, however. The patients with the Arg325Trp mutation in our study did not have a history of glaucoma.

The other changes observed here (Leu199Pro in lumican; Pro46Leu, Gly147Asp, and Arg324Thr in fibromodulin) may relate to the onset of high myopia, since the amino acids concerned are highly conserved between species and are located in the conserved LRR domains. The PolyPhen analysis also showed the Leu199Pro change to be possibly damaging. Further research would be needed to demonstrate the impact of these changes on protein structure and function.

All four SLRP genes studied here are expressed in the developing and adult eye. Opticin is the predominant SLRP in the vitreous and both opticin and PRELP are expressed by the nonpigmented ciliary epithelium. Lumican and fibromodulin are expressed in the sclera, and lumican has been shown to be a key molecule in the neonatal development of the corneal stromal matrix [Beecher et al., 2006]. Fibromodulin, lumican, and PRELP are also expressed in cartilage tissues and, besides the eye defect, fibromodulin-deficient mice develop osteoarthritis and ectopic tendon ossification [Goldberger et al., 2005]. Since patients in this study were not analyzed for skeletal abnormalities, performing a more through clinical examination would shed further light on the significance of the unique changes in these SLRP genes.

Majority of the sequence variations detected were rare and detected only in one patient. However, the number of sequence

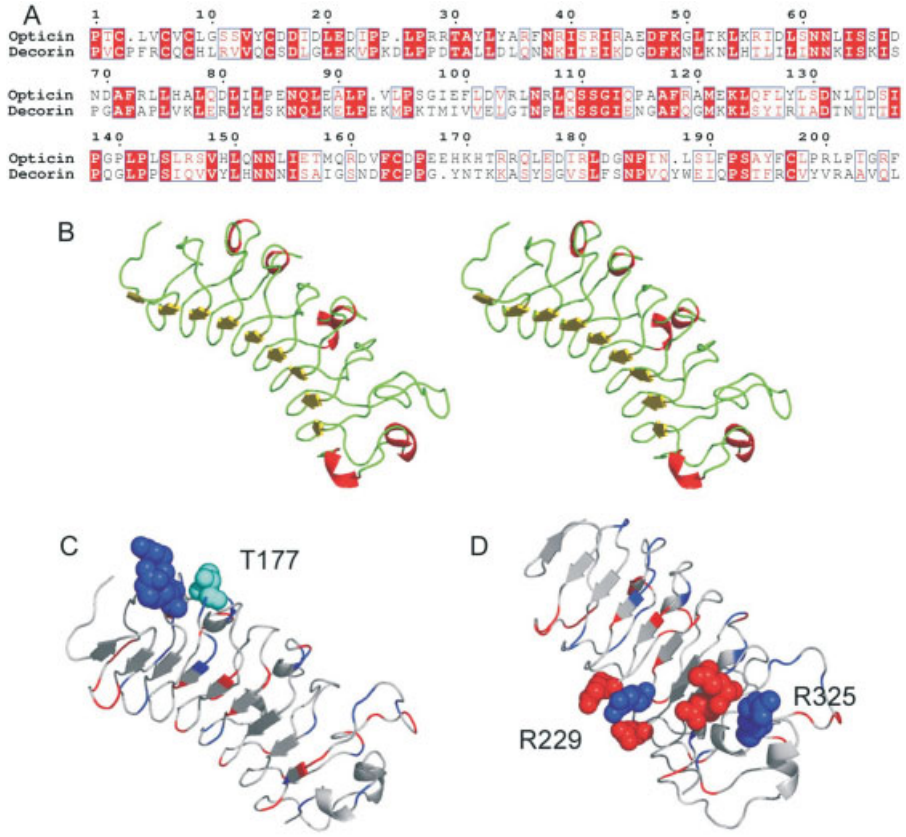

FIGURE 3. **A:** Alignment of sequences of human opticin and truncated bovine decorin used to generate a homology model for the LRR domain of opticin. **B:** Wall-eyed stereo image of the modeled LRR domain of opticin. β-strands are shown in yellow, helices in red, and loops and turns in green. **C:** Location of Thr177 (cyan spheres) in LRR2 of wild-type opticin, adjacent to Arg153 and Arg154 in LRR1 (blue spheres). **D:** Locations of Arg229 (blue spheres) in LRR4 of wild-type opticin, adjacent to the negatively charged residues E210 and D256 in LRRs 3 and 5 (red spheres), and of Arg325 in LRR7, adjacent to Glu302 and Asp303. Other basic and acidic residues shown in C and D are also colored blue and red, respectively.

TABLE 3. Summary of the Analyses Conducted for Sequence Variations

Gene	Mutation	Cosegregation	PolyPhen	Structural model	Conservativeness
OPTC	c.402C>T/p.L134L	n.a.	n.a.	No effect	–
	c.530C>G/p.T177R	+	Possibly damaging	+	+
	c.686G>A/p.R229H	n.a.	Benign	+	++
	c.803T>C/p.L268P	n.a.	Benign	No effect	n.a.
	c.810G>A/p. L270L	n.a.	n.a.	No effect	–
	c.973C>T/p.R325W	+	Probably damaging	+	++
	c.985G>A7p.G329S	+	Possibly damaging	n.a.	++
	c.989G>A/p.R330H	+	Benign	n.a.	+
LUM	c.507T>C/p.N169N	n.a.	n.a.		–
	c.596T>C/p.L199P	n.a.	Possibly damaging		++
FMOD	c.137C>A/p.P46L	n.a.	Benign		+
	c.237G>A/p.E79E	n.a.	n.a.		–
	c.440G>A/p.G147D	n.a.	Benign	.	+
	c.971G>A/p.R324T	n.a.	Benign		+
PRELP	c.97G>A/p.G33R	n.a.	n.a.		n.a.
	c.408C>T/p.N149N	n.a.	n.a.		–
	c.469A>G/p.M157V	n.a.	Possibly damaging		n.a.
	c.1042A>C/p.N348H	n.a.	Benign		n.a.

n.a., not analyzed; +, supports the role in high myopia.

variations observed in the *OPTC* gene was higher in patients than in controls ($p = 0.0422$) in the English population. Interestingly, we observed that a variation, c.893−105G>A, in the *LUM* gene was more common in controls than in patients ($p = 0.0043$), indicating that it might have a protective role or be in linkage disequilibrium with a protective allele.

Only a few candidate genes for high myopia have been studied to date. Lam et al. [2003] showed that the c.657T>C variation in TGF-β-induced factor (*TGIF*) is associated with high myopia, while Lipin 2 (*LPIN2*) was excluded as a candidate on the MYP2 locus [Zhou and Young, 2005]. Recently a 5′-regulatory region SNP in the *LUM* gene was associated with high myopia [Wang

et al., 2006]. Thus, our study provides additional new insight into the genetics of high myopia, suggesting that sequence variations in the SLRP genes expressed in the eye, especially *OPTC* and *LUM*, may be among the genetic risk factors causing the pathogenesis of high myopia. Here, we present novel findings, although replication of the study in a larger study population is needed to determine whether these genes do play a role in this trait.

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