

# Late-Onset Macular Degeneration and Long Anterior Lens Zonules Result from a *CTRP5* Gene Mutation

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**PURPOSE.** To identify the gene responsible for a complex ocular phenotype of late-onset macular degeneration, long anterior zonules (LAZ), and elevated intraocular pressure (IOP) and to study its expression.

**METHODS.** Ocular examination, visual field, fluorescein angiography, and electrophysiology testing were performed. One affected individual was treated with vitamin A. DNA from 55 family members (UM:H389) was used for linkage, mapping, and mutation analysis. Linkage analysis of macular degeneration and LAZ phenotypes was performed independently. Mutations in candidate genes were screened by sequencing. mRNA expression of *CTRP5* and *MFRP*, which are bicistronic genes, was studied by semiquantitative RT-PCR (qRT-PCR) in various human tissues. *CTRP5* expression was also evaluated by in situ hybridization.

**RESULTS.** Affected members had LAZ detectable by the third decade and/or macular degeneration by the fourth to fifth decade. A six-month treatment with vitamin A shortened dark adaptation considerably in one affected member. Both conditions mapped independently with zero recombination to 11q23, with maximum lod scores of 3.31 for macular degeneration and 5.41 for LAZ. The same *CTRP5* missense mutation was identified in all affected individuals. Retinal pigment epithelium (RPE) and ciliary epithelium (CE) showed highest *CTRP5* transcript expression, which was also true for *MFRP*. *CTRP5* tissue expression was confirmed by in situ hybridization.

**CONCLUSIONS.** A single locus at 11q23 is implicated in a complex ocular phenotype involving RPE and CE, tissues of neuroectodermal origin. All individuals with either LAZ and/or macular degeneration carry the same *CTRP5* S163R mutation, which is

transmitted in autosomal dominant manner. (*Invest Ophthalmol Vis Sci.* 2005;46:3363–3371) DOI:10.1167/iovs.05-0159

Clinical forms of macular degeneration have a broad phenotypic and genotypic spectrum. The disease is characterized by drusen, which subsequently can be associated with local tissue atrophy or with neovascularization of the choroid and lead to loss of central vision and blindness.<sup>1</sup> To date, at least 10 genes associated with various forms of macular degeneration have been identified. Seven additional loci have been mapped, but the genes are yet to be cloned.<sup>2–5</sup>

We have studied a large pedigree (UM:H389) with progressive macular degeneration that manifests primarily by the fifth decade of life.<sup>6</sup> Most of the older affected family members showed abnormally long anterior zonules (LAZ) on the anterior capsule of the crystalline lens and had a reduced zonule-free zone. Several younger family members who have not yet manifested macular degeneration have LAZ. Macular degeneration and LAZ both appear to segregate as autosomal dominant traits. Several older members with LAZ had ocular hypertension or glaucoma.

We have published the clinical characteristics of the macular degeneration and LAZ in this family.<sup>6,7</sup> The macular findings have phenotypic overlap with Sorsby fundus dystrophy,<sup>8</sup> late-onset macular degeneration associated with RDS peripherin gene mutations,<sup>9</sup> age-related macular degeneration (AMD),<sup>10</sup> and late-onset retinal degeneration (L-ORD).<sup>11</sup> Co-occurrence of LAZ with macular degeneration has not been reported previously for any of these conditions, although several individuals in families with Sorsby fundus dystrophy are reported to have glaucoma.<sup>8,12–14</sup>

We conducted a genomewide search for linkage and found that both traits of macular degeneration and LAZ map to the same region (q23) of chromosome 11 and that both traits can be accounted for by an S163R missense mutation in the complement-C1q tumor necrosis factor-related protein gene, *CTRP5*. In the process, we definitively excluded most of the previously known macular degeneration loci and approximately 90% of the genome. In addition to this genetic analysis, tissue expression of *CTRP5* and its bicistronic partner membrane-type frizzled-related protein (*MFRP*), was found to be highest in the retinal pigment epithelium (RPE) and in the ciliary epithelium (CE), tissues of neuroectodermal origin, and are implicated in the disease observed in family UM:H389.

## METHODS

### Clinical Studies

Informed consent was obtained from all participating individuals, according to protocols approved by institutional review boards at the University of Michigan, The Johns Hopkins University, and the National Eye Institute. The protocol is in accord with the tenets of the Declaration of Helsinki. Clinical examinations were performed on 42 members of family UM:H389, including all living individuals older than 48 years. The macular degeneration was described earlier in some these

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TABLE 1. Sequence of Primers Used for qRT-PCR Analysis

Primer (5'–3')	Orientation	Gene	Position
TGTATGAGACCAGCAGCTCA	Sense	<i>MFRP</i>	1229–1248 (exon 9)
ATGTCACACATCCACTGCAC	Antisense	[gi:13442819]	1453–1472 (exon 11)
TGCTGACCTGCTGGATTACATC	Sense	<i>HPRT</i>	310–332 (exon 3)
CTTGCGACCTTGACCATCTTTG	Antisense	[gi:4504482]	549–570 (exon 5)

individuals.<sup>6</sup> The family is of French and English extraction and immigrated in the 1800s.

The ocular examination included biomicroscopy, gonioscopy, intraocular pressure (IOP), and retinal funduscopy. Psychophysical testing included standard visual field measurement by Humphrey and/or Goldmann perimetry. In patient V:32, the time course of recovery of cone and rod sensitivity was evaluated in the central 10° after a 5-minute exposure to a 2700-cd/m<sup>2</sup> white bleaching light. A version of the same instrument, modified to perform von Bekesy tracking, was used to measure changes in threshold as a function of time.

The full-field electroretinogram (ERG) was recorded according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards<sup>15,16</sup> at 0.1 to 1000 Hz (–3 dB) after full pupillary dilation. Recordings were obtained with Burian-Allen bipolar corneal electrodes (Hansen Ophthalmic Instruments, Iowa City, IA) with topical anesthesia (0.5% proparacaine hydrochloride). Responses were elicited with Ganzfeld stimuli from a xenon photostrobe (PS-22 stimulator; Grass-Telefactor, West Warwick, RI), with photopic and scotopic stimuli conforming to the international ERG standard.<sup>17</sup> Dark-adapted, rod-predominant ERG responses were recorded using flash intensity of 0.42 cd-sec/m<sup>2</sup> further filtered (Wratten 47+47A+47B; Eastman Kodak, Rochester, NY) to yield dim blue flashes at 2-second intervals. Responses were then recorded under dark-adapted conditions to single, white stimuli of 4.2 cd-sec/m<sup>2</sup> per flash, which is a stimulus that gives responses roughly 75% from the rod and 25% from the cone system. Next, 30-Hz white stimuli at 4.2 cd-sec/m<sup>2</sup> per flash were used to elicit primarily cone responses. Subjects were then light adapted at 43 cd/m<sup>2</sup> for 5 minutes, after which single white flashes of 10.0 cd-sec/m<sup>2</sup> were used to elicit cone-predominant responses. Normal control ERG responses were derived from 40 control subjects.<sup>18</sup>

### Vitamin A Supplementation

One subject (V:32), with extensive drusen accumulation in the temporal perfovea of both eyes, was presymptomatic for acuity reduction but reported debilitating sensitivity to glare in daylight and reduced visual function at night and in dim light. After baseline visual function was determined, the subject was given oral 15,000 IU vitamin A palmitate daily, and visual function was evaluated again from 2 to 6 months later. This subject was selected for these measurements because the subject was considerably symptomatic but, unlike other such symptomatic family members, had preservation of central vision that allowed the performance of psychophysical tests with ease. All visual function tests were performed with natural pupils.

### Genotyping

Genotyping was performed by using microsatellite markers on leukocyte DNA collected from 48 family members and seven spouses. DNA from two subjects from the Centre d'Etude du Polymorphisme Humaine (CEPH) were analyzed concurrently as the control. A linkage-mapping program (MD-10, ver. 2.0; Applied Biosystems, Inc. [ABI], Foster City, CA) was used for genome scans. Allele sizes were estimated with a commercial standard (ROX50 standard; ABI). Genotypes were then analyzed on computer (Genescan and Genotyper software; ABI). Allele frequencies were obtained from the CEPH database.

### Linkage Analysis

For linkage analysis the two phenotypes, LAZ and macular degeneration, were treated as independent traits in this family, using the MLINK

program of the LINKAGE package (<http://linkage.rockefeller.edu/soft/linkage/>; provided in the public domain by the Rockefeller University, New York, NY) and an affecteds-only model.<sup>19,20</sup> The disease was coded as an autosomal dominant trait with 0.0001 gene frequency for the affected allele. Marker allele frequencies were taken from the genome database. Lod scores for markers near the L-ORD locus at 11q23 were also calculated in an age-dependent penetrance model, with individuals older than age 48 and without macular changes coded as unaffected.

### Mutation Analysis

The sequences containing exons and exon-intron boundaries of the *MFRP* and *CTRP5* genes were amplified using primers reported earlier.<sup>11</sup> Mutation analysis was performed by sequencing the PCR-amplified products, as previously described (3100 Sequencer; ABI).<sup>21</sup>

### RNA Isolation

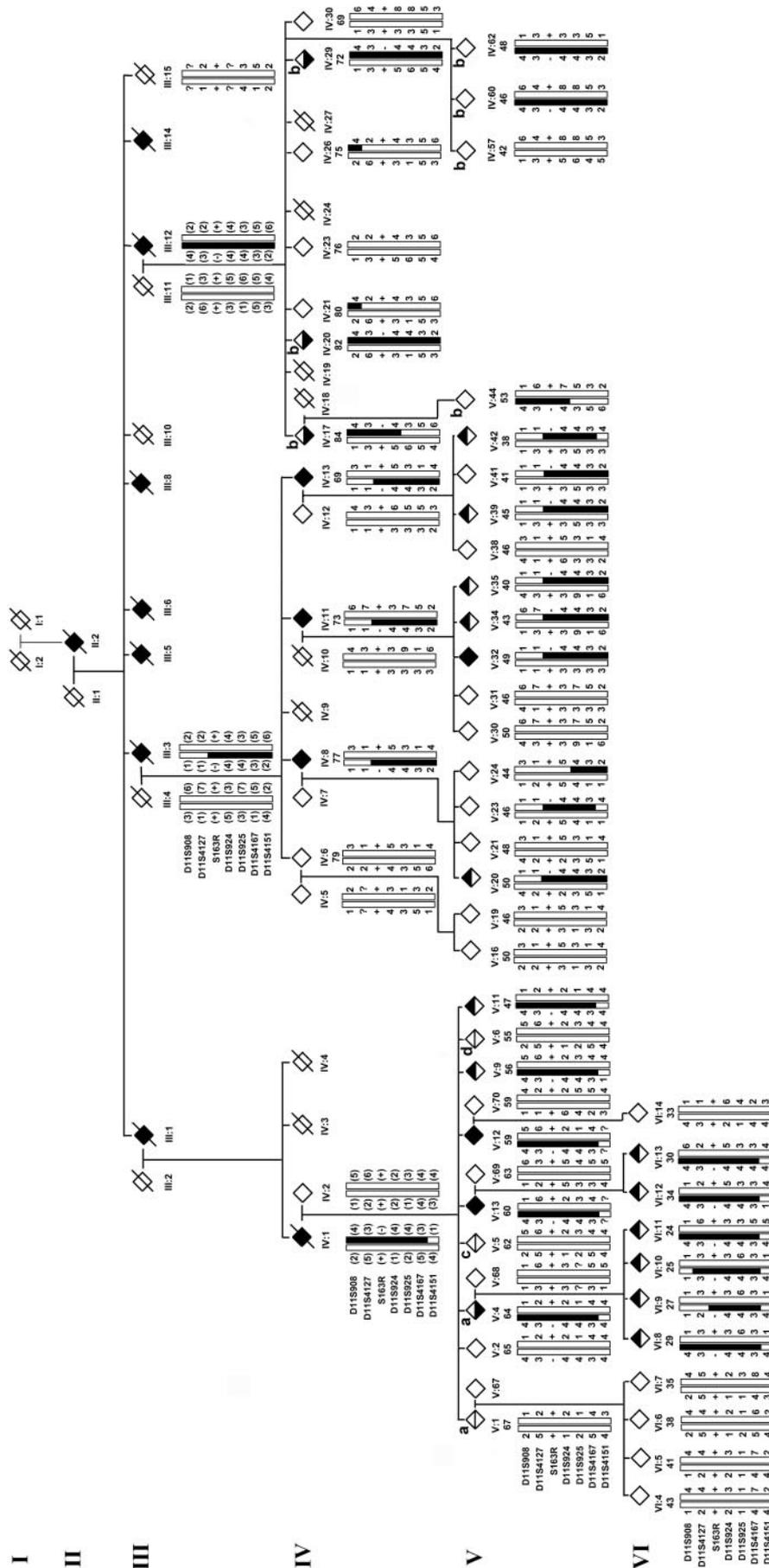
Tissues were dissected from human donor eyes obtained within 16 to 20 hours of death through the Midwest Eye Bank and Transplantation Center in Ann Arbor, MI. Retina, RPE, optic nerve (ON), and cornea were collected from a 69-year-old donor eye; iris and ciliary body (CB) were collected from a 73-year-old donor eye; and CE was dissected from a 53-year-old donor eye. After they were harvested, these tissues were stored in RNA extraction reagent (TRIzol; Invitrogen, Carlsbad, CA) at –80°C. Cultured RPE, orbital fibroblasts (OF), sclera, and Tenon's capsule (TC) were harvested and stored in the same reagent (TRIzol; Invitrogen). Similarly, cell lines derived from nonpigmented epithelium (NPE), smooth muscle (SM), trabecular meshwork (TM), lens epithelium (LE), and RPE were also harvested in the extraction reagent (TRIzol; Invitrogen) for use in this study. RNA was isolated with the reagent, according to the manufacturer's protocol.

### Real-Time Semiquantitative RT-PCR

Total RNA was treated with RNase-free DNase and purified (RNeasy minikit; Qiagen, Valencia, CA) and used for first-strand cDNA synthesis (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen). Primers for qRT-PCR were designed from regions of gene sequences that expand over at least one intron, by using commercial software (Beacon Designer 2.0 software program; Bio-Rad, Hercules, CA; Table 1). Three sets of control primers derived from the *GAPDH*, *HPRT*, and  $\beta$ -actin genes were used to normalize and validate the *CTRP5* and *MFRP* gene expression.<sup>11,22</sup> A supermix (iQ SYBR Green Supermix; Bio-Rad) and a thermocycler (iCycler; Bio-Rad) were used to perform quantitative PCR and a melt-curve analysis. The relative quantity of expression of *CTRP5* and *MFRP* in different samples was calculated by the comparative threshold cycle ( $C_t$ ) method.<sup>22</sup> Expression data were calculated from three independent qRT-PCR reactions performed for each RNA sample. These were normalized to the expression levels of three control housekeeping genes, and the mean results are presented on an arbitrary scale, to represent the relative levels of expression.

### In Situ Hybridization

Probes were generated from cDNA clone IMAGE:5278184, which contains the full-length (1.5 kb) coding sequence of human *CTRP5*. The *CTRP5* cDNA fragment was amplified with T3 and T7 primers, and T3 and T7 RNA polymerases were used to generate digoxigenin-labeled sense and antisense riboprobes, respectively (DIG-RNA label-



**FIGURE 1.** Pedigree and haplotypes of family UM:H389 with selected markers at 11q23. (◆) Individuals with LAZ; (◊) individuals with late-onset macular degeneration; (◊) individuals with macular degeneration and LAZ; (◊) unaffected. *Slashed symbols:* deceased. a, anterior capsule is absent for ascertainment of LAZ; b, not available for clinical examination; c, status of LAZ is unknown; d, status of macular degeneration is unknown. *Filled bars:* haplotype associated with the disease. Individual identification numbers are shown in roman numerals. The individual identification numbers shown for family members are the same as the identification numbers shown in our previously published pedigree of the family.<sup>6</sup> Haplotypes of individuals III:4, III:3, III:11, III:12, IV:1, and IV:2 are derived from the data of their offspring and shown in parentheses. Ages of some members are shown in arabic numerals below the identification number. The sex of the family members is not shown, to maintain the privacy of individuals at risk of development of macular degeneration later in life.

TABLE 2. Two-point lod Scores of Markers on Chromosome 11 Versus Macular Degeneration and Anterior Lens Zonules Loci

Markers	Distance* (cM)	LOD Scores at Theta							$Z_{max}$	Theta
		0	0.001	0.05	0.1	0.2	0.3	0.4		
Macular degeneration locus										
<i>D11S908</i>		-2.125	-1.083	0.535	0.73	0.712	0.486	0.192	0.73	0.1
<i>D11S4127</i>	5	-1.285	-0.248	1.19	1.256	1.054	0.686	0.262	1.256	0.1
<i>S163R</i>	1.6	3.311	3.305	3.021	2.716	2.058	1.33	0.549	3.311	0
<i>D11S924</i>	0.2	0.901	0.898	0.733	0.579	0.324	0.144	0.035	0.901	0
<i>D11S925</i>	3	-∞	-0.301	1.162	1.213	0.993	0.629	0.236	1.213	0.1
<i>D11S4167</i>	2	-2.973	-0.477	0.979	1.027	0.804	0.46	0.136	1.027	0.1
<i>D11S4151</i>	6	-∞	-3.901	-0.675	-0.234	0.039	0.064	0.023	0.064	0.3
Anterior lens zonules locus										
<i>D11S908</i>		-∞	-2.456	0.633	0.941	0.931	0.654	0.301	0.941	0.1
<i>D11S4127</i>	5	-∞	-5.3	-0.488	0.153	0.498	0.428	0.206	0.498	0.2
<i>S163R</i>	1.6	5.418	5.409	4.972	4.503	3.486	2.355	1.127	5.418	0
<i>D11S924</i>	0.2	4.618	4.609	4.169	3.705	2.737	1.726	0.718	4.618	0
<i>D11S925</i>	3	3.005	2.999	2.719	2.419	1.781	1.103	0.451	3.005	0
<i>D11S4167</i>	2	3.147	3.141	2.834	2.508	1.815	1.081	0.395	3.147	0
<i>D11S4151</i>	6	-∞	-4.08	-0.848	-0.349	-0.079	-0.011	-0.006	~	~

\* Distance to the next distal marker.

ing kit; Roche Diagnostics, Mannheim, Germany). In situ hybridization was performed on 10- $\mu$ m cryosections from anterior and posterior segments of human eyes, and also from the eyes of albino mice, according to previously published protocols.<sup>23,24</sup> Maintenance and care of the BALBc mice used in this study were in accordance with the NIH guidelines for the use of laboratory animals.

## RESULTS

### Clinical Studies

In the UM:H389 family, 55 family members were examined. Several family members affected with macular degeneration were examined on multiple occasions over more than a decade. Ten individuals in UM:H389 were affected with macular degeneration that became symptomatic in the fourth to fifth decade. The macular degeneration phenotype in this family followed an autosomal dominant inheritance across four consecutive generations (Fig. 1). In several older family members with late-onset macular degeneration and LAZ elevated intraocular pressure and glaucoma subsequently developed.

The LAZ phenotype was observed in 18 individuals between 24 to 80 years of age. All six individuals with LAZ over the age of 48 years also showed the macular degeneration phenotype. The remaining 12 with LAZ were between 24 to 47 years of age and were presumed to be too young to show definitive signs of macular degeneration. The lens and zonules could not be evaluated in four older members with macular degeneration. One died subsequent to initial retinal examination (IV:29), two were not available for evaluation of the anterior segment (IV:17 and IV:20), and one was aphakic after cataract surgery (V:4). However, all children of V:4 had LAZ, consistent with autosomal dominant inheritance.

Two of three individuals affected with both macular degeneration and LAZ had elevated IOP (IV:8 and IV:11), and in one (IV:13), glaucoma developed and was treated with topical medication. No other systemic abnormalities were noted in the medical history of members of this family. From the clinical data, it could not be determined whether macular degeneration and LAZ were associated with a single locus or were two independent traits cosegregating in this family.

### Linkage and Haplotype Analysis

On the basis of significantly negative lod scores, we excluded all the previously mapped macular degeneration loci, including

the RDS/peripherin, Stargardt macular degeneration, Stargardt-like macular degeneration, Doyme honeycomb retinal dystrophy, Best or vitelliform macular degeneration, North Carolina macular degeneration (*MCDR1*), *MCDR2*, *MCDR3*, and *ARMD1* loci.<sup>4,9,10,25-30</sup> Similarly, for the LAZ phenotype, we excluded the loci for pigmentary dispersion syndrome, Marfan syndrome, pseudoexfoliation syndrome, and previously mapped glaucoma loci.<sup>28,31-35</sup> Further, more than 90% of the genome was excluded before identifying positive lod scores, indicating linkage on the long arm of chromosome 11 for both LAZ and macular degeneration.

Further analysis of markers *D11S908*, *D11S4127*, *D11S925*, *D11S4167*, and *D11S4151* at 11q23 gave positive lod scores for both the macular degeneration and the LAZ phenotype (Table 2). All individuals affected with both macular degeneration and LAZ carried the identical haplotype at 11q23, implicating the same locus for both traits.

Recombinations observed in family members with macular degeneration localized this phenotypic trait to a 6-cM interval between the markers *D11S4127* and *D11S925*. A recombination was observed in three affected siblings (IV:8, IV:11, and IV:13) at the marker *D11S4127*, placing the gene for macular degeneration distal to this marker. A recombination in IV:17 with macular degeneration identified marker *D11S925* as the distal boundary.

Similar information was developed for the trait of LAZ. Individuals IV:8, IV:11, and IV:13, who determined the proximal boundary for the macular degeneration locus, also had LAZ, which identifies *D11S4127* as the proximal boundary for LAZ. Unfortunately, individuals IV:17 and V:44 who defined the distal boundary for the macular degeneration locus, were not available for anterior segment examination. However, recombination events in individuals V:23 and V:9 with LAZ identified *D11S4151* as the distal boundary for the LAZ locus. Individual IV:24 was clinically negative for LAZ at age 44, but inherited the haplotype associated with LAZ distal to *D11S925*, thereby identifying this marker as the distal boundary for the LAZ locus. These observations localized the loci for both the macular degeneration and the LAZ to the 6-cM interval at 11q23 between markers *D11S4127* and *D11S925*. Haplotype analysis by incorporating single-nucleotide polymorphisms (SNPs) in the region further narrowed the critical interval to a 1.35-Mb region between markers *rs3181261* (V:24) and *rs715849* (V:44; Fig. 2). The recombination observed in the

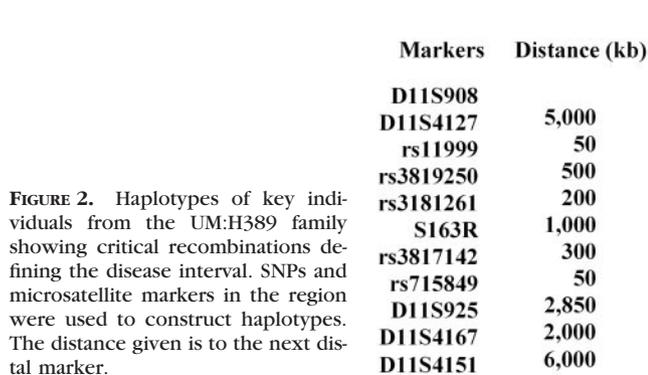
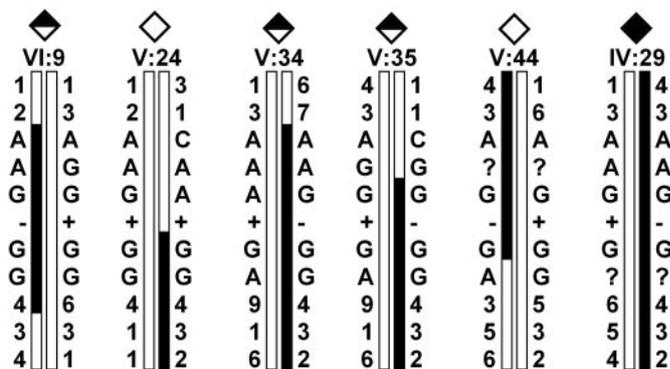


FIGURE 2. Haplotypes of key individuals from the UM:H389 family showing critical recombinations defining the disease interval. SNPs and microsatellite markers in the region were used to construct haplotypes. The distance given is to the next distal marker.



unaffected individual V:24 further supports this localization (Fig. 2).

**Mutation Analysis of Candidate Genes**

Several candidate genes were identified in the 1.35-Mb critical interval by consulting the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov/ provided in the public domain by the National Institutes of Health, Bethesda, MD). The *MFRP* gene, previously shown to carry mutations in the mouse retinal degeneration 6 (*rd6*), and the *CTRP5* gene, located in the 3'-untranslated sequence of the *MFRP* gene were identified as candidate genes, due to their location in the critical interval.<sup>36</sup> While these studies were in progress, Hayward et al.<sup>11</sup> have reported a point mutation in *CTRP5* in families with L-ORD. We performed mutation analysis on both these genes in family UM:H389 with late-onset macular degeneration or with LAZ. No mutations were found after *MFRP* was fully sequenced. In contrast, we found a serine163arginine missense change in *CTRP5*, and this mutation was not detected in 100 normal control subjects. The same mutation was observed in families with L-ORD by Hayward et al.<sup>11</sup> In addition, the change was found to be absent in 1000 normal control subjects analyzed by Hayward et al.

**CTRP5 and MFRP Expression in Human Ocular Tissues and Cell Lines**

Quantitative expression profiles of both *CTRP5* and *MFRP* were determined in the human ocular tissues by qRT-PCR. The highest level of *CTRP5* mRNA expression was observed in the RPE. Lower levels were present in the CB (Fig. 3). Cultured RPE cells also showed a significant and almost equal level of *CTRP5* expression as did native RPE. Low levels of expression were detected in the ON, sclera, and retina. Cultured human cells derived from LE showed a significant level of *CTRP5* expression. An RPE cell line also showed expression of *CTRP5*, but the amount was much lower than that in the primary RPE tissue. Low levels of *CTRP5* expression were detected in the cell lines derived from ciliary SM and TM (Fig. 3). Similar to the pattern of expression of the *CTRP5* transcript, the highest levels of *MFRP* expression were detected in the RPE and CB. No significant expression was detected in the retina, cornea, ON, and sclera. Low levels of expression were detected in CE and cultured RPE but not in other tissues and cell lines tested (Fig. 4).

**Cellular Localization of the CTRP5 Gene in Human Ocular Tissue**

*CTRP5* expression was detected in the sections of human eye by in situ hybridization using labeled riboprobes (Figs. 5A-D). Corresponding to the expression detected by qRT-PCR, the *CTRP5* gene was detected in the RPE layer in the human retinal

sections by in situ hybridization (Figs. 5B, 5C). Expression was also observed in the retinal ganglion cell layer and in a few cells of the nuclear layer, perhaps representing a subset of amacrine cells (Fig. 5B). The expression of *CTRP5* in the CB and iris could not be resolved by in situ hybridization because of interference from the pigmented cells. *CTRP5* expression in the remaining parts of the anterior segment of the human eye was not significant.

In situ hybridization of tissue sections from albino mice localized *CTRP5* expression to the RPE and CE, consistent with our observations on the expression of *CTRP5* in human ocular tissue by in situ hybridization and q-RT PCR (Figs. 5C, 5D).

**Improvement of Rod Psychophysical Function after Vitamin A Supplementation**

Individual V:32, who was the 48-year-old offspring of IV:11, reported sensitivity to daylight glare and reduced visual function at night. The subject had LAZ and extensive accumulation of drusen in a region 15° temporal from the fovea (Fig. 6), but the macula otherwise appeared clinically normal, and visual acuity was better than 20/20 in each eye. Other aspects of retinal function were also normal, including visual fields and electroretinogram responses. Although dark-adaptation testing gave normal absolute rod thresholds centrally, a 1-log-unit elevation of this threshold was measured in the area with

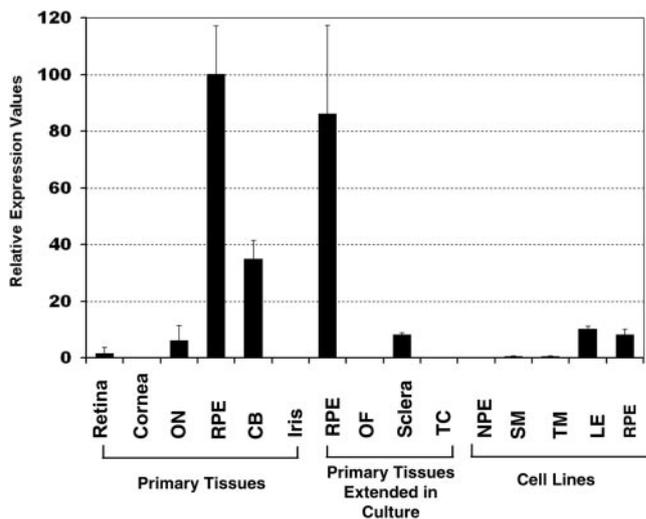


FIGURE 3. Expression levels of *CTRP5* mRNA in various ocular tissue and cultured cells, as determined by qRT-PCR. Levels were normalized and calculated by the comparative  $C_t$  method with *GAPDH*, *HPRT*, and  $\beta$ -actin expression. Relative expression levels are presented on an arbitrary scale with standard deviation.

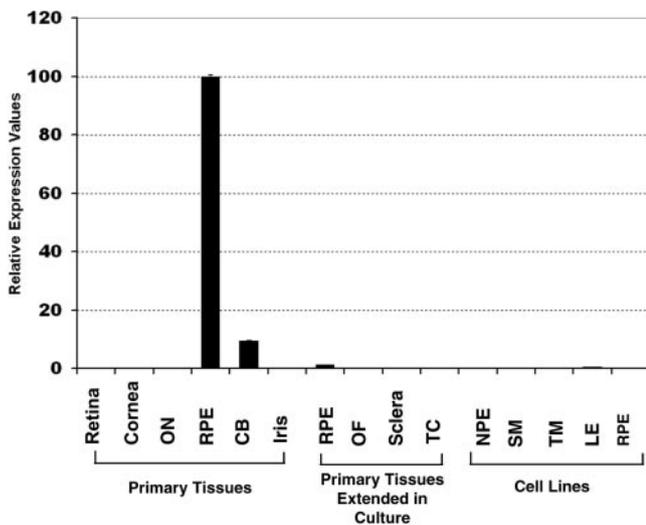


FIGURE 4. Expression profile of *MFRP* mRNA in ocular tissue and cultured cells. Levels were determined as in Figure 3.

drusen (15° nasal to fixation). The recovery of rod sensitivity from adaptation to a bleaching light was markedly delayed, as judged by the cone-rod break time of 18 minutes, which is prolonged far beyond the upper limit of the normal range seen in our control subjects (6.9 minutes; Fig. 7).

After this evaluation, the subject was started on a daily intake of 15,000 IU vitamin A palmitate and returned at 2, 4, and 6 months for reevaluation. After 2 months, this individual's dark-adaptation function had improved considerably, and the cone-rod break time was 14 minutes. After 4 months, the cone-rod break was not further shortened, but the slope of the rod limb became steeper. These improved results were main-

tained after 6 months. This also suggests that the improved dark-adaptation function was reproducible and similar to that established for control subjects. Absolute threshold remained normal and unchanged throughout the study. Concomitantly, the subject volunteered that the visual function in dim light had improved appreciably and previous sensitivity to glare was reduced.

## DISCUSSION

Family UM:H389 segregated two ocular phenotypes—macular degeneration and LAZ—both in an autosomal dominant fashion. Although several family members showed both conditions, incomplete clinical data precluded determining that the two conditions cosegregate.<sup>6</sup> After our initial description of this family,<sup>6</sup> we uncovered an additional branch of the pedigree and obtained further clinical information to initiate a genome-wide linkage study. Both conditions mapped to a 1.35-Mb interval on 11q23 that includes *CTRP5*, a gene implicated in L-ORD.<sup>11</sup> Sequencing revealed an S163R mutation in *CTRP5* in all family members who had either macular degeneration or LAZ. Complete concordance of LAZ and the disease haplotype was observed in family members aged 24 to 80 years. The youngest individual with macular degeneration in this family was 48 years old at diagnosis. LAZ were present in individuals as young as 24 years.

Based on the expression of *CTRP5* in the RPE and the CE, the next goal is to understand the role of this gene in the two tissues involved in the UM:H389 family phenotype. The *CTRP5* gene encodes a secretory protein with homology to short-chain collagens<sup>11,56</sup> and is predicted to be a member of extracellular matrix.<sup>37</sup> The retina from a *CTRP5* Ser163Arg-bearing patient with L-ORD showed retinal degeneration and a thick layer of extracellular deposits between RPE and Bruch's membrane.<sup>11,38</sup> The Ser163Arg mutation appears to cause an age-

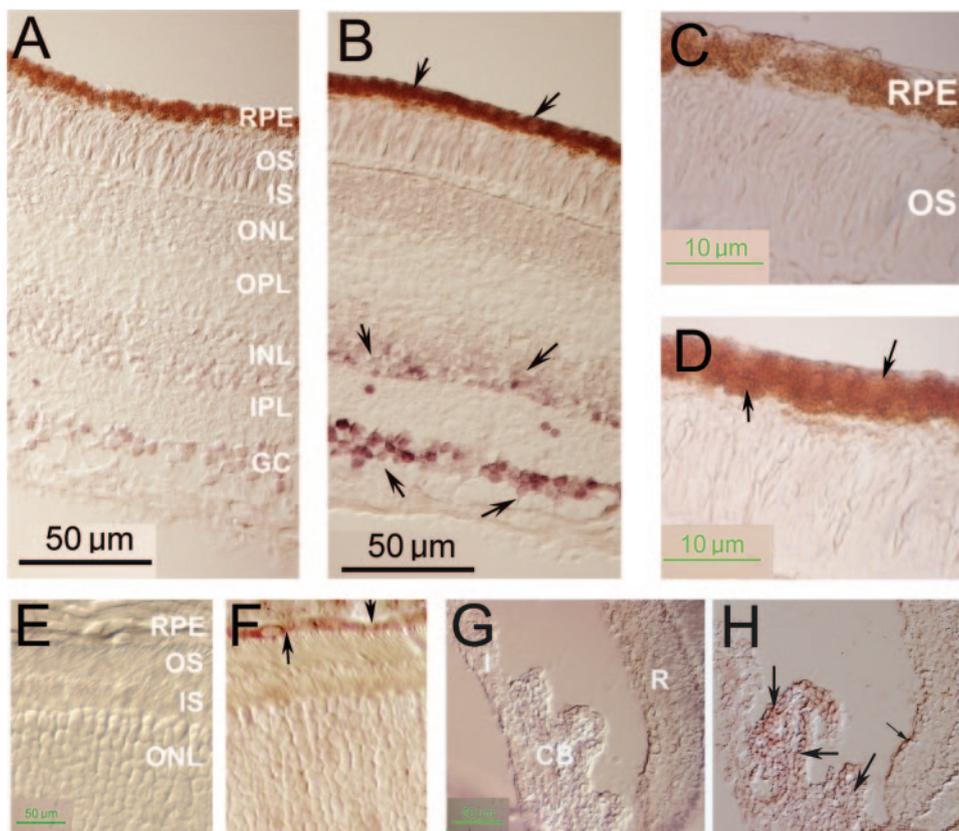
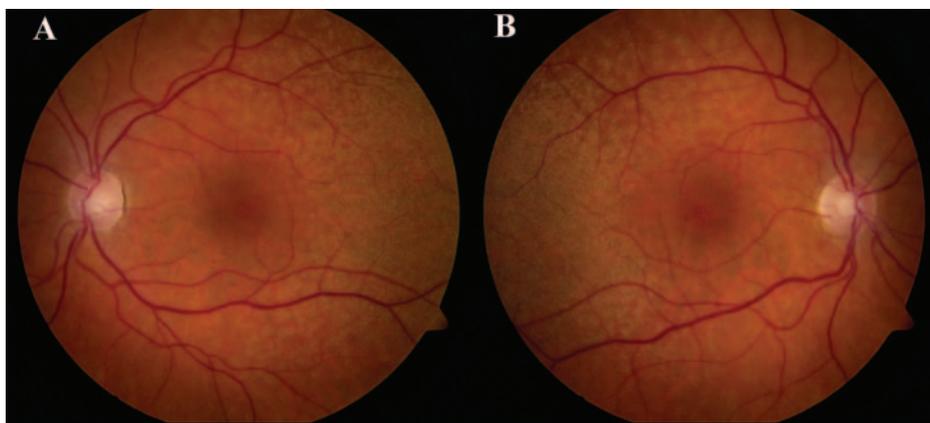


FIGURE 5. Cellular expression of *CTRP5* mRNA in human retina (A–D). (B, D, arrows) Specific expression was noted in the RPE, ganglion cell (GC) and inner nuclear (INL) layers. (A, D) Sense or control probe; (B, C) Antisense probe. A higher-magnification image of the RPE is shown in (C) and (D). Expression of *CTRP5* was noted in the RPE (F) and CB (H) of albino mice on the epithelial cell layers (arrows). (E, G) Sense or control probe; (F, H) anti-sense probe. OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; IPL, inner plexiform layer.



**FIGURE 6.** Fundus photographs of the right (A) and left (B) eyes of a 48-year-old family member (V:32) shows extensive drusen in the macula 15° temporal to the fovea.

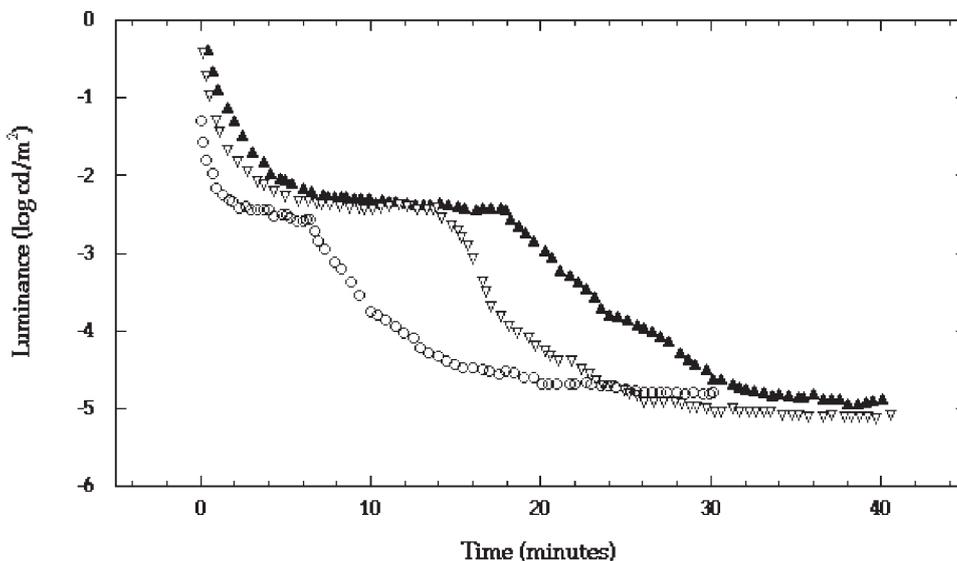
related accumulation of substances between Bruch's membrane and RPE. Similar sub-RPE deposits have also been observed in retinas from patients with mutations in the *TIMP3*, *HEMICENTIN-1*, and *EFEMP1* genes and in mouse retina lacking collagen 18A.<sup>39-43</sup> These genes, *TIMP3*, *EFEMP1*, and *HEMICENTIN-1*, encode for extracellular matrix proteins, and mutations in these genes result in macular dystrophy with subretinal deposits similar to our family UM:H389 with a mutation in *CTRP5*.<sup>10,44,45</sup> These observations suggest that the extracellular matrix proteins encoded by these genes are important in normal RPE function.

Based on the LAZ in UM:H389, it appears that *CTRP5* also has a role in the CE. Although the same S163R *CTRP5* mutation was found to be associated with L-ORD, anterior segment anomalies have not been reported in patients with L-ORD.<sup>11</sup> It is critical to screen for anterior segment changes in existing pedigrees carrying the S163R mutation in *CTRP5*. The clinical finding of LAZ has been described in both black and white populations, with and without elevated IOP and/or glaucoma.<sup>7,46</sup> Other reports on zonules relate to known abnormalities associated with pseudoexfoliation,<sup>47</sup> Marfan syndrome,<sup>48-50</sup> homocystinuria,<sup>51</sup> and ectopia lentis.<sup>41,42,52</sup> It appears that the LAZ are structurally robust, because cataract complications, spontaneous crystalline lens subluxation, or zonular dehiscence have not been reported in cases of LAZ.<sup>7,46,53</sup> Understanding the role of *CTRP5* in the CE and normal zonule anatomy is of interest for the study of the mechanism underlying LAZ phenotype.

Our study in one patient with macular degeneration with the *CTRP5* mutation may provide clues to the course of degeneration. It has been reported that delayed dark-adaptation is an early retinal phenotypic marker of L-ORD, which manifests up to a decade before macular degeneration.<sup>54</sup> A 1-month, high-dose vitamin A (50,000 IU per day) supplementation improved dark adaptation kinetics in three patients with L-ORD.<sup>54</sup> Several members of our UM:H389 family reported impaired visual function at night and in dim lighting, before onset of macular degeneration. Rod absolute-threshold sensitivity was elevated in four of five affected individuals tested, albeit after structural tissue damage had already occurred in the macula.<sup>6</sup> A 48-year-old (V:32) family member, who had macular drusen, but otherwise structurally normal macular features on clinical examination, self-reported impaired vision under scotopic conditions, which was confirmed by prolonged dark adaptation. After the patient had taken 15,000 IU daily of vitamin A palmitate for 6 months, the time course of rod dark adaptation improved in this subject, implying that there is a metabolic deficiency compromising the function of these photoreceptors. The functional improvement does not mean, however, that structural degeneration of macular tissue would benefit from a strategy of vitamin A supplementation. Reversal of night blindness by vitamin A supplementation in patients with Sorsby fundus dystrophy has also been reported.<sup>45</sup>

A slowed time course of dark adaptation and/or elevated rod dark-adapted absolute threshold sensitivity has also been found with Sorsby macular degeneration,<sup>55</sup> Malattia Leven-

**FIGURE 7.** Dark-adaptation profile of affected individual V:32 shows a prolonged time of 18 minutes to reach the cone-rod break time. (○) Control subject data (near upper normal range); (▲) patient before vitamin A supplementation; (△) patient after 6 months of vitamin A supplementation. The control time (18 control subjects) for the rod-cone break is  $5.22 \pm 0.79$  minutes (SD). This data allowed us to calculate two-tailed 90% tolerance (prediction) limits designed to include 90% of the population: lower limit: 3.53 minutes, upper limit: 6.91 minutes in identical testing conditions. After 15,000 IU daily of vitamin A palmitate supplementation for 6 months, the patient's cone-rod break time shortened to 14 minutes.



tines,<sup>56</sup> and AMD.<sup>56,57</sup> All these conditions manifest abnormal subretinal deposition of lipofuscin material and/or thickening of Bruch's membrane adjacent to the RPE.<sup>44,58,59</sup> Based on the cosegregation of both the L-ORD and LAZ phenotypes, it is likely that RPE expressing the mutant *CTRP5* secretes abnormal proteins that accumulate as subretinal deposits and lead to an age-related macular degeneration phenotype. In CE expressing mutant *CTRP5*, there appears to be an earlier phenotype manifested by LAZ that may lead to a form of pigment dispersion and elevated IOP and possibly glaucoma. Given that both the RPE and CE are derived from neuroectoderm, it is not surprising that L-ORD and LAZ phenotypes in UM:H389 cosegregate. Understanding the pathophysiology of disease manifested in both the anterior and posterior segments in patients with *CTRP5* mutations and other related extracellular matrix genes will help in unraveling the process of late-onset macular degeneration.

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