

ORIGINAL ARTICLE

Missense Variations in the Fibulin 5 Gene and Age-Related Macular Degeneration

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ABSTRACT

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N Engl J Med 2004;351:346-53.

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BACKGROUND

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the developed world. The study of a rare mendelian form of macular degeneration implicated fibulin genes in the pathogenesis of more common forms of this disease. We evaluated five fibulin genes in a large series of patients with AMD.

METHODS

We studied 402 patients with AMD and 429 control subjects from the same clinic population. Patients were examined by means of indirect ophthalmoscopy, slit-lamp microscopy, and fundus photography to establish the presence and phenotypic pattern of AMD. DNA samples were screened for sequence variations in five members of the fibulin gene family.

RESULTS

Amino acid–altering sequence variations were found in all five fibulin genes, many of which were observed only in patients with AMD. Several of the altered residues have been conserved during evolution. Seven of the 402 patients with AMD had amino acid–altering sequence variations in the fibulin 5 gene, whereas none were observed among 429 control subjects ($P < 0.01$). In addition, these seven patients all had small, circular drusen, which are commonly referred to as basal laminar or cuticular drusen.

CONCLUSIONS

Missense mutations in the fibulin 5 gene were found in 1.7 percent of patients with AMD. Many variations in other fibulin genes were also found in these patients, and the evolutionary conservation of the affected residues suggests that several of these variations may also be involved in AMD.

AGE-RELATED MACULAR DEGENERATION (AMD) is the most common cause of irreversible vision loss in the developed world.¹⁻³ In most patients, the disease is manifest as ophthalmoscopically visible yellowish accumulations of protein and lipid (known as drusen) that lie beneath the retinal pigment epithelium and within an elastin-containing structure known as Bruch's membrane. In the United States alone, more than 7 million people have drusen of sufficient size and number that they are at substantial risk for severe visual loss.⁴ AMD is likely to be a mechanistically heterogeneous group of disorders, and the specific disease mechanisms that underlie the vast majority of cases are currently unknown. However, a number of studies have suggested that both genetic and environmental factors are likely to play a role.⁵⁻⁷ On the basis of the study of other inherited retinal disorders, AMD is likely to display extensive genetic heterogeneity, involving functional sequence variations in numerous genes, sometimes singly and sometimes in combination. Because AMD is a late-onset disorder, many of these variations are likely to have subtle effects on the proteins they encode and will therefore have variable expressivity and incomplete penetrance.

In the past decade, many groups used positional cloning to try to identify genes that cause early-onset heritable macular diseases in the hope that identification of these genes would provide insight into the late-onset forms of disease. Several genes were identified with the use of this approach,⁸⁻¹⁴ but none have convincingly been demonstrated to be involved in a clinically significant fraction of late-onset macular degeneration.^{15,16} The mendelian macular disease that is arguably most similar to "typical" AMD is variably known as malattia leventinese, Doyme's honeycomb retinal dystrophy, and radial drusen¹⁷ and is caused by a single mutation (Arg345Trp) in the fibulin 3 gene (also known as *EFEMP1*).

Marmorstein et al.¹⁸ examined the eyes of an 86-year-old patient with malattia leventinese and discovered that fibulin 3 accumulates within and beneath the retinal pigment epithelium but not within the drusen themselves. These authors also showed that in patients with typical AMD, fibulin 3 accumulates between the retinal pigment epithelium and drusen, but not elsewhere. Furthermore, while investigating the potential pathophysiological mechanism of the single disease-causing missense mutation, they discovered that the mutant

fibulin 3 protein was not secreted from transfected RPE-J cells at the same rate as the wild-type protein and appeared to be misfolded.

Despite the clinical and histopathological similarities between malattia leventinese and typical AMD, variations in the coding sequence of fibulin 3 have not been found in patients with AMD.¹³ In this study, we tested the hypothesis that variations in other members of the fibulin gene family are involved in the pathogenesis of macular degeneration by examining the coding sequences of the genes for fibulin 1, 2, 4, 5, and 6 in more than 400 patients with AMD.

METHODS

A total of 402 unrelated patients with the clinical diagnosis of AMD were enrolled in the study after providing written informed consent. Among these patients, 367 were patients of the Retina Clinic of the University of Iowa, and the remaining 35 were patients of retina specialists elsewhere in the United States. All patients had been examined by fellowship-trained retina specialists and had received a diagnosis of AMD on the basis of the presence of one or more of the following features: drusen, disruption or atrophy of the retinal pigment epithelium, and choroidal neovascularization. Approximately 40 percent of the study patients had choroidal neovascularization.

Two groups of unrelated control subjects from the University of Iowa were studied. The first group consisted of 263 subjects (general-population controls) over the age of 50 years who had no history of macular degeneration. The eyes of these subjects were not examined as part of this study. The second group consisted of 166 subjects over the age of 50 years (average age, 75.5) who had no family

Table 1. Number of Different Sequence Variations.

Fibulin Gene	No. of Synonymous and Noncoding Variations	No. of Amino Acid-Altering Variations	Total Variations
Fibulin 1	21	8	29
Fibulin 2	26	13	39
Fibulin 4	6	2	8
Fibulin 5	7	7	14
Fibulin 6	11	13	24
Total	71	43	114

Table 2. Amino Acid Variants.

Gene and Variant	Nucleotide Change	Patients (N=402)	Controls (N=263)*	Degree of Conservation†
				no. of heterozygotes
Fibulin 1				
Gly96Ser	ggg→agt	0	1	0/3
Ala119Val	gcc→gtc	1	0	8/8, algae
Ile164Val	atc→gtc	0	1	3/5, sugar cane
Val428Leu	gtg→ttg	1	0	2/6, other mammals
Ala564Thr	gca→aca	1	1	6/6, bacteria
Arg577Trp	cgg→tgg	1	0	2/5, frog
His688Arg	cac→cgc	0	1	3/5, other mammals
His695Arg	cac→cgc	10	12	6/8, worm
Fibulin 2				
Glu66Lys	gag→aag	0	1	3/3, algae
Pro84Arg	ccc→cgc	0	5	4/4, wheat
His144Arg	cac→cgc	5	8	0/2
Thr210Pro	aca→cca	1	0	3/4, sea urchin
1 bp insertion (t) at codon 228	—	1	0	Frame shift‡
Ala311Thr	gcc→acc	6	0	1/4, algae
Val356Met	gtg→atg	2	0	0/2
Ser361Gly	agc→ggc	0.13§	0.11§	0/2
Asn387Thr	aac→acc	2	1	0/2
Pro409Leu	ccg→ctg	0	1	2/2, rodent
Glu556Gly	gaa→gga	1	0	1/3, horse
Arg566Leu	cga→cta	1	0	2/2, rodent
Pro1110Ala	cca→gca	1	0	1/4, bacteria
Fibulin 4				
Pro47Ser	cca→tca	3	0	4/7, fungus
Gly93Ser	ggc→agc	1	1	4/4, other mammals
Fibulin 5				
Val60Leu	gtt→ctt	1	0	6/6, fly
Arg71Gln	cgg→cag	1	0	4/4, chicken
Pro87Ser	ccc→tcc	1	0	1/3, cow
Ile169Thr	att→act	1	0	6/6, fish
Arg351Trp	cgg→tgg	1	0	6/6, chicken
Ala363Thr	gct→act	1	0	6/6, chicken
Gly412Glu	ggg→gag	1	0	6/6, algae
Fibulin 6				
Met2328Ile	atg→ata	1	0	2/2, rodent
Ile2419Thr	ata→aca	0.57¶¶	0.56¶¶	2/3, chicken
Ala2463Pro	gca→cca	2	0	8/8, barley
Glu2494Gln	gag→cag	1	0	4/4, fish
Ile4638Val	att→gtt	1	0	2/2, mouse
Gln4651His	cag→cac	0	1	2/2, mouse
Asp4744Glu	gat→gaa	1	0	1/3, cotton
Asp5088Val	gat→gtt	3	0	1/2, mouse
Arg5173His	cgc→cac	1	0	4/4, wheat
His5245Gln	cac→cag	1	0	4/5, fish
Ile5256Thr	att→act	1	0	4/4, frog
Gln5346Arg	caa→cga	2	1	10/10, fish**
Pro5506Ser	ccc→tcc	0	1	6/6, worm

* A total of 263 general-population controls were screened for coding-sequence variations in the genes for fibulin 1, 2, 4, and 6, whereas all 429 controls were screened for variations in the fibulin 5 gene.

† Values are the number of nonhuman species that share this residue with humans. The species with the greatest phylogenetic distance from humans with a sequence that is homologous to that of humans is also given.

‡ This insertion would be expected to cause the translation of 43 incorrect residues followed by a premature stop. Therefore, many highly conserved residues would be lost.

§ This common variant is reported as the allele frequency.

¶ This extremely common variant could not be reliably detected with the use of single-strand conformational polymorphism analysis, and the allele frequency was determined on the basis of sequencing data from 25 controls and 27 patients. In our screening population, the published wild-type allele (isoleucine) is the least frequent.

|| Review of fundus photographs of this patient revealed several drusen near the optic-nerve head.

**This entry combines our own homology data with those of Schultz et al.²²

history of macular degeneration and who had been examined by an ophthalmologist and found to be free of macular degeneration. The patients and controls from the University of Iowa were all enrolled during the same period by the same clinic. Over 80 percent of both groups described themselves as "caucasian." Genotype data from previous studies of large subgroups of these groups^{19,20} have shown that the patient and control groups are closely matched ethnically.

DNA was extracted from peripheral blood according to a previously described protocol.²¹ Samples from the 402 patients with AMD and the 263 general-population controls were screened for coding-sequence variations in the genes for fibulin 1, 2, 4, 5, and 6 with the use of single-strand conformational polymorphism analysis as previously described.²⁰ With the exception of a single exon each in the genes for fibulin 1 and fibulin 2 (which would not amplify reliably), the entire coding sequences of fibulin 1, 2, 4, and 5 (a total of 67 amplimers) were screened. Twenty-five of 107 exons of fibulin 6 were selected for screening on the basis of the location of known functional domains. An additional 166 controls without AMD were screened for variations in the entire coding sequence of fibulin 5. Samples from all 402 patients with AMD and all 429 controls were screened for the Gln5346Arg change in exon 104 of the fibulin 6 gene reported by Schultz et al.²² with the use of a denaturing high-performance liquid chromatography assay. The three samples found to harbor the Gln5346Arg change were confirmed by means of bidirectional automated DNA sequencing. Differences in the frequencies of coding-sequence variations between patients with AMD and controls were evaluated by means of Fisher's exact test. To evaluate the evolutionary conservation of residues with sequence variations, we used the nucleotide BLAST program and published expressed sequence tags. Each exon from the human fibulin 5 gene was used to identify homologous expressed sequence tags across multiple species. The expressed sequence tags used for subsequent analysis exhibited a minimum of 80 percent agreement with the human sequence.

For reverse-transcriptase-polymerase-chain-reaction (RT-PCR) analysis of the expression of fibulin 5, total RNA was extracted from the neurosensory retina and the retinal pigment epithelium of an eye from an adult donor with the use of Qiagen RNeasy minipreps. One microgram of DNase-treat-

ed RNA was reverse-transcribed in a random primed reaction with SuperScriptIII reverse transcriptase. Then 25-ng aliquots of this material were amplified by means of PCR.

RESULTS

We found 114 different variations in the sequences of the genes for fibulin 1, 2, 4, 5, and 6 (Table 1). Of these, 62 percent would not be expected to alter the structure of the encoded protein, whereas the remainder (38 percent) would alter one or more amino acids. Table 2 lists all the amino acid-altering variations we observed as well as their distribution in patients and controls. All but two of these changes were so rare that they were observed only in the heterozygous state. The two common changes — Ser361Gly in fibulin 2 and Ile2419Thr in fibulin 6 — were observed in the homozygous state in some subjects, but the frequencies of homozygosity were the same among patients and controls and were compatible with the presence of Hardy-Weinberg equilibrium. Table 3 shows the number of patients and controls who had one or more amino acid variants in a given fibulin gene. Only fibulin 5 showed a significant association between amino acid variations and AMD ($P < 0.01$ by Fisher's exact test). Fibulin 2 and fibulin 6 each had a very common amino acid change that was present in equal frequency among patients and controls (Table 2). After the removal of these changes from the analysis, the remaining variations in these genes were still not significantly associated with the AMD phenotype (Table 3).

Table 3. Amino Acid Variants in Patients and Controls.*

Gene	Patients	Controls	P Value
	<i>no./total no.</i>		
Fibulin 1	14/402	17/263	0.09
Fibulin 2	21/402†	17/263	0.50
Fibulin 4	4/402	1/263	0.65
Fibulin 5	7/402	0/429	0.006
Fibulin 6	14/402	3/263	0.08

* Two very common changes — Ser361Gly (in fibulin 2) and Ile2419Thr (in fibulin 6) — were not included in this analysis.

† One patient had two different changes in fibulin 2; thus, the number of patients with fibulin 2 changes is one less than the number of fibulin 2 mutations shown in Table 2.

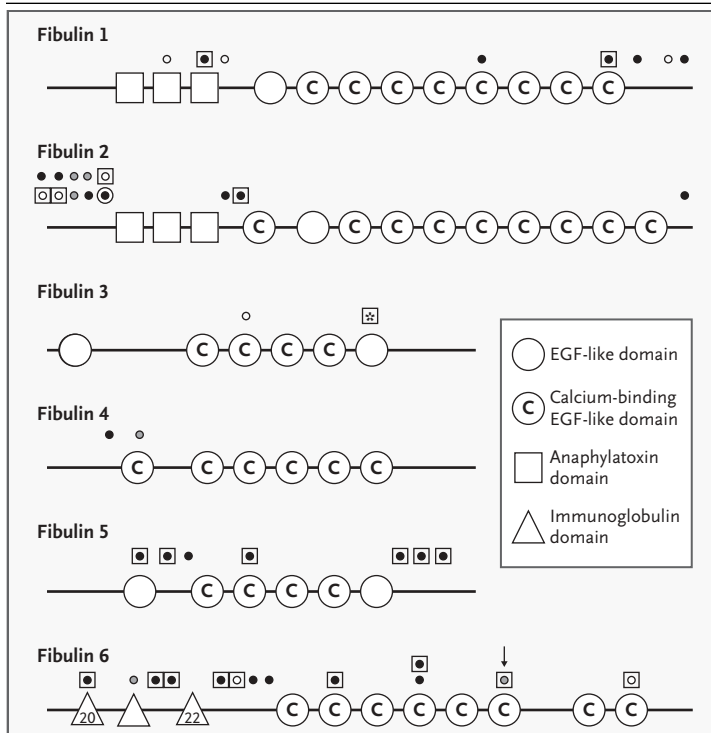


Figure 1. Locations of Amino Acid–Altering Sequence Variations in the Six Members of the Fibulin Gene Family.

The repeating domain structures of the six members of the fibulin gene family are shown: epithelial growth factor (EGF)–like domains, calcium-binding EGF-like domains, anaphylatoxin domains of fibulin 1 and fibulin 2, and immunoglobulin domains of fibulin 6. Numbers within the immunoglobulin domains indicate the number of repeats. Each of the amino acid–altering sequence variations listed in Table 2 is shown: solid circles represent variants found only in patients with AMD, hatched circles variants found in both controls and patients, open circles variants found only in controls, and boxed circles variants found in a codon that was completely conserved among all homologous expressed sequence tags (Table 2). All of the fibulin 5 changes were observed only in patients with AMD, and six of the seven changes in this sequence occurred in residues that were completely conserved. The frame-shift mutation in fibulin 2 is enclosed by a circle because it would eliminate a number of completely conserved residues. The fibulin 3 gene was not screened in the study and is included only for comparison.¹⁶ The disease-causing change in this sequence is shown as a boxed asterisk instead of a circle because it was only observed in patients with radial drusen — not typical late-onset macular degeneration.¹⁵ The Gln5346Arg change in fibulin 6 previously reported by Schultz et al.²² is marked with an arrow.

The Gln5346Arg change in fibulin 6 reported by Schultz and coworkers²² was observed in two patients with AMD and one control subject. However, this control had had photographs taken of his eyes in our glaucoma clinic in the past, and careful review of these photographs revealed several small, round drusen near the optic-nerve head that were similar in appearance to those seen in the patients with fibulin 5 changes.

Figure 1 shows the placement of the amino acid variations we observed with respect to the repeated domain structure of the fibulin gene family. The insertion of 1 bp in fibulin 2 would be expected to cause a premature truncation of the molecule before the anaphylatoxin and epidermal growth factor (EGF)–like domains. This particular variation was observed in seven of eight affected members of a large family with AMD (data not shown). Similarly, the Gln5346Arg change in fibulin 6 (previously reported by Schultz et al.²²) is found in the EGF-like domain that is nearest the carboxy terminal of a cluster of these domains — a position similar to the location of the Arg345Trp mutation in fibulin 3. Figure 1 also shows which of these variations were observed only in patients with AMD and not in controls, since these would be somewhat more likely to be true disease-causing variations than those appearing with equal frequency among patients and controls.

The seven patients with amino acid changes in fibulin 5 had all been examined and photographed in the retina clinic at the University of Iowa in the past 12 years. All seven described themselves as “caucasian.” Five had fluorescein angiograms as part of their medical record. Review of the retinal photographs revealed that these seven patients all had clusters of small, round, uniform drusen in association with variable degrees of detachment of retinal pigment epithelium. Figure 2A shows the color fundus photograph and fluorescein angiogram of the patient with the Arg71Gln change in fibulin 5. The most characteristic lesions are the numerous small, round, yellow lesions visible at the temporal edge of the macula. The larger, less distinct yellow areas nearer the center of the macula represent areas of pigment epithelial detachment. The fluorescein angiogram of this eye (Fig. 2B, 2C, and 2D) reveals these small, dot-like lesions to be brightly hyperfluorescent, whereas the areas of pigment epithelial detachment are much less visible. Three of the seven patients with fibulin 5 mutations (43 percent) had evidence of choroidal neovascularization when they were last examined. This rate was nearly identical to the rate of choroidal neovascularization in the group of patients with AMD as a whole (40 percent).

Although fibulin genes are known to be widely expressed and expressed sequence tags for fibulin 5 have been found in complementary-DNA libraries derived from eye and brain, we confirmed the expression of fibulin 5 in the retina and the retinal

Figure 2. Ophthalmoscopic and Angiographic Findings in a 64-Year-Old Woman with an Arg71Gln Variation in the Fibulin 5 Gene.

The retina of the patient's right eye has numerous small, round drusen surrounding several zones of pigment epithelial detachment, the largest of which is marked with an asterisk (Panel A). On fluorescein angiography, the small drusen fluoresce more brightly than the detached areas of retinal pigment epithelium (Panel B). Panels C and D are enlargements of the left-hand and right-hand boxed areas of Panel B, respectively. Representative drusen are marked with arrows.

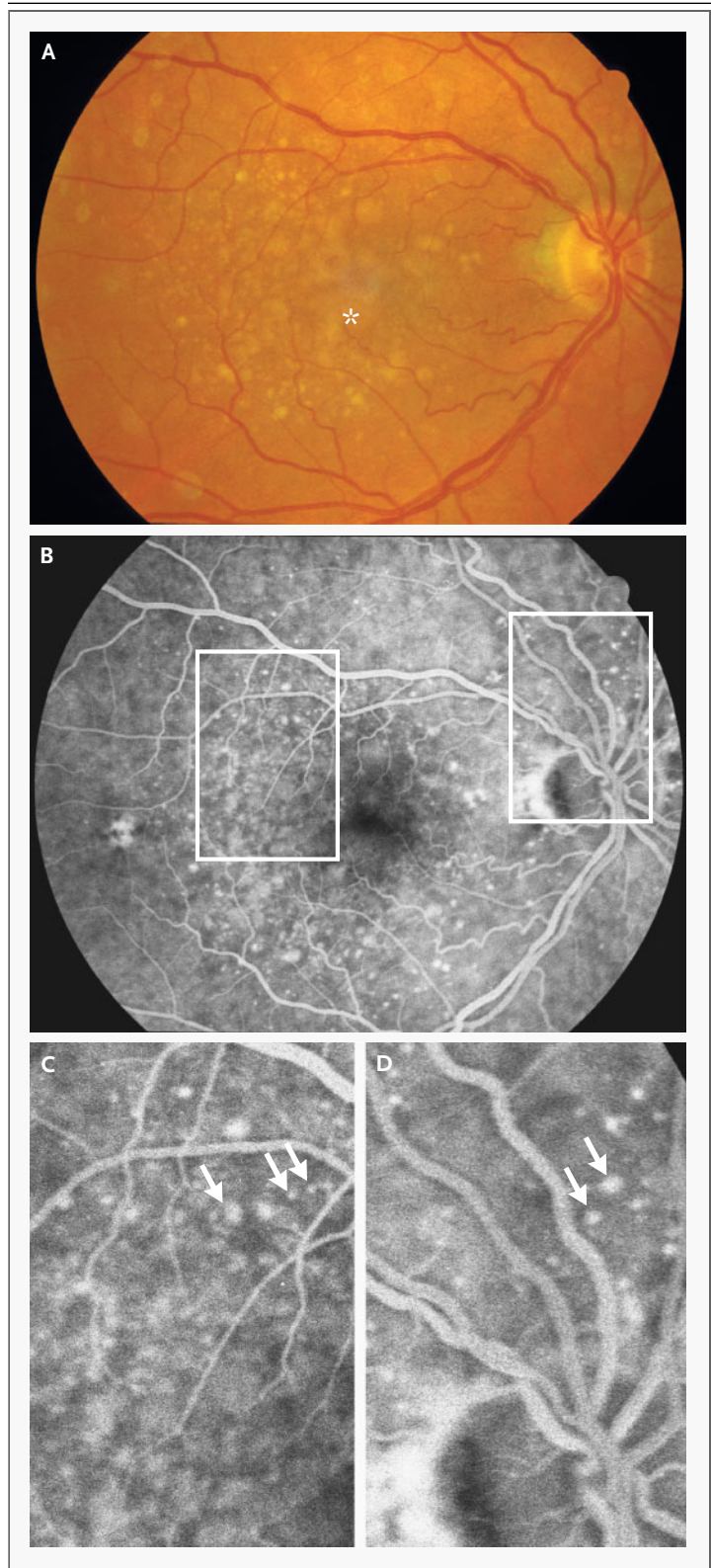
pigment epithelium by means of RT-PCR analysis (data not shown).

DISCUSSION

The fibulins are a relatively recently recognized family of extracellular proteins²³ that are widely expressed in the basement membranes of epithelia and blood vessels. Their most recognizable structural feature is the tandem array of four or more calcium-binding EGF-like domains (Fig. 1). Fibulin 3, 4, and 5 are the smallest members of the family and are nearly identical in their modular organization. In addition, fibulin 4 and fibulin 5 are over 40 percent identical to fibulin 3 at the amino acid level. All fibulins have binding sites for other basement-membrane proteins, such as fibrillin, fibronectin, proteoglycans, integrins, and tropoelastin. Fibulin 5 appears to be essential for the polymerization of elastin, because in mice and humans without functional copies of the fibulin 5 gene, tropoelastin is synthesized but is not assembled into its mature elastin fibrils.²⁴⁻²⁶

In 1999, an amino acid variation (Arg345Trp) in fibulin 3 was shown to be responsible for a specific form of drusen that are inherited in an autosomal dominant fashion.¹³ The drusen caused by this mutation are quite unusual in that they are distributed in streaks and lines that radiate from the center of the fovea. The molecular basis for this radial distribution is unknown, but it may be relevant that fibulin 6, or hemicentin, is capable of organizing cells into linear arrays during development.²⁷ In 2003, Schultz and coworkers²² found that an amino acid variation in fibulin 6 (Gln5346Arg) segregated in a large family with AMD.

Each of the five fibulin genes we examined had at least one amino acid variant in patients with AMD



that was not present in control subjects. In all, we observed 27 such changes, and many of these may cause AMD. Fifteen of these 27 changes affect residues that are completely conserved among all species for which expressed sequence tags could be identified with our search strategy. However, four of the fibulin genes also harbor one or more amino acid variations that were present in both patients and controls, and eight of these variants (present in 12 subjects) were present only in controls. Only the variations in fibulin 5 were significantly more numerous among patients than controls.

All seven of the amino acid–altering fibulin variants differed from one another. If we had observed only a single variation, there is a possibility that an unrecognized difference in ancestry might exist between the patients and controls or that the observed variation was in linkage disequilibrium with a true disease-causing variation nearby. However, given that seven different variations were observed, the most plausible explanation is that the variations themselves are actually involved in the disease. Six of these variations were completely conserved among the four to six species for which homology was detected. The expression of fibulin 5 in the retinal pigment epithelium (the tissue beneath which drusen accumulate) and the homology between fibulin 5 and fibulin 3 (a gene known to cause human macular disease) are further evidence that fibulin 5 has a role in the pathogenesis of AMD.

All seven of the patients with amino acid variations in this gene were found to have a phenotype that includes small, round, uniform drusen. First described by Gass in 1977,²⁸ such drusen (when very numerous) are referred to by clinicians as either basal laminar or cuticular drusen. Gass et al. later observed that patients with this phenotype are prone to large detachments of the retinal pigment epithelium.²⁹ This observation suggests that the molecular abnormality that gives rise to this type of drusen might also alter the attachment of the retinal pigment epithelium to Bruch's membrane. The photographs of five of the seven patients in this study with variations in fibulin 5 revealed regions of detachment of retinal pigment epithelium (Fig. 2). Cuticular drusen and detachments of retinal pigment epithelium are present in at least 20 percent of patients with AMD. Thus, missense changes in the fibulin 5 gene cannot be the sole cause of this phenotype.

The mechanism by which heterozygous mis-

sense mutations in the fibulin 5 gene could cause macular degeneration is not known. The fact that a drusen-causing missense variation in the closely related fibulin 3 gene is associated with misfolding and impaired secretion from retinal pigment epithelial cells¹⁸ raises the possibility that a similar mechanism may be operative for at least some of the variations we observed. As noted, fibulin 5 is essential for the polymerization of elastin in humans and mice.²⁴⁻²⁶ Elastin is a major component of the multilayered structure, known as Bruch's membrane, in which drusen form, and it is possible that reduced amounts of fibulin 5 protein in the extracellular space — or changes in specific residues that are important for the interaction with tropoelastin — alter the normal assembly of elastin within Bruch's membrane. It is also possible that interference with some other function of fibulin 5, such as integrin-mediated cell attachment,²⁵ will prove on further study to be the mechanism involved in fibulin 5–associated AMD. The fact that all of our patients with missense mutations in the fibulin 5 gene had some ophthalmoscopically visible detachment of the retinal pigment epithelium would support such a hypothesis.

In conclusion, we have demonstrated a significant association between sequence variations in a member of the fibulin gene family and typical AMD — the most common cause of irreversible vision loss in the developed world. In addition, we detected a number of other amino acid–altering variations in other fibulin genes in our patients with AMD, and the degree of evolutionary conservation of some of these residues suggests that they, too, may be involved in the pathogenesis of this disease. These findings should intensify interest in the components of Bruch's membrane as important participants in the pathophysiology of AMD and may facilitate the development of a murine model of AMD. Such a model would be useful in the search for drugs and other interventions for this common cause of blindness.

Supported by the Foundation Fighting Blindness, the Carver Endowment for Molecular Ophthalmology, the Grousebeck Family Foundation, the Macula Vision Research Foundation, and Research to Prevent Blindness. Drs. Stone and Sheffield are Investigators of the Howard Hughes Medical Institute.

We are indebted to J. Donald M. Gass, M.D., to whom this article is dedicated; to the patients and their families for their participation in the study; to Dr. Dennis Schultz for providing a DNA sample with the Gln5346Arg change for use as a positive control; to Heidi Haines, Gretel Beck, and Katherine Schrum for technical assistance; to Drs. Michael Grassi, Rod Philp, and Todd Scheetz for their help in preparing the manuscript; and to Linda Koser for administrative assistance.

REFERENCES

1. Tielsch JM, Javitt JC, Coleman A, Katz J, Sommer A. The prevalence of blindness and visual impairment among nursing home residents in Baltimore. *N Engl J Med* 1995;332:1205-9.
2. Klaver CC, Wolfs RC, Vingerling JR, Hofman A, de Jong PT. Age-specific prevalence and causes of blindness and visual impairment in an older population: the Rotterdam Study. *Arch Ophthalmol* 1998;116:653-8.
3. Attebo K, Mitchell P, Smith W. Visual acuity and the causes of visual loss in Australia: the Blue Mountains Eye Study. *Ophthalmology* 1996;103:357-64.
4. Friedman DS, O'Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol* 2004;122:564-72.
5. Heiba IM, Elston RC, Klein BEK, Klein R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. *Genet Epidemiol* 1994;11:51-67.
6. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol* 1997;123:199-206.
7. Klaver CC, Wolfs RC, Assink JJ, van Duijn CM, Hofman A, de Jong PT. Genetic risk of age-related maculopathy: population-based familial aggregation study. *Arch Ophthalmol* 1998;116:1646-51.
8. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 1997;15:236-46. [Erratum, *Nat Genet* 1997;17:122.]
9. Petrukhin K, Koisti MJ, Bakall B, et al. Identification of the gene responsible for Best macular dystrophy. *Nat Genet* 1998;19:241-7.
10. Weber BHF, Vogt G, Pruett RC, Stohr H, Felbor U. Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy. *Nat Genet* 1994;8:352-6.
11. Nichols BE, Sheffield VC, Vandenburgh K, Drack AV, Kimura AE, Stone EM. Butterfly-shaped pigment dystrophy of the fovea caused by a point mutation in codon 167 of the RDS gene. *Nat Genet* 1993;3:202-7.
12. Zhang K, Kniazeva M, Han M, et al. A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet* 2001;27:89-93.
13. Stone EM, Lotery AJ, Munier FL, et al. A single EFEMP1 mutation associated with both Malattia Leventinese and Doyme honeycomb retinal dystrophy. *Nat Genet* 1999;22:199-202.
14. Hayward C, Shu X, Cideciyan AV, et al. Mutation in a short-chain collagen gene, CTRP5, results in extracellular deposit formation in late-onset retinal degeneration: a genetic model for age-related macular degeneration. *Hum Mol Genet* 2003;12:2657-67.
15. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 1997;277:1805-7.
16. Stone EM, Webster AR, Vandenburgh K, et al. Allelic variation in ABCR associated with Stargardt disease but not age-related macular degeneration. *Nat Genet* 1998;20:328-9.
17. Heon E, Piguet B, Munier F, et al. Linkage of autosomal dominant radial drusen (Malattia Leventinese) to chromosome 2p16-21. *Arch Ophthalmol* 1996;114:193-8.
18. Marmorstein LY, Munier FL, Arsenijevic Y, et al. Aberrant accumulation of EFEMP1 underlies drusen formation in Malattia Leventinese and age-related macular degeneration. *Proc Natl Acad Sci U S A* 2002;99:13067-72.
19. Lotery AJ, Munier FL, Fishman GA, et al. Allelic variation in the VMD2 gene in Best disease and age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000;41:1291-6.
20. Webster AR, Heon E, Lotery AJ, et al. An analysis of allelic variation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2001;42:1179-89.
21. Buffone GJ, Darlington GJ. Isolation of DNA from biological specimens without extraction with phenol. *Clin Chem* 1985;31:164-5.
22. Schultz DW, Klein M, Humpert, AJ, et al. Analysis of the ARMD1 locus: evidence that a mutation in HEMICENTIN-1 is associated with age-related macular degeneration in a large family. *Hum Mol Genet* 2003;12:3315-23.
23. Timpl R, Sasaki T, Kostka G, Chu ML. Fibulins: a versatile family of extracellular matrix proteins. *Nat Rev Mol Cell Biol* 2003;4:479-89.
24. Nakamura T, Lozano PR, Ikeda Y, et al. Fibulin-5/DANCE is essential for elastogenesis in vivo. *Nature* 2002;415:171-5.
25. Yanagisawa H, Davis EC, Starcher BC, et al. Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* 2002;415:168-71.
26. Loeyts B, Van Maldergem L, Mortier G, et al. Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa. *Hum Mol Genet* 2002;11:2113-8.
27. Vogel BE, Hedgecock EM. Hemicentin, a conserved extracellular member of the immunoglobulin superfamily, organizes epithelial and other cell attachments into oriented line-shaped junctions. *Development* 2001;128:883-94.
28. Gass JDM. Stereoscopic atlas of macular diseases: diagnosis and treatment. 2nd ed. St. Louis: C.V. Mosby, 1977.
29. Gass JDM, Jallow S, Davis B. Adult vitelliform macular detachment occurring in patients with basal laminar drusen. *Am J Ophthalmol* 1985;99:445-59.

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