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Report

Null *RPGRIP1* Alleles in Patients with Leber Congenital Amaurosis

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We isolated and characterized the entire coding sequence of a human gene encoding a protein that interacts with RPGR, a protein that is absent or mutant in many cases of X-linked retinitis pigmentosa. The newly identified gene, called *"RPGRIP1"* for RPGR-interacting protein (MIM 605446), is located within 14q11, and it encodes a protein predicted to contain 1,259 amino acids. Previously published work showed that both proteins, RPGR and RPGRIP1, are present in the ciliary structure that connects the inner and outer segments of rod and cone photoreceptors. We surveyed 57 unrelated patients who had Leber congenital amaurosis for mutations in *RPGRIP1* and found recessive mutations involving both *RPGRIP1* alleles in 3 (6%) patients. The mutations all create premature termination codons and are likely to be null alleles. Patients with *RPGRIP1* mutations have a degeneration of both rod and cone photoreceptors, and, early in life, they experience a severe loss of central acuity, which leads to nystagmus.

One form of X-linked retinitis pigmentosa is caused by mutations in the *RPGR* gene, which normally codes for a protein with sequence homology to GTPase regulators (Meindl et al. 1996; Roepman et al. 1996; Vervoort et al. 2000). Two proteins that interact with the *RPGR* protein product have been identified. One is the δ subunit of rod cGMP-phosphodiesterase (Linari et al. 1999), and the other is called "RPGR-interacting protein" (RPGRIP1) (Boylan and Wright, 2000; Roepman et al. 2000; Hong et al. 2001). RPGR and one of its interacting proteins, δ-cGMP-phosphodiesterase, are ubiquitously expressed. Studies of mouse retinas have shown that RPGRIP1 is present only in the ciliary structures connecting the outer segments of rod and cone photoreceptors to their inner segments (Hong et al. 2001). This report describes our isolation and characterization of the human *RPGRIP1* gene and our studies of this gene as a potential cause of retinal degeneration in humans.

Starting with a murine *rpgrip* cDNA sequence (Hong et al. 2001), we scanned GenBank for a similar human genomic sequence, using BLAST software. The locus with the highest sequence homology was within a BAC fragment from human chromosome 14q11. This genomic sequence contained open reading frames that were similar to the murine *rpgrip* cDNA sequence and that were regarded, on the basis of an analysis with the exon-prediction program GENESCAN, as likely to be exons. During the course of this analysis, two other laboratories independently reported the identification of the human *RPGRIP1* cDNA sequence (Boylan and Wright 2000; Roepman et al. 2000). Our comparison with murine *rpgrip* and the GenBank genomic sequence showed that the cDNA sequence in these published reports lacked the 5′ end of the *RPGRIP1* gene (the first 10 exons). In addition, the RPGRIP1 protein predicted by those cDNA sequences was substantially smaller than that observed on western blots (Hong et al. 2001). Consequently, we determined the 5′ end of the human cDNA sequence, using reverse transcription-PCR and 5′ rapid amplification of cDNA ends (RACE). These studies indicated that the human cDNA sequence was longer than that previously reported. Its predicted protein product of 1,259 amino acids is similar in size to the 1,331-residue murine protein and has a similar primary structure (62% amino acid identity and 71% similarity). The human *RPGRIP1* gene has 25 exons (fig. 1).

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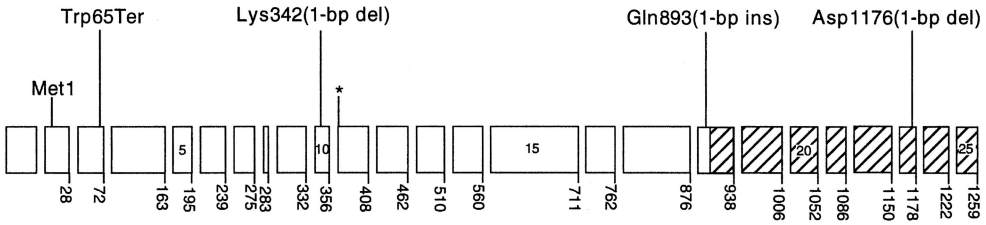

Figure 1 Schematic diagram of the human *RPGRIP1* gene, showing sizes of the exons and the locations of the mutations found in this study. Every fifth exon is numbered within its respective box, and the number of the last full codon at the end of each exon appears below each exon box. The lengths of the introns are not shown to scale. Hatched lines indicate the maximal region of the sequence encoding the domain that is known to interact with RPGR. The location of the previously reported 5′ end of the gene is shown with an asterisk. The new initiation methionine codon is indicated as Met1. The 3′ untranslated region is not shown.

To explore the possibility that *RPGRIP1* mutations are responsible for a form of retinal degeneration, we screened unrelated patients who had Leber congenital amaurosis (MIM 204000) or autosomal recessive retinitis pigmentosa (MIM 268000). The detection, early in this survey, of a null mutation in a patient who had Leber congenital amaurosis prompted us to focus our effort on a comprehensive analysis of the entire coding sequence in 57 unrelated patients with this diagnosis. Three of these patients had allelic mutations. One patient (048-044) carried the mutations Asp1176(1-bp del) and Trp65Ter heterozygously, the second patient (048-079) was homozygous for the frameshift mutation Gln893(1-bp ins), and the third patient (048-051) was homozygous for the frameshift mutation Lys342(1-bp del) (fig. 2*A*). Each of these three frameshift mutations produces premature stops within 13 codons downstream, and they are therefore likely to be null alleles, as is the nonsense mutation. None of these four mutations was found among a set of 95 unrelated control individuals without known retinal disease. Evaluation of the family members who were available and willing to participate showed that the unaffected relatives were either heterozygous carriers or homozygous wild type (fig. 2*B*). In addition, the analysis of the family (9414) of patient 048-044 showed that she was a compound heterozygote for the mutations Asp1176(1-bp del) and Trp65Ter.

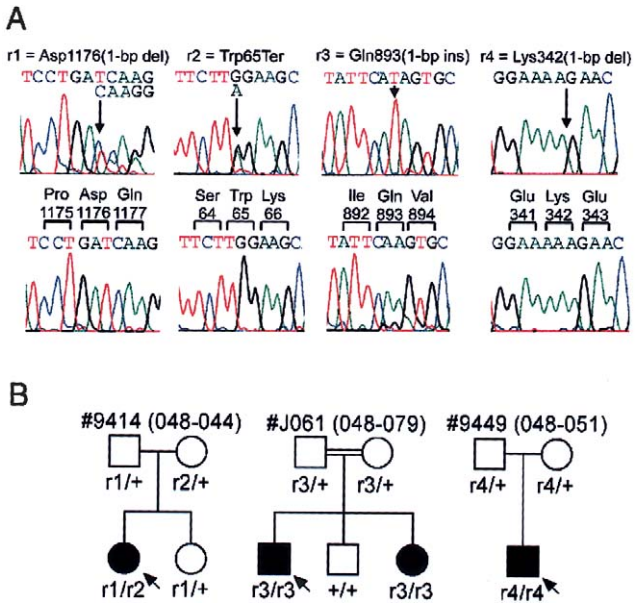

Figure 2 Sequence of *RPGRIP1* mutations and their segregation in families. *A*, The top row of sequences showing the heterozygous Asp1176(1-bp del) and Trp65Ter mutations in patient 048-044, the homozygous mutation Gln893(1-bp ins) in patient 048-079, and the homozygous mutation Lys342(1-bp del) in patient 048-051. Each mutant sequence is shown above the normal sequence of the same region in a control individual. *B*, Schematic pedigrees showing the segregation of the mutations in the families of the index patients. Alleles are labeled as follows: r1 = Asp1176(1-bp del), r2 = Trp65Ter, r3 = Gln893(1-bp ins), r4 = Lys342(1-bp del), and + = wild type. Filled symbols indicate affected individuals. Arrows point to index patient 048-044 in family 9414, to 048-079 in family J061, and to 048-051 in family 9449. The parents of patient 048-079 in family J061 are second cousins once removed.

We also found three missense variants that we interpreted as nonpathogenic, because they were so frequent and because the difference in their frequencies among 57 patients versus 88–93 controls was not statistically significant. They were Pro96Gln (CCG vs. CAG; minor allele frequency = 0.07 among patients vs. 0.08 among controls), Lys192Glu (AAA vs. GAA; 0.39 vs. 0.49), and Ala520Ser (GCT vs. TCT; 0.19 vs. 0.23). We were uncertain about the pathogenicity of three rare missense changes: Arg86Trp (CGG vs. TGG) and Asp849Gly (GAT vs. GGT) were each found in 1 heterozygous patient and in 0/88 control subjects, and Gln562His (CAG vs. CAT) was found heterozygously in 1 patient and in 1/92 control subjects. However, no variation was detected in the coding sequence of the other allele of each patient who carried one of these rare missense changes. Finally, five isocoding changes were also encountered. Allele frequencies in patients vs. controls were as follows:

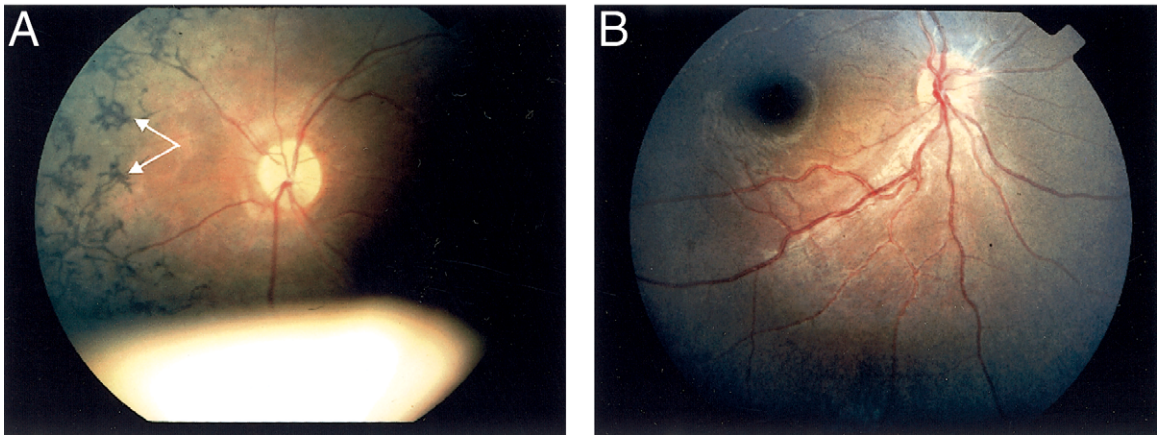

Figure 3 Fundus photographs of a 15-year-old index patient 048-079 and his affected sibling (family J061). *A*, The peripheral fundus of the left eye of patient 048-079. Numerous bone-spicule pigment deposits (*white arrows*) are present. The optic nerve head, which is normal in appearance, is visible at the center of the photograph. The retinal vessels are attenuated. The white area at the bottom is an artifact of photography. *B*, The right fundus of the index patient's 2-year-old affected sister. Slight vascular attenuation is visible, but no pigment deposits are present.

Pro175Pro (CCA vs. CCG; 0.22 vs. 0.22), Pro572Pro (CCG vs. CCA; 0.17 vs. 0.21), Glu1006Gln (GAG vs. CAG; minor allele frequency was determined only in controls: 0.33), Asp1155Asp (GAC vs. GAT; 0.04 vs. 0.01), and Ile1233Ile (ATA vs. ATT; 0.01 vs. 0.0).

The patients with frameshift or nonsense mutations in *RPGRIP1* had clinical findings characteristic of Leber congenital amaurosis. Patient 048-044 had nystagmus and vision limited to light perception since early childhood. Her fundi at age 26 years had moderate vascular attenuation and no intraretinal bone-spicule pigmentary deposits. Patient 048-079 had poor vision since early childhood; at age 15 years he had nystagmus and vision limited to light perception. At that age, he was hyperopic, with a spherical equivalent of +2.6 averaged between the two eyes. His fundi showed vascular attenuation and bone-spicule pigmentary deposits circumferentially in the midperipheral retina (fig. 3*A*). At age 15 years, this patient had a nondetectable full-field, rod-plus-cone electroretinogram (ERG) in response to single 0.5-Hz flashes of white light (amplitudes <1.0 μV; normal $\geq$350 μV). The full-field cone ERG responses to 30-Hz white flickering light, which were measured with computer averaging and narrow bandpass filtering (Berson et al. 1985; Andréasson et al. 1988), had greatly reduced amplitudes (0.34 μV, averaged between the two eyes; normal $\geq$50 μV) and delayed implicit times (56 msec; normal $\leq$32 msec). The index patient's 2-year-old affected sister also had nystagmus and was hyperopic, with a spherical equivalent of +6.9 averaged between the two eyes. She was able to follow objects with her eyes only in a well-illuminated environment. Her fundi were close to normal (fig. 3*B*). Patient 048-051 was not available for a comprehensive eye examination.

The cosegregation of the mutations with disease is of insufficient magnitude to be of statistical significance because of the rarity of the condition and the small size of the identified families. However, this cosegregation is perfect, and, in view of the *RPGRIP1* nullizygous genotypes of the affected patients and the absence of these mutations among normal control subjects, it is highly likely that these mutations are the primary cause of the patients' retinal disease. The early malfunction of both rods and cones suggests that the RPGRIP1 protein is essential to both photoreceptor types. Defects in either RPGRIP1 or its interacting protein RPGR probably alter a functional complex in the connecting cilia of rods and cones (Hong et al. 2001), and patients with defects of either protein experience a panretinal loss of photoreceptor function.

RPGRIP1 is the fifth gene identified as likely to cause Leber congenital amaurosis, after *GUCY2D-6* (which codes for guanylyl cyclase, an enzyme necessary for phototransduction in both rods and cones) (Perrault et al. 1996), *RPE65* (which codes for a protein in the retinal pigment epithelium involved in the metabolism of vitamin A) (Marlhens et al. 1997; Morimura et al. 1998), *CRX* (coding for a homeobox-type transcription factor) (Freund et al. 1998; Jacobson et al. 1998; Sohocki et al. 1998), and *AIPL1* (which codes for aryl hydrocarbon receptor–interacting proteinlike 1) (Sohocki et al. 2000). *GUCY2D-6, RPE65, CRX,* and *AIPL1* together account for ~25%–36% of the cases of this disease (Dharmaraj et al. 2000; Lotery et al. 2000; Sohocki et al. 2000, 2001). On the basis of our series of 57 patients,

we estimate that *RPGRIP1* accounts for an additional ~6% of cases.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Entrez Nucleotide Sequence Search, http://www.ncbi.nlm.nih.gov/Entrez/nucleotide.html (for the murine *rpgrip* cDNA sequence [accession number AY008297] and the human genomic RPGRIP1 sequence [accession number AL135744])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *RPGRIP1* [MIM 605446], Leber congenital amaurosis [MIM 204000], and retinitis pigmentosa [MIM 268000])

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