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Authors
Demirci, F Yesim K
Radak, Amy L
Rigatti, Brian W
et al.

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A Presumed Missense Mutation of *RPGR* Causes Abnormal RNA Splicing With Exon Skipping

F. Yesim K. Demirci, MD, Amy L. Radak, MS, Brian W. Rigatti, MS, Tammy S. Mah, MS, and Michael B. Gorin, MD, PhD

PURPOSE: A patient with retinitis pigmentosa demonstrated a novel *RPGR* mutation (213G>A, last base of exon 2) predicted to cause a missense change (G52R) in the final protein. This study was performed to determine whether this mutation altered the effectiveness of the adjacent splice site.

DESIGN: Observational case report.

METHODS: Total RNA was extracted from leukocytes of the proband and his carrier mother. Reverse transcription–polymerase chain reaction (RT-PCR) was performed by using the primers flanking exon 2 of RPGR transcript, followed by gel purification and direct sequencing.

RESULTS: Sequencing revealed skipping of exon 2 in the mutated transcript, leading to in-frame deletion of 42 amino acids affecting the critical RCC1-like domain.

CONCLUSIONS: The last base of exons is conserved as "G" in 80% of splicing consensus sequences, yet when changed, can completely disrupt constitutive splicing as in this patient. Our data confirm that the evaluation of the effects of some DNA sequence alterations at the RNA level might have important implications for appropriate genotype-phenotype correlations. (Am J Ophthalmol 2004;138: 504–505. © 2004 by Elsevier Inc. All rights reserved.)

MUTATIONS IN THE *RPGR* (RETINITIS PIGMENTOSA GTPase regulator, *RP3*, Xp21.1) are responsible for up to 70% of X-linked retinitis pigmentosa (RP).[1] *RPGR* exon ORF15 mutations also cause CORDX1 (formerly COD1) type X-linked cone-rod dystrophy (CRD) and atrophic macular degeneration.[2,3] During our *RPGR* mutation screening of male patients with X-linked or isolated forms of RP or CRD, we identified a novel mutation (213G>A) that was predicted to cause a nonconservative amino acid change (G52R) in RPGR protein in a patient with isolated RP.

Informed consent was obtained from the human subjects before participation in this study in accordance with a protocol that was approved by the University of Pittsburgh Internal Review Board and in accord with the regulations of the Health Insurance Portability and Accountability Act of 1996. The proband was first seen at age 2 years with early-onset myopia but without retinal abnormalities. At his second visit at age 7, his parents reported that he had poor night vision and was unable to maneuver in dim lights. He had a left esotropia-amblyopia and bilateral high myopia ($-11.00\ -2.00 \times 35$ diopters in right and $-7.50\ -2.00 \times 135$ diopters in left eye) with corrected visual acuities of 20/40 in the right eye (OD) and 20/200 in the left eye (OS). The fundi showed bilateral attenuated vessels and retinal thinning with some midperipheral

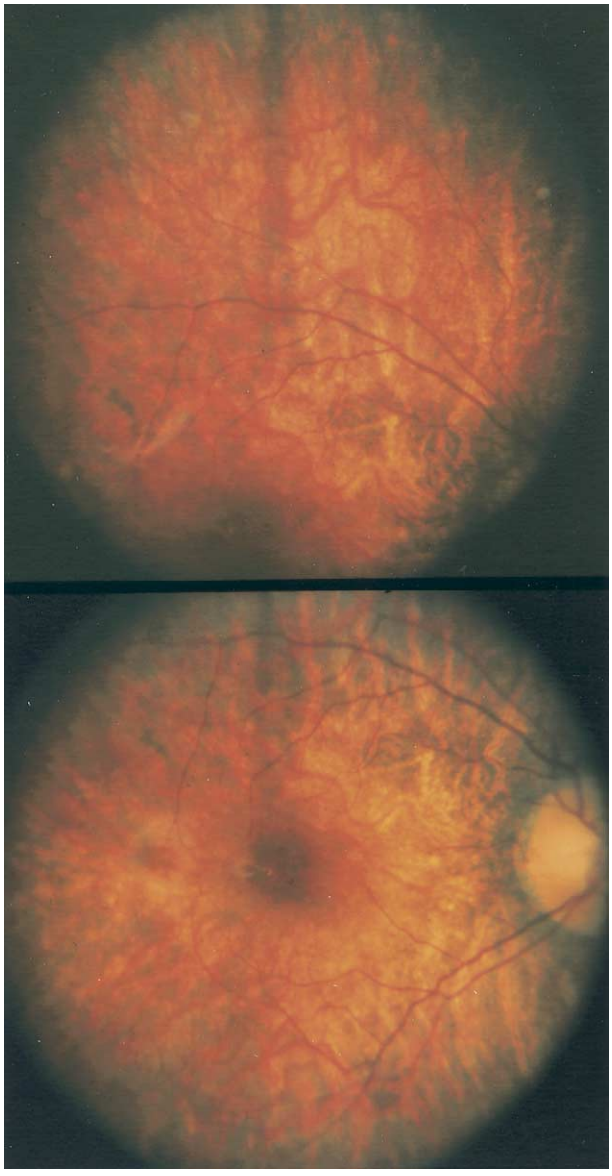

FIGURE 1. Fundus photographs of the right eye of the male retinitis pigmentosa patient. Ophthalmoscopy shows attenuated retinal vessels, retinal thinning, pigment epithelial changes, and atrophy in both the macula (bottom) and upper temporal retinal midperiphery (top).

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From the Department of Ophthalmology (F.Y.K.D., B.W.R., T.S.M., M.B.G.), School of Medicine, and Department of Human Genetics (A.L.R., M.B.G.), Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

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Inquiries to F. Yesim K. Demirci, MD, Department of Ophthalmology, University of Pittsburgh, 203 Lothrop Street, EEINS Building, Room 1027, Pittsburgh, PA 15213; fax: (412) 647-5880; e-mail: demirciyk@upmc.edu

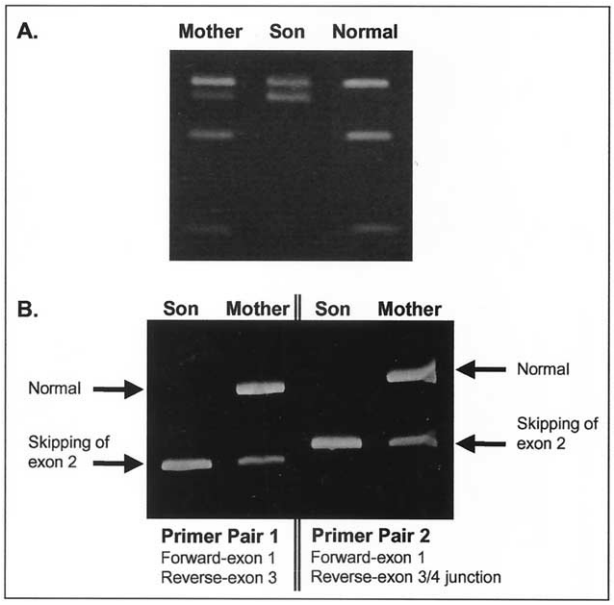

FIGURE 2. Restriction fragment length polymorphism (RFLP) and reverse transcription polymerase chain reaction (RT-PCR) analyses. (Top, 2A) Gel picture of the RFLP analysis of PCR-amplified DNA samples (using intronic primers) from the isolated retinitis pigmentosa patient with 213G>A mutation (which abolishes an *Msp*I restriction site), his mother, and a normal female. Restriction endonuclease (*Msp*I) digestion generates three fragments of 121 bp, 72 bp, and 35 bp in a normal person, but only two bands of 121 bp and 107 bp in the affected male patient with 213G>A mutation. Female carriers are expected to show four bands corresponding to both the normal and mutant alleles. (Bottom, 2B) Gel picture of the RT-PCR analysis of RNA samples (using exonic primers) from the isolated RP patient with 213G>A mutation (initially predicted to cause a missense change: G52R) and his carrier mother, by using two different primers pairs. This substitution did not act as a missense mutation but caused skipping of exon 2 at the RNA level (confirmed by sequencing), leading to an in-frame deletion of 42 amino acids affecting the initial part of the critical RCC1-like domain. The asymptomatic heterozygous carrier mother showed both normal and aberrant transcripts, although the aberrant transcript seemed relatively less abundant than the correctly spliced normal transcript. Control experiments were done to ensure that there was no contaminating genomic DNA and the primers were selected to be specific for the RNA transcripts (data not shown).

intraretinal pigment. The electroretinogram demonstrated symmetric extinguished scotopic and photopic responses. Two years later, he developed a spontaneous retinal detachment OS that was treated with scleral buckling. A 360-degree band of laser treatment was applied to the periphery of the right retina because of extensive lattice degeneration. At age 11, his corrected visual acuities were 20/50 OD and light perception OS; both retinas were flat and showed progressive thinning and retinal pigment epithelium changes (Figure 1). The left macula was significantly more atrophic than that of the right eye.

Because the pathogenic nucleotide substitution (213G>A, Figure 2A) was located in the last base of *RPGR* exon 2, we speculated that this mutation might alter splicing behavior. Total RNA samples from peripheral leukocytes of the proband and his mother were reverse transcribed and polymerase chain reaction (PCR)–amplified using primers flanking exon 2 of the RPGR transcript. Gel analysis showed that the proband had a single, smaller aberrant band, and his mother showed both normal and aberrant bands (Figure 2B). Gel-purified PCR products were directly sequenced as described.[2] Sequencing of aberrant transcript revealed skipping of exon 2 (126 nt) that would be predicted to generate an RPGR protein with an in-frame deletion of 42 amino acids, affecting the initial part of the critical RCC1-like domain.

Using a computerized MEDLINE search, we could find no reference to previous reports of a similar *RPGR* finding (an "exonic" mutation that was shown to cause missplicing with exon skipping) as demonstrated in our study. The last base of exons is conserved as "G" in 80% of 5′ splice site consensus sequences, and yet when changed, it can completely disrupt constitutive splicing, as shown in our case. Our data show the value of RNA analysis in determining the molecular pathology caused by some DNA sequence alterations. A different base change at the same nucleotide position (213G>T) has been reported and predicted to act as a nonsense mutation (G52X).[1,4,5] An alternative possibility for the major effect of this variant may be disruption of normal splicing, rather than premature termination of protein synthesis. Other presumed missense mutations may also be related to splicing if they disrupt exonic *cis*-splicing elements (splice sites, enhancers, silencers). Misclassification of mutations (as missense, nonsense, or frameshift) may occur if based solely on genomic analyses[6] and the extent of splicing-related mutations is commonly underestimated. The knowledge of exact effects of disease-causing mutations is crucial for genotype-phenotype correlations and for clarifying the molecular mechanisms of genetic diseases.

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