Novel USH2A Mutations in Israeli Patients With Retinitis Pigmentosa and Usher Syndrome Type 2

Nadia Kaiserman, MSc; Alexey Obolensky, MD, PhD; Eyal Banin, MD, PhD; Dror Sharon, PhD

Objective: To identify USH2A mutations in Israeli patients with autosomal-recessive Usher syndrome type 2 (USH2) and retinitis pigmentosa (RP).

Methods: Patients from 95 families with RP and 4 with USH2 were clinically evaluated. USH2A exons 2-72 were scanned for mutations using single-strand conformation and sequencing analyses. The frequency of novel missense changes was determined in patients and controls using restriction endonucleases.

Results: The analysis revealed 3 USH2A mutations, 2 of which are novel, in 2 families with USH2 and a large family (MOL0051) with both USH2 and RP. Compound heterozygotes for 2 null mutations (Thr80fs and Arg737stop) in MOL0051 suffered from USH2 while compound heterozygotes for 1 of the null mutations and a novel missense mutation (Gly4674Arg) had nonsyndromic RP.

Conclusions: Our results support the involvement of USH2A in nonsyndromic RP and we report here of a second, novel, missense mutation in this gene causing autosomal-recessive RP.

Clinical Relevance: Possible involvement of USH2A should be considered in the molecular genetic evaluation of patients with autosomal-recessive RP. Understanding the mechanism by which different USH2A mutations cause either USH2 or RP may assist in the development of novel therapeutic approaches.

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RETINITIS PIGMENTOSA (RP) is a group of progressive rod-cone degenerations characterized by night blindness followed by visual-field loss, resulting in severe visual impairment. Most patients have no associated systemic disease (nonsyndromic RP) while others suffer from associated extraocular diseases. The most common syndromic RP is Usher syndrome, with an estimated prevalence of 5:100,000 live births.1 Usher syndrome can be categorized into 3 clinical types: patients with Usher type 1 (USH1) typically have congenital deafness, vestibular ataxia, and night blindness noted in the first or second decade, whereas in USH2, night blindness appears in the second to fourth decade, accompanied by moderate early-onset hearing loss and no ataxia.2,3 Usher type 3 is found mostly among Finnish patients with onset of progressive hearing loss and RP in the late teens and variable vestibular dysfunction.4

At least 3 genetic USH2 loci were identified with USH2A being the most common, responsible for approximately 85% of USH2 cases.5,6 USH2A encodes for usherin, a basement membrane protein in the inner ear and retina,7 and is expressed in additional tissues as well. Recent studies suggest that usherin is integrated into a protein network formed by other USH-causing proteins.8,9 USH2A can produce a short isoform (encoded by exons 2-21) and long isoforms produced by alternative splicing of exons 2-72.10 More than 50 pathogenic mutations have been reported so far in exons 2-2111-19 and 5 mutations were reported in exons 22-72.10 Interestingly, 1 missense mutation, Cys759Phe, is responsible for approximately 7% of nonsyndromic autosomal-recessive RP (ARRP) cases, suggesting that under certain conditions USH2A can cause RP without hearing loss.14,18 We report here the identification of novel USH2A mutations causing USH2 and nonsyndromic RP in the Israeli population.

METHODS

Index patients from 95 Jewish and Muslim Israeli families with ARRP and 4 with USH2 were recruited for this study. All patients underwent clinical evaluation that included full ophthalmologic examination, assessment of refractive error, visual field testing, color vision testing, and full-field electroretinography.

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(ERG). All patients had typical RP changes to varying degrees and full-field ERG confirmed various levels of rod more than cone dysfunction. Patients with USH2 reported partial hearing loss, which was tested clinically and verified by audiometry when required. Patients with nonsyndromic ARRP were asked about hearing problems and additional testing was performed if hearing impairment was suspected. The tenets of the Declaration of Helsinki were followed and informed consent was obtained from all patients who participated in this study prior to donation of a blood sample.

The single-strand conformation polymorphism technique was used to screen genomic DNA for mutations in exons 2-21 and to perform haplotype analysis using single nucleotide polymorphisms. Mutation analysis of exons 22-72 was done by sequencing of polymerase chain reaction products (primer sequences are available on request). The frequencies of missense changes were tested in 94 ARRP index cases and 10 unrelated controls using restriction enzymes (MboI for C759F, Hpy188I for I1665T, TspRI for R2875Q, BstI for N3099S, BfaCI and Sau3AI for D3144N, NdeI for I33335M, FokI and HpaII for R4674G). In 2 sequence changes (I1665T and R4674G), where no restriction enzyme could distinguish between the wild-type and the mutant alleles, we designed a primer located adjacent to the sequence change and included a base change in the primer sequence to create a restriction site.

**RESULTS**

We studied the involvement of USH2A in 44 families with ARRP and 4 families with USH2. One of these families (MOL0051) was the focus of the study, presenting a complex inheritance pattern in which some family members suffered from USH2 while others had nonsyndromic RP (Figure 1A). MOL0051 is of Iraqi Jewish origin with no clear pattern of disease inheritance, but consanguinity in some branches of the family would suggest an autosomal-recessive inheritance. In the nuclear family, the mother (II-2) has 1 brother with USH2 and 3 siblings with RP. Three of her children manifest USH2 while 3 others were diagnosed with RP (Figure 1A). At the age of 66 years, she had advanced RP, including nonrecordable ERG responses and severely constricted visual fields (Figure 2A and B), while her audiometry findings were normal. Her 3 daughters with RP (aged 32-39 years) had somewhat less severe retinal disease, but all were legally blind, as shown in the representative clinical data of patient III-6 (Figure 2A and C). The youngest son (III-9) with USH2 still had residual cone responses on ERG, but in his 2 older siblings with USH2, ERGs were nonrecordable (Figure 2A). All 3 were found to have mild to moderate hearing loss in early childhood (a representative audiogram of patient III-4 at the age of 38 years is shown in Figure 2E). The visual field findings as well as fundus photograph and autofluorescence image for this patient are shown in Figure 2D, F, and G (note residual ring of hyperfluorescence in macular area surrounded by large patches of hypofluorescence corresponding to areas of atrophy).
**Figure 2.** Affected members of family MOL0051 manifest either retinitis pigmentosa (RP) or Usher syndrome type 2 (USH2). A, Visual acuity (VA), electroretinographic (ERG), electro-oculographic (EOG), and color vision testing findings. B and C, Marked constriction of Goldman visual fields in the 66-year-old mother (B) and her 34-year-old daughter (C) with RP. Black isopter indicates target V4e; green, III4e; blue, I4e. D-G: Visual field findings (D), audiometry results (E), fundus photograph (F), and autofluorescence image (G) of the 38-year-old son with USH2 (patient III-4). Pt indicates patient.
The segregation of both USH2 and RP in the same pedigree prompted us to study the possible involvement of USH2A by performing a haplotype analysis using 3 single nucleotide polymorphisms located within USH2A (Ala125Thr, Thr473Thr, and IVS9+33A>C; Figure 1B). The father (II-1) carried 2 identical haplotypes and was non-informative. His wife (II-2) carried 2 different haplotypes, which cosegregated with the phenotypes: haplotype 1-2-1 was inherited by the 3 children with USH2 while haplotype 2-1-2 was inherited by her daughter with RP (III-5). This cosegregation is also supported by a relative with RP type 2-1-2 was inherited by her daughter with RP (III-5). This cosegregation is also supported by a relative with RP

MUTATION ANALYSIS OF EXONS 2-21

We performed a mutation analysis of exons 2-21 in 48 index cases, including 44 with ARRP, 3 with USH2, and 2 members of family MOL0051. The analysis revealed 15 sequence changes, 2 of which are pathogenic (Table 1): a 4 base pair insertion (Thr80fs) and a novel nonsense mutation (Arg737stop). Each of these mutations was identified in 2 families: Thr80fs was found heterozygously in patients from families MOL0051 and MOL0035, and Arg737stop was found heterozygously in patients from MOL0051 and homozygously in MOL0165-1 (Figure 1B).

All patients with USH2 in MOL0051 were compound heterozygotes for either the nonsense or the frameshift mutation (Arg737stop). Each of these mutations was identified in 2 families: Thr80fs was found heterozygously in patients from families MOL0051 and MOL0035, and Arg737stop was found heterozygously in patients from MOL0051 and homozygously in MOL0165-1 (Figure 1B).

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Table 1. DNA Sequence Changes in USH2A Exons 2-21

<table>
<thead>
<tr>
<th>Source</th>
<th>DNA Change*</th>
<th>Exon</th>
<th>Protein Change</th>
<th>Mutation Type</th>
<th>Frequency†</th>
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<td>Dreyer et al</td>
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Abbreviations: ARRP, autosomal-recessive retinitis pigmentosa; FibII, fibronectin type 3; LamG, laminin G domain; ND, not done.

*Wild-type vs novel allele frequency in 188 ARRP chromosomes and 20 control chromosomes.

Table 2. Novel Sequence Changes in USH2A Exons 22-72

<table>
<thead>
<tr>
<th>Base Change</th>
<th>Exon</th>
<th>Protein Change</th>
<th>Mutation Type</th>
<th>Frequency†</th>
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Abbreviations: ARRP, autosomal-recessive retinitis pigmentosa; FibII, fibronectin type 3; LamG, laminin G domain; ND, not done.

*Wild-type vs novel allele frequency in 188 ARRP chromosomes and 20 control chromosomes.

* Nucleotide positions are based on accession number NM_206933.

† Allele frequency evaluated from a group of 88 autosomal-recessive pigmentosa alleles and 8 Usher syndrome type 2 alleles.
MOL0035 only 1 of the 2 mutations was identified and in MOL0051 2 of the 3 were identified. Sequencing analysis of exons 2-21 in members of these families did not reveal any additional sequence changes.

**MUTATION ANALYSIS OF EXONS 22-72**

To identify the remaining USH2A pathogenic mutations, we performed a sequencing analysis of exons 22-72 in families MOL0051 and MOL0035. We identified 14 novel sequence changes (Table 2), none of which is likely to create or destroy a splice site. Thirteen sequence changes were found in a heterozygous state while 6317T>C (I2106T) was homozygous in patients from both families. In addition, a human expressed sequence tag (AA883399) and all available orthologs have a cytosine at position 6317, indicating that I2106T is a common polymorphism in humans with threonine at position 2106 being the ancestral allele.

To determine which of the 7 remaining missense changes is pathogenic, we studied different aspects of these changes. First, since 6 of the missense changes were found in MOL0051, we correlated them to the haplotypes by sequencing the corresponding exons in 2 patients with RP. Both were heterozygote for all 6 changes, indicating that they are located on the RP-associated allele. Second, we examined the location of the 7 changes along the long usherin isoform and identified 3 changes (I1665T, R2875Q, and R4674G) located within functional domains (Table 2). Third, we aligned USH2A sequences from different species for each of the studied substitutions, 6 of which were evolutionary conserved (I1665T, R2875Q, and R4674G) located within functional domains (Table 2). Finally, we studied the allele frequency of the candidate sequence changes in patients with ARRP and controls. The analysis revealed that all missense changes except R4674G were prevalent in both groups (Table 2), excluding these changes as disease-causing mutations. The R4674G mutation was not found in either group and is therefore specific to the RP-associated allele in MOL0051.

**COMMENT**

We describe here 3 USH2A mutations in patients with USH2 and ARRP. Two of the mutations are null and can be found...
in patients with either phenotype while a novel missense mutation, R4674G, is specific to the RP phenotype and is likely to be pathogenic. This is the second USH2A mutation thus far reported to cause ARRP and the first to be identified on the long isoform. This indicates that the mutation interferes with the function of usherin in a retina-specific manner, perhaps affecting its interactions with retina-specific proteins while sparing the auditory system.

The first RP-causing mutation reported in USH2A, Cys759Phe, was found in approximately 7% of ARRP cases. Another study, however, reported that 2 individuals homozygotes to Cys759Phe have no retinal or hearing problems, raising the possibility that Cys759Phe is in linkage disequilibrium with another, currently unknown, mutation. Unlike previous reports, Cys759Phe could not be identified in our cohort of 95 patients with ARRP (P<.05), suggesting a low frequency in the Israeli population. Two of the mutations identified in the current study are novel, and the third was reported previously only in 1 Iranian Jewish family. All 3 mutations are therefore likely to be unique to Jews originating from the same region (Iran-Iraq-Bukhara-Afghanistan). This is in agreement with other mutations causing Usher syndrome, which were found to be relatively common and restricted to specific Jewish subpopulations.

We identified 14 novel sequence changes in exons 22-72 in patients with ARRP. Six of them were missense changes located on a single RP-associated allele and therefore could potentially be responsible for the disease. We were able to exclude 5 of these changes as pathogenic: I1206T was not conserved along evolution; I1665T, I3335M, N3099S, and D3144N were polymorphisms (common in controls as well as patients). In contrast, the R4674G mutation was found only on the RP-associated allele in MOL0051 and was negative in 208 chromosomes of patients with ARRP and controls, indicating that it is a rare variant. In addition, the arginine residue in position 4674 is perfectly conserved in a wide range of organisms (including human, chicken, and zebra fish). The combination of a highly conserved residue, a conversion of a positively charged amino acid (arginine) to a polar amino acid (glycine), and the specific association with the RP-causing allele strongly indicate that this mutation is pathogenic. The pathogenicity of this missense change will have to be verified once a functional test for usherin is available.

In conclusion, our findings support the need to consider possible involvement of the USH2A gene not only in patients with Usher syndrome but also in patients with nonsyndromic ARRP. In addition, understanding the mechanism that causes the disease to be limited only to the retina may afford new insights into the pathogenesis and can assist in the development of novel therapeutic approaches for these blinding diseases.

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REFERENCES

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Correction

Error in Acknowledgment. In the Ophthalmic Molecular Genetics article titled “Novel USH2A Mutations in Israeli Patients With Retinitis Pigmentosa and Usher Syndrome Type 2,” published in the February 2007 issue of the Archives (2007;125[2]:219-224), the Funding/Support section should have appeared as follows: “The study was supported by the Chief Scientist at the Israeli Ministry of Health (grant No. 3807) and by the Yedidut Research Grant.”