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Report

Missense Mutation in the *USH2A* Gene: Association with Recessive Retinitis Pigmentosa without Hearing Loss

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Microdeletions Glu767(1-bp del), Thr967(1-bp del), and Leu1446(2-bp del) in the human *USH2A* gene have been reported to cause Usher syndrome type II, a disorder characterized by retinitis pigmentosa (RP) and mild-to-severe hearing loss. Each of these three frameshift mutations is predicted to lead to an unstable mRNA transcript that, if translated, would result in a truncated protein lacking the carboxy terminus. Here, we report Cys759Phe, a novel missense mutation in this gene that changes an amino-acid residue within the fifth laminin–epidermal growth factor–like domain of the *USH2A* gene and that is associated with recessive RP without hearing loss. This single mutation was found in 4.5% of 224 patients with recessive RP, suggesting that *USH2A* could cause more cases of nonsyndromic recessive RP than does any other gene identified to date.

The Usher syndromes (MIM 276901) are a group of recessively inherited diseases affecting ~12,000 people in the United States alone (Boughman et al. 1983). Patients typically have progressive visual loss due to retinitis pigmentosa (RP), as well as different levels of deafness or vestibular function (Smith et al. 1994). On the basis of the severity of the sensorineural dysfunction, Usher syndrome has been subcategorized into three distinct types and has been linked to ⩾10 different loci (types IA–IF, types IIA–IIC, and type III [Hereditary Hearing Loss Home Page]). To date, only two Usher genes, *MYO7A* and *USH2A*, have been cloned, and they have been identified as responsible for Usher syndrome types IB and IIA, respectively (Weil et al. 1995; Eudy et al. 1998). In addition, mutations in *MYO7A* have been found to cause dominant or recessive nonsyndromic deafness (Liu et al. 1997*a*, 1997*b*; Weil et al. 1997). A 1.6-cM region of 11p, containing the locus responsible for type IC (*USH1C*), appeared to segregate with recessive deafness in a large consanguineous Indian family (Jain et al. 1998).

To investigate whether *USH2A* could be involved in nonsyndromic RP, we analyzed a 154-bp genomic fragment encompassing codon 759 by SSCP and direct genomic sequencing (for methods, see Hagstrom et al. 1998) in DNA samples from patients, using primers (sense, ATGTTGGATGTGAGCCCTGC; antisense, CAATTGGTGACATCTAACCC) based on the partial *USH2A* sequence that has been published (Eudy et al. 1998). We evaluated 224 unrelated patients, mostly from North America, who previously had been categorized as having recessive nonsyndromic RP. Ten of the 224 patients carried a novel missense mutation, Cys759Phe (TGC→TTC). In all 10 cases, this missense mutation was associated with an isocoding change at codon His752 (CAT→CAC; fig. 1*A*). Prior evaluations of other recessive RP genes (*PDEA, PDEB, CNGA1, TULP1, RPE65, SAG, CRALBP,* and/or *RGR*) in 7 of these 10 patients did not uncover any pathogenic mutations. Two of these 10 cases had the Cys759Phe mutation homozygously. Next we analyzed the only other regions of the *USH2A* gene with available PCR-primer sequence information (bases 2854–2962 and bases 4251–4364 [Eudy et al. 1998]). We found that two of the Cys759Phe carriers were compound heterozygotes with both that allele and one of the previously reported frameshift mutations, Glu767(1-bp del) or Leu1446 (2-bp del). The association of the Cys759Phe allele with recessive RP was investigated further by recruitment of relatives from 6 of the 10 index patients who carried this allele. In every family, including the families of the

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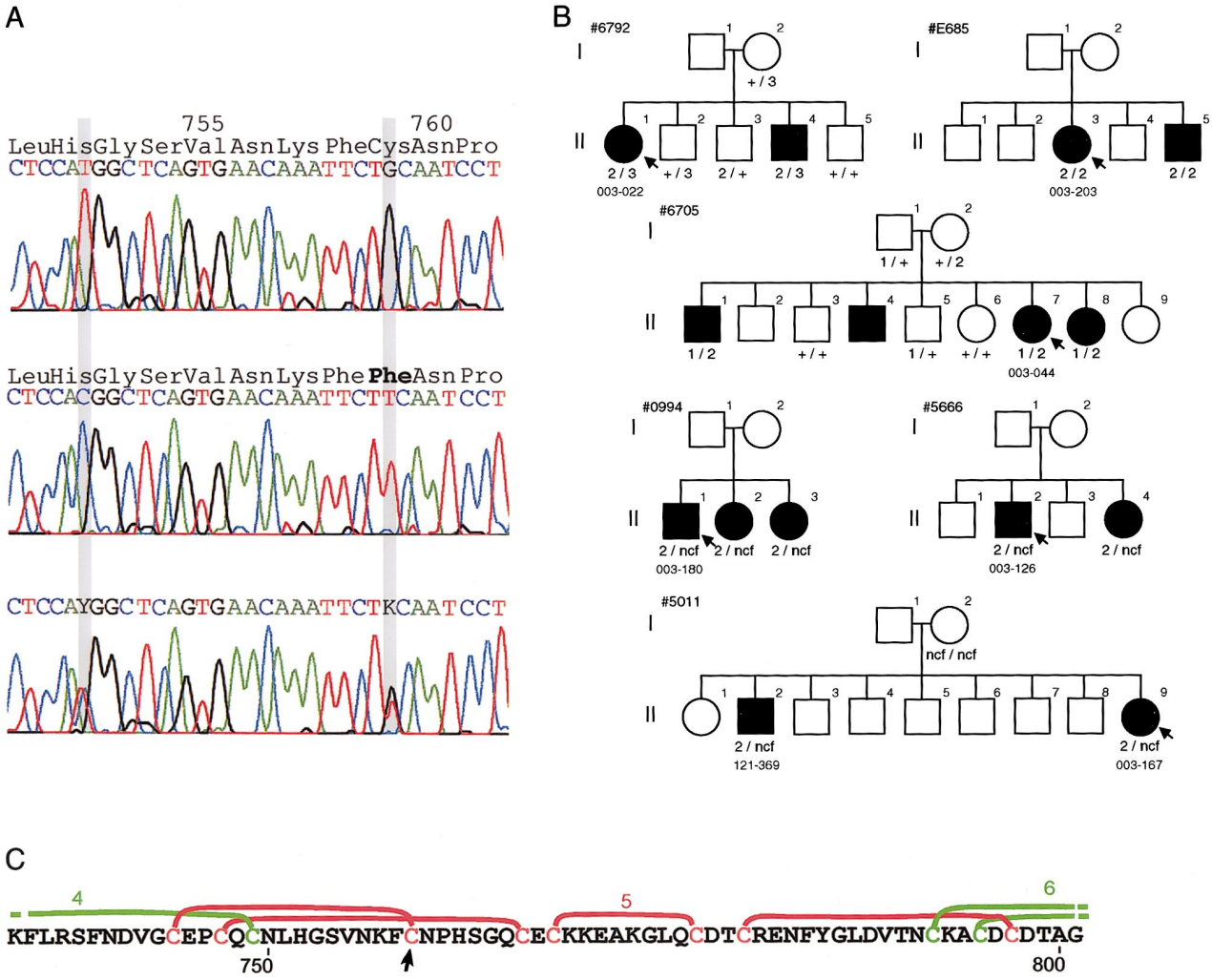

Figure 1 *A,* Nucleotide sequence of the region encompassing codons 751–761 in a normal control (*top*); in patient 003-203, who is homozygous for both the missense change Cys759Phe and an isocoding change in codon 752 (*middle*); and in patient 003-126, who is heterozygous for both changes (*bottom*). *B,* Schematic pedigrees of six families (6792, E685, 6705, 0994, 5666, and 5011) with recessive RP without hearing loss segregating the Cys759Phe allele. Arrows point to index cases. Identification numbers of the patients whose hearing was tested with audiograms are below their genotypes. 1 = 2299delG (Glu767[1-bp del]); 2 = Cys759Phe; 3 = 4337-8delTC (Leu1446[2-bp del]); ncf = no change found within the investigated regions. *C,* Primary structure of part of the USH2A protein. Lines are drawn between disulfide bridges connecting cysteine residues of the fifth LE motif (*red lines*) and the fourth and sixth LE motifs (*green lines*). An arrow points to residue 759, which participates in one of the disulfide bridges.

two index cases who were compound heterozygotes for the Cys759Phe mutation and one of the frameshift mutations in *USH2A* mentioned above (fig 1*B*), the identified *USH2A* mutations segregated with RP, as would be expected if they were pathogenic (summed $LOD_{max} = 4.4$ at recombination fraction $\theta_{max} = 0$). To determine whether Cys759Phe was specific for nonsyndromic RP and not also associated with RP with deafness, we extended the survey to include 51 unrelated patients with Usher type II and 190 normal control individuals. No patient with Usher type II carried the Cys759Phe allele; one control carried it heterozygously.

The patients with recessive RP had previously been asked to complete a questionnaire that included a question about the status of their hearing. Of the 224 patients, 194 answered this question, and 37 of those reported mild hearing impairment that was initially interpreted as not clinically significant. Nine of the 10 patients with the Cys759Phe mutation answered the question, and none reported hearing impairment (the compound heterozygote with both the Glu767[1-bp del] and the Cys759Phe allele was among the 9 patients who reported no hearing impairment; the other compound heterozygote did not answer the question). There was

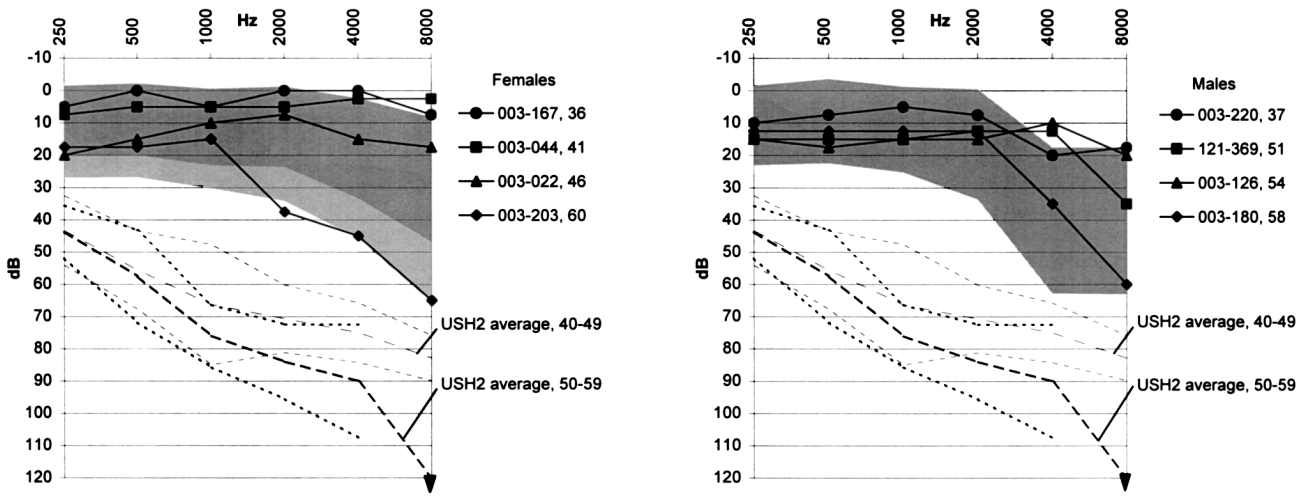

Figure 2 Pure-tone air-conduction audiograms of four female (*left*) and four male (*right*) patients carrying the Cys759Phe mutation. The data are the averages for the right and left ears. The patients' identification numbers and ages are to the right of each graph. The patients are unrelated, except for 121-369 and 003-167, who are siblings. Patient 003-203 is a homozygote; the other cases are heterozygotes. The dark- and light-gray areas are the hearing ranges (average values ± 1 SD) for normal individuals at age 48–59 and 60–69 years, respectively, reported by Cruickshanks et al. (1998). Broken and dotted lines indicate the average ± 1 SD, respectively, of the audiograms for patients with Usher type II (males + females) at age 40–49 (*thinner lines*) and 50–59 (*thicker lines*) years, on the basis of data published by Wagenaar et al. (1999). The black arrowheads at the bottom right corners indicate values that are off the scale.

no statistically significant association of the Cys759Phe allele with reported hearing loss (0/37 vs. 9/157; χ^2 = 1.12, P = .29; P = .21 by Fisher's exact test). To confirm their reported normal hearing status, 7 of the 10 index patients with the Cys759Phe allele and 1 affected sibling underwent pure-tone audiometry. Every patient had hearing acuity within the normal range for age, and, specifically, no patient had hearing impairment in the published range found in patients with Usher type II who were of comparable age (fig. 2). Included among those patients documented to have normal hearing were the two compound heterozygotes with Cys759Phe and previously reported frameshift mutations, which indicates that the frameshifts do not cause Usher type II, but only nonsyndromic RP, if they are present together with the newly identified missense mutation.

During the course of this study, we found that 14 of the 51 patients with Usher type II heterozygously carried the previously reported *USH2A* frameshift mutation Glu767(1-bp del) (Eudy et al. 1998; Liu et al. 1999). Nine of 224 patients with nonsyndromic RP also carried this mutation heterozygously, and no control did. The frequency of this allele in these groups was .14, .02, and .00, respectively. Of the nine patients with nonsyndromic RP who carried the Glu767(1-bp del) allele, eight answered the question regarding subjective hearing loss. Six of these eight patients reported hearing impairment; one of the carriers who did not was the compound heterozygote with Cys759Phe who has been mentioned above. Among patients with presupposed nonsyndromic RP, the association of Glu767(1-bp del) with reported hearing loss was statistically significant (6/37 vs. 2/157; χ^2 = 13.34, P = .0002; P = .0007 by Fisher's exact test). Our data suggest that many patients with presumed nonsyndromic RP who report mild, subjective hearing impairment actually have Usher syndrome type II.

The *USH2A* gene product is predicted to be a 171.5-kD extracellular protein with 10 laminin–epidermal growth factor (LE) and 4 fibronectin type III motifs. The residue affected by the Cys759Phe missense mutation participates in a presumed disulfide bridge in the fifth LE motif in the USH2A protein sequence (fig. 1*C*; Eudy et al. 1998). On the basis of the Cys759Phe allele's inferred alteration of the protein, its association with recessive RP, and its cosegregation with this disease, we conclude that it is pathogenic. Other known genes causing nonsyndromic, recessive RP—such as rhodopsin, the α subunit of rod cGMP-phosphodiesterase, the β subunit of rod cGMP-phosphodiesterase, the rod cGMP-gated cation channel, TULP1, CRALBP, RPE65, and arrestin—each account for $\leqslant$4% of recessive nonsyndromic RP. The *USH2A* locus might account for an even greater percentage of recessive RP, since the Cys759Phe allele by itself accounts for ~4.5% of cases.

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Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Usher syndrome type IIA [MIM 276901])

Hereditary Hearing Loss Home Page (G. Van Camp, R.J.H. Smith), http://dnalab-www.uia.ac.be/dnalab/hhh/

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