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Mutations in the Myosin VIIA Gene Cause a Wide Phenotypic Spectrum, Including Atypical Usher Syndrome

To the Editor:

Usher syndrome (USH) is the most common recessive syndromic hearing loss characterized by congenital hearing impairment associated with retinitis pigmentosa (RP). It is a clinically and genetically heterogeneous condition. Three clinical forms of USH have been described, and seven loci have been mapped (Hereditary Hearing Loss home page). The three types are distinguished by the severity and progression of the hearing impairment and by the presence of vestibular disaffection. USH1 is the most severe form and is manifested by profound congenital deafness, constant vestibular dysfunction, and RP of prepubertal onset. USH2 includes congenital moderate to severe hearing loss, normal vestibular responses, and RP. A third group of patients have been classified, in whom hearing loss is progressive and there are variable vestibular problems as well as RP. Patients in this class have been described as USH3 (Sankila et al. 1995), although, for some families, in the absence of any clear genetic distinction from other USH classifications, such individuals generally may be best classified as having an atypical USH phenotype. Five USH1 loci, USH 1A–1E, have been mapped to chromosome bands 14q32, 11q13.5, 11p15, 10q, and 21q21, respectively (Hereditary Hearing Loss home page). A locus for USH3 has been localized to chromosome 3q21-25 in Finnish families with an USH3 phenotype (Sankila et al. 1995). USH1B is encoded by the *MYO7A* gene (Weil et al. 1995), and recently a variety of mutations leading to USH1B have been reported and catalogued in the *MYO7A* gene (Weston et al. 1996; Liu et al. 1997*a*; Levy et al. 1997; Adato et al. 1997; Hasson 1997). We now report that mutations in the *MYO7A* gene can lead to atypical USH.

Seven families (six from the United Kingdom and one from China) were identified as having atypical USH phenotypes similar to that of USH3. They were examined for *MYO7A* mutations, by a combined SSCP/heteroduplex analysis method (Liu et al. 1997*a*). In family USH3.04, originating from England, the parents were unaffected and had two affected children (fig. 1*a*). Patient II.1 (39 years old) had a bilateral progressive hearing loss with onset at age 2 years. She remembered hearing well until age ~14 years (hearing thresholds in all frequencies were at 55–60 dB hearing level [HL]). Since then, she has required the use of hearing aids and her hearing thresholds have fallen to 90 dB HL, and eventually audiograms showed nonrecordable thresholds when she was age 29 years. She has normal speech and normal caloric responses. RP was diagnosed at age 38 years when she saw an ophthalmologist, since her brother had then been diagnosed with USH. Ophthalmoscopy showed typical but mild pigmentary degeneration, with bone spicules and arteriolar attenuation. The electroretinogram was mildly reduced, and visual fields were moderately constricted. Patient II.2 was found to have hearing loss at age 18 mo, with progression from moderate to severe loss. His speech is quite good. RP was diagnosed at age of 15 years. Further details of clinical evaluation of this family have been reported by Hope et al. (1997). Thus, the condition in this family with atypical USH is most closely related to USH3.

This family was tested for cosegregation of the disease with previously mapped USH3 and USH1B loci, by means of polymorphic markers specific for each locus (Hereditary Hearing Loss home page). According to the segregation analysis of the marker haplotypes (data not shown), the segregation of the disease in this family was not consistent with the involvement of the previously identified USH3 locus on chromosome 3. However, haplotype analysis did indicate that results for this family were consistent with linkage to the USH1B locus—the two affected sibs shared identical genotypes in the USH1B region, with differing paternal and maternal haplotypes inherited. Moreover, SSCP analysis of exons 17 and 35 in MYO7A (Levy et al. 1997) in this family demonstrated patterns different than those in 60 normal controls (fig. 1*b*). Sequence analysis of the SSCP variants revealed that both affected sibs were compound heterozygotes, with a Leu651Pro substitution in exon 17 in one allele, inherited from their mother, and an Arg1602Gln mutation in exon 35 in the other allele, inherited from their father (fig. 1*c*). Both mutations are believed to be pathological, first because of their location and conservation (see below) and, second, because neither change has been observed in a series of normal controls.

The mutation Leu651Pro in exon 17 lies within a crucial region in the lower 50-kD subdomain of the myosin head. This region contains the largest number of conserved residues in the motor domain and is believed to maintain structural integrity of the head domain (Cope et al. 1996). Alignment of known myosin motor domains shows that the altered leucine residue is conserved among a large number of myosin sequences (Cope et al. 1996). Interestingly, the residue is 1 of 10 amino acids deleted (del645–655) in the mouse shaker1 allele, *sh1*^{816SB} (Mburu et al. 1997) (fig. 2). This deletion removes part of the α-helix in a highly mobile region that is thought to be involved in the transduction of energy from the site of ATP hydrolysis to the regulatory domain. The identification of two pathological mutations in the same region in separate species suggests a vital function.

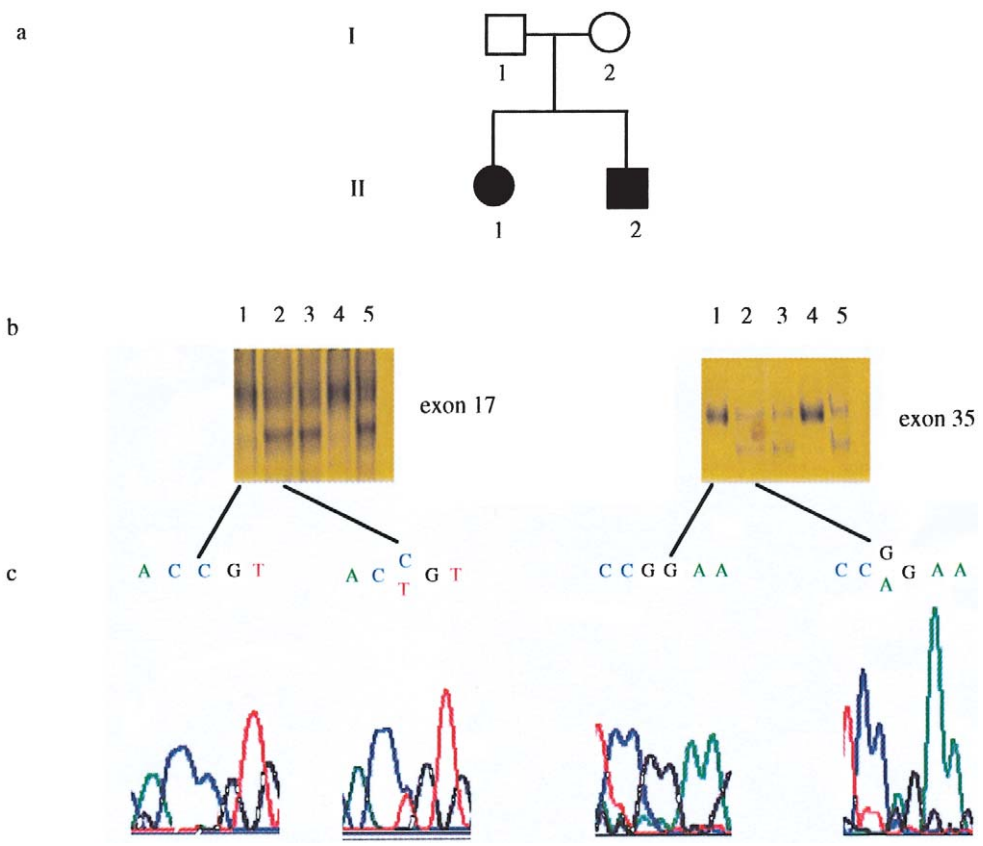

Figure 1 Analysis of family USH3.04. *a,* Pedigree. *b,* SSCP patterns of exons 17 (*left panel*) and 35 (*right panel*) of MYO7A (primers are from Levy et al. [1997]). For exon 17, a heterozygous variant (*lower and upper bands*) can be seen in lanes 2, 3, and 5 (patients II.1 and II.2 and patients' mother [I.2], respectively); results for a normal control and the patients's father (I.1) (*upper band*) also are shown, in lanes 1 and 4, respectively. For exon 35, a heterozygous variant (*lower and upper bands*) can be seen in lanes 2, 3, and 5 (patients II.1 and II.2 and patients' father [I.1]); results for a normal control and the patients's mother (I.2) (*upper band*) also are shown, in lanes 1 and 4, respectively. *c,* Direct sequence analysis of control and patient II.1, for exon 17. *Left panel,* Results for patient II.1, who shows a heterozygous C→T substitution (as does II.2 [data not shown]). The mother (I.2) is heterozygous for the mutation (data not shown). *Right panel,* Direct sequence analysis of exon 35 from the control and patient II.1, with the latter showing a heterozygous G→A substitution (as does II.2 [data not shown]). The father (I.1) is heterozygous for the mutation (data not shown).

The Arg1602Gln mutation in exon 35 occurs in a residue that is conserved in mouse and human and that lies at the C-terminal boundary of a *MYO7A* tail long direct repeat that, in both human and mouse (Chen et al. 1996; Mburu et al. 1997), shows homology to both a plant kinesin tail domain and members of the band 4.1 family, as well as to regions of other myosin tails (myosins IV, X, and XII). *MYO7A* may have the properties of a myosin motor-kinesin tail hybrid and may be involved with membrane turnover in the actin-rich environment of the apical hair-cell surface (Mburu et al. 1997). The plant kinesin and band 4.1 domains in the tail may be important for the interaction of *MYO7A* with membranes or membrane-associated proteins. Interestingly, the Arg1602Gln substitution in the atypical USH family also has been identified in patients with USH1B (Weston et al. 1998).

Genetically, USH is quite heterogeneous. Our data also show that atypical USH and USH3 are apparently genetically heterogeneous, with mutations at different loci, on 3q and 11q, in different families. In addition, it also is clear that mutations at the same locus, *MYO7A,* can give rise to both USH1, in which there is severe to profound hearing loss, and atypical USH, in which the hearing loss is progressive. Most important, the Arg1602Gln mutation identified in the atypical USH family that we have studied also has been found in patients with USH1B (Weston et al. 1998). Given both the likely severity of the nonconservative Leu651Pro change in the atypical USH family and the finding that, in some individuals, the Arg1602Gln allele can lead to severe USH1B, the data provide the first evidence suggesting that genetic background may be important in determining the severity of USH.

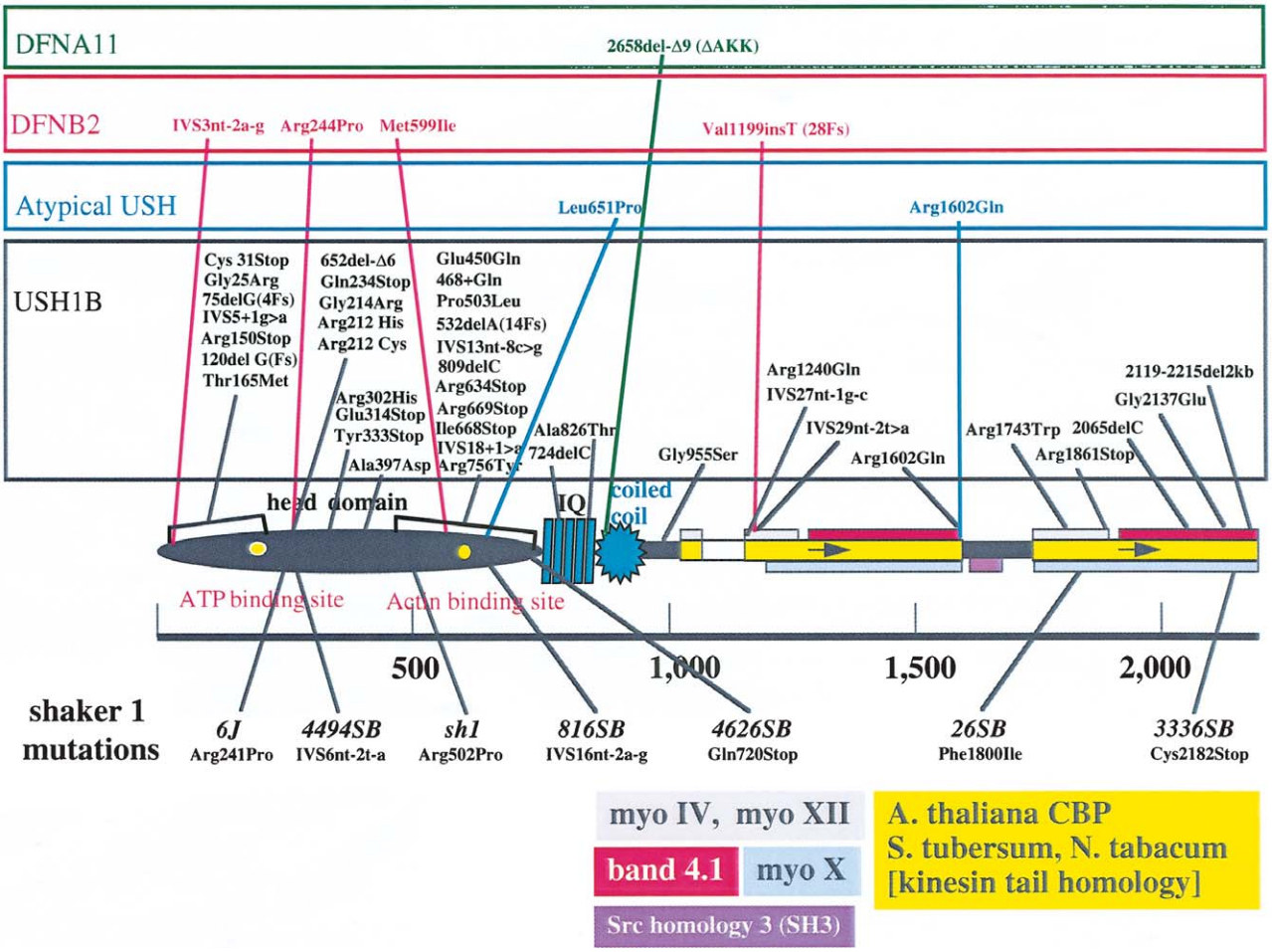

Figure 2 Spectrum of *MYO7A* mutations that lead to USH1B (Weston et al. 1996, 1998; Adato et al. 1997; Levy et al. 1997; Liu et al. 1997*a*) and atypical USH syndromic deafness (present study), as well as recessive (i.e., DFNB2 [Liu et al. 1997*b;* Weil et al. 1997]) and dominant (i.e., DFNA11 [Liu et al. 1997*c*]) nonsyndromic deafness. The positions of seven shaker1 mutant alleles are also shown (*below*) (Mburu et al. 1997), and the various homology domains in the *MYO7A* tail are indicated (Chen et al. 1996; Mburu et al. 1997).

MYO7A also recently has been shown to harbor mutations causing both recessive (DFNB2 [Liu et al. 1997*b*; Weil et al. 1997]) and dominant (DFNA11 [Liu et al. 1997*c*]) nonsyndromic deafness. When the data reported here are considered, it is now clear that different mutations in *MYO7A* can lead to a wide range of phenotypes, including both syndromic USH1B and atypical USH, as well as both nonsyndromic recessive (DFNB2) and nonsyndromic dominant (DFNA11) deafness (fig. 2). With the exception of the DFNA11 mutation, there does not appear to be a clear correlation between mutation and phenotype. The DFNA11 mutation is a 9-bp in-frame deletion in the coiled-coil region of *MYO7A* (Liu et al. 1997*c*), which is thought to be responsible for dimerization of the molecule (fig. 2). Mutations in the coiled-coil region may result in dominant hearing loss due to a dominant-negative effect. Indeed, unlike individuals with the other three forms of hearing loss caused by *MYO7A* mutations, DFNA11 patients have a less severe postlingual hearing loss (Liu et al. 1997*c*). In three DFNB2 families with *MYO7A* mutations reported to date, profound deafness with variable vestibular dysfunction is a constant phenotype. However, one of these families, which is from Tunisia, demonstrates a mutation that is known to decrease the efficiency of splicing, and affected members show a variable age at onset of deafness (Weil et al. 1997). Patients in the other two DFNB2 families, which are from China, have congenital profound deafness (Liu et al. 1997*b*).

There are many instances whereby different mutations in the same gene can result in diverse phenotypes (Romeo and McKusick 1994). Allelic heterogeneity can account for markedly different phenotypes. *MYO7A* mutations in human can lead to either an isolated inner-ear lesion, as in mouse shaker1 mutations, or a combined inner-ear and retinal pathology. It is possible that, in

human, tissue-specific differences in the function of *MYO7A* might result in specific mutations having different effects in the eye but similar effects in the inner ear. It seems likely, however, given (*a*) the wide range of *MYO7A* mutations identified for both nonsyndromic deafness and USH and (*b*) the results reported in the present study, that genetic background effects have some role to play in determining the development and severity of nonsyndromic and syndromic hearing loss.

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Electronic-Database Information

Hereditary Hearing Loss home page (Van Camp G, Smith RJH), http://dnalab-www.uia.ac.be/dnalab/hhh

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Haplotype Analysis in Icelandic Families Defines a Minimal Interval for the Macular Corneal Dystrophy Type I Gene

To the Editor:

Macular corneal dystrophy (MCD [MIM 217800]) is a rare autosomal recessive disorder that is clinically char-