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Letters to the Editor

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Possible Interaction between USH1B and USH3 Gene Products as Implied by Apparent Digenic Deafness Inheritance

To the Editor:

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The Usher syndromes (USHs; MIM 276900–276904, 601067, 60297, and 602083) are a group of autosomal recessive hereditary disorders characterized by the association of sensorineural hearing impairments and progressive visual loss due to retinitis pigmentosa. Three types of USH are distinguished on the basis of severity and onset of auditory and vestibulary dysfunctions. To date, USHs are mapped to nine different genomic loci: USH1A–F, USH2A, USH2B, and USH3 (Hereditary Hearing Loss home page). USH3 (MIM 276902), assigned to chromosome 3q, is regarded as the rarest form of USH (Sankilla et al. 1995)

The human myosin VIIA gene (MYO7A), located on 11q14, has been shown to be responsible for USH1B (MIM 276903), which is the most common USH1 subtype, accounting for ~75% of all type 1 cases (Weil et al. 1995). More recently, MYO7A has also been shown to be responsible for nonsyndromic recessive and dominant deafness (DFNB2 and DFNA11), both types having been assigned to the same 11q chromosomal region (Liu et al. 1997*a*, 1997*b*; Weil et al. 1997). These findings clearly indicate that the enzymatic activity of MYO7A is critical for normal function in the inner ear and that different mutations may cause different dysfunctions that are manifested by distinct phenotypes. Here we report on two novel MYO7A mutations that may have a synergistic effect on the symptoms of another USH different from USH1B.

Among USH-affected families recruited as part of a study on the genetics of USH, results of which were published in this journal (Adato et al. 1997), was a nonconsanguineous family of Jewish Yemenite origin that included two affected and six healthy siblings. The two affected brothers in this family have different USH phenotypes. One of the affected brothers (1549 in fig. 1) has a typical USH1 phenotype: he has a history of prelingual profound auditory impairment; he uses sign language for communication, since hearing aids are unhelpful in his case; and developmental milestones (Smith et al. 1994) in his childhood are consistent with congenital vestibular dysfunction. The other affected brother (1636 in fig. 1) has a typical USH3 phenotype: he has progressive hearing loss, with postlingual onset; he uses hearing aids and verbal communication; and he receives psychiatric therapy for mental problems. In both affected brothers, the presence of bilateral progressive pigmentary retinopathy has been diagnosed (with onset during early adolescence).

Members of this family were typed for 30 polymorphic markers spanning all nine known USH loci (USH1A-F, USH2A, USH2B, and USH3). Marker alleles were identified and arranged into the most likely haplotypes, as shown in figure 1. Haplotype segregation and linkage analysis resulted in exclusion of all USH1 and USH2 loci (LOD scores range from -1.46 to -3.72) and suggested linkage only to the USH3 locus (with a maximum LOD score of 1.35 for marker D3S1279). Both affected brothers showed homozygosity for alleles of four markers: D3S1315, D3S1279, D3S3625, and D3S1294. Homozygosity of USH3 haplotypes in the affected brothers—and the fact that, although not known to be related, both parents originate from a small Jewish community in Yemen—suggest a possible common origin for both USH3-bearing chromosomes. This "USH3 haplotype" was found to be carried (one copy) by only 2 of 54 Jewish Yemenite control subjects tested for its presence. The homozygote interval in both affected brothers and the position of recombination in the paternal chromosome of one healthy progeny (1643; see fig. 1) suggest that the USH3 gene is located between markers D3S1299 and D3S3625. This result is in agreement with the location suggested by Sankilla et al. (1995) and by Joensuu et al. (1996). The order of markers spanning the USH3 linkage region, as presented in figure 1, is cen, D3S2401, D3S1299, D3S1315, D3S1279, D3S3625, D3S1594, tel, in agreement with the order presented in the Whitehead contig (Whitehead Institute for Biomedical Research). However, in this order, the position of the markers D3S1315 and D3S1279, which our findings indicated were the most closely linked to

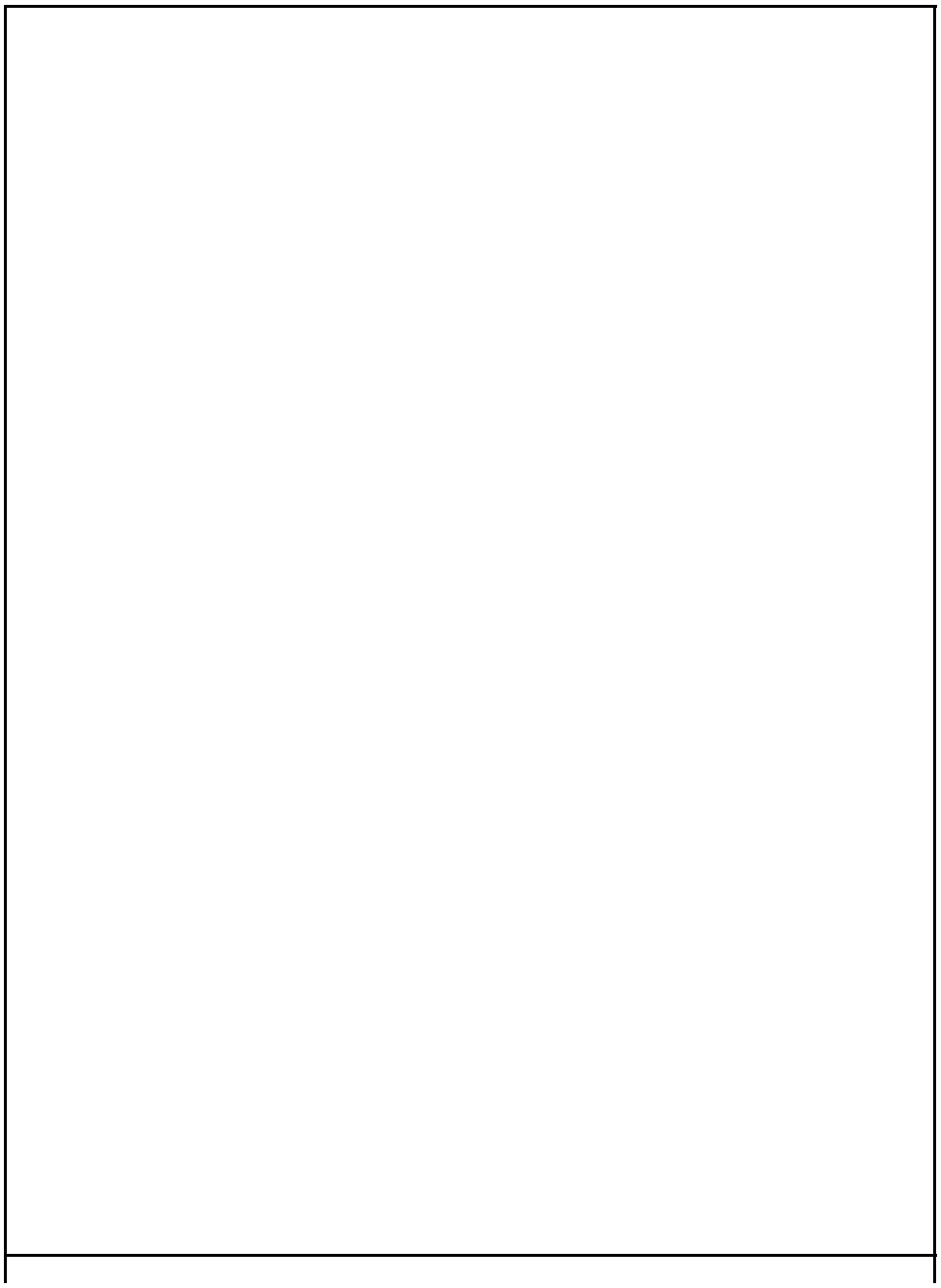

Figure 1 Genomic DNA extracted from blood of family members was used as a template for PCR amplification, which was done with 30 pairs of specific primers of markers spanning all nine USH loci. Marker alleles, identified according to their relative mobility on a denaturing formamide 4% acrylamide gel in all family members, were arranged into the most likely haplotypes. This haplotype arrangement results in exclusion of all USH1 and USH2 loci and suggests linkage only to the USH3 locus. Maternal chromosomes are gray and striped whereas paternal chromosomes are white and dotted. Blackened squares on the gray USH1B maternal chromosomes indicate the presence of the mutated MYO7A. The homozygote interval, of both affected brothers, in the USH3 locus is boxed.

USH3, differs from the one suggested by Joensuu et al. (1996).

Since one of the affected brothers had an USH1 phenotype, family members were screened for mutations in the human MYO7A gene, which has been shown to be responsible for USH1B. Two new close nucleotide changes were detected in exon 25 of the gene on one maternal chromosome: a T→C transition and a guanine deletion 5 nt upstream of this transition (fig. 2). None of these changes were found in >200 control chromosomes tested by allele-specific oligonucleotide analysis, as described by Whithney et al. (1993). This mutated MYO7A is carried by the brother with the USH1 phenotype (1549) but not by his affected brother with the USH3 phenotype (1636). The mother (1637) and two unaffected siblings (1638 and 1639), who are all double

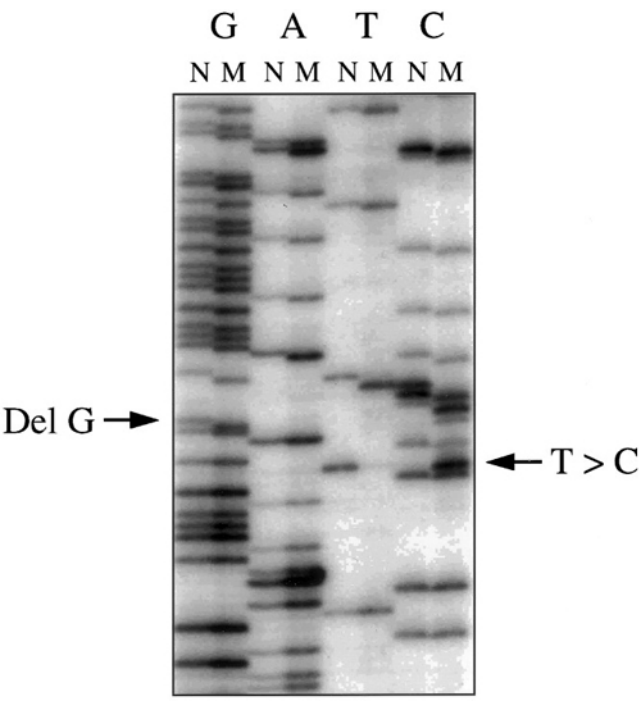

Figure 2 Comparative electrophoresis of normal (N) and mutated (M) exon 25 PCR cycle-sequencing reaction, with SequiTherm EXEL DNA Sequencing Kit (FMC). Products were electrophoresed side by side through Long Ranger Gel (Epicentre Technologies): ddGTP-terminated products of normal exon 25 appear next to ddGTP-terminated products of mutated exon 25; and ddATP-terminated products of normal exon 25 appear next to ddATP-terminated products of mutated exon 25. Arrows indicate the T→C transition and the guanine deletion (5 nt up stream of the transition).

heterozygotes for the mutated MYO7A and for a single USH3 haplotype, show no evidence of any USH symptoms or nonsyndromic deafness. This suggests a digenic inheritance pattern, with a possible synergistic interaction between MYO7A and the USH3 gene product, where presence of a single defective MYO7A allele seems to increase the severity of deafness as a part of the clinical symptoms associated with USH3.

Evidence for digenic inheritance of nonsyndromic deafness was already presented in the case of a Swedish family (Balciuniene et al. 1998), whose affected members were carriers of DFNA2 and/or DFNA12. Increased severity of deafness was found in family members that were carriers of both alleles. This clear additive effect differs from the situation in our Yemenite family, where mutated MYO7A appears to be phenotypically expressed only on the background of two defective USH3 alleles, suggesting an interaction between the MYO7A and the USH3 gene products. Digenic inheritance was also suggested as one of the possible explanations in the case of DFNB15 (Chen et al. 1997). This is an autosomal recessive nonsyndromic deafness, found in a family of Indian origin, linked to two loci on chromosomes 3q and 19p. Most interestingly in relation to our work, one of these loci, 3q21.3-3q25.2, includes the USH3 locus and the other, 19p13.3p13.1, includes (among others) the MYO1F gene (Hasson et al. 1996), which is another member of the unconventional myosin group.

Human MYO7A is a member of the unconventional myosins group (Weil et al. 1996). All myosins have three different functional domains defined within their heavy chains: an N-terminal motor domain, a regulatory (light-chain–binding) domain, and a tail domain that varies dramatically in length and in sequence among myosins (Mooseker and Cheney 1995). The functions of myosin-tail domains are largely unknown. However, a common assumption is that the tail directs the interaction of a given myosin with its cargo (Mermall et al. 1998). MYO7A is predicted to dimerize, on the basis of the coiled-coil sequence motif at the start of its tail region.

The sequence changes detected in exon 25 of MYO7A in some members of the presented family are expected to result, at the protein level, in a Leu→Pro substitution at codon 1087 and in a frameshift of the reading frame starting at codon 1089. Both these AA codons are located after the coiled-coil domain of the protein. This frameshift would result in the formation of a UGA stop codon 18 amino acids downstream from the deletion site and, therefore, in the translation of a truncated protein that lacks >50% of its normal AA sequence, which comprises most of the MYO7A tail domain. Segregation of the mutated MYO7A with healthy members of this family and, on the other hand, with the more severe USH phenotype, suggests a possible biological interaction between MYO7A and the USH3 gene products. This mutated MYO7A appears to be phenotypically expressed only on the background of two USH3 alleles.

Many disease mutations that introduce stop codons were found to lead to mRNA destabilization, as in the mouse MYO6 sv allele (Avraham et al. 1995). In such a case, segregation of the mutated MYO7A, as described above, could indicate that the normal USH3 gene product may affect the stabilization of the MYO7A mRNA or the protein.

If the mutated MYO7A in this Yemenite family was translated, then it would not lack its coiled-coil sequence motif. Unlike the case of DFNB11, where a 9 bp deletion in the coiled-coil region of MYO7A was suggested to have a dominant negative effect (Liu et al. 1997*b*), segregation of the mutated MYO7A with healthy members of our family clearly determines the recessive nature of its mutation. There are several possible explanations for this difference. The truncated MYO7A produced in our case may be incompetent for dimerization, since it is unknown whether the remainder of the tail is required for self assembly or if the truncated molecule may be destabilized. Another possible reason is the formation

of a partially functional heterodimer. These possibilities would yield ≤50% of the normal protein amounts. In any kind of direct or indirect USH3-MYO7A protein interaction, a reduced-dosage effect of active MYO7A protein is likely to have a synergistic effect on the background of the two impaired alleles of the USH3 gene product.

One possible explanation for the USH3-MYO7A interaction is that the USH3 protein might be involved in targeting or binding MYO7A to the plasma membrane. The tail of MYO7A consists of a direct repeat containing two elements. The distal element, the talinlike domain, shows significant homology to the N-terminus of talin and limited homology to the N-termini of other members of the band-4.1 superfamily of actin-binding proteins (Weil et al. 1996; Chen et al. 1997). In talin and in band 4.1, this region binds to acidic phospholipids and mediates protein-protein interactions. Therefore, it is thought that talinlike motifs serve to bind and/or target the myosin to the plasma membrane (Cheney et al. 1993; Titus et al. 1997).

It is also possible that USH3 is a cytoskeletal component and as such interacts with the MYO7A protein: Actin cytoskeleton is essential for proper the function of the inner ear, and deafness-associated genes such as MYO7A and MYO15 as well as the human diaphanous protein are assumed to be cytoskeletal components (Vasiliki and Petit 1998).

The MYO7A protein may also serve an inner hair-cell–specific role distinct from its role in actin. This role might be complementary to the function of the USH3 gene product. For example, in mice, MYO7A was found to be involved in hair-cell vesicle trafficking of aminoglycosides, which are known to induce ototoxicity (Richardson et al. 1997).

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

Hereditary Hearing Loss home page, http://dnalab-www.uia.ac.be/dnalab/hhh/index.html

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Usher syndrome subtypes [MIM 276900, 276901, 276902, 276903, 276904, 601067, 60297, and 602083])

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www.genome.wi.mit.edu/ (for Whitehead contig map)

References

Adato A, Weil D, Kalinski H, Pel-Or Y, Hammadi A, Petit C, Korostishevsky M, Bonne-Tamir B (1997) Mutation profile of all 49 exons of the human myosin VIIA gene and haplotype analysis in Usher 1B families from diverse origins. Am J Hum Genet 61:813–821

Avraham KB, Hasson T, Steel KP, Kingsley DM, Russell LB, Mooseker MS, Copeland NG, et al (1995) The mouse Snell's waltzer deafness gene encodes an unconventional myosin required for structural integrity of inner hair cells. Nat Genet 11:369–374

Balciuniene J, Dahl N, Borg E, Samuelsson E, Koisti MJ, Pettersson U, Jazin EE (1998) Evidence for digenic inheritance of nonsyndromic hereditary hearing loss in a Swedish family. Am J Hum Genet 63:786–793

Chen A, Wayne S, Bell A, Ramesh A, Srisailapathy CR, Sccott DA, Sheffield VC, et al (1997) New gene for autosomal recessive non-syndromic hearing loss maps to either chromosome 3q or 19p. Am J Med Genet 71:467–471

Cheney R, Riley MA, Mooseker MS (1993) Phylogenetic analysis of the myosin super family. Cell Motil Cytoskeleton 24: 215–223

Hasson T, Skowron JF, Gilbert DJ, Avraham KB, Perry WL, Bement WM, Anderson BL, et al (1996) Mapping of unconventional myosins in mouse and humans. Genomics 36: 431–439

Joensuu T, Blanco G, Pakarinen L, Sistinen P, Kaariainen H, Brown S, de la Chapelle A, et al (1996) Refined mapping of Usher syndrome type III locus on chromosome 3, exclusion of candidate genes, and identification of the putative mouse homologous region. Genomics 38:255–263

Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJTV,

Steel KP, Brown SDM (1997*a*) Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. Nat Genet 16: 188–190

Liu XZ, Walsh J, Tamagawa Y, Kitamura K, Nishizawa M, Steel KP, Brown SDM (1997*b*) Autosomal dominant non-syndromic deafness (DFNA11) caused by a mutation in the myosin VIIA gene. Nat Genet 17:268

Mermall V, Post PL, Mooseker MS (1998) Unconventional myosins in cell movement, membrane traffic, and signal transduction. Science 279:527–533

Mooseker MS, Cheney RE (1995) Unconventional myosins. Annu Rev Cell Dev Biol 11:633–675

Richardson GP, Froge A, Kros CJ, Fleming J, Brown SD, Steel KP (1997) Myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells. J Neurosci 17:9506–9519

Sankilla E, Pakarinen L, Kaariainen H, Aitotomaki K, Karjalainen S, Sistonen P, de la Chapelle A (1995) Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q. Hum Mol Genet 4:93–98

Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM (1993) The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics 16:325–332

Smith RJH, Berlin CI, Hejtmancik JF, Keats BJB, Kimberling WJ, Lewis RA, Moller CG, et al (1994) Clinical diagnosis of the Usher syndromes. Am J Hum Mol 50:32–38

Titus MA (1997) Unconventional myosins: new frontiers in actin-based motors. Trends Cell Biol 7:119–123

Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, et al (1995) Defective myosin VIIA gene responsible for Usher syndrome type 1B. Nature 374:60–61

Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, et al (1997) The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. Nat Genet 16:191–193

Weil D, Levy G, Shahly I, Levi-Acobas F, Blanchard S, El-Amraou A, Crozet F, et al (1996) Human myosin VIIA responsible for the Usher 1B syndrome: a predicted membrane-associated motor protein expressed in developing sensory epithelia. Proc Natl Acad Sci USA 93:3232–3237

Whithney MA, Saito H, Jakobs PM, Gibson RA, Moss RE, Grompe M (1993) A common mutation in the FACC gene causes Fancini anemia in Ashkenazi Jews. Nat Genet 4: 202–205

Vasiliki K, Petit C (1998) The fundamental and medical impacts of recent progress in research on hereditary hearing loss. Hum Mol Genet 7:1589–1597

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