ELECTRONIC LETTER

Modifier controls severity of a novel dominant low-frequency MyosinVIIA (*MYO7A*) auditory mutation

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Hearing impairment is a common sensory deficit with both genetic and environmental aetiologies. Pre-lingual hearing loss affects approximately 1 in every 1000 children in the United States with a genetic basis in about 50% of the cases.[1] An additional 1 per 1000 individuals experience auditory deficits prior to adulthood.[1] Large pedigrees with monogenic non-syndromic hearing impairment have allowed genetic mapping of at least 80 chromosomal locations harbouring auditory-related deafness (*DFN*) loci with the identification of over 30 *DFN* genes.[2] The *DFN* inheritance pattern is designated by *A* dominant, *B* recessive, and *M* modifier, with a number following *A*, *B*, or *M* indicating the relative order in which the locus was identified. For example, *DFNA1* represents the first dominantly inherited deafness locus mapped in humans.

Several myosin gene products have been implicated in hearing loss. Myosins constitute a family of motor proteins playing roles in diverse biological events such as muscle contraction, cell adhesion, organelle translocation, cytokinesis, and cell movement.[3 4] Myosin proteins share a conserved globular head domain with actin- and ATP-binding sites joined to varied amino and carboxy-terminus regions that determine the unique cellular role of each motor protein. For example, the *Drosphila* phototransduction NINAC (neither inactivation nor afterpotential C) protein couples a myosin head with a N-terminal kinase domain.[5] The first myosin discovered, myosin-II, was isolated from muscle and shown to form myosin filaments by association of bipolar tail domains.[3] Myosin-II has historically been referred to as a conventional myosin. Unconventional myosins do not form filaments and constitute a large rapidly-growing group of proteins.[6] Mutation of one conventional myosin, non-muscle myosin, heavy polypeptide 9 (*MYH9*),[7] and five unconventional myosins, myosin IA (*MYO1A*),[8] myosin IIIA (*MYO3A*),[9] myosin VI (*MYO6*),[10] myosin VIIA (*MYO7A*),[11] and myosin XV (*MYO15A*)[12] are known to cause auditory dysfunction.

Over 80 mutations in *MYO7A* have been identified,[2 13] most leading to a diagnosis of Usher syndrome type 1B (*USH1B*), a disease characterised by profound, congenital, sensorineural deafness with progressive retinitis pigmentosa leading to visual loss and vestibular areflexia. Four non-syndromic families with *MYO7A* alterations have also been reported; three with a recessive (*DFNB2*)[14 15] and one with a dominant (*DFNA11*)[16] inheritance pattern. In this article we describe a large American pedigree [referred to as HL2, for hearing loss family 2] experiencing low-frequency hearing loss associated with a novel heterozygous mutation in *MYO7A*, representing a second DFNA11 family. The severity with which this disease mutation manifests in the auditory system appears to be influenced by a genetic modifier.

Key points

- Mutations within myosin molecules can lead to syndromic and non-syndromic hearing impairment.
- We describe the genetic mapping of progressive sensorineural hearing loss first affecting low-frequency auditory thresholds within a large human pedigree to chromosome 11q13.5. A maximal pairwise LOD score of 7.23 was obtained with marker *D11S4207*.
- We identified a myosin VIIA (*MYO7A*) G2164C mutation that co-segregates with auditory dysfunction in the pedigree. The mutation results in a predicted G722R substitution at an evolutionarily conserved glycine residue in the MYO7A head domain.
- This pedigree represents a second DFNA11 family.
- The clinical severity of the G2164C mutation varies between individuals in different family branches with similar medical and noise-exposure histories, indicating involvement of a genetic modifier.
- Single nucleotide polymorphism (SNP) analysis in the family suggests that amino acid changes within the opening-reading-frame (ORF) of *MYO7A* commonly found in the general population and within the *GJB2* (connexin 26, Cx26) ORF are not responsible for the marked differences in the clinical manifestation of the G2164C mutation.

MATERIALS AND METHODS

Research subjects and controls

Under a protocol of informed consent approved by the institutional review board (IRB) of the University of Washington, Seattle, 5 ml of blood were obtained by venipuncture for high molecular weight DNA isolation using standard techniques. Control DNA samples were taken from a predominantly Caucasian population. Half of these controls underwent pure-tone air conduction screening at 250, 500, 1000, 2000, 40000, and 8000 Hz (performed by V Street, under the guidance of K Kiemele) to ensure that the control subjects' hearing levels were within normal ranges for their age.[17] The HL2 pedigree is of English decent. Male-to-male transmission is observed confirming autosomal dominant inheritance. Audiologic evaluations were either conducted as part of this study at a clinic near the HL2 family member or previous test results were released to the study. The pure-tone air and bone conduction thresholds for the HL2 family members were established by certified audiologists in sound-attenuated booths. Symmetrical hearing loss was detected in all affected HL2 family members. For clarity, only right ear responses are plotted on the audiograms in fig 1. Audiometry data for a normal hearing individual of similar age to the research subject are included on each audiogram plot.[17] Immittance testing evaluated middle ear pressures, ear canal volumes, and tympanic membrane mobility. Each study participant completed a hearing and balance questionnaire to assess their medical and noise-exposure history. For the

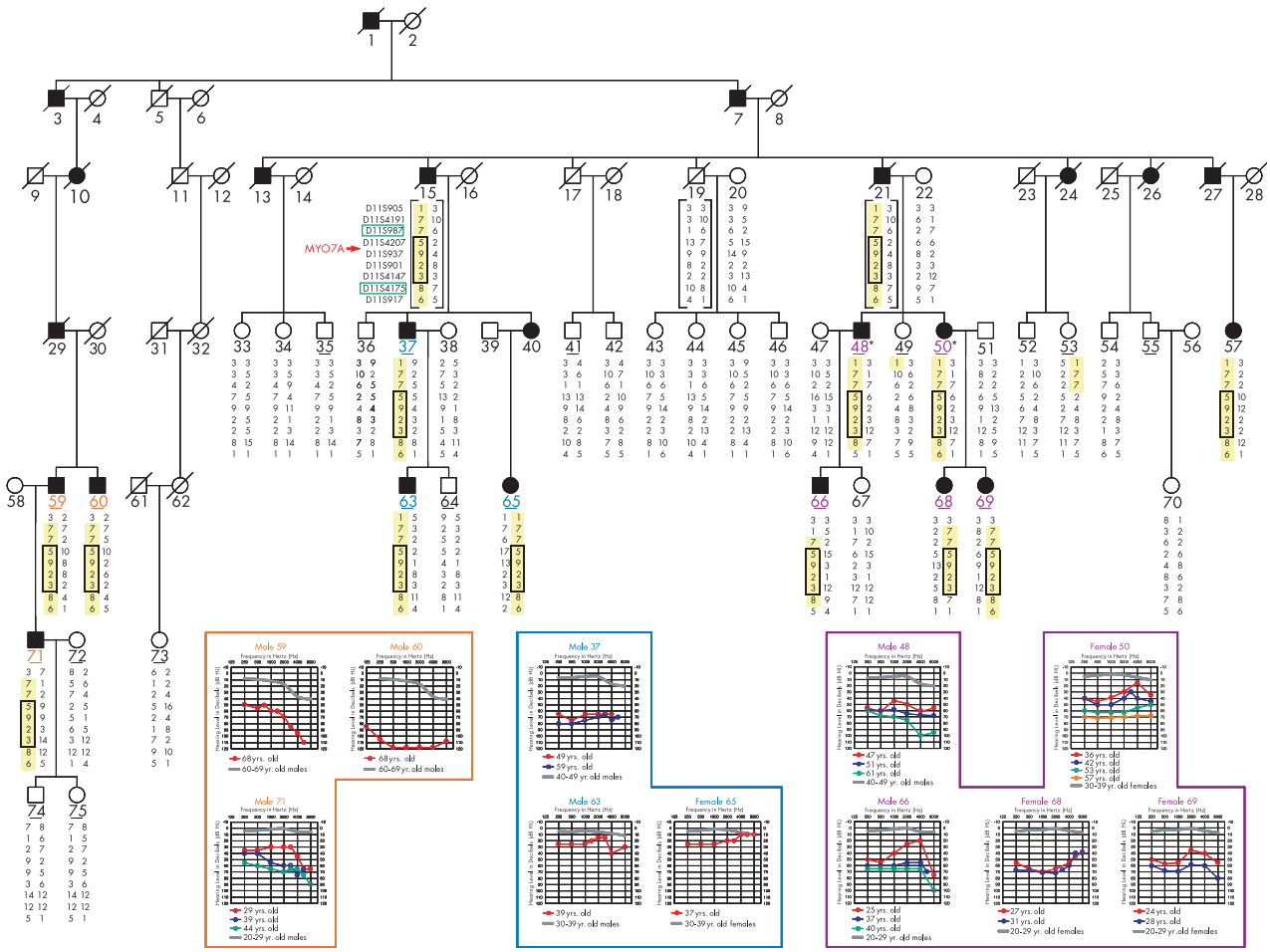


Figure 1 Haplotype and audiologic characterisation of the HL2 Pedigree. Each individual in the pedigree is assigned a number. Underlined numbers indicate the person completed an auditory evaluation. Affected individuals are denoted by blackened symbols, males are denoted by squares, females are denoted by circles, and deceased persons are indicated by a diagonal line through the symbol. Audiograms for affected individuals (shown for right ear only) are grouped as colour-coded family clusters and positioned near the appropriate family branch. Frequency in hertz (Hz) is plotted on the x-axis and hearing level in decibels (dB HL) on the y-axis. Plotted on each audiogram (grey line) are the average pure-tone air conduction thresholds for a person with normal hearing matched in age to the earliest audiogram collected for the HL2 family member. The asterisk (*) near persons 48 and 50 indicate they completed a fundus exam. Markers are listed from centromere (top) to telomere (bottom). The affected-linked haplotype is shown in yellow and the non-recombinant alleles are boxed. Inferred haplotypes for deceased individuals are shown in brackets.

fundus examination, the pupil was dilated and the retina was examined by a certified ophthalmologist.

Genotyping and linkage analysis

A genome-wide scan (ABI PRISM Linkage Mapping Set Version 2, PE Biosystems) was performed in pedigree HL2 with informative microsatellite markers[18] spaced at approximately 10 centimorgan (cM) intervals. Polymerase chain reaction (PCR) conditions were performed according to the manufacturer's recommendation (PE Biosystems). PCR products were multiplexed and separated by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (PE Biosystems). Microsatellite allele data were analysed with GENESCAN version 3.1.2 and GENOTYPER version 2.0 (PE Biosystems). Amplification products generated with the ABI panel sets were sized according to CEPH (Centre d'Etude du Polymorphisme Humain) control DNA (1347-02) and assigned allele numbers consistent with the CEPH designations (http://www.cephb.fr). New alleles not reported in the CEPH database include a 218 basepair (bp) product (designated allele 9 in our study) for marker *D11S905*, a 252, 261, 270 bp product (designated allele 15, 16, 17) for marker *D11S4207*, and a 175 bp product (designated allele 15) for marker *D11S4175*. Under a model of autosomal dominant

Table 1 PCR primers to amplify *MYO7A* genomic exons

Exon	Primer sequences	Annealing temp. (℃)
0	5′-cagagacagaccccaatgacc-3′ 5′-tcaagggtggcaggaagc-3′	64
6	5′-cagcactcgtggattgagc-3′ 5′-cagagggaagcaggcagc-3′	64
9	5′-catacgtggtccctacccтca-3′ 5′-acctgcacatggttgcttctc-3′	60
14	5′-tggcccctcactttctctatg-3′ 5′-gaggaccagatgggaacacc-3′	60
17	5′-gccctggatgtcagttgtgag-3′ 5′-tgtgctttgtaacctgtggtcagt-3′	60
28	5′-tagggaagccacagaaagca-3′ 5′-ccagaacttgagggtaggtgtc-3′	60
32	5′-aatccttgcttccctgtctct-3′ 5′-gtttccctcccctgctgt-3′	62
37	5′-cttcctgatggaggcgttg-3′ 5′-cacctggcacccaaatgac-3′	60
42	5′-gacacacagaaacccctcc-3′ 5′-agcccagacccagaggag-3′	64

*Exon 0 = 5′ non-coding exon.

inheritance, pairwise and maximal LOD scores were calculated using the LINKAGE computer program package[19] version 5.1. Equal recombination rates in males and females were assumed and a *DFN* gene frequency of 0.0005 was used for the calculations. Equal microsatellite marker allele frequencies were employed in the analyses. Haplotypes were constructed based on known marker orders.[18] The SimWalk2 program version 2.83 was employed to estimate the multipoint LOD scores.[20]

Mutation and polymorphism detection

Previously published PCR primers[21] were used to amplify the 49 (48 coding) *MYO7A* exons and immediate flanking genomic intron sequences with exceptions noted in table 1.

The previously published exon 42 primers span a region that in the HL2 family contained a C/T SNP (67 bp 3′ of exon 42 ORF). This SNP prevented the PCR amplification of both *MYO7A* alleles, therefore a new set of primers were designed for exon 42. PCR incubation mixture and thermocycling parameters were performed as noted previously.[22] The *GJB2* exon 2 was amplified as described previously.[23] PCR amplification products were separated by gel electrophoresis on 2% SeaKem LE agarose gels (BioWhittaker Molecular Applications), purified using a QIAquick Gel Extraction Kit (Qiagen) and sequenced in both directions using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems). Sequencing reactions were purified on AutoSeq G50 columns (Amersham Pharmacia Biotech) prior to analysis on an ABI 377 sequencer (PE Biosystems). Electrophoregrams were analysed using the DNASTAR software package.

Protein evolution and mutation modeling

To determine the evolution rate of the G722 residue we used the ConSurf server (http://bioinfo.tau.ac.il).[25] The Protein Data Bank (PDB) identification number for MyoIE[24] (1lkx) was entered into ConSurf. The server selected 50 myosin homologues from the PSI-BLAST database to calculate the site specific evolution rate. To model the structural impact of the G722R substitution, we utilised the DeepView (Swiss-PdbViewer) program[26 27] version 3.7 (http://www.expasy.org/spdbv). The MyoIE[24] PDB template was loaded into DeepView, the appropriate glycine residue was mutated to arginine, and the program was allowed to select the best rotamer of arginine. The image was rendered with POV-Ray version 3.5.

RESULTS

Progressive non-syndromic low-frequency hearing loss

The hearing loss onset in family HL2 is noticed generally between 20–30 years of age. Audiologic evaluation of the family members demonstrates normal immitance testing and bone conduction values that equal the air conduction measurements, suggesting sensorineural hearing impairment (data not shown). Audiograms from six affected individuals (50, 63, 65, 66, 68, 69) documenting hearing status at <40 years of age indicate that low-frequency loss is consistently present at these ages (fig 1). Individuals 50, 63, 66, and 69 show similar audiologic profiles with loss of sensitivity at both low- and high-frequency pure tone regions with relative sparing of the mid-frequencies. Family members 65 and 68 show similar S-shaped profiles with greater sensitivity at the higher frequencies compared with the low- and mid-frequency ranges. Serial audiograms from individuals 50, 66, and 69 clearly demonstrate the progressive nature of the HL2 hearing loss (fig 1). Audiograms for family member 50 are shown (right ear only) covering a 21-year span at ages 36 (red-line), 42 (blue-line), 53 (green-line), and 57 (orange-line). Between the ages of 36 and 57 a 30–55 dB worsening in pure tone thresholds is observed across the frequency ranges examined resulting in a flat moderately severe sensorineural hearing loss at 57 years of age. Audiograms for male 66 are shown over a 15-year span at ages 25 (red-line), 37 (blue-line), and 40 (green-line). Between the ages of 25 and 37 a 30-35 dB worsening in pure tone thresholds at 2000 and 4000 Hz is noted resulting in a flat moderately severe sensorineural hearing loss at 37 years of age. Female 69 demonstrates a 20–25 dB worsening of pure tone thresholds at 2000, 4000, and 8000 Hz over a 4-year time span. The documented hearing loss progression in these three individuals can not be accounted for by exposure to environmental noise or ototoxic drugs. Affected individual 60 was exposed to considerable military-related noise exposure for over 15 years and displays profound sensorineural hearing loss across all frequencies (fig 1). He received a unilateral cochlear implant at 69 years of age which has enhanced greatly his communication abilities. Affected individual 59 was exposed to recreational noise and demonstrates an audiogram with a downward sloping configuration.

The HL2 family members do not complain of night-blindness or vestibular problems. Two family members experiencing moderate to severe hearing loss (individuals 48 and 50, ages 66 and 60, respectively) underwent a fundus examination with normal retinal findings (data not shown). Given the absence of detectable ophthalmological dysfunction using these approaches, the HL2 family was considered non-syndromic.

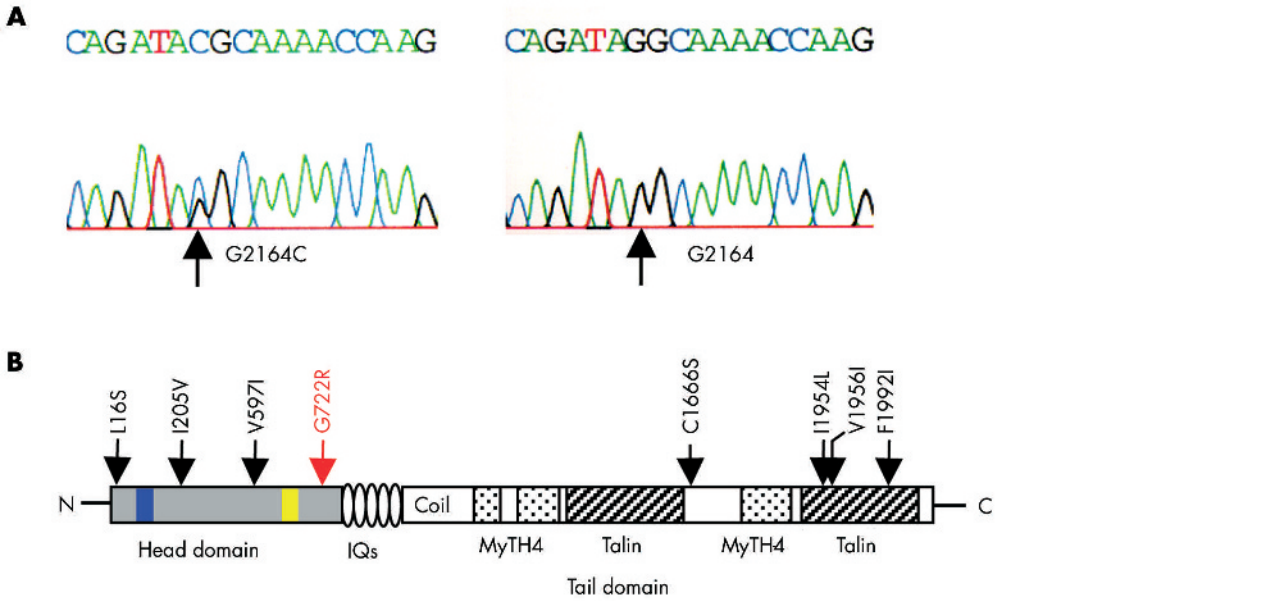
Linkage to chromosome 11q

Prior to performing microsatellite marker analysis, the linkage detection power of the HL2 pedigree was simulated using 400 replicates with the SLINK program[28 29] at a recombination frequency $\theta = 0.00$ between the disease and marker loci assuming a frequency of 0.00005 for the disease locus and five equally frequent alleles at the marker locus.

Table 2 Pairwise LOD scores between hearing loss and chromosome 11 markers

		LOD Score at θ =								
Marker*	Distance (cM)†	.0	.01	.05	.1	.2	.3	.4	Z_{max}	θ_{max}
D11S905	52.0	−∞	−3.64	−1.00	0.00	0.63	0.60	0.31	0.67	0.24
D11S4191	60.1	−∞	3.60	4.44	4.33	3.43	2.21	0.94	4.46	0.06
D11S987	67.5	−∞	4.78	5.00	4.67	3.64	2.38	1.02	5.03	0.04
D11S4207	76.1	7.23	7.10	6.56	5.88	4.44	2.92	1.32	7.23	0.00
D11S937	80.0	5.66	5.55	5.12	4.57	3.41	2.20	0.98	5.66	0.00
D11S901	85.5	5.54	5.43	4.97	4.39	3.22	2.06	0.94	5.54	0.00
D11S4147	87.9	3.98	3.90	3.57	3.15	2.29	1.44	0.66	3.98	0.00
D11S4175	91.5	−∞	3.75	4.02	3.76	2.87	1.79	0.74	4.03	0.04
D11S917	96.9	−∞	3.29	4.18	4.12	3.31	2.16	0.88	4.21	0.07

*Markers are shown in order from centromere to telomere.
†Marker map position is based on the Genethon and Marshfield human sex-averaged linkage maps.



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Organism	Protein	Sequence	Acc. #
Affected HL2	MYO7A	T H D D W Q I **R** K T K I F L K	---------
Human	MYO7A	T H D D W Q I G K T K I F L K	NM_000260
Mouse	Myo7a	T H D D W Q I G K T K I F L K	NM_008663
Zebrafish	Myo7a	R D D D W Q M G K T K I F L K	CAC05419
Fruit fly	NINAC	K M E G W A L G K T K V F L R	J03131
Worm	MHCB	S E E M F R I G L T K V F F K	P02566
Yeast	Myo1p	D T K V Y K I G N T K L F F K	NP_011888
Fungus	MYSE	D K E E I R M G K T K V F I R	Q03479
Plant	ATM1	L P E M Y Q V G Y T K L F F R	X69505

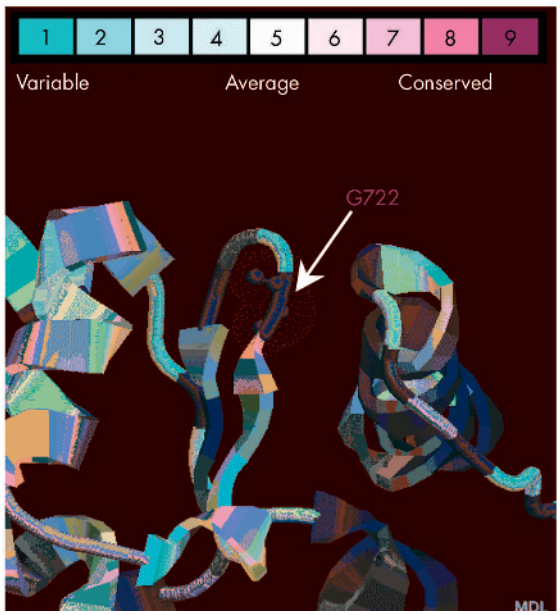

Figure 2 An evolutionarily conserved MYO7A head domain residue is mutated in the HL2 pedigree. (**A**) Electropherograms showing heterozygous G2164C mutated genomic nucleotide sequences from an affected individual compared to a homozygous G2164 wild-type unaffected family member. Nucleotide numbering starts with the ORF. (**B**) Schematic representation of MYO7A functional domains indicating the location of G722R (shown in red) within the head region (grey shading) and position of ORF SNPs resulting in amino acid changes surveyed in this study as potential modifiers. Blue and yellow box in head domain represent ATP and actin binding sites, respectively. The tail domain contains four notable regions: 1) IQs represent five light-chain-binding repeats; 2) coil indicates coiled-coil domain that may be involved in dimerization; 3) MyTH4 indicates myosin tail homology-4 domains that are regions conserved between myosins; and 4) talin represents talin-like homology domains which are predicted to bind actin. (**C**) Protein alignment shows conservation of the G722 residue during evolution. The R722 substitution in the HL2 pedigree is shown in red. Residues in the *Drosophila* NINAC myosin head domain mutagenised by Porter and Montell[39] are underlined. (**D**) A ConSurf analysis indicates that the glycine residue at position 722 is maximally conserved. ConSurf scores range from 1–9. A score of 1 (shown in turquoise) indicates that the site is evolving rapidly (variable). A score of 9 (shown in maroon) indicates that the site is evolving slowly (evolutionarily conserved).

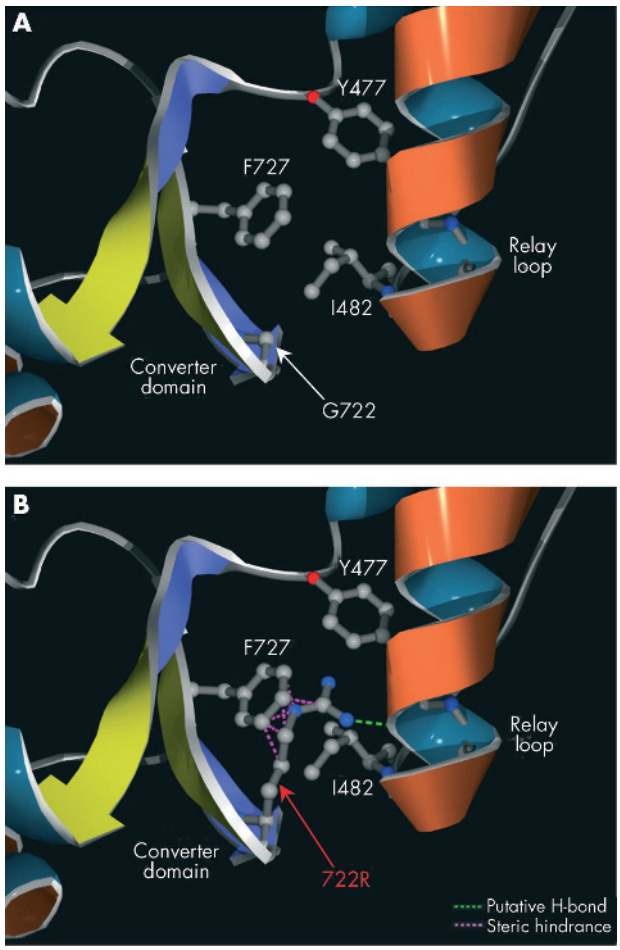

Figure 3 Structural analysis of the MYO7A G722R substitution. **(A)** Molecular ribbon model containing the normal MYO7A G722 residue. Residue numbers are given for MYO7A, not MyoIE. **(B)** Molecular ribbon model containing 722R substitution. Putative hydrogen bonds are indicated by the dashed green line. Side-chain interactions leading to steric hindrance are denoted by the dashed purple lines.

Under these conditions, a linked marker could generate a maximal pairwise LOD score (Z_{max}) of 8.3 and an average LOD score of 5.2 indicating that the HL2 pedigree is powerful enough to detect significant linkage based on the traditional LOD-score-of-3 criterion. First, linkage to two previously identified non-syndromic low-frequency hearing loss *DFN* loci, *DFNA1*[30] and *DFNA6/DFNA14*,[31 32] was excluded (LOD = −2.0) (data not shown). A genome-wide scan to detect linkage was then initiated in the HL2 pedigree. A maximal pairwise LOD score of 7.23 was obtained with marker *D11S4207* establishing linkage to chromosome 11 (table 2). Significant pairwise LOD scores $Z_{max} \geq 3$) were obtained with multiple markers from chromosome 11q13.4–q14.3 (table 2). A maximal multipoint LOD score of 10.2 for marker *D11S937* was calculated in the HL2 pedigree. Haplotype analysis indicated that four markers *D11S4207*, *D11S937*, *D11S901,* and *D11S4147* were non-recombinant with the auditory trait in family HL2 (fig 1). Individual 53 displayed a crossover between markers *D11S987* and *D11S4207*, defining *D11S987* as a proximal flanking marker. Individual 68 displayed a crossover between markers *D11S4147* and *D11S4175* defining *D11S4175* as a distal flanking marker. This haplotype analysis suggests that the HL2 *DFN* gene lies between markers *D11S987* and *D11S4175* within a 24 cM interval that contains *MYO7A* located at 11q13.5.

Novel mutation identified in conserved MYO7A head domain residue

Given the coincident map position of *MYO7A* and the HL2 gene, the 49 exons of *MYO7A* were compared between two affected (persons 48, 50) and one unaffected (person 64) HL2 family members. The sequence comparison revealed a heterozygous G-to-C transversion at position 2164 (fig 2A) in exon 17 leading to a G722R substitution within the MYO7A head domain (fig 2B). The heterozygous nucleotide change is consistent with the dominant HL2 hearing loss inheritance pattern. Direct nucleotide sequence analysis demonstrated that the mutation faithfully co-segregated with hearing loss in the HL2 family. The mutation was absent in 200 unrelated control chromosomes, supporting the hypothesis that it represents a causative mutation, not a rare polymorphism. The G722 residue is conserved across eukaryotic myosin head domains (fig 2C). The 3D structure from the recently crystallized unconventional *Dictyostelium discoideum* myosin-IE (MyoIE) motor domain[24] was employed to perform a ConSurf calculation[25] to determine the evolution rate of the homologous glycine residue. The ConSurf analysis used 50 myosin homologues to generate an evolution rate score of 9 (on a scale of 1–9) indicating that this glycine residue is evolving slowly and therefore conserved maximally (fig 2D). To our knowledge, mutations at this MYO7A G722 residue have not been described previously in USH1B[11 21 33–36] DFNB2,[14 15] or DFNA11[16] families, nor in *Myo7a shaker-1* mouse alleles.[37] Therefore, alteration of this glycine residue in the HL2 pedigree represents a novel mutation in MYO7A.

Structural impact of G722R mutation

The 3D structure from the recently crystallized MyoIE motor domain[24] served as a structural template for MYO7A comparative modelling using the DeepView program. The MYO7A G722 residue is part of the myosin head converter domain. The converter domain interacts with a distant portion of the myosin head referred to as the relay loop.[24] The relay loop communicates conformational changes caused by actin- and nucleotide-binding to the converter domain that moves along the actin filament.[24] The relay loop and converter domain are coupled to each other in part by a conserved hydrophobic pocket formed by F727 (MyoIE, F678) from the converter domain with I482 (MyoIE, I431) and Y477 (MyoIE, Y426) from the relay loop (fig 3A). The model indicates that the substituted arginine side chain projects into the conserved hydrophobic pocket creating steric hindrance with neighboring amino acid residue F727 (MyoIE, F678) (fig 3B)

Genetic modifier controlling severity differences

Whereas the HL2 family members exhibit related patterns in their audiologic profiles, some members within different branches of the HL2 pedigree show marked variation in their level of hearing impairment. First cousins 63 and 65 are the two most mildly affected family members. Female 65 clearly demonstrates the affected haplotype and the G2164C mutation. Her audiologic profile demonstrates low-frequency hearing loss greater than expected for her age; however, by definition she does not have abnormal hearing (threshold elevations >25 dB). Figure 4A compares the hearing sensitivity of female 65 (at age 37) with female 68 (at age 31) from a different family branch. Both females display an S-shaped audiogram, and neither reports a significant medical or noise exposure history. Even though female 65 was 6 years older when the hearing test was performed she demonstrates markedly better hearing responses, with differences between the two females ranging from 40–50 dB in the low- and mid-frequencies to 30 dB in the high frequencies. A striking disparity is also seen between male 63

Table 3 *MYO7A* and **GJB2* SNP analysis in individuals with phenotypic variation

Subject	L16S†§	I205V‡	V597I§	C1666S†§	I1954L‡§	V1956I¶	F1992I§	5′ 17 in**	3′ 17 in††	*M34T‡‡
63§§	L/S	I	V	C/S	L	I/V	F	g	g	M
65§§	L/S	I	V	C/S	I/L	V	F	g	a/g	M
66¶¶	L/S	I	V	S	L	V	F	g	g	M
68¶¶	L/S	I	NT	C/S	I/L	V	F	g	a/g	M/T

†Previously reported by Levy *et al* (1997).[21]
‡Previously reported by Adato *et al* (1997).[33]
§Previously reported by Janecke *et al* (1999).[35]
¶Novel to this report
**intronic g/c SNP positioned 112 bp 5′ of exon 17 ORF
††intronic a/g SNP positioned 208 bp 3′ of exon 17 ORF
§§Mild auditory phenotype
¶¶More servere auditory phenotype
‡‡Previously reported by Kelly *et al* (1998).[38]
NT = Not determined

(at age 39) and 66 (at age 37), with differences between the two males ranging from 35–50 dB in the low- and mid-frequencies (fig 4B). Salient variations in the degree of hearing loss between similarly aged G2164C individuals of the same sex with comparable medical and noise exposure histories suggests the presence of a modifier gene. To investigate the single nucleotide polymorphism (SNP) that may be responsible for the modifier affect, we first examined the opposing allele of *MYO7A* as a nucleotide change acting in *trans* could impact expression of the mutated G2164C *MYO7A* allele, especially given that MYO7A likely forms homodimers.[14] We analysed the segregation pattern of nine *MYO7A* SNPs (fig 2B): two intron SNPs flanking intron 17 containing the G2164C mutation; and seven opening-reading-frame (ORF) SNPs leading to amino acid changes commonly found in the general population (table 3). We also compared the *GJB2* (gap junction protein, beta 2) gene exon 2 sequence with known allelic heterogeneity between DNAs from mildly versus more severely affected HL2 family members. However, none of these SNPs segregated with the phenotypic variation in the pedigree (table 3).

DISCUSSION

We have provided evidence that a G2164C mutation, resulting in a predicted G722R substitution in the MYO7A head domain, leads to progressive hearing loss first affecting low-frequency auditory thresholds. The G2164C mutation co-segregates with hearing loss in the HL2 family, is not found in 200 control chromosomes, and leads to a non-conservative G722R amino acid substitution at a slowly evolving residue. The predicted arginine substitution may disrupt the conserved hydrophobic pocket formed by amino acid side chains in the converter domain and relay loop of the myosin head. This evidence strongly supports a causative role of the *MYO7A* G2164C nucleotide alteration in hearing loss. However, the HL2 *DFN* gene maps to a large 24 cM region containing a host of genes. Further confirmation of the causative nature of the *MYO7A* G2164C alteration would be provided by functional studies such as sliding-filament assays indicating altered motility, creation of a G722R MYO7A mouse mutant that recapitulates the HL2 phenotype, or discovery of an unrelated hearing impaired family demonstrating alteration of the same MYO7A glycine residue. Interestingly, mutation of the homologous glycine residue (G674) in MYO1A was found in a person suffering from moderate to severe sensorineural hearing loss.[8]

Whereas alteration of this preserved glycine negatively impacts the auditory system, the consequences appear to be milder for the visual system. Porter and Montell[39] mutagenised the *Drosophila ninaC* (human homolog is *MYO3A*) myosin head domain targeting the same glycine residue altered in the HL2 pedigree, in addition to three other highly preserved residues within this 6-amino acid region [NINAC residues 1015–1020; GXTKXF to DXAMXS] (fig 2C). The transformant line expressing the NINAC$^{1015.4}$ protein containing these four homozygous substitutions displayed temperature-dependent protein degradation. At 18℃ the NINAC$^{1015.4}$ protein showed only a twofold decrease in levels relative to wild-type NINAC. Under these conditions, the NINAC$^{1015.4}$ transformants displayed a normal ERG and only minor retinal degeneration.[39] At 29℃ the NINAC$^{1015.4}$ protein concentration was less than 5%, and the NINAC$^{1015.4}$ transformants exhibited an abnormal electroretinogram and severe retinal degeneration.[39] When present in a stable protein, these four altered residues do not appear to have a significant impact on the function of NINAC in the *Drosophila* visual system. Consistent with these findings, a fundus examination on two G2164C HL2 family members affected by hearing loss yielded normal observations. The fundus exam does not exclude the possibility of subtle changes in photoreceptor function that may be detected by electroretinography. The DFNA11 family members, with a MYO7A three-amino-acid-residue (A886-K887-K888) deletion in the coiled-coil domain, were examined by electroretinography and found to produce normal photoreceptor responses.[40] The opthalmological findings in the DFNA11 and HL2 families suggest that these two different dominant MYO7A mutations do not result in obvious damage to the eye.

Whereas the families share this feature in common, the HL2 and DFNA11 pedigrees manifest distinct audiologic profiles. DFNA11 family members demonstrate a gently downward sloping audiogram indicating that hearing loss is greatest in the high-frequency ranges.[40] The HL2 family displays a unique audiogram contour with greater low-frequency loss suggesting that the G2164C mutation first affects hair cells in the cochlear apex. This finding is noteworthy as the vast majority of non-syndromic deafness initiate with high-frequency hearing loss.[41]

The HL2 audioprofile is similar to the progressive non-syndromic low frequency hearing loss characteristic of *DFNA1* and *DFNA6/DFNA11* caused by heterozygous mutations in the diaphanous 1 (*DIAPH1*)[42–44] and Wolfram syndrome 1 (*WFS1*)[45–48] genes, respectively. The dominant *WFS1* mutations show allelic heterogeneity, but present a uniform clinical entity of low-frequency hearing loss.[45] In contrast, the two dominant *MYO7A* mutations identified in the HL2 and DFNA11 pedigrees lead to dissimilar audioprofiles. The mechanism by which the predicted MYO7A G722R substitution and A886-K887-K888 deletion mutations differentially lead to pathogenesis of the cochlear apex versus base, respectively, remains to be elucidated. In concert with our findings in the HL2 pedigree, the dominant headbanger (*Hdb*) mouse mutant has been shown recently to demonstrate low-frequency hearing loss associated with a novel

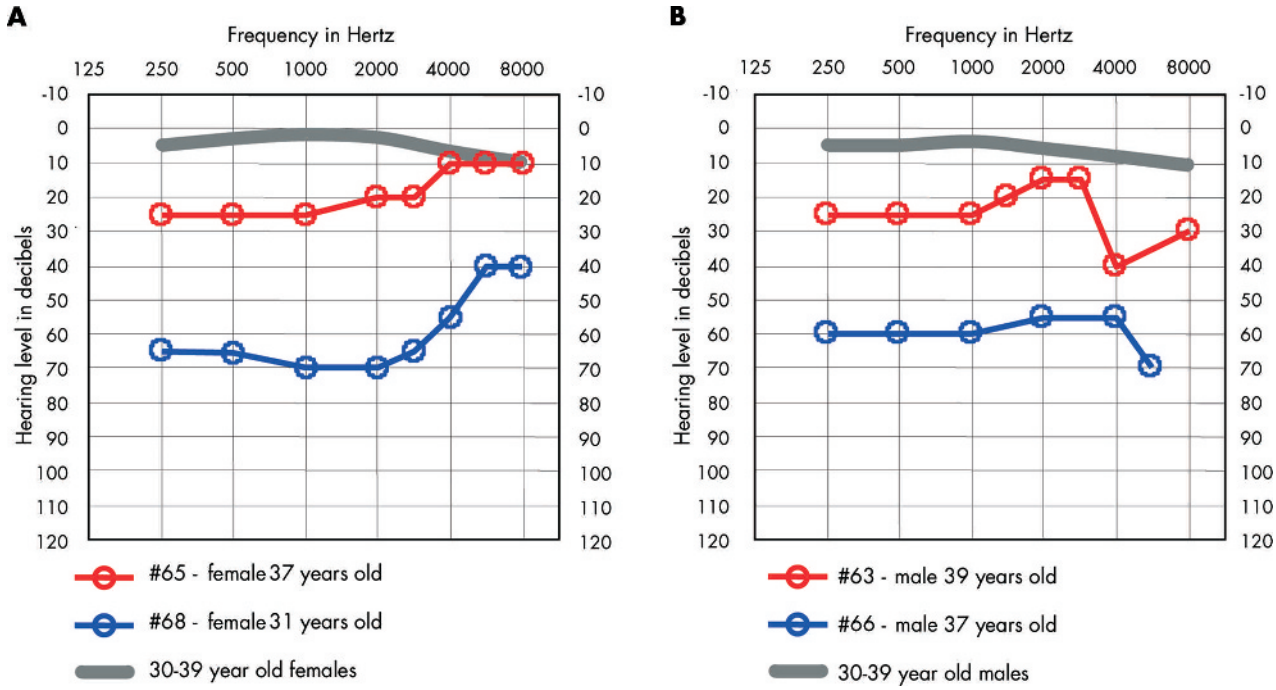

Figure 4 Variation in clinical severity between age and sex-matched HL2 family members. Pure-tone air conduction thresholds are shown for the right ears only. Responses between the right and left ears were symmetrical. Frequency in hertz (Hz) is plotted on the x-axis and hearing level in decibels (dB HL) on the y-axis. **(A)** Thresholds for female 65 are indicated by the red line, and female 68 by the blue line. **(B)** Thresholds for male 63 are shown by the red line and male 66 by the blue line.

heterozygous *Myo7a* missense mutation in the motor domain (Rhodes et al, personal communication).

The *MYO7A* G2164C mutation consistently causes low-frequency hearing loss in the HL2 pedigree; however, the clinical severity between HL2 members within two family branches is dissimilar, distinctly suggesting the presence of a genetic modifier. Based on our hearing and balance questionnaire and conversations with the HL2 family members, there is no apparent environmental influence that could result in the striking pure-tone air conduction threshold variations between different G2164C sensorineural hearing loss individuals. Previous human studies provide additional support for the role of modifier genes in the ear: intra-familial phenotypic variation has been noted for mutations in *GJB2*[49 50]; mitochondrial and nuclear auditory gene interaction has been observed;[51–53] and in one large family, a dominant modifier, *DFNM1*, that suppresses recessively inherited deafness, *DFNB26*, has been mapped in humans.[54] In mice, several genetic modifiers controlling inherited deafness have been reported as reviewed by Riazuddin.[55] Interestingly, the severity of the headbanger mouse mutant phenotype varies in different genetic background strains, suggesting interaction of the primary mutation with a modifier (Rhodes et al, personal communication).

Characterisation of genetic modifiers in humans requires exceptionally large pedigrees displaying divergent levels of hearing impairment. The HL2 pedigree is extensive with several marriages resulting in numerous offspring: couple A and B, 10 children; couple 1 and 2, 16 children; couple 3 and 4, eight children; and couple 7 and 8, 15 children. Analysis of additional family members may allow us to determine incidence of the mild auditory phenotype, mode of inheritance, and ultimately identification of the SNP(s) controlling manifestation of the *MYO7A* G2164C mutation. Whereas the *MYO7A* and *GJB2* SNPs examined in this report did not segregate with the differences in pure-tone air conduction thresholds between G2164C HL2 individuals, it is possible that SNPs within the *MYO7A* ORF not assayed in this study could partially rescue or exacerbate the mutated allele, or SNPs within the *MYO7A* promoter may control the amount of normal versus mutant gene transcribed. In addition, SNPs in other gene products known to interact with MYO7A in the ear such as harmonin (*USH1C*) and cadherin 23 (*CDH23*)[56] should also be considered as candidates. Identification of SNPs regulating the clinical severity of hearing loss will enhance understanding of gene product interactions within the auditory system and may provide predictive value in counselling patients carrying these mutations and genetic variations.

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REFERENCES

1 **Morton NE**. Genetic epidemiology of hearing impairment. *Ann NY Acad Sci* 1991;**630**:16–31.

2 **Van Camp G**, Smith RJH. Hereditary Hearing Loss Homepage. World Wide Web URL: http://dnalab-www.uia.ac.be/dnalab/hhh/, 2003.
3 **Huxley HE**. The mechanism of muscular contraction. *Science* 1969;**164**(886):1356–65.
4 **Mermall V**, Post PL, Mooseker MS. Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* 1998;**279**(5350):527–33.
5 **Montell C**, Rubin G. The Drosophila ninaC locus encodes two photoreceptor cell specific proteins with domains homologous to protein kinases and the myosin heavy chain head. *Cell* 1988;**52**(5):757–72.
6 **Berg JS**, Powell BC, Cheney RE. A millennial myosin census. *Mol Biol Cell* 2001;**12**(4):780–94.
7 **Lalwani AK**, Goldstein JA, Kelley MJ, *et al*. Human nonsyndromic hereditary deafness DFNA17 is due to a mutation in nonmuscle myosin MYH9. *Am J Hum Genet* 2000;**67**(5):1121–8.
8 **Donaudy F**, Ferrara A, Esposito L, *et al*. Multiple mutations of MYO1A, a cochlear-expressed gene, in sensorineural hearing loss. *Am J Hum Genet* 2003;**72**(6):1571–7.
9 **Walsh T**, Walsh V, Vreugde S, *et al*. From flies' eyes to our ears: mutations in a human class III myosin cause progressive nonsyndromic hearing loss DFNB30. *Proc Natl Acad Sci USA* 2002;**99**(11):7518–23.
10 **Melchionda S**, Ahituv N, Bisceglia L, *et al*. MYO6, the human homologue of the gene responsible for deafness in Snell's walter mice, is mutated in autosomal dominant nonsyndromic hearing loss. *Am J Hum Genet* 2001;**69**(3):635–40.
11 **Weil D**, Blanchard S, Kaplan J, *et al*. Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 1995;**374**(6517):60–1.
12 **Wang A**, Liang Y, Fridell RA, *et al*. Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3. *Science* 1998;**280**:1447–51.
13 **Kimberling WJ**, Pieke-Dahl S, Weston MD. Usher Syndrome Type 1. GeneReviews at GeneTests: http://www.genetests.org, 1999.
14 **Weil D**, Kussel P, Blanchard S, *et al*. The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nat Genet* 1997;**16**(2):191–3.
15 **Liu X-Z**, Walsh J, Mburu P, *et al*. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat Genet* 1997;**16**:188–90.
16 **Tamagawa Y**, Kitamura K, Ishida T, *et al*. Sensorineural hearing impairment non-syndromic, dominant DFNA11. *Adv Otorhinolaryngol* 2000;**56**:103–6.
17 **Osterhammel D**, Osterhammel P. High-frequency audiometry. *Scand Audiol* 1979;**8**:73–81.
18 **Dib C**, Faure S, Fizames C, *et al*. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996;**380**(6570):152–4.
19 **Lathrop GM**, Lalouel JM, Julier C, *et al*. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985;**37**(3):482–98.
20 **Sobel E**, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker sharing statistics. *Am J Hum Genet* 1996;**58**:1323–37.
21 **Levy G**, Levi-Acobas F, Blanchard S, *et al*. Myosin VIIA gene: heterogeneity of the mutations responsible for Usher syndrome type 1B. *Hum Mol Genet* 1997;**6**(1):111–16.
22 **Street VA**, Robinson LC, Erford SK, *et al*. Molecular genetic analysis of distal mouse chromosome 6 defines gene order and positions of the deafwaddler and opisthotonos mutations. *Genomics* 1995;**29**:123–30.
23 **Scott DA**, Kraft ML, Carmi R, *et al*. Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromic hearing loss. *Hum Mutat* 1998;**11**(5):387–94.
24 **Kollmar M**, Durrwang U, Kliche W, *et al*. Crystal structure of the motor domain of a class-I myosin. *EMBO J* 2002;**21**(11):2517–25.
25 **Glaser F**, Pupko T, Paz I, *et al*. ConSurf: identification of functional regions in proteins by surface mapping of phylogenetic information. *Bioinformatics* 2003;**19**(1):163–4.
26 **Guex N**, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 1997;**18**(15):2714–23.
27 **Schwede T**, Kopp J, Guex N, *et al*. SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res* 2003;**31**(13):3381–5.
28 **Ott J**. Computer-simulation methods in human linkage analysis. *Proc Natl Acad Sci USA* 1989;**86**:4175–8.
29 **Weeks DE**, Ott J, Lathrop GM. SLINK: a general simulation program for linkage analysis. *Am J Hum Genet* 1990;**47**(3):A204 (Suppl.).
30 **Leon PE**, Raventos H, Lynch E, *et al*. The gene for an inherited form of deafness maps to chromosome 5q31. *Proc Natl Acad Sci USA* 1992;**89**:5181–4.
31 **Lesperance MM**, Hall JW, Bess FH, *et al*. A gene for autosomal dominant nonsyndromic hereditary hearing impairment maps to 4p16.3. *Hum Mol Genet* 1995;**4**(10):1967–72.
32 **Van Camp G**, Kunst H, Flothmann K, *et al*. A gene for autosomal dominant hearing impairment (DFNA14) maps to a region on chromosome 4p16.3 that does not overlap the DNFA6 locus. *J Med Genet* 1999;**36**(7):532–6.
33 **Adato A**, Weil D, Kalinski H, *et al*. 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* 1997;**61**:813–21.
34 **Cuevas JM**, Espinos C, Millian JM, *et al*. Identification of three novel mutations in the MYO7A gene. *Hum Mutat* 1999;**14**(2):181.
35 **Janecke AR**, Meins M, Sadeghi M, *et al*. Twelve novel myosin VIIA mutations in 34 patients with Usher syndrome type I: confirmation of genetic heterogeneity. *Hum Mutat* 1999;**13**(2):133–40.
36 **Najera C**, Beneyto M, Blanca J, *et al*. Mutations in myosin VIIA (MYO7A) and usherin (USH2A) in Spanish patients with Usher syndrome types I and II, respectively. *Hum Mutat* 2002;**20**(1):76–7.
37 **Gibson F**, Walsh J, Mburu P, *et al*. A type VII myosin encoded by the mouse deafness gene shaker-1. *Nature* 1995;**374**:62–4.
38 **Kelley PM**, Harris DJ, Comer BC, *et al*. Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. *Am J Hum Genet* 1998;**62**(4):792–9.
39 **Porter J**, Montell C. Distinct roles of the Drosophila ninaC kinase and myosin domains revealed by systematic mutagenesis. *J Cell Biol* 1993;**122**(3):601–12.
40 **Tamagawa Y**, Ishikawa K, Ishikawa K, *et al*. Phenotype of DFNA11: a nonsyndromic hearing loss caused by a myosin VIIA mutation. *Laryngoscope* 2002;**112**(2):292–7.
41 **Smith RJ**, Huygen PL. Making sense of nonsyndromic deafness. *Arch Otolaryngol Head Neck Surg* 2003;**129**(4):405–6.
42 **Lalwani AK**, Jackler RK, Sweetow RW, *et al*. Further characterisation of the DFNA1 audiovestibular phenotype. *Arch Otolaryngol Head Neck Surg* 1998;**124**:699–702.
43 **Leon PE**, Bonilla JA, Sanchez JR, *et al*. Low frequency hereditary deafness in man with childhood onset. *Am J Hum Genet* 1981;**33**(2):209–14.
44 **Lynch ED**, Lee MK, Morrow JE, *et al*. Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the Drosophila gene diaphanous. *Science* 1997;**278**:1315–18.
45 **Bespalova IN**, Van Camp G, Bom SJ, *et al*. Mutations in the Wolfram syndrome 1 gene (WFS1) are a common cause of low frequency sensorineural hearing loss. *Hum Mol Genet* 2001;**10**(22):2501–8.
46 **Young TL**, Ives E, Lynch E, *et al*. Non-syndromic progessive hearing loss DNFA38 is caused by heterozygous missense mutation in the Wolfram syndrome gene WFS1. *Hum Mol Genet* 2001;**10**(22):2509–14.
47 **Lesperance MM**, Hall JW, San Agustin TB, *et al*. Mutations in the Wolfram syndrome type 1 gene (WFS1) define a clinical entity of dominant low-frequency sensorineural hearing loss. *Arch Otolaryngol Head Neck Surg* 2003;**129**(4):411–20.
48 **Pennings RJ**, Bom SJ, Cryns K, *et al*. Progression of low-frequency sensorineural hearing loss (DFNA6/14-WFS1). *Arch Otolaryngol Head Neck Surg* 2003;**129**(4):421–6.
49 **Sobe T**, Vreugde S, Shahin H, *et al*. The prevalence and expression of inherited connexin 26 mutations associated with nonsyndromic hearing loss in the Israeli population. *Hum Genet* 2000;**106**:50–7.
50 **Cohn ES**, Kelley PM, Fowler TW, *et al*. Clinical studies of families with hearing loss attributable to mutations in the connexin 26 gene (GJB2/DFNB1). *Pediatrics* 1999;**103**(3):546–50.
51 **Bykhovskaya Y**, Estivill X, Taylor K, *et al*. Candidate locus for a nuclear modifier gene for maternally inherited deafness. *Am J Hum Genet* 2000;**66**:1905–10.
52 **Abe S**, Kelley PM, Kimberling WJ, *et al*. Connexin 26 gene (GJB2) mutation modulates the severity of hearing loss associated with the 1555A-G mitochondrial mutation. *Am J Hum Genet* 2001;**103**(4):334–8.
53 **Guan MX**, Fischel-Ghodsian N, Attardi G. Biochemical evidence for nuclear gene involvement in phenotype of non-syndromic deafness associated with mitochondrial 12S rRNA mutation. *Hum Mol Genet* 1996;**5**(7):963–71.
54 **Riazuddin S**, Castelein CM, Ahmed ZM, *et al*. Dominant modifier DFNM1 suppresses recessive deafness DFNB26. *Nat Genet* 2000;**26**:431–4.
55 **Riazuddin S**, Ahmed ZM, Friedman TB, *et al*. Genetic modifiers of hereditary hearing loss. *Adv Otorhinolaryngol* 2002;**61**:224–9.
56 **Boeda B**, El-Amraoui A, Bahloul A, *et al*. Myosin VIIa, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. *EMBO J* 2002;**21**(24):6689–99.