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Mutations in the γ-Actin Gene (*ACTG1*) Are Associated with Dominant Progressive Deafness (DFNA20/26)

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Age-related hearing loss (presbycusis) is a significant problem in the population. The genetic contribution to age-related hearing loss is estimated to be 40%–50%. Gene mutations that cause nonsyndromic progressive hearing loss with early onset may provide insight into the etiology of presbycusis. We have identified four families segregating an autosomal dominant, progressive, sensorineural hearing loss phenotype that has been linked to chromosome 17q25.3. The critical interval containing the causative gene was narrowed to ~2 million bp between markers D17S914 and D17S668. Cochlear-expressed genes were sequenced in affected family members. Sequence analysis of the γ-actin gene (*ACTG1*) revealed missense mutations in highly conserved actin domains in all four families. These mutations change amino acids that are conserved in all actins, from protozoa to mammals, and were not found in >100 chromosomes from normal hearing individuals. Much of the specialized ultrastructural organization of the cells in the cochlea is based on the actin cytoskeleton. Many of the mutations known to cause either syndromic or nonsyndromic deafness occur in genes that interact with actin (e.g., the myosins, espin, and harmonin). The mutations we have identified are in various binding domains of actin and are predicted to mildly interfere with bundling, gelation, polymerization, or myosin movement and may cause hearing loss by hindering the repair or stability of cochlear cell structures damaged by noise or aging. This is the first description of a mutation in cytoskeletal, or nonmuscle, actin.

Introduction

Twenty-eight million Americans suffer from hearing impairment, including half of all octogenarians (Morton 1991). Although some degree of age-related hearing loss (ARHL, presbycusis) must be ascribed to environmental exposures, it is clear that a significant fraction—perhaps as much as 50%—is genetically determined (Karlsson et al. 1997; Gates et al. 1999; DeStefano et al. 2003). Cochlear pathologies associated with ARHL include degeneration of hair cells, neuronal loss, and atrophy of the stria vascularis (Fransen et al. 2003). Clinical correlates of these pathologies have been proposed, with sensory presbycusis—the most common type of ARHL—being defined as an elevation of high frequency pure-tone thresholds secondary to loss of hair cells at the basal end of the cochlea (Schuknecht and Gacek 1993). This region is especially vulnerable to damage by noise and aging, both of which are likely to be important components of ARHL (Fransen et al. 2003). To understand the etiology of ARHL, the physiology of hearing and the pathology of hearing loss must be understood at the molecular level. This imperative makes genes associated with late-onset nonsyndromic deafness excellent candidates for involvement in ARHL. The phenotype and progression of hearing loss are similar; only the age at onset differs (Steel 1998).

Much of the function of the inner ear relies on the specialized structure of the auditory hair cell. These structures are highly dependent on their actin cytoskeletons (Hofer et al. 1997). In the apical region of the hair cell, the projecting stereocilia contain parallel bundles of actin fibers with identical polarity (Tilney et al. 1983); in the cuticular plate immediately below the apical surface, a dense gel-like network of actin filaments anchors the stereocilia (DeRosier and Tilney 1989), and encircling the hair cell near the apical region is the adherens junction, which contains antiparallel actin filaments (Hirokawa and Tilney 1982). The basolateral region of the hair cell also contains actin structures that

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support the cell membrane and provide a track for the passage of synaptic vesicles.

The predominant actin isoform in the auditory hair cell is γ-actin, encoded by *ACTG1* (MIM 102560), one of six functional actin genes in humans. Four actin genes encode isoforms responsible for contractile movement in muscle. The remaining actin genes, *ACTG1* and *ACTB,* code for proteins found in the cytoskeleton of all mammalian cells and differ from each other by only four amino acids at or near the N-terminus. β-Actin is the predominant isoform in most cells, although γ-actin predominates in intestinal epithelial cells as well as in auditory hair cells, where it is found in stereocilia, the cuticular plate, and adherens junctions (Khaitlina 2001). It is notable that actin structures appear to be structurally damaged as a consequence of noise exposure and aging (Li and Hultcrantz 1994; Hultcrantz and Li 1995; Hu and Henderson 1997).

DFNA20 (MIM 604717) and *DFNA26* (MIM 604717) are dominant nonsyndromic deafness loci that map to the telomeric region of the long arm of chromosome 17 (Morell et al. 2000; Yang and Smith 2000). Persons affected with the DFNA20 and DFNA26 (DFNA20/26) disorders display sensorineural hearing loss that, like ARHL, begins in the high frequencies and steadily progresses to include all frequencies (Yang and Smith 2000; Elfenbein et al. 2001). Distortion product otoacoustic emission (DPOAE) data are consistent with a cochlear site of lesion (Elfenbein et al. 2001). We have determined that mutations in γ-actin are the basis for hearing loss in four families affected with DFNA20/26.

Methods

Subjects

Family MSUDF1.—Family MSUDF1 was ascertained through the Michigan State University (MSU) Genetics Clinic. Physical examination of the proband by a clinical geneticist included examination for features associated with syndromic hearing losses. An additional neurological exam provided no evidence for vestibular dysfunction. Audiologic examination of family members included otoscopy, tympanometry, and pure-tone and air- and bone-conduction thresholds. DPOAE and speech-recognition data were collected from a subset of persons. Individuals aged ⩾25 years were considered affected if they had bilateral sloping sensorineural hearing loss in the high frequencies beyond the 90th percentile of an age- and sex-dependent curve of the general population (International Organization for Standardization 1984; Cruickshanks et al. 1998). The proband's 72-year-old spouse, who had a history of noise exposure, showed this pattern but demonstrated hearing within normal limits in the low frequencies. He was considered unaffected. Information on deceased family members was obtained from relatives and census data. Written informed consent for all procedures and investigations was obtained according to the research protocol approved by the MSU Institutional Review Board.

Families 1250 and 1320.—Families 1250 and 1320 were ascertained through the Department of Otolaryngology–Head and Neck Surgery at the University of Iowa. Family histories were obtained by questionnaire and personal interviews. Information on deceased family members was obtained from relatives. Otologic examination and audiograms were performed or reviewed on most family members. Individuals were considered affected if they had bilateral sensorineural hearing loss in the high frequencies below the 95th percentile of an age- and sex-dependent control curve of the general population. Normal-hearing individuals >20 years of age were coded as unaffected if hearing thresholds at most frequencies were better than 20 dB hearing level or were above the 50th percentile. Peripheral venous blood was collected by venipuncture from consenting individuals. All procedures complied with accepted protocols from the Institutional Review Board at the University of Iowa.

Kindred 6.—Kindred 6 was ascertained at The Rockefeller University. A family history was obtained through questionnaires and oral interview. Pure-tone audiometry (air and bone conduction), a medical history and DNA samples were obtained from pedigree members in three generations. Hearing-impaired family members ⩾35 years of age presented with moderate-to-severe hearing loss in the low frequencies, which sloped to profound hearing loss in the middle-to-high frequencies. Younger hearing-impaired family members presented with normal hearing in the low frequencies, mild-to-moderate hearing loss in the middle frequencies, and moderate-to-severe hearing loss in the high frequencies. Average age at onset was 13.2 years (SD 4.6 years). The deafness locus segregating in this pedigree linked to markers located on 17q25.3, with a maximum two-point LOD score of 6.3 (DeWan et al. 2003). Informed consent for all procedures was obtained according to the research protocol that was approved by The Rockefeller University Institutional Review Board.

DNA Sequencing and Mutation Detection

Generation of somatic cell hybrids.—To efficiently screen candidate genes by sequence analysis, human-mouse somatic hybrid cell lines were used. A whole blood sample from the MSUDF1 proband was supplied to GMP Genetics for establishment of somatic hybrid cell lines containing single copies of human chromosome 17. Three cell lines were obtained, one of which contained the chromosome 17 that bears the haplotype of the critical region containing the *DFNA20* locus. These

cell lines were used to generate cDNA for RT-PCR sequencing of large genes that had <90% identity with mouse sequences to avoid coamplification of mouse cDNA.

γ-Actin mutation identification.—PCR primers synthesized in the MSU Genomic Technology Support Facility (GTSF) were used for amplification of human γ-actin exons and flanking intron sequences, as follows: exon 1, GA1453 (5′-gctttcggaaagatcgccat-3′) and GA1454 (5′-tacgtaacgtccacggctcg-3′), 390 bp; exons 2–3, GA1455 (5′-gttgcgatcttggaggccac-3′) and GA1456 (5′-acagagcctggaacagcgaa-3′), 698 bp; exons 4–5, GA1457 (5′-cactggcatgcagcatgttg-3′) and GA1458 (5′-gcacggcttcagctcgcaga-3′), 864 bp; exon 6 and 3′ UTR, either GA1459 (5′-ctctgcgagctgaagccgtg-3′) and GA1460 (5′-cgaagccaagctgagcagca-3′), 1,014 bp, or GAA04 (5′-tgaggctagcatgaggtgtg-3′, 270 bp, paired with GA1459. PCR amplification was performed under standard conditions, by use of *Taq* DNA polymerase (QIAGEN) with 60°C annealing temperature. Following amplification, the PCR products were pooled from three identical reactions and were purified using QIAquick PCR Purification Kit (QIAGEN). Purified PCR products were sequenced using the ABI Prism BigDye terminator and were analyzed on an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems) and ABI 3730 Genetic XL DNA Analyzer or ABI Prism 3700 DNA Analyzer in the GTSF. DNA sequences were analyzed using the Sequencher software (Gene Code Corporation).

γ-Actin mutation detection in control samples.—To evaluate whether the sequence variations we identified occurred in the normal hearing population, we sequenced γ-actin in unrelated individuals who had been tested and found to have normal hearing for their age. These control samples are whites and ethnically match our families, all of which are North American and of Western European descent. For the control samples, 5-μl PCRs were treated with 1 U of shrimp alkaline phosphatase and 10 U of exonuclease I (USB) at 37°C for 15 min and 80°C for 15 min and were sequenced and analyzed following the same procedures described earlier. For mutation T89I and K118M, exons 2–3 were amplified in 110 individuals by use of the primer pairs described above and were sequenced using forward primers. For P264L and P332A, primers GA1457/58 and GA1459/GAA04, respectively, were used to amplify genomic DNA from 51 individuals.

Results

DFNA20/26 Critical Interval Determination

The DFNA20 locus originally was assigned to chromosome 17q25.3 between markers D17S1806 and D17S668 (Morell et al. 2000). The MSUDF1 family (fig. 1) contained a 21-year-old member with normal hearing who carried a recombination within this interval. Two years later, this subject's hearing was still well within normal limits. Since all affected family members had clear signs of hearing loss by this age (Elfenbein et al. 2001), this subject was considered unaffected, which allowed us to refine the centromeric boundary of the DFNA20 critical interval to 50 kb telomeric to marker D17S914. The telomeric boundary remained unchanged, thereby defining a 5.3-cM region of ~2×10^6 bp.

Two families segregating autosomal dominant nonsyndromic deafness have been mapped to a region overlapping the DFNA20 interval and have been given a DFNA26 designation (families 1250 and 1320 in fig. 1) (Yang and Smith 2000). Although these were large families, they defined a critical interval that was still >6×10^6 bp. Most recently, linkage in an additional family has been described that encompasses the same critical interval as DFNA20 (kindred 6 in fig. 1) (DeWan et al. 2003). The gene for Usher syndrome 1G and the Jackson shaker mouse mutant localizes ~6×10^6 bp centromeric to the DFNA20 critical interval and has been identified as *SANS* (Kikkawa et al. 2003; Weil et al. 2003).

Phenotype of Hearing Loss

All families with DFNA20/26 display progressive, bilateral, sensorineural hearing loss that begins in the high frequencies. As age increases, the degree of hearing loss increases, with threshold shifts seen at all frequencies, although a sloping configuration is usually maintained. Age at onset varies between families, with self-reported hearing loss noticed in the 3rd decade in MSUDF1 but beginning in the 2nd decade in families 1250 and 1320 and in kindred 6 (fig. 1). Hearing threshold shifts are detectable beginning in the early teens in MSUDF1 and earlier in family 1250 and kindred 6. In families 1250 and 1320, by the 6th decade, progression of hearing loss leads to profound deafness across all frequencies. Members of both of these families have cochlear implants. In contrast, the proband in MSUDF1, at 65 years of age, had profound loss at >1000 Hz but retained thresholds at 60–80 dB in the lower frequencies.

There is no evidence of vestibular dysfunction in any persons in these families, by self-report or by testing in MSUDF1 (Elfenbein et al. 2001). Questionnaires have not revealed any additional physical problems and no abnormalities of stature, hands, or craniofacial structures have been observed. Lifespan appears normal.

Genes in the Critical Interval

The DFNA20/26 critical interval between markers D17S914 and D17S688 contains few genes with known function. Multiple builds of the Human Genome Project produced incomplete contigs across the interval. Eventually, ~20 genes were identified in the region; most of these were hypothetical proteins with no documented functions. Not all of the expressed UniGene sequences

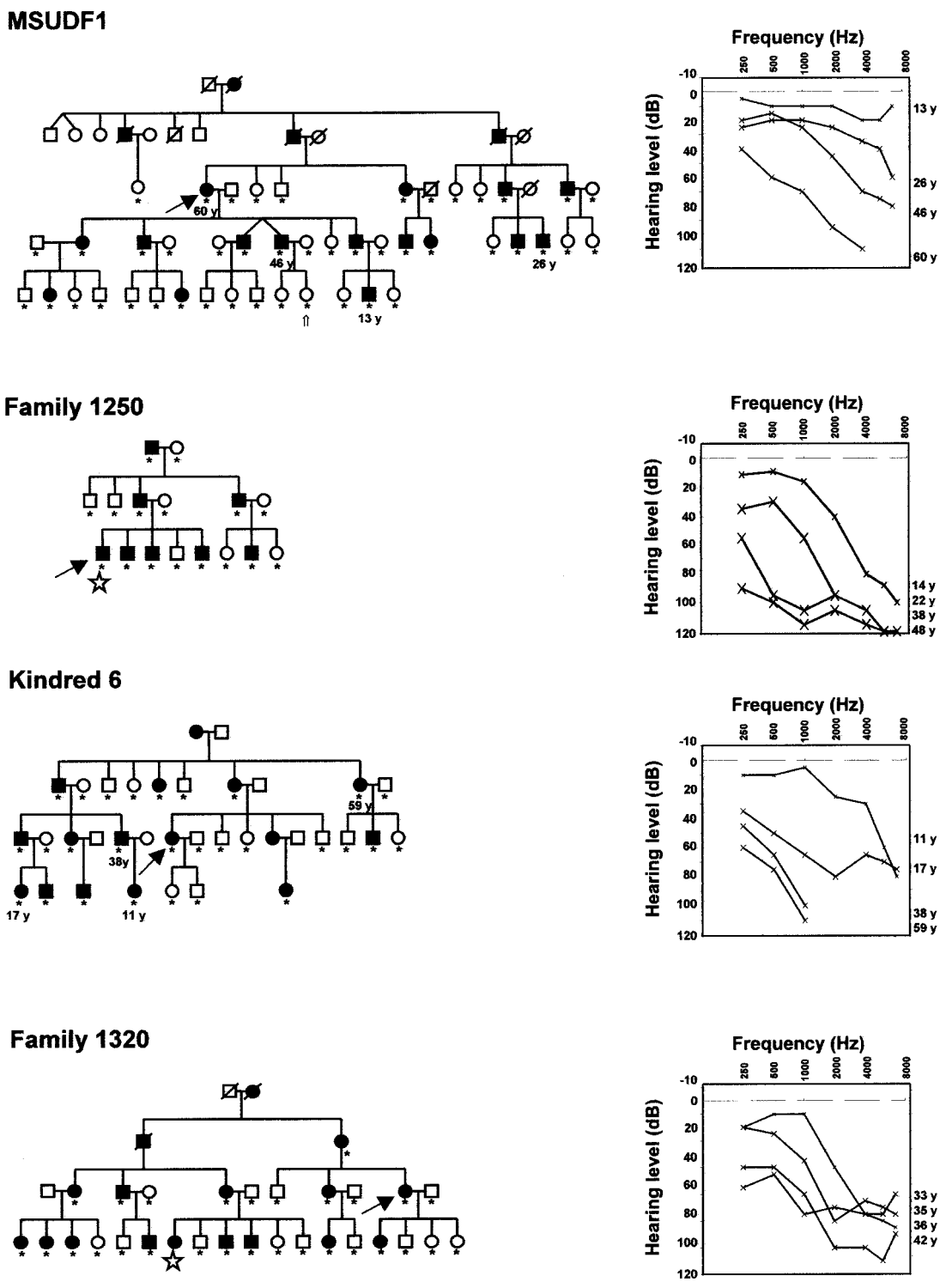

Figure 1 Families with autosomal dominant hearing loss linked to chromosome 17q25. *Left,* pedigrees of four families segregating highly penetrant autosomal dominant, sensorineural, postlingual hearing loss. Asterisks (*) indicate the persons in each pedigree who received audiological testing. The 21-year-old recombinant in family MSUDF1 who allowed refinement of the critical interval is indicated by an unfilled arrow; probands are indicated by filled arrows. *Right,* threshold data from the better ears of representative patients. Ages at testing are noted to the right of the audiograms. The individuals used to provide the graphic data are denoted by a star in families 1250 and 1320 and by their ages in family MSUDF1 and kindred 6.

were identified as RefSeq genes and not all of the genes were complete. Many of these genes were examined for expression in the inner ear, either by testing of STS markers on a human cochlear cDNA library (a gift from James Battey and Lori Hampton, National Institute on Deafness and Other Communication Disorders) or by searching the Inner Ear Gene Expression Database for the homolog in the mouse. Genes that showed expression in the cochlea or that contained domains that suggested cochlea-related functions were sequenced. There were a substantial number of genes that could be considered strong candidates.

For sequencing of genes in family MSUDF1, conversion cell hybrids were generated (GMP Genetics) that produced mouse cell lines, each carrying a single chromosome 17 from the proband. Sequencing was then carried out by PCR amplification of genomic DNA, cDNA from the hybrid cell lines, or genomic DNA from an affected individual in the family. Genes with large mRNAs and <90% human/mouse identity were sequenced from RT-PCR products. Genes sequenced in MSUDF1 included: *MGC46523* (*SLC26A11* solute transporter, similar to Pendrin), *KIAA1554* (probable thromboxane A2 receptor isoform), *KIAA1303* (raptor, G protein with WD repeats), *KIAA1118* (mouse acrosomal protein AZ1 homology, ERM domain, collagen homology domain), *FLJ31528* (hypothetical protein, mouse homolog has inner ear expression), *MGC15523* (transporter protein domain, integrin domain, strong inner ear expression), *KIAA1447* (collagen homology domain), *FLJ23058* (no known protein homologies, strong ear expression, multiple tissues), *PDE6G* (phosphodiesterase 6 G-retinal specific), *MRPL12* (mitochondrial ribosomal protein L12), and *SLC25A10* (mitochondrial solute carrier, dicarboxylate transporter). Differences from the reference sequence were evaluated for likelihood to affect protein synthesis or function, for conservation between species, and for segregation with hearing loss. Many of the genes contained polymorphisms that were found in multiple family members, both affected and nonaffected.

Numerous genes in the region flanked by D17S784 and D17S928 were screened for mutations in families 1250 and 1320. The list included *CBX4, FLJ20753, GAA, KIAA0111, CARD14, SGSH, KIAA1554, NPTX1, Raptor, BAIAP2, AATK, KIAA1118, FLJ31528, MGC15523, HGS, MRPL12, SLC25A10, SECTM1, SLC16A3,* and *CSNK1D*.

γ-Actin

Because the telomeric region of mouse chromosome 11 has homologous synteny with human chromosome 17, we were able to place several additional genes in the DFNA20/26 critical region by inspection of mouse genome data. The most interesting genes were *Fscn2* (which encodes an actin bundling protein) and *Actg* (γ-actin). Sequencing of human *FSCN2* yielded an intron polymorphism that we used to place this gene within the critical interval in MSUDF1. Actin was sequenced from genomic DNA from the hybrid cell line and from an affected individual (fig. 2), and nine differences from the reference sequence were found. When Genbank accession #M19238 was used as the reference sequence, differences included one in the 5′ transcribed noncoding region (494, G→A), six in introns (C677T, G833T, C1386A, G1432A, G1433T, and T2122A), and a synonymous change in the stop codon (TAG→TAA). None of these variations was predicted to affect function of γ-actin, and they were also found in unaffected family members and the controls. Three variants—G833T, T2122A, and TAG→TAA—were also found in reference sequence AC139149. The only sequence change unique to all 17 affected family members was the C→T transition at nucleotide 340 in exon 3 of the processed mRNA, which produces a nonconservative amino acid substitution, T89I. This amino acid is perfectly conserved in cytoplasmic actin, in species ranging from nematodes to mammals (fig. 3). In skeletal muscle actin, serine can replace threonine at this position. In comparison with 141 unique actin sequences from species including fungi, plants, protozoa, invertebrate, and vertebrates, this position is occupied by a hydroxyl-containing amino acid (Sheterline et al. 1998). This mutation was not found in 220 chromosomes from individuals with documented normal hearing.

Sequence analysis of actin from families 1250 and 1320 (Yang and Smith 2000) also revealed mutations in γ-actin. A lysine-to-methionine change was found at amino acid 118 in family 1250. The mutation segregated with hearing loss in this family (eight affected family members) and was not found in 220 chromosomes from normal hearing individuals. The amino acid in this position of actin is invariant in vertebrates and invertebrates and is replaced by arginine in a few species of plants and fungi. In family 1320, a proline-to-alanine substitution segregating with hearing loss was identified in amino acid 332. This amino acid is invariant in cytoplasmic actin (fig. 3), and, out of 141 actin sequences, it is replaced by serine in only 1 species (a protozoan). This mutation was found in the eight affected family members tested, and no change in this amino acid was found in 102 chromosomes from individuals with normal hearing.

Kindred 6 (DeWan et al. 2003) carries a proline-to-leucine mutation at amino acid 264 (fig. 2). This amino acid is perfectly conserved in the γ-actin isoforms (fig. 3), is invariant in 138/141 actin sequences, and is never leucine (Sheterline et al. 1998). The mutation cosegregates with hearing loss in 11 persons from kindred 6 and in a 20-year-old individual who carries this mutation

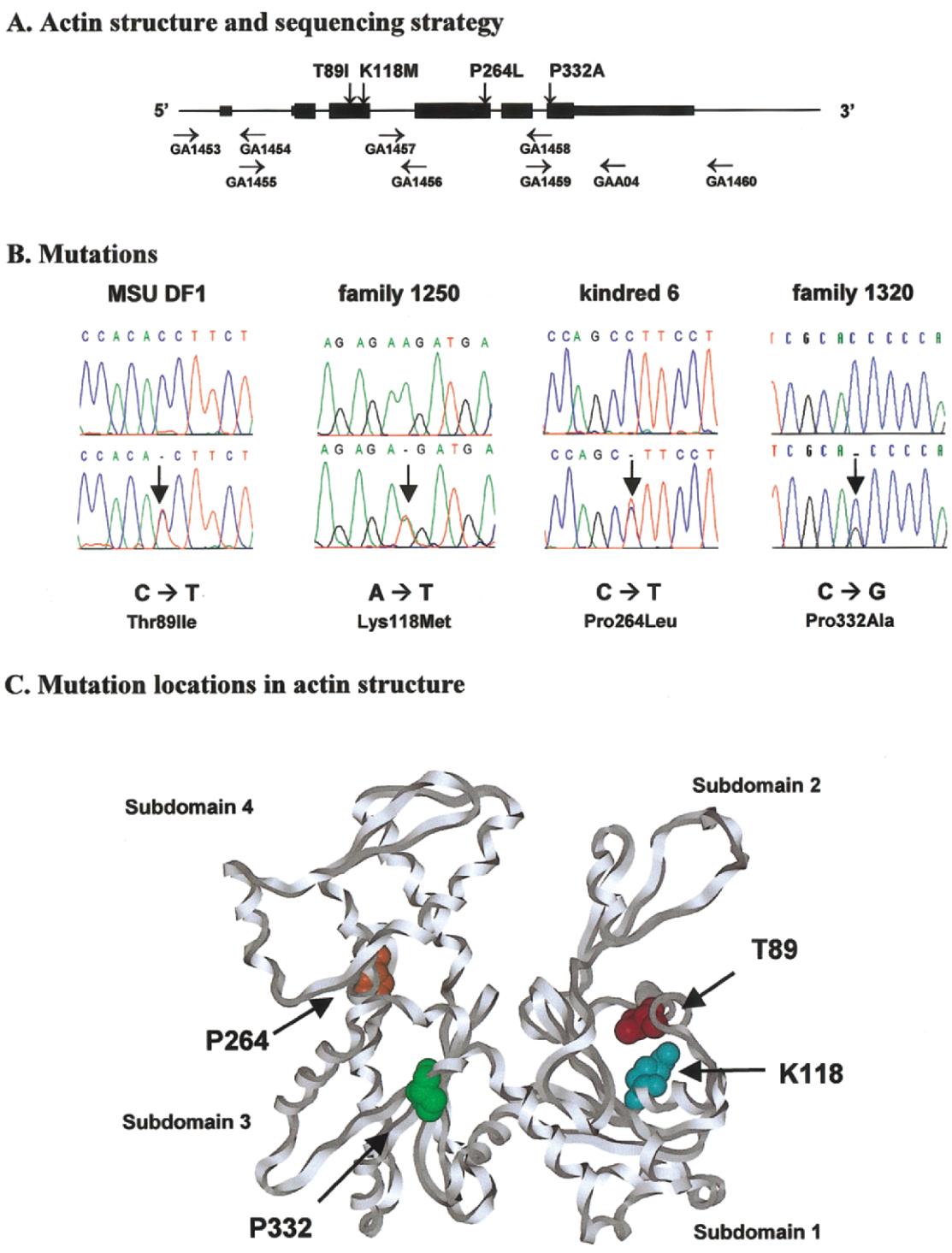

Figure 2 γ-Actin mutation analyses. *A*, Genomic structure of γ-actin showing the mature mRNA sequence in large blocks. There is a 5′ noncoding exon and a substantial 3′ UTR. The position of the mutations is identified with a down-pointing arrow (↓), and the primers used for sequencing are indicated. *B*, Sequence results for each of the mutations. Each mutation is heterozygous in affected individuals. *C*, Structural model of actin (Kabsch et al. 1990) showing the positions of the amino acids that are altered in families with DFNA20/26.

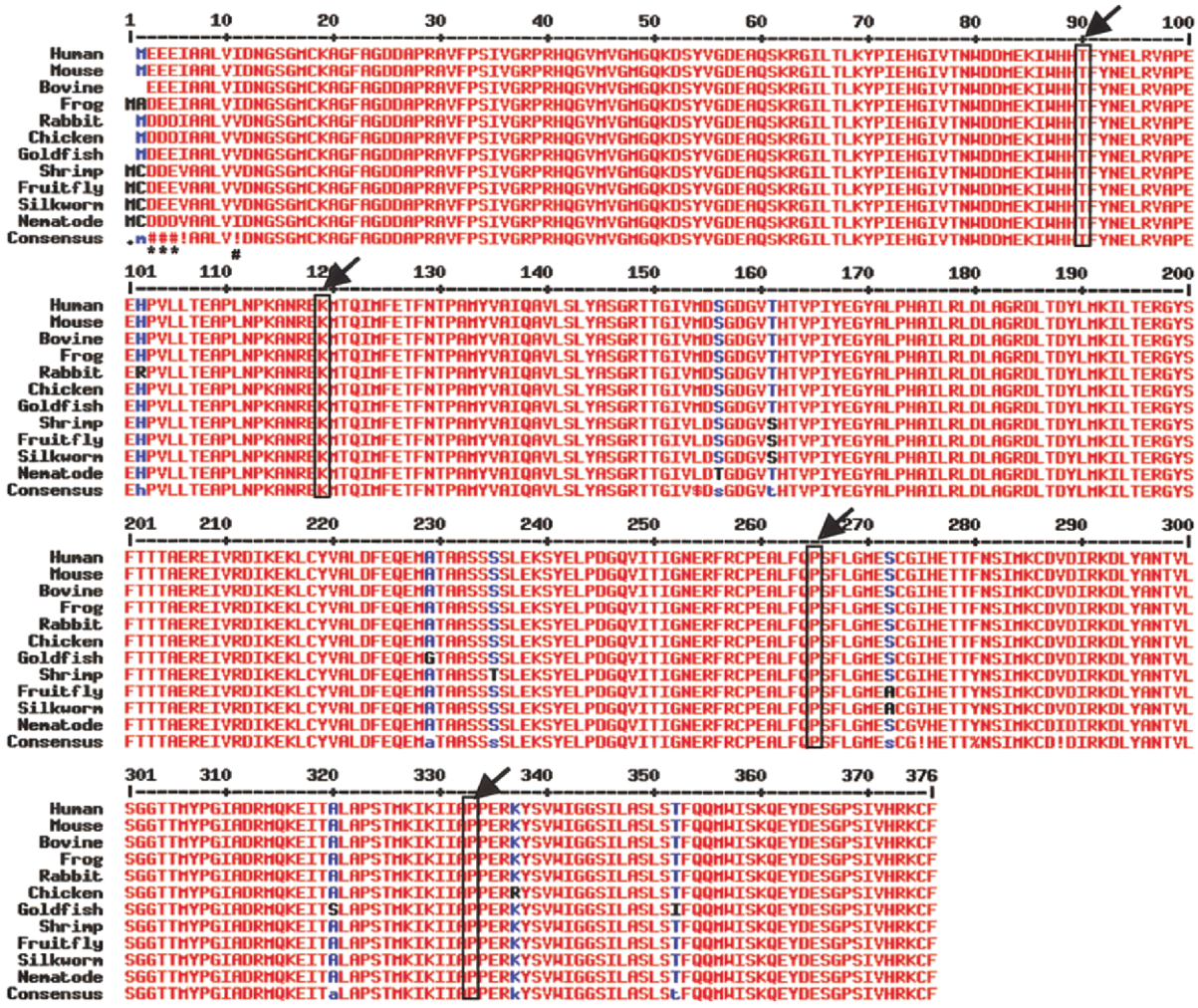

Figure 3 Conservation of actin proteins. MultAlin view of cytoplasmic actin molecules from various species. The top five sequences are γ-actin from human, mouse, bovine, frog, and rabbit, respectively, followed by chicken and goldfish β-actin, shrimp actin, fruitfly actin 5c, silkworm actin A4, and nematode actin. Human β-actin differs from γ-actin in that it carries an E at the N-terminal positions marked with asterisks (*) and a V at the position marked with a number sign (#).

but may be too young to show signs of hearing loss. The mutation was not found in 102 chromosomes from normal hearing individuals.

Discussion

γ-Actin Mutations Are Associated with Hearing Loss

DFNA20/26 hearing loss is associated with missense mutations in *ACTG1*. Actin is one of the most highly conserved proteins known, with >90% homology between the single yeast actin (*ACT1*) gene and human γ-actin. Vertebrate γ-actin proteins are identical in humans, mice, cattle, and chickens (Sheterline 1998). Until now, no mutations in vertebrate nonmuscle actins have been identified. De novo and familial mutations in heart muscle actin, *ACTC*, have been reported to cause dilated cardiomyopathy (Olson et al. 1998) and hypertrophic cardiomyopathy (Olson et al. 2000), whereas mutations in α-actin can cause nemaline myopathy (Ilkovski et al. 2001). Mutations that change the amino acid structure of actin are generally not tolerated, because actin has a remarkable number of interacting ligands and protein partners that bind noncovalently with actin to produce movement, support cellular structures, and regulate cellular responses.

We have identified four families with mutations in *ACTG1*. In these families, the only phenotype is progressive hearing loss. No other physical symptoms have been observed, and lifespan appears normal. There are, however, differences between families in average age at

onset of hearing loss and rate of progression. The four different *ACTG1* mutations occur in three of the four subdomains of the γ-actin protein (fig. 2*c*).

Two of the mutations, T89I and K118M, occur in subdomain 1. The T89I mutation is in an alpha helix that is thought to participate in binding of fimbrin, a bundling protein. An identical mutation in ACT1 in yeast has been identified as a suppressor of a mutation of SAC6 (yeast fimbrin) (Adams et al. 1989; Honts et al. 1994). The T89I mutation in ACT1 produced a mild temperature-sensitive phenotype in yeast (Adams et al. 1989). K118M is near the fimbrin-binding domain that has been implicated in interactions with fimbrin, gelsolin (Feinberg et al. 1997), and α-actinin (Fabbrizio et al. 1993; McGough et al. 1994). In the families segregating these mutations, the hearing loss is dissimilar, with family 1250 segregating a more severe phenotype that is characterized by an earlier age at onset and more rapid progression (fig. 1).

P264L is in a proposed hydrophobic plug for interstrand interactions in subdomain 4, very near the actin self-assembly site. It is not known whether this amino acid is required for actin filament assembly. The family carrying this mutation has an early age at onset of hearing loss that progresses rapidly to profound levels at the higher frequencies.

The actin mutation found in family 1320, P332A, is in a 3–amino acid loop in subdomain 3 of the actin protein (Khaitlina 2001). There is evidence that this loop is part of the primary contact site for myosin (Fabbrizio et al. 1993). The hearing loss in this family is similar to the hearing loss in family 1250 and in kindred 6 (fig. 1).

Role of Actin in Maintaining Cochlear Structure

Of the two isoforms of actin in nonmuscle cells, β-actin generally predominates. Only in intestinal cells and auditory hair cells is γ-actin more abundant (Khaitlina 2001). In tissues in which cells are frequently replaced, such as the intestinal epithelium, it is possible that the effects of these actin mutations have little consequence. However, in the cochlear hair cells, which do not regenerate, the long-term effect of subtle actin mutations may have a particular impact. In auditory hair cells, γ-actin is found in all actin-containing structures, including the cuticular plate, adherens junction, and the stereocilia (Hofer et al. 1997); β-actin is primarily restricted to the stereocilia (Schneider et al. 2002). The importance of actin to hair cell function is reflected by the fact that mutations in many of the actin-interacting proteins cause hearing loss. Included in this list are mutations in several of the unconventional myosins, espin, and harmonin that can lead to various types of dominant and recessive syndromic and nonsyndromic deafness (Petit et al. 2001; Anagnostopoulos 2002; Boeda et al. 2002; Zuo 2002).

Hair cells in mammals do not regenerate and consequently must withstand a lifetime of use and misuse. It is likely that actin and actin-interacting proteins play an important role in maintaining these cells and in repairing damage. Not unexpectedly, aging and noise damage have profound effects on the structure of the hair cells. Mice with ARHL have cochlear changes that involve actin (Hultcrantz and Li 1995; Hu et al. 2002), and humans with presbycusis show hair-cell degeneration or loss (Soucek et al. 1987). Noise-induced hair-cell damage results in scar formation necessary to retain the integrity of the reticular lamina, a process that is likely to involve the actin-myosin system (Raphael 2002) very early in the apoptotic cascade.

The mutations we have identified in families with DFNA20/26 occur in various binding domains of actin and are predicted to mildly interfere with bundling, gelation, polymerization, or myosin movement. As such, they may produce hearing loss by reducing the stability of these cochlear cell structures or by impeding the repair of structures damaged by noise or aging. Given the central role of actin in the function of the inner ear, it might not be surprising that mutations in *ACTG1* may cause deafness. The unanticipated aspect of our finding is that mutations can be found at all in this remarkably evolutionarily conserved protein and that it is possible to have mutations in this ubiquitously expressed gene that are associated only with nonsyndromic deafness. This finding may be a reflection of the fact that the cells of the organ of Corti are different from other ACTG1-expressing cells in the amount of mechanical trauma to which they are subjected and the fact that, as mechanical transducers of sound, their function is highly dependent on precise control of their form.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nih.gov/Genbank/

Genomic Technology Support Facility (GTSF) at MSU, http://genomics.msu.edu

Inner Ear Gene Expression Database, http://www.mgh.harvard.edu/depts/coreylab/genomics.html

MultAlin, http://prodes.toulouse.inra.fr/multalin

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

UniGene, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene

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