Nonsyndromic Deafness DFNA1 Associated with Mutation of a Human Homolog of the Drosophila Gene diaphanous

Eric D. Lynch,* Ming K. Lee, Jan E. Morrow, Piri L. Welch, Pedro E. León, Mary-Claire King

The gene responsible for autosomal dominant, fully penetrant, nonsyndromic sensorineural progressive hearing loss in a large Costa Rican kindred was previously localized to chromosome 5q31 and named DFNA1. Deafness in the family is associated with a protein-truncating mutation in a human homolog of the Drosophila gene diaphanous. The truncation is caused by a single nucleotide substitution in a splice donor, leading to a four–base pair insertion in messenger RNA and a frameshift. The diaphanous protein is a profilin ligand and target of Rho that regulates polymerization of actin, the major component of the cytoskeleton of hair cells of the inner ear.

DFNA1 defines the autosomal dominant, fully penetrant, sensorineural progressive hearing loss of kindred M of Costa Rica (OMIM 124900) (Fig. 1) (1, 2). In this kindred, low-frequency deafness begins at about 10 years of age and progresses by age 30 to profound, bilateral deafness involving all frequencies. The ratio of affected to unaffected children of deaf parents is 1:1; males and females are equally likely to be affected. Deafness in kindred M is a sensorineural cochleosaccular dysplasia specific to the membranous structures of the inner ear. Speech development before onset, intelligence, life expectancy, and fertility are normal. Hearing loss in this kindred has been traced eight generations to a common ancestor, born in 1713 in Cartago, Costa Rica. As with other nonsyndromic forms of deafness, identification of the gene responsible is important for the understanding of human hearing because the wild-type product of the gene is likely to be critical to development and maintenance of hearing.

DFNA1 in kindred M was mapped to a region of 1 centimorgan on chromosome 5q31 by linkage analysis, then a complete 800-kb bacterial artificial chromosome (BAC) contig was constructed of the linked region (3). In order to identify all genes in the linked region, we sequenced BACs composing the contig after shotgun subcloning each into an M13 vector (4, 5). We developed the computer program SeqHelp to organize sequences from the chromatograms, to call bases and align sequences using the computer programs PHRED and PHRAP, and to apply existing, publicly available, software to evaluate the novel genomic sequences (6). SeqHelp displayed putative coding regions, CpG islands, repeat sequences, and matches to known genes and expressed sequence tags (ESTs) from all databases in an interactive format for further analysis.

A previously unidentified human gene homologous to the Drosophila gene diaphanous (GenBank U11288) and to the mouse gene encoding p140mDia (GenBank U96963) was revealed by the genomic sequence of BACs 293C24, 45M22, and 249H5 (Fig. 2) (7). Given that the mouse and human predicted amino acid sequences are 97% identical for the regions identified from BACs, we estimated the sizes of gaps from the mouse sequence, constructed primers from the human coding sequence, and used these to amplify intervening exons from human cDNA and to carry out 5′RACE on polyadenylated [poly(A)+] RNA from lymphoblastoid lines (8). A total of 3511 base pairs (bp) of coding sequence have been identified; about 250 bp remain to be determined. The human diaphanous gene comprises at least 18 exons with ~3800 bp of coding sequence and a 3′ untranslated region (UTR) of 918 or 1891 bp (9).

To screen the DFNA1 gene for mutation in the M family, we designed primers to amplify exons and flanking splice junctions from genomic DNA of affected and unaffected members of the M family and from control individuals. Each product was screened for single-strand conformation polymorphisms (SSCPs). Each product was gel-purified, reamplified, and sequenced (10).

A guanine-to-thymine substitution in the splice donor of the penultimate exon of human DFNA1 was observed in affected members of the M kindred (Fig. 3E). The guanine-to-thymine substitution at this site disrupts the canonical splice donor sequence.

Fig. 1. The M kindred of Costa Rica. Hearing loss in this kindred is autosomal dominant, progressive and fully penetrant by age 30 and not associated with any other phenotype. Individuals with hearing loss are indicated by filled symbols and unaffected individuals by open symbols. All living individuals on the pedigree are included in the analysis. The pedigree is altered slightly, omitting young unaffected individuals, in order to protect privacy. The three-generation family drawn separately is related to the kindred, but the exact genealogy is unclear. All 78 affected individuals in the kindred share the DFNA1 mutation, and all unaffected individuals over age 30 are wild type at the comparable site.

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AAGgtaagt (Fig. 3, A and B). To determine the consequences of this mutation at the level of RNA message, poly (A)+ cDNA was prepared from lymphoblastoid cell lines of three affected members of the M kindred, from unaffected family members, and from unrelated, unaffected, controls. Insertion of TTAA was observed in cDNA of affected individuals (Fig. 3, C and D). The mechanism for the insertion was splicing at a cryptic site 4 bp 3′ of the wild-type site. The TTAA insertion leads to a frameshift, encoding 21 aberrant amino acids, followed by protein termination that truncates 32 amino acids (Fig. 4). All 78 affected members of the M kindred are heterozygous for the mutation. The site was wild type in 330 hearing, control individuals (660 chromosomes) of the following ancestries: 12 Costa Ricans unrelated to the M family, 94 Latin Americans from other countries, 32 Spanish, 154 Europeans (other than Spanish) and North Americans of European ancestry, and 38 African-Americans.

Expression of human diaphanous message in brain, heart, placenta, lung, kidney, pancreas, liver, and skeletal muscle was confirmed by Northern (RNA) hybridization (Fig. 3F). A single transcript of 4.7 kb was observed in all tissues, with highest expression in skeletal muscle. RNA from lymphoblastoid cell lines of affected and unaffected members of the M family similarly revealed a single transcript of 4.7 kb in all individuals, consistent with a 4-bp insertion in the mutant message. We confirmed expression of human diaphanous in the cochlea by reverse transcriptase–polymerase chain reaction (RT-PCR) of cochlear RNA using PCR primers that amplified the region of the gene that harbors the mutation in family M (10, 11). The sequence of the RT-PCR product from cochlear RNA was wild type. Hence, if alternate splice forms of the gene exist, normal cochlear transcripts include the region of the gene that is improperly spliced in affected members of kindred M.

The human diaphanous 1 protein, mouse p140mDia, and Drosophila diaphanous are homologs of Saccharomyces cerevisiae protein Bni1p (12). The proteins are highly conserved overall (Fig. 5). The genes encoding these proteins are members of the formin gene family, which also includes the mouse limb deformity gene, Drosophila gene cappuccino, Aspergillus nidulans gene seA, and Schizosaccharomyces pombe genes fus1 and cdc12 (13). These genes are involved in cytokinesis and the establishment of cell polarity. Rho-binding domains in the NH2-terminal regions, polyproline stretches in the central region of each sequence, and formin-homology domains in the COOH-terminal region characterize many formins (12). Multiple mutants of mouse formin have been characterized (13). A truncated mouse formin allele kdr+2 lacking the 42 COOH-terminal amino acids leads to mislocalization of the formin protein to the cytoplasm (14).

The biological role of human diaphanous 1 in hearing is likely to be the regulation of actin polymerization in hair cells. Actin polymerization involves proteins known to interact with diaphanous protein in Drosophila and mouse. The protein profilin binds actin monomers and is a regulator of actin polymerization (15). Mammalian and Drosophila diaphanous are effectors of Rho (12). Diaphanous acts in a Rho-dependent manner to recruit profilin to the membrane, where it promotes actin polymerization. As predicted by this model, transient expression of p140mDia induced homogeneous actin filament formation in COS cells (7). Rho-induced actin polymerization is conserved from yeast to mammals.

The DFNA1 mutation in the M family is relatively subtle, in that it affects only the COOH-terminal 52 amino acids. Given that human diaphanous appears to be ubiquitously expressed, and that the only ob-
erved phenotype in the M family is hearing loss, it seems likely that the hair cells of the cochlea are particularly sensitive to proper maintenance of the actin cytoskeleton. Hence, this mutation may represent a partial loss of function of the human diaphanous 1. One process in the inner ear that might be uniquely sensitive to disruption of actin polymerization is the amplification of sound reception by the inner hair cells, which is due to the concerted action of outer hair cells and pillar cells. The relay of kinetic energy from outer hair cells to inner hair cells relies critically on the presence of a rigid structure of actin fibers. Additional structural support in hair cells is provided by the cuticular plate, a dense network of actin fibers at the apical ends of hair cells into which stereocilia are anchored. Organization of the actin fiber network of the cuticular plate is not well understood but is probably dynamic. The DFNA1 mutation may impair maintenance of organization of the actin fibers of the cuticular plate.

Hair cell stereocilia may be an alternate or additional site affected by the aberrant protein. The structural support providing rigidity to the stereocilia comprises largely cross-linked actin filaments packed in a paracrystalline array (16). Upon acoustic overstimulation, the paracrystalline array becomes disordered (17). Reordering of the array is controlled by an as yet unidentified protein or proteins. It is possible that normal human diaphanous 1 is involved in this process. In the M family, mutant human diaphanous 1 might compete with the wild-type protein to repair damage from normal exposure to sound. The generation of a mouse model with the DFNA1 mutation in p140mDia, followed by testing the effects of acoustic exposures on these mice, will provide an experimental test of this hypothesis.

A second human homolog of Drosophila gene diaphanous was revealed during the cloning of DFNA1. This second human diaphanous gene maps to chromosome Xq22 (18). Nonsyndromic X-linked deafness, DFN2, also maps to Xq22 (19), suggesting the second human diaphanous gene as a candidate gene for DFN2 hearing loss.

In the past 50 years, the incidence of maternal viral disease during pregnancy has declined dramatically in most countries, with a consequent decrease in childhood deafness. Most remaining cases of deafness at birth, in childhood, or among young...
adults, are genetically influenced (20). In the past 5 years, at least 40 chromosomal loci for inherited, nonsyndromic human deafness have been mapped by linkage in families, and thus far five of the responsible genes have been identified: myosin 7A, the POU domain gene POU3F4, connexin 26, mitochondrial 12S rRNA, and mitochondrial rRNA SYN2 (21). The association of autosomal dominant, nonsyndromic deafness with the human diaphanous gene adds a new and complementary piece to this puzzle. The M kindred provides a rare mutation that may reveal universal biology and augment our understanding of hearing.

REFERENCES AND NOTES

2. This project has been approved by the Committee on Human Subjects Research of the University of Washington, Seattle. The criterion for deafness in the families, and thus far five of the responsible genes have been identified: myosin 7A, the POU domain gene POU3F4, connexin 26, mitochondrial 12S rRNA, and mitochondrial rRNA SYN2 (21). The association of autosomal dominant, nonsyndromic deafness with the human diaphanous gene adds a new and complementary piece to this puzzle. The M kindred provides a rare mutation that may reveal universal biology and augment our understanding of hearing.

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