REPORT

Mutations in *CDC14A*, Encoding a Protein Phosphatase Involved in Hair Cell Ciliogenesis, Cause Autosomal-Recessive Severe to Profound Deafness

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By genetic linkage analysis in a large consanguineous Iranian family with eleven individuals affected by severe to profound congenital deafness, we were able to define a 2.8 Mb critical interval (at chromosome 1p21.2-1p21.1) for an autosomal-recessive nonsyndromic deafness locus (DFNB). Whole-exome sequencing allowed us to identify a *CDC14A* biallelic nonsense mutation, c.1126C>T (p.Arg376*), which was present in the eight clinically affected individuals still alive. Subsequent screening of 115 unrelated individuals affected by severe or profound congenital deafness of unknown genetic cause led us to identify another *CDC14A* biallelic nonsense mutation, c.1015C>T (p.Arg339*), in an individual originating from Mauritania. *CDC14A* encodes a protein tyrosine phosphatase. Immunofluorescence analysis of the protein distribution in the mouse inner ear showed a strong labeling of the hair cells' kinocilia. By using a morpholino strategy to knockdown *cdc14a* in zebrafish larvae, we found that the length of the kinocilia was reduced in inner-ear hair cells. Therefore, deafness caused by loss-of-function mutations in *CDC14A* probably arises from a morphogenetic defect of the auditory sensory cells' hair bundles, whose differentiation critically depends on the proper growth of their kinocilium.

Almost 90% of all cases of nonsyndromic, severe to profound congenital deafness display an autosomal-recessive mode of transmission (DFNB forms). Sixty genes have already been identified, but many others remain to be discovered according to the much larger number of DFNB loci reported (Hereditary Hearing Loss website; see Web Resources).[1] With high-throughput sequencing techniques becoming available and the whole-exome sequencing approach in affected individuals, the pace of gene discovery has accelerated. Here we used a combination of genetic linkage analysis and whole-exome sequencing to identify two different nonsense mutations in *CDC14A* (OMIM: 603504).

Informed consent was obtained from all study participants. Of the eight affected individuals still alive in a consanguineous Iranian family (Figure 1A), the six that could be tested for auditory function (V.1, V.6, V.8, V.14, V.15, and V.18), aged 21–69 years, all suffered from prelingual, severe to profound deafness of cochlear origin, as shown by the markedly increased detection thresholds in pure-tone audiometry (both with air- and bone-transmitted sounds) and auditory brainstem responses and by the absence of transient evoked otoacoustic emissions (Figure 1B and data not shown).[2,3] Otoscopic examination and tympanometry with acoustic reflex testing did not show evidence of a conductive hearing impairment. General clinical examination did not find any feature of syndromic deafness, and normal age of walking onset allowed us to exclude severe congenital vestibular dysfunction. Genetic linkage analysis was carried out on 21 family members. SNP array analysis (700k Illumina OmniExpress-12) and homozygosity mapping defined a single critical region of 2.8 Mb between rs7537296 and rs950060 at chromosome 1p21.2–1p21.1 (Figure 1A). This locus (DFNB105 [OMIM: 616958]) does not match any of the previously reported human deafness loci, and the murine syntenic region at chromosome 3qF3–3qG1 does not contain a reported deafness locus either. We then carried out whole-exome sequencing in three affected individuals (V.6, V.8, and V.14) and identified a biallelic nonsense mutation in exon 11 of *CDC14A* (cell division cycle 14A; NCBI ID 8556), c.1126C>T (p.Arg376*) (NCBI RefSeq: NM_033312.2). Incidentally, one nonsynonymous and six synonymous sequence variants were also found in the critical interval; all of these were present in HapMap, 1000 Genomes, and Exome Variant Server databases. Sanger sequencing of *CDC14A* exon 11 confirmed the presence of the biallelic nonsense mutation in the eight clinically affected individuals only. In addition, all tested clinically unaffected individuals except three (V.4, V.10, and V.17) carried the mutation at the heterozygous state, as expected from the genetic linkage analysis (Figure 1A). This mutation was absent from the 1000 Genomes and Exome Variant Server databases and was not detected in

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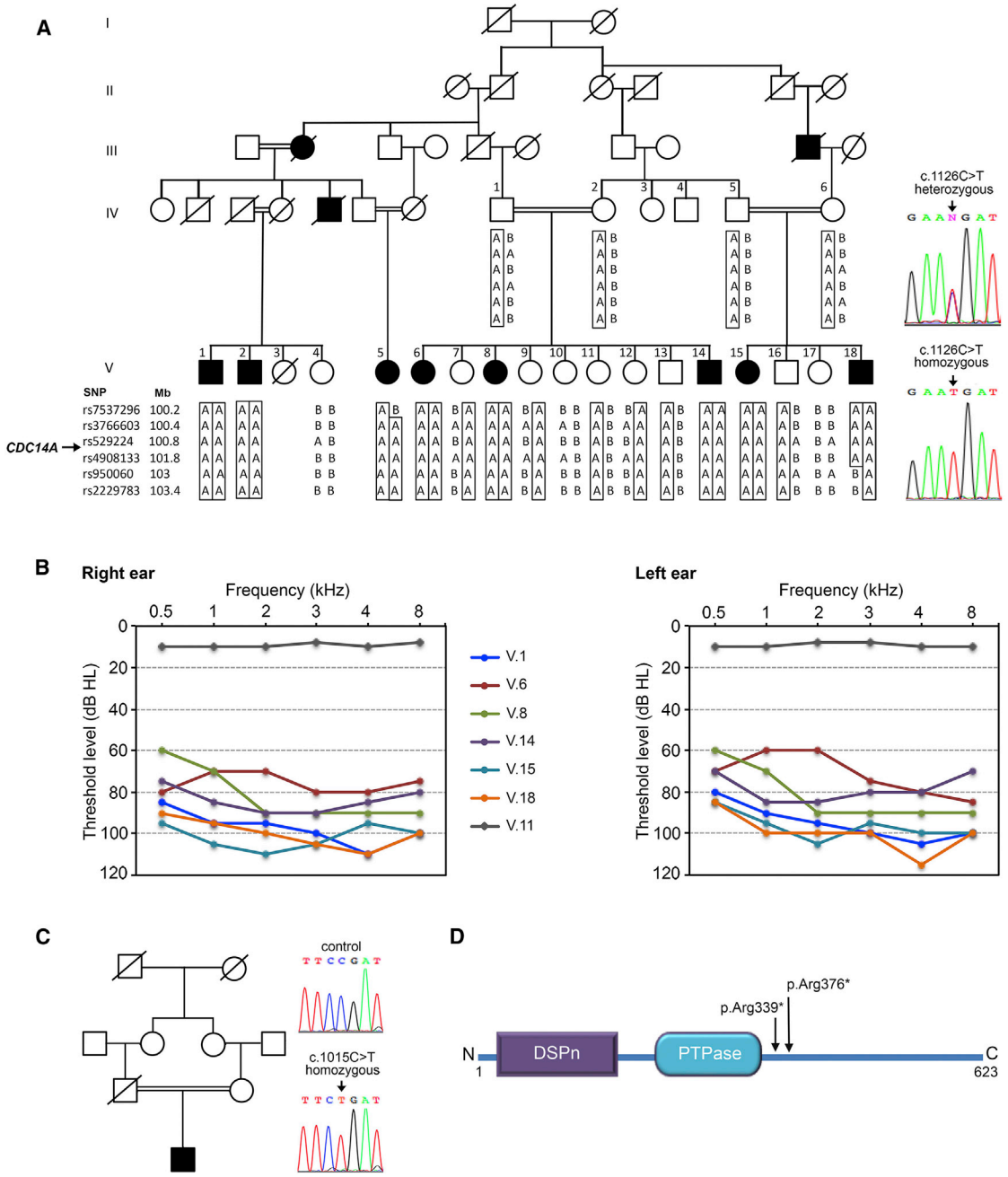

Figure 1. Phenotypic and Genotypic Analysis in the Iranian and Mauritanian Families

(A) Segregation analysis with SNP markers at chromosome 1p21.2–1p21.1 in the Iranian family. Deaf individuals and unaffected individuals are indicated by filled symbols and open symbols, respectively. The haplotype associated with the affected allele is boxed. The physical distances (in megabases, Mb) between the SNP markers and the centromere are indicated on the left (human genome reference sequence build UCSC hg19/GRCh37). Right panels: partial DNA sequence chromatograms of *CDC14A* exon 11 containing the c.1126C>T (p.Arg376*) nonsense mutation in the heterozygous state in the normal-hearing individual IV.6, and at homozygous state in the deaf individual V.18.

(B) Right- and left-ear audiograms in a normal-hearing individual (V.11, 29 years old) and in six hearing-impaired individuals (V.1, 69 years; V.6, 40 years; V.8, 34 years; V.14, 21 years; V.15, 38 years; and V.18, 31 years) of the Iranian family.

(C) A pedigree of the Mauritanian family and partial DNA sequence chromatograms of *CDC14A* exon 11 in a normal-hearing control and in the deaf proband shows the c.1015C>T (p.Arg339*) biallelic nonsense mutation in the latter.

(D) Schematic representation of the CDC14A longest isoform (623 amino acids). The dual-specificity protein phosphatase; N-terminal (DSPn) and protein tyrosine phosphatase (PTPase) domains are shown in purple and in blue, respectively. Vertical arrows indicate the positions of the two nonsense mutations.

150 Iranian individuals from the general population. Whole-exome sequencing of 115 unrelated individuals originating from Maghreb and affected by severe or profound congenital deafness allowed us to identify another biallelic nonsense mutation, c.1015C>T (p.Arg339*), in the same *CDC14A* exon in a Mauritanian individual

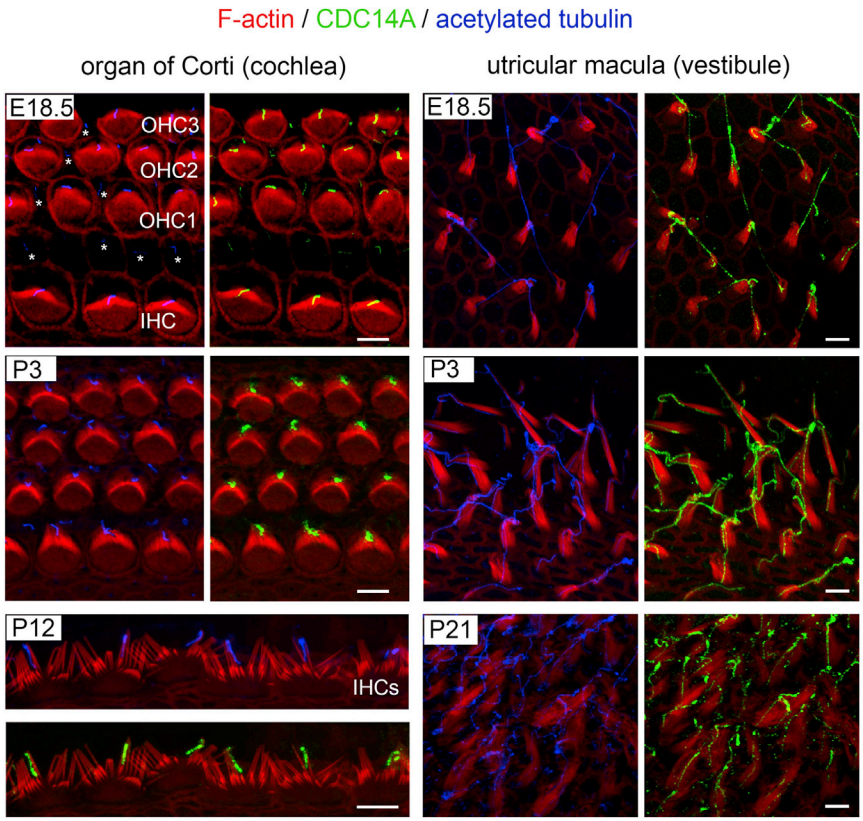

Figure 2. CDC14A in the Mouse Inner Ear
The distribution of CDC14A was analyzed in the cochlear sensory epithelium (organ of Corti) and one of the vestibular sensory epithelia (utricular macula) on E18.5, P3, P12, and P21, by whole-mount immunofluorescence. An anti-acetylated tubulin monoclonal antibody (Sigma T7451) and phalloidin (Sigma 94072) were used for labeling the hair cells' kinocilia and F-actin filled stereocilia (the modified microvilli that make the hair cells' hair bundles, which are at the core of the mechanoelectrical transduction process), respectively. CDC14A is present along the kinocilia of cochlear and vestibular hair cells at all stages analyzed. In the cochlea, the kinocilium is present only in developing hair cells, not in mature (P21) hair cells, whereas in the vestibular end organs the kinocilium persists in mature hair cells. On P12, CDC14A-immunoreactive kinocilia were still detected in inner hair cells (IHCs) of the cochlear apical region, but not in outer hair cells (OHCs), which have already lost their kinocilia at this stage (data not shown). Note that CDC14A is also detected in the primary cilia of supporting cells on E18.5 (asterisks). No labeling was observed at any stage with the rabbit pre-immune serum (data not shown). Scale bars represent 5 μm.

affected by profound deafness (Figure 1C). This mutation was not detected in 105 normal-hearing individuals, including 50 Mauritanian individuals, from this geographical region. Incidentally, neither of these nonsense mutations was detected in 195 additional congenitally deaf individuals originating from Maghreb or Iran.

CDC14A consists of 18 exons. It encodes a widely expressed protein tyrosine phosphatase (NCBI database of the transcriptome, UniGene). Six different transcripts resulting from alternative splicing have been reported (NCBI RefSeq: NM_003672.3, NM_033312.2, NM_033313.2, NM_001319210.1, NM_001319211.1, and NM_001319212.1). The transcript containing the largest open-reading frame encodes a 623 amino acid protein (NCBI RefSeq: NP_201569.1) (Figure 1D) involved in DNA repair, mitosis, meiosis, cell migration and adhesion, and ciliogenesis.[4–16] Both mutations are expected to result either in nonsense-mediated mRNA decay or in a significantly truncated protein.[17] We produced and purified two rabbit polyclonal antibodies directed against overlapping peptides (amino acids 412–489 and 412–522) from the C-terminal region of the mouse CDC14A (NCBI RefSeq: NP_001074287.1). Immunofluorescence experiments on the mouse inner ear between embryonic day 18.5 (E18.5) and postnatal day 21 (P21) showed the presence of CDC14A along both the transient kinocilia of developing cochlear hair cells and the persistent kinocilia of vestibular hair cells with either antibody. The protein was therefore detected from early stages of hair-bundle differentiation onward (Figure 2 and data not shown). Functional studies in zebrafish have shown a role of *cdc14a* in ciliogenesis in Kupffer's vesicle, the ciliated organ of body asymmetry.[16] We investigated the role of *cdc14a* in the zebrafish auditory organ by a knockdown strategy, where we used an antisense morpholino oligonucleotide (MO) targeting the splice donor site of intron 2 and a mismatch oligonucleotide (MI) as a negative control. MO injection in one- to two-cell-stage embryos resulted in two abnormal mature transcripts in the larvae: one lacking the exon 2 sequence and the other one retaining intron 2. No abnormal transcripts were detected in MI-injected larvae (Figure 3A). In the *cdc14a* knockdown larvae at 3 days post-fertilization, we did not observe gross morphological defects of the inner ear. However, there was a shortening of the hair cells' kinocilia: the mean length of the kinocilium was 4.89 ± 0.12 μm in MO-injected larvae versus 6.31 ± 0.17 μm in MI-injected and 6.17 ± 0.18 μm in non-injected control animals (unpaired two-tailed Student's t test, $p < 0.001$) (Figure 3B). No hair-bundle shape anomalies, including fragmentation, were detected by confocal microscopy (data not shown).

Together, these results establish that loss-of-function mutations of *CDC14A* cause autosomal-recessive severe to profound congenital deafness and suggest that the hearing impairment arises from abnormally short kinocilia in the differentiating hair bundles of cochlear sensory cells. The absence of an associated balance disorder, despite the detection of the defective protein in vestibular hair

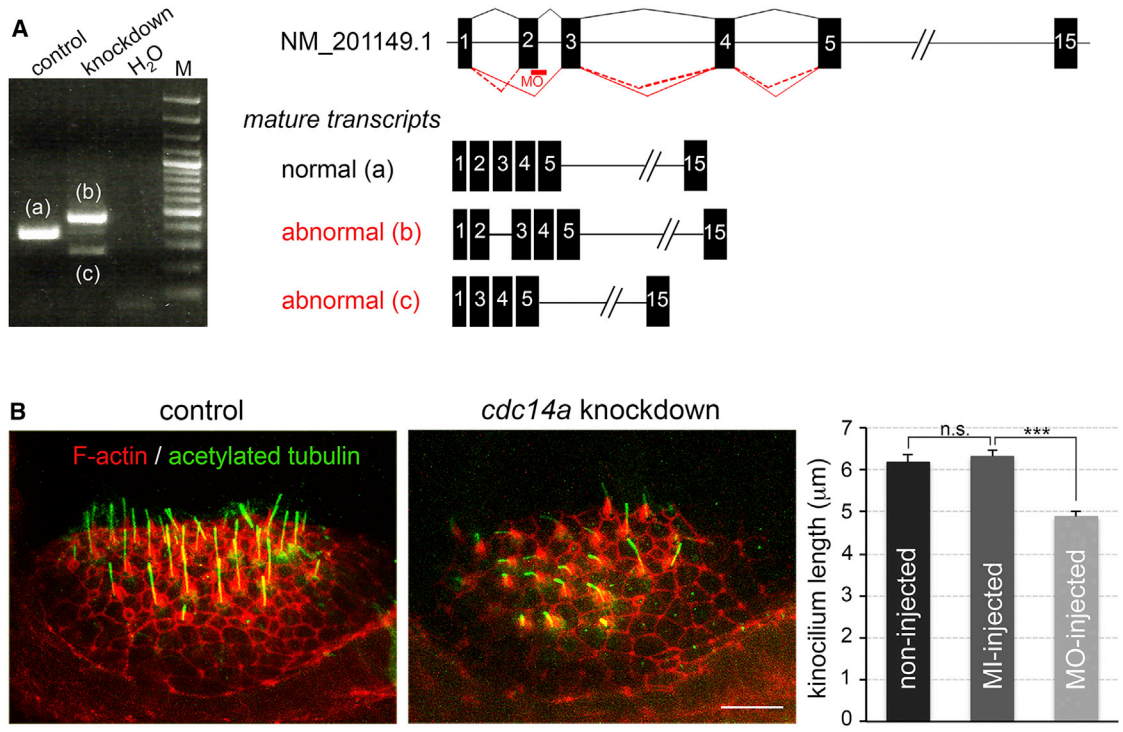

Figure 3. Knockdown of *cdc14a* in Zebrafish

(A) One- to two-cell-stage embryos of *Danio rerio* were injected with 4 ng of either a *cdc14a* splice-blocking morpholino (5′-GTTTGGGCA GACTCACTTTTCATAA-3′) or a mismatch morpholino (5′-GTaTcGGCAcACTCAgTTTTgATAA-3′; mismatched nucleotides are lowercase) as a negative control (Gene Tools), and larvae were analyzed at 3 days post-fertilization (dpf). The blocking morpholino oligonucleotide (MO, red bar) was designed to encompass the splice donor site of *cdc14a* (NCBI: NM_201149.1, 15 exons) intron 2. This resulted in abnormally spliced *cdc14a* transcripts either retaining intron 2 (transcript b) or lacking exon 2 (transcript c) in the larvae injected with the blocking morpholino (knockdown), instead of the normal transcript (transcript a) present in the larvae injected with the mismatch morpholino (control), as shown by RT-PCR analysis (with primers located in exons 1 and 5) followed by sequencing of the amplicons. M, DNA size marker: 100 bp ladder.

(B) Whole-mount immunofluorescence analysis of the inner-ear anterior macula in 3 dpf control larvae (i.e., injected with the mismatched morpholino, MI) and *cdc14a* knockdown larvae (i.e., injected with the blocking morpholino, MO); an anti-acetylated tubulin monoclonal antibody and phalloidin were used for labeling the hair cells' kinocilia and F-actin filled stereocilia, respectively (confocal microscopy). In the knockdown larva, the overall structure of the hair bundles is preserved, but the kinocilia have reduced lengths. The bar chart shows the quantitative analysis of kinocilia lengths, measured with ImageJ software, in four non-injected ($n = 91$ kinocilia), five MI-injected ($n = 119$), and ten MO-injected ($n = 227$) larvae. Data are represented as the mean ± SEM. The scale bar represents 10 μm. n.s., not significant; ***, $p < 0.001$.

cells in the mouse as well, is common in genetic forms of human deafness. It presumably results from the fact that equilibration involves multimodal perception, including vision and proprioception, which both can compensate for vestibular dysfunction. In addition, compensatory molecular mechanisms present until the full maturation of the hair cells[18,19] might continue to operate in the vestibular hair cells, which, contrary to the auditory hair cells, can regenerate. To our knowledge, *CDC14A* is the second DFNB gene reported to be involved in the control of kinocilium growth.[20,21] During hair-cell development, the growing hair bundle is connected to the kinocilium by fibrous links, which contribute to the hair-bundle integrity.[22] If these links are defective, the mature hair bundle might have an abnormal structure, thereby affecting the mechanoelectrical transduction process.[23] Alternatively, the absence of CDC14A, a protein phosphatase involved in cell signaling,[24] might directly affect the hair cells, as previously reported for the suppression of different ciliary proteins in other tissues.[25] The absence, in the Iranian and Mauritanian affected individuals, of clinical symptoms of ciliopathies other than deafness, such as recurrent respiratory infections, kidney disorders, retinal degeneration, or obesity,[26,27] suggests that the lack of CDC14A in other tissues is efficiently compensated by another protein phosphatase, possibly CDC14B.[14,16]

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Web Resources

Exome Variant Server, NHLBI Exome Sequencing Project (ESP), http://evs.gs.washington.edu/EVS/

Hereditary hearing loss homepage, http://hereditaryhearingloss.org/

Online Mendelian Inheritance in Man (OMIM). www.omim.org/

NCBI database of the transcriptome, UniGene, www.ncbi.nlm.nih.gov/unigene

The 1000 Genomes Project, http://www.1000genomes.org/

The International HapMap Project, http://hapmap.ncbi.nlm.nih.gov/

UCSC Human Genome Database Build hg19, February 2009, http://www.genome.ucsc.edu

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