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Letter to the Editor

DFNB93, a novel locus for autosomal recessive moderate-to-severe hearing impairment

To the Editor:

Hearing impairment (HI) is the most common sensory defect in human (1). The cause of HI can be environmental or genetic. Approximately 80% of hereditary non-syndromic HI is autosomal recessive (ARNSHI). Up to now, more than 70 chromosomal loci (known as DFNB) have been localized for ARNSHI and the responsible genes have been identified in about 50% of DFNB loci.

As ARNSHI in most cases is pre-lingual, non-progressive and severe-to-profound, it is often impossible to distinguish between different ARNSHI genes based on audiometric data. Thus far, among all the genes for ARNSHI, only *TECTA* (DFNB21 locus) causes moderate-to-severe HI (2, 3). Because this is a rare and recognizable phenotype caused by a single gene, it represents an important guideline for DNA diagnostics for ARNSHI.

In this study, we mapped a novel locus for ARNSHI to chromosome 11q12.3–13.3, found in an Iranian family with the distinctive audiometric profile of moderate-to-severe HI. This is the second ARNSHI locus to cause a recognizable milder phenotype. The name DFNB93 was assigned to this locus by the Human Genome Organization (HUGO) Nomenclature Committee (4).

Family IR-Sh10, living in an isolated village in the south of Iran, was ascertained (pedigree is shown in Fig. 1). The hearing impaired family members underwent clinical examinations in a local clinical center (performed by Dr M. Hassanzadeh). Air and bone conduction pure-tone audiometry was performed at frequencies ranging from 250 to 8000 Hz. Genomic DNA was extracted from EDTA-treated blood from 14 members of the family IR-Sh10 (5). Analysis of fluorescently labeled PCR-amplified short tandem repeat polymorphic (STRP) microsatellite markers was performed on an ABI 3130 DNA sequencer (XL Genetic Analyzer, ABI) using standard procedures. Alleles were assigned using GeneMapper v.3.7 software (Applied Biosystem, Foster, CA).

The genome scan analysis was performed using the Illumina SNP array utilizing 6K SNP Linkage Panel IVb (Illumina Inc., San Diego, CA). Linkage analysis was performed by calculating two-point and multi-point parametric logarithm of odds (LOD) scores using the Superlink (v.1.6) and Simwalk (v.2.91) options of EasyLINKAGE plus (version 5.01beta) (URL: http://sourceforge.net/projects/easylinkage/), respectively (6, 7). The physical positions of the STRP and SNP markers were obtained from the NCBI genome sequence Build 37.1. Haplotypes were reconstructed via Simwalk. Mistyping errors were checked and removed by looking at the Mendelian inconsistencies and double recombinations by Simwalk.

The potential candidate genes in the linked interval region on chromosome 11q were identified using the UCSC Genome Build 37.1. A set of criteria including gene functions, gene expression patterns, protein interactions and literature review were used for candidate gene prioritization. Data from the Massively Parallel Signature Sequencing (MPSS) inner ear libraries (8) and from human inner ear cDNA libraries (9) were also queried to identify potential candidate DFNB93 genes. In addition, the Endeavour Web-based software, using motif and blast score and interaction with other known HI genes, was also utilized to help prioritizing the candidate genes (10). DNA sequencing of candidate genes was performed on an ABI 3130 automated sequencer (XL Genetic Analyzer, ABI) using the big-Dye terminator cycle sequencing kit Version 3.1 (Applied Biosystem).

All 14 family members, including 4 patients, were interviewed and underwent clinical examinations. The presence of possible abnormalities present in syndromic forms of deafness was investigated. There was no obvious clinical

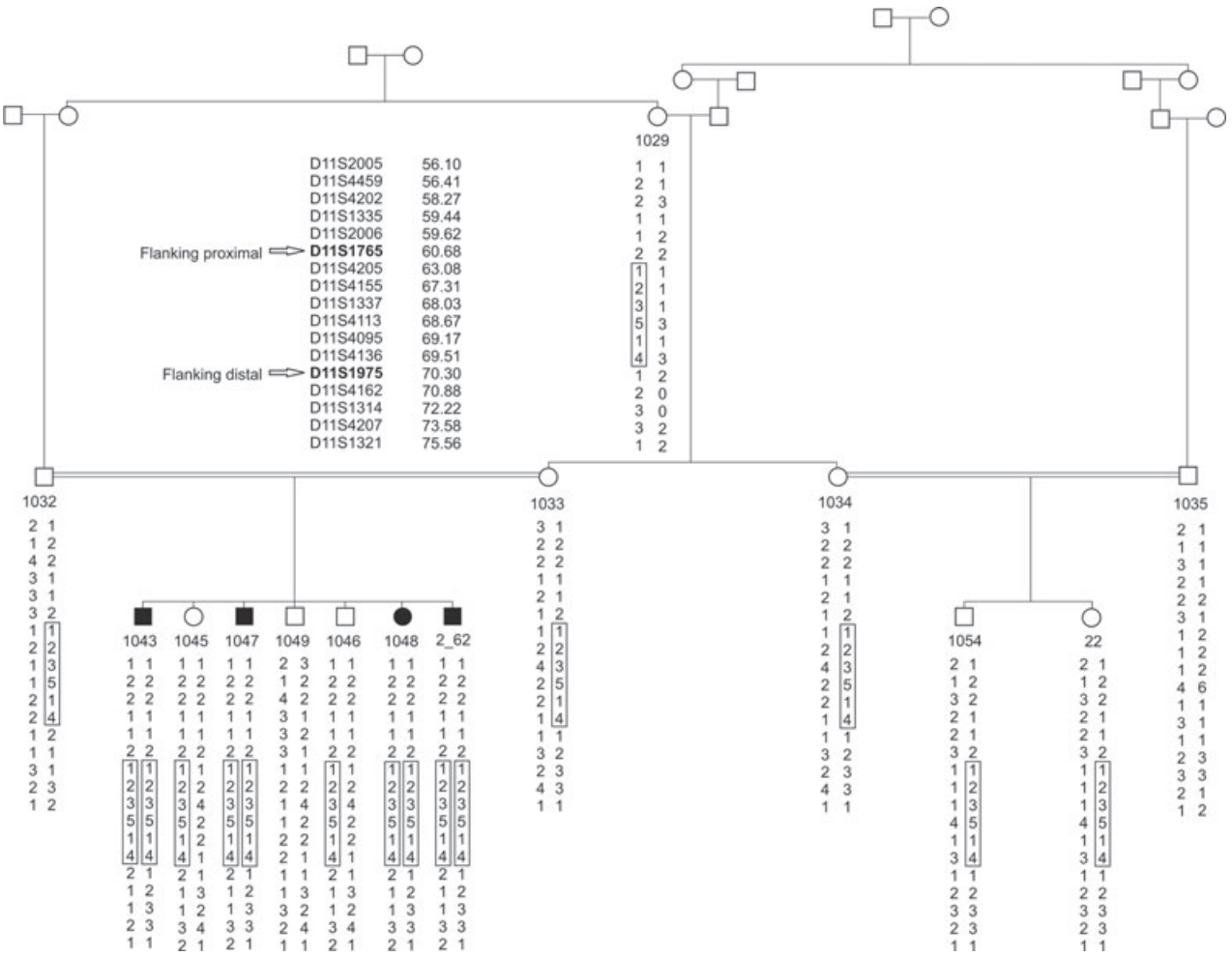

Fig. 1. Pedigree and haplotypes of the Iranian family IR-Sh10 segregating ARNSHI. A double marriage line denotes consanguinity. The STRP markers and their relative physical positions (Mb) are shown next to the pedigree. Haplotypes homozygous only in patients are boxed. The flanking markers D11S1765 (60.68 Mb) and D11S1975 (70.30 Mb), defining a critical interval of 9.62 Mb, are highlighted with an arrow. (Almost 50 microsatellite markers were run on the candidate region for this family, but only the genotypes of 17 selected markers are shown in the figure.)

manifestation co-segregating with HI in the study family, leading to a diagnosis of ARNSHI.

All 4 affected individuals showed a history of pre-lingual HI. The family patients use a combination of sign language and oral communication. The audiometric profile showed that HI is stable, bilateral, symmetric and moderate-to-severe in degree in all frequencies. The HI is slightly more pronounced in the mid-frequencies, resulting in a flat-to-shallow U-shaped audiogram. Figure 2 shows the audiograms of the right ear of all four affected family members (as the HI was symmetric, audiograms of left ears were not shown). Tympanometry showed a normal static compliance and normal middle ear pressures.

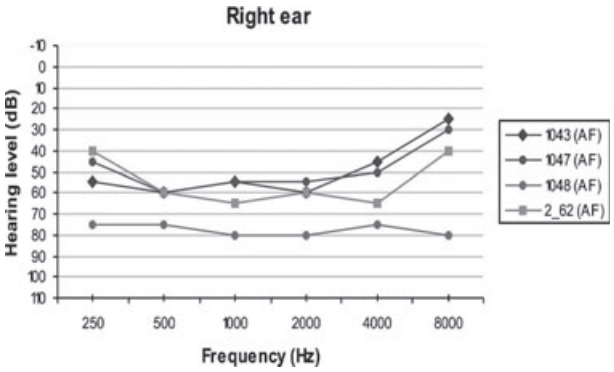

Fig. 2. Pure-tone audiograms for all four affected individuals from the family IR-Sh10 for the right ear.

Fourteen frequent ARNSHI loci among non-Caucasians (11) were ruled out as the underlying cause of HI in the family IR-Sh10. After this exclusion, a genome wide linkage scan was carried out. Significant linkage was found on chromosome 11q12.3–11q13.3 with a maximum multi-point

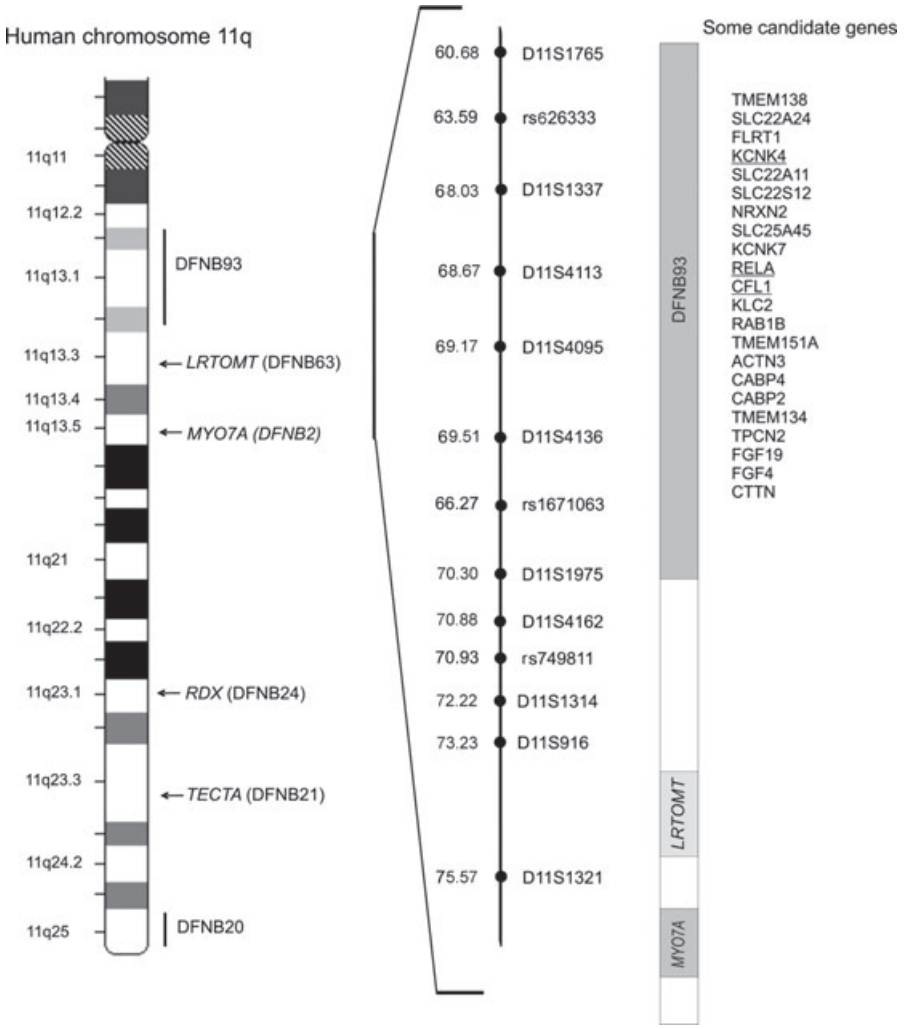

Fig. 3. Idiogram of chromosome 11q, showing the DFNB93 chromosomal map location in relation to adjacent known DFNB loci. Some STRP and SNP markers and some of the stronger candidate genes are shown. The three sequenced genes are underlined. Physical map distances (Mb) are from the UCSC Genome Bioinformatics Build 37.1. Markers D11S1765 (60.68 Mb) and D11S1975 (70.30 Mb) define the DFNB93 critical interval region. Filled circles represent STRP and SNP markers (not drawn to scale).

LOD score of 3.4 between SNPs rs1783811 (64.33 Mb) and rs1671063 (66.27 Mb). A few STRP microsatellite markers, selected from the Decode (12) and LDB (13) genetic maps in the interval were genotyped to confirm and refine the linked region. Thirty-two markers in the DFNB93 interval were genetically informative for this family and thus were included for the LOD score calculation. The candidate region is delimited by markers D11S1765 (60.68 Mb) and D11S1975 (70.30 Mb), with a maximum multi-point LOD score of 3.5 for the region between D11S1337 (68.03 Mb) and D11S4136 (69.51 Mb). The proximal recombination in the maternal chromosome at marker D11S4155 could be seen in two normal individuals, 1045 and 1046. The pedigree, linked haplotype and a genetic map of the linkage interval is shown in Fig. 1.

Five loci for ARNSHI have already been mapped to chromosome 11q, including DFNB2 (*MYO7A*), DFNB20 (no gene identification yet), DFNB21 (*TECTA*), DFNB24 (*RDX*) and DFNB63 (*LRTOMT*). Figure 3 illustrates the relative physical position of the DFNB93 locus and the neighboring known HI loci and genes.

Analysis of the DFNB93 interval at 11q12.3–11q13.3 revealed more than 300 annotated and hypothetical genes in an approximately 9.62 Mb region. Several of these genes are expressed in the human or mouse inner ear (8, 9). Three genes with strong relevance to HI were selected for DNA sequencing including: *CFL1* (14, 15), *KCNK4* (16) and *RELA* (17). However, no pathogenic variant was identified in any of these three genes. Even though *LRTOMT* is not within the candidate region (exclusion by LOD score

<-2) and the audiometric profile of the family IR-Sh10 is very different from *LRTOMT* families who segregate severe-to-profound ARNSHI (18–21), *LRTOMT* was also sequenced. However, no mutation was identified. Currently, we are considering other candidate genes within the interval. *SLC22A11*, *SLC22A12*, *SLC22A20, KCNK7*, *CABP4* and *TPCN2* are among the stronger candidate genes for DFNB93 locus.

Although the candidate region is <10.0 Mb, and we have sequenced the coding region of the most obvious candidate genes, we have not been able to identify the disease-causing gene. This is not unusual though, as there are many gene localizations for deafness in a similar situation. However, the application of next generation sequencing may be a good strategy for further analysis, leading to the identification of many of these genes.

Although most ARNSHI families have profound HI, the family IR-Sh10 presents a phenotype of moderate-to-severe HI across all frequencies, slightly more pronounced in the mid-frequencies. A similar pattern has been previously reported for the *TECTA* gene (2, 3, 22). The distinguishable phenotype in families with ARNSHI caused by a mutation in *TECTA* has led to the suggestion that mutation analysis of *TECTA* should be offered to ARNSHI families with such an audiometric profile (2, 3, 23–27). The audiometric profile of the family IR-Sh10 also provides a distinctive clinical feature that may point toward this gene for DNA diagnosis.

In summary, we have identified a novel ARNSHI locus at 11q12.3–11q13.2. It is the second locus for the ARNSHI with a distinctive audiometric profile of moderate-to-severe HI. This represents a step forward toward the possibility to predict the genetic cause based on the audiometric profile and is therefore of importance for DNA diagnostics.

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