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SHORT COMMUNICATION

A novel *DFNA5* mutation does not cause hearing loss in an Iranian family

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Abstract Mutations in *DFNA5* lead to autosomal dominant non-syndromic sensorineural hearing loss that starts at the high frequencies. To date, only three *DFNA5* mutations have been described, and although different at the genomic DNA level, all lead to exon 8 skipping at the mRNA level. This remarkable fact has led towards the hypothesis that DFNA5-associated hearing loss is caused by a gain-of-function mutation and not by haplo-insufficiency as previously thought. Here, we describe a fourth *DFNA5* mutation: the insertion of a cytosine at nucleotide position 640 (AF073308.1:_c.640insC, AAC69324.1:_p.Thr215HisfsX8). Unlike the previously described mutations, this frameshift mutation truncates the protein in exon 5 of the gene. Although the mutation was found in an extended Iranian family with hereditary hearing loss, it does not segregate with the hearing loss phenotype and is even present in persons with normal hearing. This fact provides further support for the hypothesis that DFNA5-associated hearing loss is caused by a gain-of-function mutation.

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Introduction

DFNA5 was first described in an extended Dutch family segregating autosomal dominant non-syndromic sensorineural hearing loss that started at the high frequencies in affected persons between 5 and 15 years of age and progressed to involve all frequencies. A complex intronic mutation, leading to exon 8 skipping and premature protein truncation, was identified in this family and hypothesized to result in haplo-insufficiency (Van Laer et al. 1998). To recapitulate this mechanism of action, a mouse mutant was made with a targeted deletion of exon 8 of *Dfna5*. In these mice, no Dfna5 protein could be detected by Western blotting, although RT-PCR clearly demonstrated the presence of a transcript lacking exon 8 (Van Laer et al. 2005). Unfortunately, Dfna5–/– mice did not represent a good model for DFNA5-associated hearing loss as hearing loss did not develop, even at older ages (Van Laer et al. 2005). Interestingly, 5 years after the description of the original *DFNA5* mutation, two additional *DFNA5* mutations were reported: a deletion of three nucleotides in the polypyrimidine tract of intron 7 in a Chinese family (Yu et al. 2003), and a nucleotide substitution in the splice-acceptor site of intron 7 in a Dutch family (Bischoff et al. 2004). The hearing loss in all three DFNA5 families is very similar and, although these mutations differ at the genomic DNA level, they all lead to exon 8 skipping at the mRNA level. This very remarkable fact, in addition to the fact that Dfna5–/– mice did not show any hearing loss, had led us to hypothesize that DFNA5-associated hearing loss represents a gain-of-function and not haplo-insufficiency (Van Laer

et al. 2004). This hypothesis has been supported by experimental evidence showing that mutant DFNA5 leads to cell death when transfected into yeast (Gregan et al. 2003) and mammalian cells (Van Laer et al. 2004). Here, we describe for the first time, a truncating mutation in another part of *DFNA5*. This mutation does not lead to hearing impairment, which provides further support for the gain-of-function hypothesis.

Materials and methods

Subjects

Family 741 was collected in the northern part of Iran. Pure tone audiometry was performed at 0.25, 0.5, 1, 2, 4 and 8 kHz for air conduction and at 0.25, 0.5, 1, 2 and 4 kHz for bone conduction. The hearing loss in family 741 presents as a non-syndromic autosomal dominant sensorineural loss affecting all frequencies. In addition to members of family 741, DNA was available from two affected individuals from a total of nine families with recessive hearing loss from the northern parts of Iran and from one isolated patient from the northern part of Iran with assumed recessive hearing loss. The following control subjects have been used: 89 Iranian unrelated normal hearing persons in addition to unaffected members of 35 Iranian families that had been linked to other known non-syndromic hearing loss loci. All persons gave their informed consent prior to their inclusion in the study.

Genetic and mutation analysis

Genomic DNA was extracted from 26 family members, including three spouses, using standard procedures. For linkage analysis, microsatellite markers from the ABI Prism Linkage Mapping Set version 2.5 (Applied Biosystems, Foster City, CA, USA) were amplified using fluorescently labeled primers and separated on an ABI 3100 Genetic Analyzer (Applied Biosystems). Fragments were analyzed using GeneMapper Software Version 3.0 (Applied Biosystems). Haplotypes were reconstructed using GeneScreen software (Itty Bitty Computers, San Jose, CA, USA). Two-point parametric linkage analysis was performed using the Superlink v1.4 option of the easyLINKAGE Plus v4.01beta package (URL: http://www.sourceforge.net/projects/easylinkage/), while multipoint linkage analysis was calculated using the Simwalk v2.91 option of easyLINKAGE.

For mutation analysis, PCR fragments were generated using standard methods from *DFNA5* exons 2–10 using previously described primers (Van Laer et al. 2002). PCR fragments were purified with Exonuclease I (New England Biolabs, Ipswich, MA, USA) and calf intestine alkaline phosphatase (Amersham Biosciences, Piscataway, NJ, USA). Sequencing reactions were performed according to the manufacturer's instructions using the ABI PRISM BigDye Terminator Sequencing Kit (Applied Biosystems) and separated on an ABI 3130xl Genetic Analyzer (Applied Biosystems). The resulting data were processed with Sequencing Analysis Software v5.2 Patch2 (Applied Biosystems).

Results

An extended Iranian family comprised of five generations presented with non-syndromic sensorineural hearing loss. Although several consanguineous marriages were present in the extended pedigree, the hearing loss clearly showed an autosomal dominant inheritance pattern (Fig. 1). Initially, DNA of only 14 family members, including 9 affected, was available. In order to localize the gene responsible for the hearing loss, a genome wide scan was initiated on this family subset. No regions with conclusive linkage resulted from this genome search. However, based on haplotype reconstructions, the *DFNA5* locus was indicated as potentially linked. Consequently, mutation screening of *DFNA5* was completed, using genomic primers to amplify coding exons 2–10 and their respective intron–exon borders, in two affected (IV.2 and V.8) and two unaffected (IV.1 and IV.13) family members. In one affected individual (V.8), we discovered a novel frameshift mutation in *DFNA5* exon 5 (AF073308.1:_c.640insC; AAC69324.1:_p.Thr215HisfsX8; Fig. 2). If the mRNA escapes nonsense-mediated mRNA decay, a truncated protein is predicted consisting of 214 amino acids derived from wild-type DFNA5 followed by an aberrant stretch of 8 amino acids. This mutation was not present in the other affected individual (IV.2). When the rest of the family was analyzed for the *DFNA5* c.640insC mutation, it was found in five of nine affected family members. However, none of the unaffected individuals carried the mutation. Subsequently, DNA from 12 additional family members, including three spouses, was collected and screened for the presence of the *DFNA5* c.640insC mutation. Within the entire family, 5 out of 12 affected individuals and 2 out of 11 unaffected individuals carried the *DFNA5* c.640insC mutation (Fig. 1). This inheritance pattern clearly demonstrated that the mutation did not segregate with the hearing loss in this family. Moreover, when data from the original genome scan were supplemented with new analyses on the additional samples, we localized the gene responsible for the hearing loss to chromosome 4 (unpublished results). Linkage analysis of the six markers from the *DFNA5*

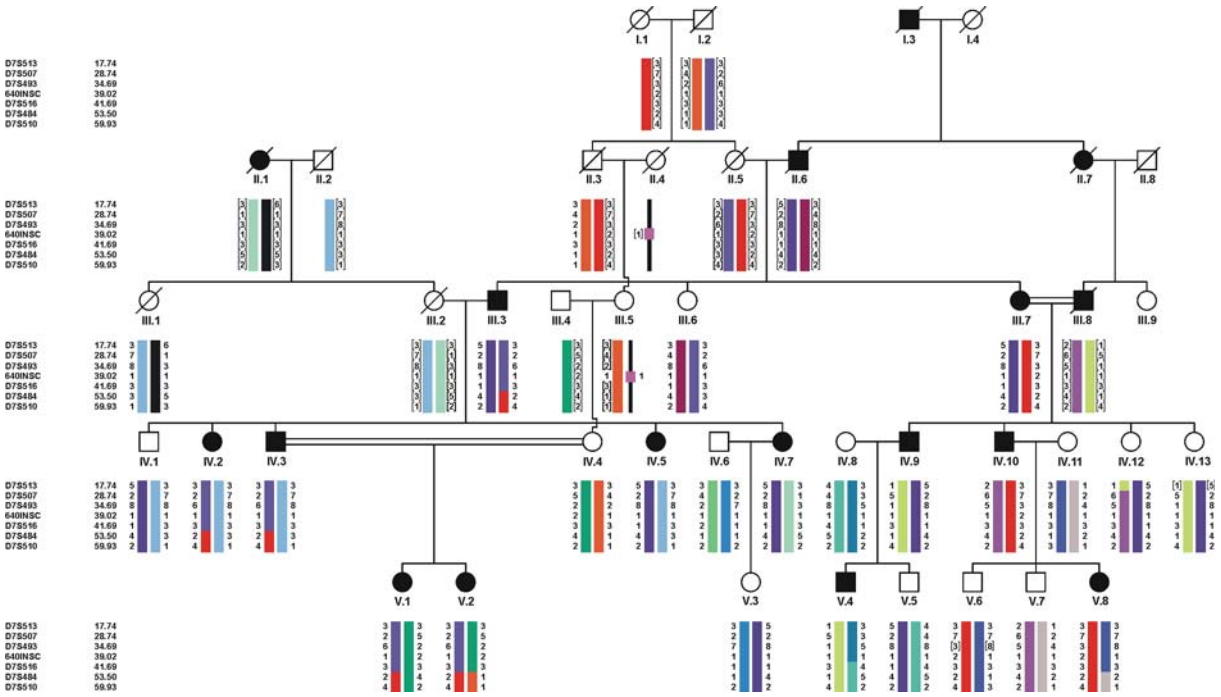

Fig. 1 Pedigree of the Iranian family. Affected individuals are represented by *solid symbols*. Haplotypes are shown beneath each subject. Alleles in *brackets* were reconstructed with easy LINKAGE, indicating that no DNA was available for these individuals. Markers derived from the *DFNA5* region and their respective positions (in cM) on chromosome 7, in addition to the *DFNA5* c.640insC mutation, are shown on the *left* next to each generation

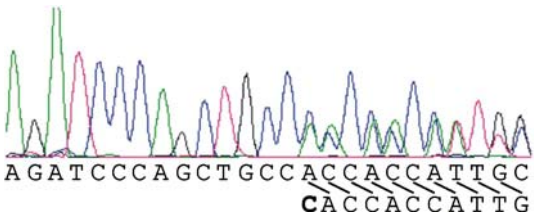

Fig. 2 Electropherogram of the *DFNA5* c.640insC mutation. The forward strand is shown. Below the sequencing profile, the wildtype DFNA5 sequence is listed. Starting from the insertion, the underlying sequence is also given. The inserted C is indicated in *bold*

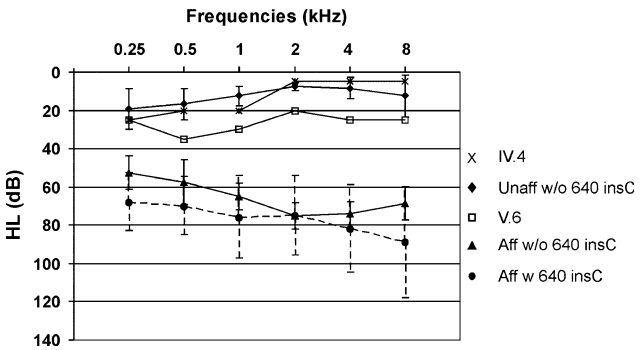

Fig. 3 Mean audiogram for all affected and unaffected family members. There is no statistically significant difference in hearing loss between affected family members who are carriers of *DFNA5* c.640insC and those who are not (Mann–Whitney *U* test). The *full line* with the *diamonds* represents the mean audiogram for all unaffected family members without *DFNA5* c.640insC ($n = 6$; mean age 24.8; age range 11–47), while the *dotted line* with the *circles* and the *full line* with the *triangles* represent the mean audiograms for affected subjects with ($n = 5$; mean age 31.4; age range 10–70) and without ($n = 4$; mean age 26.5; age range 10–39) *DFNA5* c.640insC, respectively. The individual audiograms for the unaffected subjects with *DFNA5* c.640insC are represented by a *full line* with *open squares* for V.6 and by a *full line* with *crosses* for IV.4

region on the entire family resulted in a maximum two-point LOD (log of odds) score of 0.435 for D7S516 at theta 0.00. This region was further excluded by multipoint LOD scores of less than –2 across the complete interval, indicating that *DFNA5* was indeed not the gene responsible for the hearing loss in this Iranian family.

Although we could state without any doubt that *DFNA5* c.640insC was not responsible for the monogenic hearing loss segregating in the Iranian family, we investigated whether *DFNA5* c.640insC led to any degree of hearing loss by calculating the mean audiogram for all affected (with or without *DFNA5* c.640insC) and unaffected family members (Fig. 3). When compared with unaffected family members, affected family members (with or without *DFNA5* c.640insC) showed a threshold shift of 40–60 dB (Fig. 3). There was no statistically significant difference in hearing loss between affected family members who are

carriers of *DFNA5* c.640insC and those who are not. The two unaffected individuals with *DFNA5* c.640insC (IV.4 and V.6) have either no hearing loss at all (IV.4) or a very mild type of hearing loss (V.6).

As the mutation might have entered the pedigree on two different occasions (dark green haplotype and red haplotype in Fig. 1), we investigated the possibility that *DFNA5* c.640insC might be a frequent polymorphism in Iran. However, in a total of 124 Iranian unrelated controls we did not detect a *DFNA5* c.640insC mutation carrier. We further hypothesized that *DFNA5* c.640insC, although not leading to obvious hearing loss in a heterozygotic state, might cause hearing loss in subjects who are homozygous for this mutation. Therefore, we analyzed affected probands from nine families from the same geographic region segregating autosomal recessive hearing loss, but we failed to detect the *DFNA5* c.640insC mutation.

Discussion

In this study, we have identified a novel *DFNA5* mutation which does not cause hearing loss. This mutation is only the fourth in *DFNA5* to be described. The first three mutations, although different at the genomic level, share the common feature of causing exon 8 skipping (Bischoff et al. 2004; Van Laer et al. 1998; Yu et al. 2003). The resulting aberrant mRNA escapes degradation by nonsense-mediated mRNA decay as most of the protein is left intact. That it has a novel and deleterious function is supported by transfection experiments with mutant DFNA5 that lead to cell death in yeast and mammalian cells (Gregan et al. 2003; Van Laer et al. 2004).

In our opinion, the *DFNA5* c.640insC mutation supports the hypothesis that only a very specific gain-of-function mutation caused by skipping of exon 8 can lead to DFNA5-associated hearing loss. Furthermore, as there is no obvious hearing loss in *DFNA5* c.640insC mutation carriers, a reduced amount of functional DFNA5 protein seems sufficient for adequate hearing. This observation is also supported by the Dfna5–/– and Dfna5+/– mouse mutants, which do not display hearing loss (Van Laer et al. 2005). Interestingly, Duno et al. (2004) have described two patients with a deletion of the 7p14–15 locus encompassing the complete DFNA5 gene, and both of these patients show no sign of hearing impairment.

One of the two unaffected *DFNA5* c.640insC carriers, a 10-year-old, has mild hearing loss, which is greater in the low frequencies and atypical for the hearing loss in this family. Although no previous history of infections or perinatal problems was recorded, detailed clinical data were not available for this child and so the cause of his hearing loss remains unknown. The second unaffected *DFNA5* c.640insC carrier has normal hearing for her age (33 years). There is also no significant difference in hearing thresholds between affected subjects with or without *DFNA5* c.640insC.

In conclusion, we have identified a *DFNA5* mutation that does not cause any obvious hearing loss, which further supports the hypothesis that DFNA5-associated hearing loss is caused by a gain-of-function mutation.

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