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SHORT COMMUNICATION

A canonical splice site mutation in *GIPC3* causes sensorineural hearing loss in a large Pakistani family

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With homozygosity mapping we have identified two large homozygous regions on chromosome 3q13.11–q13.31 and chromosome 19p13.3–q31.32 in a large Pakistani family suffering from autosomal recessive nonsyndromic hearing impairment (arNSHI). The region on chromosome 19 overlaps with the previously described deafness loci DFNB15, DFNB72 and DFNB95. Mutations in *GIPC3* have been shown to underlie the nonsyndromic hearing impairment linked to these loci. Sequence analysis of all exons and exon–intron boundaries of *GIPC3* revealed a homozygous canonical splice site mutation, c.226-1G>T, in *GIPC3*. This is the first mutation described in *GIPC3* that affects splicing. The c.226-1G>T mutation is located in the acceptor splice site of intron 1 and is predicted to affect the normal splicing of exon 2. With a minigene assay it was shown to result in the use of an alternative acceptor site in exon 2, resulting in a frameshift and a premature stop codon. This study expands the mutational spectrum of *GIPC3* in arNSHI.
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Hearing impairment is the most common sensory disorder worldwide. Pakistan has a high rate of consanguineous marriages and therefore this population is very suitable for collecting large families for genetic studies in recessive disorders. Many genes and loci for arNSHI have been mapped in families of Pakistani origin (hereditary hearing loss homepage). It has been shown that mutations in *GJB2* (MIM *121011) are the most prevalent cause of deafness in the Pakistani population; mutations in this gene cause deafness in about 45–50% of the families.[1] A large consanguineous Pakistani family (16DFN, Figure 1a) with prelingual arNSHI, residing in Southern Punjab, was selected for genetic screening. The study was approved by the human subjects review board of the Institute of Biomedical and Genetic Engineering, Islamabad, Pakistan. Peripheral blood was sampled from nine members of the three-generation-family, and DNA was extracted using a phenol chloroform method.[2] Mutations in the previously reported most frequently implicated genes in arNSHI, *GJB2* and *MYO15A* (MIM *602666) were excluded;[1] *GJB2* was analyzed via Sanger sequencing of both exons and *MYO15A* by genotyping microsatellite markers D17S1824 and D17S2196. Subsequently, the samples of the affected individuals IV.2, IV.4 and IV.5 were genotyped using the Human Omniexpress array (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. Homozygous regions were determined using the freely available Homozygosity Mapper software (www.homozygositymapper.org).[3] In total, three homozygous regions larger than 1 Mb were shared by the three affected individuals (Figure 1b). A small homozygous region of 1.08 Mb was identified on chromosome 6p22.1 flanked by rs2531817 and rs362529 (chr6: 28,448,894-29,530,868, hg19). Further, two large homozygous regions were identified on chromosome 3q13.11–q13.31 and chromosome 19p13.3-q13.32. The region on chromosome 3, flanked by rs1994818 and rs1347060, encompassed 13.4 Mb (chr3:103,026,304-116,455,442, hg19). The region on chromosome 19 encompassed 45.3 Mb (chr19:3,414,088-49,092,430, hg19) flanked by rs12975319 and rs12984929. The homozygous region on chromosome 3 did not contain or overlap with a known deafness locus or gene. However, several loci for arNSHI have been mapped to chromosome 19. The homozygous region identified in the present family was much larger than any of the loci described so far on chromosome 19. The present region contains the previously described DFNB68 and DFNB81 loci and partially overlaps with the DFNB15, DFNB72 and DFNB95 loci.[4–8] For DFNB68 and DFNB81 no disease-associated mutations have been reported so far. For DFNB15, DFNB72 and DFNB95 mutations in *GIPC3* (MIM *608792) were found to be causative for the disease.[5,7] As *GIPC3* resides within the homozygous region of chromosome 19, all exons and exon–intron boundaries were screened by Sanger sequencing in affected individual IV.2. Primer sequences and PCR conditions have been described previously.[5] The previously reported mutations (Supplementary Table 2) were not present in affected individual IV.2. However, a canonical splice site mutation was identified in intron 1 at genomic position Chr19(GRCh37):

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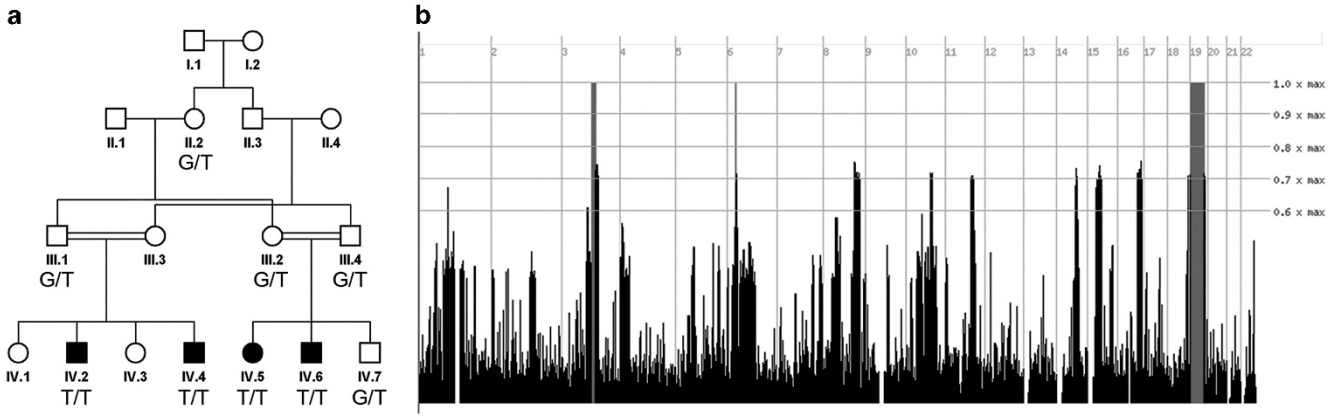

Figure 1 Pedigree and homozygosity mapping (**a**) Only those family members of the pedigree are depicted who were relevant for the study. The segregation of the c.226-1G>T is presented in the pedigree. (**b**) Graphical representation of the homozygous regions identified with the Homozygosity Mapper software.

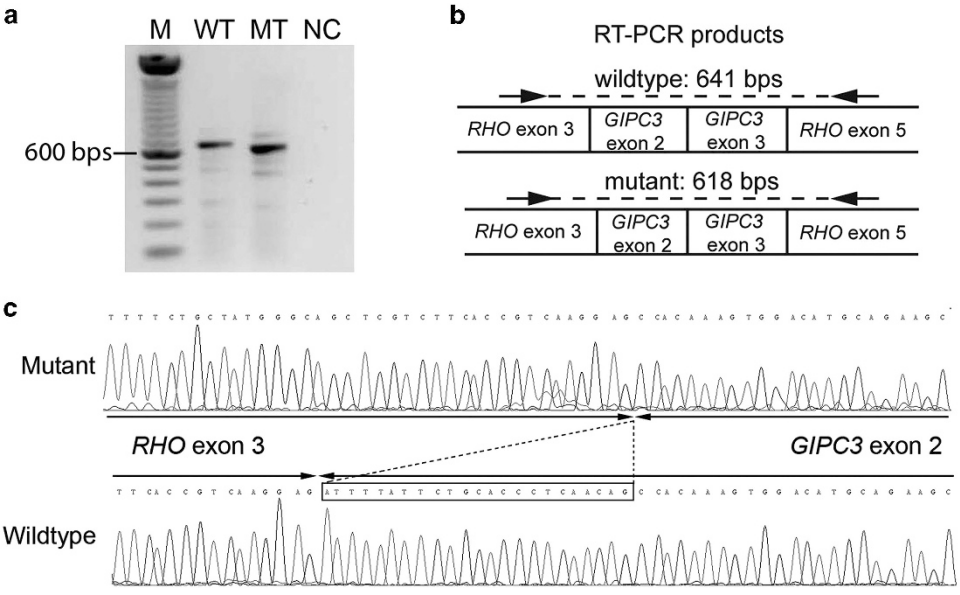

Figure 2 Splicing effect of the c.226-1G>T mutation (**a**) An agarose gel containing Reverse transcription-PCR products detected from HEK293T cells transfected with the wild-type and mutant minigene construct. (**b**) A schematic representation of the identified splicing products. The RT-PCR products were verified with sequence analysis. (**c**) Electropherogram of the partial cDNA sequence of RNA derived from cells transfected with the pCI-NEO with either the mutant or wild-type *GIPC3* exon 2. The c.226-1G>T mutation leads to the use of an alternative splice acceptor site in exon 2 and to the deletion of the first 23 nucleotides of exon 2.

g.3586492G>T (c. 226-1G>T, reference sequence NM_133261.2) segregating with the hearing impairment in the family (Figure 1a and Supplementary Figure 1). The altered nucleotide is highly conserved and this variation has not been reported in any database so far, not even in the Exome Variant Server, which demonstrates the uniqueness of this variant.

The c.226-1G>T mutation abolishes the original splice acceptor site of intron 1, going from a score of 76.0 (calculated by the Human Splicing Finder, range 1–100) in the wild-type situation to no score at all for the mutation (Supplementary Figure 2). This will affect the normal splicing of exon 2; however, the resulting effect of the mutation is difficult to predict. One possibility is the use of an alternative splice acceptor site located within exon 2. This alternative splice acceptor site has a consensus value of 83.4, which is even higher than that of the original splice acceptor site (76.0, Supplementary Figure 2). Another possibility is that exon 2 will be skipped completely. This exon consists of 186 nucleotides and encodes 62 amino acids. To determine the effect of the c.226-1G>T mutation on splicing, a minigene approach was used as previously described.[1] Primer sequences to amplify a fragment containing exon 2 and 3 of *GIPC3* are shown in Supplementary Table 1. The minigene assay revealed correct splicing of the wild-type *GIPC3* exon 2, whereas the c.226-1G>T mutation resulted in the use of the predicted alternative acceptor site in exon 2 (Figure 2). This leads to absence of the first 23 nucleotides of exon 2 in the mRNA and causes a frameshift and a premature stop codon. The predicted truncated protein does not have the PDZ domain and thus loses its function.

In total 12 mutations have been described in *GIPC3*, including the mutation identified in the current study, which all cause arNSHI

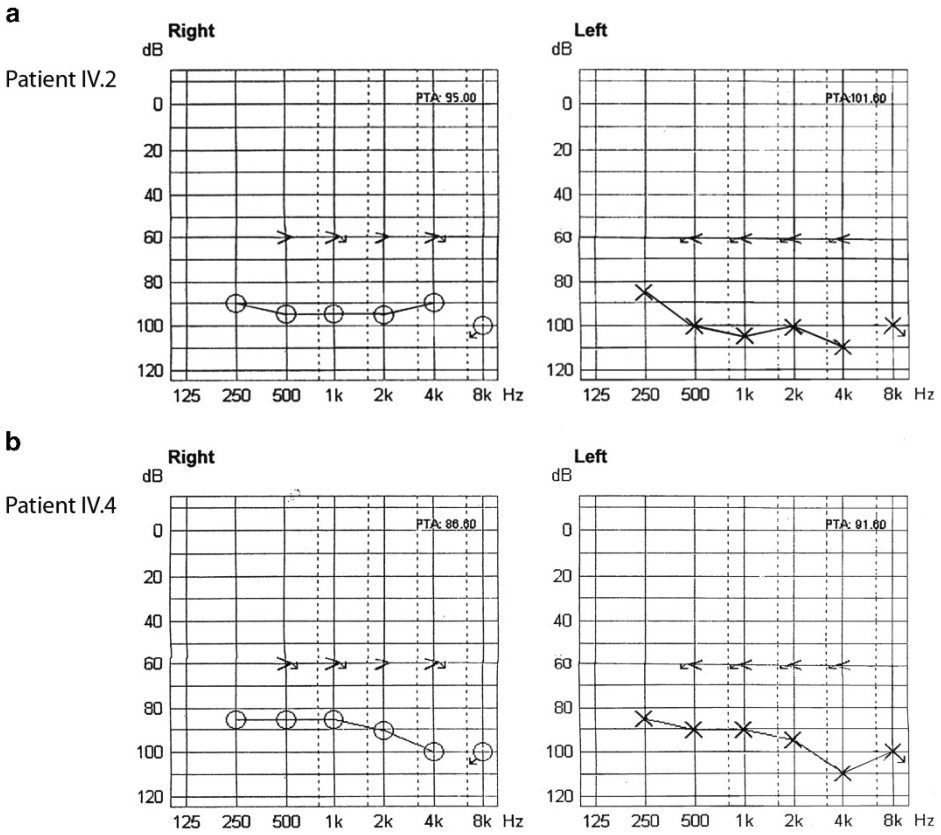

Figure 3 Pure tone air-conduction thresholds are shown of two affected members of family 16DFN. (**a**) Individual IV.2 (17 years of age) (**b**) Individual IV.4 (14 years of age).

(Supplementary Table 2).[5,7,9–11] Most of these mutations were found in the Pakistani population thus showing the major contribution of this gene to deafness in this population. The mutations described for *GIPC3* include three truncating mutations, and nine missense mutations. Two missense mutations have been reported so far in the PDZ domain of *GIPC3*.[7,11] The other disease-related mutations described so far are in regions of unknown function or in low complexity regions (Supplementary Table 2).[5,7,10]

Different degrees of hearing impairment have been reported in families with *GIPC3* mutations, ranging from mild-to-severe to profound.[5,7,9–11] No clear relation is evident between the type or location of the mutations in *GIPC3* and the degree of hearing loss. Pure-tone air conduction audiometry was available from affected individuals IV.2 and IV.4 of family 16DF. Both individuals show severe to profound hearing impairment affecting all frequencies at the age of 17 and 14, respectively (Figure 3). The hearing impairment was self-reported to be congenital and non-progressive. Progression of hearing loss has been reported in one individual of a Dutch family, who had a mutation in *GIPC3*.[5]

Mouse studies have suggested that GIPC3 is required for the postnatal maturation of hair bundles in the inner ear and long-term survival of hair cells and spiral ganglion.[5] *Gipc3* mutant mice showed defects in mechanotransduction in both inner and outer hair cells that were associated with defects in potassium channel activity.[5] Identification of novel mutations in *GIPC3* may further elucidate the role of GIPC3 in various pathways of sound transduction and processing. Also, it may aid to further define the phenotype associated with *GIPC3* mutations.

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Web resources. The URLs for the data presented herein are as follows: The Hereditary Hearing loss Homepage, http://hereditaryhearingloss.org/. HomozygosityMapper, http://www.homozygositymapper.org/. Polymorphism Phenotyping (Polyphen-2), http://genetics.bwh.harvard.edu/pph2/. Sorting Intolerant from Tolerant (SIFT), http://sift.jcvi.org/. UCSC Human Genome Database, Build hg19, http://www.genome.ucsc.edu/. Exome variant Server, http://evs.gs.washington.edu/EVS/. 1000 Genome projects, http://browser.1000genomes.org/. National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/

Author contributions: SS and JO performed the experiments, SM and MI provided the family for the study. SS did the data analysis. HK provided all reagents/software. SS wrote the paper. RQ, AM, HK and MS supervised the work and critically read the manuscript.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)