Published in final edited form as:

Clin Genet. 2012 May ; 81(5): 498–500. doi:10.1111/j.1399-0004.2011.01729.x.

***DFNB86*, a novel autosomal recessive nonsyndromic deafness locus on chromosome 16p13.3**

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To the Editor:

We identified linkage of recessive pre-lingual deafness to a new locus, designated *DFNB86*. This linkage was identified in a large consanguineous family PKDF799 with eleven affected individuals (Fig. 1a). Family PKDF799 was recruited in Pakistan after obtaining Institutional Review Board (IRB) approvals from the National Center of Excellence in Molecular Biology, Lahore (FWA00001758), the Combined Neuroscience IRB at the National Institutes of Health (OH-93-N-016), and the IRB Committee at the Cincinnati Children's Hospital Research Foundation (2009-0684). Written informed consent was obtained from all participating individuals and parents of minor subjects. The proband was V:6 (Fig. 1a). Four affected individuals and three unaffected siblings from this family were tested for hearing ability using pure-tone air and bone conduction audiometry. All of the affected individuals evaluated showed profound sensorineural hearing loss across all tested frequencies (Fig. 1b). There was no history of vestibular difficulties among the deaf individuals in this family, but we were not able to perform sophisticated tests of the status of their vestibular system using posturography or a rotary chair, for example. We tentatively concluded that hearing loss segregating in family PKDF799 is nonsyndromic.

We initially observed that deafness in family PKDF799 did not co-segregate with short tandem repeat (STR) markers for 63 of the reported recessive nonsyndromic deafness loci (data not shown). A genome wide linkage analysis using 388 fluorescently labeled microsatellite (STR) markers determined that the phenotype showed significant evidence of

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Conflict of interest statement

Authors declare no conflict of interest

linkage to STR markers on chromosome 16p13.3. In addition to the chromosome 16p13.3 locus, two point LOD scores of 1.54 for marker *D11S1338* and 1.48 for marker *D17S1852* were also found. However, genotyping additional DNA samples from family PKDF799 excluded linkage to both chromosomes 11 and 17. Additional STRs on 16p were then genotyped, and haplotype analysis revealed a 3.09 Mb linkage interval extending from the telomere of chromosome 16p to marker *D16S3070* (Fig. 1a). Under a model with a disease allele frequency of 0.001 and a fully penetrant disorder, a maximum two-point LOD score of 8.54 ($\theta=0$) was obtained for the marker *D16S3024*. These results define and delimit *DFNB86* (HUGO approved locus symbol), a novel recessive deafness locus on chromosome 16p13.3.

There are few possible interpretations of the haplotype data (Fig. 1a) as to the number of mutant alleles segregating in family PKDF799. If there is a single mutant allele, then all affected individuals are likely to be homozygous for a region of the disease haplotype. Under this model, the causative gene resides in a 1.44 Mb interval between markers *D16S3024* and *D16S3070*. The proximal meiotic recombination at marker *D16S3024* occurred in individuals IV:5 and IV:6, while the distal recombination at marker *D16S3070* is observed in normal hearing individual V:8 (Fig. 1a). However, if there are two different mutant alleles of the same deafness gene segregating in this family, the *DFNB86* gene is located telomeric to marker *D16S3395*, and affected individuals IV:5 and IV:6 are compound heterozygotes (Fig. 1a). Under this assumption, a linkage region of 3.09 Mb extends from the telomere of chromosome 16p to marker *D16S3070*. Although unusual, consanguineous families segregating two different mutant alleles have been reported (1, 2). Other possibilities for the haplotype data are that individuals IV:5 and IV:6 have hearing loss due to a mutation at different locus, or are phenocopies. We will be able to distinguish between these various interpretations of the haplotype data once we have identified the *DFNB86* gene.

The *DFNB86* locus does not overlap with any published mutated gene associated with a hearing phenotype or other reported deafness loci on chromosome 16. There are 167 candidate genes in the 3.09 Mb *DFNB86* linkage interval including *MSRB1*, *NTN3*, *GFER*, *SYNGR3*, *CLDN6*, and *CLDN9*. Methionine Sulfoxide Reductases (MSRs) are enzymes involved in repair of proteins damaged by oxidative stress. We recently reported that mutations of Methionine Sulfoxide Reductase B3 cause nonsyndromic deafness DFNB74 (3). Therefore, *MSRB1* was sequenced using genomic DNA of an affected individual from family PKDF799. We did not find a causative variant in the coding exons, 3' and 5' untranslated regions (UTR) as well as several conserved intronic regions of *MSRB1*. Similarly, a likely causative variant was not found in *SYNGR3*, *NTN3*, and *GFER* encoding a synaptic vesicle protein, an axon guidance protein, and a growth factor, respectively. *CLDN6* and *CLDN9* are also located in the *DFNB86* interval. Since claudins 9, 11 and 14 have been shown to be necessary for inner ear function (4–8), we sequenced the coding exons of *CLDN6* and *CLDN9*, but did not find a potential pathogenic mutation. When we sequenced these six genes using genomic DNA from affected individuals from family PKDF799, the only variants we found were known SNPs. Rather than continue hierarchical sequencing of candidate genes based on function or expression in the inner ear, future studies will employ massively parallel sequencing of genomic DNA (9) enriched for the entire *DFNB86* critical interval.

Acknowledgments

The authors are grateful to the family for contributing to this study. We thank Alejandro Schaffer for suggestions regarding statistical analysis of our linkage data. We also thank Dennis Drayna and Changsoo Kang for their critiques. This study was supported by Cincinnati Children's Hospital Research Foundation intramural research funds to SiR and ZA, the National Institute on Deafness and Other Communication Disorders (NIDCD/NIH)

research grant R00-DC009287-03 to ZA. ZA is also a recipient of RPB Career Development Award. Work in Pakistan was supported by the Higher Education Commission, Islamabad, EMRO/WHO-COMSTEC, Ministry of Science and Technology, Islamabad and the International Center for Genetic Engineering and Biotechnology, Trieste, Italy under project CRP/PAK08-01 Contract no. 08/009 to ShR. This study was also supported by intramural funds from the NIDCD/NIH DC00039-14 to TBF.

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Fig. 1. *DFNB86*-linked pedigree. (a) Chromosome 16p13.3 haplotypes in family PKDF799. Filled symbols represent deaf individuals. The linked haplotype is boxed and/or shaded grey in all generations. All affected individuals are homozygous below marker *D16S3024*. The haplotype between markers *D16S521* and *D16S3024* (shaded grey) represents a region for which affected individuals V:2–4 are homozygous for a different haplotype than other affected individuals in this family. The STR markers and physical map positions in megabases (Mb, February 2009 human reference sequence GRCh37, hg19) are shown on the left of the pedigree. A two-point LOD score at recombination fraction $\theta=0$ for each marker is also provided. Haplotype analysis shows a linkage region of 3.09 Mb extending

from the telomere of chromosome 16p to marker *D16S3070*. The distal breakpoint at marker *D16S3070* was provided by a normal hearing individual V:8. **(b)** Pure tone audiograms for family PKDF799 V:8 (18 yo female), V:5 (15 yo male), V:6 (19 yo male) and IV:5 (60 yo male).