

SHORT REPORT

# DFNB79: reincarnation of a nonsyndromic deafness locus on chromosome 9q34.3

Shahid Yar Khan<sup>1,4</sup>, Saima Riazuddin<sup>2,4,5</sup>, Mohsin Shahzad<sup>1</sup>, Nazir Ahmed<sup>1</sup>, Ahmad Usman Zafar<sup>1</sup>, Atteeq Ur Rehman<sup>1,2</sup>, Robert J Morell<sup>2</sup>, Andrew J Griffith<sup>3</sup>, Zubair M Ahmed<sup>2,5</sup>, Sheikh Riazuddin<sup>1</sup> and Thomas B Friedman<sup>\*,2</sup>

Genetic analysis of an inbred Pakistani family PKDF280, segregating prelingual severe to profound sensorineural hearing loss, provided evidence for a *DFNB* locus on human chromosome 9q34.3. Co-segregation of the deafness trait with marker *D9SH159* was determined by a two-point linkage analysis (LOD score 9.43 at  $\theta=0$ ). Two additional large families, PKDF517 and PKDF741, co-segregate recessive deafness with markers linked to the same interval. Haplotype analyses of these three families refined the interval to 3.84 Mb defined by *D9S1818* (centromeric) and *D9SH6* (telomeric). This interval overlaps with the previously reported *DFNB33* locus whose chromosomal map position has been recently revised and assigned to a new position on chromosome 10p11.23–q21.1. The nonsyndromic deafness locus on chromosome 9q segregating in family PKDF280 was designated *DFNB79*. We are currently screening the 113 candidate *DFNB79* genes for mutations and have excluded *CACNA1B*, *EDF1*, *PTGDS*, *EHMT1*, *QSOX2*, *NOTCH1*, *MIR126* and *MIR602*.

European Journal of Human Genetics (2010) 18, 125–129; doi:10.1038/ejhg.2009.121; published online 15 July 2009

**Keywords:** hereditary deafness; *DFNB79*; *DFNB33*; Pakistan; chromosome 9q34.3

## INTRODUCTION

The genetic heterogeneity of nonsyndromic recessive deafness (*DFNB*) reflects the complexity of structure and function of the vertebrate inner ear. Fifty-three *DFNB* loci have been mapped and reported.<sup>1,2</sup> The rate at which novel *DFNB* loci have been mapped over the past 10 years has been relatively constant. Approximately five new *DFNB* loci have been reported each year since 1998 despite the frequent assignment of linkage of deafness segregating families to known *DFNB* loci. Thus, it has become necessary to ascertain more and more large families to identify a novel *DFNB* locus. To date, a gene has yet to be identified for 60% of the *DFNB* loci.

Medlej-Hashim *et al.*<sup>3</sup> reported the mapping of *DFNB33* to chromosome 9q34.3 in a single family from Jordan with a LOD score of 3.38. However, the linkage of *DFNB33* deafness segregating in the Jordanian family on 9q34.3 was recently reported to be spurious, and subsequently *DFNB33* was reassigned to chromosome 10p11.23–q21.1.<sup>4</sup> Here, we report three Pakistani families with prelingual hearing loss supporting the existence of a *DFNB* locus, *DFNB79*, on chromosome 9q34.3.

## MATERIALS AND METHODS

### Study participants

This study was approved by the Institutional Review Board (IRB) at the National Centre of Excellence in Molecular Biology (NCEMB), Lahore, Pakistan and the Combined Neuroscience IRB at the National Institutes of Health, USA (OH-93–N-016). Written informed consent was obtained from all of the

study participants. Hearing was evaluated by pure-tone (air conduction) audiometry at octave frequencies from 250 to 8000 Hz. Vestibular function was evaluated by tandem gait and Romberg testing. The ocular evaluation comprised slit lamp and funduscopic examinations.

### Genotyping and sequencing

Blood was obtained through venipuncture and genomic DNA was extracted using a standard protocol.<sup>5</sup> We initially excluded linkage to the reported *DFNB* loci in the year 2001 using STR (short tandem repeat) markers. Subsequently, a genome-wide scan was undertaken by using 388 fluorescently labeled microsatellite markers spaced at an average interval of 10 cM across the genome (ABI Prism Linkage Mapping Set, v2.5 Applied Biosystems, Foster City, CA, USA). Markers were amplified by polymerase chain reaction (PCR) on a Gene Amp PCR 9700 (Applied Biosystems) and were analyzed on an ABI Prism 3100 Genetic Analyzer. Alleles were assigned using Genescan and Genotyper software (Applied Biosystems). The FASTLINK (<http://www.ncbi.nlm.nih.gov/CBBresearch/Schaffer/fastlink.html>) computer package was used to calculate LOD scores.<sup>6</sup> MLINK was used for two-point LOD scores. Marker order and map distances were obtained from the Marshfield genetic map (<http://research.marshfieldclinic.org>). An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were used for linkage analysis.

### Candidate gene screening

Positional candidate genes were identified using the UCSC Genome Bioinformatics web browser (UCSC Genome Bioinformatics Build 36.1: <http://genome.ucsc.edu/>). Primers used for PCR amplification and sequencing of *CACNA1B*, *EDF1*, *PTGDS*, *EHMT1*, *QSOX2*, *NOTCH1*, *MIR126* and *MIR602* from the flanking region of each exon were designed using Primer3.

<sup>1</sup>National Center of Excellence in Molecular Biology, Punjab University, Lahore, Pakistan; <sup>2</sup>Laboratory of Molecular Genetics, Section on Human Genetics, Rockville, MD, USA; <sup>3</sup>Otolaryngology Branch, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, MD, USA

\*Correspondence: Dr TB Friedman, Laboratory of Molecular Genetics, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, 5 Research Court, Room 2A-19, Rockville, MD 20850, USA. Tel: +1 301 496 7882; Fax: +1 301 402 7580; E-mail: [friedman@nidcd.nih.gov](mailto:friedman@nidcd.nih.gov) <sup>4</sup>These authors contributed equally to this work. <sup>5</sup>Current address: Divisions of Pediatric Otolaryngology-Head and Neck Surgery and Ophthalmology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH 45219, USA.

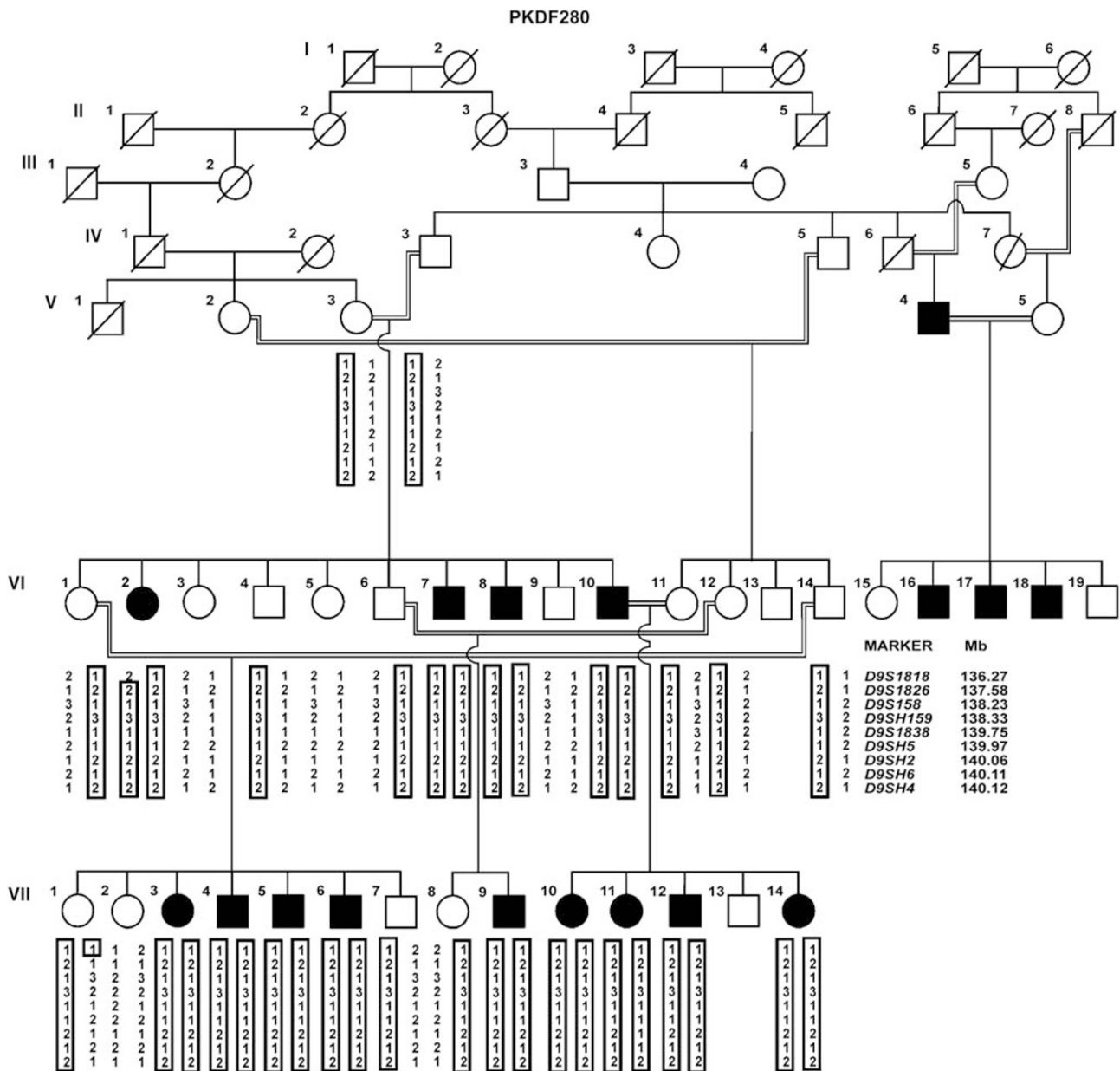
Received 18 March 2009; revised 11 June 2009; accepted 11 June 2009; published online 15 July 2009

## RESULTS

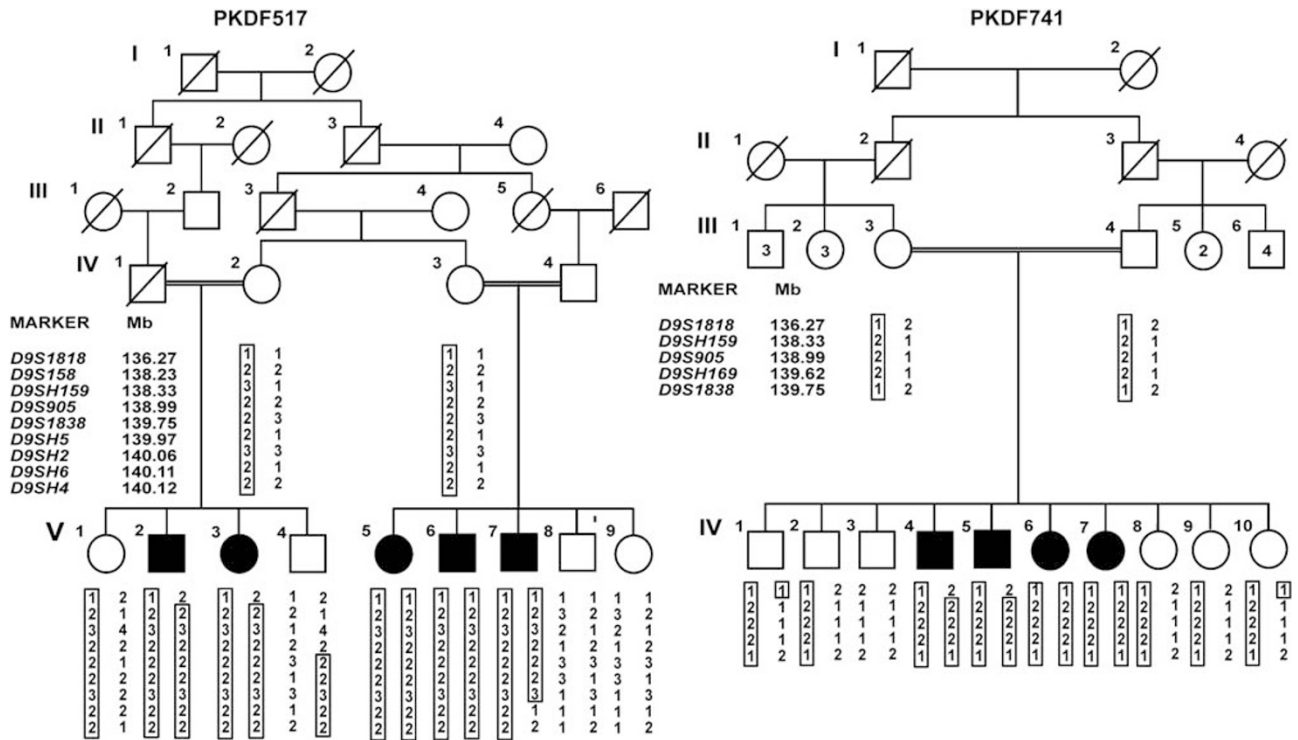
The segregation of hearing loss in families PKDF280, PKDF517 and PKDF741 is consistent with autosomal recessive inheritance (Figures 1 and 2). Affected individuals displayed prelingual, bilateral and severe to profound hearing loss. There is some variability in the severity of hearing loss observed among the affected individuals of *DFNB79* families (Figure 4). Only air conduction thresholds were available, and thus we cannot rule out a conductive component of the hearing loss. Tandem gait and Romberg testing were normal in affected individuals indicating that there is no obvious vestibulopathy. We identified no evidence of bone, skin or renal anomalies or a vision disorder. We found no other obvious clinical manifestations co-segregating with hearing loss in the study participants.

A preliminary linkage survey of deafness segregating in family PKDF280 revealed no linkage to any known *DFNB* loci known in 2001. Evidence of linkage to chromosome 9q34.3 was obtained through a subsequent genome-wide linkage analysis. Mapping with additional STR markers and haplotype analysis defined a linkage interval of 3.84 Mb (Figure 1), without a telomeric meiotic breakpoint in PKDF280. However, *D9S1818* (136.27 Mb) defined the centromeric boundary in affected individual VI:2 (Figure 1). A significant two-point LOD score ( $Z_{\max}$ ) of 9.43 at ( $\theta=0$ ) was obtained for the marker *D9SH159* (138.33 Mb; Table 1).

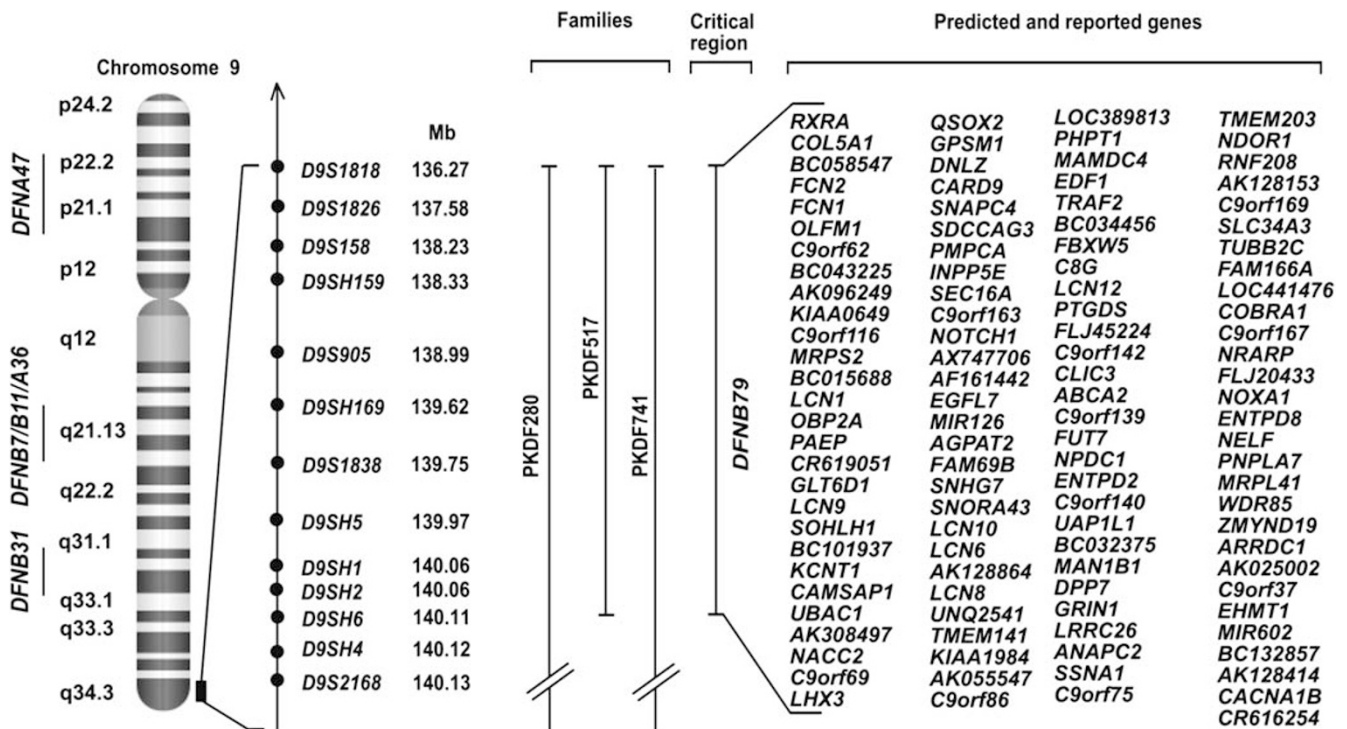
A cohort of more than 700 consanguineous *DFNB* families has been ascertained in Pakistan at the NCEMB. We screened these families using STR markers and found two additional families segregating



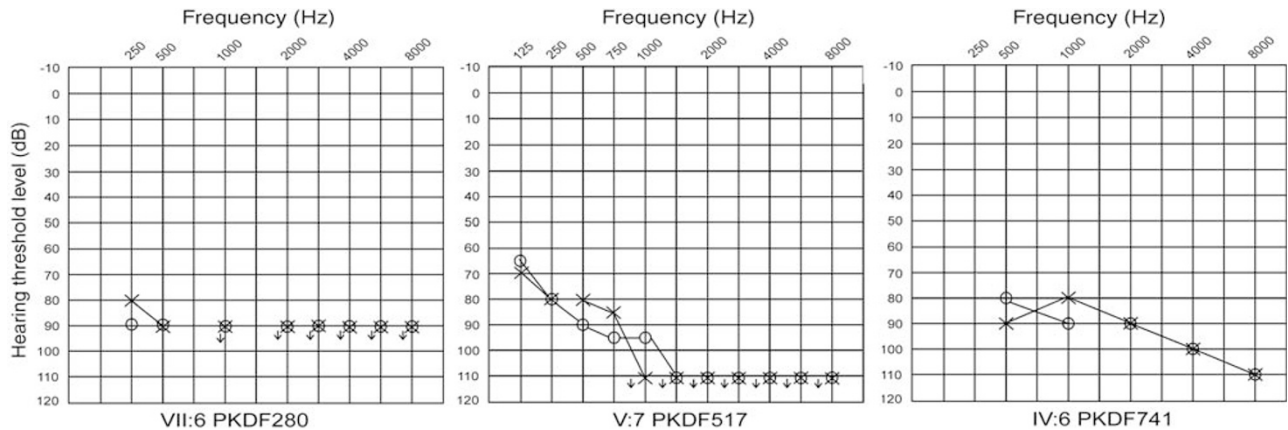
**Figure 1** Pedigree of family PKDF280. Filled symbols represent hearing-impaired individuals. The linked haplotypes are boxed. The STR markers and their relative map positions (Mb) according to UCSC Genome Bioinformatics Build 36.1 (<http://genome.ucsc.edu/>) are shown along with the pedigree. Haplotype of PKDF280 defines a linkage region of ~3.84 Mb. Affected individual VI:2 provided the proximal recombination breakpoint at marker *D9S1818* (136.27 Mb).



**Figure 2** Pedigrees of families PKDF517 and PKDF741. The linked haplotypes are boxed. Haplotype analysis of family PKDF517 defined a linkage interval of 3.84 Mb bounded by marker *D9S1818* (136.27 Mb) and *D9SH6* (140.11 Mb). In family PKDF741, the centromeric boundary was established by meiotic recombinations in two affected individuals (IV:4 and IV:5) at *D9S1818* (136.27 Mb). The *DFNB79* linkage interval in family PKDF741 extends to the telomere.



**Figure 3** *DFNB79* chromosomal map location at 9q34.3. Physical map distances are from the UCSC Genome Bioinformatics Build 36.1. STR markers are represented by filled circles. All of the known genes and predicted transcription units in this interval are shown. Candidates are ordered from the proximal gene (*RXRA*) in column 1 to the distal gene (*CR616254*) at the bottom of column 4. Markers *D9S1818* (136.27 Mb) and *D9SH6* (140.11 Mb) define the *DFNB79* critical linkage interval.



**Figure 4** Audiograms from an affected individual from each of the three DFNB79 families PKDF280, PKDF517 and PKDF741. The ages at the time of audiological examination are ~15, 21 and 9 years, respectively. Symbols 'o' and 'x' denote air conduction pure-tone thresholds at different frequencies in the right and left ear.

**Table 1** LOD scores for markers on chromosome 9q34.3 in three DFNB79 families

Markers	Mb	Two-point LOD scores <sup>a</sup>		
		PKDF280	PKDF517	PKDF741
D9S1818	136.27	-4.34	-1.42	-4.34
D9S1826	137.58	7.08		
D9S158	138.23	7.14	2.90	
D9SH159	138.33	9.43	4.30	3.25
D9S905	138.99		2.90	3.25
D9SH169	139.62			3.25
D9S1838	139.75	3.47	4.16	3.25
D9SH5	139.97	9.42	4.30	
D9SH2	140.06	9.42	2.90	
D9SH6	140.11	5.61	-4.34	
D9SH4	140.12	7.08	2.90	

<sup>a</sup>Recombination fraction  $\theta=0$ .

deafness (PKDF517 and PKDF741) linked to DFNB79 (Figure 2). PKDF741 yielded a maximum two-point LOD score ( $Z_{\max}$ ) of 3.25 for markers D9SH159, D9S905, D9SH169 and D9S1838 (Table 1). The maximum two-point LOD score ( $Z_{\max}$ ) for family PKDF517 was 4.30 at  $\theta=0$  for markers D9SH159 and D9SH5 (Table 1). Meiotic recombinations in affected individuals from each DFNB79 family delineate the centromeric boundary at marker D9S1818 (136.27 Mb; Figures 1 and 2). The inferred haplotypes of affected individual V:7 in PKDF517 provided evidence for the telomeric boundary of the DFNB79 locus at marker D9SH6 (Figure 2). Assuming locus homogeneity, these three DFNB79 families define a critical linkage interval of 3.84 Mb on chromosome 9q34.3 (Figure 3), and each of the three DFNB79 families has a distinct haplotype across the refined linkage interval, suggesting different mutant alleles.

Three deafness loci have been mapped earlier to chromosome 9, including DFNA36/DFNB7/DFNB11<sup>7</sup>, DFNB31<sup>8,9</sup> and DFNA47.<sup>10</sup> The genetic interval of DFNB79 does not overlap with any of these three deafness loci (Figure 3). The DFNB79 locus has 113 annotated and hypothetical genes located in an approximately 3.84 Mb interval (Figure 3). The coding exons and flanking intronic sequence of CACNA1B, EDF1, PTGDS, EHMT1, QSOX2, MIR126, MIR602 and NOTOCH1 were sequenced in two affected individuals from each of

the three DFNB79 families analyzed in this study, and no pathogenic sequence variants were identified.

## DISCUSSION

Families PKDF280, PKDF517 and PKDF741 provide evidence for DFNB79, a novel nonsyndromic recessive deafness locus on chromosome 9q34.3. Each of the three families reported here has a unique DFNB79 haplotype suggesting a multiple allelic series, assuming a single mutated gene is responsible for this phenotype.

There are 113 candidate genes in the DFNB79 critical linkage interval and several of them are expressed in the inner ear,<sup>11</sup> including QSOX2, NOTCH1, EDF1, PTGDS, EHMT1 and CACNA1B.<sup>12</sup> CACNA1B encodes a voltage-dependent calcium channel. Calcium homeostasis is crucial for mechano-electrical transduction,<sup>13</sup> but no mutations were found in the 46 exons of this gene. A transmembrane receptor, NOTCH1, is crucial for inner ear development.<sup>14</sup> However, we did not find a pathogenic mutation in the 34 exons and flanking intronic sequence of NOTCH1 in affected individuals of our DFNB79 families. We also did not find a mutation in EDF1, PTGDS, EHMT1 and QSOX2, which encode a transcription co-activator, an enzyme necessary for the synthesis of prostaglandin, a histone methyltransferase and neuroblastoma-derived sulfhydryl oxidase, respectively. Recently, a dominant mutation of MIR96 responsible for human DFNA50 nonsyndromic deafness<sup>15</sup> and a semidominant mutation of Mirn96 causing rapid progressive hearing loss phenotype in the diminuendo mouse<sup>16</sup> were reported. The DFNB79 interval includes two microRNAs genes, MIR126 and MIR602, but no mutant alleles were found.

## ACKNOWLEDGEMENTS

We are grateful to the participants of this study. We also thank Drs Melanie Barzik, Tamar Ben-Yosef, Byung Yoon Choi, and Dennis Drayna for suggestions regarding this article. We also thank Barbara Ploplis and Erich Boger for their technical assistance. This study was supported by the Higher Education Commission, Islamabad, Pakistan; EMRO/WHO-COMSTECH (RABGH 06-07\_24), Ministry of Science and Technology (MoST), Islamabad, and intramural funds from the National Institute on Deafness and Other Communication Disorders, NIH (1 Z01 DC000039-12) to TBF and (Z01 DC000064) to AJG.

**Electronic database information**

Hereditary Hearing Loss Homepage: <http://www.uia.ac.be/dnalab/hhh/>  
Primer3 Web-Based Server: [http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\\_www.cgi](http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)

UCSC Genome Bioinformatics: <http://genome.ucsc.edu/>  
Morton Fetal Cochlea cDNA Library: [http://www.brighamandwomens.org/bwh\\_hearing/InnerEarCDNAArrays.aspx](http://www.brighamandwomens.org/bwh_hearing/InnerEarCDNAArrays.aspx)  
NEIBank (select cochlear library): <http://neiblast.nei.nih.gov/>

- 1 Friedman TB, Griffith AJ: Human nonsyndromic sensorineural deafness. *Annu Rev Genomics Hum Genet* 2003; **4**: 341–402.
- 2 Morton CC, Nance WE: Newborn hearing screening—a silent revolution. *N Engl J Med* 2006; **354**: 2151–2164.
- 3 Medlej-Hashim M, Mustapha M, Chouery E *et al*: Non-syndromic recessive deafness in Jordan: mapping of a new locus to chromosome 9q34.3 and prevalence of DFNB1 mutations. *Eur J Hum Genet* 2002; **10**: 391–394.
- 4 Belguith H, Masmoudi S, Medlej-Hashim M *et al*: Re-assigning the DFNB33 locus to chromosome 10p11.23-q21.1. *Eur J Hum Genet* 2009; **17**: 122–124.
- 5 Grimberg J, Nawoschik S, Belluscio L, McKee R, Turck A, Eisenberg A: A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res* 1989; **17**: 8390.
- 6 Schaffer AA: Faster linkage analysis computations for pedigrees with loops or unused alleles. *Hum Hered* 1996; **46**: 226–235.
- 7 Kurima K, Peters LM, Yang Y *et al*: Dominant and recessive deafness caused by mutations of a novel gene, TMC1, required for cochlear hair-cell function. *Nat Genet* 2002; **30**: 277–284.
- 8 Mburu P, Mustapha M, Varela A *et al*: Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31. *Nat Genet* 2003; **34**: 421–428.
- 9 Mustapha M, Chouery E, Chardenoux S *et al*: DFNB31, a recessive form of sensorineural hearing loss, maps to chromosome 9q32-34. *Eur J Hum Genet* 2002; **10**: 210–212.
- 10 D'Adamo P, Donaudy F, D'Eustacchio A, Di Iorio E, Melchionda S, Gasparini P: A new locus (DFNA47) for autosomal dominant non-syndromic inherited hearing loss maps to 9p21-22 in a large Italian family. *Eur J Hum Genet* 2003; **11**: 121–124.
- 11 Peters LM, Belyantseva IA, Lagziel A, Battey JF, Friedman TB, Morell RJ: Signatures from tissue-specific MPSS libraries identify transcripts preferentially expressed in the mouse inner ear. *Genomics* 2007; **89**: 197–206.
- 12 Fettiplace R, Hackney CM: The sensory and motor roles of auditory hair cells. *Nat Rev Neurosci* 2006; **7**: 19–29.
- 13 Street VA, McKee-Johnson JW, Fonseca RC, Tempel BL, Noben-Trauth K: Mutations in a plasma membrane Ca<sup>2+</sup>-ATPase gene cause deafness in deafwaddler mice. *Nat Genet* 1998; **19**: 390–394.
- 14 Lanford PJ, Kelley MW: Notch signaling and cell fate determination in the vertebrate inner ear. Chapter 3 in development of the inner ear. in Kelley MW, Wu DK, Popper AN, Fay RR (eds): *Springer Handbook of Auditory Research*. Springer: New York, 2005. vol 26, pp 122–157.
- 15 Mencia A, Modamio-Hoybjor S, Redshaw N *et al*: Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. *Nat Genet* 2009; **41**: 609–613.
- 16 Lewis MA, Quint E, Glazier AM *et al*: An ENU-induced mutation of miR-96 associated with progressive hearing loss in mice. *Nat Genet* 2009; **41**: 614–618.