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Autosomal Recessive Deafness is Heterogeneous in Pakistani Pakhtun Population

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Abstract: The aim of this study was to elucidate the genetic cause of non-syndromic autosomal recessive deafness in Pakistani Pakhtun ethnic group. Hearing loss is one of the most common hereditary disorders in humans affecting approximately 1/1000 children at birth or during early childhood. Seven Pakhtun families containing at least three members affected with pre-lingual, severe to profound sensorineural deafness were enrolled from different regions of the Khyber Pakhtunkhwa province of Pakistan. Detailed history of each family was taken for the confirmation of consanguineous marriages and pedigree drawing. Blood samples from all participants were obtained for genetic analysis by genotyping, haplotype analysis and LOD score calculation. Four among the seven families showed linkage to known loci/genes. PKDF935 family showed linkage to the markers flanking *DFNB9*/*OTOF* and was defined to the boundaries of critical region at 2p22-p23. The affected individuals of the family PKDF941 were homozygous for markers used for the screening of *DFNB49/TRIC*. PKDF950 family showed linkage to *DFNB37/MYO6*-linked STRs markers. The affected individuals of the family PKDF953 showed linkage with chromosome 7 markers harboring recessive deafness loci *DFNB4/PDS* and *DFNB14*. The present study shows the presence of different loci in Pakhtun ethnic group that indicates genetic heterogeneity in autosomal recessive deafness in Pakhtun population of Pakistan. This study will also help to reduce the incidence of deafness in Pakistani population by carrier screening within the families with multiple affected individuals to identify the persons at a high risk.

Key words: Consanguineous marriages, deafness, genetic analysis, hereditary disorder

INTRODUCTION

Approximately one in 1000 children are affected by severe or profound hearing loss at birth or during early childhood which is defined as pre-lingual deafness (Petersen and Willems, 2006). It is a major public health concern because it affects 6 to 8% of the population in developed nations (Petit *et al*., 2001; Petit, 2006). Hearing loss is caused by both genetic and environmental factors (Willems, 2000) and in case of hereditary deafness, the non-syndromic is responsible for 70% and that syndromic for only 30% of the cases (Piatto *et al*., 2005). Among non-syndromic hearing loss, autosomal recessive is the most frequent trait contributing 75-85% of cases followed by dominant trait (12-13%) and X-linked or mitochondrial, with 2-3% of the cases (Bitner-Glindzicz, 2002; Van *et al*., 2003).

Most of the recessively inherited forms of hearing impairment cause a phenotypically identical severe to profound, pre-lingual hearing loss, but mutations at a few loci - DFNB2 (*MYO7A*) (Liu *et al*., 1997), DFNB8/10 (*TMPRSS3*) (Veske *et al*., 1996) and DFNB16 (*STRC*) (Verpy *et al*., 2001) cause a delayed, childhood-onset hearing impairment. All types of early-onset hereditary deafness currently observed are due to monogenic defects (Petit *et al*., 2001; Friedman and Griffith, 2003) with the exception of a few cases in which digenic inheritance has been suggested (Petit, 2006).

Molecular genetics of deafness has experienced remarkable progress in the last decade. Genes responsible for hereditary hearing impairment are being mapped and cloned progressively (Piatto *et al*., 2005). However, there is no data that describe genetic variation in autosomal recessive deafness in Pakhtun population of Pakistan. The present study aims to determine the genetic cause of autosomal recessive deafness in large Pakhtun families with multiple affected individuals and to identify the persons at high risk, which may help to reduce the incidence of deafness in Pakistani population.

MATERIALS AND METHODS

The present study was performed at National Centre of Excellence in Molecular Biology, University of the

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Table 1: STRs markers used for linkage analysis of *DFNB4/PDS*, *DFNB9/OTOF*, *DFNB37/MYO6* and *DFNB49/TRIC* locus

Locus	Markers	cM distance	DYE	PCRprogram	PCR conditions	Productsize (bp)
DFNB4/PDS	D7S821	109.12	VIC	Julie 54	0.3mlPrimers,1.5mMMgCl2, Spermine	238-270
	D7S518	112.32	NED	Julie 54	0.2mlPrimers,2.5mMMgCl2, Spermine	179-201
	D7S2453	115.96	VIC	Julie 54	0.2mlPrimers,1.5mMMgCl2, Spermine	167-199
	D7S2420	119.81	FAM	Touch-Down 65-55	0.2mlPrimers,2.5mMMgCl2, Spermine	240-292
	D7S2459	119.81	VIC	Multiplex 52	0.2mlPrimers,2.5mMMgCl2, Spermine	140-152
	D7S2456	120.61	NED	Julie 54	0.2mlPrimers,2.5mMMgCl2, Spermine	238-252
	D7S2847	125.15	NED	Julie 54	0.3mlPrimers,2.5mMMgCl2, Spermine,	174-201
	D7S480	125.95	FAM	Touch-Down 67-57	0.3mlPrimers,2.5mMMgCl2, Spermine	189-206
	D7S1842	128.41	FAM	Julie 54	0.3mlPrimers, 2.5mMMgCl2	114-154
DFNB9/OTOF	D2S305	38.87	NED	Multiplex 54	0.1ulPrimer,2.5mM MgCl2	269-283
	D2S2150	40.47	VIC	Multiplex 54	0.1ulPrimer,2.5mM MgCl2	154-186
	D2S174	46.90	NED	Multiplex 54	0.2mlPrimers,2.5mMMgCl2	203-221
	D2S2144	46.37	FAM	Multiplex 54	0.2mlPrimers,2.5mMMgCl2	217-245
	D2S165	47.43	FAM	Multiplex 54	0.1ulPrimer,2.5mM MgCl2	81-111
DFNB37/MYO6	D6S1031	88.63	NED	Touch-Down 65-55	0.4ulPrimer,2.5mMMgCl2,Spermine	251-266
	D6S1589	89.23	NED	54	0.4ulPrimer,2.5mMMgCl2	170-188
	D6S286	89.83	FAM	54	0.3ulPrimer,1.5 mMMgCl2	206-232
DFNB49/TRIC	D5S629	75.89	FAM	57	0.1ulPrimer,2.0mMMgCl2	233-253
	GATA141B10	75.89	FAM	54	0.1ulPrimer,2.0mMMgCl2	104-116
	D5S637	75.89	FAM	54	0.1ulPrimer,2.0mMMgCl2	246-254

Punjab, Lahore, Pakistan from January, 2009 to April, 2010. Special children schools in different Pakhtun populated area of Khyber Pakhtunkhwa province, Pakistan were visited and information about the deaf student families was collected. Families with 3 or more affected individuals (with deafness) in 2 or more loops were selected for the study. Informed consent was obtained from participants in study. Detailed history was taken from each family to minimize the presence of other abnormalities and environmental causes for deafness. Families were questioned about skin and hair pigmentation, problems relating to balance, vision, thyroid, kidneys, heart and infectious diseases like meningitis as well as antibiotic usage. Pure tone audiometry with air conduction with 250, 500, 1000, 2000, 4000, 8000 Hz was performed with Siemens, SD-25 or Beltone 112 audiometer. Pedigrees of the enrolled families were drawn using Cyrillic® program for Windows.

Genomic DNA was extracted from the blood samples following an inorganic method (Grimberg *et al*., 1989). Plate map was designed that consisted of all the affected individuals with parents and normal siblings from the family. Replicates of the designed master plate were made with 50 ng of DNA for linkage studies and overlaid with 10 mL mineral oil.

Haplotype analysis: Families were screened for all the microsatellite markers for the characterization of autosomal recessive deafness. Here only the linked markers (Table 1) were used for haplotype analysis. All the markers for linkage analysis were dinucleotide repeats except D7S821, D7S2847, D7S1842, GATA141B10 (Tetra repeats), and D6S1031 (Tri repeat) and were chosen from the Marshfield Comprehensive Human Genetic Maps (http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp). The primer sequences for amplification of each marker are listed in the genome database (http://genome.ucsc.edu/cgi-bin/hgGateway). Genotyping was performed by using ABI PRISM® 3100 Genetic Analyzer.

A haplotype representing an individual's chromosomal segment is the set of genotyped alleles arranged according to the cM distance along a chromosome. Alleles were arranged in ways that confirm the inheritance pattern of segregating disease. Linkage to a particular locus was confirmed when homozygous data of affected members correlated with the disease pattern in the family tree.

LOD score calculations: Two-point LOD scores were calculated for deafness linked markers by using EASYLINKAGE software as described by Ott (1991) and Terwillger and Ott (1994). Deafness was assumed to be inherited in an autosomal recessive manner with complete penetrance. Recombination frequencies were assumed to be equal in both males and females. Genetic distances were based on Marshfield human genetic map.

RESULTS

Seven Pakhtun families with a history of deafness and recessive mode of inheritance were collected from different areas of KP (Khyber Pakhtunkhwa Province: Formerly known as NWFP). All affected members of these families had pre-lingual, severe to profound sensorineural deafness. Physical and clinical evaluation was performed to rule out any extra-auditory phenotype. Detailed medical history was taken to exclude environmental causes. Written informed consent was obtained from all participants. These families were screened for known deafness loci through linkage analysis

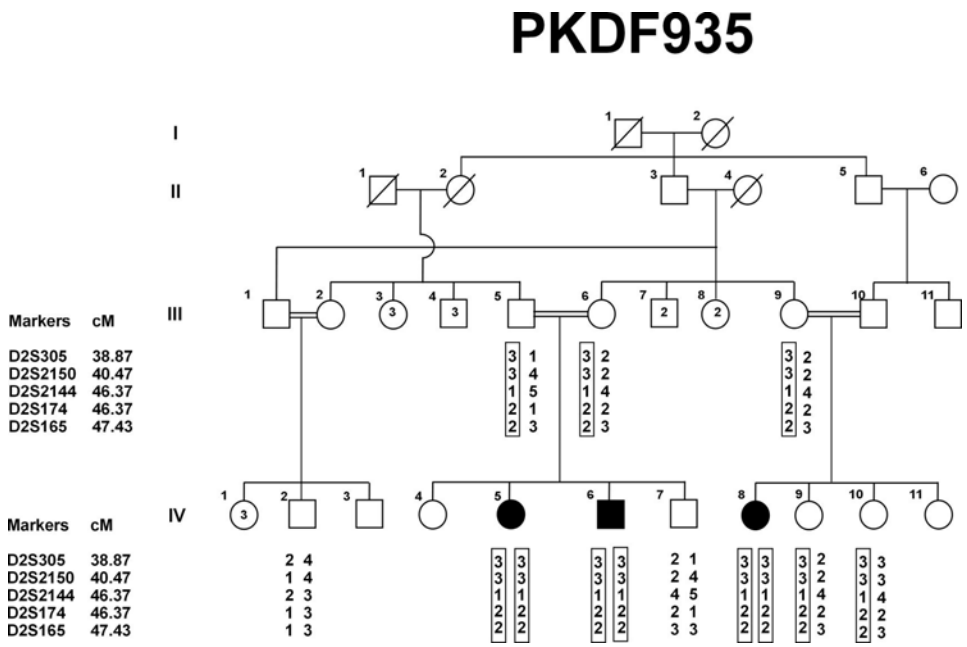

Fig. 1: Pedigree drawing of PKDF935 showing linkage to *DFNB9/OTOF*

and four families PKDF935, PKDF941, PKDF950 and PKDF953, showed linkage to four different genes/loci *DFNB9/OTOF*, *DFNB49/TRIC*, *DFNB37/MYO6*, and *DFNB4/PDS/DFNB14*, respectively.

Medical profile of the affected individuals: Physical and clinical evaluation of all the affected individuals ruled out the syndromic as well as environmental causes of deafness in these families. Affected individuals had no signs of goiter, night blindness, balance problems, diabetes, and mental retardation along with deafness indicating segregation of non-syndromic deafness in all the enrolled families. Tandem gait test was performed to rule out balance dysfunction in affected individuals.

PKDF935: This family was a large consanguineous family enrolled from district Malakand, KP (Fig. 1). This family belonged to the Musha Khail caste with Pakhtun ethnicity. Detailed pedigree was drawn after interviewing multiple members of the family. The pedigree consists of three affected individuals (IV: 5, IV: 6, and IV: 8) (two female and one male) having congenital hearing loss. At the time of enrolment blood of three deaf and seven normal individuals were collected.

Linkage analysis: After screening, the family showed linkage to the markers flanking *DFNB9*/*OTOF*. To define the boundaries of critical region at 2p22-p23, a set of five STRs markers, D2S305 (38.87cM), D2S2150 (40.47cM), D2S2144 (46.37cM), D2S174 (46.90cM), and D2S165 (47.43cM) were genotyped in the family. From the haplotype analysis, it was found that all deaf individuals were homozygous for all above mentioned markers flanking *DFNB9*. The haplotype analysis showed 8.56cM linkage interval, delineated by markers, D2S305 (38.87cM), which lies above the gene and D2S165 (47.43cM), locating below the gene. Among the phenotypically normal individuals, individuals, III:5, III:6, III:9, IV:9, and IV:10 were carrier for the diseased haplotype, while individuals IV: 2, and VI: 7 were phenotypically and genotypically normal (Fig. 1). The two-point LOD score calculation for the family data produced a maximum LOD score (Zmax) of 2.8619 with marker D2S2144 (46.37cM).

PKDF941: Highly inbred pedigree PKDF941 enrolled from district Malakand belongs to Pakhtun background. PKDF941 consists of six affected individual in two loops (Fig. 2). Pedigree was drawn by consulting several members of the family to confirm the consanguinity. The ages of the affected individuals (V: 1, V: 2 V: 3, V: 6, V: 7, and V: 8) were 17, 10, 15, 10, 25, and 9 at the time of enrolment. All the affected members displayed progressive hearing loss.

Linkage analysis: Haplotype analysis revealed that all affected individuals (V: 1, V: 2, V: 3, V: 6, V: 7, and V: 8) were homozygous for markers, D5S629 (75.89cM), GATA141B10 (75.89cM), and D5S637 (75.89cM) used for the screening of *DFNB49/TRIC*. The individuals V: 4, V: 11, V: 14, IV: 1, IV: 6 and IV: 7 were phenotypically normal but genetically carrier for the disease allele and

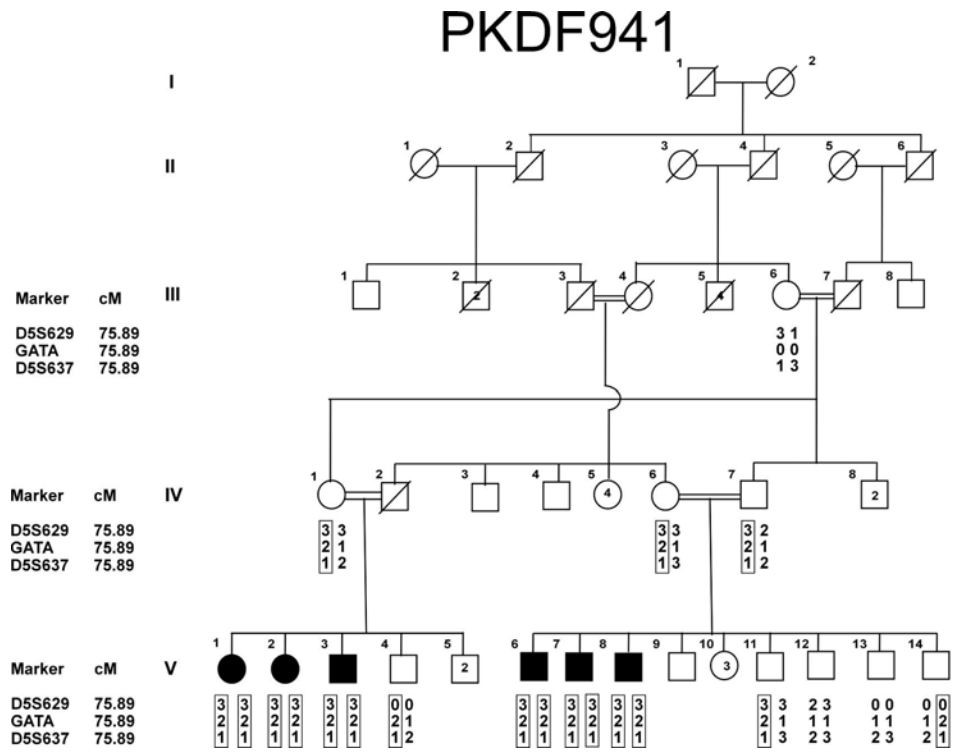

Fig. 2: Pedigree drawing of PKDF941showing linkage to *DFNB49/TRIC*

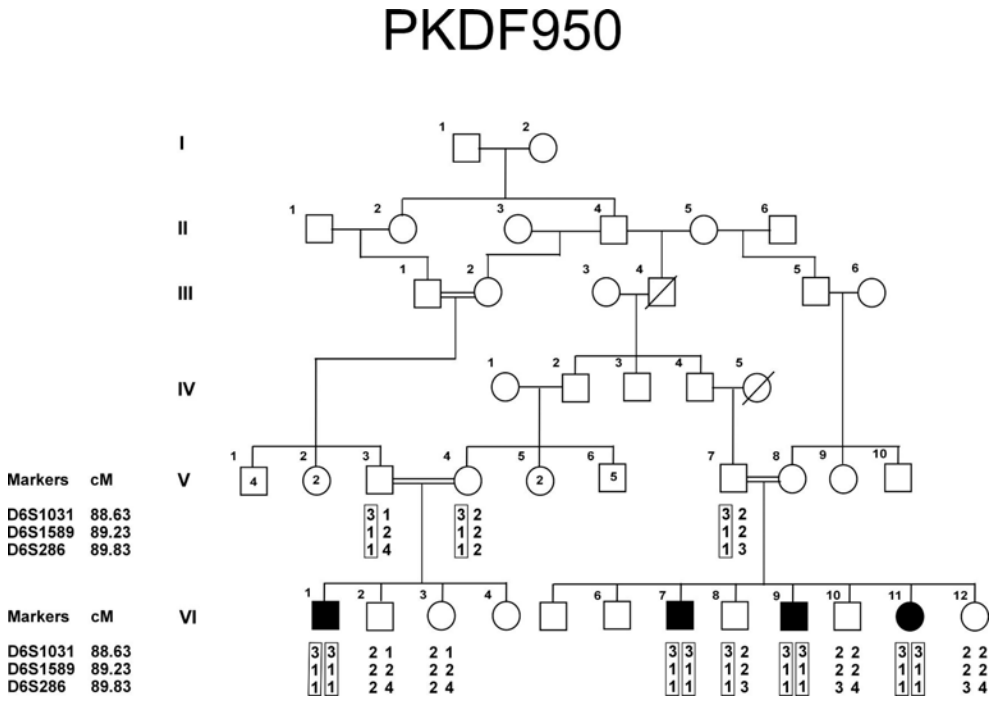

Fig. 3: Pedigree drawing of PKDF950 showing linkage to *DFNB37/MYO6*

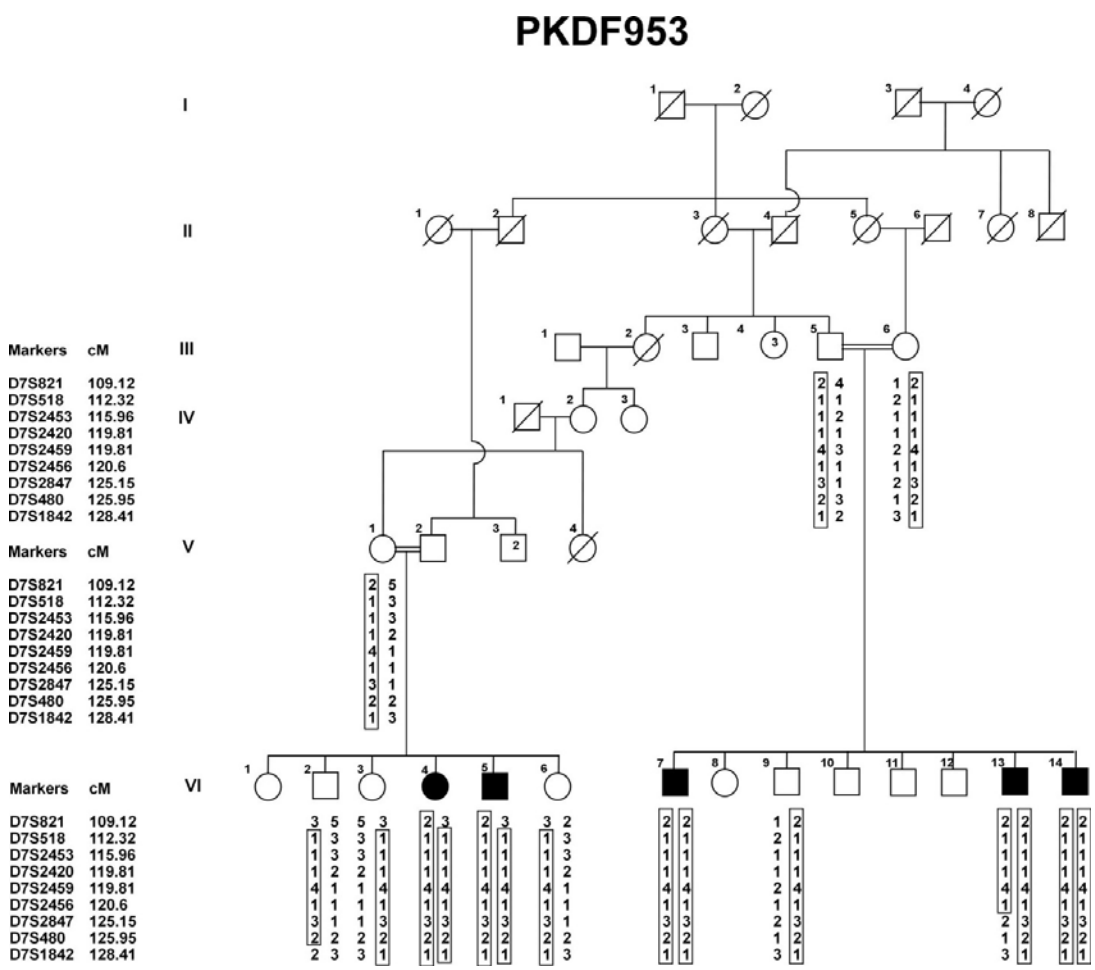

Fig. 4: Pedigree drawing of PKDF953 showing linkage to *DFNB4/SLC26A4* and DFNB14 at chromosome 7

the individuals V: 12 and V: 13 were found normal both phenotypically and genotypically (Fig. 2). Two-point LOD score was calculated for family that yielded a maximum LOD score (Zmax) of 4.0280 for marker D5S637 (75.89cM).

PKDF950: This consanguineous family, PKDF950 ascertained from "Mardan" belongs to caste "Uthman Khail" Pakhtun. It consists of four affected individuals (VI: 1, VI: 7, VI: 9 VI: 11) in two loops (Fig. 3). Blood samples of deaf individuals, normal and their parents were taken with their consent at the time of enrolment.

Linkage analysis: Linkage analysis to known loci was performed by genotyping three microsatellite markers. Four deaf and eight normal individuals of two loops were included in the linkage study. All affected individuals showed linkage to *DFNB37/MYO6*-linked STRs markers, D6S1031 (88.63cM), D6S1589 (89.23cM), and D6S286 (89.83cM). All deaf were homozygous for the diseased haplotype and the individuals VI: 8, V: 3, V: 4 and V: 7 were phenotypically normal, but were found genetically carrier (Fig. 3). The individuals VI: 2, VI: 3, VI: 10 and VI: 12 showed no linkage and were found normal both phenotypically and genotypically. A maximum two-point LOD score (Zmax) of 3.1159 was obtained for marker D6S286 (89.83cM).

PKDF953: This was a consanguineous Madoo Khail (Pakhtun) family enrolled from district Dir Lower containing five affected individuals in two loops (Fig. 4). Blood samples of five deaf and seven normal individuals were taken with their consent at the time of enrolment. The affected individuals (VI: 4, VI: 5, VI: 7, VI: 13 and VI: 14) aged 11, 6, 24, 22 and 18 years, respectively at the time of enrollment.

Linkage analysis: During screening, all the affected individuals of PKDF953 showed linkage with chromosome 7 markers harboring two recessive deafness

loci (*DFNB4/PDS* and *DFNB14*). Additional STR markers were genotyped to confine the linkage interval and define the proximal and distal boundaries. Haplotype analysis defined the proximal breakpoint at D7S821 (109.12cM) in affected individual (VI: 4 and VI: 5) and D7S2847 (125.15cM) defined the distal breakpoint in affected individual (VI: 13) (Fig. 4). These two meiotic recombinations define a critical linkage interval of 16.03cM. Unaffected individuals (III: 5, III: 6, V: 1, VI: 2, VI: 3, VI: 6, and VI: 9) were phenotypically normal and genetically carrier for the diseased allele. A maximum two-point LOD score (Zmax) of 3.9748 was obtained for marker D7S2459 (119.81cM).

DISCUSSION

A large number of genes are anticipated to be responsible for controlling the anatomic and physiologic function of the ear (Friedman and Griffith, 2003). Phenotypically, hearing impairment is classified as syndromic and non-syndromic. Non-syndromic forms of deafness transmitted as a recessive trait are the most common cause of hereditary hearing loss and often exhibit the most severe hearing phenotype (Cohen and Gorlin, 1995). Non-syndromic autosomal recessive deafness is usually clinically homozygous and is progressive in nature (Van-Camp *et al*., 1997). Recessive deafness is more prevalent in endogenous and isolated populations (Friedman and Griffith, 2003) and Pakistan represents a true treasure for molecular dissection of hearing disorder because 60% marriages are consanguineous and out of those approximately 80% are between first cousins (Hussain and Bittles, 1998).

DFNB4/PDS is the most common locus in Pakistani population with the prevalence of 7.2% (Anwar *et al*., 2009). One large consanguineous family, PKDF953, with profound hearing loss was found linked with *DFNB4/PDS* and *DFNB14* with significant two-point LOD scores of 3.9748 and 3.0788 for markers D7S2459 and D7S518. Ages of all the affected individuals were below thirty and they showed no signs of goiter. Mutational analysis is required to find out whether this family is linked with *DFNB4* or *DFNB14.* Further more, if the affected individuals develop goiter later in their life (above thirty years), then it would be confirmed that the hearing loss is syndromic and is inherited as recessive. *DFNB4* was first described in a deaf Israeli Druze family with pre-lingual, severe deafness and found linked to a 5-cM region on human chromosome 7 (Baldwin *et al*., 1995). Mutant alleles of *SLC26A4* are responsible for non-syndromic, *DFNB4* (Li *et al*., 1998), Pendred syndrome (Everett *et al*., 1997) as well as Enlarged Vestibular Aqueduct (EVA) syndrome (Usami *et al*., 1999). *SLC26A4* mutations account for approximately 10% of hereditary deafness in diverse populations that include eastern and southern Asians (Park *et al*., 2003).

Another consanguineous family, PKDF935, with five affected individuals in two loops was found linked with *DFNB9/OTOF*. *DFNB9/OTOF* was mapped first time in a large consanguineous Lebanese family through genome-wide linkage analysis at chromosome 2p23-p22 (Chaïb *et al*., 1996). Mutations of *OTOF* account for hereditary deafness in diverse populations (Yasunaga *et al*., 1999; Houseman *et al*., 2001; Leal *et al*., 1998; Mirghomizadeh *et al*., 2002; Migliosi *et al*., 2002). *DFNB9/OTOF* is infrequent cause of deafness in Pakistani population with 557 families screened, only 13 showed linkage to *DFNB9/OTOF*, thus contributing only 2.3% (Choi *et al*., 2009).

A large inbred Pakhtun family from district Mardan, PKDF950, with four affected individuals in two loops showed linkage with *DFNB37/MYO6*. *DFNB37* locus was first identified in a large consanguineous Pakistani family with profound, sensorineural, pre-lingual hearing loss that co-segregated at 6q13 (Ahmed *et al*., 2003). The linkage region included the *MYO6* gene encoding *myosin VI* and which was previously found to be responsible for an autosomal-dominant form of post-lingual progressive deafness (*DFNA22*) in Italian kindred (Melchionda *et al*., 2001). Up till now, three recessive *MYO6* mutations (36-37insT, 3496C→T, and E216V) have been identified in Pakistani families (Ahmed *et al*., 2003). *DFNB37/MYO6* is a rare cause of deafness in Pakistani population.

DFNB49, a recessive hearing disorder, was first mapped in two large Pakistani families at chromosome 5q13 (Ramzan *et al*., 2005). Mutations within the TRIC gene, which encodes tricellulin, are responsible for *DFNB49* hearing loss (Riazuddin *et al*., 2006). Tricellulin is a tricellular tight-junction (tTJ) protein, which is key in the formation of barriers between tricellular contacts of epithelial cells throughout the body (Ikenouchi *et al*., 2005). A large consanguineous family *PKDF941* from district Malakand with six affected individuals in two loops was found linked with three microsatellite markers D5S629 (75.89cM), GATA141B10 (75.89cM) and D5S637 (75.89cM) used for screening of *DFNB49/TRIC*. The relative contribution of *DFNB49* in Pakistani deaf population is approximately 1.06%, a significant proportion considering the extensive genetic heterogeneity of deafness in this population (Chishti *et al*., 2008).

CONCLUSION

The presence of different genes/loci associated with autosomal recessive deafness in Pakhtun ethnic group indicates genetic heterogeneity. *DFNB4/PDS* is the most common locus in Punjabi ethnic group and it is expected that it might be the most frequent locus present in Pakhtun ethnic group as well, but needs to be further investigated. The benefit of this study is to reduce the incidence of

deafness in Pakistani population by providing knowledge and awareness through carrier screening within the families with multiple affected individuals to identify the persons at a high risk. Marriages within the family are quite common in Pakistan, therefore identification of carriers, increasing awareness about the possible effects of consanguineous families, offering the genetic counseling and prenatal diagnosis to the families can help to reduce the incidence of hereditary deafness in our population. For obtaining this objective, the need is to further characterize the deafness at molecular level and identify the particular genes/loci that contribute most to hearing loss in the concerned population.

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REFERENCES

Ahmed, Z.M., R.J. Morell, S. Riazuddin, A. Gropman, S. Shaukat, M.M. Ahmad, S.A. Mohiddin, L. Fananapazir, R.C. Caruso, T. Husnain, S.N. Khan, S. Riazuddin, A.J. Griffith, T.B. Friedman and E.R. Wilcox, 2003. Mutations of MYO6 are associated with recessive deafness, DFNB37. Am. J. Hum. Genet., 72: 1315-1322.

Anwar, S., S. Riazuddin, Z.M. Ahmed, S. Tasneem, Ateeq-ul-Jaleel, S.Y. Khan, A.J. Griffith, T.B. Friedman and S. Riazuddin, 2009. SLC26A4 mutation spectrum associated with DFNB4 deafness and Pendred's syndrome in Pakistanis. J. Hum. Jenet., 54: 266-270.

Baldwin, C.T., S. Weiss, L.A. Farrer, A.L.D. Stefano, R. Adair, B. Franklyn, K.K. Kidd, M. Korostishevsky and B. Bonné-Tamir, 1995. Linkage of congenital, recessive deafness (DFNB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. Hum. Mol. Genet., 4: 1637-1642.

Bitner-Glindzicz, M., 2002. Hereditary deafness and phenotyping in humans. Br. Med. Bull., 63: 73-94.

Chaïb, H., C. Place, N. Salem, S. Chardenoux, C. Vincent, J. Weissenbach, E. El-Zir, J. Loiselet and C. Petit, 1996. A gene responsible for a sensorineural non-syndromic recessive deafness maps to chromosome 2p22-23. Hum. Mol. Genet., 5: 155-158.

Chishti, M.S., A. Bhatti, S. Tamim, K. Lee, M. McDonald, S.M. Leal and W. Ahmad, 2008. Splice-site mutations in the TRIC gene underlie autosomal recessive nonsyndromic hearing impairment in Pakistani families. J. Hum. Genet., 53(2): 101-105.

Choi, B.Y., Z.M. Ahmed, S. Riazuddin, M.A. Bhinder, M. Shahzad, T. Husnain, S. Riazuddin, A.J. Griffith and T.B. Friedman, 2009. Identities and frequencies of mutations of the otoferlin gene (OTOF) causing DFNB9 deafness in Pakistan. Clin. Genet., 75(3): 237-243.

Cohen, M.M. and R.J. Gorlin, 1995. Epidemiology, Etiology and Genetic Patterns. In: Gorlin, R.J., H.V. Toriello and M.M. Cohen, (Eds.), Hereditary Hearing Loss and its Syndromes. Oxford University Press, pp: 43-61.

Everett, L.A., B. Glaser, J.C. Beck, J.R. Idol, A. Buchs, M. Heyman, F. Adawi, E. Hazani, E. Nassir, A.D. Baxevanis, V.C. Sheffield and E.D. Green, 1997. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat. Genet., 17: 411-422.

Friedman, T.B. and A.J. Griffith, 2003. Human nonsyndromic sensorineural deafness. Ann. Rev. Genom. Hum. Genet., 4: 341-402.

Grimberg, J., S. Nawoschik, L. Belluscio, R. McKee, A. Turck, and A. Eisenberg, 1989. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acids Res., 17: 83-90.

Houseman, M.J., A.P. Jackson, L.I. Al-Gazali, R.A. Badin, E. Roberts and R.F. Mueller, 2001. A novel mutation in a family with non-syndromic sensorineural hearing loss that disrupts the newly characterised OTOF long isoforms. J. Med. Genet., 38: e25.

Hussain, R. and A.H. Bittles, 1998. The prevalence and demographic characteristics of consanguineous marriages in Pakistan. J. Biosoc. Sci., 30: 261-275.

Ikenouchi, J., M. Furuse, K. Furuse, H. Sasaki, S. Tsukita and S. Tsukita, 2005. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. J. Cell Biol., 171: 939-945.

Leal S.M., F. Apaydin, C. Barnwell, M. Iber, T. Kandogan, M. Pfister, U. Braendle, M. Cura, H.P. Zenner and E. Vitale, 1998. A second Middle Eastern kindred with autosomal recessive non-syndromic hearing loss segregates DFNB9. Eur. J. Hum. Genet., 6: 341-344.

Li, X.C., L.A. Everett, A.K. Lalwani, D. Desmukh, T.B. Friedman, E.D. Green and E.R. Wilcox, 1998. A mutation in PDS causes non-syndromic recessive deafness. Nat. Genet., 18: 215-217.

Liu, X.Z., J. Walsh, P. Mburu, J. Kendrick-Jones, M.J.T.V. Cope, K.P. Steel and S.D.M. Brown, 1997. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. Nat. Genet., 16: 188-190.

Melchionda, S., N. Ahituv, L. Bisceglia, T. Sobe, F. Glaser, R. Rabionet, M.L. Arbones, A. Notarangelo, E.D. Iorio, M. Carella, L. Zelante, X. Estivill, K.B. Avraham, and P. Gasparini, 2001. MYO6, the human homologue of the gene responsible for deafness in Snell's waltzer mice, is mutated in autosomal dominant nonsyndromic hearing loss. Am. J. Hum. Genet., 69: 635-640.

Migliosi, V., S. Modamio-Heybjer, M.A. Moreno-Pelayo, M. Rodriguz-Ballesteros, M. Villamar, D. Telleria, I. Menendez, F. Moreno, and I.D. Castillo, 2002. Q829X, a novel mutation in the gene encoding otoferlin (OTOF), is frequently found in Spanish patients with prelingual non-syndromic hearing loss. J. Med. Genet., 39: 502-506.

Mirghomizadeh, F., M. Pfister, F. Apaydin, C. Petit, S. Kupka, C.M. Pusch, H.P. Zenner, and N. Blin, 2002. Substitutions in the conserved C2C domain of otoferlin cause DFNB9, a form of nonsyndromic autosomal recessive deafness. Neurobiol. Dis., 10: 157-164.

Ott, J., 1991. Analysis of Human Genetic Linkage. Johns Hopkins University Press.

Park, H.J., S. Shaukat, X.Z. Liu, S.H. Hahn, S. Naz, M. Ghosh, H.N. Kim, S.K. Moon, S. Abe, K. Tukamoto, S. Riazuddin, M. Kabra, R. Erdenetungalag, J. Radnaabazar, S. Khan, A. Pandya, S.I. Usami, W.E. Nance, E.R. Wilcox, S. Riazuddin and A.J. Griffith, 2003. Origins and frequencies of SLC26A4 (PDS) mutations in East and South Asians: global implications for the epidemiology of deafness. J. Med. Genet., 40: 242-248.

Petersen, M.B. and P.J. Willems, 2006. Non-syndromic, autosomal-recessive Deafness. Clin. Genet., 69: 371-392.

Petit, C., 2006. From deafness genes to hearing mechanisms: Harmony and counterpoint. Tre. Mol. Med., 12: 57-64.

Petit, C., J. Levilliers and J.P. Hardelin, 2001. Molecular genetics of hearing loss. Ann. Rev. Genet., 35: 589-646.

Piatto, V.B., E.C.T. Nascimento, F. Alexandrino, C.A. Oliveira, A.C.P. Lopes, E.L. Sartorato and J.V. Maniglia, 2005. . Molecular genetics of non-syndromic deafness. Rev. Bras. Otorrinolaringol., 71(2): 216-223.

Ramzan, K., R.S. Shaikh, J. Ahmad, S.N. Khan, S. Riazuddin, Z.M. Ahmed, T.B. Friedman, E.R. Wilcox and S. Riazuddin, 2005. A new locus for nonsyndromic deafness DFNB49 maps to chromosome 5q12.3-q14.1. Hum. Genet., 116: 17-22.

Riazuddin, S., Z.M. Ahmed, A.S. Fanning, A. Lagziel, S. Kitajiri, K. Ramzan, S.N. Khan, P. Chattaraj, P.L. Friedman, J.M. Anderson, I.A. Belyantseva, A. Forge, S. Riazuddin and T.B. Friedman, 2006. Tricellulin is a tight-junction protein necessary for hearing. Am. J. Hum. Genet., 79(6): 1040-1051.

Terwillger, J.D. and J. Ott, 1994. Handbook of Human Genetic Linkage. Baltimore, Johns Hopkins University Press.

Usami, S., S. Abe, M.D. Weston, H. Shinkawa, G. Van-Camp and W.J. Kimberling, 1999. Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by PDS mutations. Hum. Genet., 104: 188-192.

Van, L.L., K. Cryns, R.J. Smith and G. Van-Camp, 2003. Nonsyndromic hearing loss. Ear Hear., 24: 275-288.

Van-Camp, G., P.J. Willems and R.J.H. Smith, 1997. Nonsyndromic hearing impairment unparalleled heterogeneity. Am. J. Hum. Genet., 60: 758-764.

Verpy, E., S. Masmoudi, I. Zwaenepoel, M. Leibovici, T.P. Hutchin, I.D. Castillo, S. Nouaille, S. Blanchard, S. Lainé, J.L. Popot, F. Moreno, R.F. Mueller and C. Petit, 2001. Mutations in a new gene encoding a protein of the hair bundle cause non-syndromic deafness at the DFNB16 locus. Nat. Genet., 29: 345-349.

Veske, A., R. Oehlmann, F. Younus, A. Mohyuddin, B. Muller-Myhsok, S.Q. Mehdi and A. Gal, 1996. Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan. Hum. Mol. Genet., 5: 165-168.

Willems, P.J., 2000. Genetic causes of hearing loss. N. Engl. J. Med., 342: 1101-1109.

Yasunaga, S., M. Grati, M. Cohen-Salmon, A. El-Amraoui, M. Mustapha, N. Salem, E. El-Zir, J. Loiselet and C. Petit, 1999. A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. Nat. Genet., 21: 363-369.