Molecular Distribution of Deafness Loci in Various Ethnic Groups of the Punjab, Pakistan

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ABSTRACT

Objective: To determine the existence of autosomal recessive deafness loci in different ethnic tribes of the Punjab.
Study Design: Descriptive observational study.
Place and Duration of Study: Department of Human Genetics and Centre of Excellence in Molecular Biology, University of Health Sciences, Lahore, from July 2009 to March 2012.
Methodology: Healthy willing subjects with autosomal recessive deafness loci were studied for selected deafness loci. Those who were unhealthy and gave history of infectious disease were excluded. DNA extraction was carried out using the inorganic method. Fluorescently labeled microsatellite markers were used for amplification of desired regions by PCR (Polymerase Chain Reaction). Automated allele assignment was performed using the ABI PRISM GeneScan Analysis Software Version 3.7 for Windows NT Platform. Two-point LOD scores were calculated using the FASTLINK computer package (Schaffer 1996) and MLINK was used for calculation and 95% CI (confidence intervals) were calculated.
Results: One hundred and thirty two individuals of 8 families were analyzed. Three families (SAPun-03, SAPun-10 and SAPun-15) were found linked to DFNB12; two families (SAPun-05 and SAPun-17) were found linked to DFNB8/10, while three families (SAPun-06, SAPun-13 and SAPun-19) were found linked to DFNB29, DFNB36 and DFNB37 respectively.
Conclusion: The genotyping results revealed that DFNB12 locus was the most common followed by DFNB8/10 locus, while the Loci DFNB29, DFNB36 and DFNB37 were less common.


INTRODUCTION

Among the diverse sensory defects in human, deafness constitutes the major part.¹ Deafness is defined as partial or complete hearing loss that leads to impaired speech, language and effective communication skills. Almost 1 in 1000 infants is affected by severe or profound hearing loss at birth or during early childhood.² Regardless of the cause, deafness may be classified into three categories: conductive hearing loss, sensorineural hearing loss and mixed. Of the total hereditary hearing loss, 30% is syndromic,³ while 70% of genetically determined cases are non-syndromic.⁴ Non-syndromic and syndromic co-localizations include DFNB12 and Usher1B (Myo7A), DFNB12 and Usher1D (CDH23), DFNB18 and Usher1C (USH1C), DFNB23 and Usher1F (PCDH15), DFNB31 and Usher2D (WHRN), and DFNB4/Pendred syndrome (SLC26A4). DFNB8/10, an autosomal recessive deafness loci, was independently mapped in two consanguineous families from Palestine (DFNB10) and Pakistan (DFNB8) to chromosome 21q22.3,⁵,⁶ DFNB12 was mapped to chromosome 10q21-q22 in consanguineous kindred from Syria.⁷ Usher syndrome type 1D (USH1D) locus was subsequently mapped in Pakistani population and suggested that DFNB12 and USH1D might be due to allelic mutation.⁸ It is estimated that approximately 75% of cases of deafness show autosomal recessive inheritance, 12 - 24% of cases are autosomal dominant and 1 - 3% cases are X-linked.⁹

Consanguinity is a major factor in higher ratio of autosomal recessive disorders including deafness, in developing countries. Consanguinity appears to be quite common in the Indo-Pakistan subcontinent. The first ever study on consanguineous marriages in rural Pakistan, revealed 100% isonym or caste group marriages, with 80 percent consanguinity and about 64 percent first / second cousin marriages.¹⁰ Inter-caste marriages were advocated as the primary method of prevention of disability.¹¹ Custom and tradition favouring cultural consanguinity, however, is a hindrance in the prevention of disability due to recessive mode of inheritance.¹²

In this project, the authors studied Punjabi population to find out the prevalence of deafness in different districts of Punjab and enrolled 08 consanguineous families with multiple affected individuals belonging to different tribes of the Punjab. The primary aim was to screen out deafness loci in various isonym groups in order to
elicidate the genetic and molecular basis of the deafness.

**METHODOLOGY**

This study was conducted at the University of Health Sciences, Lahore, from July 2009 to March 2012. Families with three or more deafness-affected individuals were identified through descriptive observational study from different areas of the Punjab, Pakistan. The contacted families were briefed about the research program on deafness. A detailed history was taken from each family to establish the nature of phenotype and to rule out any environmental cause for deafness. The affected individuals had conductive, sensorineural and mixed type of hearing loss. Recruited families were given ID number SAPun, denoting the initial of the workers and the Punjab. Audiometric testing was performed for all deaf individuals. A detailed pedigree was drawn for each family using Cyrillic program and Macromedia FreeHand software by interviewing multiple members of the family to confirm consanguinity. The enrolled families provide convincing evidence for autosomal recessive mode of inheritance.

Blood samples (05 - 10 ml) were collected from the families depending on their willingness and availability. Informed written consent approved by our Institutional Review Committee (IBC) was obtained from all family members for participating in this study. Blood samples were stored in an ice box at 4°C during sampling and transport. On arrival in the laboratory, the collected blood samples were kept frozen at -20°C. Separate isolated bench space was allotted for blood handling and DNA extraction.

In case of elderly people or very young children, where it was difficult to obtain blood samples, buccal swabs were collected, as it was simple and noninvasive technique. Cheek cells were collected by means of Master Amp TM Buccal Swab Brushes (EPICENTRE Biotechnologies W1, Medical Package Co-operation, CA, USA). Two swabs were taken from each individual by swirling each brush firmly on the oral mucosa for 30 seconds, air dried and then stored in the original packaging at room temperature.

Genomic DNA was extracted from the WBC (white blood cells), which are the only nucleated cells present in the blood and an easy source of DNA. DNA extraction was carried out using the inorganic method.13 DNA was extracted from these cells (WBC) by following the protocol of Walker and colleagues.14 The yields of the DNA with this method are usually 2 - 8 ng/µl. Initially, 5 - 6 individuals from each family were selected for automated fluorescent genotyping. A 96 well master plate was prepared using 25ng/µl DNA of each individual in separate well. Replicas of this master plate were made with 2 µl of DNA dispensed into each well overlaid with 12 µl mineral oil.

Fluorescently labeled microsatellite markers were used for amplification of desired regions by PCR (Polymerase Chain Reaction) using genomic DNA as a template in Gene Amp PCR system 9700 and 2700 (Perkin Elmer). The forward primers used for this purpose were labeled with one of the five fluorescent dyes, FAM, VIC, NED, TET or HEX, while the reverse primers were unlabeled. The markers used for the linkage analysis encompassed the chromosomal locations reported for deafness loci and were chosen from the Marshfield Comprehensive Human Genetic Maps. These markers were mostly dinucleotide or trinucleotide repeats and the primer sequences for amplification of each marker are listed in genome database.

An aliquot of 2µl of the PCR products of different sizes labeled with different dyes were pooled together in a 96 well pooling plate using 12 capillary Hamilton Syringe. 11.7 µl of deionized formamide containing 0.3 µl of ROX (GS-500 ABI), LIZ or TAMRA (Perkin Elmer) size standard was added to the pooled amplicons. The samples were denatured at 95°C for 5 minutes followed by quick chilling on ice for 5 minutes before running in the ABI Prism 3100 genetic analyzer.

After running samples on ABI Prism 3100 genetic analyzer, alleles in base pairs for each marker were recorded. Automated allele assignment was performed using the ABI PRISM GeneScan Analysis Software Version 3.7 for Windows NT Platform. For initial screening, few members from each family were genotyped for three markers for a locus. Additional markers from the corresponding regions were also typed if some of these markers were uninformative. Markers were run to define the region of homozygosity and all family members were genotyped and haplotype generated to either include or exclude the linkage region.

A haplotype representing an individual's chromosomal segment is the set of genotyped alleles arranged according to the cM (centi Morgan) distance along a chromosome. Alleles were arranged in a way that confirms the inheritance pattern along a chromosome and the inheritance pattern of segregating disease. If three polymorphic (fully confirmative) markers located in the linkage interval of a DFNB locus did not show homozygosity among the affected person of a family, the locus was considered unlinked. Linkage to a particular locus was confirmed when homozygous data of affected members correlates with the disease pattern in the family tree. If the data indicated linkage to some locus, all family members including affected and normal individuals were genotyped to confirm the linkage in whole family.
A complete medical history was obtained from each affected individual to exclude the possibility of infectious or environmental causes of hearing impairment. These individuals showed no evidence of external ear abnormality, mental retardation, loss of vision, renal anomaly or integumentary disorder. In addition, no apparent balance problems or vertigo and no delay in the age of walking were noted.

RESULTS

The study was conducted to find out the deafness loci in isolated ethnic tribes of the Punjab, Pakistan. A total of 132 individuals were analyzed comprising of 8 families with multiple affected individuals identified and collected from different districts of the Punjab. These families were screened for linkage to selected deafness loci (DFNB8/10, DFNB12, DFNB29, DFNB36 and DFNB37) by using informative STR (short tandem repeat) markers. The results are given in Table I. These results indicate molecular heterogeneity of the deafness loci in the Punjab population. These families were from different ethnic groups. The most frequently occurring locus among studied families of the Punjab was DFNB12.

Table I: Analysis of deafness known genotypes in various casts of the Punjab.

<table>
<thead>
<tr>
<th>Family name</th>
<th>Linked genotype</th>
<th>Total persons</th>
<th>Expired</th>
<th>Affected</th>
<th>Analyzed</th>
<th>Cast</th>
<th>Districts</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAPun03</td>
<td>DFNB12</td>
<td>27</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>Rajput</td>
<td>Sialkot</td>
</tr>
<tr>
<td>SAPun05</td>
<td>DFNB8/10</td>
<td>34</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>Pukhtoon</td>
<td>Attock</td>
</tr>
<tr>
<td>SAPun06</td>
<td>DFNB29</td>
<td>27</td>
<td>0</td>
<td>5</td>
<td>6</td>
<td>Jutt</td>
<td>Sialkot</td>
</tr>
<tr>
<td>SAPun10</td>
<td>DFNB12</td>
<td>31</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>Jutt</td>
<td>Sahiwal</td>
</tr>
<tr>
<td>SAPun13</td>
<td>DFNB36</td>
<td>33</td>
<td>0</td>
<td>4</td>
<td>9</td>
<td>Rajput</td>
<td>Sahiwal</td>
</tr>
<tr>
<td>SAPun15</td>
<td>DFNB12</td>
<td>22</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>Warayaha</td>
<td>Gujrat</td>
</tr>
<tr>
<td>SAPun17</td>
<td>DFNB8/10</td>
<td>17</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>Boota-Jutt</td>
<td>Tobateksingh</td>
</tr>
<tr>
<td>SAPun19</td>
<td>DFNB37</td>
<td>19</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>Jawar</td>
<td>Bhakkar</td>
</tr>
</tbody>
</table>

Genotype analysis of markers in the DFNB12 region was performed on all participating members of this family. Genotype and haplotype data reveals that three deaf individuals (IV:4, IVI:, V:6) are homozygous for all three DFNB12 markers D10S606, D10S1694, and D10S1432. Maximum two-point LOD score of 3.12 was observed for the marker D10S606 and 2.70 for the marker D10S1694 respectively. One normal hearing individual IV:3 was genetically carrier of the diseased allele.

The family SAPun15 was enrolled from Gujrat belonging to sub-caste Warayaha of Jutt (Jat). It was a large consanguineous family comprising of three deaf individuals in two loops. The pedigree is shown in Figure 3. All deaf individuals were genotyped. During screening, linkage was observed with chromosome 10q22.1 markers.

All the deaf individuals were found to be genetically homozygous for DFNB12 linked markers, D10S606, D10S1694, and D10S1432. A maximum 2.25 LOD score was obtained for the marker D10S606. This family was mapped to the same region as CDH23 interval known to cause DFNB12.

SAPun05 Family was a large Pakhtoon family residing in Attock district, which migrated from Pashto speaking area of Pakistan/Afghanistan, containing four affected individuals in one loop, as shown in Figure 4. Blood samples were collected from all these individuals and used in linkage analysis. The ages of affected individuals IV:1, IV:2, IV:5 and IV:6 were 9, 6, 16, and 18 years respectively at the time of enrollment.

Four affected individuals, two normal siblings and their normal parents were included in linkage study. Haplotype of markers D21S1225 and 994GCA50, bounded causative region of DFNB8/10 at chromosome position 21q22. Individuals IV:1, IV:2, IV:5 and IV:6, were homozygous for affected haplotype. A maximum two-point LOD score of 2.75 at θ=0 was observed for the marker 994G8CA50.
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SAPun17 Family was a consanguineous Boota-Jutt family enrolled from Toba Tek Singh containing three affected individuals in two loops (Figure 5). The affected individuals III:1, III:2 and IV:1, were aged 6, 12 and 10 years respectively at the time of enrollment.

It was confirmed about all affected individuals of the family that no associated symptoms with deafness such as external ear abnormality, mental retardation, goiter, eye, renal or integument disorder was present. Also none of these congenitally deaf children had a balance problem. No evidence for any acquired risk factor.

Profound hearing loss was noticed in affected individuals. Three affected and three normal individuals of two loops were included in linkage study. Haplotype of markers D21S1225 and 994GCA50 bounded the causative region of DFNB8/10 at chromosome position 21q22. A maximum two-point LOD score of 2.44 at \( \theta = 0 \) was observed for the marker 994G8CA50. Individuals III:1, III:2, and IV:1 were homozygous for affected haplotype. Individuals III:1, III:2 and IV:4, were heterozygous carrier.

SAPun06 family was a small consanguineous family enrolled from Sialkot, containing four affected individuals in two loops as shown in Figure 6. The age of affected individuals was 24, 22, 20, and 19 years respectively at the time of enrollment. The family belonged to caste Jutt.

Deafness was found to be non-syndromic as none of the individuals carried any associated symptom such as external ear abnormality, mental retardation, goiter, eye, renal or integumentary disorder. Balance problem was also absent from these affected individual. It was ascertained that no environmental factor was involved to cause hearing loss in affected individuals.

Four affected individuals and their parents were included in linkage study. Haplotype marker (D21S2078 and D21S2080) bounded the causative region of DFNB29 at chromosomal position 21q22. Individuals V:1, V:2 and V:4 were homozygous for affected individual. Individual V:3, IV:5 and IV:6 were heterozygous carrier. A maximum two-point LOD score of 3.56 at \( \theta = 0 \) was obtained for the marker D21S2078.

SAPun13 was a small consanguineous Rajput family enrolled from Sahiwal, containing three affected individuals in as single loop as shown in Figure 7, at the time of enrollment all the three deaf individuals and three normal sibs and their parents were available whose blood samples were taken with consent. DNA was extracted from the blood sample and used in linkage analysis. The age of affected individuals, IV:1, IV:2, and IV:3 was 15, 12 and 10 years respectively at the time of enrollment.

Four affected and four normal individuals of two loops were included in linkage study. Haplotype of markers (D1S2870 and D1S3774) bounded the causative region of DFNB36 at chromosomal position 1p36.3. Individuals IV:1, IV:2, and IV:3 were homozygous while individuals IV:4 were heterozygous affected. A maximum two-point LOD score of 2.60 was obtained from the markers D1S2870 and D1S3774 at the recombination fraction at \( \theta = 0 \).

SAPun19 was a small consanguineous family of Jarwar enrolled from Bhakkar, containing three affected individuals in single loop. At the time of enrollment all three deaf individuals and one normal sib along with their parents were available whose samples were taken and used in linkage analysis. The age of affected individuals IV:1, IV:2 and IV:3 were 13, 17 and 7 years respectively at the time of enrollment (Figure 8).

Three affected and three normal individuals from one loop were included in linkage study. Haplotype of 3 markers D6S1838, D6S1589 and D6S286 bounded the causative region of DFNB37 at chromosome position 6q13. Individuals IV:1, IV:2 and IV:3 were homozygous for affected haplotype. The gene in this locus is located between first two markers. Individuals III:5, III:6 and IV:4 were heterozygous carrier. Two point linkage analysis of linked microsatellite markers generates a maximum two-point LOD scores of 2.44 at recombination fraction \( \theta = 0 \) for D6S286 respectively (89.83cM).

**DISCUSSION**

The genetic heterogeneity of Pakistani population is manifest from the different deafness loci. While 47 recessive deafness loci have been published and 21 genes discovered from all over the world, 28 loci have been found to occur in Pakistani population, moreover four deafness causative genes TMRSS3, PCDH15, ESPN and MYO6 were mapped in the loci DFNB8/10, DFNB23/USH1F, DFNB36 and DFNB37 respectively.\(^{15,16}\) But they did not clearly mention their castes and locations within Pakistan. We are reporting very first time the linkage of consanguineous families along with their castes and locations within Punjab Pakistan.

Out of the eight linked families, four families (SAPun-03, SAPun-10 and SAPun-15) were found to be linked to DFNB12; two families (SAPun-05 and SAPun-17) were found linked to DFNB8/10, remaining three families SAPun-06, SAPun-13 and SAPun-19 were found to be linked to DFNB29, DFNB36 and DFNB37 respectively. The linkage of different deafness loci was a proof of heterogeneous genetic makeup of population of the Punjab.

DFNB8/10 was identified originally from Pakistani and Palestinian population.\(^{6}\) Genetic hearing loss is most often monogenic.\(^{17}\) Ibrahim studied different loci in Pakhtoon ethnic group that indicated genetic heterogeneity in autosomal recessive deafness in Pakhtoon population of Pakistan.\(^{18}\) DFNB36 is relatively less common recessive deafness locus and was identified from Pakistani population by Zheng.\(^{19}\)
DFNB12 is a common deafness locus found in Pakistani population. The gene for the overlapping locus is CDH23. The mutated gene causes stereo cilia abnormalities in number, organization, shape and position relative to the Kino cilium. The Usher type ID (USHID) locus was also mapped to chromosome 10q. Overlapping the previously mapped DFNB12 non-syndromic deafness locus at chromosome 10q21-q22 with 15-cM interval.

The genetic studies of deafness will help in understanding the auditory function and will be used to evolve ways and treatments to combat the malformation of deafness that is affecting a large population of the world and may significantly influence the diagnosis and treatment of deafness worldwide.

The highest percentage of congenital deafness was in Rajput families followed by Jutt, Warayah, Pakhtoon and Jawar. The reported results may be helpful in monitoring the disease trend and predisposing effective and targeted ethnic tribes and regional prevention plans. No doubt, the high rate identified in certain tribes in the Punjab needs to be further studied in depth in order to corroborate the existence of possible genetic clusters. This study has a unique advantage in deafness study of large Ethnic tribes of Punjab Pakistan.

CONCLUSION

The genotyping results revealed that DFNB12 locus was the most common followed by DFNB8/10 locus, loci DFNB29, DFNB36 and DFNB37 were less common. The data about the occurrence of different deafness loci strengthen the rhetoric of prevalent genetic heterogeneity for deafness in the Punjab population of Pakistan, making it more easy and attractive for research work on the molecular basis of deafness. The study of heterogeneity of deafness genes is also useful for genetic counseling strategies.

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REFERENCES


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