Biomedical Letters ISSN 2410-955X– An International Biannually Journal

Research Article
2019 | Volume 5 | issue 1 | Pages 53-66

ARTICLE INFO

Received
July 31, 2019
Revised
September 27, 2019
Accepted
October 27, 2019

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Keywords
DFNB1
DFNB2
GJB2
MYO7A
Hearing impairment
Pakistan

How to Cite
Tariq H, Zaigham K. Genetic contribution of GJB2 gene and DFNB2 locus to hearing impairment in Kashmiri and Pakistani families. Biomedical Letters 2019; 5(1):53-66.

Both authors contributed equally.

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Genetic contribution of *GJB2* gene and *DFNB2* locus to hearing impairment in Kashmiri and Pakistani families

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Abstract

Hearing impairment, the inability to hear has heterogeneous etiology. One hundred seventeen genes have been mapped for hearing impairment to date. The present study was aimed to determine the major contribution of the *GJB2* and *MYO7A* for hearing loss in Pakistani and Kashmiri families belonging to different ethnic groups. Twenty-one families were ascertained from different areas of Pakistan and Azad Kashmir. The contribution of *GJB2* and *MYO7A* was initially studied by linkage analysis using short tandem repeats microsatellite markers. Sanger sequencing was further performed for *GJB2* (Gap junction beta-2 protein) *gene* to identify the causative variants. Phenotypes of five families were found linked with these two genes by linkage analysis study (two families with *GJB2* and three families with *MYO7A*). Sanger sequencing of *GJB2* resulted in identification of two homozygous recurrent mutations c.71G>A (p. Trp24*) and c.231G>A (p. Trp77*) in two families supporting previous studies. The genetic causes of hearing incapability in 23.80% of all the studied families (5/21) were determined by genotypic and phenotypic expression of autosomal recessive hearing impairment. In this study we established the founder effect for intragenic single nucleotide polymorphism (SNP) haplotypes in families sharing *GJB2* gene in Pakistani hearing-impaired families. The founder effect in *GJB2* gene is being reported for the very first time in a Kashmiri family.

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Introduction

Hearing loss, the most common form of sensory deficit, has worldwide prevalence affecting 1 in 650 infants, having frequency of 2.7/1000 in minors and 3.5/1000 in adolescents [1, 2]. Hearing loss has multifactorial etiologies including genetic or environmental factors. Any disruption in highly synchronized mechanism of hearing leads to impairment of this sense. Almost 60 % cases of profound hearing loss are due to genetic causations [3].

Hearing impairment exhibits extreme genetic heterogeneity. Hearing loss with distinctive clinical features is called syndromic and hearing loss without clinical anomaly is termed as non-syndromic hearing loss [4]. So far 152 DFNB loci have been mapped linked with hearing impairment (http://deafnessvariationdatabase.org/).

Pakistani masses is one of the richest genetic asset to study hereditary diseases due to its distinctive societal norms of consanguineous unions [5]. In Pakistan the prevalence of hearing impairment is 1.6 per 1,000 live births which is higher than world average of 1 per 1000 live births [6, 7].

Out of the disorders allied with syndromic hearing loss the most common is Usher syndrome (USH). A total of 14 chromosomal loci are assigned to three clinical USH types, namely USH1B-H, USH2A-D, and USH3A-B. Mutations in USH type 1 genes cause the most severe form of Usher syndrome with congenital hearing loss, pre-pubertal onset of retinitis pigmentosa (RP) and severe vestibular dysfunctions. Less severe than USH1, USH2 shows moderate to severe congenital hearing loss, vestibular dysfunction and a later onset of RP. USH3 is characterized by variable onset of RP, variable impairment of vestibular function combined with progressive hearing loss [2].

This study was carried out to identify Pakistani families suffering from hearing impairment and their genetic analysis for the most prevalent hearing impairment loci and the causative mutations in Pakistani families. Three families were found linked with DFNB2 harboring *MYO7A* gene, while sequencing of *GJB2* gene located at DFNB1 locus revealed two known mutations in two families.

The frequency of the genetic variation varies according to ethnicity and population. The most common cause of non-syndromic hearing loss is *GJB2* and *MYO7A* variants in South Asia [8]. The frequency of *GJB2* gene is up to 38% in different regions of South Asia. The other most common variant is *MYO7A* prevailed in South Asia with 5% frequency [9]. Study of mutational spectrum of hearing impairment in a particular population provides a background data for the development of molecular genetic screening techniques for that population [10]. The mutational spectrum of hearing impairment in Kashmiri population of Pakistan has not been well studied to the best of our knowledge. The present study revealed strong founder mutations in Kashmiri population for the first time.

Materials and Methods

The approval for this study was taken by institutional review board at the National Centre of Excellence in Molecular Biology, Lahore, Pakistan. Informed written consents and clinical information was taken from all the participants of the study. 5-10 ml of blood sample was taken from all participating members in 50 ml Sterilin® polypropylene tubes having 400 µl of 0.5 M anticoagulant EDTA.

Genomic DNA extraction

The genomic DNA was extracted by a modified version of non-organic method [11]. The concentration of extracted genomic DNA was estimated by SmartSpec plus Bio-Rad Spectrophotometer (Bio-Rad, Hercules, CA).

Exclusion analysis, PCR amplification and genotyping

Exclusion studies were performed to screen the most common hearing impairment loci prevalent in Pakistani population *i.e.* DFNB1 and DFNB2. Linkage analysis was done with the help of highly polymorphic fluorescent STR markers. Mixtures of PCR amplified products and size standards were prepared and run in Applied Biosystems 3100 DNA Analyzer. To align genotypes GeneMapper® software from Applied Biosystems was used.

Sanger Sequencing

Sequencing PCR was carried out for one coding exon of *GJB2* gene by using Big Dye Terminator fluorescent dideoxynucleoside triphosphates provided by Applied Biosystems. ABI PRISM 3100 DNA analyzer (Applied Biosystems) was used to run the sequencing products. The sequencing data was

analyzed with Chromas software version 2.6.6 by Technelysium.

Results

In this study we performed linkage analysis of most common hearing impairment loci DFNB1 harboring *GJB2* gene and DFNB2 carrying *MYO7A* gene. A cohort of 21 hearing impaired families belonging to diverse ethnicities was ascertained from diverse zones of Pakistan and Azad Kashmir. After sample collection 21 hearing impaired families were subjected to linkage studies out of which three families showed linkage with DFNB2 and two families showed linkage with DFNB1. Out of three families linked to DFNB2 two families *i.e.* family 01 and family 02 exhibited non-syndromic hearing loss and family 03 exhibited syndromic form *i.e.* hearing loss with night blindness, characteristic feature of Usher syndrome type 1 (USH1) [14]. The two families *i.e.* family 04 and family 05 linked with DFNB1 were then subjected to Sanger sequencing to identify the causative variants in *GJB2* gene. Sequencing data revealed the two most frequent variants c.71G>A (p.W24X) and c.231G>A (p.W77X) in these families. Both variants are disease causing recurrent mutations in Pakistani population [19]. The mutation c.231G>A (p.W77X) having founder effect is reported for the first time in the present study in Kashmiri population. The evolutionary conservation of amino acid Trp24 (W24) (**Table 1**) and Trp77 (W77) (**Table 2**) was also compared in other *GJB2* orthologs. The amino acid tryptophan at position 24 and at position 77 is conserved along with other amino acids present in the vicinity.

Table 1: Sequence conservation of amino acid W24

Species	Protein Sequence						
	G_{21}	K_{22}	I_{23}	W_{24}	L_{25}	T_{26}	V_{27}
Human	G	K	I	**W**	L	T	V
Rhesus	G	K	I	**W**	L	T	V
Mouse	G	K	I	**W**	L	T	V
Dog	G	K	I	**W**	L	T	V
Elephant	G	K	I	**W**	L	T	V
Chicken	G	K	I	**W**	L	T	V
X_tropicalis	G	K	I	**W**	L	S	V
Zebrafish	G	R	I	**W**	L	S	V
Lampray	G	R	V	**W**	L	S	V

Note: Sequence conservation of amino acid Tryptophan at position 24 among different species of vertebrates. (G: Glycine, K: Lysine, I: Isoleucine, W: Tryptophan, L: Leucine, T: Threonine, V: Valine).

A map of single nucleotide polymorphism (SNP) flanking the causative mutations was constructed and the established haplotype (CGGAGG), suggested the common founder for the c.71G>A and c.231G>A transition in *GJB2* gene (**Table 3**). The detrimental effects of these two recurrent mutations *i.e.* c.71G>A (p.W24X) and c.231G>A (p.W77X) were recorded using different bioinformatics tools like mutation taster (http://www.mutationtaster.org/), likelihood ratio test (LRT) (https://www.itl.nist.gov) and genomic evolutionary rate profiling (GERP) score (SidowLab/downloads/gerp/index.html) are mentioned in (**Table 4**). Both of these variants were classified as disease causing [9].

Table 2: Sequence conservation of amino acid W77

Species	Protein Sequence						
	I_{74}	R_{75}	L_{76}	W_{77}	A_{78}	L_{79}	Q_{80}
Human	I	R	L	**W**	A	L	Q
Rhesus	I	R	L	**W**	A	L	Q
Mouse	I	R	L	**W**	A	L	Q
Dog	I	R	L	**W**	A	L	Q
Elephant	I	R	L	**W**	A	L	Q
Chicken	I	R	L	**W**	A	L	Q
X_tropicalis	I	R	L	**W**	C	L	Q
Zebrafish	I	R	L	**W**	A	L	Q
Lampray	V	R	L	**W**	A	L	Q

Note: Sequence conservation of amino acid Tryptophan at position 77 among different species of vertebrates. (I: Isoleucine, R: Arginine, L: Leucine, W: Tryptophan, A: Alanine, Q: Glutamine).

Family 01

The family 01 (**Fig. 1A**) is a consanguineous family registered from Lahore belonging to Punjabi ethnicity. Medical profile of the affected individuals showed severe to profound hearing loss. The affected individuals (VI: 4, VI: 5, VI: 6) of pedigree were tested for audiometry (**Fig. 2A**) and they had no associated syndrome with hearing loss.

Haplotype Analysis

Screening revealed disease phenotype segregating in family 01 was linked with chromosome 11 harboring *MYO7A* gene. Linkage was ascertained to chromosome 11q at 79.98 cM with markers D11S4186 and D11S1789 which is the candidate region of DFNB2. All three affected individuals (VI: 4, VI: 5, VI: 6) were homozygous for the two DFNB2 markers however, affected individuals along with mother gave proximal cross with marker D11S4196. Haplotype analysis revealed that both parents (V: 5, V: 6) were carriers having a normal allele and an affected allele while the individual (VI: 3) was genotypically and phenotypically normal

having both copies of normal alleles one from each parent. The maximum 2-point Lod Score (Zmax) of 1.69 was calculated for D11S4186 at recombination fraction θ=0 (**Fig. 3A**).

Table 3: SNP haplotypes of affected individuals in Family 04 and 05

		SNP Haplotype (rs No.)					
Family	**Individual**	**529500747**	**1801002**	**2274084**	**2274083**	**111033196**	**111033186**
Family 04	IV:5	C	G	G	A	G	G
Family 05	IV:3	C	G	G	A	G	G

Note: Intragenic SNP haplotypes of families sharing *GJB2* mutations in affected individuals of family 04 and 05.

Table 4: List of recurrent mutations in *GJB2*

Mutation	Mutation Taster	*GERP Score	LRT
c.71G>A, p.W24X	Pathogenic effect	5.33	Deleterious
c.231G>A, p.W77X	Pathogenic effect	5.33	Deleterious

Note: *GERP score ranges from -12.3 to 6.17. Greater the score, higher will be the amino acid conservation

Family 02

The family 02 (**Fig. 1B**) is a consanguineous family enrolled from Layyah belonging to Saraiki ethnic group. The affected individuals (V: 5, V: 6, V: 7, V: 8) of pedigree were tested for audiometry (**Fig. 2B**) and they had no other clinical manifestations except congenital bilateral profound hearing loss.

Haplotype Analysis

In family 02 linkage was ascertained to the candidate region of DFNB2, with markers D11S4207, D11S4186, D11S1789, D11S4079 located on q arm of the chromosome 11 at 79.98 cM. All four affected individuals of the family (V: 5, V: 6, V: 7, V: 8,) were homozygous for all four DFNB2 markers. After constructing haplotypes, it was found that both parents (IV: 4, IV: 5) and a child (V: 12) were carriers. One parent of the loop (IV: 3) was normal and the other parent (IV: 2) along with a child (V: 3) were carriers, children of the loop (V: 1, V: 2, V: 4) were genotypically and phenotypically normal. one affected individual (V: 8) and one carrier individual (V: 12) gave distal cross with marker D11S901 85.48 cM suggesting that the candidate region to which the family was linked is 79.98 cM *i.e.* the location of *MYO7A* gene. The maximum 2-point Lod Score (Zmax) of 2.56 was calculated for D11S4207 at recombination fraction θ=0 (**Fig. 3B**).

Family 03

The family 03 (**Fig. 1C**) is a consanguineous family enrolled from Khlil-abad, Kot chutta belonging to ethnic group Saraiki Baloch. Medical profile showed that four individuals were affected with profound hearing loss and had a history of night blindness. The affected individuals (V: 1, V: 3, V: 6, V: 7) of pedigree were tested for audiometry (**Fig. 2C**). Clinical tests confirmed that affected individuals were suffering with Usher syndrome, type 1B.

Haplotype Analysis

On screening of family 03, linkage was ascertained to the candidate region of DFNB2, with markers D11S4186, D11S1789, D11S4079 and D11S901. All four affected individuals (V: 1, V: 3, V: 6, V: 7,) were homozygous for DFNB2 markers. Two individuals (V: 2) and (V: 5) were carriers *i.e.* they were phenotypically normal but genotypically carried one normal and one affected allele. The individual (V: 4) was normal because she carried both copies of normal alleles. The maximum 2-point Lod Score (Zmax) of 2.44 was calculated for D11S901 at recombination fraction θ=0 (**Fig. 3C**).

Family 04

This family (**Fig. 1D**) was registered from Mianwali. It has two hearing disabled individuals in main loop and one effected individuals in other loop. All hearing-impaired persons (IV:2, IV:5, IV:6) and normal individual (IV:1, IV:3 and IV:4) along with parents (III:1 and III:2, III:3 and III:4) were enrolled in this research.

Haplotype Analysis

The Haplotype of all the deaf persons showed linkage to DFNB1 locus. All the affected individuals in both loops were having both mutant alleles (homozygous) to markers GJB2, D13S175 and D13S1275 and normal persons were having different

alleles (heterozygous) as it carries different combination of alleles from both parents. A maximum 2-point Lod Score (Zmax) of 1.27 was observed with markers GJB2 at recombination fraction (θ) = 0 (**Fig. 3D**).

Mutational Analysis

Sanger sequencing of *GJB2* gene revealed a nonsense mutation due to transition from G to A (c.71G>A). Due to this alteration the amino acid tryptophan TGG at position W24 is changed into stop codon TAG resulting in truncated protein (p. Trp24*). This single base pair change G>A at position 71 (p.W24X) shows penetrance with hearing loss phenotype in family 04 (**Fig. 4A & B**).

Family 05

This family (**Fig. 1E**) was registered from Azad Kashmir region of Pakistan having three deaf individuals in main loop and two hearing disabled individuals in other loop. All affected (IV:2, IV:3, IV:4, IV:6 and IV:7) and normal individual (IV:1, IV:5 and IV:8) along with parents (III:2 and III:3, III:5 and III:5) were enrolled in this research.

Haplotype Analysis

The Haplotype of all the affected individuals showed linkage to DFNB1 locus. All the affected individuals in both loops were homozygous to markers GJB2, D13S175 and D13S1275 and normal individuals were heterozygous as it carries different combination of alleles from both parents. A maximum 2-point Lod Score (Zmax) of 3.06 was observed with markers D13S1275 and 2.32 and 1.96 for markers D13S175, GJB2 respectively, at recombination fraction (θ) = 0 (**Fig. 3E**).

Mutational Analysis

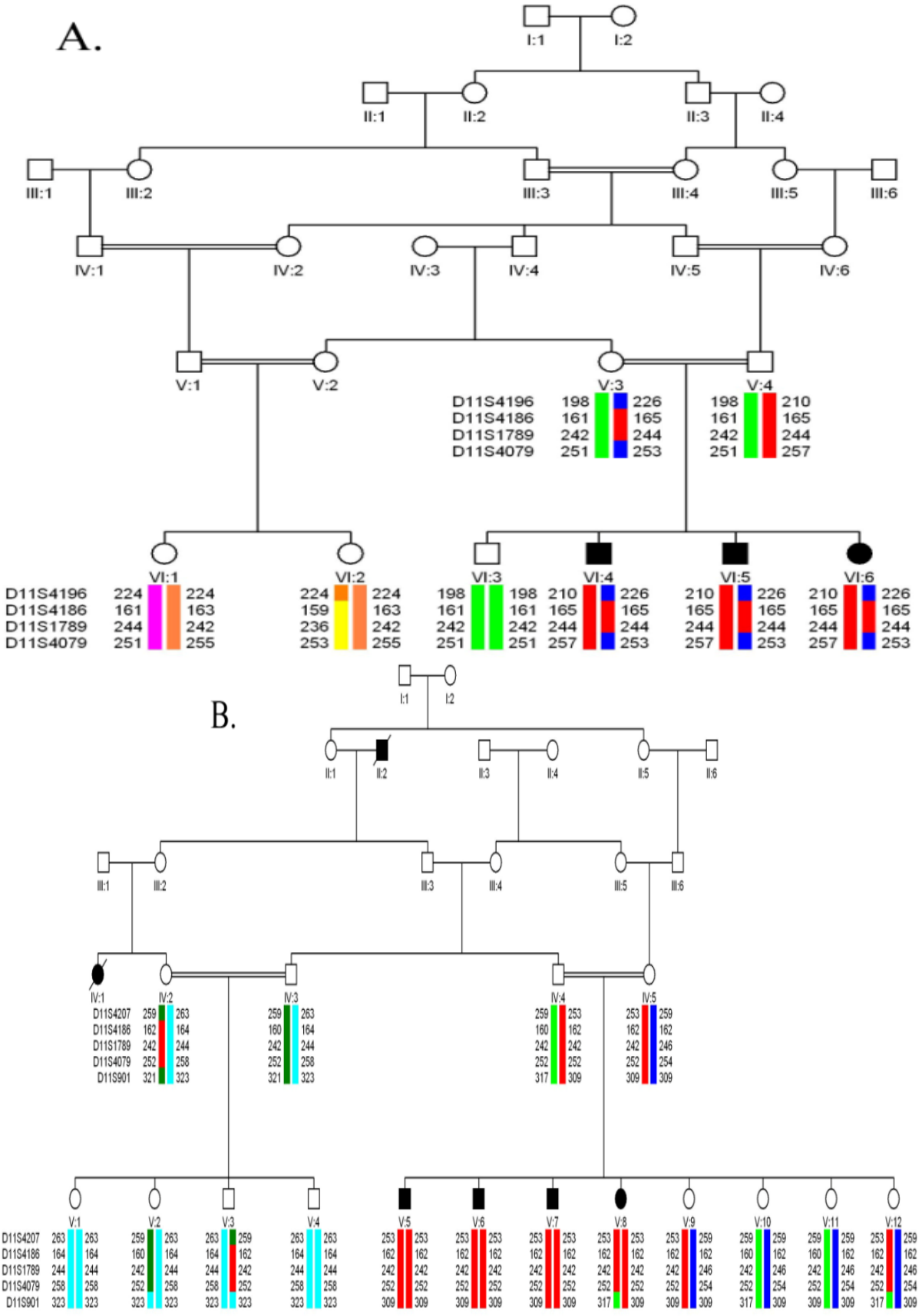
Sanger sequencing of *GJB2* gene revealed a nonsense mutation due to transition from G to A (c.231G>A). Due to this alteration the amino acid tryptophan TGG at position W77 is changed into stop codon TAG resulting in truncated protein (p. Trp77*). This single base pair change G>A at position 231 (p.W77X) shows penetrance with hearing loss phenotype in family 05 (**Fig. 4C & D**).

Discussion

The commonest sensory polygenic disease in humans having genetic contributors is hearing loss. Among all forms of hearing inability autosomal recessive non-syndromic form is most frequent with 70% genetic causations [8]. Frequently occurring autosomal recessive sensorineural hearing loss is due to DFNB1. The *GJB2* gene at DFNB1 locus encodes a gap junction protein Connexin26 [12]. DFNB1 locus having *GJB2* gene is responsible for 20% of all child hood deafness and may have a carrier rate as high as 2.8% [13].

Hearing loss is presented with extreme allelic and locus heterogeneity. The results of the screening studies carried out worldwide suggest that *GJB2* is more frequently mutated gene than any other counterpart [14]. Similarly, 50% of the hearing compromised individuals in Pakistani population carry mutations in *GJB2* gene [15]. The two most commonly prevalent causative variants of *GJB2* gene in Pakistani population are c.71G>A (p.W24X) and c.231G>A (p.W77X) [16] which is in line with the results of present study *i.e.* family 04 with c.71G>A (p.W24X) and family 05 with c.231G>A (p.W77X). The most frequent *GJB2* mutation in European, Middle Eastern and African ancestries is c.35delG (p.G12V) [16, 17]. However, c.235delC (p.L79Cfs) is the major contributing *GJB2* mutation in East Asian lineages [18].

The locus DFNB2 is also prevalent in Pakistani population and the mutations in the underlying gene *MYO7A* are responsible for syndromic as well as non-syndromic form of hearing loss. Our study identified two pedigrees *i.e.* family 01 and family 02 linked with DFNB2. The *MYO7A* gene encodes for protein myosin which is important for optimal hearing and retinal cell functionality [19]. Usher type 1 (USH1), an associated syndrome of hearing loss is the outcome of recessive mutations in *MYO7A* gene. One pedigree of the present study i.e. family 03 showed characteristic features of Usher syndrome type 1. Several hundred causative mutations in *MYO7A* gene have been compiled in deafness variation database (http://deafnessvariationdatabase.org/gene_page/MYO7A). Heterogeneous expression of USH1 in Pakistani population is comparable to other states like USA and UK [2, 14, 20]. The dominant allelic pattern of *MYO7A* is expressed as non-syndromic hearing loss, DFNA11 (MIM# 601317). The recessive allelic pattern of *MYO7A* is responsible for DFNB (MIM# 600060) [21]. Therefore it can be said that the majority of the recessive mutations in *MYO7A* gene are major contributors of syndromic form of hearing loss *i.e.* Usher syndrome type 1 (USH1) on the other hand *MYO7A* gene mutations also have a minor contribution in phenotypic expression of hearing loss [22].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II:4
III:1
III:2
III:3
III:4
III:5
III:6
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
V:1
V:2
V:3
V:4
D11S4196 198 226
D11S4186 161 165
D11S1789 242 244
D11S4079 251 253
D11S4196 198 210
D11S4186 161 165
D11S1789 242 244
D11S4079 251 257
VI:1
VI:2
VI:3
VI:4
VI:5
VI:6
D11S4196 224 224
D11S4186 161 163
D11S1789 244 242
D11S4079 251 255
224 224
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236 242
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B.
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II:4
II:5
II:6
III:1
III:2
III:3
III:4
III:5
III:6
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IV:2
IV:3
IV:4
IV:5
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D11S4186 162 164
D11S1789 242 244
D11S4079 252 258
D11S901 321 323
259 263
160 164
242 244
252 258
321 323
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160 162
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317 309
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242 246
252 254
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V:1
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V:12
D11S4207 263 263
D11S4186 164 164
D11S1789 244 244
D11S4079 258 258
D11S901 323 323
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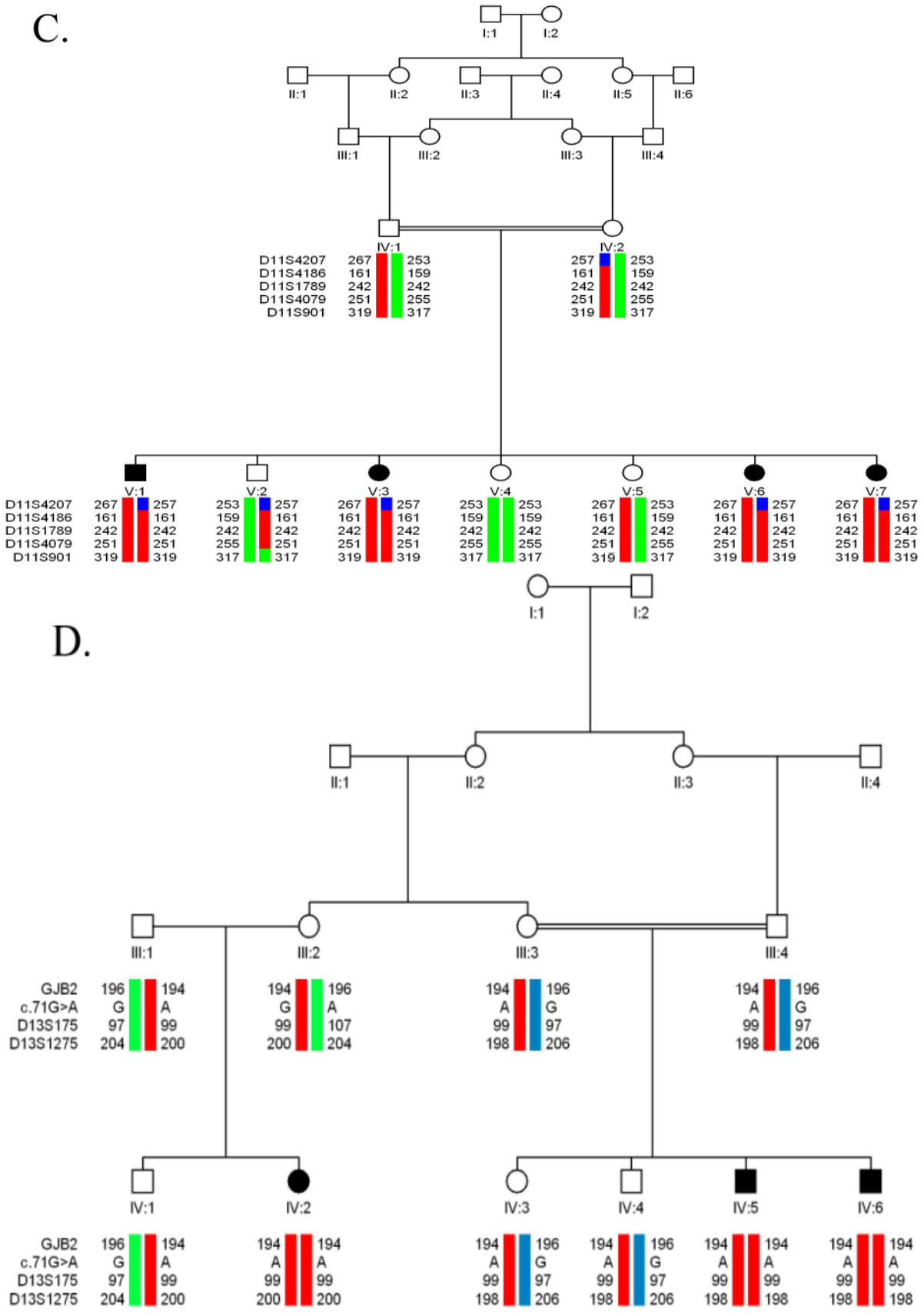
C.
I:1
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II:2
II:3
II:4
II:5
II:6
III:1
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IV:1
IV:2
D11S4207 267 253 257 253 267 253 253 267 257 267 257
D11S4186 161 159 161 159 161 159 159 161 161 161 161
D11S1789 242 242 242 242 242 242 255 242 242 242 242
D11S4079 251 255 251 255 251 255 255 251 251 251 251
D11S901 319 317 319 317 319 317 317 319 319 319 319
V:1
V:2
V:3
V:4
V:5
V:6
V:7
D.
I:1
I:2
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II:3
II:4
III:1
III:2
III:3
III:4
GJB2 196 194 194 196 194 196 194 196
c.71G>A G A G A A G A G
D13S175 97 99 99 107 99 97 99 97
D13S1275 204 200 200 204 198 206 198 206
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
GJB2 196 194 194 194 194 196 194 196 194 194 194 194
c.71G>A G A A A A G G G A A A
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D13S1275 204 200 200 200 198 206 198 206 198 198 198 198

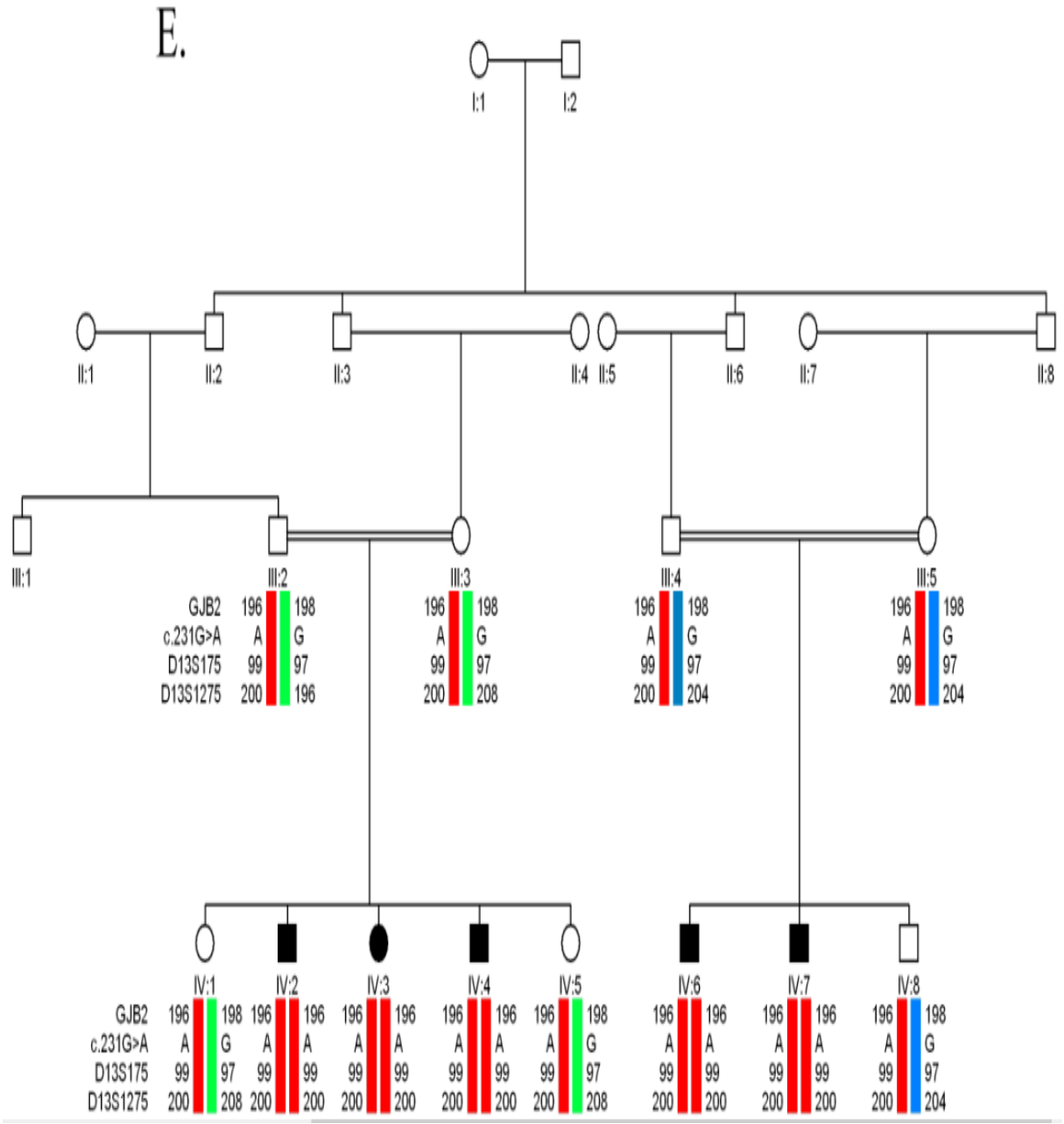


Fig. 1: Pedigree of family 01 (A), family 02 (B), family 03 (C) showing structure and haplotypes of chromosome 11q STR markers and family 04 (D), family 05 (E) showing structure and haplotypes of chromosome 13q STR markers flanking *GJB2* mutations.

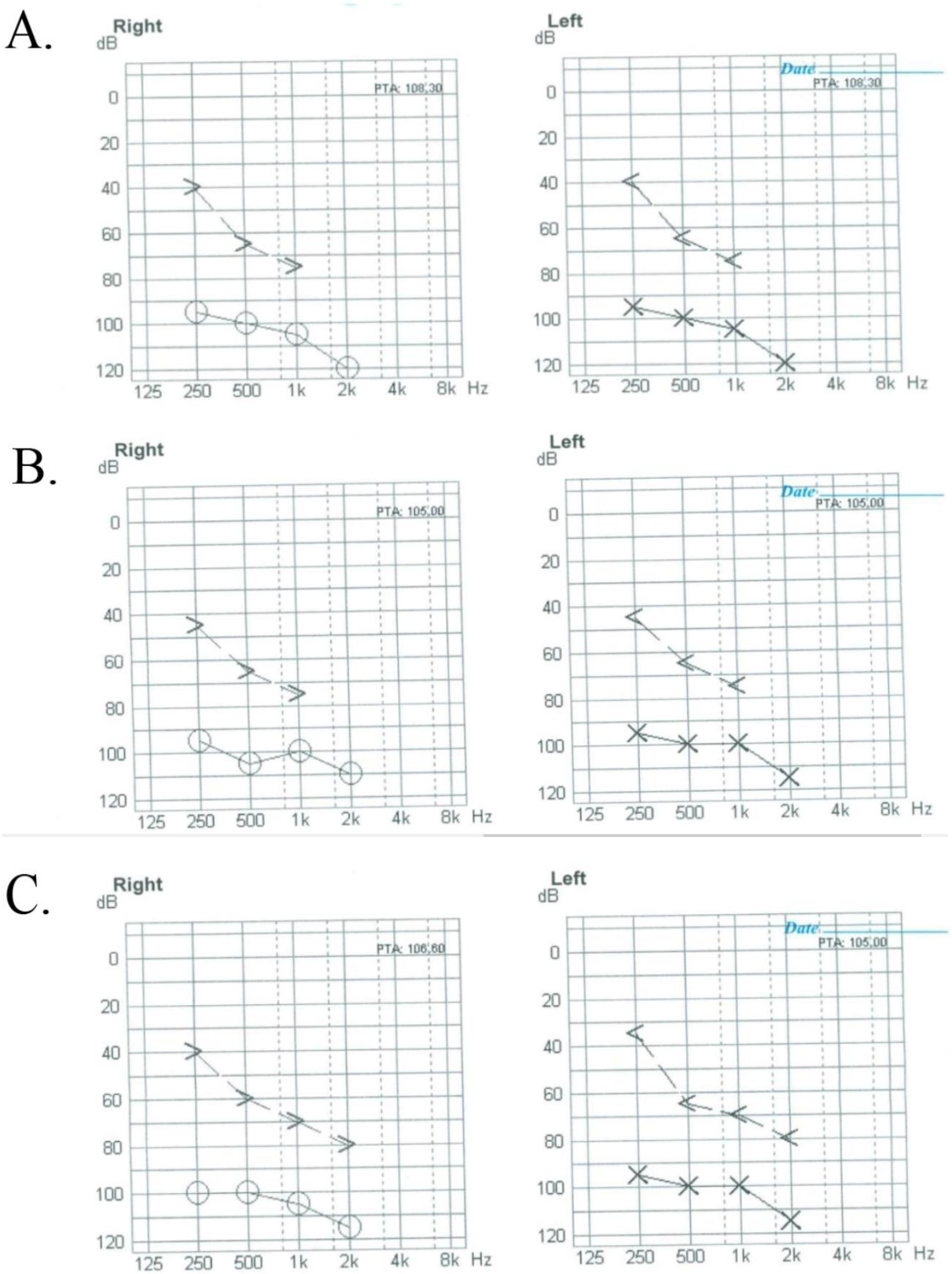
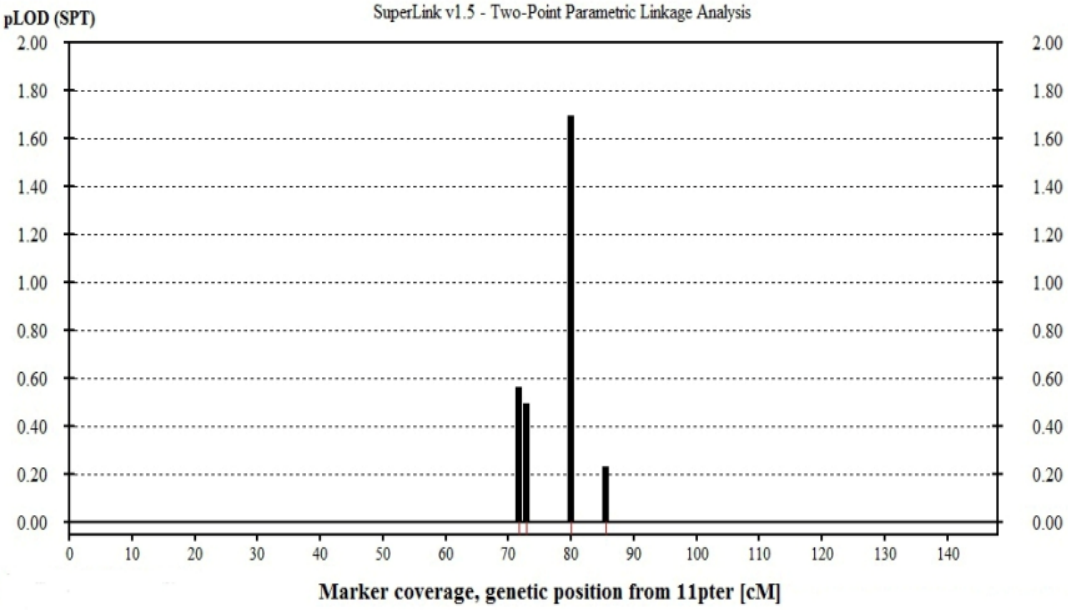


Fig. 2: Audiograms of IV: 4 of family 01 (A), V: 6 of family 02 (B), V: 7 of family 03 (C) showing bilateral profound (more than 80 db) hearing impairment.

A.

Project:	FAMILY01	Inheritance:	Recessive
Family name:	TOTALS	Common allele:	99.90 %
Used map:	Marshfield (sex averaged)	Disease allele:	0.10 %
Marker positions:	6 ok / 0 ? / 0 outside	LC1 PCOPY rate:	0.00 %
Allele frequencies:	All individuals (marker file)	LC1 PENET wt/mt:	0.00 %
CALC interval:	Entire chromosome	LC1 PENET mt/mt:	100.00 %

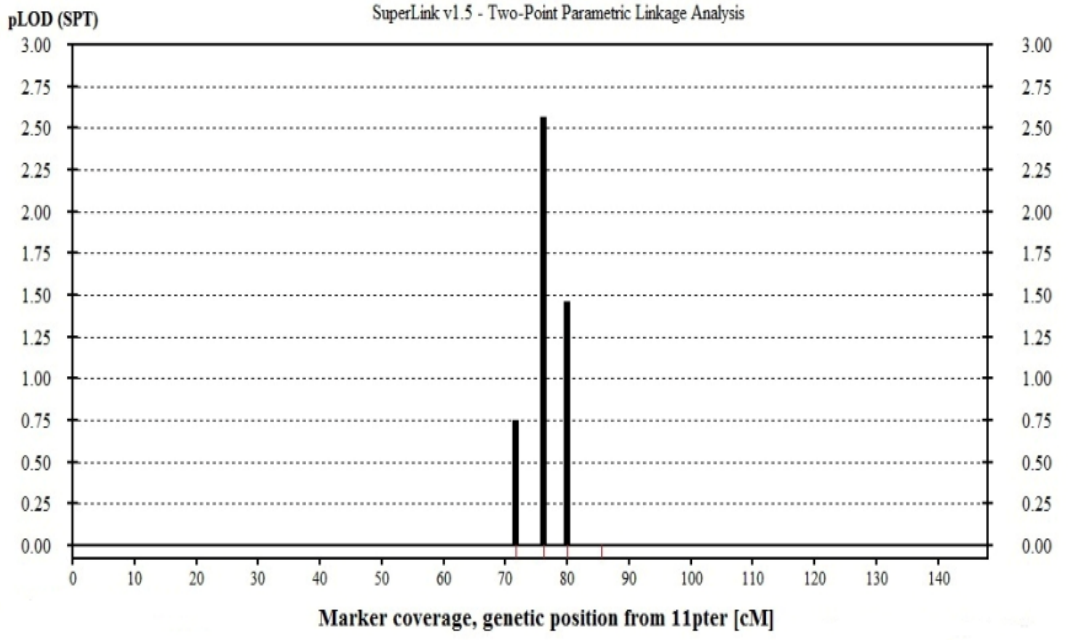
Marker	CHR	cM	LOD	Theta
1.D11S4186	11	79.98	1.6957	0.0000
2.D11S1789	11	79.98	1.5907	0.0000
3.D11S4136	11	71.60	0.5654	0.1000
4.D11S4196	11	72.82	0.4923	0.1000
5.D11S901	11	85.48	0.2302	0.1000



B.

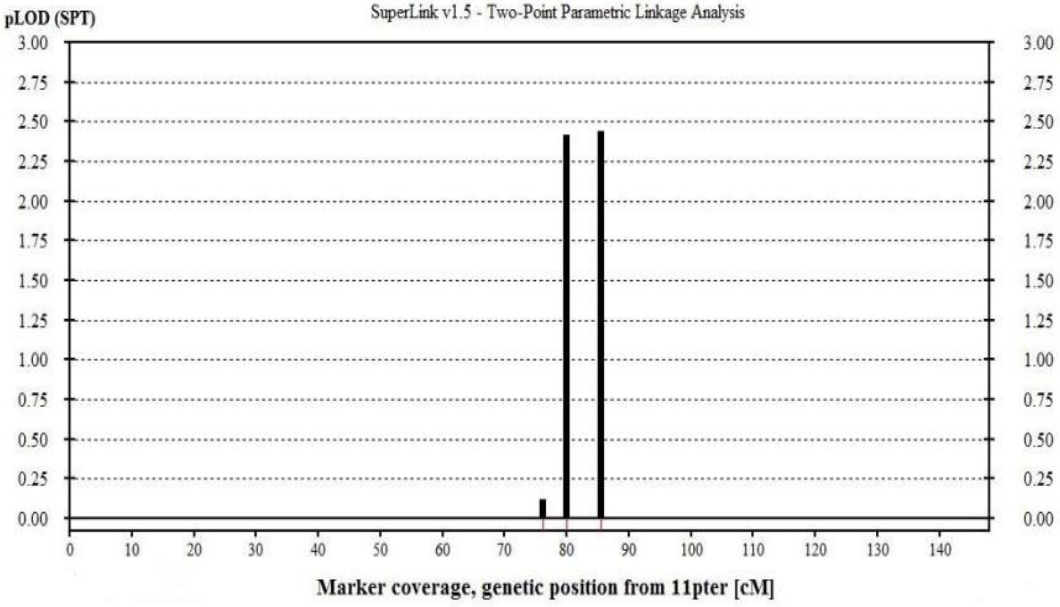
Project:	FAMILY02	Inheritance:	Recessive
Family name:	TOTALS	Common allele:	99.90 %
Used map:	Marshfield (sex averaged)	Disease allele:	0.10 %
Marker positions:	6 ok / 0 ? / 0 outside	LC1 PCOPY rate:	0.00 %
Allele frequencies:	All individuals (marker file)	LC1 PENET wt/mt:	0.00 %
CALC interval:	Entire chromosome	LC1 PENET mt/mt:	100.00 %

Marker	CHR	cM	LOD	Theta
1.D11S4207	11	76.13	2.5622	0.0000
2.D11S4079	11	79.98	1.4628	0.0000
3.D11S1789	11	79.98	1.4627	0.0000
4.D11S4186	11	79.98	1.0267	0.0000



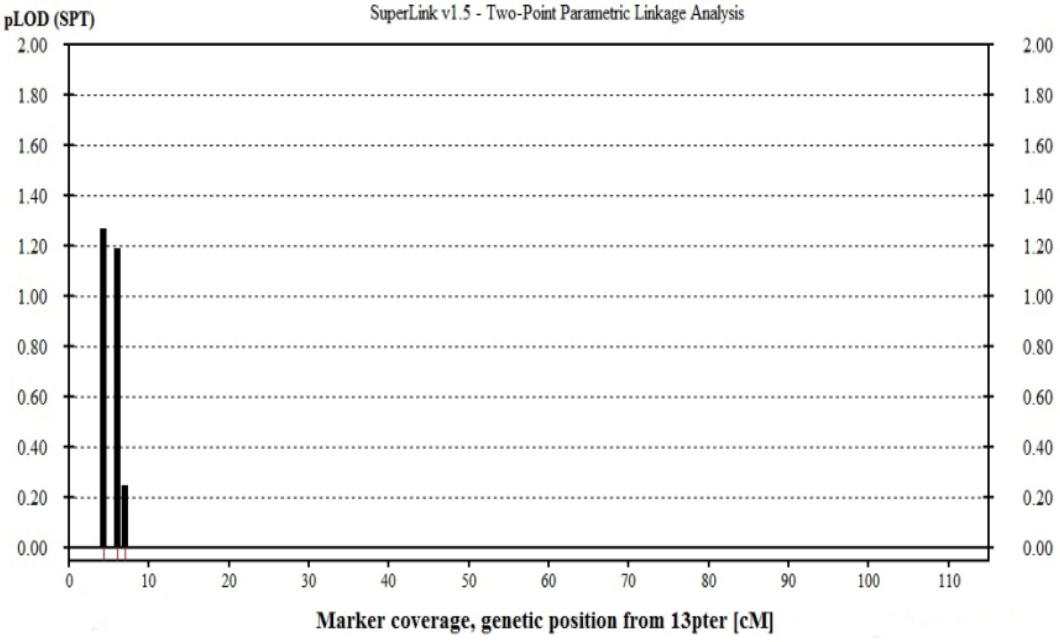
C.

				Marker	CHR	cM	LOD	Theta
Project:	FAMILY03	Inheritance:	Recessive					
Family name:	TOTALS	Common allele:	99.90 %	1.D11S901	11	85.48	2.4434	0.0000
Used map:	Marshfield (sex averaged)	Disease allele:	0.10 %	2.D11S4186	11	79.98	2.4121	0.0000
Marker positions:	5 ok / 0 ? / 0 outside	LC1 PCOPY rate:	0.00 %	3.D11S4079	11	79.98	2.4121	0.0000
Allele frequencies:	All individuals (marker file)	LC1 PENET wt/mt:	0.00 %	4.D11S4207	11	76.13	0.1197	0.3000
CALC interval:	Entire chromosome	LC1 PENET mt/mt:	100.00 %	5.D11S1789	11	79.98	0.0000	0.5000



D.

				Marker	CHR	cM	LOD	Theta
Project:	FAMILY04	Inheritance:	Recessive					
Family name:	TOTALS	Common allele:	99.90 %	1.GJB2	13	4.24	1.2708	0.0000
Used map:	Marshfield (sex averaged)	Disease allele:	0.10 %	2.D13S175	13	6.03	1.1918	0.0000
Marker positions:	3 ok / 0 ? / 0 outside	LC1 PCOPY rate:	0.00 %	3.D13S1275	13	6.99	0.2498	0.1500
Allele frequencies:	All individuals (marker file)	LC1 PENET wt/mt:	0.00 %					
CALC interval:	Entire chromosome	LC1 PENET mt/mt:	100.00 %					



E.

Project:	FAMILY05	**Inheritance:**	Recessive	**Marker**	**CHR**	**cM**	**LOD**	**Theta**
Family name:	TOTALS	**Common allele:**	99.90 %	1.D13S1275	13	6.99	3.0633	0.0000
Used map:	Marshfield (sex averaged)	**Disease allele:**	0.10 %	2.D13S175	13	6.03	2.3206	0.0000
Marker positions:	3 ok / 0 ? / 0 outside	**LC1 PCOPY rate:**	0.00 %	3.GJB2	13	4.24	1.9635	0.0000
Allele frequencies:	All individuals (marker file)	**LC1 PENET wt/mt:**	0.00 %					
CALC interval:	Entire chromosome	**LC1 PENET mt/mt:**	100.00 %					

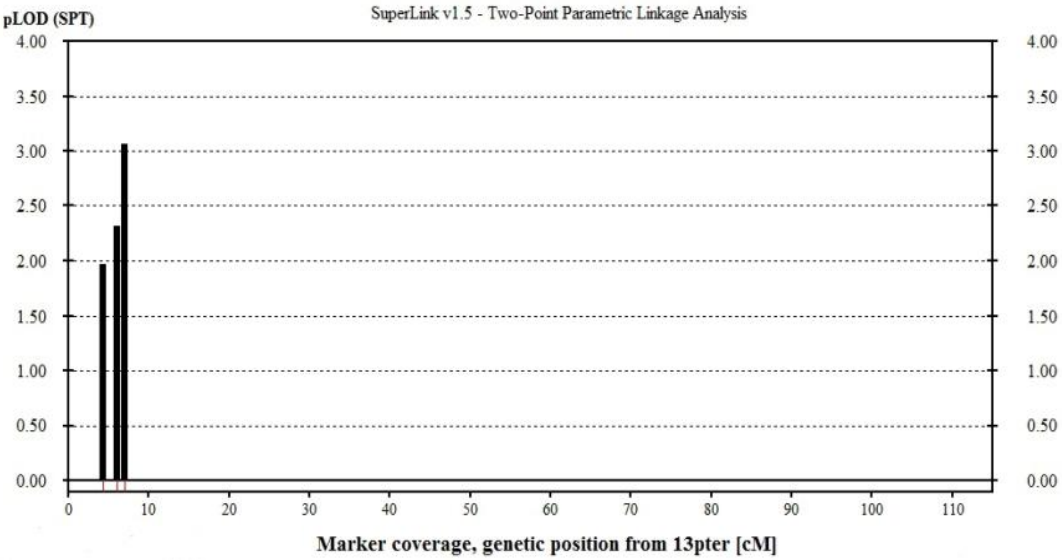


Fig. 3: Two-point Lod score of family 01 (A), family 02 (B), family 03 (C), family 04 (D), family 05 (E) showing linkage with locus DFNB1 (*GJB2*) and DFNB2 (*MYO7A*) STR markers.

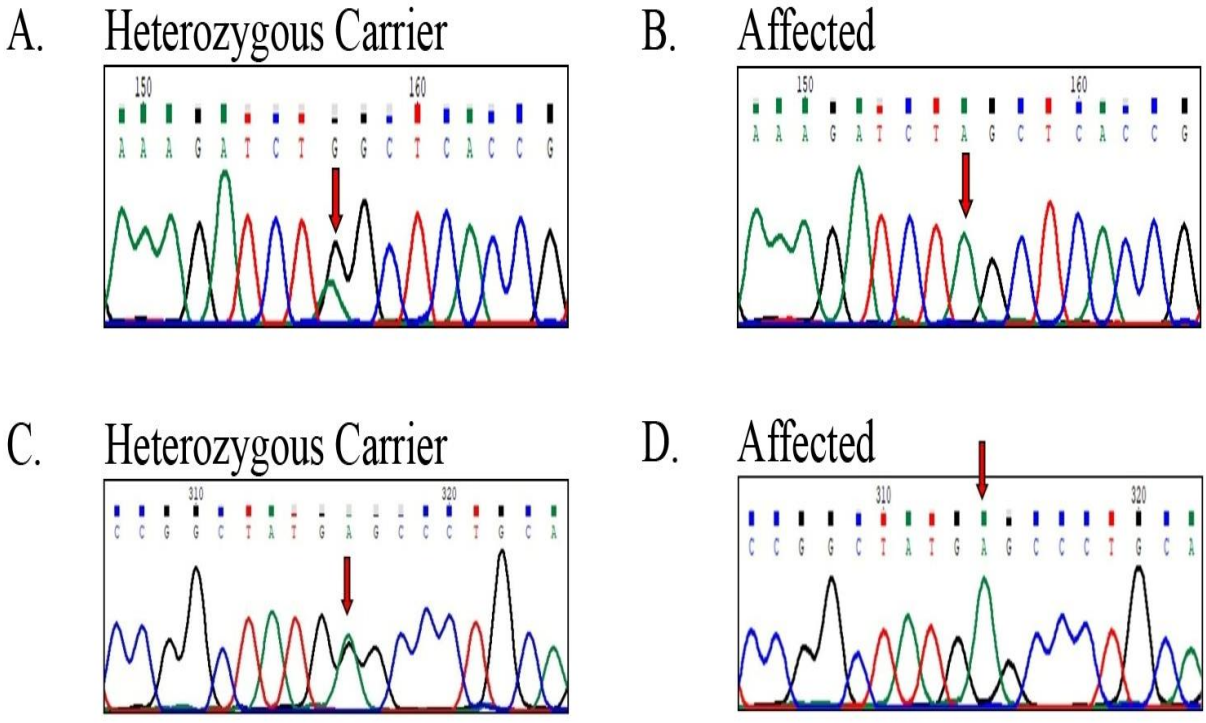


Fig. 4: Chromatograms (A & B) of the mutation c. 71G>A, (p.W24X) and (C & D) of the mutation c. 231G>A, (p.W77X). The position of mutations is indicated by arrow in chromatogram (A), (B), (C) and (D).

Conclusion

The findings of this study proposed that consanguineous Pakistani families exhibit mutations in *GJB2* and *MYO7A* genes irrespective of the ethnicities. However, this is the first study to report founder effect of *GJB2* gene in a Kashmiri family. Kashmiri population has not been targeted previously to study the genetic contributors of hearing impairment. Therefore, this is the paramount research suggesting that Kashmiri population share the same genetic makeup with Pakistani population. By designing and practicing similar studies on different ethnicities and different populations inhabiting different provinces of Pakistan and Azad Kashmir will provide population specific knowledge for clinical diagnosis and genetic counseling of the families. By providing carrier status to these families we can play a vital role to lower the disease burden of hearing impairment in Pakistan and Azad Kashmir.

Abbreviations

GERP: Genomic evolutionary rate profiling
GJB2: Gap junction beta 2
HI: Hearing impairment
LOD: Logarithm of the odds
LRT: Likelihood ratio test
MYO7A: Myosin 7A
SNP: Single nucleotide polymorphism
STR: Short tendem repeats
USH: Usher

Declarations

Ethics approval and consent to participates

Ethical approval was obtained from the institutional review board, National Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan. After explanation of the objectives of this project, written informed consent was obtained from all participating individuals.

Consent for publication

Written informed consent to publish findings of this study was acquired from each participant.

Availability of data and material

All the data generated or analyzed during this study have been included in this manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by the indigenous fellowship of Higher Education Commission (HEC), Islamabad Pakistan. The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We are highly thankful to Higher Education Commission (HEC) of Pakistan for funding this work and Centre of excellence in Molecular Biology for providing research facilities. The authors are also thankful to all the participating families for their cooperation in this study.

Databases

http://deafnessvariationdatabase.org/
http://deafnessvariationdatabase.org/gene_page/MYO7A
http://www.mutationtaster.org/
(https://www.itl.nist.gov)
SidowLab/downloads/gerp/index.html

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