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# Evaluation of a recurrent mutation in *HGF* gene responsible for non-syndromic hereditary deafness in Kashmiri population

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## Abstract

Background: Mutations in gene coding for hepatocyte growth factor protein, HGF are responsible for hereditary deafness worldwide. Evaluation of recurrent variations displays prevalent heredity diversity of a specific population. Mutational screening of *HGF* was aimed to ascertain the causative recurrent variations in Kashmiri families.

Methods: Kashmiri families were enrolled from different divisions of Azad Jammu and Kashmir. By employing linkage analysis all the families were screened for loci common in Pakistani population. Families linked with DFNB39 locus were subjected to direct sequencing for mutational analysis of variants prevalent in Pakistani population.

Results: Sanger sequencing identified a noncoding c.482+1986_1988delTGA variant of *HGF* as recurrent mutation in Kashmiri population. These findings implicate this *HGF* variant as major contributing variant of hearing impairment in Kashmiri families with a frequency of 8.8%.

Conclusion: This is the first study conducted to elucidate the founder effect and prevalence of *HGF* variants in Kashmiri population. This study increases the prevalence of *HGF* variants associated with hearing impairment in the Kashmiri families.

## Introduction

Hearing impairment (HI) is a heterogeneous infirmity worldwide. In Pakistan incidence of bilateral HI is 1.6 per 1000 individuals [1-3]. The Kashmiri population of Pakistan is an invaluable resource to study the recessive mode of genetic disorders like HI due to high incidence rates of consanguineous marriages or mating within the same ethnic groups [4-6]. The hearing loss related gene mutation patterns vary significantly among different races and regions. In Kashmiri population almost 70% of the HI appears due to consanguineous reunions, consequently as compared to other populations of the world, the number of hearing impaired families in this population is much higher [7].

Deafness is caused by hundreds of genes that when get mutated cause pathogenicity [8-12]. Of the 93 non-syndromic hearing deafness (NSHD) loci that recorded so far, the pathogenic variations have been known for 75 genes (Hereditary Hearing Loss Homepage: https://hereditaryhearingloss.org/). This varies from cochlear specialized genes performing inner ear specific functions to housekeeping genes with ubiquitous expression [13,14]. For many monogenic disorders extensive genetic analysis studies have presented variations in noncoding part of the genes. One such gene, hepatocyte growth factor gene *HGF* has been reported to cause non syndromic deafness in large cohort of hearing impaired families due to variations present in noncoding region of the gene [15].

Human hereditary deafness (DFNB39) is concomitant with noncoding variations in the 3'UTR and intron of an isoform of *HGF*. The growth factors like hepatocyte growth factors (HGF) are essential for the development of auditory system and for the maintenance of hearing process in humans. In many different tissues this growth factor is involved to perform intricate signal transduction mechanism. Mouse model studies revealed abnormal cochlear tissue growth in inner ear in the absence of HGF. HGF is essential for integration of sensory crest cells into the central stria vascularis layer during the development of inner ear of mice. The stria retains an endocochlear potential (+80 to +120 mV) and high potassium concentration necessary for mechanotransduction of sound by inner hair cells of ear [16]. Using a *HGF* mutant mouse in a study [15] with a ten base pair deletion recapitulating a human *DFNB39* noncoding variant. The findings of the study demonstrated failure of migration of neural crest cells to stria vascularis middle layer causing significant reduced endocochlear potential in inner ear hair cells.

Regulatory putative *HGF* variants have been reported to cause surprisingly a specific phenotype, non-syndromic deafness. HGF levels must be fine-tuned for normal hearing as an over or less *HGF* expression results in loss of hearing. In this study we identified one regulatory variant (c.482+1986_1988delTGA) in the non-coding region (intron 4) of the *HGF* as recurrent mutation in four Kashmiri families of Pakistan.

## Methods

An approval from Institutional Review Board was obtained for this study from National Centre of Excellence in Molecular Biology, University of the Punjab Lahore Pakistan. A total of 45 families with three or more affected individuals were visited and collected from different divisions of Azad Jammu and Kashmir region of Pakistan. Pedigrees were constructed for the confirmation of inheritance pattern and initial medical information. Detailed medical histories were recorded to rule out environmental factors and any associated syndromes.10 ml of blood sample was taken from all the participants of the study. Blood samples were preserved at -20 ºC for long term storage before DNA extraction.

### Extraction and Quantification of Genomic DNA

For the extraction of DNA standard protocol of DNA extraction was performed [17]. The integrity and quantification of DNA was estimated by using Agarose gel electrophoresis.

### Screening of Common Deafness Loci

Linkage analysis was performed initially for all the families to screen common prevalent deafness loci (DFNB1, DFNB2, DFNB3, DFNB4, DFNB12 and DFNB39) by using STR markers (fluorescently labeled). The pooled PCR products were evaluated on 3130 DNA genetic analyzer. Gene mapper software was used for allele calling. Cyrillic software was used for constructing pedigrees and Haplotypes. LOD (logarithm of odds) score was calculated by FASTLINK [18].

### Mutational Screening

PCR (polymerase chain reaction) amplification following Sanger sequencing was done for one primer pair of *HGF* with most common non coding mutation in intron 4 (Table 1). The sequencing results were analyzed with Sequencher® 5.4.6 software (Gene Codes Corporation).

| Primer Name | Primer Sequence | Annealing Temperature (°C) | Product Size (bp) |
|---|---|---|---|
| Intron 4 HGF (F) | 5'-GGCCGAGAGGATCCAGTATATTA-3' | 60 | 496 |
| Intron 4 HGF (R) | 5'-GGCAAGGCTTTAAGAGAGACAAG-3' | | |

**Table 1:** Primer Sequences for Amplification of *HGF* Intron 4.

## Results

In the present study four consanguineous Kashmiri kindred, Family1563, Family1577, Family1587 and Family1589 with hearing impairment, were linked to DFNB39 locus with recurrent noncoding mutation in intron 4 of *HGF*. The families belonged to same ethnic (Kashmiri) background and caste (Jutt). Autosomal recessive inheritance pattern was found in all the four families (Figure 1). No affected individual from four families had complaint of night blindness or any other clinical manifestation. The haplotype of all hearing impaired individuals of families showed linkage to DFNB39 locus at chromosome 7q21.11. In Family1563 a maximum two point LOD score ($Z_{max}$) of **2.10** at θ= 0 was observed with marker D7S660 and D7S2540. Linkage was sustained to this region at distal (D7S660) and proximal side (D7S2540) for other three families with maximum two point LOD score ($Z_{max}$) of 2.19 (with

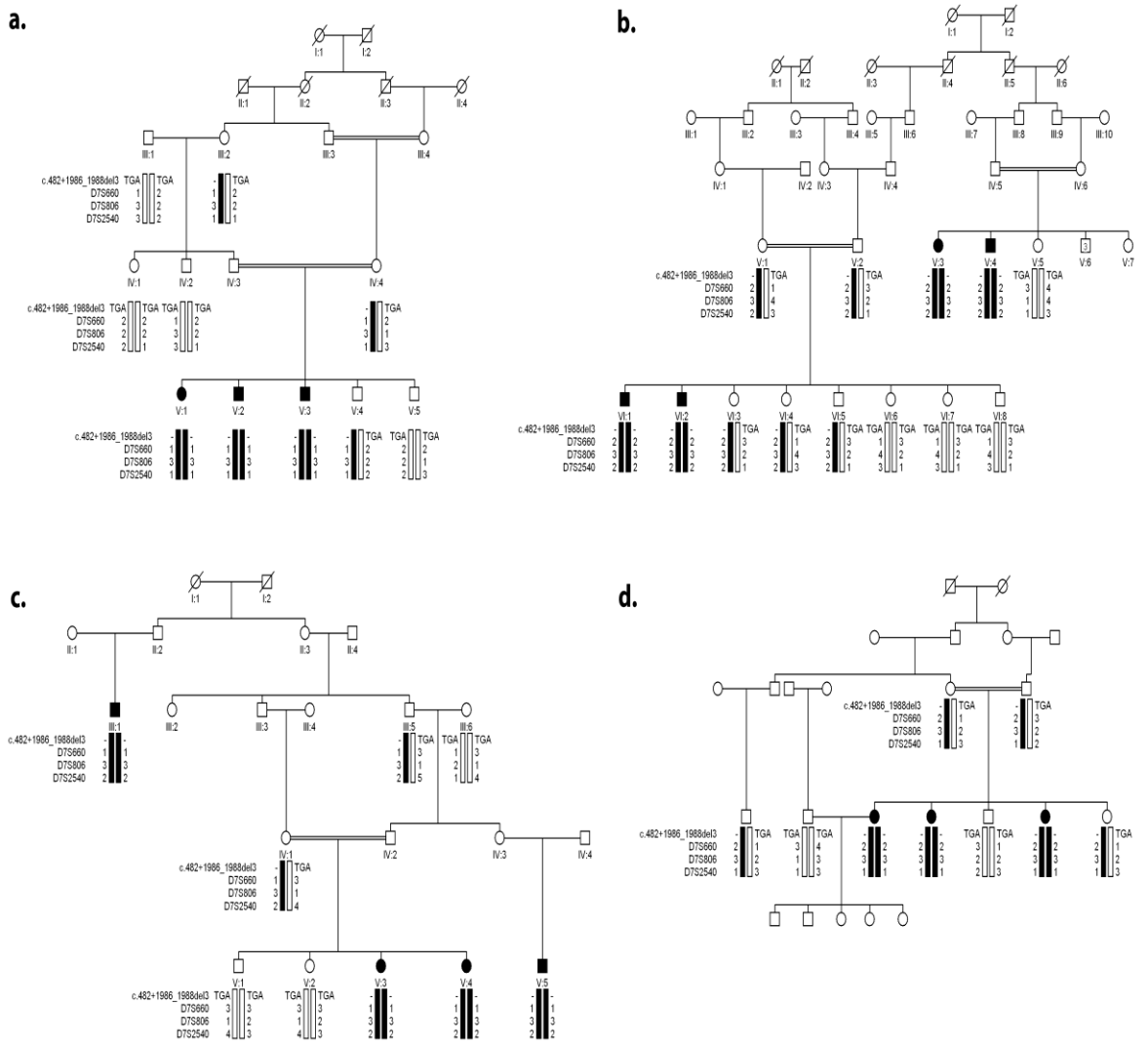


**Figure 1:** Pedigrees of Family1563 **(a)**, Family1577 **(b)**, Family1587 **(c)** and Family1589 **(d)** with the haplotypes of alleles on chromosome 7q21.11 and segregation of c.482+1986_1988delTGA *HGF* variant. Alleles forming the risk haplotype are shaded black and alleles not co-segregating with HL are shown in white. Square: male; circle: female; filled symbol: affected individual; the double line between individuals: consanguineous marriage; diagonal line through a symbol: deceased individual.

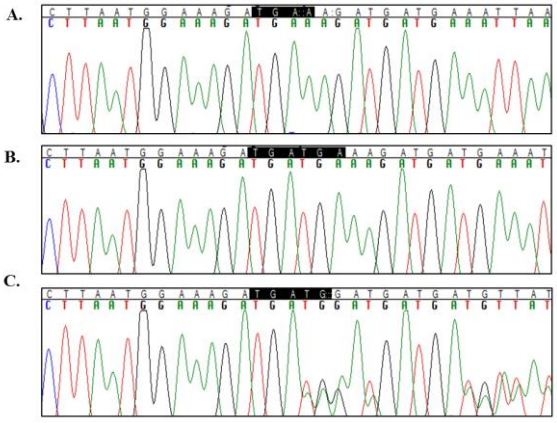


**Figure 2:** Sequence chromatograms of mutations identified in patients with HL. (A) Chromatogram of affected individuals homozygous for c.482+1986_1988delTGA mutation in *HGF*; (B) unaffected individual homozygous for wild type allele. (C) Heterozygous carriers for c.482+1986_1988delTGA in *HGF*.

| Family | Markers | cM | Mb | 0 | 0.01 | 0.03 | 0.05 | 0.07 | 0.09 | 0.1 | 0.2 | 0.3 | $Z_{max}$ | $\theta_{max}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Family1563 | D7S660 | 93.63 | 80.59 | 2.10 | 1.55 | 1.94 | 1.83 | 1.72 | 1.61 | 1.55 | 1.00 | 0.49 | 2.10 | 0 |
| | D7S806 | 94.87 | 81.36 | 2.18 | 1.68 | 2.03 | 1.95 | 1.83 | 1.73 | 1.68 | 1.16 | 0.65 | 2.18 | 0 |
| | D7S2540 | 97.38 | 83.11 | 2.10 | 1.59 | 1.95 | 1.85 | 1.74 | 1.64 | 1.59 | 1.06 | 0.55 | 2.10 | 0 |
| Family1577 | D7S660 | 93.63 | 80.59 | 2.19 | 2.14 | 2.06 | 1.97 | 89 | 1.80 | 1.75 | 1.27 | 0.78 | 2.19 | 0 |
| | D7S806 | 94.87 | 81.36 | 2.19 | 2.14 | 2.06 | 1.97 | 89 | 1.80 | 1.75 | 1.27 | 0.78 | 2.19 | 0 |
| | D7S2540 | 97.38 | 83.11 | 2.19 | 2.14 | 2.06 | 1.97 | 89 | 1.80 | 1.75 | 1.27 | 0.78 | 2.19 | 0 |
| Family1587 | D7S660 | 93.63 | 80.59 | 2.06 | 2.00 | 1.90 | 1.80 | 1.70 | 1.59 | 1.54 | 1.02 | 0.53 | 2.06 | 0 |
| | D7S806 | 94.87 | 81.36 | 2.83 | 2.78 | 2.67 | 2.56 | 2.44 | 2.33 | 2.27 | 1.66 | 1.04 | 2.83 | 0 |
| | D7S2540 | 97.38 | 83.11 | 2.83 | 2.78 | 2.66 | 2.54 | 2.42 | 2.29 | 2.23 | 1.60 | 0.97 | 2.83 | 0 |
| Family1589 | D7S660 | 93.63 | 80.59 | 2.68 | 2.63 | 2.53 | 2.42 | 2.32 | 2.21 | 2.16 | 1.61 | 1.04 | 2.68 | 0 |
| | D7S806 | 94.87 | 81.36 | 2.49 | 2.44 | 2.34 | 2.24 | 2.13 | 2.03 | 1.97 | 1.42 | 0.85 | 2.49 | 0 |
| | D7S2540 | 97.38 | 83.11 | 2.68 | 2.63 | 2.53 | 2.42 | 2.32 | 2.21 | 2.16 | 1.61 | 1.04 | 2.68 | 0 |

**Table 2:** Two-point LOD scores localized to chromosome 7q21.11.

| Family Designation | Ethnicity | Cast | STR haplotypes linked to DFNB39 | | | Variant | *In silico tools* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | D7S660 | D7S806 | D7S2540 | | *Mutation Taster* | *EVS Frequency* | *EXAC* | *TGP* | *LRT* | *GERP Score* |
| Family1563 | Kashmiri | Jutt | 184 | 132 | 190 | Δ3 | Splice site changes/Disease causing | Absent | Absent | Absent | Absent | Absent |
| Family1577 | Kashmiri | Jutt | 184 | 132 | 190 | Δ3 | | | | | | |
| Family1587 | Kashmiri | Jutt | 184 | 132 | 190 | Δ3 | | | | | | |
| Family1589 | Kashmiri | Jutt | 184 | 132 | 190 | Δ3 | | | | | | |

Δ3=c.482+1986_1988delTGA

**Table 3:** STR Haplotype Linked To DFNB39 And *In Silico* Validation.

| Family | rs1421134578 | rs904221567 | rs1371361043 | rs1208755637 | B39 | rs1296475387 | rs1030487619 | rs1464051447 | rs963677172 |
|---|---|---|---|---|---|---|---|---|---|
| Family1577 | GG | CC | TT | GG | Δ3 | GG | TT | TT | CC |
| Family1587 | GG | CC | TT | CC | Δ3 | GG | TT | TT | CC |
| Family1589 | GG | CC | TT | CC | Δ3 | GG | TT | TT | CC |
| Family1563 | GG | CC | TT | CC | Δ3 | GG | TT | TT | CC |

SNP Single nucleotide polymorphism

Δ3=c.482+1986_1988delTGA

**Table 4:** SNP Haplotypes of Affected Individuals Harboring Non Coding Splice Site C.482+1986_1988deltga Mutation in *HGF.*

markers D7S660, D7S806 and D7S2540), 2.83 (with markers D7S806 and D7S2540) and 2.68 (with markers D7S660 and D7S2540) at θ= 0 for Family 1577, Family 1587 and Family 1589 respectively (Table 2). Zmax was obtained with D7S2540 in all four families. The assembled haplotype also displayed same genotype pattern i.e., 184, 132 and 190 (Table 3) for all the four families revealing founder effect.

Bidirectional Sanger sequencing revealed that all the four families harbored common recurrent c.482+1986_1988delTGA mutation in intron 4 of *HGF*. All the affected individuals showed homozygosity for the identified mutation. Intronic noncoding three base pair (TGA) deletion was present in splice site of *HGF* gene as a result of which protein features might be affected. Consequently, the genetic expression of c.482+1986_1988delTGA relates to the phenotypic manifestation of hereditary deafness in all four families.

The damaging effects of this noncoding mutation were verified by using different *in silico tools* i.e., *Mutation Taster*, *ExAC* (exome aggregation consortium), *LRT* (likelihood ratio test), *EVS* (exome variant server), *TGP* (1000 genome project) and *GERP* (genomic evolutionary rate profiling) score (Table 3). The variant was classified as disease causing according to Mutation Taster. The occurrence of same causative pathogenic mutation (c.482+1986_1988delTGA) in all ethnically matched families inhabiting Kashmir region pave the

mode to probe the founder effect of pathogenic noncoding mutation among these four Kashmiri families belonging to Jutt caste. All normal wild type bases flanking the detected mutation were assembled where established haplotype around site of mutation revealed the common founder effect of c.482+1986_1988delTGA mutation (Table 4).

## Discussion

In current study noncoding c.482+1986_1988delTGA *HGF* variant is reported in four consanguineous Kashmiri families with NSHD (non-syndromic hereditary deafness). The families were linked to DFNB39 locus at chromosome 7q21.11. Linkage was persistent to this region at distal (D7S660) and proximal side (D7S2540) with Zmax obtained with D7S2540 in all four families. The findings were consistent with the work of [15] ascertaining three base pair deletion in intron 4 (c.482+1986_1988delTGA) in thirty six Pakistani and two Indian families suffering from hearing loss. For this deletion mutation heterozygosity was detected in 2 out of 429 control samples of Pakistani population. While none of control sample out of 830 Indian, Caucasian, and human diversity panel control chromosomes revealed heterozygous genetic status for the same mutation. This noncoding deletion mutation vested in a region of putative splice enhancer sites (exonic) contiguous to an anticipated binding site of splice factor. The findings of present and previous study propose that the deletion might result in dysregulation of *HGF* isoforms [19].

All four families with same ethnicity and caste linked to DFNB39 locus presented same alleles haplotype strongly predicted this variant sharing common ancestral inheritance. Inconstant incidence of recurring variations in genes has been studied among ethnically diverse groups [20]. Three mutations (non-coding) in *HGF* have been reported in Pakistani and Indian population resulting in non-syndromic deafness. The two non-coding *HGF* variants in intron 4 were c.482+1986_1988delTGA and c.482+1991_2000delGATGATGAAA. The 3$^{rd}$ was c.495G>A; p.S165S synonymous splice site variant present in exon 5 [15]. In the present study, four out of forty five families suffering from hearing loss had non-coding deletion mutation, c.482+1986_1988delTGA, estimating 8.8% frequency of *HGF* variation in Kashmiri population which is in line with the previous study describing same frequency of *HGF* variation in a study on Pakistani families [21].

Our study encapsulates the genetic mutational spectrum concluding founder effect of detected *HGF* non-coding variation among hearing impaired families from Azad Jammu and Kashmir region of Pakistan. Increasing the screening of such variants will lessen disease burden and will further sweep the way of diagnostic therapeutics. This study outlines the mutational spectrum of *HGF* in Kashmiri population first time.

**Abbreviations**

HI: Hearing impairment
NSHD: Non syndromic hereditary deafness
HGF: Hepatocyte growth factor
ExAC: Exome aggregation consortium
LRT: Likelihood ratio test
EVS: Exome variant server
TGP: 1000 genome project
GERP: Genomic evolutionary rate profiling

**Ethics approval and consent to participate**

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.

**Competing interests**

The authors declare that they have no conflict of interest.

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## Authors' Contributions

KZ: study concept and design; identification and enrolment of families, implementation of experiments; data acquisition; analysis and interpretation of data; drafting of the manuscript. HT: analysis and interpretation of data; revising the manuscript critically for important intellectual content. SAM performed some lab tasks: AAK: supervised the work. All authors read and approved the final manuscript.

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## Competing Interest

The authors declare that they have no competing interests.

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