



## Analysis of *SLC26A4* Gene in Individuals with Non Syndromic Hearing Impairment in Relation with *GJB2* Associated Mutations

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### Abstract

**Background:** Hearing Loss (HL) is the most common sensory disorder. HL commonly ranges from mild to severe. Persons with HL face difficulty in hearing conversations or sounds through one ear or both ears, which impacts one's ability to interact with others. Hence it is a communicable disorder that makes people socially isolated, lonely, and frustrated. HL in children severely affects language development. The people who are referred to as 'Deaf' with very little or no hearing capabilities, are considered as having profound hearing loss. More than 124 genes are causative for Non-Syndromic HL (NSHL) with varying inheritance, among which the *SLC26A4* mutations are the second commonest cause of hereditary HL across the globe.

**Methods:** Samples from 70 NSHL patients were analyzed through *Next-Generation Sequencing* (NGS) and generated five pathogenic variants [N246fs (rs918684449), K564fs (rs746427774), F122fs, V239D (rs111033256), T721M (rs121908363)] each with frequency of 1.42%. Three missense variants [S399P (rs747431002), L597S (rs55638457), and G6V (rs111033423)] were reported under the "uncertain" category. All the collected samples were further genotyped to look for the possibility of having *GJB2* and HL-associated mutations.

**Results:** Out of five *SLC26A4* pathogenic mutations N246fs (rs918684449) and K564fs (rs746427774) were observed in samples which were positive for *GJB2*-HL associated candidate mutations [W24X (rs104894396), Q124X (rs397516874) and W77X (rs80338944)]. Similarly, pathogenic variants F122fs, V239D (rs111033256) and T721M (rs121908363) were observed in patient samples which were negative for *GJB2*-HL associated mutations.

**Conclusion:** Our data will expand the list of variants underlying NSHL and encourage further genotype *SLC26A4* gene concerning the south Indian population with a large sample size.

**Key words:** Child, Genotype, Hearing loss, High-throughput nucleotide sequencing, Humans, Mutation

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### Introduction

Among different sensory disorders, Hearing Loss (HL) is the most common one, and over 5% of the

world's population suffers from one or more forms of hearing impairment <sup>1</sup>. On average, abnormal changes in

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genetic makeup are solely responsible for 50% of hearing impairment<sup>2</sup>. HL commonly ranges from mild to severe. People with HL face difficulty in hearing conversations or sounds through one ear or both ears, which impacts one's ability to interact with others. Hence it is a communicable disorder that makes people socially isolated, lonely, and frustrated. HL in children severely affects language development. People who are referred to as 'Deaf' with very little or no hearing capabilities, are considered as having profound hearing loss. More than 124 genes are found to be causative for Non-Syndromic Hearing Loss (NSHL), with varying inheritance such as 51 genes for DFNA or autosomal dominant, 78 genes for DFNB or autosomal recessive, and 5 genes for DFNX or X-linked inheritance<sup>3</sup>. In addition, some mutations within mitochondrial DNA are associated with HL<sup>4</sup>. Interestingly, mutations in these genes do not occur at the same frequency across different populations or ethnic groups.

The *SLC26A4* gene or Solute carrier family 26, member 4 encodes pendrin, a highly inner ear localized anion transporter<sup>5</sup>. *SLC26A4* mutations are the second most common cause of hereditary hearing loss across the globe<sup>6</sup>. *SLC26A4* mutations include non-syndromic recessive deafness and Pendred syndrome which is a combination of sensorineural hearing loss and goiter. LitVar listed 289 *SLC26A4* variants including 50 with HL association<sup>7</sup>. *SLC26A4* genotyping has become an important part of molecular genetic testing for HL. In spite of numerous studies, the association of *SLC26A4* with HL in different populations remains elusive. This is due to the heterogeneity of the studied cohorts and/or methods of analysis, and studies that either target screening of only the most common *SLC26A4* mutations or coding and adjoined regions or the entire *SLC26A4* gene<sup>8</sup>.

Studies have shown variations in frequency distribution of *SLC26A4* linked to HL among different populations across India. Five *SLC26A4* mutations (G6V, I363V, S399P, I455F, and K715) were reported from the West Bengal population, and variant Y556X was detected in the Jammu and Kashmir geographic location<sup>9</sup>. Variants S90L, V239D, V359E, G389fs, T410M, N457K, and p.K715N were reported from South India<sup>10</sup>. The variant K715N was reported in both Jammu and Kashmir<sup>9</sup> and in the South Indian population<sup>10</sup>. However, given its high heterogeneity, the number of new impairment causing gene variants for NSHL continues to grow.

The recent technological advances in target enrichment methods and Next Generation Sequencing (NGS) have overcome the barriers possessed by the earlier methods such as Sanger sequencing and laid the path for comprehensive analysis of all the known genes causing NSHL. Recently, Lin *et al* demonstrated the use of NGS in genetic testing of hearing impaired patients with non-confirmative *GJB2* genotypes on conventional genetic examinations<sup>11</sup>. In view of this, we

have performed whole *SLC26A4* gene sequencing by NGS among the South Indian patients with NSHL. Further, we investigated *GJB2*-associated mutations in individuals who are positive for *SLC26A4* pathogenic mutations.

## Materials and Methods

### Subjects, sample collection and NGS analysis

This study was carried out at the Unit for Human Genetics at the All India Institute of Speech and Hearing. Institutional ethical clearance was obtained prior to commencing the study (Ethical clearance number SH/CDN/ARF-Aud-2/2017-18). Seventy unrelated individuals (n=40 males, n=30 females) visiting our institute for hearing impairment were enrolled for the study. Parents of NSHL children were interviewed regarding the pre, peri and postnatal history, family history and other related medical records and explained the purpose of the study. Subjects with bilateral, severe to profound hearing loss with no birth trauma, neonatal infections, physical damage or any other known etiology that can cause HL were included in the study.

After receiving the informed consent, an approved and structured questionnaire was used to obtain the general demographic information such as age, severity, type with the education and rehabilitation history. To determine the degree of HL, audio logical tests including Behavioral Observation Audiometry (BOA), Pure Tone Audiometry (PTA), Speech Audiometry (SA), Tympanometry, Acoustic Reflex Threshold (ART), Distortion Product Otoacoustic Emissions (DPOAEs) and Transitory Evoked Otoacoustic Emission (TEOAEs) were performed. All audio logical assessment tests were performed in sound treated rooms as per the standards of ANSI S3.1 (1999).

Their blood samples were collected between January 2018 to March 2019 and screened for variants in the *SLC26A4* gene through NGS technology. The current study employed MiSeq, Illumina's integrated NGS instrument for data acquisition. The \*.bcl files generated through the machine were uploaded to BaseSpace platform (<https://basespace.illumina.com>) which converts it into FastQ files. It is with the help of germline Dragon pipeline, a part of BaseSpace, suite that converts it into VCF files. Pathogenic and other significant variants reported in the VCF files under ClinVar column were selected further (Figure 1).

## Results

### *SLC26A4* genotyping

Variant analysis of *SLC26A4* gene among study population was carried out. The MiSeq Illumina platform generated 254 variants which included 15 missense variants, 5 synonymous variants, 9 frame shift mutations, 2 in-frame insertions and 222 variants pertaining to intronic and other regions (Supplementary data Table S1). Most significant variants with dbSNP numbers and ClinVar accession numbers were listed in

Table 1. Variants observed in *SLC26A4* gene among the South Indian NSHL patients

Mutation	Type	Zygosity	dbSNP	clinvar	Category	Allele Frequency (n) in percentage
N246fs	Frameshift	Heterozygous	rs918684449	RCV000806485.1	Pathogenic	1.42
K564fs	Frameshift	Heterozygous	rs746427774	RCV000795419.1	Pathogenic	1.42
F122fs	Frameshift	Homozygous	-	VCV001069540.1	Pathogenic	1.42
V239D	Missense	Homozygous	rs111033256	RCV000724118.2	Pathogenic	1.42
T721M	Missense	Heterozygous	rs121908363	VCV000004826.10	Pathogenic	1.42
S399P	Missense	Heterozygous	rs747431002	RCV000669625.1	Uncertain	1.42
I455F	Missense	Heterozygous	rs375576481	RCV000710343.3	Benign	4.28
L597S	Missense	Heterozygous	rs55638457	VCV000043525.10	Uncertain	1.42
G6V	Missense	Heterozygous	rs111033423	RCV000169379.1	Likely benign	1.42
1544+9C>T	Intron	Heterozygous	rs368970459	RCV000835659.1	Likely benign	1.42
1708-18T>A	Intron	Heterozygous	rs55701254	RCV000244473.1	Benign	1.42
1438-7delT	Intron	Heterozygous	rs753586849	RCV000668233.1	Uncertain	1.42
*868G>A	3' UTR	-	rs2712218	VCV000358515.2	Benign	81.42

UTR=Untranslated Region.

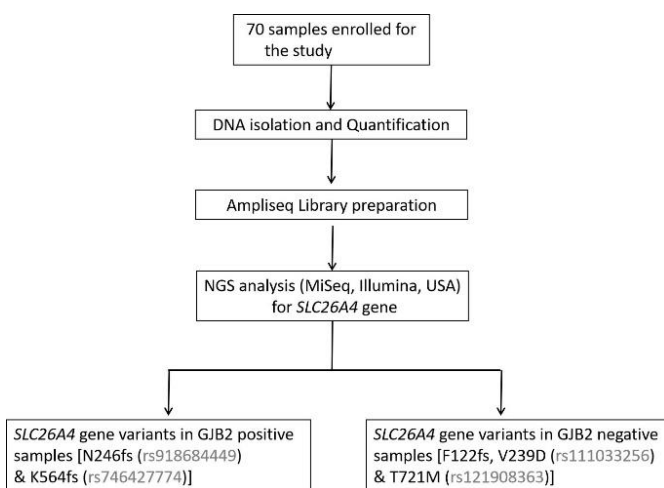


Figure 1. The overall workflow and detailing of different stages and processes used in the current investigation.

table 1. Interestingly, variants N246fs (rs918684449), K564fs (rs746427774), F122fs, V239D (rs111033256), and T721M (rs121908363) were the pathogenic mutations observed in the present study each with frequency of 1.42%. Three missense variants [S399P (rs-747431002), L597S (rs55638457) and G6V (rs-111033423)] were reported under "uncertain" category. One benign variant I455F (rs375576481) was observed with frequency 4.28%. Three intronic variants (rs-368970459, rs55701254 and rs753586849) with frequency of 1.42%, and one 3' UTR variant rs2712218 with frequency of 81.42% was also evident by the current study.

#### Association of *GJB2* variants

All the collected samples were further genotyped to look for the possibility of having *GJB2* and HL associ-

ated mutations. Out of five *SLC26A4* pathogenic mutations, N246fs (rs918684449) and K564fs (rs-746427774) were observed in samples which were positive for *GJB2* candidate mutations [W24X (rs-104894396), Q124X (rs397516874) and W77X (rs-80338944)]. Similarly, pathogenic variants F122fs, V239D (rs111033256) and T721M (rs121908363) were observed in samples which were negative for *GJB2* associated mutations [(W24X (rs104894396), Q124X (rs397516874) and W77X (rs80338944)].

#### Discussion

At present, there are more than 124 genes related to deafness, many of which are involved in inner ear function, with mutations affecting the physiology and structure of the inner ear. The most commonly identified genes are *GJB2*, *GJB6*, *SLC26A4*, *OTOF*, *MYO15A*, *MYO7A*, *POU3F4*, *TMC1*, *CDH23* and mitochondrial 12S rRNA<sup>12</sup>. *SLC26A4* mutations are the second commonest cause of hereditary HL across the globe<sup>6</sup>. It is a major gene identified to be causative for deafness among Chinese population<sup>13</sup>. *SLC26A4* gene found to be located at 7q31, and contains 21 exons and encodes 'pendrin' with a 780 amino acid residues acting as a polytopic transmembrane protein<sup>5</sup>. Pendrin is essential for endolymphatic fluid resorption in the inner ear<sup>14</sup>. Mutations in *SLC26A4* may lead to an imbalance of ion circulation in internal lymphatic fluid, resulting in deafness. The variations in *GJB2* and *SLC26A4* in infants with HL among Chinese population with positive genotypes, account for 77.6% (52 of 67) of cases<sup>15</sup>. As per the Indian scenario, *SLC26A4* associated variants were also reported from West Bengal, Jammu and Kashmir<sup>9</sup> and South Indian region<sup>10</sup>. Despite numerous studies, the association between

*SLC26A4* and HL remains elusive in different populations due to heterogeneity of cohorts and/or methods of analysis. The screening method either targets only the most prevalent mutations of *SLC26A4* or coding and adjacent regions<sup>8</sup>. Our variant analysis of the *SLC26A4* gene using NGS revealed five pathogenic variants [N246fs (rs918684449), K564fs (rs746427774), F122fs, V239D (rs111033256), and T721M (rs121908363)]. Among the five variants V239D mutation was previously reported in South Indian cohort<sup>10</sup>. Due to the high prevalence of *GJB2* and *SLC26A4* mutations and the rich literature on this topic, variants in these two genes are studied. To establish an association between *SLC26A4* and *GJB2*, all the collected samples were further genotyped to look for the possibility of having *GJB2* and HL-associated mutations. Out of five *SLC26A4* pathogenic mutations, N246fs (rs918684449) and K564fs (rs746427774) were observed in samples that are positive for *GJB2* candidate mutations [W24X (rs104894396), Q124X (rs397516874), and W77X (rs80338944)]. Similarly, pathogenic variants F122fs, V239D (rs111033256), and T721M (rs121908363) were observed in samples that were negative for *GJB2* [(W-24X (rs104894396), Q124X (rs397516874), and W77X (rs80338944)] associated mutations. A genetic counsellor or risk assessor could benefit from these findings if they work with families living in this region of India.

### Conclusion

In conclusion, we performed NGS analysis of the *SLC26A4* gene among the South Indian cohort in comparison with *GJB2* associated variants. Our data expands the list of variants pertaining to the *SLC26A4* gene underlying NSHL and encourage further genotyping of the *SLC26A4* gene with respect to the South Indian population with a large sample size.

### Statement of Ethics

All the study procedures were adhered to the principles of the Declaration of Helsinki and approved by the Institutional Ethical Committee (SH/CDN/ARF-Aud-2/2017-18).

### Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### Conflict of Interest

The authors have no conflicts of interest to declare.

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