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RESEARCH NOTE

Low incidence of *GIPC3* variants among the prelingual hearing impaired from southern India

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Abstract. The broad spectrum of causal variants in the newly discovered *GIPC3* gene is well reflected in worldwide studies. Except for one missense variant, none of the reported variants had reoccurred, thus reflecting the intragenic heterogeneity. We screened all the six coding exons of *GIPC3* gene in a large cohort of 177 unrelated prelingual hearing impaired after excluding the common *GJB2*, *GJB6* nuclear and A1555G mitochondrial variants. We observed a single homozygous pathogenic frameshift variant c.685dupG (p.A229GfsX10), accounting for a low incidence (0.56%) of *GIPC3* variants in south Indian population. *GIPC3* being a rare gene as a causative for deafness, the allelic spectra perhaps became much more diverse from population to population, thus resulting in a minimal recurrence of the variants in our study, that were reported by authors from other parts of the globe.

Keywords. prelingual deafness; c.685dupG; consanguinity; south India; *GIPC3* gene.

Introduction

Deafness variants are recurrently identified in *GJB2*, *SLC26A4*, *TMC1*, *OTOF* and *CDH23* genes (Hilgert *et al*. 2009; Sloan-Heggen *et al*. 2016; Azaiez *et al*. 2018). Some gene variants are exceedingly rare and do not occur at same frequencies across ethnicities. This work focusses on one such gene, GAIP-interacting protein C terminus-3 (*GIPC3*) located at 19p13.3. Although *GIPC3* was first localized (DFNB95) in an Indian family with recessive inheritance using linkage approach (Charizopoulou *et al*. 2011), it was not well explored later. Most of the studies on *GIPC3* were in Asian populations predominately from Pakistan (table 1). *GIPC3* extends over 8 kbp (six coding exons) encoding 312 amino acids. Involvement of GIPCs in signalling, trafficking and recycling of receptor tyrosine kinases, G-protein coupled receptors, integrins and other transmembrane proteins are very well established. GIPCs are proteins with central PDZ domain and flanking conserved GIPC homology (GH1 and GH2) domains (Katoh 2002).

In the past two decades, tremendous efforts have been made to unravel the rich locus heterogeneity with respect to autosomal recessive nonsyndromic hearing loss (ARNSHL) on chromosome 19. Telomeric region of chromosome 19p includes three closely linked hearing loss loci DFNB72, DFNB81 and DFNB68 (Katoh 2013). Germline variants of human *GIPC3* have been reported in ARNSHL involving DFNB15 (Chen *et al*. 1997), DFNB72 (Rehman *et al*. 2011), and DFNB95 (Charizopoulou *et al*. 2011). Studies suggest that this gene is required for postnatal maturation of the hair bundle and long-term survival of hair cells and spiral ganglion in the ear (Charizopoulou *et al*. 2011) and regulate sensorineural signalling in the cochlea (Katoh 2013). In this study, we have focussed on the frequency of *GIPC3* auditory gene variants and its genetic heterogeneity among the prelingual hearing impaired (HI) from southern India.

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Table 1. Worldwide compilation of *GIPC3* gene mutation studies in autosomal recessive nonsyndromic hearing loss.

	Ethnicity	Pathogenic mutations in *GIPC3* gene	Variant type	Amino acid change	Exon	No. of families	Type of hearing loss	Method of detection	Reference
1	India	785T>G	Missense	p.L262R	5	Single family	Prelingual–profound	Sanger sequencing	Charizopoulou *et al.* (2011)
2	Dutch	903G>A	Nonsense	p.W301X	6	Single family	Prelingual and postlingual –profound		
3	Pakistan	136G>A	Missense	p.G46R	1	Seven families	Prelingual to postlingual, mild to profound	Sanger sequencing	Rehman *et al.* (2011)
4		264G>A	Missense	p.M88I	2				
5		281G>A	Missense	p.G94D	2				
6		565C>T	Missense	p.R189C	3				
7		662C>T	Missense	p.T2211	4				
8		685dupG	Frame shift	p.A229GfsX10	4				
9		767G>A	Missense	p.G256D	5				
10	Turkey	508C>A	Missense	p.H170N	3	1/20 (5%)	Prelingual–profound	Whole exome sequencing	Sirmaci *et al.* (2012)
11	Saudi Arabia	122C>A	Missense	p.T41K	1	1/100 (1%)	Prelingual–profound	Sanger sequencing	Ramzan *et al.* (2013)
12	Pakistan	226-1G>T	Splice site	NA	Splice site of exon 1	Single family	Prelingual severe to profound	Sanger sequencing	Siddiqi *et al.* (2014)
13	Algeria	c.764T>A	Missense	p.M255L	5	1/33 (3.03%)	Prelingual severe to profound	Whole exome sequencing	Ammar-Khodja *et al.* (2015)
14	India	c.662C>T	Missense	p.T221I	4	1/23 (4.35%)	Profound	Next generation sequencing (NGS) (for a panel of 180 deafness genes using MiamiOtoGenes)	Yan *et al.* (2016)
15	Iran	c.472G>A	Missense	p.G158L	3	Single family	Prelingual severe to profound	NGS (for a panel of 129 deafness genes)	Asgharzade *et al.* (2018)

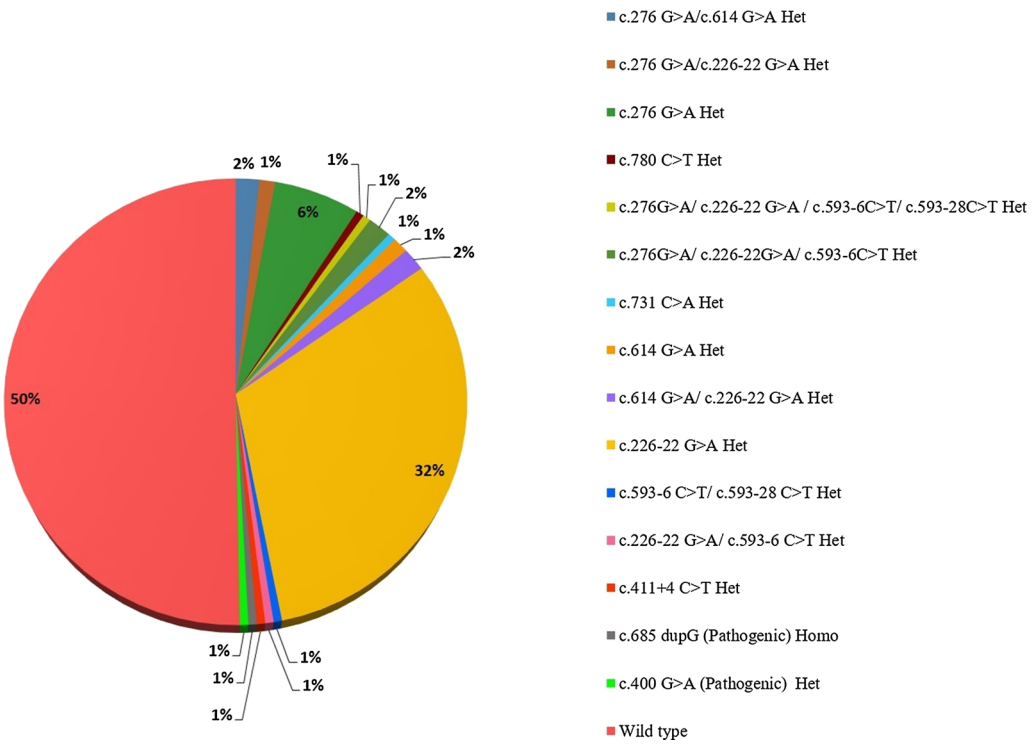

Figure 1. Overall distribution of *GIPC3* auditory gene variants observed among the 177 probands.

Table 2. Coefficient of inbreeding among the parents of the 177 HI probands.

Type of marriages	HI probands (n=177)	Coefficient of inbreeding
Nonconsanguineous	67 (37.85%)	–
Consanguineous	110 (62.15%)	–
Uncle niece	26 (14.69%)	0.0184
First cousin	51 (28.81%)	0.0180
First cousin once removed	11 (6.21%)	0.0019
Second cousins	22 (12.43%)	0.0019

Materials and methods

Our DNA repository consists of 469 prelingual HI hailing from educational/printing schools for deaf, aged between 5 and 22 years (n=437) and adult deaf associations (5 to 60 years; n=32) from southern India. Of the 469, about 18.34% (86/469) of the HI had genetic diagnosis due to the common *GJB2* variants and 0.4% (2/469) had mitochondrial A1555G pathogenic variant (Selvakumari 2015; Amritkumar *et al.* 2018). Overall, 88 prelingual HI were excluded and 381 were included for the randomized selection of 177 probands. Detailed flowchart depicting the selection of the probands is shown in figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet. When individuals tested positive for pathogenic variants, they were comprehensively examined for otoscopy, temporal bone CT and MRI scan, fundoscopy and dermatology. Further, the pathogenic variants were screened in available family members for segregation analysis. Fifty unrelated normal hearing controls (20–60 years) were recruited.

Six *GIPC3* coding exons were amplified using IDT primers (Integrated DNA Technologies, USA). Primers were designed for exon 1 with the annealing temperature of 63°C whereas other primer sequences (exons 2&3, 4 and 5&6) were adapted from Charizopoulou *et al.* (2011). All the purified PCR products were bidirectionally sequenced to confirm the complete coverage of the coding and splice site regions. The FASTA sequences were aligned and compared with appropriate reference sequence (NG_031943.1) using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UCSC Genome Browser BLAT (https://genome.ucsc.edu/cgi-bin/hgBlat) programs to identify the variants. The identified variants were evaluated using *in silico* tools such as Mutation Taster, PROVEAN and I-mutant 2.0.

Results

This study aims to assess the contribution of *GIPC3* variants among the 177 prelingual HI probands born to both consanguineous and nonconsanguineous parents (table 1 in electronic supplementary material). The coefficient of inbreeding is shown in table 2.

Table 3 shows the genotypes (homozygous/heterozygous) and corresponding frequencies of the *GIPC3* variants

Table 3. Genotype frequencies of *GIPC3* gene variants among the prelingual HI probands.

	Genotype	Locale		No. of HI	% Among the proband (177)	% Among total *GIPC3* variants (88)
		Exon	Intron			
1	**c.276 G>A**/+	2	–	11	6.21	12.5
2	**c.400 G>A*** **/+**	2	–	1	0.56	1.14
3	**c.614 G**>A/+	4	–	2	1.13	2.27
4	**c.685 dupG /c.685dupG***	4	–	1	0.56	1.14
5	**c.780 C>T**/+	5	–	1	0.56	1.14
6	**c.731 C**>A/+	5	–	1	0.56	1.14
7	**c.276 G>A/c.614 G>A**	2/4		3	1.69	3.41
8	**c.276 G**>A/*c.226-22 G>A*	2	1	2	1.13	2.27
9	**c.276G**>A/ *c.226-22G>A/ c.593-6C>T / c.593-28C>T*	2	1/3	1	0.56	1.14
10	**c.276G>A**/ *c.226-22G>A/ c.593-6C>T*	2	1/3	3	1.69	3.41
11	**c.614 G>A**/ *c.226-22 G>A*	4	1	3	1.69	3.41
12	*c.226-22 G>A*/+	–	1	56	31.64	63.64
13	*c.411+4 C>T***/+	–	2	1	0.56	1.14
14	*c.226-22 G>A/ c.593-6 C>T*	–	1/3	1	0.56	1.14
15	*c.593-6 C>T/ c.593-28 C>T*	–	3	1	0.56	1.14
16	**+/+**			89	50.28	-
	Total			177	99.94	100

Exonic variants are in bold; intronic variants in italics.
*Pathogenic variants.
**Potential splice site donor variant.
+/+ Wild type.

observed among the 177 HI probands; 50.28% (89/177) of the cohort did not have any changes in both exonic and flanking intronic regions. In the remaining 50%, nine variants were identified that include missense, frameshift and flanking intronic variants. The observed variants were limited to exons 2, 4 and 5; 9.60% (17/177) of the probands carried exclusively one exonic variant that was found to be either polymorphic or pathogenic (table 3).

Only one proband (KAV-1) was found to carry a homozygous pathogenic variant c.685dupG in exon 4 (0.56%, 1/177). Duplication of a guanine nucleotide at position 685 of the mRNA resulted in a frameshift at codon 229. The mutant transcript translates nine missense amino acids followed by a premature stop codon at position 239 (p.A229GfsX10). This variant was not previously reported in 1000 genomes and ExAC database. None of the controls harboured this variant.

Polymorphisms in exons 2 and 4 as compound heterozygotes is accountable for 1.69% (3/177). A single variant (c.226-22G>A) in intron 2 was preponderant in 31.63% of probands (56/177) as well as in controls (16%, 8/50). Only one proband 0.56% (1/177) was found to carry an intronic splice site donor variant (c.411+4C>T). The combination of both intronic and exonic variants were not so common, 5.08% (9/177). The overall distribution of *GIPC3* gene variants that include both exonic and intronic are shown in figure 1.

The genotype–phenotype correlation with respect to the homozygous pathogenic variant c.685dupG (p.A229GfsX10) (figure 2) is shown in the four generation family pedigree (figure 2 in electronic supplementary material). The clinical examinations of 21-year-old proband (KAV-1) born of an uncle niece marriage are summarized here. CT scan showed mastoid air cells and middle ear ossicles appearance as normal on both sides. The cochlea, cochlear aqueduct, vestibule, vestibular aqueduct, cochlear promontory, round and oval windows, internal auditory canal, ganglion, facial nerve canal, eustachian tube, external auditory canal and the semicircular canals were also normal. No evidence of otosclerosis. No fluid or abnormal soft tissue in the middle ear or mastoid on the right side but minimal soft tissue thickening was seen adherent to the left tympanic membrane and incus. The EEG wave patterns showed normal response to eyes opening and photic stimulation and there were no abnormal electrical activity discharges seen in the brain. The complete blood profile including biochemical, hormonal and urine analyses were normal.

Genotype analysis was extended to the unaffected parents and two unaffected elder sisters; all of them were heterozygous for c.685dupG (figure 2). The clinical characterization revealed variable findings. Proband's father

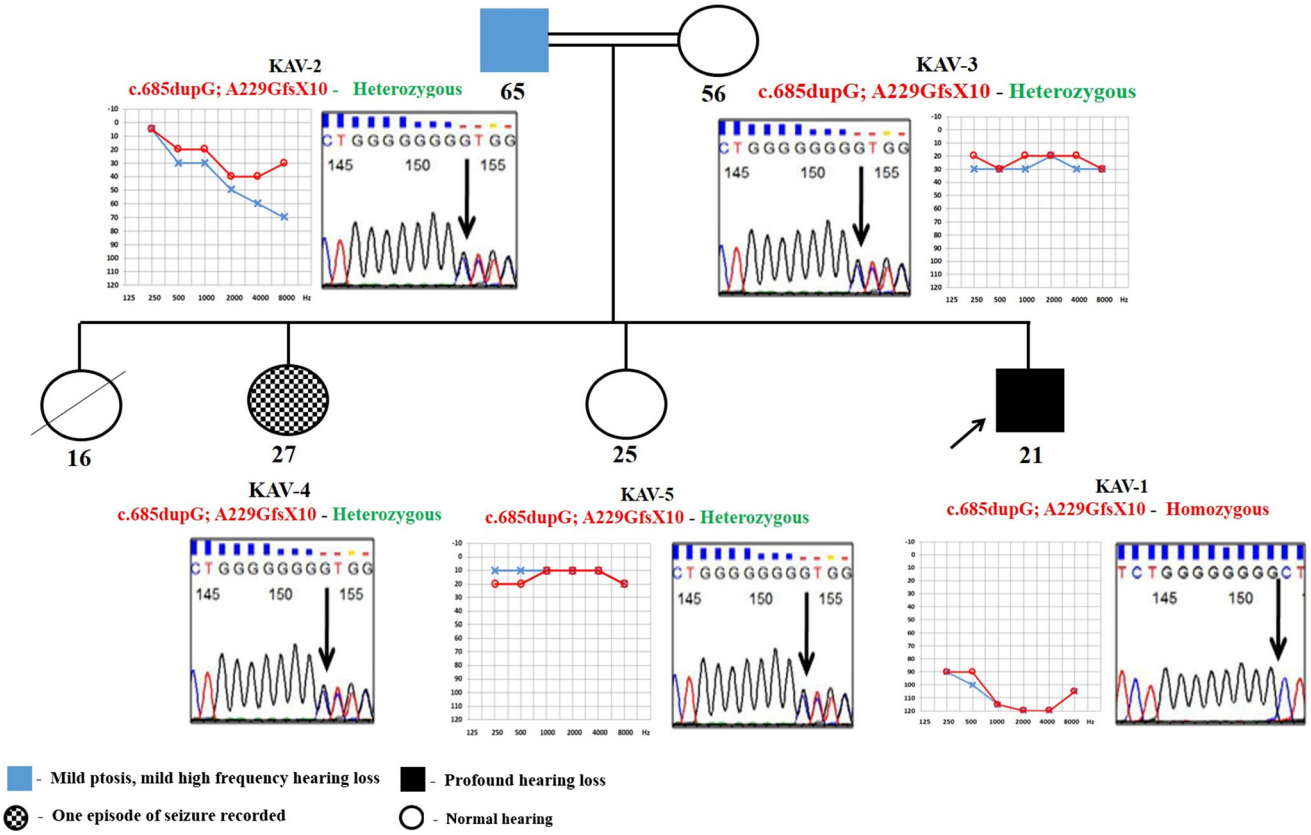

Figure 2. Pedigree of the KAV family showing the segregation pattern of *GIPC3* pathogenic variant in partial chromatograms along with their respective audiograms.

(KAV-2) had age related high frequency hearing loss (normal speech), partial ptosis (right) since childhood with normal visual acuity (6/6). His mother (KAV-3) and sister (KAV-4) did not have any other abnormalities. However, second sister (KAV-5) had one episode of seizure but was not cooperative for further clinical documentation.

The consequences of this variant was predicted to be 'disease causing', 'decreased stability' and 'deleterious' by three *in silico* tools used. However, the impact of this putative disease causal frameshift variant should be validated using *in vivo* studies to assess the functional changes.

Discussion

To this date only nine reports are available on *GIPC3* gene related deafness worldwide (table 1). Among them, seven reports essentially address large consanguineous Muslim families from Pakistan (Rehman *et al*. 2011; Siddiqi *et al*. 2014; Khan *et al*. 2019), Turkey (Sirmaci *et al*. 2012), Saudi Arabia (Ramzan *et al*. 2013), Algeria (Ammar-Khodja *et al*. 2015) and Iran (Asgharzade *et al*. 2018).

Our study reports a homozygous pathogenic frameshift variant c.685dupG with profound sensorineural hearing loss (SNHL) from a consanguineous Hindu family. This variant renders in loss of about 85% of the conserved C-terminal GH2 domain of GIPC3 due to nonsense mediated mRNA decay. This might be important for interactions with actin-based molecular motor-like myosins in inner ear hair cells. It has been reported that GH2 is the MYO6-interacting domain in GIPC1 during endocytic vesicle trafficking. This GIPC/myosin complex acts as an anchor on the cell surface, which is critical for maintaining the structural integrity of stereocilia in inner ear (Katoh 2013). It is also shown that *MYO6* causes ARNSHL (Ahmed *et al*. 2003). These evidences support the pathogenicity of frameshift variant c.685dupG in *GIPC3*. Establishing precisely the molecular mechanisms, functions and regulation of *GIPC3* in auditory system will help our understanding of its pathogenicity and heterogeneity.

This frameshift variant was previously reported in a Pakistani family (Rehman *et al*. 2011) where the affected individual had moderate to severe SNHL. None of the variants reported so far had recurrence in any two studies concurrently, reflecting the intragenic heterogeneity. As an exception, Yan *et al*. (2016) reported the recurrence of one

missense variant c.662C>T (p.T221I) in an Indian family using MiamiOtoGenes panel but this was absent in our study. The present study will be the second one to report the recurrence of c.685dupG variant in an Asian population.

Charizopoulou *et al*. (2011) determined the significance of variants in *Gipc3* as the cause for progressive SNHL (age-related hearing loss 5, ahl5) and audiogenic seizures (juvenile audiogenic monogenic seizure 1, jams1) in a mouse model. They localized *Gipc3* to inner ear sensory hair cells and spiral ganglion. The mutant model $Gipc3^{343A/A}$ was found to interrupt the structure of the stereocilia bundle affecting the long-term function of auditory hair cells and spiral ganglion neurons. *Gipc3* has an essential role in acoustic signal acquisition and propagation in cochlear hair cells. They identified a homozygous missense variant, c.785T>G, in exon 5 in a consanguineous Indian HI family. In a Dutch family, they found a homozygous region at 19p13.11-13.3 encompassing *GIPC3* common for two affected siblings (rs11880407–rs6512152; assigned locus symbol: DFNB95). On variant analysis, they found homozygous nonsense variant c.903G>A (p.W301X) in exon 6. This family presented with a wide range of phenotypic variability from progressive to profound HL.

Later, Rehman *et al*. (2011) added four more Pakistani families to Ain *et al*. (2007) collection who had bilateral HL ranging from mild to profound. The linkage region of this family further refined the critical DFNB72 interval to that between markers D19S209 and D19S894. This refined interval spans 1.08 Mb and contains at least 36 genes including *GIPC3*. The coding region of *GIPC3* was sequenced in all the seven families. One homozygous frameshift c.685dupG and six different homozygous missense variants c.136G>A, c.264G>A, c.281G>A, c.565C>T, c.662C>T, c.767G>A were identified in the affected individuals in these seven families.

Screening 23 HI from India, Yan *et al.* (2016) reported a frequency as high as 4.35% (1/23) for *GIPC3*, while we observed only 0.56% (1/177). In fact, their study was on a multi-ethnic cohort comprising of native families, 90 from Nigeria, 53 from USA (south Florida), 91 from South Africa, 38 from Tunisia, 23 from India, 19 from Turkey, 21 from Iran, and seven from Guatemala. None of the seven ethnic cohorts apart from India were found to have *GIPC3* variants. A large-scale screening of HI probands is required to further establish such a high incidence of *GIPC3* variants in India.

Worldwide reports (table 1) implicate 15 *GIPC3* pathogenic variants that are notated as DFNB15/95/72 aetiology (https://hereditaryhearingloss.org/). Interestingly the HI probands have been predominantly recruited from large consanguineous Muslim families. Contemporary attention on consanguineous marriage continues to be largely focussed on the expression and identification of rare autosomal recessive alleles in the indigenous population (Hilgert *et al*. 2009; Dror and Avraham 2010).

Considering the consanguineous marriages and heterogeneous population, many other genes are involved in ARNSHL in addition to *GJB2*. Several studies have reported new genes in HL patients such as *USH2A*, *OTOA*, *CABP2*, *TMPRSS3*, *GIPC3*, *ADGRV1*, *LHFPL5*, *MYO6*, *PTPRQ*, *CIB2*, *POU3F4*, *TMIE*, *ESPN*, *GPSM2*, *GRXCR1*, *KCNQ1*, *MTRNR1*, *OTOGL*, *PRPS1*, *TRIOBP*, *WFS1*, *DFNB31*, *USH1C* (Finsterer and Fellinger 2005; Yan *et al*. 2016; Khan *et al*. 2019), but there is no information about the frequency of these gene variations. Evaluation of the frequencies should be conducted to clearly apportion for each of the gene in HL and its occurrence in a specific ethnicity. Our study will be the first documentation of mutation burden in *GIPC3* in the south Indian population. Gene hunting for the second tier of HL causative genes after excluding *GJB2/6* and mitochondrial gene variants (A1555G) showed very less contribution from *GIPC3* (0.56%). *GIPC3* cannot be placed in the list of second tier of genes due to its low incidence.

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