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Case Report
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Paternal Disorder of Connexin 26 Gene Mutation Increase Risk of Developing Sensory Loss in Non-Syndromic Offspring: An Interesting Case Study

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Abstract

Neurosensory congenital deafness in non-syndromic cases occurs due to the mutation (235delC) of Connexin 26 gene (GJB2) and is mapped at chromosome 13q11-q12 that encodes large family protein involved in the formation gap junction. Earlier study shows genetic diversity in the frequency of 235delC between Asians, Caucasians populations. Present study of connexin 26 mutations were carried out in clinically diagnosed families and individuals which totals consisted of total n=17 that included, father (four), mother (four) and siblings (seven including male & female). PCR based study on agarose (1.5%) gel showed the disappearance of Cx48/Cx1040L (1000bp) and GJB2 (900bp) band in father (one family) and lacked in both mother and off springs. These findings reflect that congenital hearing loss (25%) of the offspring is paternal in origin in non-syndromic cases. Since, this is a developmental defect, curiosity has been generated to analyze pluripotent stem cell Oct4, Nanog and Sox4 marker using PCR technique with specific forward /reverse primers and again showing disappearance of band Oct4 (577bp) and Sox9A (823bp) in father of the same family, suggesting failure to maintain pluripotency during organogenesis.

Conclusion: Present case study reflects that the mutation of connexin 26 gene has positive correlation with early transcription factors Oct4 and Sox9A that might have originated truncated protein synthesis.

Keywords: Connexin 26 gene; Stem cell marker; Oct4 and Sox9A and Non-syndromic cases

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Introduction

Epidemiological studies reveal that congenital deafness or sensorineural disorder varies in 1:500 newborns involving 46 genes. The Hearing Loss (HL) varies from age specific i.e. prelingual to post lingual (after speech development) and involving either inner or middle ear. Most of the HL cases either hereditary (70%) and environmental (non-genetic) factors due to the congenital infection of *Cytomegalovirus* [1-2]. Genetics screening study shows different frequency in monogenic (non-syndromic) cases of HL which is either autosomal recessive (80%) or dominant constitutes only 20%. Less than 1% cases of HL are X chromosome linked [3]. Connexin 26 is a member protein that is involved in gap-junction and is associated with the direct transfer electrical synapses (ionic current) to the neighboring cells. The most common gene mutation is GJB2 (35delG), whose frequency varies between two different populations i.e. Caucasians and European [4]. Recent study shows mutation of SLC26A and FOX1 act as transcriptional regulatory gene in syndromic and non-syndromic heterozygous condition [5]. Present study includes genetics screening of connexin 26 gene (Cx48U/Cx1040L) and GJB2 in family as well as individuals in clinically diagnosed cases of HL. Since, this study fall under the category of "Birth defects", hence it becomes imperative to characterize early transcription factors- Oct4, Nanog and Sox9A to determine pluripotency using Polymerase Chain Reaction (PCR) with specific forward/reverse set of primers on agarose gel electrophoresis in the family (father, mother and proband) or individual cases of HL. The identification of Cx48U/Cx1040L and GJB2 genes mutation help to provide better understanding and their correlation with stem cell biology and that might help to develop future strategies in the management of such common genetic disorder.

Case Presentation

The peripheral blood samples (n=18) were collected from clinically diagnosed cases from the

Table 1: Expression of Connexin 26 gene (Cx48U/Cx1040L & GJB2) and stem cell Sox 9A, Oct4 & Nanog in the family of congenital deafness.

S. No.	Patient (ID)	Family	Age/sex	Connexin 26 gene		Stem cell marker		
				Cx48U/Cx1040L (1000bp)	GJB2 (900bp)	SOX 9A (823 bp)	Oct4 (577 bp)	Nanog (151 bp)
1	P1044	Mother		+	+	+	+	+
		Father		-	-	-	-	+
		Case	3Y/M	+	+	+	+	+
2	P1060	Case (twin sisters)	1½ Y/F	+	+	+	+	+
3	P1069	Mother	1 ½Y/M	+	+	+	+	+
		Father		+	+	-	-	+
		Case		+	+	+	+	+

proband along with mother and father after ethical approval from the IEC (Institute Ethical Committee) and consent from the patients or individuals visiting the OPD of department of otorhinolaryngology, AIIMS-Patna. Genomic DNA was isolated as per the prescribe protocol of the kit (Promega) and quantified by nano drop spectrophotometer (Thermo USA). Cx48U/Cx1040L, *GJB2*, Oct4, Nanog and Sox9A genes were amplified using Polymerase Chain Reaction (PCR) with specific forward & reverse primers on agarose (1.5%) gel, and bands were characterized and visualized on Gel doc system (Bio rad USA) after staining with ethidium bromide.

Analysis of Connexin 26 gene mutation: The details of Polymerase Chain Reaction (PCR programs of an individual gene amplification including annealing temperature, meting point and cycling conditions are described as follows using forward and reverse primers F TGC-TTA-CCC-AGA-CTC-AGA-GAA and R CGA-CTG-AGC-CTT-GAC-AGC-TGA for Cx48U/Cx1040L gene consist of 1000bp and for *GJB2* gene F5′TTGGTGTTTGCTCAGGAAGA3′ and R5′GGCCTACAGGGGTTTCAAAT3 with annealing temperature 60°C were used for analysis of PCR amplification after confirmation of sequences from NCBI (BLAST/http://blast.ncbi.nlm.nih.gov.). The PCR reaction was achieved in a 25 µl mixture containing 5X Green GoTaq PCR reaction buffer, dNTPs Mix (10 mM), 1 µl each of 10 pmol of specific primer i.e. forward and reverse, 0.2 µl of GoTaq DNA polymerase (5 U/µl). The cycle conditions consist of 35 cycles comprising, initial denaturation at 95°C for 5 min, followed by denaturation 95°C for 1.0 min, annealing at 60°C for 1.0 min and elongation at 72°C for 1 min, followed by final elongation at 72°C for 5 min for connexin 26 gene.

Analysis of stem cell marker study: PCR products were characterized on agarose gel electrophoresis using highly specific forward and reverse sets of primers sequences for Oct4 F5'GACCATCTGCCGCTTGAG3'; R5'CCCCCTGCCCCATTCCTA3' (577bp) with annealing temperature 60°C, and for Nanog F5'CTGTGATTTGTGGGGCCTGA3' and reverse TGTTTGCCTTTGGGACTGGT3' (151bp) having annealing temperature 56°C with 35 cycles. The forward and reverse primers were used for Sox9A F5'CGGGTGGCTCTAAGGTG3; and reverse 5'TTGTGCAAGTGCGGGTA3', and the detailed laboratory protocols are adapted [6-8].

Results and Discussion

The different frequency of connexin 26 gene mutation leading to neuro sensory loss in non-syndromic cases between different population is a debatable question. Polymerase Chain Reaction (PCR) based analysis were carried out using specific forward and reverse set of primers for Cx48U/Cx1040L and *GJB2* gene in both families as well as individual cases of congenital deafness as documented in Table 1. There is lack of mutation were observed in both male and females (n=7) probands as well as in mother including twin sisters. Interestingly, father of the one family showed complete disappearance of band *GJB2* gene (900bp) and Cx48U/Cx1040L (1000bp) as depicted in Figure 1A, 1 B.

Since, congenital deafness fall under the category of Birth Defects, the biology of pluripotent stem cell markers Oct4, Nanog and Sox9A becomes relevant to determine the physiological role during ontogenetically development, if it exist in the same family (n=4) and individual cases. Further analysis was carried out in the same family,

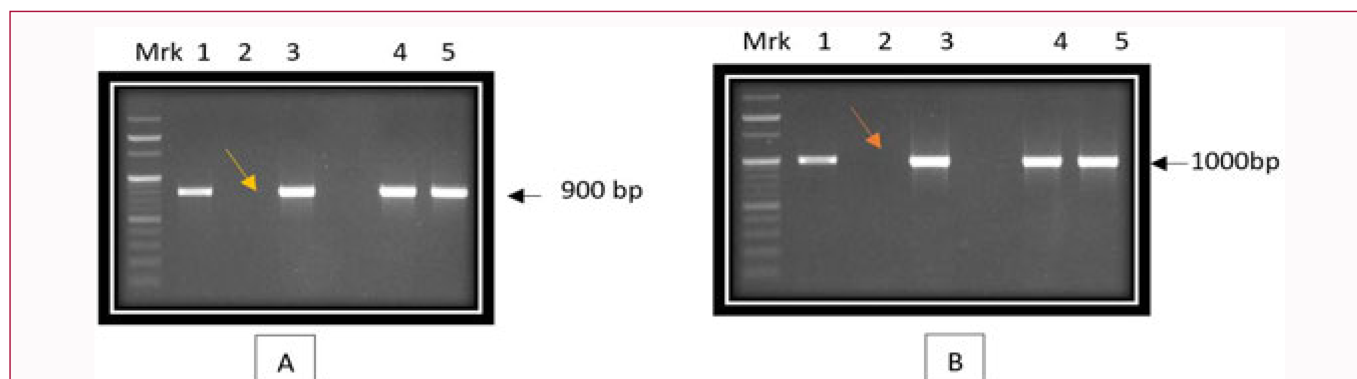

Figure 1A, 1B: PCR based analysis of GJB2gene (A) and Cx48U/Cx1040 (B) showing complete disappearance band consist 900bp and 1000bp on agarose gel (1.5%), characterized on Gel Doc system after staining with fluorescence dye (ethidium bromide) considered as mutation in the father as shown in lane-2 (arrow). There is a lack of mutation in mother (lane-1) and proband (lane-3) in the family of congenital deafness. Individually, twin sisters of congenital deafness also showing lack of mutation (lane-4 & 5).

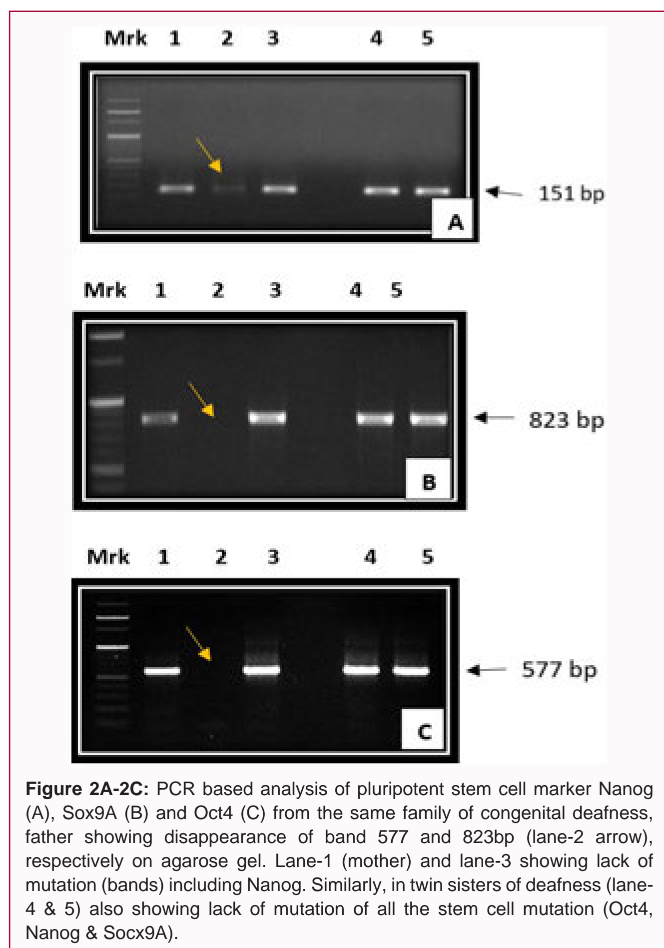

Figure 2A-2C: PCR based analysis of pluripotent stem cell marker Nanog (A), Sox9A (B) and Oct4 (C) from the same family of congenital deafness, father showing disappearance of band 577 and 823bp (lane-2 arrow), respectively on agarose gel. Lane-1 (mother) and lane-3 showing lack of mutation (bands) including Nanog. Similarly, in twin sisters of deafness (lane-4 & 5) also showing lack of mutation of all the stem cell mutation (Oct4, Nanog & Socx9A).

interestingly showing the disappearance of Oct4 (577bp) and Sox9A (823bp) in the father, but lacking both in mother and proband.

The etiopathology of congenital deafness is highly complex due to involvement of genetics and epigenetics factors. Connexin 26 gene belongs to gap-junctions transmembrane that mediates electrical coupling between adjacent cells. These *GJB2* (Cox26) genes are associated with syndromic and non-syndromic family, that involved in neurosensory epithelium of cochlear fibrocytes [9]. Earlier studies shows that more than seventy mutations of *GJB2* have been reported in deafness and is the most common ancient mutation that arises after the mutation is 235delC in Asian population, whose frequency varying in different population [10].

The present study showed lack of mutation of both the genes in the proband as well as in mother. This might be either due to small sample size or other unknown environmental factors. These findings in non-syndromic family confirm the paternal mode of inheritance, because father showed more than 33% mutation frequency of the genes (Table 1). However, this preliminary data of stem cell Oct and Sox9A also support our finding that early transcription factors increased "risk" of developing congenital deafness to the offspring during organogenesis because that failed to maintain pluripotency. Although, authors failed to concludes that the function of these two genes mutation work together either in synergistic manner or independently during ontogenetically development. Secondly, the mode of transmission is seemed to be X-linked or act as carrier. Nance et al. [11], hypothesize that such a high frequency of *GJB2* 235delC gene mutation leading to deafness in Asians population may be either due to consanguineous or population diversity between Mongoloids and Caucasians [12].

Conclusion

However, the present study of the biology of deafness is based on connexin 26 gene mutation and showed synergism with stem cell gene Oct4 and Sox9A, reporting first time in Indian cases, where the paternal disorder increase "risk factor" by influencing neurosensory potassium influx disorder in the inner ear. No doubt, present illustration is small, but authors try to make more interesting and failed to conclude the exact mode of inheritance. However, more sample sizes are required for the authenticity of the findings otherwise the study will be remain inconclusive.

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