第 49 卷 第 11 期
2022 年 11 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)
http://jonuns.com/index.php/journal/index

Vol. 49 No. 11
November 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.11.6

Highly Prevalent GJB2 Gene Mutations among Congenital Nonsyndromic Hearing Impairment in a South Indian Population

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Abstract: Hearing impairment (HI) is due to genetic (autosomal recessive) having a frequency of one in 1000 children. Hearing impairment has a widespread gene involvement of various chromosomes. Mutations in the GJB2 gene on chromosome 13 were frequent findings for congenital non-syndromic hearing impairment. To make it more complicated, it has been observed that these mutations show population variations. Objective: To determine the prevalence of the most common mutations in the GJB2 gene among the Indian population. Methods: In this study, 38 subjects (22 cases and 16 controls) enrolled, and the degree of hearing loss was assessed. To identify the involvement of GJB2 gene mutations p.W24X and c.235delC among the populations, PCR Technique and RFLP were adopted. Results: The mutations under investigation were at a higher prevalence rate, i.e., 45.45% for c.235delC and a lower prevalence rate of 18.18% for p.W24X, and both the mutations had heterozygous natures. Conclusion: This paper is the first of this kind that tries to identify a prevalent mutation in the GJB2 gene in the population of Karnataka state. The results are supported to some extent by previous Indian studies involving other than the current population. It is also noteworthy that the mutations identified are heterozygous and, therefore, are not pathogenic. This implies either the existence of potential mutations in the gene's unexplored region or the possible co-implication of another connexin gene, i.e., the digenic origin of the hearing loss, which could be related to the putative formation of heteromeric connexons or heterotypic channels.

Keywords: congenital nonsyndromic hearing loss, p.W24X, c.235delC mutations.

南印度人群先天性非綜合徵性聽力障礙中高度流行的国杰宝2基因突變

摘要：聽力障礙是由於遺傳（常染色體隱性遺傳）導致的，頻率為千分之一。聽力障礙廣泛涉及各種染色體的基因。13號染色體上国杰宝2基因的突變是先天性非綜合徵性聽力障礙的常見發現。更複雜的是，已經觀察到這些突變顯示出種群變異。目的：確定印度人群中国杰宝2基因最常見突變的流行程度。方法：在本研究中，納入了38名受試者（22名病例和16名對照組），並評估了聽力損失的程度。為了確定国杰宝2基因突變p.W24X和c.235刪除C在人群中的參與，採用聚合酶链反应技術和RFLP。結果：被調查的突變具有較高的流行率，即c.235delC為45.45%，p.W24X的流行率為18.18%，且兩種突變均具有雜合性。結論：本文是此類嘗試確定卡納塔克邦人群中国杰宝2基因中普遍存在的突變的首篇論文。這些結果在一定程度上得到了之前印度研究的支持，這些研究涉及的不是當前人口。還值得注意的是，鑑定出的突變是雜合的，因此不是致病性的。這意味著該基因的未探索區域存在潛在突變或另一個連接蛋白基因可能共同暗示，即聽力損失的雙基因起源，這可能與異聚連接子或異型通道的假定形成有關。

关键词：先天性非綜合徵性聽力損失，p.W24X，c.235delC突變。

Received: July 15, 2022 / Revised: September 12, 2022 / Accepted: October 10, 2022 / Published: November 30, 2022

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1. Introduction

Hearing impairment (HI) is an autosomal recessive sensory disorder. It is estimated that 0.7% of newborns are deaf [1, 2]. 50% of childhood deafness is because of genetics, which is autosomal dominant (20%), autosomal recessive (80%), X-linked (1%), and mitochondrial (1%) [3]. Congenital Hearing impairment, syndromic forms constitute one-third, and non-syndromic constitute two-thirds [4]. There are many genes associated with HI. DFNB1 (13q11-q12) locus, comprising the GJB2 gene, accounts for up to 50% of non-syndromic autosomal recessive disorders. The GJB2 gene codes the Gap junction connexin 26 (CX26), which transports potassium ions and certain small molecules.

A wide ethnic diversity exists in the mutation rates in the GJB2 gene [5-10] and there are very few reports regarding the mutations in Indian population [1, 2, and 11] and there were differences of opinions within the specific populations about the prevalence and causative mutations in the GJB2 gene for hearing impairment. This study attempts to determine the prevalence of most commonly found mutations like p.W24X and c.235delC of GJB2 gene.

2. Materials and Methods

The deaf, or parents of deaf children, were informed about the study and requested to sign consent forms. 24 subjects and 16 controls were enrolled voluntarily. Complete clinical and familial data were collected from family members. The Institutional ethical Committee certified the study design, material and methods adopted in the study.

2.1. Audiological Assessment

Otoscopic examination, immittance audiometry, tympanometry and acoustic reflex measurements were carried out to analyze the middle ear functioning. Tympanogram type was noted and the acoustic reflexes were obtained. Pure-tone audiometric thresholds were measured using a Grason Stadler Incorporation, Model 68, diagnostic audiometer to determine the degree and type of hearing loss. The audiometric thresholds were estimated according to the modified Hughson-Westlake method as proposed in [18].

2.2. Molecular Analysis

The isolation of DNA was performed by the salting-out technique. Quantification of the genomic DNA was measured using a Perkin Elmer Lambda 35 UV-VIS Spectrophotometer, and quality of the DNA was confirmed by running the 0.8% agarose gel electrophoresis.

2.2.1. GJB2 Mutational Analysis of PCR

The Polymerase Chain Reaction for GJB2 mutation analysis was performed to identify p.W24X and c.235delC mutations using the primers shown in the Table 1.

Table 1 Primers used

Mutation	Primers
p.W24X	1F: TCTTTTCCAGAGCAAACCGC
	1R: GACACGAAGATCAGCTGCAG
c.235delC	1F: TGTGTGCATTCGTCTTTTCCAG
	1R: GGTTGCCTCATCCCTCTCAT

2.2.2. GJB2 Mutational Analysis of RFLP

For p.W24X mutations Alu1 enzyme and c.235delC mutations Apa1 enzyme were used and RFLP analysis was performed out by agarose gel electrophoresis of the digested products. DNA fragments were observed using ultraviolet light and photographs were obtained using the Molecular Imager Gel Doc TM Image Lab Software.

3. Results

The RFLP analysis revealed a band at 280 bp in all the subjects and controls which were normally expected, except in 4 subject samples (1,15,19 and 21) there were two more additional bands, one at the level of 100 bp and another at 180 bp, indicating heterozygous condition for the mutation (Fig. 1).

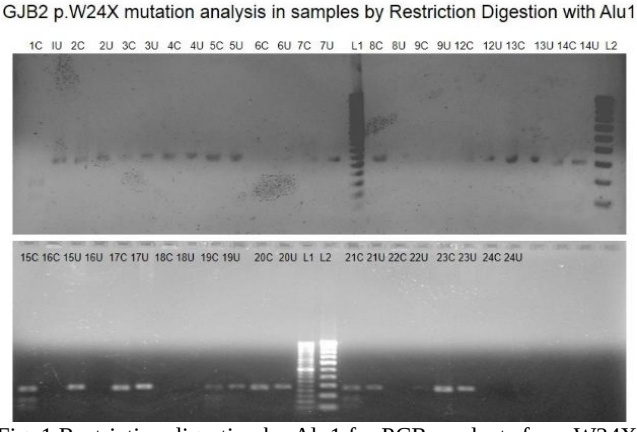

Fig. 1 Restriction digestion by Alu1 for PCR products for p.W24X mutation

The number of each well indicates the identification number of samples and control groups, respectively. The suffix C and U indicate RFLP and control conditions, respectively. L1 and L2 indicate 50 bp and 100bp ladder, respectively.

RFLP was performed for the c.235delC mutation as detailed in methods (section 2.2.2). In the case of subject samples 10 were (subjects nos. 8, 13, 14, 15, 16, 17, 20, 22 and 23) exhibited the additional band at 780 bp along with a bands at 300 bp and 500bp indicating heterozygous condition for the mutation (Fig. 2). The RFLP for the c.235delC mutation in

control group samples showed normal bands at 300 bp and 500bp as expected (Fig. 2).

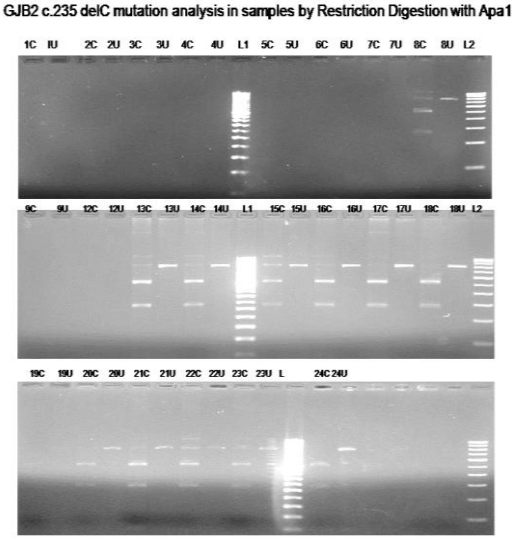

Fig. 2 Subject nos. 8, 13, 14, 15, 16, 17, 20, 21, 22 and 23 showing mutations

The suffix C and U indicate RFLP and control conditions, respectively. L1 and L2 indicate 50 bp and 100bp ladder, respectively.

4. Discussion

Many factors exist to be considered for a missense mutation to be described as pathogenic. These include the position of the mutation whether at coding or noncoding region leading to the formation of abnormal or malfunctioning proteins. A change or abnormal amino acid at a key position in a complex of protein structure can cause the functional efficacy of the protein structure.

As the gap junction proteins are highly complex in their protein structure and functions, it is very difficult to presume or assume the pathogenicity of some missense mutations unless they are proven with functional studies.

This study is an attempt to emphasize the knowledge of the connexin 26 mutations (p.W24x and c.235delC) and their prevalence in the Indian population. Till date, very few studies have been conducted in some regions of India and none from Karnataka state, which tries identifying the causative genes and its mutations and polymorphisms. More than 80 different GJB2 mutations have been identified. Nevertheless, 35delG, 167delT, 235delC, and V37I account for 50% of mutant alleles in Caucasians, Ashkenazi Jews and Asians, respectively [12].

The mutations in the coding region p.W24x, described in this study have been reported in few studies from the Indian subcontinent, including India, Pakistan, Bangladesh, and Sri Lanka [13-15]. GJB2 gene shows the p.W24X, most common (95%) among the mutations found in the Indian population and probably a founder effect for causing the HI [16]. Another study shows 6.5% homozygous and 0.5% heterozygous conditions. The prevalence of the same was 13.33% in North India [17], 18.1% in south India [16]. Our results, i.e., 18.18% of p.W24X mutation show the contrary to the studies as all the mutations were heterozygous.

Another frequent mutation found in GJB2 is c.235delC, which is a frame shift mutation, was screened with Apa1 restricted enzyme, which identifies the restriction site, which is normally present in the wild type of gene and cuts it into two fragments of size 300 bp and 500 bp. In the present study, 10 of 22 (45.45%) subjects found the presence of one more band at 780 bp along with 300 bp and 500bp (Fig. 2). This indicates that there is mutation vanishes the restriction site due to which the restriction enzyme is not able to identify it and cut the gene.

5. Conclusion

In our study, 22 subjects with nonsyndromic congenital hearing loss were selected from the school for hearing impairment and were subjected to mutational analysis for p.W24X and c.235delC types of GJB2 mutation. It was seen that out of 22 subjects, 4 probands (18.18%) were heterozygous for W24X and 10 probands (45.45%) were heterozygous for the c.235delC type. The control group showed no mutation and carrier frequency for both type of mutation. It is also noteworthy that the mutations identified are heterozygous hence are not pathogenic. This implies the possibility of the presence of potential mutations in the unexplored region of the gene or due to the other mutations at the different gene, which is related to the hearing loss. This compound or multiple mutations could be involved in the nonfunctional heteromeric or heterotypic channels.

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