



# A screening analysis of the *GJB2* c.176 del 16 mutation responsible for hereditary deafness in a Chinese family

Hao Jiang<sup>a</sup>, Xi Shi<sup>b</sup>, Shiwei Qiu<sup>b</sup>, Yanfen Dong<sup>b</sup>, Yuehua Qiao<sup>c,\*\*</sup>, Dongzhi Wei<sup>a,\*</sup>

<sup>a</sup> New World Institute of Biotechnology, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, 200237, Shanghai, China

<sup>b</sup> The Institute of Audiology and Balance Science of Xuzhou Medical University, Xuzhou, 221004, China

<sup>c</sup> Clinical Hearing Center of Affiliated Hospital of Xuzhou Medical University, Xuzhou, 221006, China

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## Abstract

**Objective:** To determine whether a new-born child from a family carrying a deafness gene needs cochlear implantation to avoid dysphonia by screening and sequencing a deafness-related gene.

**Results:** Both screening and sequencing results confirmed that the new born child had a normal *GJB2* gene despite the fact that she has a brother suffering from hearing loss triggered by an allelic *GJB2* c.176 del 16 mutation. We cloned the *GJB2* genes derived from their respective blood genomic DNA into GFP fused plasmids and transfected those plasmids into the 293T cell line to test for gene function. While the mutated *GJB2* gene (*GJB2* c.176 del 16) of her deaf brother was found to be unable to form the gap junction structure between two adjacent cells, the baby girl's *GJB2* gene ran into no such problems.

**Conclusion:** The screening and sequencing as well as the *GJB2* gene function tests invariably showed results consistent with the ABR tested hearing phenotype, which means that the child, with a normal wild type *GJB2* gene, does not need early intervention to prevent her from developing hearing loss and dysphonia at a later stage in life.

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**Keywords:** Deafness gene screening; *GJB2*; Gap junction

## 1. Introduction

Hearing loss, known as a partial or total loss of the ability to hear, can often be attributed to multiple causes such as ageing, genetics, perinatal problems and external causes like

noise and illness (Deafness, 2011). In general, hereditary deafness is mostly the result of mutations in certain genes playing a decisive role in the physiological mechanism of hearing. While around 75–80% of cases of hereditary deafness are known to have been caused by recessive genes, the pathogenic cause of 20–25% of them can be attributed to dominant genes. Among all such cases, 1–2% can be explained by X-linked patterns and another 1% by mitochondrial inheritance (Rehm).

Gap junction beta-2 (*GJB2*), whose corresponding protein is connexin 26 (Cx26), is one of the leading genetic causes of deafness. Connexins, a type of gap junction proteins, are a family of structurally related transmembrane proteins that can

\* Corresponding author.

\*\* Corresponding author. Fax: +86 21 64250068.

E-mail addresses: [shixi19850112@163.com](mailto:shixi19850112@163.com) (X. Shi), [18361337211@163.com](mailto:18361337211@163.com) (S. Qiu), [732379051@qq.com](mailto:732379051@qq.com) (Y. Dong), [yuehuaqiao001@163.com](mailto:yuehuaqiao001@163.com) (Y. Qiao), [dzhwei@ecust.edu.cn](mailto:dzhwei@ecust.edu.cn) (D. Wei).

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help form vertebrate gap junctions. Each gap junction is made up of two hemi-channels, or connexons, whose structure commonly contains six connexin molecules. Together with other proteins, they can dock with the hemi-channels of adjacent cells to form gap junctions (Jongen et al., 1991). The gap junctions formed as a result consist of cell-to-cell channels crucial to multiple physiological processes, for example, the transmission of Potassium ions in the inner ear, meaning that defects in this gene can lead to one of the most common forms of congenital deafness (Kelsell et al., 1997).

In this research, we helped the parents of a 5-year-old deaf son, who recently gave birth to a new baby girl to determine whether their second child needs artificial cochlear implantation to prevent her developing hearing loss by screening the genomic DNA of the entire family. Research results showed that the second new born child was fortunate enough to have a properly-functioning (wild type) *GJB2* gene and run no risk of suffering from congenital hearing disabilities linked to a defective *GJB2* gene.

## 2. Material and methods

### 2.1. Clinical analysis

All members of the studied family underwent consecutive auditory brainstem response (ABR) tests. Their medical histories showed that they had not exhibited any abnormality during pregnancy or delivery, nor had they been exposed to amino-glycosides.

### 2.2. Molecular analysis

We used DNA Isolation kits (Tiangen, china) to isolate blood genomic DNA from the blood samples of the children and their parents. Nine hotspot mutations of deafness genes commonly found among Chinese populations were screened by adopting a method called multiplex allele specific PCR-based universal array (ASPUA) (Li et al., 2008). These mutations include the four mutations of c.35delG, c.176del16bp, c.235delC and c.299delAT in the *GJB2* gene as well as c.538C > T in the *GJB3* gene, c.IVS7-2A > G and c. 2168A > G in the *SLC26A4* gene, and m.1555A > G and m.1494C > T in the *RNR1* gene of mitochondrial DNA (mtDNA). The family was then put through bi-directional sequencing of the coding region of the *GJB2* gene to exclude rare or novel pathogenic mutations.

### 2.3. Localization analysis of wild-type and mutated Cx26 protein in HEK293

The HEK293 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The plasmids encoding WT or mutated Cx26 tagged with fluorescent protein markers were transfected to the HEK293 cells using Shining Jet transfection reagent (Shing, China) under the manufacturer's instructions. The intracellular localization of WT and mutant Cx26 proteins was studied through fluorescence microscopic analysis 24 h after transfection.

## 3. Results

### 3.1. Molecular analysis

The blood genomic DNA from all members of the family were used for ASPUA molecular screening. 8 of 9 potential hotspot mutations in *SLC26A4*, *GJB2*, *GJB3* and mtDNA *RNR1* genes were excluded as causative factors of hearing loss except c.176 del 16, a prevalent mutation in the *GJB2* gene. The existence of this mutation was subsequently reconfirmed during the sequencing process (Fig. 1). Our screening and sequencing results showed that while the heterozygous mutation of c.176 del 16 is found in the couple's *GJB2* genes, the deaf boy carries a homozygote mutation in *GJB2*; c.176 del 16. Fortunately, the new born child did not inherit any of such mutations in her allelic *GJB2* genes.

### 3.2. Intracellular gene function test

To see whether the *GJB2* gene from the new born baby was functional, we cloned the *GJB2* genes from the two children's blood genomic DNA and constructed two respective *GJB2*-GFP fused plasmids. Then we transfected HEK293 cells with these two expression plasmids, which allowed the researchers to track the intracellular location of Cx26.

In our test, the normal Cx26-GFP protein cloned from the second child was successfully formed into some small foci localized in the cell membrane, indicative of involvement of the gap junction between two adjacent cells (Fig. 2B arrow). By contrast, the mutated Cx26 protein (*GJB2* c.176 del 16) from the deaf child was defused on the full cells evenly without any specific localization on the cell membrane and no foci (gap junctions) were observed in these cells (Fig. 2A).

Therefore, we reached the conclusion that the *GJB2* c.176del16 mutation might potentially lead to the boy's hearing loss by undermining the ability of mutant Cx26 proteins to form the gap junction structure in the cell membrane.

## 4. Discussion

As many studies have shown, *GJB2* mutations, as one of the predominant causes of non-syndromic hearing loss, are either dominant or recessive (Han et al., 2008; Abe et al., 2000; Dai et al., 2009). In this study we first screened and sequenced the 5-year-old child diagnosed with profound hearing loss for hot-point genes (Li et al., 2008) related to the symptom. Then the same procedures were performed on the other child of the family, a healthy one born recently, in an attempt to seek out the same gene. This process was considered essential to excluding the possibilities of her developing the same hearing disabilities at a later stage in life. Luckily enough, both screening and sequencing results confirmed that the second child had a normal *GJB2* gene, an indication that this new born child is unlikely to develop the same gene type of hearing disabilities as did her elder brother whose genomic type is homozygosity for the *GJB2* c.176 del 16 mutation.

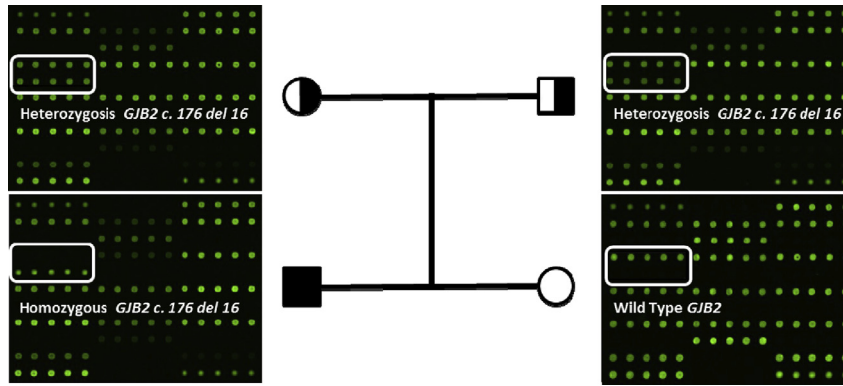


Fig. 1. Genotype screening showing heterozygous or homozygous *GJB2* c.176del16 mutations and wild type *GJB2* in the family. Chip images show the results of screening described in Material and Methods. White box contains information of the newborn girl (with wild type *GJB2*), the deaf boy (with homozygous 176 del C mutation) and parents (with heterozygosis 176 del C mutation).

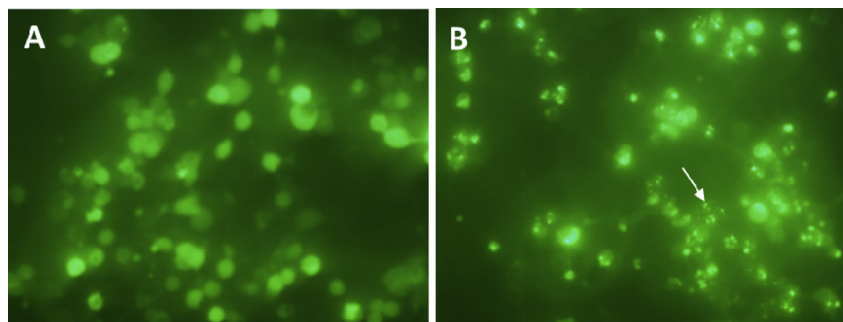


Fig. 2. GFP fused plasmid transfected into the 293T cell line to test gap junction assembly function of the mutated *GJB2* (c.176del16). (A) *GJB2* gene cloned from the deaf boy; (B) *GJB2* gene cloned from the newborn girl; arrowhead: small foci localized in cell membrane including CX26-GFP proteins.

To make a further comparison of their *GJB2* genes, we cloned the sequences of their *GJB2* genes derived from their respective blood genomic DNA into GFP fused-plasmids. Then 293T cells were used to measure gene function, showing that the mutated *GJB2* gene (*GJB2* c.176 del 16) from the first child could not form the gap junction structure between two adjacent cells, indicative of a dysfunctional *GJB2* gene. This is consistent with the phenotype of the child's deafness determined by the screening and sequencing procedures. In contrast, the *GJB2* gene from the second child has no problems helping form functional gap junctions needed for cell communication and is therefore considered healthy. The screening and sequencing results are both consistent with her ABR tested phenotype.

Our results also echoed previous studies in that the frame shift mutation of *GJB2* c.176 del 16 (Mikstiene et al., 2016; Kudo et al., 2000) is sited in the second topological domain and can erase the second transmembrane domain of Cx26. With such mutations at play, a stop codon (TGA) introduced into the *GJB2* gene and helps form a truncated CX26 protein comprising 75 amino acids. This abnormal protein can seriously inhibit the formation of hexamers (Zhang, 2009), a process reenacted by our cell line experiments (Fig. 2).

In summary, our study helped determine the possibility of a child developing hearing loss in a family carrying a deafness-

inducing gene. The methodologies adopted in this study are suitable for the identification of any hot-point or rare-point mutation in the *GJB2* gene. In our case, the child was found to have a normal (wild type) *GJB2* gene and we presume that no preemptive intervention was needed to prevent the development of hearing loss and language learning problems caused by mutated *GJB2*. However, it is impossible to exclude the existence of other causative genes in the genome of this child. So we recommend that further screening of deafness genes based on the next generation of sequencing technology should be carried out if the patients can afford it.

#### Conflicts of interest

The authors declare no conflict of interest.

#### Acknowledgments

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