Brief Report

The Anticipation and Inheritance Pattern of c.487A>G Mutation in the GJB2 Gene

Masoumeh Falah MSc[1], Massoud Houshmand PhD[2], Saeid Mahmoudian MSc[1], Hessamaldin Emamdjomeh MSc[1], Yaser Ghavami MD[1], Mohammad Farhadi MD•[1]

Abstract

Mutations in the GJB2 gene are the most common causes of hereditary hearing loss. This study reveals some facts about the inheritance pattern of M163V in the GJB2 gene.

This study was performed on two different families with non-syndromic hearing loss. We screened the GJB2 coding region with direct sequencing.

There was a substitution of A to G in exon 2 at nucleotide 487 (M163V). This mutation was heterozygous in fathers and children while mothers were normal. Fathers of both families showed late onset hearing impairment, but there was early onset hearing loss in the children, which was more severe compared to the fathers.

M163V has been reported as an unknown heterozygous mutation that leads to failure of the homotypic junctional channel formation. Another mutation in this codon is M163L, with an autosomal dominant inheritance, which impairs trafficking through the plasma membrane, resulting in cell death. Assessment of the familial pedigree has revealed anticipation in phenotype and autosomal dominant inheritance. These data in addition to the high conservation of methionine residue in mammalian species suggest that M163V is inherited with an autosomal dominant pattern. Therefore, the risk of inheritance will increase. Genetic counselors and otologists should prioritize the evaluation and prevention of this disorder in patients.

Keywords: Connexin26, DFNB1, dominant, GJB2, gap junctions

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Introduction

Hearing loss is the most frequent inherited sensory disorder in humans.[1] In previous studies, about 130 loci have been described in non- syndromic hearing loss and 47 related genes have been mapped so far.[2]

The first locus, DFNB1 was identified by Guilford et al. in 1994. Three years later, mutations in the GJB2 gene, which encodes the gap junction protein connexin26 (Cx26), were shown to be responsible for deafness at this locus.[3]

In the cochlea, Cx26 proteins have important roles in the recirculation of potassium ions.[4]

This cyclic ionic flux is crucial for the auditory process since it enables the transduction of sound into nerve impulses which are then directed to the brain for processing.

GJB2 is a small gene about 5500-bp length with 2 exons, of which only 1 contains the coding region.[5]

Hereditary hearing loss can be inherited as an autosomal dominant, autosomal recessive, X-linked or mitochondrial pattern.[2] Most of the GJB2 mutations described so far are recessive but a few dominant patterns variations associated to hearing impairment have also been identified.[6]

Identifying the inheritance pattern of these mutations could help genetic counselor for setting priority in evaluation, and prevention of the disease. The aim of the current study is to confirm M163V mutation causes a disease with the autosomal dominant pattern.

Authors' affiliations: [1]Department and Research Centre of ENT and Head and Neck Surgery Tehran University of Medical Sciences, Tehran, Iran, [2]National Institute for Genetic Engineering and Biotechnology, Tehran, Islamic Republic of Iran.
•**Corresponding author and reprints:** Mohammad Farhadi MD, Department and Research Centre of ENT and Head and neck Surgery, Tehran University of Medical Sciences, Niayesh St., Sattarkhan St., Tehran, Iran.
Tel: +98-21-665-52828, Fax: +98-21-665-25329,
E-mail: mfa.ent@gmail.com
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Materials and Methods

Two different Iranian families with non-syndromic hearing loss were studied. This study was approved by the Ethics Committee of Tehran University of Medical Sciences and signed informed consents were obtained from the patients.

A total of six family members underwent otoscopic examination and audiometric testing. Hearing levels were determined by pure-tone audiometry with frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz at intensities up to120 dB.

Environmental causes (such as infectious diseases and ototoxic drugs) were excluded by interviewing the patients. Peripheral blood samples were taken from each member of the families and genomic DNA was isolated using a FlexiGene DNA Kit (Cat. No. 51204, QIAGEN, U.S.) according to the manufacturer's instructions.

An 806 bp DNA fragment that contained the coding exon of the GJB2 gene (exon 2) was amplified by PCR using the following primers: forward: 5'-CTC CCT GTT CTG TCC TAG CT-3' and reverse: 5'-CTC ATC CCT CTC ATG CTG TC-3' (Gene Bank: NG_008358.1).

PCR was performed under the following conditions: initial denaturation at 96°C for 3 min, 5 cycles of denaturation at 95°C for 1 min, annealing at 59ºC for 1 min, extension at 72°C for 1 min, 26 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 45 sec, extension at 72°C for 45 sec, followed by 8 min of final extension at 72°C.

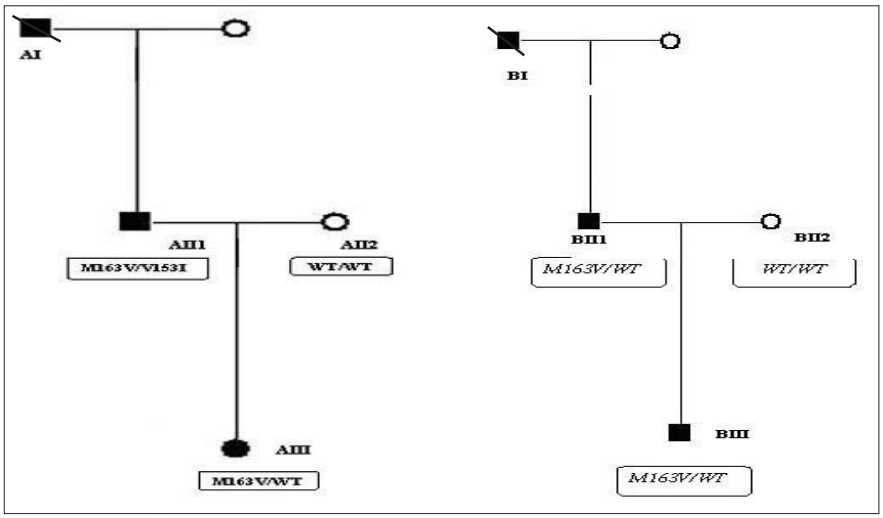

Figure1. Pedigree of the family A and B.

The PCR product was subjected to direct sequencing by the chain termination method on an ABI 3730 XL sequencer. The sequencing results were analyzed by Codon Code Aligner 3.5.2.

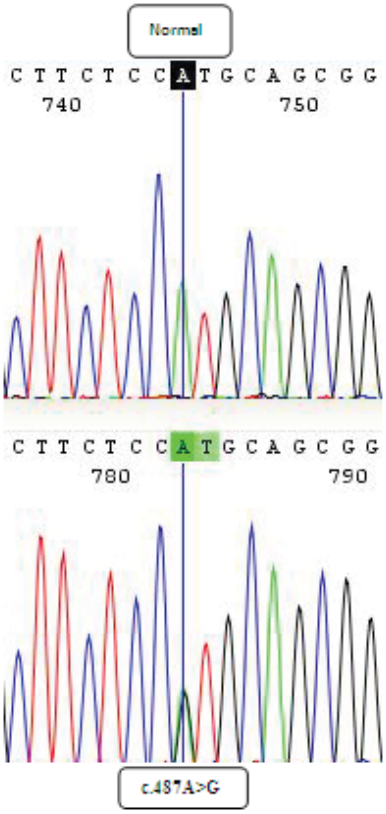

Figure 2. Nucleotide sequence of the heterozygous variant c.487A>G compare with normal control.

Results

The phenotypes and genotypes of the members in each studied family are as follows: in family A, the deaf child (AIII) was heterozygous for the c.487A > G mutation (Figure 1). This mutation causes the substitution of the methionine amino acid residue at position 163 of the Cx26 protein for a valine (p.M163V; Figure 2). Compound heterozygous M163V/V153I was associated with a moderate hearing impairment in the child's 34 year-old father (AII1). V153I is a polymorphism associated with the GJB2 gene.[7] In this family, the grandfather (AI) also had a hearing impairment but he was not alive to be screened. The mother of the family (AII2) was normal for pure-tone test and was Wt/Wt for the GJB2 gene.

In family B, the proband (BIII) was a nine year-old boy with a mixed severe hearing impairment, heterozygous for M163V/WT (Figure 1). The child's father (BII1) had the same heterozygous genotype with mild high frequency hearing impairment that began one year ago. As with family A, the grandfather was also positive for a hearing impairment. The mother (BII2) was normal for pure-tone test and was Wt/Wt for the GJB2 gene.

The fathers and grandfathers of both families showed late onset hearing impairment. In fathers, this began in the third or fourth decades of their lives, whereas in the grandfathers the onset was in the sixth decade of their lives.

Discussion

A broad spectrum of GJB2 mutations associated with hearing loss have been reported.[2] The c.487A > G mutation in its heterozygous form was found in both families screened in this study. This mutation causes the substitution of methionine amino acid at position 163 of the Cx26 protein, which is located in the second extracellular loop with valine (p.M163V). This methionine residue is highly conserved among most mammalian species.[6,7]

Assessment of the familial pedigrees in our study revealed anticipation in phenotype and autosomal dominant inheritance. In the two studied families, the fathers also had M163V mutations with late onset of hearing impairment during the third or fourth decades of their lives, whereas their children exhibited early onset hearing impairment. The children had more severe impairment than the fathers.

Several different studies have shown that the M163V mutation is heterozygous.[7–11] Dalamon et al. in 2005 described this mutation in a 46-year-old male whose brother and parents also had the

mutation with late onset hearing loss that occurred around the fifth decade of life.[10]

In 2003, Bruzzone et al. studied the functional effects of M163V and showed that the GJB2 gene with this mutation could be translated but failed to form homotypic junctional channels.[12]

On the other hand, Matos et al. reported a new autosomal dominant mutation that changes the methionine amino acid residue at position 163 of the Cx26 protein to a leucine (c.487A > C). The resultant mutant protein causes defective trafficking through the plasma membrane and is associated with increased cell death.[6]

In conclusion, this residue (M163V) has an important role in the functional protein and is conserved between different mammalian species. This might explain why its mutations have always been observed in the heterozygous form, not as compound heterozygous. In addition to the aforementioned studies, the autosomal dominant inheritance pattern in our families is another proof that confirms this mutational pattern.

Although anticipation was observed in both families, this may be a result of a three nucleotide repeat in an unknown hearing loss associated gene or a risk modifying allele from the mother that collaborates with M163V. Until now, there has been no gene with a three nucleotide repeat in the hearing loss area. This needs additional research in the future.

Most GJB2 mutations are inherited as an autosomal recessive pattern, and if these mutations are found by themselves (heterozygous), they are less important for the genetic counselor. However, when proven to be a mutation that belongs to an autosomal dominant pattern, the risk of inheritance will increase. Therefore, genetic counselors and otologists should pay more attention to prioritize the evaluation and prevention of this disorder in patients.

Conflict of interest

The authors report no conflict of interest.

Acknowledgment

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References

1. Kudo T, Ikeda K, Kure S, Matsubara Y, Oshima T, Watanabe K, et al. Novel mutations in the connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. *Am J Med Genet*. 2000; **90:** 141 – 145.
2. Guy Van Camp RS. The hereditary hearing loss homepage. Available from: URL: http://hereditaryhearingloss.org/main.aspx?c=.HHH&n=86307
3. Schrijver I.Hereditary non-syndromic sensorineural hearing loss: transforming silence to sound. *J Mol Diagn*. 2004; **6:** 275 – 284.
4. Kikuchi T, Kimura RS, Paul DL, Takasaka T, Adams JC. Gap junction systems in the mammalian cochlea. *Brain Res Brain Res Rev*. 2000; **32:** 163 – 166.
5. Kelley PM, Cohn E, Kimberling WJ. Connexin 26: required for normal auditory function. *Brain Res Brain Res Rev*. 2000; **32:** 184 – 188.
6. Matos TD, Caria H, Simões-Teixeira H, Aasen T, Dias O, Andrea M, et al. A novel M163L mutation in connexin 26 causing cell death and associated with autosomal dominant hearing loss. *Hear Res*. 2008; **240:** 87 – 92.
7. Marlin S, Garabédian EN, Roger G, Moatti L, Matha N, Lewin P, et al. Connexin 26 gene mutations in congenitally deaf children: pitfalls for genetic counseling. *Arch Otolaryngol Head Neck Surg*. 2001; **127:** 927 – 933.
8. Yilmaz A, Menevse S, Bayazit Y, Karamert R, Ergin V, Menevse A, et al. Two Novel Missense Mutations in the Connexin 26 Gene in Turkish Patients with Nonsyndromic Hearing Loss. *Biochem Genet*. 2009; **48:** 248 – 256.
9. Hamid M, Karimipoor M, Chaleshtori MH, Akbari MT. A novel 355-357delGAG mutation and frequency of connexin-26 (GJB2) mutations in Iranian patients. *J Genet*. 2009; **88:** 359 – 362.
10. Dalamón V, Béhèran A, Diamante F, Pallares N, Diamante V, Elgoyhen AB, et al. Prevalence of GJB2 mutations and the del(GJB6-D13S1830) in Argentinean non-syndromic deaf patients. *Hear Res*. 2005; **207:** 43 – 49.
11. Bayazit YA, Cable BB, Cataloluk O, Kara C, Chamberlin P, Smith RJ, et al. GJB2 gene mutations causing familial hereditary deafness in Turkey. *Int J Pediatr Otorhinolaryngol*. 2003; **67:** 1331 – 1335.
12. Bruzzone R, Veronesi V, Gomès D, Bicego M, Duval N, Marlin S, et al. Loss-of-function and residual channel activity of connexin 26 mutations associated with non-syndromic deafness. *FEBS Lett*. 2003; **533:** 79 – 88.