Iranian J Publ Health, Vol. 43, No.12, Dec 2014, pp.1710-1713

Case Report

A Novel De Novo Dominant Mutation in *GJB2* Gene Associated with a Sporadic Case of Nonsyndromic Sensorineural Hearing Loss

***Habib ONSORI**[1], **Mohammad RAHMATI**[2], **Davood FAZLI**[3]

1. *Dept. of Genetics, Marand Branch, Islamic Azad University, Marand, Iran*
2. *Dept. of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*
3. *Dept. of Biology, Payame Noor University (PNU), Tehran, Iran*

***Corresponding Author:** Email: onsoribiomol@marandiau.ac.ir

(Received 10 Mar 2014; accepted 11 Sep 2014)

Abstract

Mutations in the *GJB2* gene are the most common known cause of hereditary congenital hearing loss. Rapid genomic DNA extraction (RGDE) method was used for genomic DNA extraction. After amplification of coding region of CX26 gene with specific primers, expected PCR products with 724bp length were subjected to direct sequencing in both directions. We describe here a novel heterozygous -T to -C transition at codon 202 (TGC→CGC) of the *GJB2* gene in a patient, 40-year-old Iranian woman, which replaces a cysteine with an arginine residue (C202R). The dominant mutation C202R associated with non-syndromic sensorineural hearing loss. This mutation has not previously been described in affected or control samples from other populations investigated for GJB2 mutations, indicating that it is a rare substitution. This dominant mutation was recorded in NCBI GenBank with accession number KF 638275.

Keywords: *GJB2* gene, Dominant mutation, Hearing loss

Introduction

Deafness is a frequent disorder that affects about 1/1000 newborns (1). Approximately 80% of congenital hearing loss cases are recessively inherited and 15% dominantly inherited. Mutations of the *GJB2* gene, encoding gap junction protein Connexin 26 (Cx26), are the most common cause of hereditary congenital hearing loss in many countries (2).

Cx26 is a member of the connexin family of gap junction proteins, which facilitate intercellular communication by encoding channels that directly link the cytoplasm of adjacent cells (3, 4). In spite of contribution of several different genes as causative agents of deafness, mutations in one gene encoding Connexin 26 (GenBank M86849, MIM 121011) with chromosomal location 13q11-12 known as DFNB1 (OMIM 220290) responsible for half of severe to profound autosomal recessive non syndromic deafness in many populations (5-8). *GJB2* is a small gene about 5500-bp length with two exons, of which only one (exon 2) contains the coding region. The coding region consists of 681 bp that encodes a gap-junction protein with 226 amino acids (6).

In the present study, we report a novel dominant mutation (C202R) in an Iranian patient with bilateral non-syndromic sensorineural hearing loss.

Case Report

A 40-year-old woman with bilateral hearing loss, for molecular analysis of deafness, was referred to Welfare Organization of Marand, Iran in August 2012. Her parents were clinically normal.

DNA extraction and PCR

For molecular analysis, genomic DNA was extracted from 1 ml of EDTA anticoagulated peripheral blood by rapid genomic DNA extraction (RGDE) method (9). Polymerase chain reaction of coding region of *CX26* gene was performed using cx26F: 5'-TCT TTT CCA GAG CAA ACC GC-3' as a forward primer and cx26R: 5'-TGG GCA ATG CGT TAA ACT GGC-3' as a reverse primer. PCR reactions were carried out in 25 µL reaction mixture containing 1 × PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 10 pmoles of each primer, 0.5 U of Taq DNA polymerase and about 1µg of genomic DNA on a SENSOQUEST (Labcycler/Germany) Thermal Cycler. PCR was performed under the following conditions: initial denaturation at 95°C for 5 min, 34 cycles of denaturation at 95°C for 45 sec, annealing at 60°C for 1 min, extension at 72°C for 1 min, followed by 8 min of final extension at 72°C. The amplified fragments were detected on 1.5% agarose gel by safe dye staining.

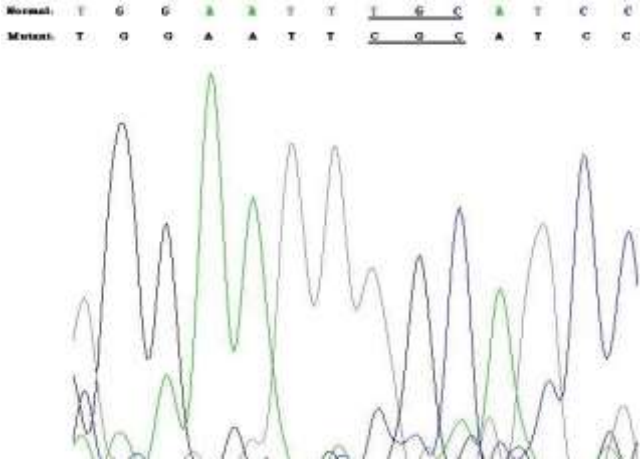

Fig. 1: DNA sequence of the *GJB2* coding exon for the heterozygous -T to -C transition mutation. Position of mutation indicated with underlining

Sequencing and Sequence analyzing

Expected PCR products with 724bp length were subjected to direct sequencing in both directions. The sequencing results were analyzed by sequencing-analysis Chromas Lite 2.1 software and were compared with the wild type.

Direct sequencing of PCR products in both directions revealed a novel heterozygous -T to -C transition at nucleotide 604 in codon 202 (TGC→CGC) of the *GJB2* gene which replaces a cysteine with an arginine residue (C202R) (Fig. 1).

Discussion

In the present study, we report very rare, novel and dominant mutation C202R in *GJB2* gene that has not previously been reported in the CX26-deafness database. Gap junctional intercellular communication (GJIC) fulfills a multitude of different functions, tailored to meet the specific needs of organs, tissues or groups of cells in which Cx are expressed. In the auditory system, intercellular channels formed predominantly by Cx26 but also Cx30 and Cx31 seem crucial for maintaining a high extracellular electrical potential in the cochlea by facilitating the local circulation of K^+ ions (2). The identification of mutations in the connexin 26 gene (GJB2: MIM# 121011) as a cause for profound sensorineural hearing impairment prompted a series of studies on *GJB2* in affected families and subjects from Europe, North America, the Near East, North Africa, and Japan (10, 11). Mutations in the *GJB2* gene are the cause of an important number of cases of non-syndromic recessive deafness but are not as common in non-syndromic dominant deafness cases (2). A few DFNB1 mutations have been related to dominantly inherited hearing impairment, both non-syndromic [R184Q (11), W44C (12), C202F (1) and R143Q (13)] and syndromic with accompanying skin disease [G12R (14), G59A (15), delE42 (16)].

To date, more than 14 different types of connexins have been identified, and each one contains four transmembrane domains (TM1–TM4), two extracellular domains (EC1–EC2), one cyto-

plasmatic loop (CL), and N and C-cytoplasmatic termini (NT–CT). The N-terminal domain is involved in the insertion of the nascent polypeptide chain into the endoplasmic reticulum and, along with the first transmembrane domain, determines voltage gating. The extracellular loops regulate the connexon- connexon interactions, including heterotypic channel formation; each loop contains three cysteine residues, conserved across all connexins that form essential intramoleculardisulphide bonds. The intracellular loop and C-terminal domain regulate pH gating (17). C202R substitution, which lies in the fourth (TM4) transmembrane domain of Cx26, may impair connexinoligomerisation. This cysteine residue is highly conserved among most mammalian species (Fig. 2).

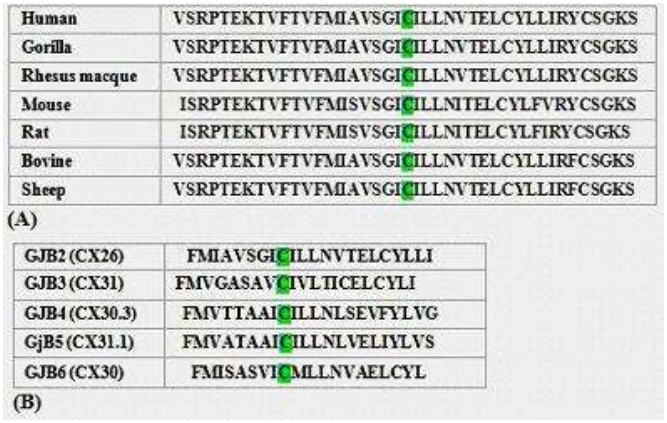

Fig. 2: Alignments of the residue conservation of the mutated area of the *GJB2* gene among the species (A) and different gap junction genes (B)

Conclusion

Dominant mutation, C202R, which associated with non-syndromic sensorineural hearing loss, has not previously been described in affected or control samples from other populations, indicating that it is a rare substitution. This mutation was recorded in NCBI GenBank with accession number KF 638275. Probably this novel mutation has prevalence in hearing loss patients in Marand region and it seems that study of causative mutations in *GJB2* gene and other genes related to deafness is necessary in this region.

This work was helpful in providing genetic counseling to the affected family and helps in confirming the clinical diagnosis.

Ethical consideration

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the author.

Acknowledgements

This study was supported by a Grant of Marand branch, Islamic Azad University. We thank our patient and her family for their cooperation in this research. We are also most grateful to the head of Welfare Organization of Marand, and Mr. Mohammad Hossein-zadeh for their help in collecting samples on hereditary deafness. The authors declare that there is no conflict of interests.

References

1. Morle L, Bozon M, Alloisio N, Latour P, Vandenberghe A, Plauchu H, Collet L, Edery P, Godet J, Lina-Granade G (2000). A novel C202F mutation in the connexin 26 gene (GJB2) associated with autosomal dominant isolated hearing loss. *J Med Genet,* 37: 368-70.
2. Rabionet R, Morales-Peralta E, Lopez-Bigas N, Lourdes Arbones M, Estivill X (2006). A novel G21R mutation of the GJB2 gene causes autosomal dominant non-syndromic congenital deafness in a Cuban family. *Genet MolBiol,* 29: 443-5.
3. Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fischer R (1998). Mutations in the connexin 26 gene (GJB2) among Ashkenazi Jews with non syndromic recessive deafness. *N Engl J Med*, 339: 1500-5.
4. White TW (2000). Functional analysis of human Cx26 mutations associated with deafness. *Brain Res Rev,* 32: 181-183.

5. Achkar WA, Moassass F, Halabi BA, Ablog AA (2011). Mutations of the Connexin 26 gene in families with non-syndromic hearing loss. *Mol Med Reports*, 4: 331-5.
6. Hamid M, Karimipoor M, Hashemzadeh M, Akbari MT (2009). A novel 355–357del GAG mutation and frequency of connexin-26 (GJB2) mutations in Iranian patients. *J Genet*, 88(3): 359-62.
7. Hashemzadeh CM, MontazerZohour M, Hoghooghi Rad L, Pour-Jafari H, Farhud DD, Dolati M (2006). Autosomal Recessive and Sporadic Non Syndromic Hearing Loss and the Incidence of Cx26 Mutations in a Province of Iran. *Iranian J Publ Health,* 35(1): 88-91.
8. Hwa HL, Ko KM, Hsu CJ, Huang CH, Chiang YL, Oong JL (2003). Mutation spectrum of the connexin 26 (GJB2) gene in Taiwanese patients with prelingual deafness. *Genet Med,* 5(3): 161–5.
9. Saremi MA, Saremi M, Tavallaei M (2008). Rapid Genomic DNA Extraction (RGDE). *Forensic SciInt,* 1: 63–5.
10. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, Leigh, IM (1997). Connexin 26 mutations in hereditary non-syndromicsensorineural deafness. *Nature*, 387: 80-3.
11. Hamelmann C, Amedofu GK, Albrecht K, Muntau B, Gelhaus A, Brobby GW, Horstmann RD (2001). Pattern of connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. *Hum Mutat*, 18: 84-5.
12. Denoyelle F, Lina-Granade G, Plauchu H, Bruzzone R, Chaib H, Levi-Acobas F, Weil D, Petit C (1998). Connexin 26 gene linked to a dominant deafness. *Nature,* 393: 319-20.
13. Loffler J, Nekahm D, Hirst-Stadlmann A, Gunther B, Menzel HJ, Utermann G. Janecke AR (2001). Sensorineural hearing loss and the incidence of Cx26 mutations in Austria. *Eur J Hum Genet,* 9: 226-30.
14. Richard G, Rouan F, Willoughby CE, Brown N, Chung P, Ryynanen M, Jabs EW, Bale SJ, DiGiovanna JJ, Uitto J, Russell L (2002). Missense mutations in GJB2 encoding connexin-26 cause the ectodermal dysplasia keratitisichthyosis-deafness syndrome. *Am J Hum Genet*, 70: 1341-48.
15. Heathcote K, Syrris P, Carter ND. Patton MA (2000). A connexin 26 mutation causes a syndrome of sensorineural hearing loss and palmoplantar hyperkeratosis (MIM 148350). *J Med Genet,* 37: 50-1.
16. Rouan F, White TW, Brown N, Taylor AM, Lucke TW, Paul DL, Munro CS, Uitto J, Hodgins MB, Richard G (2001). Trans-dominant inhibition of connexin-43 by mutant connexin-26: Implications for dominant connexin disorders affecting epidermal differentiation. *J Cell Sci,* 114**:** 2105-13.
17. Primignani P, Trotta L, Castorina P, Lalatta F, Cuda D, Murri A, Ambrosetti U, Cesarani A, Curcio C, Coviello D, Travi M (2007). A New De Novo Missense Mutation in Connexin 26 in a Sporadic Case of Nonsyndromic Deafness. *Laryngoscope*, 117: 821-24.