A novel G21R mutation of the GJB2 gene causes autosomal dominant non-syndromic congenital deafness in a Cuban family

Raquel Rabionet1, Estela Morales-Peralta2, Núria López-Bigas1, Maria Lourdes Arbonés1 and Xavier Estivill1

1Center for Genomic Regulation, Genes and Disease Program, Barcelona, Spain.
2National Center of Medical Genetics, Havana, Cuba.

Abstract

Deafness is a complex disorder affecting 1/1000 infants. In developed countries, more than 50% of deafness cases are thought to have a genetic cause. At least 40 loci for dominant non-syndromic deafness and another 30 for recessive non-syndromic deafness have been described. 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. We describe here a new dominant mutation (G21R) in the GJB2 gene which causes deafness and has been identified in a three generation Cuban family with dominant non-syndromic congenital sensorineural profound deafness.

Key words: connexin 26, GJB2, DFNA3, hearing impairment.

Received: July 11, 2005; Accepted: December 14, 2005.
formed consent was obtained from each participating member of the family or their legal guardian. Screening for mutations in the \textit{GJB2} coding region was performed using single-strand conformation polymorphism (SSCP) analysis as previously described (Rabionet \textit{et al.}, 2000), followed by the sequencing of abnormal banding patterns on an ABI 377 automated sequencer with ABI BigDye Terminators. A single G to A mutation was identified at position 61 leading to the substitution of a glycine residue at \textit{GJB2} position 21 to an arginine residue (the G21R mutation). The G21R mutation segregates with the deafness phenotype as indicated by the fact that it was present in all the affected family members investigated but absent in their non-affected relatives (Figure 1A). The G21R mutation has not previously been described in affected or control samples from other populations that have been investigated for \textit{GJB2} mutations, indicating that it is a rare substitution. In order to rule out a possible case of ‘pseudo-dominant’ inheritance, the samples were also tested for the presence of the two \textit{GJB6} deletions, del(GJB6-d13s1854) (del Castillo \textit{et al.}, 2005) and del(GJB6-D13S1830) (del Castillo \textit{et al.}, 2002), using the polymerase chain reaction (PCR) described elsewhere (del Castillo \textit{et al.}, 2005; del Castillo \textit{et al.}, 2002). The results showed that both deletions were absent, indicating that G21R is a dominant mutation.

Only 15 out of the more than 100 \textit{GJB2} mutations so far identified cause dominantly inherited hearing impairment. Syndromic deafness, accompanied by skin disease, has been reported to be caused by ten of these mutations [G12R, S17F and D50N (Richard \textit{et al.}, 2002), delE42 (Rouan \textit{et al.}, 2001), N54K (Richard \textit{et al.}, 2004), G59A (Heathcote \textit{et al.}, 2000), D66H (Maestrini \textit{et al.}, 1999), R75Q (Uyguner \textit{et al.}, 2002), R75W (Richard \textit{et al.}, 1998), and G130V(Snoeckx \textit{et al.}, 2005)]. The other reported dominant mutations in the \textit{GJB2} gene [W44C (Denoyelle \textit{et al.}, 1998), C202F (Morle \textit{et al.}, 2000), R143Q (Loffler \textit{et al.}, 2001), D179N (Primignani \textit{et al.}, 2003) and R184Q (Hamelmann \textit{et al.}, 2001)] and the G21R mutation cause non-syndromic deafness. Most of the mutations causing syndromic deafness lie in the connexin 26 first extracellular domain (Figure 2). G21R, instead, is located in the first intracellular domain, which has been proposed to be involved in voltage gating polarity (Bruzzone \textit{et al.}, 1996). The glycine at position 21 is a highly conserved amino acid, both between species and beta-connexins (Figure 1B). The non-conservative substitution of the small and neutral glycine for the bulkier and charged amino acid arginine could cause the resulting channel to be non-functional without preventing the formation of the channel itself. On the other hand, dominant mutations affecting the first extracellular domain probably interfere with hemichannel coupling.

The deafness phenotype observed in this family, congenital sensorineural profound deafness, is very similar to that described by Denoyelle \textit{et al.} (1998) in several individuals carrying the \textit{GJB2} gene W44C mutation which is also related to dominant non-syndromic hearing impairment. These patients also showed profound loss at high frequencies and moderate hearing impairment at lower frequencies.

Although \textit{GJB2} dominant mutations are less common than recessive mutations, analysis of the \textit{GJB2} gene should be considered in diagnostic tests for both syndromic and non-syndromic dominantly inherited deafness. Functional studies on this and other \textit{GJB2} dominant mutations should allow comparison of the amino acid changes that lead to syndromic and non-syndromic deafness (Richard \textit{et al.}, 2002; Rouan \textit{et al.}, 2001) and should provide a better understanding of the molecular mechanisms underlying this disorder.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A: Pedigree of the family carrying the \textit{GJB2} G21R mutation. The DNA for individuals with no genotype was not available. B: Comparison of the conservation of position 21 and surrounding amino acids in different connexins and in different species.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Dominant mutations in the connexin 26 protein. Non-syndromic mutations are underlined and are represented by dark circles while syndromic mutations are represented by white circles.}
\end{figure}
Acknowledgments

We thank the family for their cooperation. RR and NLB were supported by a BEFI grant from the FISS (98/9207 and 00/9379). This work was supported by La Marató TV3 (993610) and FIS-ISCIII (G03/203). NLB is supported by the Human Frontiers Science Program.

References

Abe S, Usami S, Shinkawa H, Kelley PM and Kimberling WJ (2000) Prevalent connexin26 gene (GJB2) mutations in Japanese. J Med Genet 37:41-3.

Bruzzone R, White TW and Goodenough DA (1996) The cellular Internet: On-line with connexins. Oxford University Press, Oxford, pp 9-21.

Cohen MM and Gorlin RJ (1995) Epidemiology, etiology and genetic patterns. In: Gorlin RJ, Toriello HV and Cohen MM (eds) Hereditary Hearing Loss and its Syndromes. Oxford University Press, Oxford, 18:709-18.

del Castillo FJ, Rodriguez-Ballesteros M, Alvarez A, Hutchin T, Leonardi E, de Oliveira CA, Azaiez H, Brownstein Z, Avenarius MR, Marlin S, Pandya A, Shahin S, Siemering KR, Wei D, Wuys W, Aguirre LA, Martin Y, Moreno-Pelayo MA, Villamar M, Avraham KB, Dahl HH, Kanaan M, Nance WE, Petit C, Smith RJ, Van Camp G, Sartorato EL, Murgia A, Moreno F and del Castillo I (2005) A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFN1 non-syndromic hearing impairment. J Med Genet 42:588-94.

del Castillo I, Villamar M, Moreno-Pelayo MA, del Castillo FJ, Alvarez A, Telleria D, Menendez I and Moreno F (2002) A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. N Engl J Med 346:243-9.

Denoyelle F, Lina-Granade G, Plauchu H, Bruzzone R, Chaib H, Levi-Acobs F, Wei D and Petiti C (1998) Connexin 26 gene linked to a dominant deafness. Nature 393:319-20.

Estivill X, Fortina P, Surrey S, Rabionet R, Melchionda S, D’Agruma L, Mansfield E, Rappaport E, Govea N, Mila M, Zelante L and Gasparini P (1998) Connexin-26 mutations in sporadic and inherited sensorineural deafness. Lancet 351:394-8.

Gasparini P, Rabionet R, Barbujani G, Melchionda S, Petersen M, Brondum-Nielsen K, Metspalu A, Oitmaa E, Pisano M, Fortina P, Zelante L and Estivill X (2000) High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 35delG. Eur J Hum Genet 8:19-23.

Hamelmann C, Amedouf GK, Albrecht K, Muntau B, Gelhaus A, Brobby GW and Horstmann RD (2001) Pattern of connexin 26 (GJB2) mutations causing sensorineural hearing impairment in Ghana. Hum Mutat 18:84-5.

Heathcote K, Syrris P, Carter ND and 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.

Loffler J, Nehaem D, Hirst-Stadlmann A, Gunther B, Menzel HJ, Utermann G and Janecke AR (2001) Sensorineural hearing loss and the incidence of Cx26 mutations in Austria. Eur J Hum Genet 9:226-30.

Maestrini E, Kerge BP, Ocana-Sierra J, Calzolari E, Cambiaghi S, Scudder PM, Hovnanian A, Monaco AP and Munro CS (1999) A missense mutation in connexin26, D66H, causes mutilating keratoderma with sensorineural deafness (Vohwinkel’s syndrome) in three unrelated families. Hum Mol Genet 8:1237-43.

Morle L, Bozon M, Allolio S, Latour P, Vandenberge A, Plauchu H, Collet L, Edery P, Godet J and Lina-Granade G (2000) A novel C202F mutation in the connexin26 gene (GJB2) associated with autosomal dominant isolated hearing loss. J Med Genet 37:368-70.

Nadol JB (1993) Hearing Loss. N Engl J Med 329:1092-1102.

Primignani P, Castorina P, Sironi F, Curcio C, Ambrosetti U and Covilli DA (2003) A novel dominant missense mutation D1-79N-in the GJB2 gene (Connexin 26) associated with non-syndromic hearing loss. Clin Genet 63:516-21.

Rabionet R, Zelante L, Lopez-Bigas N, D’Agruma L, Melchionda S, Restagno G, Arbines ML, Gasparini P and Estivill X (2000) Molecular basis of childhood deafness resulting from mutations in the GJB2 (connexin 26) gene. Hum Genet 106:40-4.

Richard G, Brown N, Ishida-Yamamoto A and Krol A (2004) Expanding the phenotypic spectrum of Cx26 disorders: Bart-Pumphrey syndrome is caused by a novel missense mutation in GJB2. J Invest Dermatol 123:856-63.

Richard G, Rouan F, Willoughby CE, Brown N, Chung P, Rynnanen M, Jabs EW, Bale SJ, DiGiovanna JJ, Uitto J and Russell L (2002) Missense mutations in GJB2 encoding connexin-26 cause the ectodermal dysplasia keratitis-ichthyosis-deafness syndrome. Am J Hum Genet 70:1341-8.

Richard G, White TW, Smith LE, Bailey RA, Compton JG, Paul DL and Bale SJ (1998) Functional defects of Cx26 resulting from a heterozygous missense mutation in a family with dominant deaf-mutism and palmoplantar keratoderma. Hum Genet 103:393-9.

Rouan F, White TW, Brown N, Taylor AM, Lucke TW, Paul DL, Munro CS, Uitto J, Hodgens MB and 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.

Snoeck RL, Hassan DM, Kamal NM, Van Den Bogaert K and Van Camp G (2005) Mutation analysis of the GJB2 (connexin 26) gene in Egypt. Hum Mutat 26:60-1.

Sobe T, Erlich P, Berry A, Korostichevsky M, Vreugde S, Avraham KB, Bonne-Tamir B and Shohat M (1999) High frequency of the deafness-associated 167delT mutation in the connexin 26 (GJB2) gene in Israeli Ashkenazim. Am J Med Genet 86:499-500.

Uyguner O, Tukel T, Baykal C, Eris H, Eminoeglui M, Hafiz G, Ghanbari A, Baserer N, Yuksel-Apak M and Wollnik B (2002) The novel R75Q mutation in the GJB2 gene causes autosomal dominant hearing loss and palmoplantar keratoderma in a Turkish family. Clin Genet 62:306-9.

Editor: Angela M. Vianna-Morgante