



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

Raquel Rabionet<sup>1</sup>, Estela Morales-Peralta<sup>2</sup>, Núria López-Bigas<sup>1</sup>, Maria Lourdes Arbonés<sup>1</sup> and Xavier Estivill<sup>1</sup>

<sup>1</sup>Center for Genomic Regulation, Genes and Disease Program, Barcelona, Spain.

<sup>2</sup>National 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.

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Deafness is a frequent disorder that affects about 1/1000 newborns, and up to 4% of people younger than 45 years old (Nadol 1993). In developed countries, more than 50% of congenital deafness is thought to have a genetic etiology. Approximately 80% of these cases are recessively inherited and 15% dominantly inherited (Cohen and Gorlin 1995).

More than 60 different mutations of the *GJB2* gene have been described as causing recessive deafness in several populations ([www.crg.es/deafness](http://www.crg.es/deafness)). In various populations the *DFNB1* gene has been shown to be the most important recessive locus (Abe *et al.*, 2000; Estivill *et al.*, 1998; Sobe *et al.*, 1999), with the *DFNB1* gene 35delG mutation being the most frequent mutation of this gene in European populations (Gasparini *et al.*, 2000). Although mutations implicated in dominantly inherited hearing impairment are not as common as those implicated in recessively inherited hearing impairment, a few *DFNB1* mutations have been related to dominantly inherited hearing impairment, both non-syndromic [W44C (Denoyelle, *et al.*, 1998), R184Q (Hamelmann *et al.*, 2001), C202F (Morle *et al.*, 2000) and R143Q (Loffler *et al.*, 2001)] and syndromic with accompanying skin disease [G12R (Richard *et al.*,

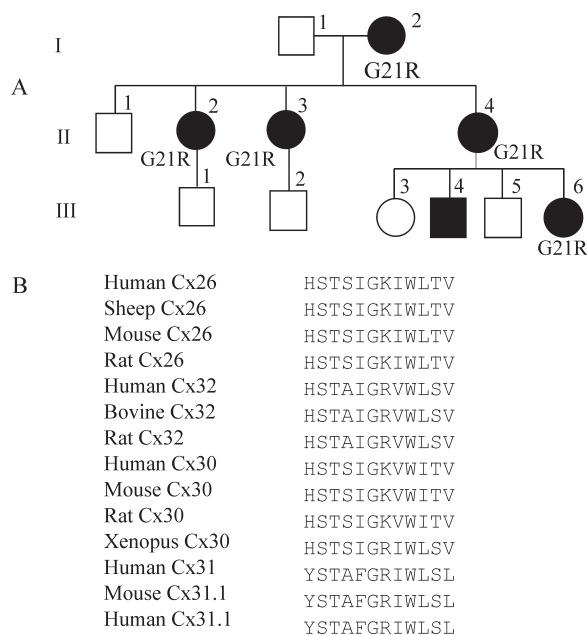
2002), delE42 (Rouan *et al.*, 2001), G59A (Heathcote *et al.*, 2000), D66H (Maestrini *et al.*, 1999) and R75W (Richard *et al.*, 1998)]. The identification of additional dominant *GJB2* mutations might help in understanding the relationship between this gene and disease phenotypes. We have identified a new *GJB2* mutation, G21R, which is responsible for a dominant non-syndromic hearing impairment phenotype segregating in a Cuban family.

We studied a Cuban family in which five individuals from three generations were affected with pre-lingual hearing impairment. Information on the progression of hearing impairment was obtained by interview. All affected members in this family presented congenital non-progressive sensorineural profound deafness. Audiometric studies were obtained for individuals III-3 and III-4 and showed profound hearing impairment at higher frequencies and a milder degree of hearing loss at lower frequencies. Environmental causes (*e.g.* infectious diseases, ototoxic drugs) for the hearing impairment in this family were excluded by interview.

Peripheral blood was obtained from five affected individuals and two non-affected relatives and DNA was extracted according to standard protocols. Samples for other members of the family were not obtained because they either could not be contacted or declined participation in the study. This study was approved by the Medical Ethics Committee of the Oncological Research Institute and in-

formed consent was obtained from each participating member of the family or their legal guardian. Screening for mutations in the *GJB2* coding region was performed using single-strand conformation polymorphism (SSCP) analysis as previously described (Rabionet *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 *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 *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 *GJB6* deletions, del(GJB6-d13s1854) (del Castillo *et al.*, 2005) and del(GJB6-D13S1830) (del Castillo *et al.*, 2002), using the polymerase chain reaction (PCR) described elsewhere (del Castillo *et al.*, 2005; del Castillo *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 *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 *et al.*, 2002), delE42 (Rouan *et al.*, 2001), N54K (Richard *et al.*, 2004), G59A

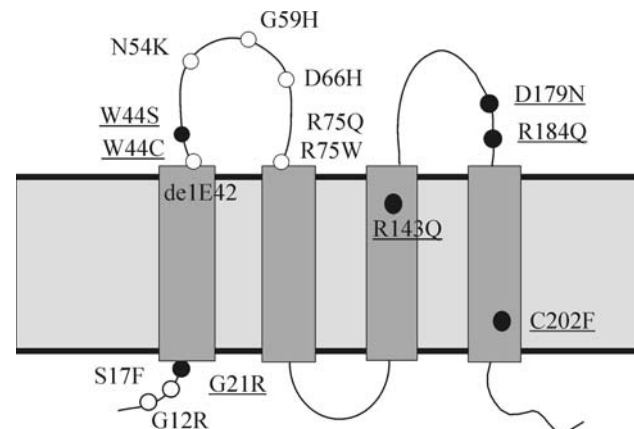


**Figure 1** - A: Pedigree of the family carrying the *GJB2* G21R mutation. The DNA for individuals with no genotype was not available. B: Comparison of the conservation of position 21 and surrounding amino acids in different connexins and in different species.

(Heathcote *et al.*, 2000), D66H (Maestrini *et al.*, 1999), R75Q (Uyguner *et al.*, 2002), R75W (Richard *et al.*, 1998), and G130V(Snoeckx *et al.*, 2005)]. The other reported dominant mutations in the *GJB2* gene [W44C (Denoyelle *et al.*, 1998), C202F (Morle *et al.*, 2000), R143Q (Loffler *et al.*, 2001), D179N (Primignani *et al.*, 2003) and R184Q (Hamelmann *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 *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 *et al.* (1998) in several individuals carrying the *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 *GJB2* dominant mutations are less common than recessive mutations, analysis of the *GJB2* gene should be considered in diagnostic tests for both syndromic and non-syndromic dominantly inherited deafness. Functional studies on this and other *GJB2* dominant mutations should allow comparison of the amino acid changes that lead to syndromic and non-syndromic deafness (Richard *et al.*, 2002; Rouan *et al.*, 2001) and should provide a better understanding of the molecular mechanisms underlying this disorder.



**Figure 2** - 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.

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