

## Brief Research Communication

# No Association Between *DFNA6* and Pro250Arg Mutation in *FGFR3*

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*DFNA6* is a locus mapped to 4p16.3 in a family with dominant, delayed-onset, low-frequency, progressive, sensorineural, nonsyndromic hereditary hearing impairment [Lesperance et al., 1995]. The fibroblast growth factor receptor 3-gene (*FGFR3*), which also maps to 4p16.3, has been proposed as a candidate gene for *DFNA6* [Colvin et al., 1996; Lesperance et al., 1995; Muenke et al., 1997]. Recently, the Pro250Arg mutation in *FGFR3* has been found to cosegregate with autosomal dominant craniosynostosis and bilateral sensorineural hearing loss in a large Australian family [Hollway et al., 1998]. Because the penetrance of the craniosynostosis was low and its manifestations often subtle, it was hypothesized that some families with the Pro250Arg mutation could present with deafness alone. The hearing loss in the reported family resembled that of the *DFNA6* family in that it was bilateral, sensorineural, of moderate degree, and predominantly low-frequency. However, the hearing loss affecting the *DFNA6* family differed in that it was delayed in onset (age 5–15 years) as opposed to congenital, and progressive, although usually not to profound hearing loss.

The point mutation Pro250Arg in the *FGFR3* gene causes a C-to-G transversion at nucleotide 749 and results in a Pro250Arg amino acid substitution in the extracellular domain of the *FGFR3* protein. We analysed DNA from 16 members of the *DFNA6* family for the presence of the mutation. Of the 16, nine were affected family members, four were unaffected, and three were spouses. Amplification of genomic DNAs was performed using the forward and reverse polymerase chain reaction (PCR) primers described by Bellus et al. [Bellus et al., 1996]: 5'-CGGCAGTGACGGTGGTGTGA-3' and 5'-CCAAATCCTCACGCAACCC-3', respectively. Two hundred nanograms of genomic DNA was used as a template in 25  $\mu$ L PCRs consisting of 10

mM Tris-HCL pH 8.3, 50 mM KCL, 1.5 mM MgCl<sub>2</sub>, 5 mM  $\beta$ -mercaptoethanol, 0.001% (w/v) gelatin, 10% DMSO, 0.25 mM dNTPs, 1 mM of each primer and 5 U KlenTaq I polymerase (Ab Peptides, Saint Louis, MO.). PCRs were performed in a PTC-100™ Programmable Thermal Controller (Model 96V, MJ Research, Watertown, MA) under the following conditions: denaturation (94°C, 3 min), 30 cycles of amplification (94°C for 1 min, 60°C for 1 min, and 72°C for 3 min), extension (72°C, 15 min). The final products were digested with the restriction enzyme NciI and analysed in a 1.5% agarose gel. The final PCR products (341 bp) were cleaved into 218 bp and 123 bp fragments in all individuals. To rule out partial digestion, the digested products were transferred from an agarose gel onto a Hybond-N+ nylon membrane (Amersham Life Science Inc., Cleveland, OH), and the Southern blot was hybridized against the 341 base pair PCR fragment. No additional fragments of 151 bp and 67 bp, which would be created by the Pro250Arg mutation, were observed. Thus, we found no evidence for the Pro250Arg mutation in *DFNA6* patients. Further studies will be necessary to rule out other mutations in the *FGFR3* gene as the underlying cause of hearing loss in this family.

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