Letter to the Editor

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Mutations in the Mitochondrial tRNA Ser(UCN) and in the *GJB2* (Connexin 26) Gene Are Not Modifiers of the Age at Onset or Severity of Hearing Loss in Spanish Patients with the 12S rRNA A1555G Mutation

To the Editor:

Late-onset deafness is likely to be the result of complex interactions between genetic susceptibility factors and the environment. Although enormous progress is being achieved in the identification of genes that cause congenital deafness, less is known about the progressive forms of hearing loss. The A1555G mutation in the mitochondrial 12S rRNA has been associated with aminoglycoside-induced and nonsyndromic sensorineural deafness (Prezant et al. 1993). We have demonstrated (Estivill et al. 1998*b*) that the A1555G mutation is also present in a large proportion of families and patients with deafness who were not treated with aminoglycosides; it has also been shown (Torroni et al. 1999) that many independent origins account for the high frequency of this mutation in patients with hearing loss.

The difference in the expression of the deafness phenotype in families with the A1555G mutation could be explained by different levels of exposure to environmental agents, other than aminoglycosides, that have not yet been determined. However, it could also be due to additional mutations in a nuclear gene, to other variants in the mitochondrial DNA, or, more likely, to complex interactions of genetic and environmental factors.

To date, efforts to identify a nuclear gene acting as a modifying factor for deafness in patients with the A1555G mutation have not been successful (Guan et al. 1996; Bykhovskaya et al. 1998). Recently, Pandya et al. (1999) demonstrated the coexistence of two mitochondrial mutations—G7444A in the tRNA Ser(UCN) and A1555G—in Mongolian students who were deaf. They suggested that patients with both mutations in the mitochondrial DNA may present earlier onset and an increased severity of hearing loss than other patients with the A1555G mutation alone. They proposed digenic epistasis for A1555G and G7444A, especially in the absence of aminoglycoside exposure.

We have studied the relationship between A1555G and other mutations in the mitochondrial DNA affecting the tRNA Ser(UCN) and mutations in exon 2 of the connexin 26 gene, for which a high carrier frequency has been described in the Mediterranean population (Zelante et al. 1997; Estivill et al. 1998*a*; Gasparini et al. 2000). After analyzing 42 unrelated families with the A1555G mutation, we report evidence that these genes do not modify the severity and age at onset of hearing loss in Spanish patients.

In an earlier work we identified 19 families with maternally transmitted deafness that had the A1555G mutation (Estivill et al. 1998*b*). Now we have collected data on 139 new families with sensorineural hearing loss: 109 multiplex families (43 families with compatible autosomal dominant hearing loss, 53 with compatible autosomal recessive hearing loss, and 13 with maternally inherited hearing loss) and 30 simplex families. These new families were tested for the A1555G mutation, as described elsewhere (Estivill et al. 1998*b*). We found the A1555G mutation in 23 of these families (11 maternally inherited, 7 autosomal dominant, 2 autosomal recessive, and 3 sporadic). By combining the families presented here with those described earlier, we identified a total of 209 families with deafness, 42 (20%) having the A1555G mutation. Of these 42 families, a diagnosis of deafness was established in 338 of 668 subjects (50%) with the mutation, indicating an incomplete penetrance of the A1555G mutation, in agreement with our earlier observations (Estivill et al. 1998*b*). The age at onset of deafness in these patients ranged widely (0–65 years). Only ~20% of patients with the A1555G mutation and deafness were treated with aminoglycosides prior to their hearing loss. Negative data about treatment with aminoglycosides were obtained from the patients and their parents. This negative history included being given any drug by injection or having undergone major surgery. These data indicate the strong role of factors other than antibiotics in the development of deafness.

We have analyzed the presence of mutations in the mitochondrial tRNA Ser(UCN) gene by digestion with the *Xba*I restriction enzyme, as described by Reid et al. (1994). Loss of the *Xba*I site can be due to mutations A7443G and A7445C, described by Pandya et al. (1999); to mutation G7444A, reported in association with Leber's Hereditary Optic Neuropathy (Brown et al.

1995); or to mutation A7445G, initially identified in a Scottish pedigree with sensorineural hearing loss (Reid et al. 1994). We have not detected any of these changes in a total of 42 unrelated patients with hearing loss, representing all of the A1555G-positive families, nor in 112 additional deaf patients with A1555G from these families (a total of 154 subjects with hearing loss who were ages 0–65 years at the onset of their deafness). In contrast with previous findings about deaf students from Mongolia (Pandya et al. 1999), the G7444A mutation was not associated with A1555G in the 42 Spanish families with deafness. Thus, G7444A is unlikely to be a modifier of the wide clinical variability of the deafness phenotype due to the A1555G mutation in Spanish patients.

Mutations in the connexin 26 gene (*GJB2*) are found in a large proportion of patients with autosomal recessive and dominant deafness (Kelsell et al. 1997; Zelante et al. 1997; Denoyelle et al. 1998). Mutation 35delG in *GJB2* accounts for 41% of deafness with childhood onset in European populations (Estivill et al. 1998*a*). Carrier frequencies for 35delG of 1/75 in central and northern Europe and of 1/35 in the Mediterranean region have been found (Gasparini et al. 2000).

We have studied the *GJB2* 35delG mutation in the same sample set of 154 deaf patients with A1555G that were analyzed for tRNA Ser(UCN); the analysis of the mutation was performed as described in the study by Rabionet and Estivill (1998). We also analyzed the coding region of *GJB2* (exon 2) in the sample set of 42 unrelated individuals representing each of the A1555G-positive families by SSCP analysis. We detected only one family with the 35delG mutation in heterozygosity, including four patients also having the A1555G mutation and two patients with A1555G alone. None of these individuals was treated with aminoglycosides. All had moderate deafness, except for the youngest member of the family (age 16 years) who had the A1555G mutation alone. The onset of deafness in the other patient carrying the A1555G mutation alone was at age ~20 years, whereas the onset of deafness in the patients with both mutations was at ages 16–30 years. Thus, in this family, 35delG mutation does not seem to modify the deafness phenotype due to the A1555G mutation. No other changes in the *GJB2* gene were found in this sample of 42 unrelated patients with A1555G.

Since the frequency of 35delG was similar in patients with A1555G (1 in 42) and in the Spanish general population (1 in 40) (Estivill et al. 1998*a*), it is unlikely that the *GJB2* gene is a major modifier for hearing loss due to the A1555G mutation in Spanish patients.

In conclusion, we demonstrate that mutations in the connexin 26 gene or in the mitochondrial tRNA Ser(UCN) gene are not modifying factors for the wide variability of the age at onset and severity of hearing loss in Spanish patients with the A1555G mutation. The identification of the different agents that participate in hearing loss in the presence of the A1555G mutation should help in the understanding of the complex interactions between environment and genes in hearing and may facilitate the development of prevention and therapeutic interventions.

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N. López-Bigas,[1] R. Rabionet,[1] E. Martinez,[1] O. Bravo,[2] J. Girons,[2] A. Borragan,[4] M. Pellicer,[3] M. L. Arbonés,[1] and X. Estivill[1]
[1]*Medical and Molecular Genetics Center, Hospital Duran i Reynals, L'Hospitalet,* [2]*Otolaryngology Service, Bellvitge Hospital, L'Hospitalet de Llobregat, and* [3]*Pediatrics Otolaryngology Service, Hospital de la Vall d'Hebron, Barcelona; and* [4]*Centro de Foniatría y Logopedia, Santander, Spain*

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Address for correspondence and reprints: Dr. Xavier Estivill, Medical and Molecular Genetics Center, Hospital Duran i Reynals, Autovia Castelldefels Km 2.7, 08907 L'Hospitalet de Llobregat, Barcelona, Catalonia, Spain; e-mail: estivill@iro.es

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