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Apparent phenotypic anticipation in autosomal dominant connexin 26 deafness

Abstract

Background: Connexin 26 (*GJB2*) mutations are associated with various types of hearing loss, either without associated symptoms or with skin disease, constituting a form of syndromic hearing loss. These mutations can lead to deafness in either a recessive or a dominant autosomal form of inheritance.

Methods: Ascertainment of a Jewish Ashkenazi family with nonsyndromic hearing loss led to the construction of a pedigree for a four-generation family, with hearing loss detected in three successive generations. The entire coding region of the *GJB2* gene was amplified and sequenced by Sanger sequencing.

Results: Audiological analysis revealed that the age of onset and severity of hearing loss were earlier and more severe, respectively, in each successive generation of an Ashkenazi Jewish family. A mutation, c.224G>A, leading to missense p.Arg75Gln was detected only in the affected members of the family.

Conclusions: The entire coding region of *GJB2* should be checked in hearing-impaired patients by Sanger sequencing, rather than examination only of the two most prevalent mutations, regardless of mode of inheritance or ethnicity. Furthermore, predictions regarding phenotype based on genotype can be difficult to make due to clinical

variability in multigenerational families, as demonstrated in the family presented in this study.

Keywords: cochlea; Cx26; *GJB2*; hearing impairment; hearing loss.

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Introduction

Hearing loss (HL) is the most frequent sensory disorder in humans, with about half of the cases due to genetic causes. *GJB2* was the first gene found to be involved in nonsyndromic HL (NSHL), discovered in 1997 [1]. Up to 50% of autosomal recessive sensorineural HL (SNHL) is caused by mutations in the *GJB2* gene, which encodes the connexin 26 protein (Cx26). To date, more than 100 mutations in *GJB2* have been discovered (Connexin-Deafness Homepage, <http://davinci.crg.es/deafness/>). The *GJB2* c.35delG mutation accounts for up to 70% of all *GJB2* mutations for autosomal recessively inherited HL [2]. Among the Jewish Ashkenazi population, the c.167delT mutation in *GJB2* is most prevalent [3]. *GJB2* is also involved in dominant forms of deafness [4] and in syndromic HL (SHL), specifically with skin disorders, in addition to deafness [5]. The c.224G>A mutation, which leads to an exchange of arginine to glutamine at position 75 of the protein, p.Arg75Gln (also referred to as R75Q), was first described in a Turkish family with autosomal dominant hearing impairment and palmoplantar keratoderma (PPK) [6]. This mutation was also described in Italian and French families with mild to profound SNHL, inherited in an autosomal dominant fashion, either with or without skin disease [7, 8]. In the present study, we describe an Ashkenazi Jewish family with NSHL inherited in an autosomal dominant manner and associated with the *GJB2* R75Q mutation. The phenotype in this family was marked by variable severity and varying age at onset with apparent anticipation, with a more severe HL from generation to generation.

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Materials and methods

The study was approved by the Israel Ministry of Health Helsinki Committee. Seven members of a Jewish Ashkenazi family residing in Israel, including five individuals with different degrees of NSHL, were ascertained, and a pedigree was constructed. Blood samples were collected from individuals of three generations: II – 1, 2, 3; III – 2, 3; and IV – 1, 2. Genomic DNA was extracted from peripheral blood samples using standard procedures. The entire coding region of the *GJB2* gene (exon 2) was amplified and sequenced as described previously [9]. Environmental causes such as infectious diseases and ototoxic drugs were excluded by interviewing the patients. Audiograms were obtained for all hearing-impaired individuals (II – 2; III – 2, 3; and IV – 1, 2), as well as auditory brainstem response (ABR) results for the two children (IV – 1, 2).

Results

The *GJB2* c.224G>A mutation was detected by Sanger sequencing in all affected family members and was not identified in the two hearing family members, II-1 and II-3 (Figure 1A). No other variant was found in the *GJB2* coding sequence on the second allele of the patients. There was no presence of the *GJB6* deletion [10]. The proband III-3 was diagnosed as an infant with bilateral moderate to severe HL, which deteriorated by the age of 31 to severe to profound SNHL. By the age of 3, her pure-tone average (PTA), reflecting the average of the four speech frequencies of 500, 1000, 2000, and 4000 Hz, was 75 dB in the better

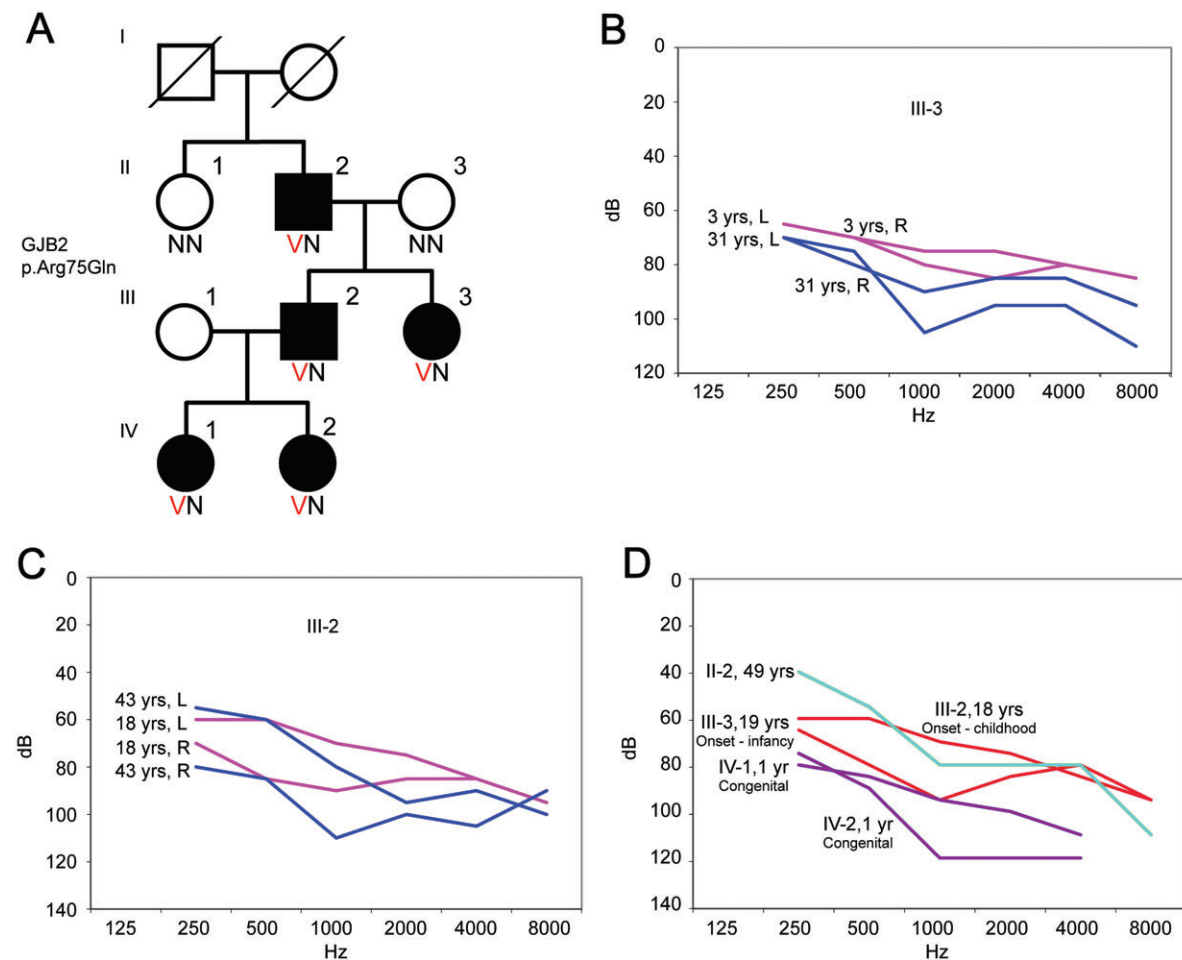


Figure 1 The multigenerational family exhibits HL with a dominant mode of inheritance.

(A) Pedigree of the family with HL (black symbols). A c.224G>A mutation was identified in the *GJB2* gene encoding the connexin 26 protein, predicted to lead to a p.Arg75Gln missense mutation, by Sanger sequencing. N, no variant; V, variant. (B) Pure-tone audiogram of proband III-3 demonstrating progression of HL between the ages of 3 (pink lines, left, L, and right, R, ears) and 31 (blue lines, left and right ears). (C) Pure-tone audiogram of III-2, demonstrating progress of HL between the ages of 18 (pink lines, left and right ears) and 43 (blue lines, left and right ears). (D) Pure-tone audiograms of all hearing-impaired family members, demonstrating a decline in hearing from generation to generation. Each individual is represented by an audiogram from the better ear, with onset of HL and age of test indicated. Audiograms at similar ages in each generation were selected, with each generation shown in a different color.

ear and 80 dB in the other ear, while at 31 years of age, it reached 85 dB in the better ear and 95 dB in the other ear (Figure 1B). Her older brother, III-2, was diagnosed at the age of 5 with moderate HL by report, and at the age of 18, he felt a decline in his hearing in his right ear, which was confirmed by an audiogram showing a PTA of 85 dB in this ear, compared with 72.5 dB in the left ear. By the age of 43, his PTA deteriorated to 80 dB in the left ear and 90 dB in the right ear (Figure 1C). Their father, II-2, was diagnosed in his early 20s and at the age of 64 showed a symmetric sloping audiogram of bilateral moderate to severe SNHL, with a PTA of 75–80 dB (Figure 1C), similar to the audiogram obtained at the age of 49. The two daughters of III-2, IV-1 (currently aged 7) and IV-2 (currently aged 5), have congenital profound SNHL that was diagnosed immediately after birth by ABR. For both siblings, no responses were obtained for the 90-dB maximum output of the ABR device. Later, audiograms showed a PTA of over 110 dB for both children (Figure 1D). Both children have cochlear implants from 1 year of age, with successful outcomes. Figure 1D shows audiograms of all impaired family members, demonstrating the decline in hearing with each subsequent generation. No information is available regarding the hearing status of generation I, now deceased.

Discussion

The c.224G>A mutation in the *GJB2* gene leads to an exchange of arginine to glutamine at position 75 of the protein (p.Arg75Gln). Another mutation is known at the same site, p.Arg75Trp, also associated with dominant HL and PPK [6]. Both these mutations were shown to have a dominant-negative effect on wild-type connexin 26. It was demonstrated that these connexin 26 mutants form gap junction plaques with the wild-type connexin 26, suggested to cause defective docking or gating, leading to the disruption of the proper function of the channels [11]. In another study, it was found that the dominant-negative p.Arg75Trp mutation in mice, which causes failure of intercellular communication, delays programmed cell death around the organ of Corti, resulting in the collapse of the organ of Corti [12]. It was suggested that this outcome is true for all dominant-negative *GJB2* mutations, strongly supporting the deleterious impact of the p.Arg75Gln mutation.

The *GJB2* p.Arg75Gln mutation was detected in our research study in an Ashkenazi Jewish family for the first time. Moreover, this is also the first dominant mutation detected in *GJB2* in the Jewish population. These findings emphasize that it is essential not only to check for the common *GJB2* mutations in the Jewish population but also

to sequence the entire coding region of *GJB2*. We recommend that Sanger sequencing be used as a routine diagnostic method in all hearing-impaired patients, including those with a suspected recessive or dominant mode of inheritance.

Assessment of the phenotypes of the patients in our study was marked by variable age of onset with apparent anticipation. Phenotypic anticipation is the tendency for some genetic disorders to manifest themselves at an earlier age and/or to increase in severity with each succeeding generation [13]. In the studied family, the grandfather (II-2) noticed his HL in his early 20s with a PTA of 75–80 dB, whereas his daughter (III-3) had severe SNHL with a PTA of 85 dB at 3 years of age and his son (III-2) had a severe to profound HL with a PTA of 80 dB in the better ear since childhood. His granddaughters (IV-1, IV-2) had congenital profound SNHL and obtained cochlear implants at the age of one. The children exhibited a more severe HL than their father and his sister did, who in turn had a more severe HL than their grandfather did (Figure 1C). These differences in severity and onset emphasize the inability to predict the levels of HL associated with *GJB2* mutations. Genotype-phenotype correlations have been found in a multicenter study of *GJB2*-mutation patients, but a large phenotypic variability still exists, making predictions difficult [14]. While the phenotypic variability may be due to modifier genes, in a study on patients with homozygous 35delG mutations, no major modifier was found [15].

Manifestation at an earlier age and/or an increase in severity with each succeeding generation was previously seen in other families with deafness associated with the p.Arg75Gln dominant mutation in *GJB2*. Two French families presented with autosomal dominant HL due to the p.Arg75Gln mutation. In the first family, a mother and her son both presented with congenital SNHL with no apparent skin abnormalities [8]. However, while the hearing defect was moderate/severe in the mother, it was profound in the child. In the second family, the father developed PPK during infancy and was diagnosed with mild SNHL at the age of 18. His elder daughter had a progressive mild HL detected at the age of 10, associated with bilateral palm ichthyosis. The younger daughter had a progressive moderate SNHL, diagnosed at the age of 4, with PPK diagnosed at age 2. Another *GJB2* dominant missense mutation, p.Met163Val, was associated with genetic anticipation [16]. The authors described two families in which the fathers had late-onset HL, beginning in the third or fourth decade, whereas their children exhibited onset at a younger age, with a more severe hearing impairment.

Anticipation is prevalent in trinucleotide repeat disorders such as Huntington's disease and myotonic dystrophy, where a dynamic mutation in DNA occurs [13]. This

phenomenon, while less common, has been demonstrated in families with diseases segregating with missense mutations. For example, autosomal dominant inheritance with genetic anticipation of missense mutations was observed in familial Ménière's disease [17]. Missense mutations in SOD1, leading to familial amyotrophic lateral sclerosis, were associated with anticipation [18]. A missense mutation in the hTERT gene was associated with anticipation in a three-generation family with dyskeratosis congenita [19]. The mechanisms for the phenotypic variability and apparent anticipation are unclear and may be due to the presence of other genetic variants or represent ascertainment bias or more awareness of symptoms in the present time [13].

In conclusion, we identified a dominantly inherited c.224G>A mutation in the *GJB2* gene for the first time in a Jewish Ashkenazi family, leading to p.Arg75Gln, emphasizing the need to sequence the entire coding region of *GJB2* in each deaf patient, including those manifesting dominant inheritance, on a routine basis. We provide evidence for phenotypic anticipation with a decrease in the age of diagnosis and increase in severity of HL in successive generations. The molecular mechanism behind the apparent anticipation in this family, as in other families with the same p.Arg75Gln mutation or with other missense mutations, is unclear. Nevertheless, these findings may have important implications for genetic counseling, molecular testing, and clinical management.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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