International Journal of Pediatric Otorhinolaryngology (2009) **73**, 437—443

ELSEVIER

International Journal of Pediatric Otorhinolaryngology

www.elsevier.com/locate/ijporl

High frequency of connexin26 (GJB2) mutations associated with nonsyndromic hearing loss in the population of Kerala, India

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Received 23 September 2008; received in revised form 27 November 2008; accepted 27 November 2008

KEYWORDS
GJB2;
Hearing loss;
Kerala population;
Mutation detection

Summary

Objective: Mutations in connexin 26 gene (GJB2) are the most common cause of hearing loss in different populations. The aim of our study was to determine the prevalence of GJB2 mutations in the population of Kerala, India.
Methods: This study was conducted on the genomic DNA of 86 affected subjects and their relatives from 59 families of Kerala, India. Mutation detection was done by sequencing and PCR—RFLP.
Results: 36% of the probands had mutations in the GJB2 gene. We found that 45% (15/33) of the families that had a family history of deafness had mutations in GJB2 gene. Two different mutations were identified. W24X mutation was detected in 32.5% of the affected patients. Analysis of control samples revealed a carrier frequency of 0.0357 for this mutation. The estimation of haplotype frequency revealed that there was a significant association between the W24X mutation and the haplotype in this region with respect to the markers, D13S143 and D13S175 suggesting a founder effect for this mutation in this population. A novel mutation, R32L was detected in 3.5% of the affected patients. Structural prediction revealed that this mutation alters the helical structure of the first transmembrane domain of GJB2 protein resulting in defective gap junctions.
Conclusion: Mutations in connexin26 is responsible for 36% of non-syndromic sensorineural deafness in the population of Kerala, India.

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0165-5876/$ — see front matter
doi:10.1016/j.ijporl.2008.11.010

1. Introduction

Congenital deafness is a major malady affecting one in 1000 newborns across different populations. Roughly, half of severe childhood deafness is attributed to genetic causes [1]. The spectrum of hereditary deafness is broad and ranges from simple deafness without other clinical abnormalities to genetically determined syndromes in which deafness is one of a number of clinically recognizable signs. Non-syndromic hearing impairment (NSHI) accounts for two thirds of the genetic deafness identified so far. Among NSHI, the most common forms are those transmitted as an autosomal recessive trait (Non Syndromic Recessive deafness or NSRD) accounting for 75—80% cases of childhood prelingual deafness.

Connexin 26 (GJB2) was the first gene reported to be responsible for autosomal recessive non-syndromic deafness [2]. Mutations in this gene account for up to 50% of all cases of prelingual deafness in tested populations [3—5]. It causes both autosomal dominant and autosomal recessive forms of deafness. It has been established that mutations in GJB2 gene are a major cause of inherited and sporadic non-syndromic deafness in various populations. The relatively small size of the GJB2 gene resulted in the rapid identification of mutations in this gene. One of the most common GJB2 variants identified is 35delG. It has been found to be the most common hearing loss associated GJB2 allele in Caucasian population [6]. In a population study conducted in the Mediterranean population, 35delG was found to account for 82% of all GJB2 deafness alleles [4]. The 167delT, 235delC and R143W alleles are the most common hearing loss associated GJB2 alleles in the Ashkenazi Jewish, Japanese and Ghanian population respectively [5,7,8]. Although there are population differences in the distribution of various GJB2 alleles, there is a relatively high carrier rate of GJB2 alleles in all described populations.

Connexin 26 is a member of a large family of proteins involved in the formation of gap junctions which allow the direct transfer of small molecules and ions between neighbouring cells. Gap junctions are numerous in the cochlear and vestibular portion of the inner ear where GJB2 is one of the major connexins expressed [9]. It has been suggested that these gap junctions are involved in the local circulation of potassium ions between the fluids of the inner ear [10]. The network of connexin channels in the fibrocytes and epithelial supporting cells of the inner ear may be involved in the recirculation of potassium ions to the stria vascularis where potassium ions are pumped back into the cochlear endolymph to restore a high potassium level. Defects in connexins may therefore reduce the efficiency of potassium ion circulation and consequently lead to impaired hearing sensitiviy.

Hearing impairment affects a relatively large percentage of the population of India. Among the different types of deafness observed, congenital non syndromic sensorineural deafness accounts for a significant number deafness cases. Mutations in GJB2 have been previously reported in Indian population [11,12]. This study was conducted to estimate the frequency of connexin26 mutations and to identify novel mutations associated with non syndromic sensorineural deafness in the population of the South Indian state of Kerala.

2. Materials and methods

2.1. Family data

For this study, 59 families with nonsyndromic congenital deafness were enrolled for genetic analysis. Twenty of these families were consanguineous. Blood samples were collected from 86 probands and their parents and siblings with normal hearing. We presumed the pattern of inheritance in these families with two hearing parents and at least one affected child to be autosomal recessive. Diagnosis of non-syndromic deafness among the participants was established by accepted clinical criteria. Infection, oto-trauma and pharmaceutical treatment as the cause of deafness was excluded by collecting the detailed clinical history. The ethical committee related to the institution approved the work. Written informed consent was obtained from all the participants.

2.2. Audiology

Audiometric assessment of the affected individuals and their families was done by National Institute of Speech and Hearing (NISH), Thiruvananthapuram. Various audiometric tests such as behavioral observation audiometry (BOA), tympanometry, reflexometry and brainstem evoked response audiometry (BERA) were conducted for children below 3 years. Conditioned audiometry was done for children above 3 years. Hearing loss was assessed as severe to profound sensorineural by these tests.

2.3. Mutation detection

Genomic DNA was prepared from blood. The full-length GJB2 coding sequence was amplified using the primers GJB2F (5′-TCT TTT CCA gAg CAA ACC gC-3′) and GJB2R (5′-gggCAATgCgTTAAACTggC-3′). The