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Main Article

Screening for gap junction protein beta-2 gene mutations in Malays with autosomal recessive, non-syndromic hearing loss, using denaturing high performance liquid chromatography

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Abstract

Objective: To determine the frequency and type of gap junction protein beta-2 gene mutations in Malay patients with autosomal recessive, non-syndromic hearing loss.

Methods: A total of 33 Malay patients with autosomal recessive, non-syndromic hearing loss were screened for mutations in the Cx26 coding region. Deoxyribonucleic acid was extracted from buccal swab samples and subjected to polymerase chain reaction. Slow-reannealing was performed, followed by screening using denaturing high performance liquid chromatography.

Results: Eight of the samples (24.2 per cent) showed heterozygous peaks, and further sequencing of these samples revealed four patients (50.0 per cent) with the W24X mutation, two (25.0 per cent) with the V37I mutation and another two (25.0 per cent) with the G4D mutation.

Conclusions: Analysis of buccal swab samples by denaturing high performance liquid chromatography is noninvasive and suitable for rapid and reliable screening of gap junction protein beta-2 gene mutations in patients with autosomal recessive, non-syndromic hearing loss. Malay patients with autosomal recessive, non-syndromic hearing loss have different kinds of gap junction protein beta-2 gene mutations which are rarely found in other populations.

Key words: GJB2 Protein, Human; Sensorineural Hearing Loss; Congenital; Malaysia; Human Genetics

Introduction

Hearing loss is the most common congenital sensory deficit in humans. Roughly one to three children in a thousand are born with hearing impairment.[1–5] The occurrence of hearing loss is considerably greater in certain sub-populations; for example, it is present in one to five in 100 neonatal intensive care patients and infants selected from at-risk registers.[3,6–11] Hearing loss can be caused by environmental factors as well as genetic factors. Genetic causes represent 50–70 per cent of hearing loss, with autosomal recessive inheritance representing approximately 80 per cent of this total.[12] It is believed that more than 100 genes may be involved in hearing impairment. Several of these genes have been identified, one of which is the gap junction protein beta-2 gene. The identification of this deafness gene has facilitated understanding of the molecular process of hearing, and it offers prospects for deoxyribonucleic acid (DNA) testing.

Mutations in the gap junction protein beta-2 gene (also know as the connexin 26 gene) are responsible for half of the cases of autosomal recessive, non-syndromic hearing loss. The 35delG mutation of this gene has been reported to be common in several countries.[13] However, in non-white populations, the 35delG mutation is either absent or very rare, with other common mutations prevailing, such as the 235delC mutation in the Japanese,[14] the V37I mutation in Malaysians[15] and the 167delT mutation in Ashkenazi Jews.[16]

The increasing demand for gap junction protein beta-2 gene mutation detection warrants the need for a rapid and accurate method of screening for these mutations. This study was undertaken in order (1) to investigate the types and frequencies of gap junction protein beta-2 gene mutations in Malay patients with autosomal recessive, non-syndromic hearing loss, and (2) to assess the effectiveness of buccal smears and denaturing high

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performance liquid chromatography in screening for such mutations.

Materials and methods

This study received approval from the Universiti Sains Malaysia research and ethics committee.

The study group comprised 33 Malay patients with autosomal recessive, non-syndromic hearing loss. The exclusion criteria were acquired hearing loss and mixed race Malay origin. Written consent was obtained from all patients before sampling.

Samples were collected by gently rubbing the patient's inner cheek with a sterile cotton swab. Genomic DNA was extracted from the swab using the GeneAll® DNA extraction kit (General Biosystem, Seoul, South Korea), according to the manufacturer's protocol.

The collected DNA was then subjected to polymerase chain reaction, using a first primer pair as described by Zelante *et al.*, [17] and electrophoresed on 1.5 per cent agarose gel to confirm the presence of amplicons. The polymerase chain reaction product was then mixed with wild-type amplicons in a ratio of 1:1. Slow-reannealing was performed before screening for mutations, using denaturing high performance liquid chromatography. Samples which showed heterozygous peaks were then sequenced, using an automated DNA sequencing system.

Results

Buccal smears were taken in all subjects, without complication. In all 33 patients, the buccal smear sample provided sufficient genomic DNA to enable polymerase chain reaction of the second exon of the gap junction protein beta-2 gene, and subsequent DNA sequencing.

Following denaturing high performance liquid chromatography, eight (24.2 per cent) of the 33 samples showed heterozygous peaks. Sequencing

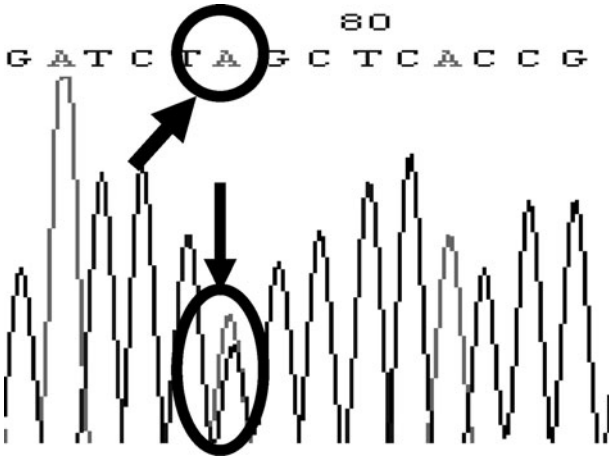

FIG. 1

Sequencing analysis of a patient's deoxyribonucleic acid, showing a heterozygous G/A nucleotide at position 71G→A (W24X) of the gap junction protein beta-2 gene. G = guanine; A = adenine; T = thymine; C = cytosine

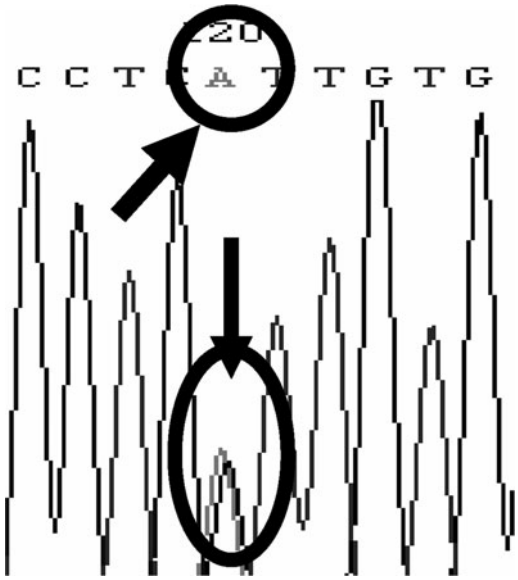

FIG. 2

Sequencing analysis of a patient's deoxyribonucleic acid, showing a heterozygous G/A nucleotide at position 109G→A (V37I) of the gap junction protein beta-2 gene. C = cytosine; T = thymine; A = adenine; G = guanine

results indicated that four patients (50.0 per cent) were carrying the W24X mutation, two (25.0 per cent) the V37I mutation and another two (25.0 per cent) the G4D mutation. Figures 1, 2 and 3 show the sequencing results for samples with mutations.

Discussion

Severe to profound congenital deafness is a common genetic disorder. Mutations in the gap junction protein beta-2 gene, encoding the connexin 26 gap-junction protein, account for a significant proportion

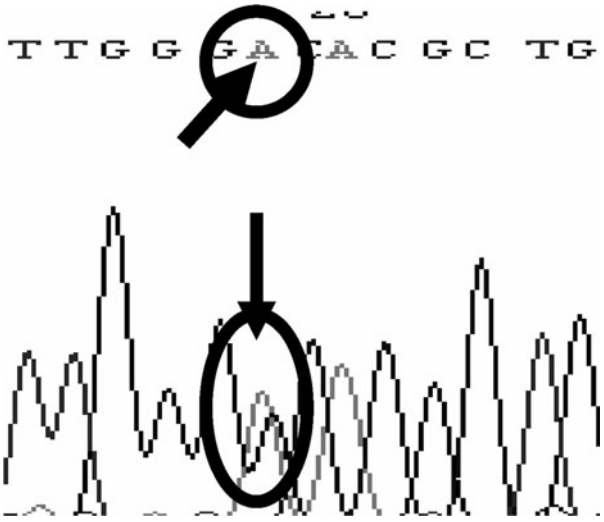

FIG. 3

Sequencing analysis of a patient's deoxyribonucleic acid, showing a heterozygous G/A nucleotide at position 11G→A (G4D) of the gap junction protein beta-2 gene. T = thymine; G = guanine; A = adenine; C = cytosine

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of autosomal recessive, non-syndromic hearing loss. The substantial contribution to gap junction genes encode beta connexins of several nuclear genes[18] has been established in some populations. More than 20 gap junction protein beta-2 gene mutations have been described, in different population distributions. The prevalence of some gap junction protein beta-2 gene mutations is known to depend on the population's ethnic origin.

Data concerning the molecular basis of autosomal recessive, non-syndromic hearing loss in the Malaysia population are scarce. The present study was designed to explore the effectiveness of denaturing high performance liquid chromatography as a molecular scanning method for analysis of gap junction protein beta-2 gene mutations, and also to assess the pattern of this mutation within a Malay population.

Buccal smears were able to be taken conveniently without complication in all subjects. This non-invasive method provided sufficient genomic DNA for subsequent analysis. Analytical results suggested that denaturing high performance liquid chromatography screening followed by DNA sequencing was adequate for identification of all sequence alterations in Cx26. All eight samples found to have heterozygous peaks on denaturing high performance liquid chromatography analysis were found to have gap junction protein beta-2 gene mutations. Our results show that denaturing high performance liquid chromatography is a highly specific technique for mutation detection, and is applicable for the detection of gap junction protein beta-2 gene mutations. Lin *et al.*[19] found that both the sensitivity and specificity of denaturing high performance liquid chromatography in detecting gap junction protein beta-2 gene sequence alterations were 100 per cent. Gurtler *et al.*[20] reported these values as 100 and 83 per cent, respectively.

In our analysis, gap junction protein beta-2 gene mutations were observed in 24.0 per cent of patients. Three different types of mutations were detected – W24X, V37I and G4D – of which W24X was the most common (50 per cent), followed by V37I and G4D in equal frequencies (both 25 per cent).

Mutation of the W24X gene sequence is very rare in many populations. A very low prevalence has been reported in Thais,[21] Greeks,[22] Iranians[23] and Germans.[24] However, it is common in other populations, such as in India[25] and Pakistan.[26] Minarik *et al.*[27] and Alvarez *et al.*[28] found the W24X mutation to be the commonest mutation in Solvak Romany and Spanish Romani (i.e. Gypsy) ethnic groups, both of which originated in India. These ethnic groups accounted for 23.2 and 79.0 per cent of autosomal recessive, non-syndromic hearing loss patients, respective to Solvak Romany[27] and Spanish Romani[28]. Kalay *et al.* observed that the W24X mutation was the second most common gap junction protein beta-2 gene mutation in a group of Turkish autosomal recessive, non-syndromic hearing loss sufferers[29] (the majority of whom originated from Middle Asia). These findings support the suggestion that the W24X mutation originates from Asian populations.

The V37I mutation was observed in 25.0 per cent of our patients. Dahl *et al.* observed that this mutation was the commonest gap junction protein beta-2 gene mutation in patients from the west coast of Malaysia.[15] However, this study did not specify the ethnic origin(s) of the population studied. Our study focussed on patients of Malay origin. The V37I mutation has been found to be relatively common in Chinese Australians,[30] and has also been detected at low percentages in Japanese[14] and Chinese populations.[31]

- **This study assessed the frequency and type of gap junction protein beta-2 gene mutations in Malay patients with autosomal recessive, non-syndromic hearing loss**
- **Increasing demand for gap junction protein beta-2 gene mutation detection warrants the need for a rapid and accurate method of screening for such mutations**
- **The 35delG mutation is not common in Malays with autosomal recessive, non-syndromic hearing loss. The most common gap junction protein beta-2 gene mutation is W24X**
- **The G4D mutation is a new type of gap junction protein beta-2 gene mutation found in Asian populations with autosomal recessive, non-syndromic hearing loss**

Two out of eight patients (25.0 per cent) in our sample group were found to carry the G4D type of gap junction protein beta-2 gene mutation. To our knowledge, there have been only two previously reported cases of the G4D mutation. In Texas, Tang *et al.*[32] reported one case of G4D mutation out of 46 patients with hearing impairment and gap junction protein beta-2 gene mutations. In an African American population with congenital cytomegalovirus infection in Alabama, Ross *et al.*[33] found the G4D mutation in a normal hearing subject and reported it as a novel mutation. We believe that this very rare mutation is one of the important causes of deafness within the Malay population of Malaysia.

Our findings on gap junction protein beta-2 gene mutations in Malay patients suggest that the mutation pattern differs from that found within other Asian populations, e.g. in Japan,[14] Thailand,[21] China[31] and even in other parts of Malaysia.[15] Even though the most common gap junction protein beta-2 gene mutation (i.e. W24X) has also been found in Indian[25] and Pakistani[26] populations, the V371 and G4D mutations have not been reported in those countries. However, we suggest that further study, of a larger population, is needed in

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order to confirm the pattern of gap junction protein beta-2 gene mutations in the Malay population of Malaysia.

Conclusion

In this study, we found buccal swabbing to be a good alternative to blood testing when collecting samples for genomic DNA analysis. Denaturing high performance liquid chromatography analysis was found to be a suitable method for rapid and reliable screening of gap junction protein beta-2 gene mutations in patients with autosomal recessive, non-syndromic hearing loss. Malay patients with this type of hearing loss had different kinds of gap junction protein beta-2 mutations, some of which have been rarely reported in other populations.

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