Prevalence Of The Connexin 26 Mutation 35delG In Non-Syndromic Hearing Loss In Egypt

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Citation

Abstract
The prevalence of the 35delG mutation in Connexin 26 gene-in thirty-one Egyptian families diagnosed with NSHL-was examined. Each nuclear family had between two to five cases of sensorineural hearing loss, with no symptoms or features suggestive of any other possible etiology. Control subjects were studied to determine the carrier rate of connexin 26 in the general population. All DNA samples were screened for the 35delG mutation using an allele-specific polymerase chain reaction (AS-PCR). We found that the 35delG mutation is much less common in Egypt (~10%) than in many other parts of the world, with a carrier rate of 2.7%.

PUBLICATION NOTE
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INTRODUCTION
Hearing loss may have deleterious effects on a person’s life. Social, educational and vocational aspects are all affected according to the degree of hearing loss. Severe prelingual hearing loss has a great impact on speech and language development in hearing-impaired children. Children with severe to profound hearing loss lack constant auditory stimulation, as well as accurate feedback of their own speech.

Hearing loss as a consequence of genetic defects is one of the most frequent forms of hearing impairment, affecting approximately 1 in 1,000 children in many parts of the world. In Egypt, this number rises significantly due to high rates of consanguinity (Country Profile on Disability, Arab Republic of Egypt, Japan International Cooperation Agency, http://www.jica.go.jp/english/global/dis/profile.html). Approximately 70% of genetic hearing loss cases are isolated or nonsyndromic (i.e., deafness is the only symptom), and 30% are syndromic [i.e., deafness is part of a larger set of medical symptoms].

Introducing molecular diagnosis and clinical genetic testing to medical practice makes precise identification of hearing impairment and genetic counseling possible. In the past two years, considerable progress has been made in the mapping and cloning of human deafness genes. Up to 90 genes are known to be involved in hearing loss, designated as DFNB1-B39 for autosomal recessive hearing loss and DFNA1-A51 for autosomal dominant hearing loss (Hereditary Hearing Loss Homepage, http://www.uia.ac.be/dnalab/hhh/). However, mutations in the connexin 26 (locus designation GJB2) gene at the DFNB1 locus, located on the long arm of chromosome 13, may account for half of all early-onset cases of hereditary nonsyndromic hearing loss (NSHL).

Furthermore, the 35delG mutation in GJB2 is documented as the most common mutation in patients with NSHL in many parts of the world. NSHL is highly prevalent in Egypt due to the phenomenon of consanguineous marriage, which reaches up to 90% in rural areas. Therefore, we performed a systematic screening program to evaluate the etiology of hearing loss in the prelingually deaf population. Our attention was directed to molecular diagnosis of the most prevalent mutation encountered as a cause of DFNB1, the 35delG mutation, which has not been previously studied in the Egyptian population.

SUBJECTS AND METHOD
SUBJECTS
A total of 31 Egyptian families were included in our study.
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Each nuclear family had between 2 to 5 cases of NSHL with no history suggestive of any other possible etiology. All deaf cases, numbering a total of 83, were examined medically and had no other associated clinical findings other than hearing loss. Our evaluation included a meticulous history taking, a pedigree analysis as well as a full audiological evaluation (pure-tone audiometry, speech audiometry and auditory brainstem response when needed) at the Audiology Unit, Sohag University Hospitals. We consulted other departments, e.g. Ophthalmology and Nephrology, when suspicions of syndromic hearing loss arose. The neighbors of each family, having no family history of deafness of any cause, were included as controls in our study (74 subjects). Blood samples were taken after obtaining written informed consent from each individual and, in the case of individuals under 18, from their parents.

MUTATION DETECTION

Blood samples were taken for genomic DNA extraction and molecular studies from all deaf people and the control group. All DNA samples were screened for the 35delG mutation using an allele-specific polymerase chain reaction (AS-PCR). Experiments were performed at a private laboratory in Cairo. The AS-PCR was performed using 40 ng of DNA in a 8.5 µl PCR reaction mixture as follows: 1.25 µl PCR buffer (100 mmol tris-hydrochloride, pH 8.8, 500 mmol potassium chloride, 15 mmol magnesium chloride, 0.01% w/v gelatin); 200 mmol each of dATP, dCTP, dGTP, dTTP; 0.25 µl Taq polymerase (5 U/µl), and 15 pmol each of either normal or mutant primer and, the common primer; and 15 pmol each of control primer A, 5'CCCACCTTCCTCCTCCAGCACAATGGG-3', and control primer B, 5'GGGCCTAGTCCCCACATGCTCAAGAGGT-3' 13. For the 35delG AS-PCR, we used the normal primer 5'TTGGGGCACGCTGCAGACGATCCTGGGGAG-3'; the mutant primer 5'TTGGGGCACGCTGCAGACGATCCTGGGGAT-3'; and the common primer 5'GAAGTAGTGATCGTAGCACACGTTCTTGCA-3'. Samples were denatured at 95°C for 5 minutes, followed by 34 cycles of 95°C for 40 seconds, 60°C for 30 seconds and 72°C for 1 minute. The PCR products were analyzed by electrophoresis on a 1.0% agarose gel containing ethidium bromide.

RESULTS

Only 14 of the tested subjects (N=83) were found to have a 35delG mutation, as revealed by AS-PCR; 8 were homozygous and 6 were heterozygous. Five families had both homozygous and heterozygous mutations, 3 families had only homozygous mutations and one family had only heterozygous mutations. The heterozygous group with a 35delG mutation had better hearing thresholds by 5-10 dBs than the homozygous group. Two carriers were observed among the control group.

DISCUSSION

In Egypt, the prevalence of inherited prelingual hearing loss is among the highest in the world. This could be attributed to the extended consanguineous marriages in the Egyptian community. We carried out a screening program for the most common mutation known to cause autosomal-recessive NSHL, the 35delG mutation. We found that the 35delG mutation is much less common in Egypt (~10%) and the carrier rate is low (2.7%). These results are comparable to the results of similar studies conducted in the Middle East region. The 35delG mutation does not appear to be the leading mutation in Turks, Palestinians, Iranians and Israelis. The high prevalence of deafness, high levels of consanguinity encountered in our study, and the low number of individuals with 35delG connexin 26 mutations, suggest that there are several other genes responsible for deafness in
the Egyptian population. In regions with high rates of congenital deafness such as Egypt, genetic diagnosis is crucial for genetic counseling and further management. Screening for hereditary deafness in this population may aid in the early diagnosis and rehabilitation of very young children.

As a consequence of the above mentioned results, many future goals arose:

1. Determination of other connexin 26 mutations that exist.
2. Determine if connexin 26 heterozygotes have connexin 30 deletions on the 2nd allele.
3. Identification of any new deafness genes in Egyptian population.

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References
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