Connexin 26 gene in non-syndromic hearing loss

Thesis

Submitted for partial fulfillment of MD degree in Audiology

by

Mona Ahmed El-Akkad

M.B, B.CH., MSc.

Supervisors

Prof.Dr.Ahmad Sameh Farid

Professor of ENT and Audiology

Faculty of Medicine

Cairo University

Prof.Dr.Mohamed Ibrahim Shabana

Professor of Audiology

Faculty of Medicine

Cairo University

Prof.Dr.Maha Hassan Abou-Elew

Professor of Audiology

Faculty of Medicine

Cairo University

Prof. Dr.Olfat Gamil Shaker

Professor of Medical Biochemistry

Faculty of Medicine

Cairo University

2012

ABSTRACT

Background: Mutations in *GJB2* gene, encoding the gap-junction protein connexion 26 (Cx26) are the leading cause of deafness in autosomal recessive nonsyndromic sensorineural hearing loss (ARNSHL) and the 35delG mutation is the most common in many ethnic groups. **Aim:** To evaluate the extent of contribution of the 35delG mutation of *GJB2* gene to ARNSHL in the Egyptian population as well as to correlate the clinical relevance of this mutation to the severity of hearing loss (HL). **Methodology:** Thirty seven patients with congenital ARNSHL and 30 control subjects with normal hearing were enrolled in the study. Patients had variable degrees of bilateral sensorineural hearing loss (SNHL) ranging in severity from mild to profound. Screening for 35delG mutation was performed by allele specific-PCR (AS-PCR). **Results:** The 35delG mutation was found in 7 patients (18.9 %), 5 homozygous and 2 heterozygous for this mutation. The frequency of the mutant allele among patients was 16.21% (12/74 chromosomes).Genotype-phenotype relation of the 35delG mutation in our patients revealed that bilateral profound SNHL was detected in 5 patients: 3 homozygous and 2 heterozygous for the mutation. In the remaining 2 patients with homozygous alleles: one had severe and the other suffered from moderate SNHL. The allelic frequency of 35delG mutation among control group was 1.7% (1/60 chromosomes). **Conclusion:** The 35delG mutation of *GJB2* gene is an important contributor to ARNSHL among Egyptian population. It has a considerable phenotypic variation on the degree of HL. Further studies are needed on larger scale to identify other mutations in *GJB2* gene as well as other genes implicated in the development of ARNSHL.

Keywords: Nonsyndromic autosomal recessive sensorineural hearing loss, *GJB2*, Connexin 26, 35delG mutation.

Summary

Genetic hearing loss approximately accounts for a considerable percent of deafness that approximately to 50% in children (Willems, 2000). The aim of this studey was to detect 35delG mutation in children and adults with early onset of non syndromic autosomal recessive SNHL in addition to correlation with degree and configuration of audiogram. Thirty-seven patients (17 males, 20 females) with age range from 5 to 32 years and 30 age and sex matched controls were selected from the Audiology Unit Outpatient Clinic at Kasr Al-Aini hospital. Full history taking was obtained from patients/ parents and full audiological investigations (hearing threshold level test, speech audiometry and immittancemetry) after being classified as non syndromic hearing loss.

The inclusion criteria in this study were: (1)Bilateral sensorineural hearing loss (SNHL) dating since birth or early childhood of variable degree of severity.(2)Normal otological otoscopic examination, ophthalmological and neurological examination.(3)Pattern of inheritance in familial cases was of the recessive type.

The exclusion criteria were: (1) Any accompanying stigmata for syndromic hearing loss. (2) An autosomal dominant pattern of inheritance (parents complain of hearing loss). (3) Abnormal radiological cochlear anatomy. (4) Those that were showing other causes of acquirad SNHL, for example, pre, peri, and postnatal problems, otitis media, meningities, cytomegalovirus etc. were excluded.

At biochemistry department laboratory; a blood sample (5ml) was drawn from the patients in EDTA tube or a CBC tube and then genomic

DNA was extracted from each sample using a “Quiagen kit”. Allele specific PCR (AS-PCR) was used to detect 35delG mutation ***(Scott et al., 1998).***

The 35delG mutation was detected in 7 out of 37 patients (18.9%), 5 of them (71.4%) carried the mutation in a homozygous state (13.5%) while 2 patients were heterozygous (5.4%) for the mutation. The frequency of the mutant allele among patients was 16.2% (12/74 chromosomes). Out of the 30 controls; only one had heterozygous 35delG mutations denoting a carrier rate of 1.7 %(1/60 chromosomes)**.**

Positive 35delG mutations were found in 4 females (57.1%) and 3 males (42.9%) with their age ranging from 5-31 years with a mean of 7.37 ± 9.08. Four positive familial (13.3%) and 26 (86.4 %) sporadic patients were found in negative 35delG patients. Out of seven patients positive for 35delG mutation; 3 had a familial and 4 had a sporadic hearing loss. A positive consanguinity was found in 6 patients.

Regarding genotype-phenotype relation of the 7 patients carrying the 35delG mutation, five (71.4%) patients had bilateral profound SNHL, one (14.3%) patient had bilateral severe SNHL and one (14.3%) patient had bilateral moderate SNHL. Only one of the 30 control subjects (3.3%) had heterozygous 35delG mutation. The allelic frequency of 35delG mutation among control group was 1.67% (1/60 chromosomes).

Results revealed 5 patients (13.5%) with 35delG were homozygous, while 2 patients (5.4%) were heterozygous. Where 30 patients (81.1%) were negative 35delG, with only 1 (3.3%) control was homozygous. For the homozygous cases, mostly profound SNHL, with positive consanguinity and progressive type of hearing loss. As regards gender there were no differences reported significantly through history and examination.

The 35delG mutation is a contributor to autosomal recessive non syndromic SNHL. It represents 18.9% in the studied sample. The allelic mutation was 16.2%. The allelic frequency of 35delG mutation in normal population of the sample studies was 1.7%. Homozygous mutation was found more than heterozygous in the positive 35delG mutation. Although all 35delG mutation positive patients were found in consanguineous marriages sporadic HL was significantly higher in both study and control groups. The 35delG mutation has a considerable phenotype-genotype variation. No statistical difference was found between positive and negative 35delG mutation as regards course, degree and configuration of HL. Further studies are needed on a larger scale to identify other mutations in *GJB2* gene as well as other genes implicated in the development of ARNSHL.