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Hearing impairment in Estonia: An algorithm to investigate genetic causes in pediatric patients

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ABSTRACT

Purpose: The present study was initiated to establish the etiological causes of early onset hearing loss (HL) among Estonian children between 2000-2009.

Methods: The study group consisted of 233 probands who were first tested with an arrayed primer extension assay, which covers 199 mutations in 7 genes (*GJB2, GJB6, GJB3, SLC26A4, SLC26A5* genes, and two mitochondrial genes – *12S rRNA, tRNA*$^{Ser(UCN)}$). From probands whose etiology of HL remained unknown, DNA analysis of congenital cytomegalovirus (CMV) infection and G-banded karyotype and/or chromosomal microarray analysis (CMA) were performed.

Results: In 110 (47%) cases, the etiology of HL was genetic and in 5 (2%) congenital CMV infection was diagnosed. We found mutations with clinical significance in *GJB2* (100 children, 43%) and in 2 mitochondrial genes (2 patients, 1%). A single mutation in *SLC26A4* gene was detected in 5 probands (2.2%) and was considered diagnostic. In 4 probands a heterozygous IVS2-2A>G change in the *SLC26A5* gene was found. We did not find any instances of homozygosity for this splice variant in the probands. CMA identified in 4 probands chromosomal regions with the loss of one allele. In 2 of them we were able to conclude that the found abnormalities are definitely pathogenic (12q13.3-q14.2 and 17q22-23.2 microdeletion), but the pathogenity of 2 other findings (3p26.2 and 1p33 microdeletion) remained unknown.

Conclusion: This practical diagnostic algorithm confirmed the etiology of early onset HL for 115 Estonian patients (49%). This algorithm may be generalized to other populations for clinical application.

Key words: : Sensorineural hearing loss; *GJB2* gene; congenital cytomegalovirus; chromosomal microarray analysis

INTRODUCTION

Hearing loss (HL) is a sensory condition affecting millions of people worldwide, and although not life-threatening may impact social and professional life [1]. The global prevalence rate of children born with HL is approximately 1 to 2 per 1000 [2-5]. The etiology of HL is extremely heterogeneous. While environmental factors such as congenital cytomegalovirus (CMV) infection, prenatal rubella infection, prematurity and meningitis are thought to be the cause of 40-50% of HL cases, the remainder are genetic and result from mutations involving any one of numerous loci [2,6]. The incidence of genetic HL is increasing because acquired impaired hearing

from meningitis is decreasing as a consequence of improved prenatal and neonatal care, antibiotic therapy and vaccination programs [2,7-9]. Current research estimates that 1% of the 30,000—50,000 human genes are necessary for hearing, of which more than 95 independent genes and 170 loci have been identified as causes of HL [8-10].

The majority of deaf children are born to normal hearing parents (90-95%) and in most of these families there is no history of HL [7,8,11,12]. It is caused by the fact that the autosomal-recessive forms of HL are most common and usually more severe than the other forms of sensorineural hearing loss (SNHL) [13]. Mutations in the *GJB2* gene, which have been mapped to 13q11-q12 and encode the gap junction protein connexin 26 (MIM 121011), represent a major cause of pre-lingual, non-syndromic, recessive deafness [13-15]. One specific mutation, c.35delG, accounts for the vast majority of the *GJB2* mutations detected in Caucasian populations and represents one of the most frequent disease-associated mutations identified so far [13].

To identify the genetic basis of HL, mutation screening of certain genes is offered to patients with HL. In children with early onset HL, *GJB2* and *GJB6* gene are tested most often [13]. During the last fifteen years, major achievements have been made in detecting new deafness genes. Unfortunately, most diagnostic tests are still performed using the classical sequencing technology, which is expensive and time consuming. For this reason, only a very small set of genes is routinely screened for mutation with result that in a large percentage of individuals with HL, no genetic cause is identified [5]. An additional problem for extended diagnostic screening is that most genes for autosomal-recessive SNHL lead to congenital severe-to-profound hearing impairment (HI) that is indistinguishable between different genes [5].

The present study, was initiated to establish the genetic and congenital causes of early onset HL among Estonian children. Due to very heterogeneous genetic etiology of early onset HL, we decided to work-out diagnostic algorithm, which has been practically applied to care for children who are deaf or hard of hearing in Estonia.

MATERIAL AND METHODS

Study group

The overall study group consisted of 233 probands (children ranging in age from 0-18 years) who were referred to genetic evaluation between 2000-2009 from the whole of Estonia, with early or childhood onset HL as a main complaint. The diagnosis of HL was confirmed by audiologists in the hearing centers of Estonia. All the probands were selected from children who were referred to an otorhinolaryngologist due to a suspicion of HL or were selected from the newborn hearing screening (NBHS) program.

The NBHS program started in Estonia in 2004 and by 2009 88% of all newborns were included in the program. In Estonia, we have three-stage NBHS. All children whose HI with a pure tone average $(PTA)_{0.5\text{-}4kHz}$ is less than 40 dB in the better ear should be identified by NBHS. The definition of the degree and type of HI was based on the most recent audiogram available. The severity of HI was graded by the degree of HL in the better ear as mild (21-40 dB), moderate (41-70 dB), severe (71-95 dB) and profound (greater than 95 dB).

All children were evaluated in one of two tertiary education hospitals, Tallinn Children's Hospital for northern and western Estonia, and Tartu University Hospital for south eastern Estonia based on investigation program presented in the *Fig. 1.* In all cases of HL, family histories were collected, focusing particularly on the potential occurrence of HL in multiple generations. The clinical examination was performed with particular attention to dysmorphic features including growth parameters, facial phenotype, external ears, neck, skin, hair, eyes and digits, to exclude syndromic causes of HL.

Cytogenetic and molecular investigations

The detailed pathway of all investigations is presented in *Fig 1.*

All probands were tested between 2005 and 2009 with an arrayed primer extension (APEX) assay (Asper Biotech, Tartu, Estonia) [16] in the Department of Genetics of the United Laboratories of Tartu University Hospital. This microarray is capable of simultaneous evaluation of 199 mutations: several connexin genes (*GJB2, GJB6, GJB3*), mutations in 2 SLC26 anion transport genes (*SLC26A4* and *SLC26A5*), and mutations in 2 mitochondrial genes (*12S rRNA* and $tRNA^{Ser(UCN)}$). A complete description of this APEX assay including a list of the 199 mutations covered is published by Gardner *et al.* [16] and by Teek *et al.* [17]. Thirty-two patients were analyzed before 2005 for c.35delG mutation in *GBJ2* gene by PCR analysis; if the patients were homozygous for mutation c.35delG, APEX array analysis was not performed.

In 15 probands, who were heterozygous for c.35delG or p.M34T mutation in *GJB2* gene, the whole *GJB2* gene was sequenced. Five probands, who had heterozygous mutation in *SLC26A4* gene, the *SLC26A4* gene was sequenced, and multiplex ligation-dependent probe amplification (MLPA) analysis was performed.

In 55 children, a geneticist decided that regular G-banded chromosomal analysis should be performed from peripheral blood lymphocytes.

From 126 probands whose etiology of HL remained unknown after DNA testing with the APEX method, 96 patients were chosen for the DNA analysis of CMV infection from neonatal screening cards–blood stored on Guthrie cards. Since the neonatal screening program for the whole of Estonia for phenylketonuria started in 1993, neonatal