Significance of heterozygosis M34T mutation of GJB2 gene in non-syndromic congenital deafness. Retrospective analysis of 12,472 samples of amniotic fluid

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Summary

Objective: **to determinate the role of heterozygosis of M34T mutation of GJB2 gene in non syndromic congenital deafness.**
Methods: **retrospective study between March 2010 and June 2013. Molecular screening for 35delG and M34T mutations of the GJB2 gene was offered to all women undergoing to second trimester genetic amniocentesis. Patients were excluded from the study group if one of the following conditions were present: infections, fetal abnormalities, family history for congenital deafness, diagnosis of chromosomal abnormalities, and consanguinity between parents.**
Results: **a total of 12.472 Caucasian women gave informed consent for this test. Seventy-seven cases were excluded. From the 12.395 amniotic fluid analysis remained, the following was found: 2 cases of 35delG homozygosis and 352 cases of heterozygous carriers (42 M34T mutation, 298 35delG mutation, 12 double heterozygosis M34T/35delG). The follow up in first year of life in the 42 newborns with heterozygosis for M34T mutation showed a mild deafness in 23 cases.**
Conclusions: **in our series, presence of heterozygosis M34T mutation is associated in more than 50% of cases to mild congenital deafness.**

Key words: congenital deafness, prenatal diagnosis, M34T mutation, amniocentesis.

Introduction

Deafness occurs in 1/1000 neonates (1) and the cause is hereditary in about half. This type of hearing impairment is sometimes referred as prelingual, as it affects the child before the age of speech development. There are two types of deafness: syndromic deafness (30%), in which the deafness is accompanied by other specific abnormalities, and non-syndromic deafness (70%) in which there are no additional abnormalities. More than half of non-syndromic deafness forms are caused by a recessive disorder (2). The human gap junction β-2 gene (*GJB2*) that encodes the protein connexin 26, was the first autosomal gene associated with the type of autosomal recessive non-syndromic sensorineural deafness known as "DFNB1" (3), even if it is involved in an autosomal dominant form of deafness (DFNA3) too. Connexin 26 is part of a large family of gap junction proteins involved in direct cell-to-cell transfer of small molecules and ions, and is considered to be vital for the recycling of endolymphatic potassium ions in the endolymph of the cochlea (4). Numerous GJB2 amino acid substitutions have been reported as dominant and recessive deafness alleles at the DFNA3 and DFNB1 loci (5) and 101 T→C (M34T), encodes a methionine-to-threonine substitution at amino acid 34, was one of the first mutations linked to deafness (3).
All data presents in literature, about the correlation between genotype-phenotype and neurosensorial hearing loss in composed heterozygous for M34T mutation and of 35delG mutation are controversial.
In particular, same authors (6,7) have been indicated for the M34T mutation a pathogenetic role also in heterozygous and indicated for the same mutation a dominant activity. Later, this dominant role has been retracted by indicating, on the other hand, an interface of the same mutation in association with 35delG mutation in the development of neurosensorial hearing loss with variable gravity. With this retrospective study of 12.472 cases of prenatal genetic, we want to demonstrate the role of heterozygosis of M34T mutation in NSSNHL (non-syndromic sensorineural hearing loss).

Materials and methods

From March 2010 to June 2013, in Prenatal Diagnosis Center, Artemisia, Rome, Italy, molecular screening for 35delG and M34T mutations of the GJB2 gene, was offered to all the women undergoing second trimester genetic amniocentesis.
A total of 12.472 Caucasian women gave informed consent for this test. A wide range of information was collected about the mothers and their offspring, especially about deafness, including information about the mother during pregnancy.
Patients were excluded from the study group if one of the following conditions were present: infections, fetal abnormalities detected at ultrasound scan, family history for congenital deafness, diagnosis of chromosomal abnormalities, sibling relationships between parents.
In cases heterozygous for M34T mutation, a post-natal screening with audiometric objective methods for deafness (TEOAE - Transiently evoked otoacoustic emissions - and ABR - Auditory Brainstem Response) was performed before the end of first year of life.

Genetic analysis
Genomic DNA was isolated from 5 cc of amniotic fluid using QIAGEN DNA isolation kit. The fragment spanning the region of mutations M34T and 35delG were PCR amplified by using reference primers. The quality and quantity of purified genomic DNA were determined by running a 0,8 agarose gel and spectrophotometry. All fragments were analyzed by direct sequencing in an Applera, Life Tecnologies, 3130 automated DNA sequencer.

Results

From a total of 12.472 amniotic fluid samples, 28 cases were excluded for the presence of one or more exclusion criteria. From the 12.395 amniotic fluid analysis remained, the following information have been detected: 352 carriers of heterozygosis for GBJ2 mutation and 2 cases of homozygosis (Tab. 1).
Of 352 cases of heterozygous carriers, 42 presented M34T mutation, 12 M34T/ 35delG, 298 35delG mutation (Tab. 2).
The follow up in first year of life of the 42 newborns with heterozygosis for M34T mutation showed a mild deafness in 23 cases (Tab. 3).

Table 1. Cases included in the study.

Total cases	*heterozigous foetus*	*homozygous foetus*
12.395	352	2

Table 2. Distribution of GJB2 gene mutations in cases of heterozygous carriers.

Cases of heterozygous carriers	*N 352*
M34T mutation	42
M34T mutation + 35delG in trans mutation	12
35delG mutation heterozygous	298

Table 3. Frequency of deafness in the study group (M34T heterozigous mutation).

M34T heterozigous mutation	*N 42*
mild deafness	23
conserved acoustic activity in the first year of life	19

Discussion

Mutations in GJB2 gene account for more than 70% of non-syndromic deafness in the white population (6). Numerous deafness causing by mutations of this gene have been reported, although a single mutation, 35delG, predominates (8-10).
Our data obtained from amniotic fluid from a generic low risk population, also showed that 35delG mutation is the most frequent one.
We reported also evidence for another frequent mutation in congenital non-syndromic deafness: M34T. This analyzed mutation presents an incidence in general population of 0,3 %.
To determine an exact correlation between M34T mutation and deafness is extremely important. There are many well-known papers dealing with M34T mutation and its pathogenetic role in non-syndromic deafness (11,12). In 2002 D'Andrea et al. (6) described M34T as an autosomal dominant mutation; whereas many other studies attributed to the M34T mutation an autosomal recessive role, because of its occurrence with other GJB2 mutations or as homozygous in deaf individuals (7,13-18). Finally, Cucci et al. (14) proposed that the effect of the M34T allele might be dependent on the mutation present in trans.
These results of patogenetic role of M34T are substantially in agreement with our study, performed on amniotic fluids from Mediterranean, Caucasian and general low risk women, showing a prevalence of 1:300 for the heterozygous condition.
In fact, from a total of 352 heterozygous carriers, 42 M34T mutations were observed, and of these, 23 presented unilateral or bilateral mild deafness versus 19 cases in which acoustic activity was conserved in the first year of life.

Conclusions

In our series, presence of heterozygosis M34T mutation is associated in more than 50% of cases to mild congenital deafness detected within the first year of life. Our detection gives a convincing example of the potential efficacy and advantage of a prenatal screening test.

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