MOLECULAR INVESTIGATION OF PEDIATRIC PORTUGUESE PATIENTS WITH SENSORINEURAL HEARING LOSS

Nogueira C[1], Coutinho M[2], Pereira C[1], Tessa A[3], Santorelli FM[3], Vilarinho L[1]

[1]Genetics Department, R&D Unit, National Institute of Health, INSA, Porto, Portugal; [2]Otorhinolaryngology Department, Children's Hospital Maria Pia, Porto, Portugal; [3]Molecular Medicine & Neurogenetics, IRCCS Fondazione Stella Maris, Pisa, Italy

INTRODUCTION

Sensorineural hearing loss (SNHL) is one of the most common disabilities in human, and genetics is an important aspect in research and clinical practice for SNHL. One in 1000 children is born with bilateral SNHL, and 50-70% of them have monogenic causes for their deafness [1].

Hereditary hearing loss can be classified into syndromic and nonsyndromic (NSSNHL) depending on the associated features. Whilst over 400 genetic syndromes have been described in association with mono or bilateral deafness, syndromic conditions account for about 30% of hereditary congenital hearing loss whereas the relative contribution to all deaf people is much higher (>70%) for nonsyndromic subtypes. NSSNHL is predominantly inherited in an autosomal recessive patterns (DFNB loci) (80%) but can be also autosomal dominantly (DFNA) (15–20%), X-linked (DFN) (2–3%), or maternally (1%) transmitted. A polygenic or multifactorial pattern of inheritance should be postulated for late onset cases of hearing impairment [2, 3]. To date, 134 deafness loci (77 DFNB and 57 DFNA), have been reported, with more than 40 genes cloned. Mutations in the *GJB2* and *GJB6* genes on the DFNB1 responsible for up to 50% of NSSNHL [4].

The understanding of the molecular genetics in SNHL has advanced rapidly during the last decade but the molecular etiology of hearing impairment in the Portuguese population has not been investigated thoroughly.

PATIENTS AND METHODS

We analyzed the whole mitochondrial genome in 95 unrelated children with SNHL (53 non-syndromic and 42 syndromic) [5] and searched for variations in two frequent mutated genes, *GJB2* and *GJB6*, in the non-syndromic patients.

Sequencing analysis was performed to identify disease-causing mutations.

RESULTS

Collectively, about 17% (16/95) of the Portuguese children analyzed in the present study were molecularly characterized whereas a single variant was identified in four patients (Table 1). We identified in *GJB2* two novel mutations (p.P70R and p.R127Qfs*84) and the c.35delG accounted for 40% (16/40) of all mutant alleles. Up to half of mutations occurring in connexin-related genes. Additionally, 4.2% of studied patients (4/95) presented mutations in mtDNA, including a undescribed change in the mtDNA-tRNA^{Trp} gene (m.5558A>G) (Figure 1).

Table 1 – Genotype of patients with mutations in nuclear genes (*GJB2* and *GJB6*) and in mitochondrial genome.

Number of patients	*GJB2*	*GJB6*	mtDNA
4	c.35delG/ c.35delG	--	--
1	p.W24X/ p.W24X	--	--
1	c.35delG/ p.E47X	--	--
1	c.35delG/ p.L76P	--	--
1	c.35delG/ p.V95M	--	--
1	p.M34T/wt	--	--
1	p.R127QfsX84/ p.R127QfsX84	--	--
3	c.35delG/wt	--	--
1	p.P70R /wt	--	--
2	c.35delG	del(GJB6-D13S1830)	--
1	--	--	m.7445A>G
1	--	--	m.1555A>G
1	--	--	m.3243 A>G
1	--	--	m.5558 A>G

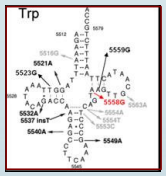

Figure 1 – Schematic secondary structure of human mitochondrial tRNA^{Trp} gene. Black stands for pathogenic mutations, gray stands for polymorphic mutations and red stand for the novel mutation described in our study.

DISCUSSION / CONCLUSION

Our data suggest that analysis of the *GJB2* gene may have clinical implications in the diagnosis of deaf Portuguese children. The relatively higher incidence of mtDNA mutations also suggests that screening for variations in the mitochondrial genome should always be considered unless mitochondrial inheritance can be excluded for certain. The molecular diagnosis will permit more accurate genetic counseling for family members, monitor possible multisystem complications, and avoid usage of aminoglycosides if infections occur.

REFERENCES

[1] T. Matsunaga, "Value of genetic testing in the otological approach for sensorineural hearing loss", Keio J Med., vol 58, no. 4, pp. 216-222, 2009.
[2] R. Utrera et al., "Detection of the 35delG/GJB2 and del(GJB6-D13S1830) mutations in Venezuelan patients with autosomal recessive nonsyndromic hearing loss," Genet Test, vol. 11, no 4, pp. 347-352, 2007.
[3] O. Vele and I. Schrijver, "Inherited hearing loss: molecular genetics and diagnostic testing," Expert Opin. Med. Diagn., vol. 2, pp. 231–248, 2008.
[4] G.V. Putcha, B.A. Bejjani, S. Bleoo, et al., "A multicenter study of the frequency and distribution of GJB2 and GJB6 mutations in a large North American cohort," Genet. Med., vol. 9, pp. 413–426, 2007.
[5] Grant of the Comissão de Fomento da Investigação em Cuidados de Saúde do Ministério da Saúde – Proj.254/2001.