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European Journal of Medical Genetics, Issy les Moulineaux, v.57, n.4, p.125-128, 2014
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Deletion of the entire POU4F3 gene in a familial case of autosomal dominant non-syndromic hearing loss

Érika L. Freitas a,*, Jeanne Oiticica b, Amanda G. Silva d, Roseli S.M. Bittar b, Carla Rosenberg a, Regina C. Mingroni-Netto a

a Department of Genetics and Evolutionary Biology, Institute of Bioscience, University of São Paulo, São Paulo, Brazil
b Department of Otolaryngology, Medical School, University of São Paulo, São Paulo, Brazil

corrresponding author. Departamento de Genética e Biologia Evolutiva, Instituto de Ciências Biológicas, Universidade de São Paulo, São Paulo, SP, Brazil. Tel./fax: +55 11 30917573.
E-mail address: erikafreitas@gmail.com (É.L. Freitas).

European Journal of Medical Genetics 57 (2014) 125–128

Article info
Article history:
Received 24 April 2013
Accepted 8 February 2014
Available online 18 February 2014

Keywords:
Autosomal dominant non-syndromic hearing loss
ADNSHL
DFNA15
5q32 deletion
POU4F3

Abstract
In 20% of cases, hereditary non-syndromic hearing loss has an autosomal dominant inheritance (ADNSHL). To date, more than 50 loci for ADNSHL have been mapped to different chromosomal regions. In order to verify whether genomic alterations contribute to the hearing loss etiology and to search for novel deafness candidate loci, we investigated probands from families with ADNSHL by oligonucleotide array-CGH. A deletion in the 5q32 region encompassing only one gene, POU4F3, which corresponds to DFNA15, was detected in one family. POU4F3 protein has an important role in the maturation, differentiation and survival of cochlear hair cells. Defects in these cells may therefore explain sensorineural hearing loss. Mutations in this gene have already been associated with autosomal dominant hearing loss but this is the first description of a germline POU4F3 deletion associated with hearing impairment.

1. Clinical description
Hereditary hearing loss is a clinically and genetically heterogeneous disorder that can present several inheritance patterns, including autosomal-dominant inheritance (ADNSHL), which is observed in about 20% of the cases. In this report case there are only two ADNSHL affected members in the family, the proband and his father (Fig. 1). Concomitant disease, surgery, immunization and any other possible causes of acquired deafness were ruled out. After ear nose and throat (ENT) examination, pure-tone audiometry (PTA) was performed along with additional tests, including speech reception threshold (SRT), word recognition score (WRS) and tympanometry. Although none of the affected people reported vestibular symptoms, vestibular function was tested by Electrooculography (EOG) and Sinusoidal Harmonic Acceleration Test (SHAT), aiming to reveal any signal of vestibular hair cell damage. The EOG test incorporated the evaluation of oculomotor tests including gaze, smooth pursuit, random horizontal saccade and optokinetic nystagmus responses; and it also evaluated the vestibulo-ocular reflex by caloric stimulus. The SHAT allows evaluation of bilateral vestibular response at high frequencies of angular acceleration (0.02, 0.04, 0.16 Hz), complementing the EOG in which only low frequencies are tested. Otoacoustic emissions (OAE) and Brain Evoked Response Audiometry (BERA) tests were also performed. Computed tomography (CT) scanning of the temporal bone and Magnetic Resonance Imaging (MRI) with a contrast (gadolinium) were obtained.

The proband reported that his hearing loss began at the age of 11 years old, although there is no audiogram confirming a normal hearing before that age. One year later he had an episode of appendicitis that was treated by surgical removal of the appendix (laparotomy) and systemic antibiotic therapy with amikacin for at least one month. After this episode the family realized that, coincidentally or not, he could not hear as well as before. In the following years, there was a progressive worsening of the hearing on both ears documented by audiometry (Fig. 2). At the age of 25, after an episode of pneumonia treated with antibiotics, the patient referred additional worsening in hearing loss and a new symptom, tinnitus, on both ears. There were no complaints of vestibular dysfunction or ear fullness. The EOG showed 1.8/second spontaneous left beating nystagmus; normal gaze; symmetrical gain and phase on the smooth pursuit; latency, velocity and precision in normal range at the random horizontal saccades; optokinetic with asymmetrical gain in 35/second stimulation, with higher frequencies to the left, and normal range gain in 20 and 50/second stimulations; and symmetric responses...
after caloric test stimulus on both ears. SHAT showed gain and phase in normal range, and asymmetrical response in the 0.040 Hz frequency. OAE and BERA performed at the age of 27, showed, on both ears, no response on distortion-product and no response on click-evocated brainstem potential at 90dBHL, respectively.

The father of the proband referred bilateral hearing loss that has begun at the age of 13. He was seized with convulsions episodes during his adolescence, with no apparent etiology and was treated with trimuride. He denies additional symptoms such as vertigo, tinnitus or ear fullness. OAE and BERA tests showed, at the age of 59, no response on distortion-product on both ears and no response on click-evocated brainstem potential at 90dBHL, respectively. The EOG showed a slight spontaneous left beating nystagmus (1.1/second) with normal ocular motor tests (normal gaze; symmetrical gain and phase on the smooth pursuit; latency, velocity and precision in normal range at the random horizontal saccades), except for an asymmetric gain in 20, 35 and 50/seconds stimulations at the optokinetic test (higher frequencies to the left); and symmetric responses after caloric test stimulus on both ears. SHAT showed gain, symmetry and phase in normal range at all tested frequencies.

CT scanning of the temporal bone excluded inner ear malformations; and MRI ruled out membranous labyrinth, eighth nerve affection or other brain tumors, as source of symptoms in both affected members.

2. Methods

After obtaining informed consent, peripheral blood was collected and genomic DNA was isolated from family members. Mutations often associated with hearing loss were discarded, specifically: c.35delG and c167delT GJB2 mutations, delGJB6 [D13S1830] and delGJB6 [D13S1854], mitochondrial A1555G mutation and other mutations in the entire coding region of the GJB2 gene.

Array-CGH was performed by using genomic DNA from the index patient and a platform from Oxford Gene Technology (CytoSure ISCA v2 - 4x180k) containing 180,000 oligonucleotides as described by the manufacturer. Data were processed with Feature Extraction software (Agilent Technologies) and subsequently analyzed with the Genomic Workbench software (Agilent Technologies). Gains and losses of genomic sequences were called using an aberration detection statistical algorithm ADM-2, with a sensitivity threshold of 6.7. Detected copy number variations (CNVs) were compared to data from oligoarray studies documented in the Database of Genomic Variants [DGV; http://projects.tcag.ca/variation/]. All variants present in at least four different studies in DGV were considered as normal population variants (common), and subsequently disregarded. Chromosome regions not previously reported as CNVs were singled out for further investigations, which included confirmatory studies by real-time quantitative PCR (qPCR) using the TaqMan® Copy Number Assays system (Applied Biosystems). A pre-designed probe specific for the POU4F3 gene was used (assay ID HS02668483_cn) along with a control probe (Rnase P gene).

**Fig. 1.** Family pedigree. The arrow indicates the proband.

**Fig. 2.** Pure-tone Audiometry Data Analysis Follow up of the proband. **A.** PTA at the age of 12 years old. **B.** PTA at the age of 14 years old. **C.** PTA at the age of 20 years old. **D.** PTA at the age of 23 years old. **E.** PTA at the age of 25 years old. **F.** PTA at the age of 26 years old. **SRT:** Speech Reception Threshold; **SDT:** Speech Detection Threshold; **WRS:** Word Recognition Score; **RE:** Right Ear; **LE:** Left Ear.
3. Genomic rearrangement

Array-CGH analysis of the proband showed a deletion at 5q32 (145,682,536 bp to 145,738,485 bp region – NCBI Build 36, Feb 2007). The proximal breakpoint mapped between 145,658,445 bp (A_16_P17337086 – last probe not deleted) and 145,682,536 bp (A_16_P01358616 – first deleted probe); while the distal breakpoint was located between 145,738,485 bp (A_16_P17337308) and 145,796,051 bp (A_16_P17337434) (respectively, the last deleted probe and the first non-deleted probe) (Fig. 3A and B). The minimal size of the deletion is 56 kb and the maximal size is 137 kb. Real-time quantitative PCR analysis confirmed the alteration and revealed that the deletion is likely to be inherited from the affected father (Fig. 3C).

4. Discussion

Here we report the first ADNSHL familial case with a germline deletion of the entire POU4F3 gene. The POU4F3 (POU class IV homeobox 3; OMIM 602460) gene is a member of the POU-domain class IV transcription factor family, and it has 14 members in humans. All these genes are characterized by the presence of two DNA binding domains: a POU-specific domain and a POU homeodomain. The various POU proteins are involved in tissue-specific gene regulation. The transcription factors POU4F1, POU4F2 and POU4F3 are essential for the maturation and survival of motor and sensory neural cells, retinal ganglion cells, and cochlear hair cell, respectively [Vahava et al., 1998].

So far, few mutations in the POU4F3 gene have been described in patients with ADNSHL. The first mutation, c.884del8, was found in one Israeli pedigree [Vahava et al., 1998], two missense mutations (p.L289F and p.L223P) were subsequently identified in Dutch families [Collin et al., 2008] and one (c.662del14) in a Korean patient [Lee et al., 2010]. Recently with the advances in new technologies such as Next Generation Sequencing, two novel mutations were described. A missense mutation, p.E232K, was identified by Baek et al. [2012 Sep 3] and a novel c.603_604delGG was predicted to lead to a truncated protein by Yang et al. [2013 Jun 14]. However, there are no reports of mutations in POU4F3 in Brazilians, and no report of ADNSHL caused by germline large
deletions. All mutations described previously resulted in protein with decreased binding to consensus DNA, reduced capability to activate reporter gene expression, and partial mislocalization in the cytoplasm. These functional studies indicate that both missense as well as the frameshift mutations affects proper functioning of the POU4F3 protein. Based on these results, haploinsufficiency seems to be the most-likely mechanism underlying the hearing impairment in DFNA15-patients, and the deletion of an entire copy of the POU4F3 gene in our patient is also in agreement with this hypothesis. Apparently, even very low levels of the protein are sufficient to ensure proper vestibular function in these individuals [Vahava et al., 1998].

According to the literature, DFNA15 patients have shown varying rates of progression, as well as varying ages of onset for hearing impairment and vestibular defects. In the Israeli family [Vahava et al., 1998], hearing impairment appeared to be moderate to severe, audiometric configuration varied between flat and downsloping. The patients presented adult-onset progressive hearing loss, beginning between the ages of 18 and 30 years, without concomitant vestibular complains. Unlike the Israeli family, some of the patients from the Dutch family [Collin et al., 2008] presented vestibular symptoms, including instability, vertigo, and a tendency to fall; and a mean subjective onset of the hearing loss at the age of 35 years. The Korean patient demonstrated a severe to profound sensorineural hearing loss with no additional clinical symptoms [Lee et al., 2010]. In the family described in this study, the phenotype onset occurred between the ages 11–13 years for the two affected members and progressed from moderate to severe deafness by mid-age in the father, and from moderate to profound by adulthood in the proband. Even in the absence of vestibular complains, we decided for a more accurate vestibular investigation, since the previous clinical findings regarding POU4F3 gene mutations were variable. The main findings at the vestibular tests points out to some degree of visual-vestibular system asymmetry, but the findings are so subtle that are in agreement with the absence of vestibular complains described in other reports.

According to the clinical evaluation, the proband presented profound sensorineural hearing loss and the father presented moderate to severe sensorineural hearing loss. Although similar molecular mechanisms underlie hearing loss in the patient and his father, the clinical and audiometric phenotype were distinct in the two affected members. A steeply downsloping audiogram was noticed for the proband, with tinnitus, and a flat to gently downsloping audiometric configuration was observed for the father. Despite the similar early onset of the bilateral sensorineural hearing loss, the proband presented a significant faster progression on hearing loss along the years, unlike his father who showed slower progression with advancing age. Amikacin is known to be ototoxic and the exposure to this drug may somehow explain why the proband hearing loss worsened so precociously. However, this possible ototoxic effect alone does not explain the hearing loss progression documented long after the treatment.

The differences in the clinical presentation in DFNA15 patients reinforce the possibility that genetic profile is under influence of environmental factors, acting together to determine diversity of clinical phenotypes.

Our results lend support to the relevance of CNVs in the etiology of ADNSHL and they also support the importance of the array-CGH technique. Sanger Sequencing is not suitable for quantifications and, therefore, does not detect duplications and heterozygous deletions. MLPAs, in contrast, allows detection of large deletions and duplications. There are several commercial MLPA kits for ADNSHL, but none of them include the POU4F3 gene. Next generation sequencing technologies (NGS) provide an alternative method for detecting copy number by the counting of sequence reads in non-overlapping windows between patient and control samples. However, due to the relatively young age of the procedures, their performance is not fully understood. As more mutations contributing to hearing loss are identified, and thorough audiological analysis are performed, comprehensive genotype-phenotype correlations can be made for inherited non-syndromic hearing loss.

Acknowledgments

The authors are grateful to the family members for their enrollment in this study and would like to thank laboratory fellows for their collaboration, especially Maria Teresa de Mello Auricchio for technical support. This work was financially supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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