When a pseudogene hides a gene: STRC, implication in childhood isolated deafness

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Introduction

Deafness is the most common sensory disability, occurring in one in 1000 children at birth. It can be isolated (non-syndromic deafness) or associated with many other signs (syndromic deafness). All inheritance modes are reported: autosomal dominant, autosomal recessive, maternal, X or Y related.

Of the genes known to be responsible for isolated perception deafness, STRC has the particularity of possessing a particularly homologous pseudogene (99.6% identity in the coding sequence) and of being subjected to frequent gene rearrangements.

CNVs (Copy Number Variations) are a common cause of bilateral non-syndromic deafness without malformation of the inner ear (Shearer et al., 2014). The genomic region most commonly involved in deafness is the STRC gene locus (Figure 1).

![Figure 1: Chromosome organization of STRC gene locus. From the website www.genoscope.cic.cn](image1)

STRC gene (29 exons) is located on chromosome 15q15.3. It is responsible for autosomal recessive isolated hearing loss DFNB16 (MIM #603720). Stereocilin is expressed at the level of stereocilia of the outer hair cells (OHC). It is involved in the cohesion of the ciliary bundles of OHC and in the attachment of OHC to the tectorial membrane.

Patients and Methods

The study of STRC gene was carried out by different techniques on a cohort of 423 unrelated index cases presenting with the following phenotype: mild to moderate bilateral hearing loss between 21 and 40 dBHL for mild deafness) 41 and 70 dBHL for moderate deafness, obtained by averaging the hearing loss on conversational frequencies (500, 1000, 2000 and 4000 Hz), onset before 10 years old, normal inner ear CT-scan and no mutation in GJB2-GJB6.

We searched for a deletion of STRC gene by PCR-specific exons 20 allele using modified primers (STRC-Ex20F: 5’-CCAAATCTTGAGCTCTTC-3’ and STRC-Ex20R: 5’-CTTGAAGGTTCGAAGTCC-3’) for 360 patients. These primers now the preferential amplification of the gene with respect to the pseudogene. (Examples of profiles: Figure 2).

![Figure 2: Sanger sequencing of exons 20 of the STRC gene with modified primers. A. Normal profile. B. Heterozygous deleted. C. Homozygous deleted.](image2)

We then analyzed 122 index cases using the MLPA® P461-A1 kit (MRC-Holland), testing both STRC and OTOA genes. The P461-A1 kit contains 5 probes for STRC, 5 probes for CATSPER2 and 6 probes for OTOA gene. Multiple flanking probes are included for multiple genes in the 15q15.3 and 16q12.2 regions, indicating the extent of possible deletions / duplications. In addition, this kit contains 12 reference probes distributed on the genome.

This MLPA® kit is designed to semi-quantitatively detect deletions / duplications of one or more sequences in STRC and OTOA genes in a DNA sample. A heterozygous deletion of a recognition sequence gives a relative peak height reduced by 35 to 50% of the amplification product of this probe. Point mutations are not detectable by this technique. (Examples of profiles: Figure 3).

![Figure 3: Examples of MLPA profiles obtained with Coffalyser software. A. Normal profile. B. Heterozygous deleted for STRC gene. C. Homozygous deleted for STRC gene.](image3)

We then analyzed by high throughput sequencing (NGS) using a Custom Amplicon TruSeq kit on MiSeq (Illumina), STRC gene in 16 patients with a heterozygous deletion detected in MLPA (further analyzes planned). Finally, the potentially causal variants were confirmed by Sanger sequencing.

Results

Among 423 index cases tested, we found 59 patients with a homozygous deletion of STRC gene and 13 heterozygous composite patients for a STRC gene deletion and a point mutation in trans (identified by NGS sequencing); STRC mutation rate is 17% (72/423).

It is important to remember that large deletions of chromosome 15q15.3 carrying STRC and CATSPER2 in the homologous state are responsible for a combination of deafness-infertility (contiguous gene syndrome) responsible for early deafness in boys and girls and exclusively male infertility (by oligospermia) (MIM #611002) and that they are not uncommon.

For some families, family segregation study is complicated by the existence of STRC gene duplication (observed in 8 out of 122 index cases tested). Thus, it may be necessary to study grandparents to confirm the genotype responsible for the index case. Finally, other complex rearrangements of these regions make their study delicate.

Conclusion

CNVs are common causes of bilateral deafness isolated from the child, without malformation of the inner ear, and the use of MLPA is interesting in first intention (before NGS study) or in second intention to confirm a deletion observed in NGS or by a cytogenetic approach.

STRC gene rearrangements are frequently involved in cases of isolated deafness in children and should be investigated by appropriate technique. The existence of a pseudogene greatly complicates the study of the regions concerned.

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