IDENTIFICATION AND FUNCTIONAL ANALYSIS OF THAP1 GENE SEGREGATING VESTIBULAR PHENOTYPE IN MONOZYGOTIC TWINS WITH MENIERE’S DISEASE

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Background

- Meniere’s disease (MD) is a complex disorder, characterized by sensorineural hearing loss, episodic vertigo and tinnitus.
- Familial MD is found in 8-10% of cases in European population (1-3).
- Genetic heterogeneity with different patterns of inheritance is observed.
- We obtained biological samples from a family with two monzygotic twins affected by MD (Figure 1).
- The age of onset was 38 years in both patients presenting sensorineural hearing loss in the left ear and ipsilateral vestibular hypofunction. Patients have two healthy sister and one sister affected with episodic vertigo and juvenile myoclonic epilepsy (JME).

Methods

We performed Whole-Exome Sequencing (WES) analysis in 5 individuals of the same family: two monzygotic twins with MD, two healthy individuals and the sister affected with JME in SOLID S5500xl platform.

We have also performed different bioinformatics analyses:

1. We followed the workflow on Figure 2 to identify candidate Single Nucleotide Variants (SNVs) associated with MD.
2. We looked for copy number variations (CNVs) in the genome of affected individuals using CNOFER (a command-line python program) (Figure 3).
3. To study the possibility of a recessive pattern we performed Homozygosity mapping assay in order to find homozygosity regions (Figure 4).
4. We used the sister affected with JME to distinguish the phenotype segregated by the variants.

Results

- We did not find any CNV with significant Z-score associated with MD phenotype. Recessive pattern was discarded because none of the SNVs identified were in any of the regions of interest.
- We have selected a novel heterozygous SNV in THAP1 gene present in cases and in the sister affected with JME, among the best candidates SNVs resulting from bioinformatic analyses, segregating the vestibular phenotype.
- A novel heterozygous variant in ARNT2 gene present in cases but not in the sister affected with JME was selected as the best candidate variant segregating SNHL phenotype.
- Both variants were validated by Sanger sequencing (Figure 5 and 6).
- RNA extraction from one monzygotic twin was performed to determine relative fold change in THAP1 expression by qPCR method (Figure 8).
- Four plasmids were nucleofected into a Jurkat cell line to perform an apoptosis assay (Figure 9) to detect a functional effect of the mutation identified.
- We observed that cells with the mutated protein stayed more time in apoptosis (Figure 10).

Conclusions

We have identified two novel missense variants segregating the vestibular and cochlear phenotypes in the THAP1 and ARNT2 genes, respectively. Further studies are required to evaluate the functional effect of these novel variants in these genes and its effect on MD phenotype.