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Functional Analysis of Variants Implicated in Hearing Loss in a Cohort from Argentina: From Molecular Diagnosis to Pre-Clinical Research

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Abstract : Hearing loss (HL) is the most prevalent sensorineural disorder affecting about 10% of the global population, with more than half due to genetic causes. About 1 in 500-1000 newborns present congenital HL. Most of the patients are non-syndromic with an autosomal recessive mode of inheritance. To date, more than 100 genes are related to HL. Therefore, the Whole-exome sequencing (WES) technique has become a cost-effective alternative approach for molecular diagnosis. Nevertheless, new challenges arise from the detection of novel variants, in particular missense changes, which can lead to a spectrum of genotype-to-phenotype correlations, which is not always straightforward. In this work, we aimed to identify the genetic causes of HL in isolated and familial cases by designing a multistep approach to analyze target genes related to hearing impairment. Moreover, we performed in silico and in vivo analyses in order to further study the effect of some of the novel variants identified in the hair cell function using the zebrafish model. A total of 650 patients were studied by Sanger Sequencing and Gap-PCR in GJB2 and GJB6 genes, respectively, diagnosing 15.5% of sporadic cases and 36% of familial ones. Overall, 50 different sequence variants were detected. Fifty of the undiagnosed patients with moderate HL were tested for deletions in STRC gene by Multiplex ligation-dependent probe amplification technique (MLPA), leading to 6% of diagnosis. After this initial screening, 50 families were selected to be analyzed by WES, achieving diagnosis in 44% of them. Half of the identified variants were novel. A missense variant in MYO6 gene detected in a family with postlingual HL was selected to be further analyzed. A protein modeling with AlphaFold2 software was performed, proving its pathogenic effect. In order to functionally validate this novel variant, a knockdown phenotype rescue assay in zebrafish was carried out. Injection of wild-type MYO6 mRNA in embryos rescued the phenotype, whereas using the mutant MYO6 mRNA (carrying c.2782C>A variant) had no effect. These results strongly suggest the deleterious effect of this variant on the mobility of stereocilia in zebrafish neuromasts, and hence on the auditory system. In the present work, we demonstrated that our algorithm is suitable for the sequential multigenic approach to HL in our cohort. These results highlight the importance of a combined strategy in order to identify candidate variants as well as the in silico and in vivo studies to analyze and prove their pathogenicity and accomplish a better understanding of the mechanisms underlying the physiopathology of the hearing impairment.

Keywords : diagnosis, genetics, hearing loss, in silico analysis, in vivo analysis, WES, zebrafish
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