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A New Mutation in FGFR1 Gene (P.M771I) With No Pathogenic Effect On Hearing Loss Found by Whole Exome Sequencing in an Iranian Family

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1. Abstract

1.1. Objectives: Hearing loss, the second most frequent sensorineural impairment, could be associated with missense mutations in several genes involved in the development of hearing parts. The receptor tyrosine kinase fibroblast growth factor receptor 1 (FGFR1) is known to be expressed in the inner ear and plays an important role in the formation of auditory-sensory epithelium. There have been some reports of FGFR1 gene mutations causing hearing loss. Using Whole Exome Sequencing (WES), we identified a novel mutation in the FGFR1 gene in a 30-year-old Iranian woman.

1.2. Material and Methods: Whole Exome Sequencing (WES) was performed on a 30-year-old woman, followed by sanger sequencing to check for the new mutation in her parents. PolyPhen-2 and Mupro in silico studies were performed to identify probable changes in wild-type and mutant structures.

1.3. Results: WES analysis showed a novel mutation in FGFR1 (NM 023110: exon18:c.G2313A:p.M771I) in the case. Her parents' Sanger sequencing revealed heterozygosity in her father and homozygosity of the normal allele in her mother. In silico investigations revealed no significant differences in pathogenic effects.

1.4. Conclusion: Altogether, our findings revealed no pathogenic effect of the new mutation (M771I) of the FGFR1 gene in an Iranian 30-year-old woman. Because of hearing loss importance in preclinical diagnosis, this benign variant could help with the FGFR1 role on hearing loss in future pregnancies.

2. Introduction

Hearing loss is the second most prevalent sensorineural impairment, affecting about one in every 500 people [1,2]. This deficiency might also affect 2.7 out of every 1,000 children under the age of five, and 3.5 out of every 1,000 individuals. Furthermore, about 66% of people with hearing loss live in developing countries [3,4]. Furthermore, hearing loss may be classified depending on ear involvement, which indicates impairments of inner, middle, or outer ear sensory neurons, or even two parts [5]. Fibro-

blast growth factor receptor 1 (Fgfr1), a receptor tyrosine kinase, is expressed in the inner ear and is required for the development of the auditory-sensory epithelium [6]. Fibroblast growth factors (FGFs) activate FGFR1, causing it to dimerize and auto-phosphorylate, transforming it into its active conformation. The stimulated receptor interacts with several pathways, including Ras/MAP kinase and the PLC/IP3 pathways [8]. Previous studies have demonstrated that Fgfr1 is involved in a wide range of activities, such as the development of sensory organs [6,8]. A research found that a missense mutation in the FGFR1 gene (Y766) causes an amino acid substitution in the receptor's kinase domain. The mutant protein was identified as normal at the cell membrane; however, functional tests revealed that the mutation results in a lack of Ras/MAP kinase and PLC/IP3 pathway activation. The severity of the heterozygote phenotype suggested that the mutant protein had a dominant negative impact, most likely by dimerizing with and inhibiting the function of the wild-type protein [9]. Thus, utilizing WES and Sanger sequencing, the current work discovered a novel mutation in the FGFR1 gene and investigated if it was a pathogenic or benign variant causing hearing loss.

3. Case Presentation

A 30-year-old woman with a history of unrelated marriage of her parents came to genetic counseling to find the possible cause of her deafness. She was deaf from birth and was treated for cochlear implants as a child. Although the transplant was successful, speech disorders were also observed in this patient. The main reason for referring this person was to have children in the future and get married. The family was trying to figure out what the genetic cause of this deafness was and what the risk would be for future pregnancies and marriages. Genetic counseling with the possibility of mutation or missense mutations in potential genes involved in the formation of the inner and middle ear, as well as proteins involved in signaling pathways in this area, prescribed a WES test and then to evaluate the effect of inheritance pattern and inheritance risk. Tested for mutation confirmation by sanger sequencing.

The patient's WES analysis revealed a missense variant in the FGFR1 gene, which is associated with an autosomal dominant type of deafness. This mutation was eventually sequenced and evaluated in his parents to confirm and examine if it was de novo. Because the father of the family also had the same variant and their phenotypic was normal, this variant is hereditary and is classified as a polymorphic variant with no clinical relevance. Because the pathogen cannot be seen and her parents are not related, their future pregnancies can be considered routine high-risk pregnancies. There is a major emphasis on genetic counseling and prenatal screening.

4. Material and Methods

The SureSelect Human All Exon Kit version 6 was applied to fragment and enrich genomic DNA for exome sequences, and sequencing was done on an Illumina HiSeq 4000 platform with a minimum average coverage of 90X. The Illumina Whole-Genome Sequencing Service in Macrogen (Seoul, Korea) performed the initial sequencing component of this test, as well as the alignment, variant calling, data filtering, and interpretation. In summary, reads were aligned to the human reference sequence (GRCh37) using the Burrows-Wheeler Aligner (BWA), and variants were identified using the Genomic Analysis Tool Kit (GATK). Variants were filtered to identify the most potential candidate variants. The evidence for phenotype-causality was then assessed for each variant acquired from the filtering strategies. Only those variants with evidence for causing or contributing to disease were reported. Every variant was evaluated according to the available information from the following: databases including HGMD, ClinVar, LSDBs, NHLBI Exome Sequencing Project, 1000 Genomes, and dbSNP, published literature, clinical associations and its predicted functional or splicing impact by evolutionary conservation analysis and computational tools including AlignGVGD, MAPP, MutationTaster, PolyPhen-2, SIFT, and SNAP. Structure analyses and homology modeling on the FGFR1 sequence (amino acids 1-822) were performed using UniProt, PDB, PolyPhen-2, and MuPro tools to test for any alterations in mutant forms of the FGFR1 protein. Assessments were done for the putative changes between the two structures for a primary prediction by Poly-Phen2 (http://genetics.bwh.harvard.edu/pph2/) and MuPro (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index).

5. Results

5.1. In Vitro Results

With the patient's complete consent, all personal files were archived after a comprehensive examination of the case's clinical features. The diagnosis for the patient was a normal phenotype. The case performed WES test, and new variants in the FGFR1 gene's exon 18 (FGFR1:NM 023110:exon18:c.G2313A:p.M771I), which are thought to be associated to hearing impairment, were identified.

Due to the case's heterozygosity, sanger sequencing was done on the case's parents to screen for the mutation. Confirmation of the FGFR1 variant mutation indicated that her mother was homozygous for the normal allele, and her father was heterozygous for the mutant allele. These results showed that, there is no cause for concern because the M771I mutation in the FGFR1 gene is novel and not pathogenic (Figure 1). This gene inherited in an autosomal dominant pattern. According to these findings, this mutation does not induce hearing loss, which is a novel report in the literature. Some in silico investigations for M771I in the FGFR1 gene were performed for additional confirmation.

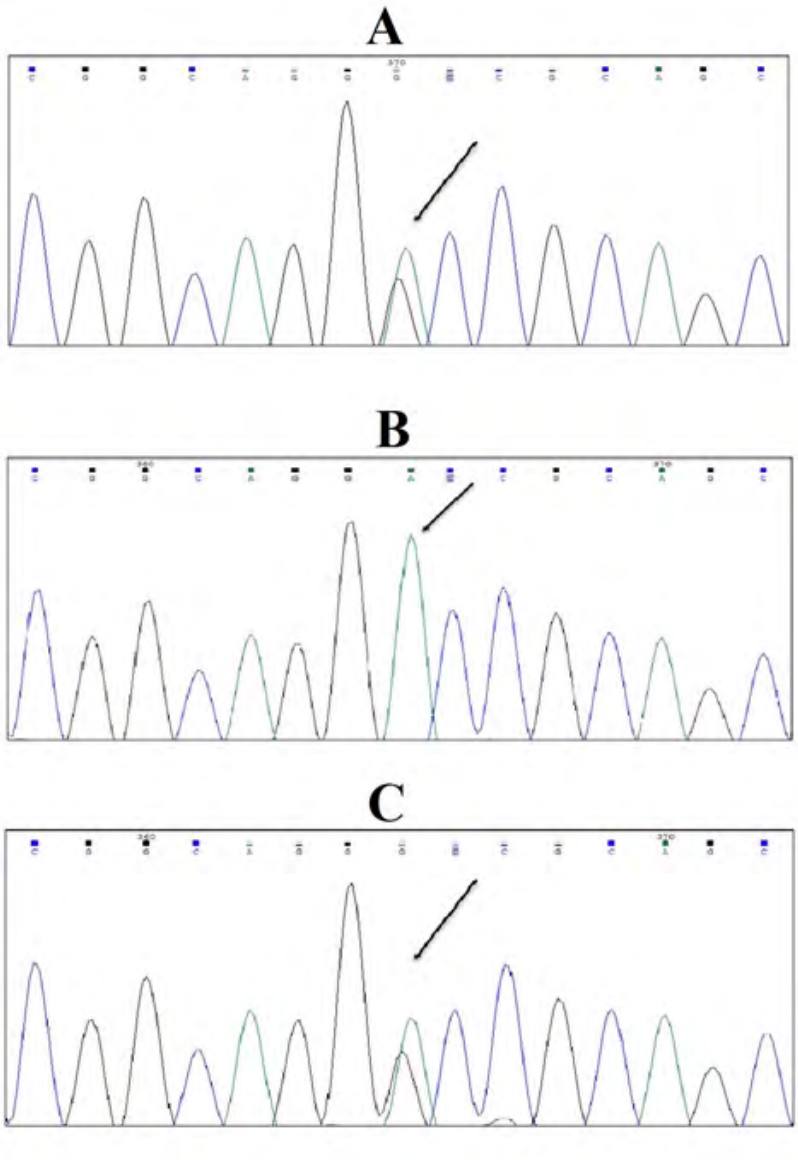

Figure 1: FGFR1 gene mutation confirmation of heterozygote proband (A), her homozygote mother (B), and her heterozygote father (C).

5.2. In silico Results

With a score of 0.004, PolyPhen-2 predicted M771I new mutation as a Benign variant (sensitivity: 0.97; specificity: 0.59). Furthermore, MuPro predicted both the value and sign of energy change using SVM, whereas sequence information only predicted a decrease in protein structural stability (G= 0.89). PROSITE Expasy study on P11362 Uniprot ID for human FGFR1 suggested amino acid range 478-767 as a protein kinase domain, which is the protein's critical site. SWISS-MODEL homology modeling did not display amino acids exceeding 762, hence structural analysis was inapplicable.

6. Discussion

In the current paper, we report a novel mutation in the FGFR1 gene in an Iranian female by WES and sanger sequencing tests. A 30-year-old woman was refereed for genetic counseling with hearing loss from childhood. She came for genetic counseling because of her future marriage. WES test was recommended for her to find plausible pathogenic mutations. WES results showed one missense mutation predicted with benign impact. This novel mutation in the FGFR1 gene (M771I) was concerning because of the FGFR1 role in the formation of the inner ear; therefore, mutation conformations were performed on her parents. Sanger sequencing results revealed that the M771I mutation is not pathogenic. Also, further in silico analyses represented no change in the protein structure mutant model compared with the wild-type one. Increasing reports of hearing impairments because of genes involved in auditory developments make hearing impairments a critical challenge that needs to be diagnosed in the prenatal diagnosis.

In silico analyses on human FGFR1 (Uniprot ID: P11362) showed M771I is in the neighboring of a protein kinase domain (residues range from 478 to 767) known as the key site of Fgfr1. Based on the previous repoers, there is an important residue near the Met771 documented as Tyr766. Auto-phosphorylation on Tyr766 in the C- terminal of FGFR1 makes a specific-binding site for the SH2 domain of phospholipase Cγ (PLCγ) [10]. Previous reports

revealed that Y766 mutation substituted to phenylalanine cannot activate the hydrolysis of Phosphatidylinositol and Ca2+ secretion in response to FGF stimulation suggesting PI hydrolysis is unimportant for FGF-induced mitogenic stimulation of cultured cells. Although, the analysis of ‘‘knock-in’’ mice carried mutated Y766 indicated that this tyrosine residue is essential for a negatively regulated signal during anteroposterior modeling of mouse embryos [11].

There are few reports on the association of FGFR1 gene missense mutations with hearing loss. Miraoui et al. reported heterozygosity for a c.1042G-A transition in exon 8b of the FGFR1 gene, resulting in a Gly348Arg substitution in a female patient with congenital hypogonadotropic hypogonadism, suffering from anosmia and also displayed hearing loss, abnormal dentition, and low bone mass [12]. Dode et al found homozygosity for an FGFR1 mutation (Ala167Ser) with Kallmann syndrome who also showed a unilateral hearing loss, cleft palate, corpus callosum agenesis, and fusion of the fourth and fifth metacarpal bones [13].

7. Conclusion

In conclusion, the WES test and mutation conformations for a 30-year-old Iranian woman and her parents showed a missense mutation (M771I) in the FGFR1 gene with uncertain significance. Due to the importance of hearing loss in genetic counseling, both pathogenic and benign variants are important for future pregnancies. Finally, the novel-found missense mutation M771I in the FGFR1 gene in described Iranian woman is not pathogenic and will be considered a normal change in the prenatal diagnosis and genetic counseling in the future.

8. Declaration Statement

The authors declare that there is no conflict of interest to disclose.

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