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Case Report

Identification of a novel missence mutation in *FGFR3* gene in an Iranian family with LADD syndrome by Next-Generation Sequencing

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ABSTRACT

Lacrimo-auriculo-dento-digital syndrome (LADD) is a multiple congenital anomaly and a genetically heterogeneous disorder. The aim of this study was to identify the pathogenic gene in an Iranian family with LADD syndrome and review the literature on reported mutations that involved in pathogenesis of LADD syndrome. One novel variant, c.1882 G > A, in fibroblast growth factor receptor 3 (FGFR3) was identified by next generation sequencing and Sanger sequencing. The heterozygous FGFR3 c.1882 G > A variant results in substitution of aspartic acid with asparagine at amino acid 628 (p.D628N) and co-segregated with the phenotype in the LADD family. Our findings suggest that the heterozygous FGFR3 c.1882 G > A variant might be the pathogenic mutation, because this amino acid is conserved in several species. Our data extend the mutation spectrum of the FGFR3 gene and have important implications for genetic counseling for the families. This is the second report of FGFR3 involvement in syndromic deafness in humans, and confirms the gene's positive role in inner ear development. In addition, this is the first FGFR3 mutation recognized in the Iranian LADD family.

1. Introduction

Autosomal dominant Lacrimo-auriculo-dento-digital syndrome [LADD (MIM 149730)] is a rare Multiple Congenital Anomaly (MCA) disorder. MCA disorders are typically infants with three or more minor malformations such as a club foot, syndactly, and abnormally formed pinnae or with two or more major malformations such as cardiac defect, neural tube defect, missing limb. LADD is characterized by dental and digital anomalies, aplasia, atresia or hypoplasia of the lacrimal and salivary systems, cup-shaped ears and hearing loss in humans [1,2].

Numerous familial and sporadic cases of this evidently rare and highly variable syndrome have been described [3–12]. LADD syndrome can be caused by heterozygous mutations in the genes encoding fibroblast growth factor receptors 2 (*FGFR2*) and 3 (*FGFR3*), and *FGF10* gene [13]. Identification of the responsible gene/mutation in any family with LADD syndrome, especially in the absence of any additional identifiable feature is hampered because the genetic heterogeneity of LADD syndrome, significant phenotypic overlap between LADD syndrome and other disorders such as ALSG and the variable expression of these disorders [14].

Next Generation Sequencing (NGS) has recently been used as an alternative approach to more traditional methods. NGS allows for a targeted enrichment and resequencing of almost all exons of protein-coding genes. Thus, next generation technology presents a transformational approach for detecting causative mutations in Mendelian disorders such as, LADD syndrome. In the present study, we applied NGS in an Iranian family with LADD syndrome. The systematic use of NGS reveals that this approach is a rapid, accurate, and cost-effective method to recognize causative mutations in affected family that was referred to our centre.

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2. Materials and methods

2.1. Human subjects

The proband of the Iranian family (male, 23 year-old) and his affected mother suspected of having LADD syndrome as well as his unaffected father and 200 unrelated unaffected Iranian individuals as a healthy control group were recruited for the study. Before testing, informed consent has been obtained from the proband, his consanguineous parents and 200 healthy controls, according to the guidelines of the Ethic Committee of Iran's Ministry of Health and Medical Education. The family history was notable only for a history of hearing disability in the proband's maternal uncle. The patients (II-5 and III-1) underwent complete clinical examinations including audiologic (Fig. 1b) and ophthalmologic evaluations, laboratory tests, physical exam, and radiographic findings. The primary clinical diagnosis of hearing disability for each patient was mainly established via standard audiometry according to current clinical standards in a sound-proofed room.

2.2. Next Generation Sequencing (NGS) and variant analysis

Two patients (Fig. 1a: II-5 and III-1) were selected for genetic sequencing test. Peripheral blood samples were obtained from each participant and genomic DNA was isolated from peripheral blood leukocytes according to standard methods [15]. Libraries were prepared following standard Illumina protocol. In summary, 3mg of genomic DNA was randomly fragmented to 200–300 base pairs. Terminal A residues were added following the incubation with the Klenow 3′-5′ exo-enzyme and dATP. Then, adapters were ligated to 3′ and 5′ ends of the resulting fragments. Next, the 200–300 bp product was selected for further PCR amplification. A customized Human capture array (Roche NimbleGen) was designed to capture all coding regions and the intron/exon boundaries of the 66 target genes (supplementary information) that involved in pathogenesis of syndromic hearing impairment followed by Next Generation Sequencing (NGS) approach (BGI-Shenzhen, Guangdong, China).

Sequence reads were aligned to the reference human genome (UCSC hg19). Indels and SNPs were recognized using GATK software. Previously recognized common variants (frequency > 1%) and synonymous substitutions were filtered out by using public databases including the 1000 Genome Project and HapMap samples. Potential disease-causing variants were evaluated by using reference tools such as Polymorphism Phenotyping v2 [16], Sorting Intolerant From Tolerant [17], as well as Mutation Taster predictions [18].

2.3. Sanger sequencing

To validate true positive of the novel mutation identified by NGS, Sanger sequencing was carried out in two subjects (II-5 and III-1). The specific PCR primers (Forward primer 5′- CTGGCAGCCCGTCTGAGGAGC-3′, Reverse primer 5′- CTGCTCCCAGCATCTCAGGGCA-3′) were used for the amplification of target gene site based on the reference sequences of Human Genome from GenBank in NCBI (NM_000142) [19]. PCR products were directly sequenced on ABI Prism 3100 automated genetic analyzer (ABI 3100; Applied Biosystems). We confirmed that both patients had a heterozygous c.1882G > A, p.D628N mutation in the FGFR3 gene (Fig. 1c). We additionally genotyped 200 healthy unrelated Iranian individuals for the mutation using Sanger sequencing. No mutated alleles were identified, suggesting that the c.1882G > A, p.D628N mutation is not present in the normal Iranian population at higher than 0.1% allele frequency.

3. Results

The 66-gene panel yielded an average of 619,167 reads per sample, with approximately 99.15% coverage of targeted regions. The average sequencing depth of targeted regions was 203.17. We successfully recognized 2287 homozygous and heterozygous variants by NGS. Using the candidate genes information for hearing impairment, we detected 26 heterozygous variants (11 genes) of which 1 variant (1 gene) is functional mutation. This variant was then filtered to select for rare variant with minor allele frequency <1% in the 1000 Genome project and HapMap.

The sequence analysis by NGS reveals a novel heterozygous missense mutation: c.1882G > A, p.D628N in exon 14 of the *FGFR3* gene in the proband (Fig. 1c). This mutation was predicted to cause the deleterious aspartic acid to asparagine substitution at position 628 (PolyPhen2, benign; SIFT, tolerated; Mutation Taster, disease causing).

We confirmed that the proband's mother (II-5) had a heterozygous c.1882G > A, p.D628N mutation in the *FGFR3* gene with the dominant inheritance pattern (Fig. 1a and b). In addition, we genotyped 200 healthy unrelated Iranian individuals for this mutation using Sanger sequencing. No mutated alleles were identified, suggesting that the c.1882G > A, p.D628N mutation is not present in the normal Iranian population.

4. Discussion

In present study, we made the exact genetic diagnoses on an Iranian family with LADD syndrome due to typical clinical symptoms combined with detection of one causative variant of *FGFR3* gene via targeted next-generation sequencing. The present patients were associated with a missence heterozygous D628N mutation in *FGFR3* and LADD syndrome. Seven mutations have been previously reported in *FGFR2*, *FGFR3* and *FGF10* genes with LADD phenotype in HGMD, a great number of which have been missence mutations (Table 1). The c.1882G > A, p.D628N mutation in the FGFR3 gene is not reported in the HGMD as either a mutation or a polymorphism. Additionally, this mutation was not identified in 400 control chromosomes.

The fibroblast growth factor receptor 3 (*FGFR3*, OMIM 134934) gene, located on 4p16.3, contains 19 exons and covers a genomic fragment of over 16.5-kD [20]. *FGFR3* encodes a highly conserved protein, the fibroblast growth factor receptor 3, which comprises 806 amino acids and is involved in a variety of important cellular processes including proliferation, wound healing, angiogenesis and embryo development. FGF receptors 3, contains the extracellular domain, the transmembrane domain, and the cytoplasmic tyrosine kinase domain [20].

Previous studies demonstrated that activating *FGFR3* missense mutations leads to short-limbed bone dysplasias and craniosynostosis syndromes, including severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN; OMIM 134934), thanatophoric dysplasia I and II (OMIM 187600), Muenke syndrome (OMIM 602849), achondroplasia (OMIM 100800), hypochondroplasia (OMIM 146000), and Crouzon syndrome with acanthosis nigricans (OMIM 134934) [21].

The proband and his mother carried a single base substitution (c.1882G > A, GAC > AAC) that substitutes aspartic acid 628 with asparagine (D628N) in the conserved cytoplasmic tyrosine kinase domain of *FGFR3* (Fig. 2). The aspartic acid to asparagine substitution result in the loss of negatively charged and acidic polar side chains and replacement with residues containing neutral charged and polar side chains. In addition, multiple sequence alignment of human *FGFR3* protein by PolyPhen, SIFT and Mutation Taster Conseq Server software revealed high conservation of this substitution