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PAPER

Whole-exome sequencing reveals an association between TJP2 and Hypercholanemia Familial 1

Onna Leyva[1]

[1]International Laboratory for Human Genome Research - National Autonomous University of Mexico

*onnaleyva@gmail.com

Abstract

Familial hypercholanemia-1 (FHCA1) is an autosomal recessive disorder characterized by elevated concentrations of bile acids, itching, and fat malabsorption. In this case study, we present a male patient exhibiting an unusual combination of high bile acids, itchiness, and hypercholesterolemia. To investigate the underlying genetic cause, whole-exome sequencing was performed on the proband, revealing homozygosity for a nonsynonymous single nucleotide variant (SNV) in the *TJP2* gene, known to be a causative factor for *FHCA1*.

Key words: Familial hypercholanemia-1; nonsynonymous SNV; fat malabsorption; hypercholesterolemia.

The patient, or proband, is a male displaying three primary symptoms: elevated bile acids, itching, and hypercholesterolemia. His two brothers do not exhibit any symptoms, and their parents are also symptom-free; it is unknown whether they are carriers, as depicted in Figure 1.

To investigate the aforementioned symptoms, the patient underwent whole-exome sequencing (WES). Following data processing, annotation, and segregation filtering (as explained in the supplementary material), it was discovered that the patient was the only family member homozygous for a *c.T155C* (nonsynonymous single nucleotide variant) in exon 3 of the *Tight Junction Protein 2 (TJP2)* gene located at 9q21.11. This gene encodes a zonula occludens that functions as a component of the tight junction barrier in epithelial and endothelial cells, essential for the proper assembly of tight junctions (see Figure 2). Mutations in this gene have been identified in patients with hypercholanemia, specifically *FHCA1*. Genomic duplications result in autosomal dominant deafness-51, among other associations.

Due to the proband's symptoms, the significant diagnosis is *FHCA1*, an autosomal recessive disorder with incomplete penetrance, with a prevalence in the population of less than 1 in 1,000,000 individuals. It is characterized by elevated concentrations of bile acids, itching, and fat malabsorption, leading to poor overall growth and deficiencies of fat-soluble vitamins. This deficiency results in rickets, and vitamin K deficiency leads to coagulopathy [1, 2, 3]. Additionally, fat malabsorption can lead to the development of nonalcoholic fatty liver disease (NAFLD), a condition in which excess fat accumulates in the liver, subsequently causing complications associated with the metabolic syndrome, such as high blood pressure and abnormal levels of triglycerides and cholesterol in the blood [4]. The latter is crucial because, although it is not a direct symptom of FHCA1, it can be a secondary consequence.

Methods

Participants

Adult members of the study family, which consisted of the proband, mother, father, and two male brothers provided written informed consent for themselves (and their children or themselves) for the WES and subsequent enrollment in the Centers for Mendelian Genomics research program, to identify the molecular cause of the patient's phenotype. The study protocols were approved by the institutional review board of the International Laboratory for Human Genome Research

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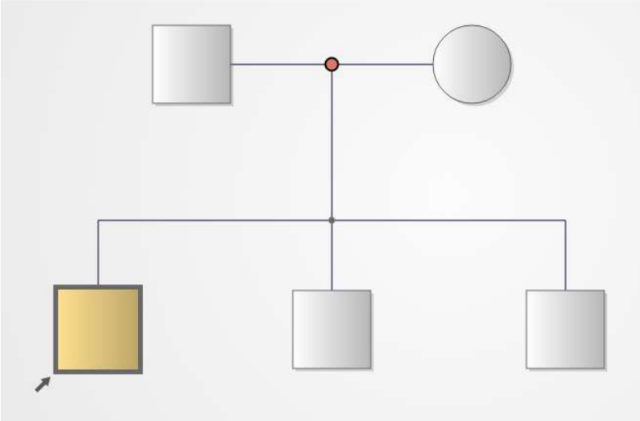

Figure 1. Pedigree of the Family

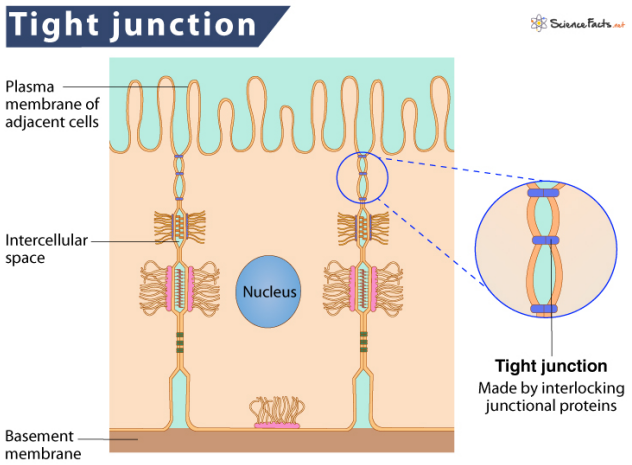

Figure 2. Pedigree of the Family

(LIIGH).

Whole-Exome Next-Generation Sequencing

Two milliliters of peripheral blood samples were collected from each family member. Genomic DNAs were extracted using Universal Genomic DNA Kit. Whole-exome capture was carried out using the Agilent SureSelect Human All Exon Kit and high-throughput sequencing by the Illumina HiSeq 2000 sequencer instrument. All variants were identified through Genome Analysis Toolkit (GATK). Mapping and alignment were performed using a pipeline to map to the human genome reference assembly GRCh37 (hg19). A total of 8.5 Gb of sequence data were produced. Variant calling from the aligned BAM file was performed using the ATLAS [5] and SAMtools [6] suites. Annotation and variant filtering were performed using ANNOVAR [7] and additional databases for informing variant annotation.

Whole-Exome Next-Generation Sequencing Reveals a Mutation in TJP2

WES identified 22,668 variants in coding regions, of which 21,344 had coverage greater than or equal to 10, genotype quality greater than or equal to 30, and allelic balance greater than or equal to 0.20. Among these, 10,793 were nonsynonymous variants. After filtering variants with alternative allele frequencies less than or equal to 0.01, we narrowed down to 661 variants.

Subsequently, we applied a recessive disease model and identified 11 variants. Among these, a predicted deleterious nonsynonymous single nucleotide variant (c.T155C) was found in exon 3 of the TJP2 gene located at 9q21.11. Although this variant coincided with the observed symptoms, we also explored heterozygous, compound heterozygous, and X-linked disease models briefly. However, none of these models aligned with the observed symptoms.

The code of the above analysis is in the supplementary material.

Check the Diagnosis

To check the diagnosis we could do a polymerase chain reaction (PCR) amplification of exon 3 followed by Sanger dideoxy sequencing in the proband and both parents confirmed the variants and their inheritance.

Analyses

The association between TJP2 and FHCA1 has been previously documented. Several mutations in this gene have been reported, and FHCA1 has also been linked to other genes such as BAAT, among others. In certain cases, FHCA1 has been identified as an oligogenic disease involving multiple genes.

However, it's worth noting that there have been reported cases of affected individuals without mutations in genes previously associated with the condition. Additionally, it is important to mention that there are other related genes that independently cause the same set of symptoms [3].

Treatments

There are several treatments available for the principal symptoms. To address high bile acid levels and itching, there are medications designed to reduce or control these symptoms, respectively [8]. Additionally, for patients with elevated cholesterol levels, there are different medications aimed at lowering these levels. However, it is crucial to consider that these patients experience this symptom due to fatty liver disease. Therefore, before addressing cholesterol levels, the primary focus should be on treating fat malabsorption. Initially, a diet high in calories and vitamins is recommended. If necessary, injections of digestive enzymes or medications to slow down normal bowel movement can be utilized [9].

Conclusion

Based on a bioinformatic analysis of the proband's whole-exome sequence, along with the symptoms and previous investigations, we conclude that the proband is very likely to have Familial hypercholanemia-1, which is caused by a mutation in the Tight Junction Protein 2 (TJP2) gene.

Supplementary Material

This GitHub LINK has the code of the bioinformatic analysis.

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