Annals of Clinical Case Reports

Case Report
Published: 23 Oct, 2023

Mutations in the CFAP74 Gene Explains the Variation of the Phenotype in a Family Quartet with PCD

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Abstract

Primary Ciliary Dyskinesia (PCD) is a rare genetic disease in which patients suffer from recurrent airway infections and other pulmonary conditions as well as other cilia-related problems such as sterility and situs inversus. There are many genes that are associated with PCD and these genes are responsible for either the structure or the function of the cilia. In this study, a family quartet consisting of unaffected parents and their offspring with PCD was examined. One of the children shows more severe symptoms of PCD, while the other child has a milder phenotype. Two variants in the CFAP74 gene were identified as a compound heterozygous state in the child with severe PCD and one of these mutations is found in the child with milder PCD. Our results demonstrate the importance of carrying another causal variant on the severity of the phenotype.

Introduction

Primary Ciliary Dyskinesia (PCD) is one of the rare ciliopathies causing chronic lower and upper airway infections, bronchiectasis, sinusitis, Situs Inversus (SI), hearing loss and sterility. Caused by congenital ciliary dysfunction, PCD is distinguished from secondary ciliary problems. Diagnosis is complex, relying on nNO measurement, ciliary beat frequency, cilia ultrastructure, PICADAR scoring and genetic testing. Early diagnosis enhances the quality of life with preventive measures and airway clearance [1].

From the nose to the furthest portion of the conducting airways (the terminal bronchioles), the human respiratory system is lined by a mucociliary epithelium. Through the constant trapping and upward movement of inhaled particles and pathogens, mucus-producing cells and ciliated cells with numerous motile cilia work together to form the mucociliary clearance system, which is an essential component of the Airway Defense system [2].

Cilia are hair-like, microtubule-based organelles that extend from the surface of many types of cells. They are composed of a cylindrical arrangement of microtubule doublets, which are made up of tubulin monomers. The microtubules are organized in a 9+2 pattern in motile cilia, meaning that there are nine microtubule doublets surrounding a central pair of microtubules. Non-motile and nodal cilia have a 9+0 configuration, meaning that they lack the central pair of microtubules and the dynein arms that are present in motile cilia.

Motile cilia beat in a coordinated, wave-like pattern, which is essential for their function in processes such as mucociliary clearance and hearing. The coordinated beating of these cilia is regulated by a complex system involving the dynein arms, which are molecular motors that drive the sliding of microtubules against each other. Non-motile and nodal cilia, on the other hand, do not have dynein arms and are unable to move. Instead, their function is primarily related to sensory and signaling processes. In the case of nodal cilia, their spinning motion generates fluid flow, which is essential for determining left-right asymmetry in early embryos. The appropriate form and function of cilia are essential for a range of physiological processes, and their dysfunction can lead to various diseases, collectively known as ciliopathies.

There are already more than 40 genes that have been linked to PCD due to the intricacy of motile cilia and ciliogenesis. Dynein-related genes such as the Dynein Axonemal Assembly Factor (DNAAF) family genes, the Dynein Axonemal Heavy Chain (DNAH) family genes, the Dynein Axonemal Intermediate Chain (DNAI) family genes, the Dynein Axonemal Light Chain (DNAL) family genes and Outer Dynein Arm Docking Complex (ODAD) subunit genes which have integral roles in assembly and motility of dynein arms have been associated to PCD. Mutations in these genes may cause defects in ciliary movement, leading to impaired mucociliary clearance and

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Received Date: *03 Oct 2023*
Accepted Date: *17 Oct 2023*
Published Date: *23 Oct 2023*

Citation:
Öz E, Sever EA, Eralp EE, Karadağ B, Gökdemir Y, Sezerman OU. Mutations in the CFAP74 Gene Explains the Variation of the Phenotype in a Family Quartet with PCD. Ann Clin Case Rep. 2023; 8: 2505.
ISSN: 2474-1655.

increased susceptibility to respiratory infections. In addition, various Coiled-Coil Domain genes such as CCDC103, CCDC39, CCDC40, and CCDC65 also have regulatory and structural roles in dynein assembly [3,4].

On the other hand, there are genes that are associated with PCD, affecting different pathways in ciliogenesis and cilia function as well. To illustrate, the Cilia and Flagella Associated Protein (CFAP) gene family is one of the major gene families implicated in PCD pathogenesis. CFAP genes encode proteins that are involved in the assembly and maintenance of cilia, as well as in the regulation of ciliary motility. Recent studies have shown that mutations in CFAP genes cause defects in cilia structure and function, leading to impaired mucociliary clearance and recurrent infections [5-8]. One of the genes that belong to this family is the CFAP74 gene which is predicted to be involved in axoneme assembly. CFAP74 gene has been associated with PCD with minor ciliary beating irregularities and a reduced ability to remove mucus even though it has normal ciliary ultrastructure. in a number of studies [9-12].

Case Presentation

The case consists of a family of 2 children who are PCD patients and their unaffected parents. Affected siblings are encoded as EPIX53 and EPIX56, whereas unaffected parents are encoded as EPIX54 and EPIX55. EPIX53 exhibits more severe symptoms of PCD even though both of the siblings have the disease. The clinical table of the family is shown in Table 1 (Figure 1).

Five ml (5 ml) Blood samples taken from the children and the parents were isolated with the Zymo Research Quick-DNA blood isolation kit following the user manual. DNA concentrations were measured by the IMPLEN PEARL nanophotometer. Whole exome sequencing was performed on the DNAs with a >100 (ng/µl) using the Illumina Hiseq 2500 platform and Agilent SureSelect XT_V6 Post cap kit. Adapters on the reads were removed by Trimmomatic (v 0.36). The sequencing data were processed according to GATK best practices version 4.0. The reference genome hg38 was used for alignment with the help of BWA mem v0.7.12. Afterwards, the annotations including the dbSNP database, pathogenicity predictions from several in silico tools, and Minor Allele Frequencies (MAF) were added by using the ANNOVAR program.

Table 1: Clinical information of family.

ID	PICADAR	Recurrent Lower Respiratory Infection	Recurrent Upper Respiratory Infection	Dexrocardy	BE
EPIX53	8	Yes	Yes	No	Yes
EPIX56	5	Yes	Yes	No	Yes
EPIX54	0	No	No	No	No
EPIX55	0	No	Yes	No	No

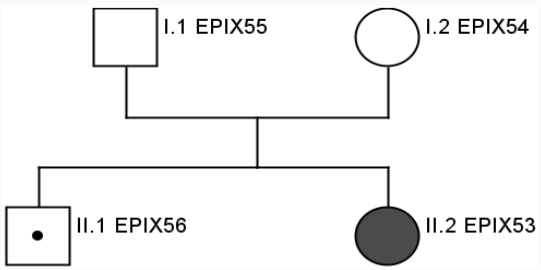

Figure 1: Pedigree of family.

In the variant filtration step, prioritizing variants is a crucial step in identifying potential disease-causing mutations. To accomplish this, various criteria are used to filter through the vast amount of genomic data generated from sequencing. One such criterion is the Minor Allele Frequency (MAF) threshold. In this case, an MAF threshold of 0.01 was used. Additionally, only variants located within exonic and splicing regions were extracted for further analysis. Synonymous variants were also eliminated from consideration. To further refine the analysis, multiple analyses were conducted using both autosomal dominant and compound heterozygous disease models. Finally, analyses were repeated both with and without EPIX56. By conducting multiple analyses using different models and criteria, it has been aimed to identify the most likely disease-causing variant.

After the causal variants were identified, they were confirmed by Sanger sequencing. Primers that are designed specific to regions of mutation are shown in Table 2. Images of mutations are shown in Figure 2.

Discussion

The study conducted on two siblings has shed light on a genetic mutation that has not been reported in ClinVar before. Albeit the frequency of this mutation is not low, with 0.00771 in the gnomAD exome and 0.00795 in the gnomAD genome, the CFAP74:c.G3206T:p.G1069V mutation found in the siblings is absent in their parents, indicating that it is a *de novo* mutation. This finding suggests that the mutation may have arisen spontaneously in the siblings.

It is noteworthy that the sibling who suffers from more severe PCD symptoms, EPIX53, has another heterozygous mutation in the same gene, as a compound heterozygous state, CFAP74:c.G1163A:p.R388Q. This mutation is rarer than the other mutation and there is not any healthy individual that carries it homozygous in gnomAD database, albeit the data in the region have a good coverage. The mutation is also found in a heterozygous state in the mother, EPIX54, but not in other members of the family. There is not any other possibly causing variant that may explain the patient's phenotype better.

The CFAP74 gene has been previously linked to PCD, and studies have shown that individuals with mutations in this gene may exhibit minor ciliary beating irregularities and a reduced ability to remove mucus despite having normal ciliary ultrastructure. The findings of this study add to the growing body of evidence that suggests that mutations in the CFAP74 gene play a role in the development of PCD [12]. In addition, our results demonstrate the importance of carrying another causal variant on the severity of the phenotype.

Conclusion

In conclusion, the identification of the CFAP74:c.G3206T:p.

Table 2: Primer sequences for regions of mutations.

Mutation	Forward	Reverse
CFAP74:c.G3206T:p.G1069V	TGAAACCCTGTGGTTAAGGGTG	ACGGCCCTATACGACACCT
CFAP74:c.G1163A:p.R388Q	TCCGTAGTGTGTCTTGTCCC	TGCACTCATCTGAGAAGCGA

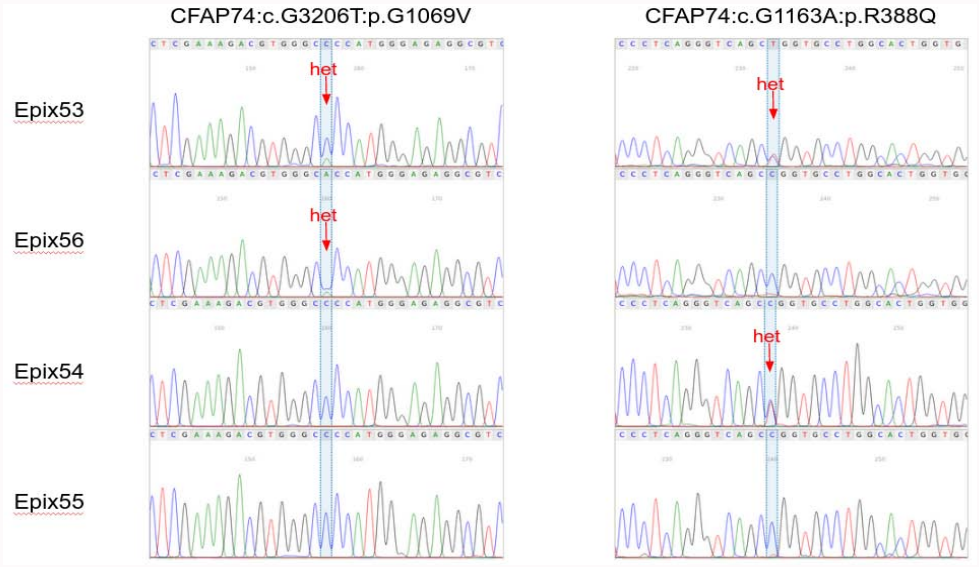

Figure 2: Chromatography images for regions of mutations.

G1069V mutation in both siblings and the fact that the second mutation CFAP74:c.G1163A:p.R388Q causes a more severe phenotype highlights the importance of genetic testing in the demonstration of the fact that impact of additional mutations in the same gene may explain variations of the phenotype. Functional studies are needed to understand the whole mechanism by which mutations in the CFAP74 gene lead to the development of PCD and to develop effective treatments for it.

Acknowledgement

This project is funded by TÜBİTAK 1001 grant (No 119S713).

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