Two novel mutations in TTC21B gene, c.497del and c.2323-3T>A, identified in a Chinese patient

Xia Gao (gaoxiagz@vip.163.com)
Guangzhou Women and Children's Medical Center
Lingna Shi
Lanzhou University Second Hospital
Xin Liao
Guangzhou Women and Children's Medical Center
Qing Du
Gansu Province People's Hospital
Haiyan Zhao
Gansu Province People's Hospital
Lili Wang
Gansu Province People's Hospital
Jing Zhang
Ningxia Medical University

Case Report

Keywords: Nephronophthisis, TTC21B, ciliopathies, end-stage renal disease

Posted Date: July 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-39200/v1

Abstract

Methods

A whole-exome sequencing end stage renal disease patient whose original renal disease is unknown. Swiss model predict the 3D structure of the protein. The related literature was searched by using search terms “NPHP” in PubMed CNKI and VIP database from January 2000 to January 2020.

Results

The whole exome by next-generation sequencing and found two unreported TTC21B mutation sites, c.497del (p.Lys166Serfs) and c.2323−3T > A. The c.497del (p.Lys166Serfs) variant, which is a frame shift mutation, suggested deletion of a single nucleotide. A at position 497 from the TTC21B gene CDS results in replacement of lysine by serine at codon 166 of the TTC21B protein, as well as premature translational stop at position 201. The c.2323−3T > A variant indicates that the antepenultimate base of intron upstream of nucleotide 2323 in the CDS region of TTC21B gene changes from T to A. The change in the three-dimensional structure of the protein caused by the mutations in TTC21B may affect the functions associated with the protein length shorten. Further, this study summarized 25 NPHP gene mutations and the phenotypes that have been reported.

Conclusion

This study reported two novel mutations in the TTC21B gene resulting in NPHP in a Chinese patient and expanded on the spectrum of known causative mutations of TTC21B gene.

Introduction

Nephronophthisis (NPHP) is a cystic renal disease caused by autosomal recessive inheritance of the tubular interstitium [1]. NPHP is a relatively rare disorder with a prevalence of 1/900,000 to 1/50,000 in the world, 1/61,800 in Finland, and 1/1,000,000 in the United States [2-4].

Recent studies found that the protein products of all NPHP genes are expressed in the primary cilia of most mammalian cells [5]. Therefore, the associated diseases are sometimes called renal ciliopathies. Cilium refers to the microscopic digitation from the cellsurface [5]. It is a type of projection organ similar to an antenna, which is involved in the transmission of intracellular and intercellular information [6]. Therefore, cilia play a key role in normal cellular function. Almost all NPHP-related genes encode cilia-related proteins, and pathogenic mutations in these genes may lead to defects in ciliary protein formation or transport [6].

Until now, there were 15 NPHP reports from China [7]. The main genotypes were NPHP4, NPHP8, NPHP12, NPHP2, NPHP3, and NPHP5. Recently, a case of NPHP was diagnosed by our team using whole-exome sequencing. The patient harbored c.497del (p.Lys166 Serfs) and c.2323-3T>A mutations in the TTC21B gene. Mutations in these two loci of TTC21B gene have not been reported. This study also summarize and analyze the newly reported NPHP genotype data.

Subjects And Methods

Patient

The proband was an 7-year-old Chinese female, who was hospitalized at the Gansu Provincial Hospital (Lanzhou, China) because of chronic renal failure. The proband's 5-year-old brother and parents were healthy. Analysis of the medical family history found the family had a history of marriage between close relatives for three generations. The patient’s paternal cousin died of unexplained uremia at the age of 23. Blood samples of the proband and her parents were collected for further analysis.

The proband underwent a series of clinical examinations to assess the presentation of renal failure, including biochemical blood analysis, echocardiography，blood gas analysis, parathyroid hormone，electrolyte, and so on. But, the proband’s parents refused renal biopsy. In order to clarify the cause of renal failure, the whole-exome sequencing (WES) was recommended to analyze the genotypic characteristics of the patient and her parents.

Whole-exome sequencing

Fresh peripheral blood samples were sent for WES and disease-related mutation analysis (Shanghai Gemple Biotechnology Co. Ltd). Total DNA was isolated using the UnigeneDx Blood DNA Extraction Kit (UnigeneDx) and DNA concentration was determined using the Qubit dsDNA HS Assay Kit (Life Technologies). Exome sequencing libraries were prepared using the KAPA HyperPlus Prep Kit (KAPA Biosystem, Roche), and enrichment was performed with the SeqCap EZ MedExome Kit (NimbleGen, Roche) according to the manufacturer's instructions. Libraries were sequenced using the Illumina Hiseq X Ten platform by 150 bp paired-end sequencing.

WES data processing and analysis

Fastq data were translated into vcf files using an integrated bioinformatic pipeline. First, the paired-end reads were subjected to quality control by FASTQC version 0.11.5. Second, Burrows-Wheeler Aligner (BWA) version 0.7.15 [8] was used to align sequencing reads to the reference human genome GRCh19. SAM format files were generated by BWA. Third, the SAM format files were further processed to BAM files using Samtools version 1.3.1 [9], followed by the removal of duplicates using Picard version 2.5 [10]. After these processes, variant calling was performed by GATK-HaplotypeCaller version 4.0 [11] and the Vcf file was generated. Finally, the Vcf data were analyzed on the Translational Genomics Expert (TGex) platform featuring the VarElect scoring system. Candidate variants were manually analyzed according to the basic criteria specified by American College of Medical Genetics and Genomics (ACMG) guideline [12].

Sanger sequencing validation

The two candidate variants of the TTC21B gene were validated by conventional polymerase chain reaction amplification and Sanger sequencing in both the case and the parents (Table. 1).

Three-dimensional (3D) structure of the ATM protein

The 3d structure of the protein was predicted by Swiss Model (https://www.swissmodel.expasy.org/), and that of its mutated form was predicted based on the cdna nucleotide sequences of the patients. The wild-type and mutated form were compared to determine the effect of the mutation on its structure [13, 14].

Results

Clinical features

Physical examination data of the patient at the time of admission were as follows : blood pressure 160/100 mmHg; height 106 cm (below the third percentile), and weight 22 kg (below the third percentile). The hands were larger than those of her age. The phalanges were thick and flat. In addition, clubbing of fingers was observed. The toes of both feet were significantly short and small, hypertrophic, and without deformity. The palms of the upper limbs and lower extremities below the joint showed slightly pitting edema. The eyelids also showed slight edema. Renal function parameters were as follows: Blood urea nitrogen 45.3 mmol/L, Serum creatinine 643.1 μmol/ L, and Uric acid 399 μmol/L. The other laboratory positive tests including: proteinuria 1.9008 g/24 h, albuminaemia 46.1 g/L, and cholesterolemia 4.09 mmol/L. B-mode ultrasound showed the right kidney dimensions of 74 mm × 24 mm and left kidney dimensions of 69 mm × 32 mm. The cardiac ultrasound showed left atrioventricular cavity enlargement, main pulmonary artery widening, and aortic valve insufficiency (mild). MRU showed peritoneal and pelvic effusion（Fig. 1a）. Renal dynamic imaging showed left and right kidney glomerular filtration rates of 13 ml/min and 16 ml/min, respectively, indicating that the functions of both kidneys were severely impaired（Fig 1b）. Electrocardiogram showed sinus arrhythmia and ST-T changes (suggesting hyperkalemia).

Genetic analysis

Genotypic analysis of the patient was performed with an average sequencing depth of 182.66x and 98.86% coverage of targeted variants at least 20x depth. The data were analyzed on the Translational Genomics Expert (TGex) platform featuring the VarElect scoring system [15] (Table. 2). Two mutations c.497del (p.Lys166Serfs) and c.2323-3T>A were selected as candidate mutations in the TTC21B gene. Sanger sequencing of the parents'DNA samples were performed to confirm the origin of both mutations (Fig. 2). The results showed that the two variants of the proband were complex heterozygous variants, and the proband's father was the carrier of the variant c.2323-3T>A, while the proband's mother was the carrier of the variant c.497del (p.Lys166Serfs) (Table. 3).

The pathogenicity of these variants referred to the ACMG guidelines [12]. The c.497del (p.Lys166Serfs) variant, which is a frameshift mutation, was classified as likely pathogenic based on the following evidences.

Deletion of a single nucleotide a at position 497 from the TTC21B gene CDS results in replacement of lysine by serine at codon 166 of the TTC21B protein, as well as premature translational stop at position 201. It is predicted that the mutant allele eventually encodes a truncated

protein of 200 amino acids as compared to the wild-type protein of 1316 amino acids. Thus, this variant is predicted to be a loss-of-function (LoF) variant, either through protein truncation or nonsense-mediated mRNA decay (NMD). Since multiple loss-of-function variants of TTC21B are known to be pathogenic, TTC21B is proposed to cause diseases through a LoF mechanism. The c.497del (p.Lys166Serfs) variant is not observed in large population cohorts (1000 Genomes, ExAC, gnomAD). In addition, there is no information on this variantinthe available literature.

The c.2323-3T>A variant indicates that the antepenultimate base of intron upstream of nucleotide 2323 in the CDS region of TTC21B gene changes from T to A. The c.2323-3T>A variant is not reported in large population cohorts (1000 Genomes, ExAC, gnomAD). In addition, there is no information on this variant in the available literature. Splicing variant prediction analysis methods (dbscSNV) suggests that this variant may cause abnormal gene splicing, but this prediction has not been confirmed by published transcriptional studies. This variant is found in compound heterozygous individuals with a likely pathogenic variant in this case. Therefore, we interpret c.2323-3T>A as a variant of uncertain significance.

The proband's mother carried the same heterozygous mutation, whereas the father did not, the father possessed wild-type gene. This indicates that the heterozygous mutation c.497del (p.Lys166Serfs) in the TTC21B gene of the patient was inherited from her mother. The c.2323-3T>A heterozygous variant detected in the TTC21B gene of the patient was also present inthe father. This mutation was not detected in the mother, who possessed the wild-type gene, indicating that the heterozygous mutation 2323-3T>A in the TTC21B gene was paternally inherited（Fig. 2）.

3D structure of TTC21B and its variant form.

The structure of the protein predicted by Swiss Model indicated that the mutation resulted in protein structure changed. According to the Smart (smart. embl-heidelberg.de/) and Pfam (http://pfam.xfam.org/) data- bases, the mutation due to an obvious shorten in the protein. The wild type and mutation's amino aids are showed in table 4. And protein 3D structure was showen in Fig 3.

Summary of reports on NPHP-related gene mutations

This study reviewed the reported NPHP-related pathogenic genes and found that a total of 25 gene mutations could lead to NPHP. As shown in Table 5, different gene mutations could lead to different or identical clinical phenotypes. Mutations in the same gene may lead to a variety of severe phenotypes. Missense mutations may result in isolated NPHP or Senior-Loken syndrome (SLS, retinitis pigmentosa), whereas truncation mutations may lead to Meckel-Gruber syndrome (MKS) [16]. In addition, some NPHP genes may be related to extra-renal characteristics. In addition, patients with Bardet-Biedl syndrome (BBS), a cilia-related disease, show the characteristics of "modifier gene" in which multiple genes have pathogenic mutations. This phenomenon is called genetic heterogeneity [17].

At present, NPHP-2, 3, 4, 8, and 12 have been reported in China. It is found that NPHP-8 and NPHP-4 are co-located in the transition zone of cilia base, while NPHP8 plays an important role in cilia formation and cilia-mediated chemoreceptors [18]. Genotype to phenotype correlations were observed in NPHP3 gene mutations, all of which showed hepatorenal phenotype [7].

Discussion

The earliest clinical symptom of NPHP is polyuria, which is caused by renal resistance to vasopressin and impairment of urine concentration. During the disease progression, anemia may occur earlier. Loss of sodium through urine may also occur, which is often accompanied by developmental retardation and osteopathy. Disease progression is slow in many patients, and renal insufficiency is difficult to detect clinically until they are diagnosed with uremia.

About 15% of patients could exhibit extrarenal phenotypes affecting the eyes, liver, skeleton, central nervous system, etc. Eye damage is mainly manifested as retinopathy in NPHP, and patients may exhibit coarse nystagmus, retinal degeneration, optic atrophy, retinal defects, and even blindness [19]. A small number of patients may also have abnormal manifestations such as liver fibrosis and conical epiphyseal separation. Therefore, it is very difficult to diagnose NPHP based on clinical phenotypes, and it is easy to be overlooked and misdiagnosed.

It's well known that the disease is associated with relatively clear pathogenic genes, and WES has helped clinicians enhance their understanding of the disease and improve the detection rate of the disease. For example, in children with end-stage renal disease (ESRD), NPHP accounts for 5% [19] of cases in the United States and 6.5% [20, 21] of cases in the United Kingdom. However, there arelimited reports on NPHP cases in China. In recent years, with the widespread application of genomics technology, the genotype data of NPHP patients is increasing lyused for diagnosis in China.

The cause of the present case was not easy to identify. The proband was admitted to hospital with renal failure; at the time of admission, the kidney size was consistent with age, and renal failure was considered as an acute onset. Routine urine analysis indicated presence of urinary protein (+++), suggesting a possible history of chronic renal disease. To determine the causes of proteinuria, renal biopsy was recommended. Due to the family members refused a renal biopsy. The patient showed characteristics, such as acromegaly facies, and thick, short, and flat phalanges. The family had a history of marriage between close family members for three generations. After multiple disciplinary team (MDT) discussion, we decided to make a definite diagnosis by sequencing the whole exome. The results revealed c.497del (p.Lys166Serfs) and c.2323-3T>A mutations in the TTC21B gene; the genotype was highly correlated with the clinical phenotype of the patient. Further, sanger sequencing was performed to verify that the two variants of the mutation in the patient were inherited from herparents, who were compound heterozygous in dividuals, this was consistent with the autosomal recessive inheritance model. The two mutation sites were retrieved from databases including PubMed, EMBASE, HighWire, the Cochrane Library, and CNKI. The retrieval time was from January 1992 to January 2020. The search terms included “NPHP”, “ciliopathies”, “TTC21B”, and “adolescent kidney depletion disease”. Retrospective retrieval and citation retrieval of references were carried out. Manual retrieval was also carried out when necessary. It was finally confirmed that the two genetic loci of the patient carried mutations.

This case highlights three major points: 1. genetic testing should be carried out as early as possible for children in high-risk family to facilitate early diagnosis, especially those who have lost the chance of renal biopsy; 2. the understanding of NPHP has been significantly improved by WES, although only about 30% of NPHP patients have identifiable mutations [22]. This suggests that more NPHP genes are yet to be discovered. The NPHP genes will give us a better understanding of the pathological mechanisms of this disease and the relationship between cilia and cyst development. In addition to conventional treatment and renal replacement therapy, the new therapies are expected to replace the old ones; 3. it is very important to strengthen prenatal education and premarital examination in poverty-stricken areas and avoid the transmission of inherited metabolic diseases.

Although 25 genetic loci related to NPHP are reported, they are only found in approximately 30% of NPHP cases [22]. Different gene loci have different clinical manifestations, which are related to the pathogenesis of gene loci. However, the same gene loci may have different clinical manifestations, such as different TTC21B loci mutations resulting in clinical manifestations with significant differences. A heteromorphic allele of TTC21B may cause Jeune asphyxiating thoracic dystrophy (JATD), while a similar genotype (c.2758-2A>G-het; p.Pro209Leu-het or p.Cys552X-het; p.Pro209Leu-het) leads to early onset of NPHP; however, homozygous p.Pro209Leu leads to significantly isolated NPHP [23]. Moreover, the same p.Pro209Leu allele can also exist in the heterozygous in dividuals with BBS having cystic nephropathy (BBS prevalence < 24%) [24].

NPHP1 is the first identified NPHP gene, accounting for the majority of known NPHP cases (20-25%) [25, 26]. NPHP2 is observed in infants, and may lead to established renal failure (ERF) [27]. Mutations in NPHP3 may lead to a variety of phenotypes, including adolescent NPHP, NPHP with liver fibrosis, NPHP with retinitis pigmentosa (RP), infantile NPHP, and MKS [28, 29]. NPHP4 mutation is most frequently associated with retinal phenotypes [30]. In patients with NPHP5 and NPHP10, abnormal expression of renal cyst protein connecting cilia may increase the risk of retinitis pigmentosa by 100% and 80% respectively. In addition, NPHP5 and NPHP6 were also found to be directly related to the function of the retinitis pigmentosa GTPase regulator (RPgr) [31, 32]. Other reported NPHPs that can be detected in photoreceptors include NPHP-1, 8, 11, and 12 [23, 33-35]. NPHP7 is associated with Gli-similar protein 2 transcription factor, and the deletion of this transcription factor results in an increased risk of fibrosis and apoptosis [36]. The deletion of NPHP9 is associated with increased DNA damage in the pathogenesis of NPHP [37]. NPHP-5, 6, 9, 10, 13, and 15 have been reported in SLS syndrome case reports [38-41]. Mutations in NPHP-6 and NPHP-11 may cause Joubert syndrome [42]. In some cases, the type of mutation in the disease may affect the phenotype, for example, the reported truncation mutations of NPHP11 [43, 44] and NPHP5 [45] often lead to a clinical phenotype that is more severe than that caused by missense mutations.

In conclusion, the two novel mutations in TTC21B gene, c.497del and c.2323-3T>A, identified in a Chinese patient. The deletion mutation in the TTC21B gene resulted in a change of the 3D structure of the protein, which may have impaired the functions. And, this study highlighted the importance of genetic testing in the diagnosis of this disease. The related gene mutation review contributed to the spectrum of known pathogenic variants of TTC21B, expanded the current understanding on the NPHP.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

XG , LNS and XL conceived and designed the study. LNS, HYZ and QD were responsible for the acquisition of patient information and communication with the patients' families. JZ and LLW collected clinical data from the NPHP family. XL contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors have read and approved the final manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 81660130) and the Natural Science Foundation of Gansu Province, China (Grant No. 18JR3RA045).

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standard of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Informed written consent for publication was obtained from the patient. A copy of the consent form is available

Competing interests

The authors declare that they have no competing interests.

References

1. Konig J, Kranz B, Konig S, Schlingmann KP, Titieni A, Tonshoff B, et al. Phenotypic Spectrum of Children with Nephronophthisis and Related Ciliopathies. Clin J Am Soc Nephrol. 2017;12(12):1974-83.
2. Ala-Mello S, Koskimies O, Rapola J, Kaariainen H. Nephronophthisis in Finland: epidemiology and comparison of genetically classified subgroups. European journal of human genetics : EJHG. 1999;7(2):205-11.
3. Ala-Mello S, Sankila EM, Koskimies O, de la Chapelle A, Kaariainen H. Molecular studies in Finnish patients with familial juvenile nephronophthisis exclude a founder effect and support a common mutation causing mechanism. J Med Genet. 1998;35(4):279-83.
4. Blowey DL, Querfeld U, Geary D, Warady BA, Alon U. Ultrasound findings in juvenile nephronophthisis. Pediatr Nephrol. 1996;10(1):22-4.
5. Stokman M, Lilien M, Knoers N. Nephronophthisis. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA): University of Washington, Seattle University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
6. Bredrup C, Saunier S, Oud MM, Fiskerstrand T, Hoischen A, Brackman D, et al. Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the IFT-A gene WDR19. Am J Hum Genet. 2011;89(5):634-43.
7. Sun L, Tong H, Wang H, Yue Z, Liu T, Lin H, et al. High mutation rate of NPHP3 in 18 Chinese infantile nephronophthisis patients. Nephrology (Carlton). 2016;21(3):209-16.
8. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics (Oxford, England). 2009;25(14):1754-60.
9. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics (Oxford, England). 2009;25(16):2078-9.
10. http://broadinstitute.github.io/picard/.
11. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 2010;20(9):1297-303.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(5):405-24.

13. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic acids research. 2014;42(Web Server issue):W252-8.
14. Kiefer F, Arnold K, Kunzli M, Bordoli L, Schwede T. The SWISS-MODEL Repository and associated resources. Nucleic acids research. 2009;37(Database issue):D387-92.
15. Stelzer G, Plaschkes I, Oz-Levi D, Alkelai A, Olender T, Zimmerman S, et al. VarElect: the phenotype-based variation prioritizer of the GeneCards Suite. BMC genomics. 2016;17 Suppl 2:444.
16. Coppieters F, Lefever S, Leroy BP, De Baere E. CEP290, a gene with many faces: mutation overview and presentation of CEP290base. Human mutation. 2010;31(10):1097-108.
17. Badano JL, Kim JC, Hoskins BE, Lewis RA, Ansley SJ, Cutler DJ, et al. Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. Human molecular genetics. 2003;12(14):1651-9.
18. Liu L, Zhang M, Xia Z, Xu P, Chen L, Xu T. Caenorhabditis elegans ciliary protein NPHP-8, the homologue of human RPGRIP1L, is required for ciliogenesis and chemosensation. Biochem Biophys Res Commun. 2011;410(3):626-31.
19. Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy. J Am Soc Nephrol. 2009;20(1):23-35.
20. Lewis M, Shaw J, Reid C, Evans J, Webb N, Verrier-Jones K. Demography and management of childhood established renal failure in the UK (chapter 13). Nephrol Dial Transplant. 2007;22 Suppl 7:vii165-75.
21. Lewis MA, Shaw J, Sinha M, Adalat S, Hussain F, Inward C. UK Renal Registry 11th Annual Report (December 2008): Chapter 13 Demography of the UK paediatric renal replacement therapy population. Nephron Clinical practice. 2009;111 Suppl 1:c257-67.
22. Hurd TW, Hildebrandt F. Mechanisms of nephronophthisis and related ciliopathies. Nephron Experimental nephrology. 2011;118(1):e9-14.
23. Davis EE, Zhang Q, Liu Q, Diplas BH, Davey LM, Hartley J, et al. TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum. Nat Genet. 2011;43(3):189-96.
24. Beales PL, Elcioglu N, Woolf AS, Parker D, Flinter FA. New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. J Med Genet. 1999;36(6):437-46.
25. Hildebrandt F, Otto E, Rensing C, Nothwang HG, Vollmer M, Adolphs J, et al. A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. Nat Genet. 1997;17(2):149-53.
26. Saunier S, Calado J, Heilig R, Silbermann F, Benessy F, Morin G, et al. A novel gene that encodes a protein with a putative src homology 3 domain is a candidate gene for familial juvenile nephronophthisis. Human molecular genetics. 1997;6(13):2317-23.
27. Haider NB, Carmi R, Shalev H, Sheffield VC, Landau D. A Bedouin kindred with infantile nephronophthisis demonstrates linkage to chromosome 9 by homozygosity mapping. Am J Hum Genet. 1998;63(5):1404-10.
28. Bergmann C, Fliegauf M, Bruchle NO, Frank V, Olbrich H, Kirschner J, et al. Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. Am J Hum Genet. 2008;82(4):959-70.
29. Tory K, Rousset-Rouviere C, Gubler MC, Moriniere V, Pawtowski A, Becker C, et al. Mutations of NPHP2 and NPHP3 in infantile nephronophthisis. Kidney Int. 2009;75(8):839-47.
30. Hoefele J, Sudbrak R, Reinhardt R, Lehrack S, Hennig S, Imm A, et al. Mutational analysis of the NPHP4 gene in 250 patients with nephronophthisis. Human mutation. 2005;25(4):411.
31. Otto EA, Loeys B, Khanna H, Hellemans J, Sudbrak R, Fan S, et al. Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. Nat Genet. 2005;37(3):282-8.
32. Chang B, Khanna H, Hawes N, Jimeno D, He S, Lillo C, et al. In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. Human molecular genetics. 2006;15(11):1847-57.
33. Fliegauf M, Horvath J, von Schnakenburg C, Olbrich H, Muller D, Thumfart J, et al. Nephrocystin specifically localizes to the transition zone of renal and respiratory cilia and photoreceptor connecting cilia. J Am Soc Nephrol. 2006;17(9):2424-33.
34. Murga-Zamalloa CA, Desai NJ, Hildebrandt F, Khanna H. Interaction of ciliary disease protein retinitis pigmentosa GTPase regulator with nephronophthisis-associated proteins in mammalian retinas. Molecular vision. 2010;16:1373-81.
35. Collin GB, Won J, Hicks WL, Cook SA, Nishina PM, Naggert JK. Meckelin is necessary for photoreceptor intraciliary transport and outer segment morphogenesis. Investigative ophthalmology & visual science. 2012;53(2):967-74.
36. Attanasio M, Uhlenhaut NH, Sousa VH, O'Toole JF, Otto E, Anlag K, et al. Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. Nat Genet. 2007;39(8):1018-24.

37. Choi HJ, Lin JR, Vannier JB, Slaats GG, Kile AC, Paulsen RD, et al. NEK8 links the ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies. Molecular cell. 2013;51(4):423-39.
38. Chaki M, Airik R, Ghosh AK, Giles RH, Chen R, Slaats GG, et al. Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. Cell. 2012;150(3):533-48.
39. Valente EM, Silhavy JL, Brancati F, Barrano G, Krishnaswami SR, Castori M, et al. Mutations in CEP290, which encodes a centrosomal protein, cause pleiotropic forms of Joubert syndrome. Nat Genet. 2006;38(6):623-5.
40. Schaefer E, Zaloszyc A, Lauer J, Durand M, Stutzmann F, Perdomo-Trujillo Y, et al. Mutations in SDCCAG8/NPHP10 Cause Bardet-Biedl Syndrome and Are Associated with Penetrant Renal Disease and Absent Polydactyly. Molecular syndromology. 2011;1(6):273-81.
41. Halbritter J, Porath JD, Diaz KA, Braun DA, Kohl S, Chaki M, et al. Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. Human genetics. 2013;132(8):865-84.
42. Fleming LR, Doherty DA, Parisi MA, Glass IA, Bryant J, Fischer R, et al. Prospective Evaluation of Kidney Disease in Joubert Syndrome. Clin J Am Soc Nephrol. 2017;12(12):1962-73.
43. Mougou-Zerelli S, Thomas S, Szenker E, Audollent S, Elkhartoufi N, Babarit C, et al. CC2D2A mutations in Meckel and Joubert syndromes indicate a genotype-phenotype correlation. Human mutation. 2009;30(11):1574-82.
44. Bachmann-Gagescu R, Ishak GE, Dempsey JC, Adkins J, O'Day D, Phelps IG, et al. Genotype-phenotype correlation in CC2D2A-related Joubert syndrome reveals an association with ventriculomegaly and seizures. J Med Genet. 2012;49(2):126-37.
45. Iannicelli M, Brancati F, Mougou-Zerelli S, Mazzotta A, Thomas S, Elkhartoufi N, et al. Novel TMEM67 mutations and genotype-phenotype correlates in meckelin-related ciliopathies. Human mutation. 2010;31(5):E1319-31.
46. Tory K, Lacoste T, Burglen L, Moriniere V, Boddaert N, Macher MA, et al. High NPHP1 and NPHP6 mutation rate in patients with Joubert syndrome and nephronophthisis: potential epistatic effect of NPHP6 and AHI1 mutations in patients with NPHP1 mutations. J Am Soc Nephrol. 2007;18(5):1566-75.
47. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, et al. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet. 2003;34(4):413-20.
48. Olbrich H, Fliegauf M, Hoefele J, Kispert A, Otto E, Volz A, et al. Mutations in a novel gene, NPHP3, cause adolescent nephronophthisis, tapeto-retinal degeneration and hepatic fibrosis. Nat Genet. 2003;34(4):455-9.
49. Gattone VH, 2nd, Wang X, Harris PC, Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. Nature medicine. 2003;9(10):1323-6.
50. Bakkaloglu SA, Kandur Y, Bedir-Demirdag T, Isik-Gonul I, Hildebrandt F. Diverse phenotypic expression of NPHP4 mutations in four siblings. The Turkish journal of pediatrics. 2014;56(4):423-6.
51. Wolf MT, Hildebrandt F. Nephronophthisis. Pediatr Nephrol. 2011;26(2):181-94.
52. Sayer JA, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, et al. The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. Nat Genet. 2006;38(6):674-81.
53. Wolf MT, Saunier S, O'Toole JF, Wanner N, Groshong T, Attanasio M, et al. Mutational analysis of the RPGRIP1L gene in patients with Joubert syndrome and nephronophthisis. Kidney Int. 2007;72(12):1520-6.
54. Otto EA, Trapp ML, Schultheiss UT, Helou J, Quarmby LM, Hildebrandt F. NEK8 mutations affect ciliary and centrosomal localization and may cause nephronophthisis. J Am Soc Nephrol. 2008;19(3):587-92.
55. Otto EA, Tory K, Attanasio M, Zhou W, Chaki M, Paruchuri Y, et al. Hypomorphic mutations in meckelin (MKS3/TMEM67) cause nephronophthisis with liver fibrosis (NPHP11). J Med Genet. 2009;46(10):663-70.
56. Fehrenbach H, Decker C, Eisenberger T, Frank V, Hampel T, Walden U, et al. Mutations in WDR19 encoding the intraflagellar transport component IFT144 cause a broad spectrum of ciliopathies. Pediatr Nephrol. 2014;29(8):1451-6.
57. Park E, Lee JM, Ahn YH, Kang HG, Ha, Il, Lee JH, et al. Hepatorenal fibrocystic diseases in children. Pediatr Nephrol. 2016;31(1):113-9.
58. Cheng LE, Zhang J, Reed RR. The transcription factor Zfp423/OAZ is required for cerebellar development and CNS midline patterning. Developmental biology. 2007;307(1):43-52.
59. Ramachandran H, Engel C, Muller B, Dengjel J, Walz G, Yakulov TA. Anks3 alters the sub-cellular localization of the Nek7 kinase. Biochem Biophys Res Commun. 2015;464(3):901-7.
60. Taskiran EZ, Korkmaz E, Gucer S, Kosukcu C, Kaymaz F, Koyunlar C, et al. Mutations in ANKS6 cause a nephronophthisis-like phenotype with ESRD. J Am Soc Nephrol. 2014;25(8):1653-61.
61. Halbritter J, Bizet AA, Schmidts M, Porath JD, Braun DA, Gee HY, et al. Defects in the IFT-B component IFT172 cause Jeune and Mainzer-Saldino syndromes in humans. Am J Hum Genet. 2013;93(5):915-25.

62. Failler M, Gee HY, Krug P, Joo K, Halbritter J, Belkacem L, et al. Mutations of CEP83 cause infantile nephronophthisis and intellectual disability. Am J Hum Genet. 2014;94(6):905-14.
63. Schueler M, Braun DA, Chandrasekar G, Gee HY, Klasson TD, Halbritter J, et al. DCDC2 mutations cause a renal-hepatic ciliopathy by disrupting Wnt signaling. Am J Hum Genet. 2015;96(1):81-92.
64. Macia MS, Halbritter J, Delous M, Bredrup C, Gutter A, Filhol E, et al. Mutations in MAPKBP1 Cause Juvenile or Late-Onset Cilia-Independent Nephronophthisis. Am J Hum Genet. 2017;100(2):372.
65. Utsch B, Sayer JA, Attanasio M, Pereira RR, Eccles M, Hennies HC, et al. Identification of the first AHI1 gene mutations in nephronophthisis-associated Joubert syndrome. Pediatr Nephrol. 2006;21(1):32-5.
66. Tuz K, Hsiao YC, Juarez O, Shi B, Harmon EY, Phelps IG, et al. The Joubert syndrome-associated missense mutation (V443D) in the Abelson-helper integration site 1 (AHI1) protein alters its localization and protein-protein interactions. The Journal of biological chemistry. 2013;288(19):13676-94.
67. O'Toole JF, Liu Y, Davis EE, Westlake CJ, Attanasio M, Otto EA, et al. Individuals with mutations in XPNPEP3, which encodes a mitochondrial protein, develop a nephronophthisis-like nephropathy. The Journal of clinical investigation. 2010;120(3):791-802.
68. Hurd TW, Otto EA, Mishima E, Gee HY, Inoue H, Inazu M, et al. Mutation of the Mg2+ transporter SLC41A1 results in a nephronophthisis-like phenotype. J Am Soc Nephrol. 2013;24(6):967-77.
69. Gorden NT, Arts HH, Parisi MA, Coene KL, Letteboer SJ, van Beersum SE, et al. CC2D2A is mutated in Joubert syndrome and interacts with the ciliopathy-associated basal body protein CEP290. Am J Hum Genet. 2008;83(5):559-71.
70. Sang L, Miller JJ, Corbit KC, Giles RH, Brauer MJ, Otto EA, et al. Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. Cell. 2011;145(4):513-28.
71. Bizet AA, Becker-Heck A, Ryan R, Weber K, Filhol E, Krug P, et al. Mutations in TRAF3IP1/IFT54 reveal a new role for IFT proteins in microtubule stabilization. Nature communications. 2015;6:8666.

Tables

Table. 1 TTC21B Sequencing of TTC21B gene mutation sites

Mutation	Primer sequence	Product size (bp)
TTC21B	F:5'TATAGTTCCCAAGACACTAGGCT3'	462
c.497del / p.Lys166fs	R:5'CCTACACAAATGCCAGTCTACC3'	
TTC21B	F:5'CATACTTAATTGACAGGCAGGTGT3'	218
c.2323-3T>A /splicing	R:5'TCAGCCAGGTCATAGCAAAGAT3'	

Table. 2 List of candidate variants associated with clinical phenotypes

Gene (Transcript)	Chromosome Position hg19CDS	Nucleotide change	Amino acid change	Functional change	Heterozygosity	Variation classification	Source of variation	Related diseases (OMIM number, genetic model)
TTC21B NM_024753.4	chr2:166799784 Exon5	c.497del	p.Lys166Serfs	Frameshift variation	hybrid	Suspected pathogenicity	mother	NPHP12 type 613820, AD/AR
TTC21B NM_024753.4	chr2:166767978 Intron17	c.2323-3T>A	-	Splicing mutation	hybrid	Unclear meaning	father	NPHP12 type 613820, AD/AR

Table. 3 The samples information

Sample number	The sample name	Sample type	Clinical phenotype	Site 1 verification result	Site 2 verification result
GPD-B1830145	proband	peripheral blood	NPHP	heterozygous variation	heterozygous variation
GPD-B1830146	father of the proband	peripheral blood	normal	wild type	heterozygous variation
GPD-B1830147	mother of the proband	peripheral blood	normal	heterozygous variation	wild type

Table. 4 The amino aid sequence with wild type and the mutation

Types	Amino aids sequence
Wild type	MDSQELKTLINYYCQERYFHHVLLVASEGIKRYGSDPVFRFYHAYGTLMEGKTQEALREFEAIKNKQDVSLCSLLALIYAHK MSPNPDREAILESDARVKEQRKGAGEKALYHAGLFLWHIGRHDKAREYIDRMIKISDGSKQGHVLKAWLDITRGKEPYTKK ALKYFEEGLQDGNDTFALLGKAQCLEMRQNYSGALETVNQIIVNFPSFLPAFVKKMKLQLALQDWDQTVETAQRLLLQDSQ NVEALRMQALYYVCREGDIEKASTKLENLGNTLDAMEPQNAQLFYNITLAFSRTCGRSQLILQKIQTLLERAFSLNPQQSEFA TELGYQMILQGRVKEALKWYKTAMTLDETSVSALVGFIQCQLIEGQLQDADQQLEFLNEIQQSIGKSAELIYLHAVLAMKKN KRQEEVINLLNDVLDTHFSQLEGLPLGIQYFEKLNPDFLLEIVMEYLSFCPMQPASPGQPLCPLLRRCISVLETVVRTVPGLLQT VFLIAKVKYLSGDIEAAFNNLQHCLEHNPSYADAHLLLAQVYLSQEKVKLCSQSLELCLSYDFKVRDYPLYHLIKAQSQKKM GEIADAIKTLHMAMSLPGMKRIGASTKSKDRKTEVDTSHRLSIFLELIDVHRLNGEQHEATKVLQDAIHEFSGTSEEVRVTIAN ADLALAQGDIERALSILQNVTAEQPYFIEAREKMADIYLKHRKDKMLYITCFREIAERMANPRSFLLLGDAYMNILEPEEAIVA YEQALNQNPKDGTLASKMGKALIKTHNYSMAITYYEAALKTGQKNYLCYDLAELLLKLKWYDKAEKVLQHALAHEPVNEL SALMEDGRCQVLLAKVYSKMEKLGDAITALQQARELQARVLKRVQMEQPDAVPAQKHLAAEICAEIAKHSVAQRDYEKAIK FYREALVHCETDNKIMLELARLYLAQDDPDSCLRQCALLLQSDQDNEAATMMMADLMFRKQDYEQAVFHLQQLLERKPDN YMTLSRLIDLLRRCGKLEDVPRFFSMAEKRNSRAKLEPGFQYCKGLYLWYTGEPNDALRHFNKARKDRDWGQNALYNMIEIC LNPDNETVGGEVFENLDGDLGNSTEKQESVQLAVRTAEKLLKELKPQTVQGHVQLRIMENYCLMATKQKSNVEQALNTFTEIA ASEKEHIPALLGMATAYMILKQTPRARNQLKRIAKMNWNAIDAEEFEKSWLLLADIYIQSAKYDMAEDLLKRCLRHNRSCCKA YEYMGYIMEKEQAYTDAALNYEMAWKYSNRTNPAVGYKLAFNYLKAKRYVDSIDICHQVLEAHPTYPKIRKDILDKARASLRP
mutation	MDSQELKTLINYYCQERYFHHVLLVASEGIKRYGSDPVFRFYHAYGTLMEGKTQEALREFEAIKNKQDVSLCSLLALIYAHKMSP NPDREAILESDARVKEQRKGAGEKALYHAGLFLWHIGRHDKAREYIDRMIKISDGSKQGHVLKAWLDITRGKEPYTKKAL**S**ILKR DSKMGMILLLCWVRHNALRCARIIQVPWRL

Notes: TTC21B: NM_024753: exon5:c.497delA:p.K166Sfs*36. https://web.expasy.org/translate/?_ga=1.91521050.1804783454.1476928400

Table. 5 The genetic classification of NPHP and NPHP-associated syndromes

Genetype	HGNC gene symbol	Chromosome	Disorders associated with mutation	Protein	Reference
NPHP1	NPHP1	2q13	NPHP, SLSN, JBTS	Nephrocystin-1	[46]
NPHP2	INVS	9q31	NPHP/SLSN (including infantile NPHP) situs inversus	Inversin	[47]
NPHP3	NPHP3	3q22.1	SLS, HF, MKS and situs- inversus	Nephrocystin-3	[48,49]
NPHP4	Nephrocystin-4	1p36.31	Juvenile NPHP, RP, OMA, SLSN, liver	Nephrocystin-4	[50]
NPHP5	IQCB1	3q13.33	SLSN/LCA	Nephrocystin-5 or IQ motif containing B1	[31,51]
NPHP6	CEP290	12q21.32	JBTS/BBS/MKS/LCA/SLSN	Centrosomal protein 290	[39,51-52]
NPHP7	GLIS2	16p13.3	–	GLI similar 2	[53]
NPHP8	RPGRIP1L	16q12.2	SLS, JS and MKS	RPGRIP1-like	[39,53]
NPHP9	NEK8	17q11.1	SLS	NIMA-related kinase 8	[54]
NPHP10	SLSN7	1q43-q44	SLS and BBS-like	Serologically defined colon cancer antigen 8	[54]
NPHP11	TMEM67	8q22.1	JS, HF, MKS	Transmembr-ane protein 67	[55]
NPHP12	TTC21B	2q24.3	JS, MKS, BBS and JATD	Intraflagellar transport protein 139	[23]
NPHP13	WDR19	4p14	NPHP, JS, RP, Caroli, Sensenbrenner syndrome	Nephrocystin-13/ WD repeat domain 19/IFT	[56,57]
NPHP14	ZNF423	16q12.1	JBTS	Nephrocystin-14/ Zinc finger protein 423	[37]
NPHP15	CEP164	11q23.3	RP, JBTS, LF, obesity	Nephrocystin-15 centrosomal protein 164	[58]
NPHP16	ANKS6	9q22.33	LF, situs inversus, Cardiovascular abnorm.	Nephrocystin-16/ ANKS6	[59,60]
NPHP17	IFT172	2p23.3	JATD, MZSDS, JBTS	Nephrocystin-17/IFT172	[61]
NPHP18	CEP83	12q22	Learning disability,hydrocephalus, LF	Nephrocystin-18/centrosomal protein 83 kDa	[62]
NPHP19	DCDC2	6p22.3	NPHP, renal-hepatic ciliopath	Doublecortin domain-containing protein 2	[63]
NPHP20	MAPKBP1	15q15.1	NPHP	Mitogen-activated protein kinase	[64]
JBTS3	AHI1	6q23.3	JBTS, RP	Jouberin	[65,66]
NPHP1L	XPNPEP3	22q13	NPHP, myocardiosis, epilep	Nephrocystin-1L/XPNPEP3	[67]
NPHP2L	SLC41A1	1q32.1	NPHP, bronchiectasia	Solute carrier family 41 member 1	[68]
CC2D2A	MKS6	4p15.32	MKS, COACH, JBST	Coiled coil and C2 domain containing 2	[69]
ATXN10	-	22q13.3	NPHP/spinocerebellar ataxia	-	[70]

TRAF3IP1	-	2q37.3	NPHP, SLSN, RP	TRAF3 interacting	[71]

BBS, Bardet–Biedl syndrome; COACH, cerebellar vermis hypo/aplasia, oligophrenia, congenital ataxia, ocular coloboma an-d hepatic fibrosis; JATD, Jeune asphyxiating thoracic dysplasia; JBTS, Joubert syndrome; LCA, Leber congenital amaurosis;HF, hepatic fifibrosis; JS, Jeune syndrome; LF, liver fibrosis; MZSDS, Mainzer-Saldino syndrome; MKS, Meckel syndrom-e; OMA, oculomotor aprakip; SLNS, Senior–Løken syndrome.

Figures

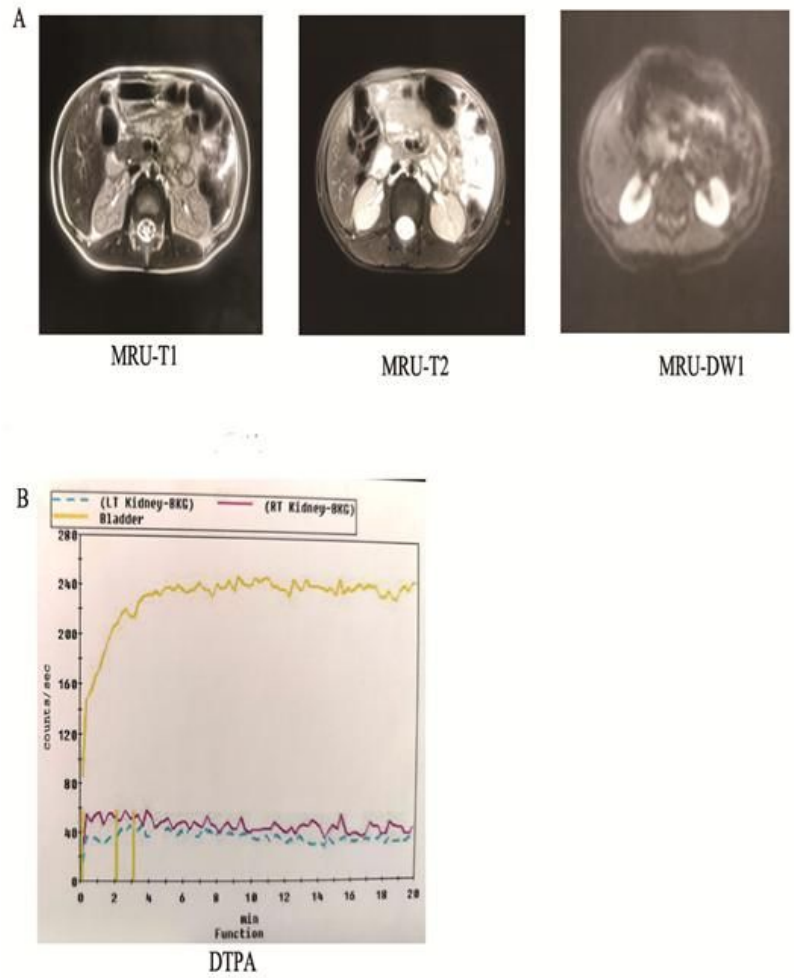

Figure 1

MRU and DTPA of the proband. a. The MRU showedperitoneal and pelvic effusion. b. Renal dynamic imaging showed that the functions of both kidneys were severely impaired.

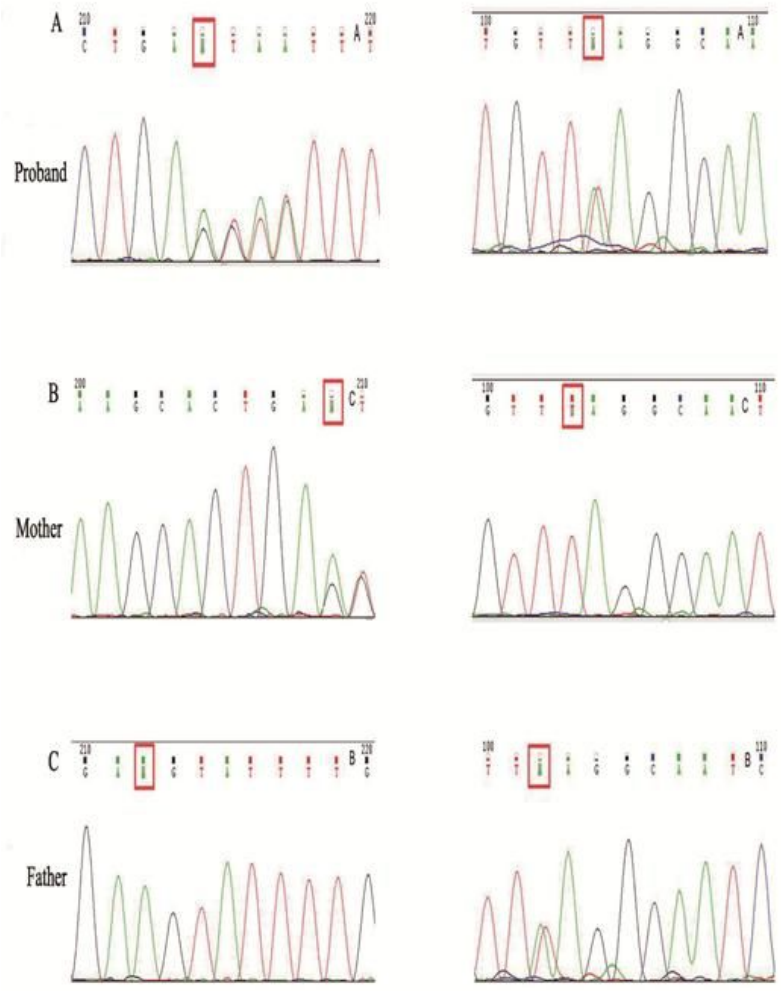

Figure 2

Data from Sanger sequencing the proband and her parents. The heterozygous mutation c.497del (p.Lys166Serfs) in the TTC21Bgene of the patientwas inherited from her mother. The c.2323-3T>A heterozygous variant detected in the TTC21B gene of the patient was also present in the father.

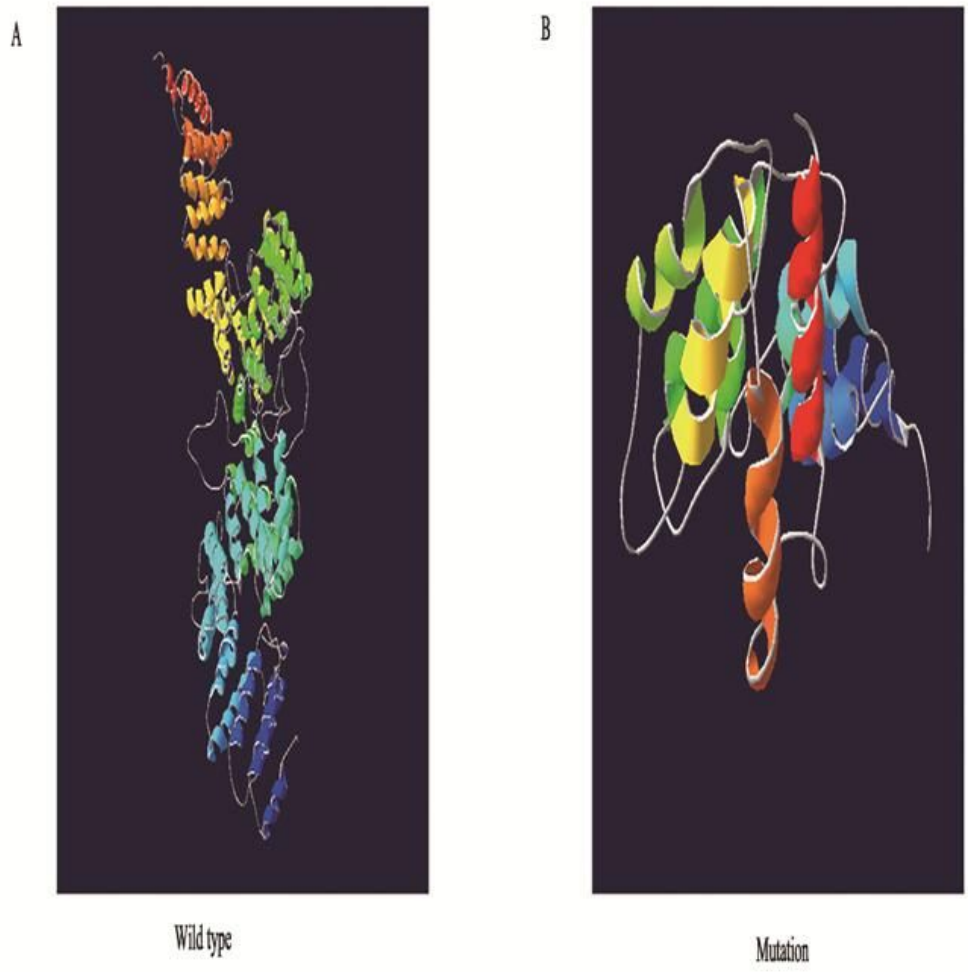

Figure 3

3-Dstructure of the TTC21B protein. Structure of the (a) wild-type and (b) mutated protein.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- coverletter.docx
- coverletter.docx