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Original Article

Two Chinese nephronophthisis pedigrees harbored a compound heterozygous deletion with a point mutation in *NPHP1*

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Abstract: *NPHP1* is the most prevalent genetic factor in the development of juvenile nephronophthisis (NPHP). In our previous study, *NPHP1* homozygous point mutations were detected by Sanger sequencing in three cases from two nonconsanguineous pedigrees. However, mutant sites were detected in only one parent from each respective pedigree. To investigate whether other disease-causing mutations were present, targeted exome sequencing (TES) of 63 ciliopathy genes was performed in the probands of the two pedigrees. In addition to the previously detected point mutations, a complete heterozygous deletion of *NPHP1* (1-20 exons) in the other allele was found in each of the three patients. The deletions were inherited from one parent of each pedigree. These is the first report of Chinese NPHP patients harboring a complete heterozygous deletion of *NPHP1* in one allele and a point mutation in the other one. The study demonstrated that TES is helpful in identifying complicated mutations in patients with NPHP.

Keywords: Nephronophthisis, *NPHP1*, heterozygous deletion, targeted exome sequencing

Introduction

Nephronophthisis (NPHP) is an autosomal recessive hereditary cystic kidney disease and is the main genetic factor leading to the development of end-stage renal disease (ESRD) during childhood [1-3]. To date, more than 20 causative genes have been identified, including *NPHP1–NPHP20* [4-6]. *NPHP1* is the most common pathogenic variant of NPHP [2]. Using polymerase chain reaction (PCR) amplification of satellite markers and Sanger sequencing, we previously identified seven patients with *NPHP1* mutations among 27 Chinese juvenile NPHP cases [7]. Three patients from two nonconsanguineous pedigrees were indicated harboring homozygous point mutations by Sanger sequencing. However, a heterozygous mutation at the same site was detected in only one parent in each pedigree. The sources of the observed homozygous status in these patients were not completely clarified. In the present study, we applied targeted exome sequencing (TES) to investigate whether other disease-causing mutations were harbored in these two pedigrees that were presumed to have *NPHP1* homozygous point mutations.

Materials and methods

The study, in accordance with the Declaration of Helsinki, was approved by the ethics committee of the First Affiliated Hospital, Sun Yat-sen University. Written informed consent was obtained from all subjects or their parents. Blood samples and clinical data were collected from the two NPHP pedigrees. DNA was extracted from peripheral blood using a QIAamp DNA Blood Mini Kit (QIAGEN, Germany).

Targeted exome sequencing (TES) was carried out in the two probands (patient F1-II2 and

patient F2-II1) of the two pedigrees using a gene panel comprising 63 known ciliopathy associated genes, including *NPHP1-16*, *PKD1-2,* and *PKHD1*, and causative genes of Bardet-Biedl syndrome, Meckel-Gruber syndrome, and Joubert syndrome. The minimum average sequencing depth was 150 ×. Briefly, 3-5 μg of qualified DNA was fragmented and exome enrichment was conducted using a kit (GenCap, MyGenostics, Beijing). The libraries were then sequenced on an Illumina HiSeq X Ten platform. Using Burrows-Wheeler Aligner (BWA) software, high-quality sequence reads were mapped against the human genome build37 (hg19). Variants were called by SOAPsnp and Genome Analysis ToolKit (GATK), and then annotated by ANNOVAR. Candidate variants were validated by Sanger sequencing. Copy number variants (CNV) were detected by applying a depth-based method. Heterozygous deletions/duplications determined were then subsequently confirmed by Quantitative PCR (Q-PCR).

Results

Clinical manifestations

The two pedigrees were both of Chinese Han ethnicity and from South China, and the patients were children from nonconsanguineous marriages. All parents were healthy (**Figure 1**).

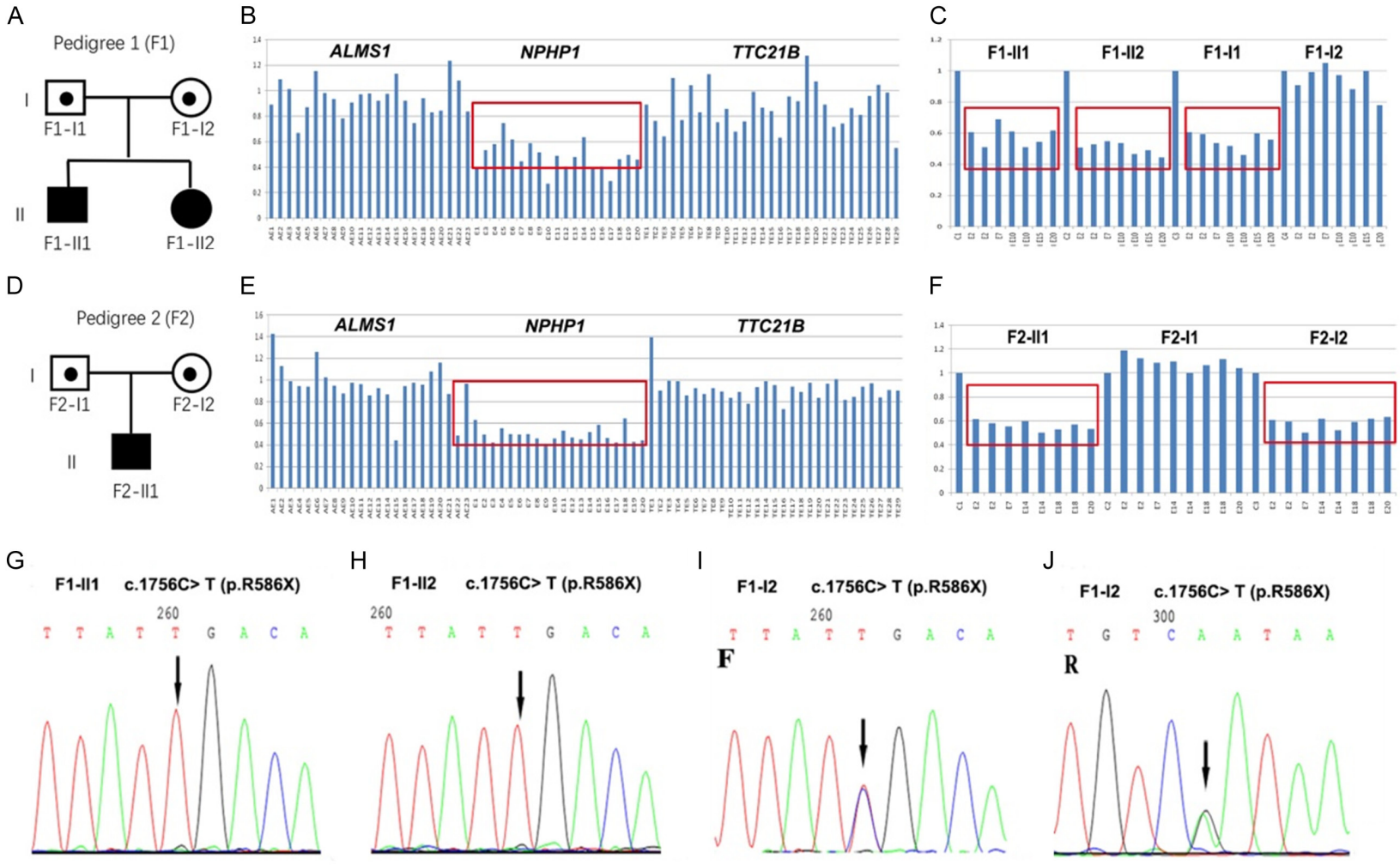
Pedigree 1 (F1): Patient F1-II2 (the proband) was a female showing normal vision and intelligence. She exhibited fatigue and anorexia at the age of 11 years and 4 months. Laboratory data revealed that her serum creatinine (Scr) was 155 μmol/L, and her hemoglobin was 84-114 g/L. She was 140 cm [-2.7 standard deviation score (SDS)] tall and weighed 29.5 kg (-2.7 SDS). Her blood pressure was 130/80 mmHg. Ultrasonography and magnetic resonance imaging (MRI) revealed normal-sized kidneys (left: 97 mm × 33 mm, right: 101 mm × 34 mm) with increased echogenicity and loss of corticomedullary differentiation. Two cysts (12-14 mm in diameter) in the right kidney and one (15 mm × 13 mm) in the left kidney were observed. Liver ultrasound revealed no abnormalities. She progressed to ESRD at the age of 15 years and 2 months, with an Scr level of 1200 μmol/L.

Patient F1-II1, the older brother of patient F1-II2, was born at full gestational age without asphyxia. Amblyopia was observed at the age of three. Polydipsia, weight loss, and fatigue were observed at the age of 12. He did not visit a doctor until he was 13 years and 7 months old, at which point he was found to have renal impairment (Scr 166.8 μmol/L; estimated glomerular filtration rate (eGFR) 36.4 mL/min/1.73 m^2) and anemia (hemoglobin 98-103 g/L). Urinalysis was normal except for decreased urinary gravity (1.000). The patient exhibited normal intelligence, was 147 cm (<-3 SDS) tall, and weighed 31.5 kg (<-3 SDS). His blood pressure was 110-120/65-70 mmHg. Ultrasonography showed that his right kidney was normal in size (92 mm × 33 mm), while his left kidney was dysplastic (32 mm × 38 mm). Increased echogenicity and a loss of corticomedullary differentiation were observed in both kidneys. No cysts were found in either kidney, and his liver was normal based on what was revealed by ultrasonography. He progressed to ESRD at the age of 16 years and 7 months, with an Scr level of 1400 μmol/L.

Pedigree 2 (F2): Patient F2-II1 (the proband) was a male showing normal vision and intelligence. He had anorexia, fatigue, and growth retardation at the age of nine. Laboratory data revealed renal impairment (Scr 240 μmol/L) and anemia (hemoglobin 60 g/L). His blood pressure was 150/100 mmHg. One year later, he progressed to ESRD (Scr 900 μmol/L). The patient was 125 cm (-3.1 SDS) tall and weighed 24 kg (-2.2 SDS) at 11 years and 9 months. Urinalysis showed proteinuria 1+. Ultrasonography of the kidneys revealed reduced size (left: 66 mm × 29 mm, right: 60 mm × 27 mm), hyperechogenicity, and loss of corticomedullary differentiation. A single cyst (5 mm × 5 mm) was found in his right kidney. His liver function was normal.

TES results and bioinformatic analysis

TES was performed in the probands of the two pedigrees (patient F1-II2 and patient F2-II1). The percentage of the target region captured with more than 10 × was 92.38% in the proband of pedigree 1 and 97.3% in the proband of pedigree 2. Apart from the point mutations consistent with our previous Sanger sequencing results (**Figure 1**), an entire heterozygous dele-


A
Pedigree 1 (F1)
I
F1-I1
F1-I2
II
F1-II1
F1-II2
B
ALMS1
NPHP1
TTC21B
C
F1-II1
F1-II2
F1-I1
F1-I2
D
Pedigree 2 (F2)
I
F2-I1
F2-I2
II
F2-II1
E
ALMS1
NPHP1
TTC21B
F
F2-II1
F2-I1
F2-I2
G
F1-II1 c.1756C> T (p.R586X)
260
T T A T T G A C A
H
F1-II2 c.1756C> T (p.R586X)
260
T T A T T G A C A
I
F1-I2 c.1756C> T (p.R586X)
260
T T A T T G A C A
F
J
F1-I2 c.1756C> T (p.R586X)
300
T G T C A A T A A
R

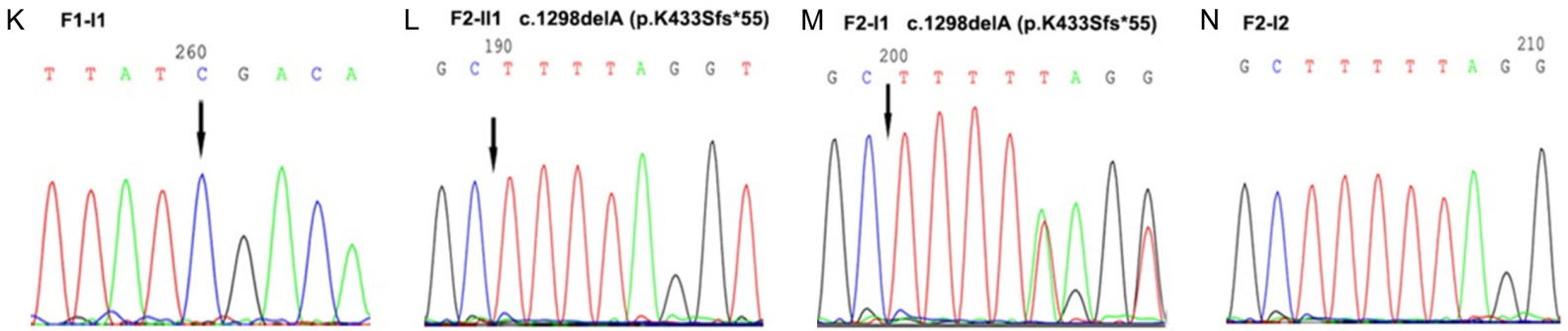


Figure 1. *NPHP1* complete heterozygous deletion identified in two pedigrees and point mutations verified by Sanger sequencing. A-C (pedigree 1), A: The filled square and circle denote the patients in the pedigree. B: A complete heterozygous deletion of *NPHP1* was detected in F1-II2 by TES (red rectangle). C: A complete heterozygous deletion of *NPHP1* in patients F1-II1 and F1-II2 and their father (F1-I1) was confirmed by Q-PCR (red rectangle); the mother was spared. D-F (pedigree 2): A complete heterozygous deletion of *NPHP1* in patient F2-II1 and his mother (F2-I2) detected by TES, and was confirmed by quantitative polymerase chain reaction. (AE1-23: Exon 1-23 of *ALMS1*, E1-20: Exon 1-20 of *NPHP1*, TE1-29: Exon 1-29 of *TTC21B*. C1-C4 are the control samples). G-N: Point mutations verified by Sanger sequencing in two pedigrees.

tion in the other allele of *NPHP1* was detected by TES in both probands (F1-II2 and F2-II1). This deletion was confirmed by Q-PCR and was also detected in another patient (F1-II1) (**Figure 1**). The deletions originated from one of the respective parents: the father of pedigree 1 (F1-I1) and the mother of pedigree 2 (F2-I2).

The complete deletion in *NPHP1* identified in both pedigrees would lead to the loss of expression of its encoded protein, nephrocystin 1. The nonsense mutation (c.1756C>T, p.R586X) of *NHPH1* in pedigree 1 and the frameshift mutation (c.1298delA, p.K433Sfs*55) in pedigree 2 were both predicted to create a premature stop codon and cause truncation of nephrocystin 1.

The original data from TES are available in the NCBI Sequence Read Archive (SRA) (accession number: SRP100717).

Discussion

NPHP1 was the first NPHP gene identified [8], and its mutations have been identified in 19.7%-29.4% of all NPHP cases [2, 8]. Most patients with *NPHP1* mutations manifest with juvenile NPHP [9, 10]. Clinically, homozygous deletions are the most frequent type of *NPHP1* mutation and are observed in approximately 85% of *NPHP1* mutant cases [11]. Compound point mutations and compound heterozygous deletions with point mutations are not common [12]. The compound heterozygous deletion with a point mutation in *NPHP1* identified in two pedigrees in the present study was determined to be disease-causing based on bioinformatics analysis. Both of the point mutations identified in the two pedigrees were truncation mutations that had been reported previously in other patients with NPHP [2, 13]. Compound heterozygous deletion with a point mutation in *NPHP1* alleles has not been reported before in Chinese NPHP patients.

Non-quantitative PCR and Sanger sequencing cannot distinguish compound heterozygous deletion in one allele with a point mutation in the other from cases with homozygous point mutations [14]. When the source of the point mutation cannot be identified in either parent, other causative mutations including heterozygous fragment deletions and CNVs of the pathogenic gene or mutations in other causative genes should be considered. In the present study, 62 additional candidate ciliopathy genes were screened simultaneously by TES and were excluded as causative genes in these two pedigrees.

Large heterozygous *NPHP1* deletions could also be detected by other methods, such as in situ hybridization and single-strand conformation polymorphism analysis [13, 15]. However, it is difficult to ascertain the location and the extent of the deleted fragment with these methods. Quantitative multiplex PCR of short fluorescent fragments was also recently applied for the detection of *NPHP1* heterozygous deletion [16].

In conclusion, we identified a complete heterozygous deletion with a point mutation in *NPHP1* on the second allele in two Chinese NPHP pedigrees. In patients with a homozygous mutation indicated by direct sequencing for whom the source of each copy of the mutation is not confirmed, further exploration of the causative mutations is warranted. The results of this study demonstrated that TES is helpful in identifying complicated mutations in patients with NPHP.

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Disclosure of conflict of interest

None.

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