Whole Exome Sequencing Reveals a XPNPEP3 Novel Mutation Causing Nephronophthisis in a Pediatric Patient

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ABSTRACT

Background: NPHP is a progressive tubulointestinal kidney condition that demonstrates an AR inheritance pattern. Up to now, more than 20 various genes have been detected for NPHP, with NPHP1 as the first one detected. XPNPEP3 mutation is related to NPHP-like 1 nephropathy and late onset NPHP. Methods: The proband (index patient) had polyuria, polydipsia and chronic kidney disease and was clinically suspected of NPHP. After the collection of blood sample from proband and her parents, WES was performed to identify the possible variants in the proband from a consanguineous marriage. The functional importance of variants was estimated by bioinformatic analysis. In the affected proband and her parents, Sanger sequencing was conducted for variants’ confirmation and segregation analysis. Results: Clinical and paraclinical investigations of the patient was not informative. Using WES, we could detect a novel homozygous frameshift mutation in XPNPEP3 (NM_022098.2: c.719_720insA; p. Q241Tfs*13), and by Sanger sequencing, we demonstrated an insertion in XPNPEP3. Conclusion: The homozygous genotype of the novel p.Q241Tfs*31 variant in XPNPEP3 may cause NPHP in the early childhood age. DOI: 10.29252/ibj.24.6.400

Keywords: Nephronophthisis, Whole exome sequencing, XPNPEP3

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INTRODUCTION

Nephronophthisis was first described by Smith and Graham in the mid-40s. This disorder was first called MCKD, but in the early 50’s, the term NPHP was first used by Fanconi.[1] NPHP and related diseases are considered as ciliopathy[2] and lead to ESRD in individuals up to 10 years of age.[3] NPHP is an AR disorder, considered as the most prevalent cause of renal diseases in the first two decades of life, accounting for about 2.4-15% of ESRD.[4] Unlike MCKD, ESRD occurs in fourth decade of life or later[5]. MCKD shows an autosomal dominant pattern, and in this sense, it is distinct from NPHP[1].

After the introduction of NPHP by Fanconi, in 1982, around 300 cases were reported, and 10%-15% of them showed extra renal symptoms such as retinitis pigmentosa (Senior-Loken syndrome), cerebellar vermis aplasia (Joubert syndrome), liver fibrosis, skeletal changes, congenital cardiac malformations, obesity, central nervous system abnormalities, and situs inversus[2,3-7]. In 1997, NPHP1 was identified as
the first gene in which pathogenic variants can cause NPHP. NP
duced nephropathy, the major cause of NPHP,
was detected in almost 20% of the cases. Up to now,
pathogenic variants in more than 20 various genes,
including NPHP1, TMEM216, CC2D2A, TTC21B,
TMEM67, INPP5E, MKS1, ARL13B, CEP290, GLIS2,
IQCB1, NPHP3, NPHP4, CEP290, INVS, RPGRIP1L,
NEK8, AH11, and XPNPEP3\cite{18,24,25}, are associated with
NPHP\cite{22,23}.

Previous studies have demonstrated that the genes
associated with NPHP encode nephrocystin, and the
majority of them are mostly located in primary cilia
and cilia extended to the kidney tubular lumen; there
fore, NPHP was classified as cilipathies\cite{26}. Based
on the incidence of ESRD, the NPHP is categorized
into three clinical forms: infantile, juvenile, and
adolescent, of which the juvenile form is most
common. The juvenile NPHP incidence occurs mostly
at age 13, but the symptoms of the disease begin
between the ages of four and six\cite{27,28}.

Herein, we report the causative variant in the gene
belonging to the family of XPNPEP3. The encoded
protein is localized in the mitochondria of renal
cells\cite{29}. Disorders related to XPNPEP3 comprise
NPHP-like 1 nephropathy and late onset NPHP. Owing
to the genetic heterogeneity and various genes involved
in NPHP, this disease is a major clinical finding in
multiple syndromes such as Senior Løken, Meckel-
Gruber, Cogan, Sensenbrenner, and Joubert\cite{30}. The
symptoms of NPHP includes polydipsia, polyuria,
growth retardation, anemia, and secondary enuresis\cite{31}.
The present study showed a novel homozygous
frameshift mutation in XPNPEP3 (NM_022098.2:
c.719_720insA; Q241Tfs *13) in an Iranian patient
using WES.

MATERIALS AND METHODS

Subjects and clinical assessment

The patient was referred for the genetic diagnosis of
NPHP by a medical geneticist. An informed written
consent was obtained from the parents of the proband,
before blood collection from proband and her parents
as reference points. Clinical assessment consisted of
standard history, physical examination, renal
scintigraphy, abdominal and pelvic sonography, and
metabolic profiling.

DNA extraction

Peripheral blood samples were collected from the
proband (index patient) and her parents for the
extraction of genomic DNA by using the Exgene
Clinic SV kit (GeneAll Biotechnology Co. Ltd., Seoul,
Korea), according to the manufacturer’s manual. The
DNA from parents was used for the segregation
analysis.

WES

To identify the underlying genetic causes of the
disease, WES was performed by Macrogen, Korea.
Exons of DNA sample were captured using the
insolution SureSelect Target Enrichment System
(Agilent, Human All Exon KI v6; Agilent Technologies,
Inc., Santa Clara, CA, USA), followed by a paired-end high-throughput sequencing on reads
of 100 bp using Illumina HiSeq 4000 (Illumine Inc.,
San Diego, CA, USA). A 7.15-gigabase sequence
was generated with at least 98.08% coverage for 4x,
91.86% for 20x, and 85.66% for 30x of the sample.
The coverage of the target region was 98.93%, and the
mean depth was 90.58x. To confirm the candidate
variants found in WES, Sanger sequencing was
performed.

In silico pathogenicity assessment of variants

The obtained variants were annotated with wANNOVAR
(http://wannovar.wglab.org/), and the
process was followed by the variant
frequency analysis (i.e. minor allele frequency <0.02)
reported in the Single Nucleotide Polymorphism
database (http://www.ncbi.nlm.nih.gov/SNP/), the
1000 genome project dataset (http://www.
1000genomes.org), the ESP6500, gnomAD,
and ExAC (http://exac.broadinstitute.org).
Bioinformatics analyses were conducted to confirm the
pathogenic novel variants obtained from WES. The damaging
effects of the novel variant were assessed by using
bioinformatics tools, including SIFT (sift.jcvi.org)\cite{32},
PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2)\cite{33},
CADD\cite{34}, and Mutation Taster (http://www.
mutationtaster.org)\cite{35}, based on Standards and
Guidelines for the Interpretation of Sequencing
Variants (ACMG-AMP guidelines)\cite{36}. Among the
detected variants, frameshift variants or variants
predicted to be damaging were considered as the most
promising candidates. PubMed and OMIM databases
were reviewed for publications regarding candidate
genes as well as functional and expression data.
Sequencing the limited coding region of XPNPEP3
(sequence reference: NM_022098.2) as well as Sanger
sequencing confirmed our result and demonstrated an
insertion in XPNPEP3 in the affected proband.

Ethical statement

The above-mentioned sampling or treatment or both
protocols were approved by the Research Ethics
Committee of Iran University of Medical Sciences,
Tehran, Iran (Ethical code: IR.IUMS.FMD.
REC.1399.144). An informed consent form was
obtained from the parents of the proband.
RESULTS

Clinical findings
The proband case was a 13-year-old female who was born via normal vaginal delivery from an 18-year-old mother. The parents were first-cousin. In prenatal history, threats of abortion occurred at 6th months of pregnancy, and there was oligohydramnios at the third trimester of gestation. By 6th month of age, although she had normal neurodevelopment, growth failure and anemia were obvious. The proband showed polyurea and polydipsia as well as chronic kidney disease at 3rd years of age. Renal ultrasonography indicated an increased parenchymal echogenicity, and the size of kidneys was within the lower limit of normal.

Genetic findings
WES was performed on proband by focusing on the genes involved in ciliopathies, especially NPHP. According to the data obtained from WES, 11644 indel variations and 104245 single nucleotide variants were detected. We applied filtration process on exome data sequence to define the pathogenic mutation. Finally, by using the zygosity and CADD phred score (cut-off = 15), we arranged the filtered variants. We detected 18 homozygous variants in genes such as XPNPEP3, ACO2, DNAH2, MUC4, DARC, SLC25A5, KCNM1, ARAF, and MPST. XPNPEP3 that caused NPHP was the only mutation matched with the clinical findings of the proband. The mutation of XPNPEP3 gene was a frameshift in exon 4 (NM_022098.2: c.719_720insA; Q241Tfs*13). Also, Mutation Taster predicted that the mutated XPNPEP3 was a disease-causing variant. The identified variant was absent in the 1 k Human Genome Database, the ESP6500, gnomAD, and ExAC. Findings from WES were confirmed by Sanger sequencing (Fig. 1).

DISCUSSION
NPHP is an AR cystic kidney disease, leading to renal fibrosis and ESRD in affected cases. In addition to symptoms of kidney, nearly 15% of NPHP cases manifest some external symptoms of ciliopathy, including retinal defects, liver fibrosis, skeletal abnormalities, and brain developmental disorders[9]. The diagnosis of NPHP requires clinical characteristics (a renal concentrating defect that leads to polyuria and polydipsia) that have to be confirmed by the biochemical assessment (urine test), renal ultrasound scan, detection of mutation in NPHP-related genes by NGS and WES, and renal biopsy (when diagnosis is not clear or genetic diagnosis is impossible)[4,16].

In the present study, WES was used to investigate the genetic cause of a case suspected of NPHP, in which chronic kidney disease and polyurea and polydipsia
were not adequately specific for the NPHP diagnosis. We conducted WES on the affected proband, in reference to her parents, to find potentially damaging gene variants and also homozygous variants. Analysis of WES revealed unknown novel homozygous frameshift mutation (NM_022098.2: c.719_720insA; p.Q241Ts*13) in exon 4 of the XPNPEP3 gene associated with NPHP. This frameshift mutation was shown to disrupt the normal reading frame, leading to a PTC. PTC is recognized by the nonsense-mediated mRNA decay, a mechanism that degrades mRNA harboring PTC.\(^{7}\)

Based on our bioinformatics survey, p.Q241Ts*13 was the probable pathogenic candidate variant that was identified by WES. This variant did not exist in 1000 genome project databases and ExAC. The allele also was not found in gnomAD browser with any reported homozygotes. Referring to the latest ACMG-AMP guidelines scoring system/classification, this variant includes one very strong pathogenic evidence (PVS1 score) for null variant (frameshift, affecting gene XPNPEP3 which is a known inducer of disease), one moderate pathogenic (PM2 score), and one supporting pathogenic (PP4 score) evidence. Therefore, according to the ACMG-AMP guidelines, this variant may be classified as a pathogenic variant.

In conclusion, our findings revealed that the p.Q241Ts*13 in XPNPEP3 is possibly one of the potential pathogenic variants for NPHP.

**CONFLICT OF INTEREST.** None declared.

**REFERENCES**