

# IDENTIFICATION OF A MUTATION IN A VIETNAMESE FAMILY WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE USING WHOLE EXOME SEQUENCING

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*Autosomal dominant polycystic kidney disease (ADPKD) is a form of polycystic kidney disease (PKD) in which cysts develop within the kidneys, causing the kidneys to enlarge and lose function over time. ADPKD is caused by mutations in two major genes: PKD1 and PKD2. By whole exome sequencing (WES), we identified a heterozygous missense PKD1 variant (NM\_001009944: c.10529C>T, p.T3510M) in a proband from a 2-generation Vietnamese family, in which the proband presented a bilateral renal enlargement and multiple bilateral renal cysts. The segregation of this known mutation was confirmed using Sanger sequencing. The proband and his affected mother were carriers for the mutation, while his father and his sister were wild types. The findings of this study enable doctors to comprehend the underlying genetic cause of polycystic kidney disease and elect appropriate treatment for the patients.*

**Keywords:** PKD1, Polycystic kidney disease, Sanger sequencing, Vietnamese, WES.

## I. INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited form of kidney disease, with a prevalence of 1:500 to 1:1000, and is observed in approximately 7% - 10% of patients with end-stage renal disease (ESRD).<sup>1,2</sup> Typical ADPKD kidneys have multiple bilateral renal cysts along with associated renal enlargement.<sup>3</sup> The development of the cysts and renal enlargement over time leads to inflammation, tubular obstruction, and loss of kidney function. ADPKD is shown to be more common than hemophilia, sickle cell disease, cystic fibrosis, and Huntington's disease, thus becoming a

major concern for nephrologists around the world.<sup>2</sup>

At the molecular level, genetic testing has identified mutations in two major genes that are responsible for ADPKD: (1) *PKD1* (responsible for ~85% of cases) and (2) *PKD2* (responsible for ~15% of cases).<sup>1,4</sup> *PKD1* maps to chromosome 16 (16p13.3) and encodes the protein polycystin-1 (PC1), while *PKD2* maps to chromosome 4 (4q21–23) and encodes the protein polycystin-2 (PC2).<sup>5,6</sup> PC1 and PC2 are involved in establishing planar cell polarity, an important organizer of organogenesis.<sup>7</sup> In ADPKD patients, the amount of functional PC1 or PC2 drops significantly, causing the activation of protein kinase A (PKA) and the loss of calcium inhibitory effect on cAMP signaling.<sup>7,8</sup> The consequences of this aberrant change impair tubulogenesis, cell proliferation, increased fluid secretion, and interstitial inflammation.

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Researchers worldwide have carried out several studies to investigate the mutations in the *PKD1* and *PKD2* genes. By applying many different methods, these studies have summed up 1243 pathogenic variants out of the 2055 listed variants for the *PKD1* gene and 374 pathogenic *PKD2* variants out of the 463 listed variants for the *PKD2* gene.<sup>9</sup> Whole exome sequencing (WES) has contributed significantly to the methods utilized to discover these variants.<sup>9,10</sup> It can detect genetic variants in the entire exonic regions of the genome, where about 85% of the disease-causing mutations are located.<sup>11</sup> The application of WES in identifying mutations in ADPKD has been widely performed by researchers worldwide; however, in Vietnam, the utilization of WES in ADPKD studies is still limited. In this study, we applied WES to identify pathogenic variants for Vietnamese ADPKD patients and validated the obtained variants in the family using Sanger sequencing.

## II. METHODS

### 1. Study subject

The study included four members from a 2-generation Vietnamese family. Herein, the two ADPKD patients were the mother and the son diagnosed by the internal medicine clinic of the Center for High Technology and Gastroenterology, Hanoi Saint Paul Hospital, on May 19, 2022. Blood samples from all family members were collected after receiving informed consent from the proband's parents. The genetic testing was performed at the Institute of Genome Research, Vietnam Academy of Science and Technology, from April 1, 2022, to Sept 30, 2022. The study was approved by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology (No: 2-2019/NCHG-

HĐĐĐ).

### 2. Methods

#### *Whole exome sequencing (WES)*

Genomic DNA was extracted from whole blood obtained from the proband and other family members using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (ThermoFisher Scientific, USA) following the manufacturer's protocol. WES was performed on the proband II.1. A DNA hybrid library was prepared by shearing genomic DNA into 150 – 200bp fragments and ligating them to Illumina sequencing adapters. The adaptor-ligated library was amplified according to the instruction of the Agilent SureSelect Target Enrichment kit (Illumina, USA) and sequenced through the HiSeq Illumina platform (Illumina, USA). The FASTA file of the raw WES data was evaluated for quality control and preprocessed by using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and PRINSEQ (<https://prinseq.sourceforge.net/>). Exome data were mapped onto the human reference genome (UCSC hg19, NCBI build 37.1) using Novoalign (<http://www.novocraft.com/products/novoalign/>). Variant callings were performed using Genome Analysis Toolkit (GATK) Best Practices in GATK pipeline (<https://www.broadinstitute.org/gatk/index.php>) after filtering out unmapped, low-quality reads and PCR duplications by Bamtools (<https://bio.tools/BamTools>) and Picard version 2.18.7 (<http://broadinstitute.github.io/picard/>). All variants were annotated via ANNOVAR (<https://annovar.openbioinformatics.org/en/latest/>).

#### *PCR and Sanger sequencing*

To validate the causative variant, PCR was used to amplify the flanking region of the *PKD1* gene-containing variant using DNA samples from the proband (II.1) and three other family

members (I.1, I.2, II.2) and specific primers (Table 1). PCR condition consisted of an initial denaturation at 95°C for 5 minutes, followed by 15 cycles of 95°C for 30 seconds, 62°C for 20 seconds, 72°C for 30 seconds, accompanied by an additional round of 25 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and a final extension at 72°C for

4 minutes. PCR products were subsequently purified and sequenced using ABI Big Dye Terminator v3.1 Sequencing Standard Kit (Applied Biosystems, CA) on ABI 3500 Genetic Analyzer sequencer (Applied Biosystems). The sequencing results were compared with the reference sequence (NM\_001009944) of *PKD1* using SnapGene Viewer.

**Table 1. Primer sequences used for amplifying exon 35 of *PKD1***

Name	Sequence	Amplicon size (bp)
PKD1_F	5'-GGAAGCAGAGACAGACCTGT-3'	294
PKD1_R	5'-ACCCAAGAGGCTCAAGAAAC-3'	

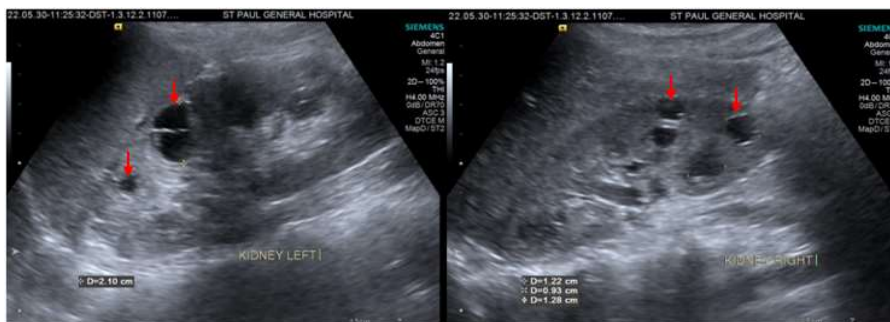
**Prediction tools**

The amino acid conservation at position 3510 across different species was evaluated using the UCSC Genome Browser Group (<https://genome.ucsc.edu/>). In addition, the pathogenicity of the targeted variants was

predicted using in silico programs, including PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>) and PredictSnp (<https://loschmidt.chemi.muni.cz/predictsnp1/>).

**III. RESULTS**

**1. Case presentation**



**Figure 1. Ultrasonography result of the proband II.1. The image showed multiple cysts with different sizes scattering in bilateral kidneys**

The proband II.1 was a 23-year-old male. He was diagnosed by ultrasonography with a bilateral renal enlargement (right: 125mm x 48mm, left: 130mm x 58mm) and multiple bilateral renal cysts, in which the largest cyst was 21mm x 25mm (Figure 1). The proband mother (I.2) was also diagnosed with ADPKD. She used to have two large kidneys with multiple

bilateral renal cysts and markedly reduced kidney function before her nephrectomy. She is undergoing treatment with intermittent hemodialysis and waiting for a kidney transplant. In addition, the proband showed no symptoms of hypertension (~120/80mmHg), while his mother was diagnosed with hypertension (~150/90mmHg).

Table 2. List of variants in *PKD1* and *PKD2* identified by WES

Chr	Start	End	Genotype	Region	Gene	Amino acid change	Polyphen2	SIFT
16	2144182	2144182	C/T	exonic	<i>PKD1</i>	c.C10529T;p.T3510M	deleterious.	deleterious
16	2152427	2152427	C/T	exonic	<i>PKD1</i>	c.C9156T;p.G3052G	.	.
16	2154461	2154461	C/T	intronic	<i>PKD1</i>	.	.	.
16	2154478	2154478	A/G	intronic	<i>PKD1</i>	.	.	.
16	2164808	2164808	A/A	exonic	<i>PKD1</i>	c.G2216A;p.R739Q	Benign	Benign
16	2140623	2140623	_/AGCTG	intronic	<i>PKD1</i>	.	.	.
4	88959381	88959381	G/A	intronic	<i>PKD2</i>	.	.	.

Note: Chr: Chromosome, "-": Wildtype allele, ".": not determined

## 2. Genetic analysis

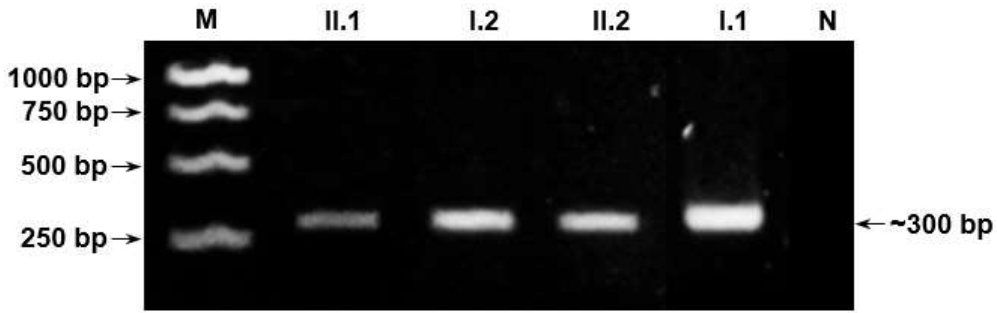
Whole exome sequencing was used to detect pathogenic variants of the proband. After filtering out variants that were not associated with ADPKD, a total of seven remained within the two genes *PKD1* and *PKD2* (Table 2). Based on the function of the variant, a missense variant NM\_001009944: c.10529C>T, p.T3510M in exon 35 of the *PKD1* gene was the most promising candidate.

The DNA region containing the exon 35 of the *PKD1* gene was amplified by PCR reaction using specific primers *PKD1\_F* and *PKD1\_R*. The PCR products were loaded on 1.5% agarose gel. The electrophoresis outcomes indicated one specific, sharp, and bright band with a molecular weight of about 300bp (Figure 2).

The presence of the obtained variant in the proband and all other family members was further verified using Sanger sequencing. The sequencing results confirmed the presence of the heterozygous variant c.10529C>T, p.T3510M in the proband (II.1) and his affected mother (I.2). His father (I.1) and his sister (II.2) did not possess the variant (Figure 3A - B).

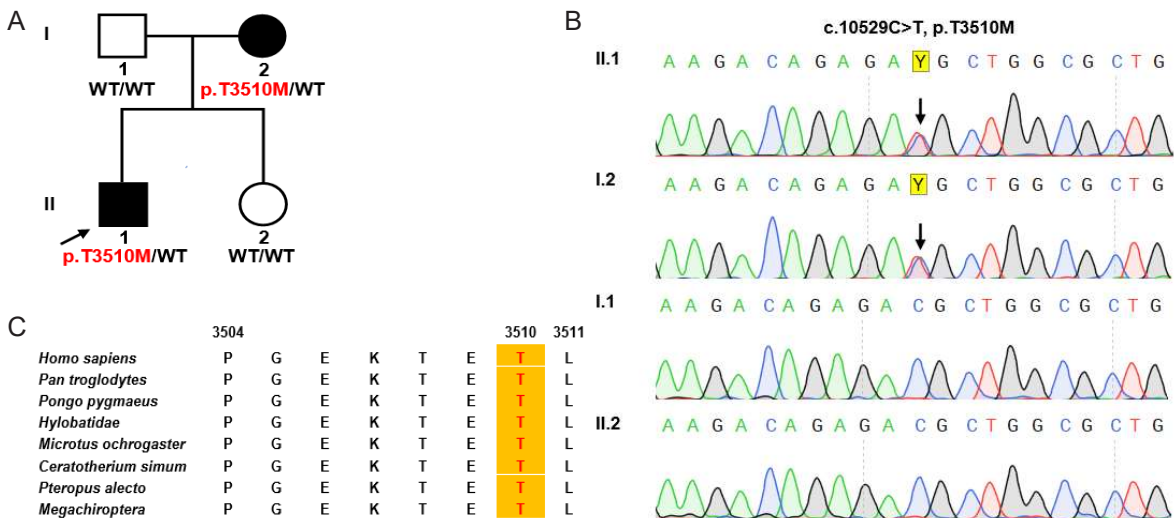
## 3. In silico analysis

The c.10529C>T variant leads to the conversion of the amino acid Threonine to Methionine at position 3510 (p.T3510M), which is located in a highly conserved region of the PC1 protein, indicating a pathogenic effect (Figure 3C). Thus, the position of the mutated amino acid might have a damaging impact on the protein integrity. In addition, the mutation of the amino acid was predicted to be deleterious by SIFT, and PolyPhen-2.



**Figure 2. PCR products of DNA region containing exon 35 of the *PKD1* gene**

*M*: Marker 1 kb, *II.1*: PCR product obtained from the proband, *I.2*: PCR product obtained from the proband's mother, *II.2*: PCR product obtained from the proband's sister, *I.1*: PCR product obtained from the proband's father, *N*: Negative control



**Figure 3. Pedigree analysis of the family in the study**

(A) Family pedigree of the proband, (B) Sanger sequencing results of the proband and family members, (C) Multiple sequence alignment at amino acid position 3510 (highlighted in red)

#### IV. DISCUSSION

Using WES, a missense variant of the *PKD1* gene (NM\_001009944: c.10529C>T, p.T3510M) was detected in the proband. This missense mutation has been reported in several patients with ADPKD.<sup>12-15</sup> One study showed the presence of this variant in a large Chinese family with ADPKD.<sup>12</sup> Using NGS and Sanger sequencing, the variant was pointed out in all six patients with ADPKD, while it was

not detected in the other 17 unaffected family members, along with 300 unrelated controls. In another study, the variant c.10529C>T was found in the Japanese population.<sup>13</sup> The study was conducted in 69 families and 50 controls using PCR-direct sequencing and allele-specific oligonucleotide hybridization. The variant was only segregated with samples diagnosed with ADPKD. In addition, the c.10529C>T variant was

also reported in other non-Asiatic populations, such as the Czech ADPKD population with an allele frequency of 3.3% and the Finnish ADPKD population with an allele frequency of 0.7%.<sup>14,15</sup> In this study, the pathogenic effect of the mutation has been confirmed by showing the co-segregation of the disease phenotypes with the missense p.T3510M mutation. In contrast, the father and sister of the index case who did not exhibit the mutation were unaffected. In addition, the genetic testing from the present study can further accomplish the clinical results about the complication of the two ADPKD patients since the obtained mutation is considered to be also involved in hypertension and cerebrovascular disease.<sup>12</sup> In our case, the genetic testing has confirmed the cause of hypertension in the mother of the index case and anticipated the potential to possess hypertension and cerebrovascular disease in the future for the proband who has not yet shown the symptoms of hypertension.

In recent years, biotechnology has developed significantly in Vietnam. DNA sequencing technologies have been widely applied in biomedical and biological research and in the diagnosis of genetic diseases. Preimplantation genetic testing (PGT), a technique used in reproductive medicine to identify genetic abnormalities in embryos produced through in vitro fertilization (IVF), has been utilized to diagnose many genetic diseases such as thalassemia or polycystic kidney disease, etc. In our study, we identified and analyzed a mutation of the *PKD1* gene in two ADPKD patients from a 2-generation Vietnamese family. The results would be helpful for genetic counseling to assist the patients choose appropriate therapies to prevent the disease to escalate to kidney failure. By detecting the mutation in the patients, we could

also advice the patients to test for abnormal embryos before IVF to produce healthy children who do not carry the mutated gene.

## V. CONCLUSION

We present a known mutation (NM\_001009944: c.10529C>T, p.T3510M) in the *PKD1* gene of a Vietnamese family in an autosomal dominant inheritance using whole-exome sequencing. The segregation of the mutation was confirmed in the family using Sanger sequencing. The result is significant for early identification of the underlying genetic disorder. Combined with clinical findings, our study provides solid evidence for the diagnosis process, genetic counseling, and management strategy.

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## REFERENCES

1. Al-Muhanna FA, Al-Rubaish AM, Vatte C, et al. Exome sequencing of Saudi Arabian patients with ADPKD. *Ren Fail.* 2019;41(1):842-849. doi:10.1080/0886022X.2019.1655453
2. Saini AK, Saini R, Singh S. Autosomal dominant polycystic kidney disease and pioglitazone for its therapy: a comprehensive review with an emphasis on the molecular pathogenesis and pharmacological aspects. *Molecular Medicine.* 2020;26(1):128. doi:10.1186/s10020-020-00246-3

3. Rahbari-Oskoui F, Mittal A, Mittal P, et al. Renal Relevant Radiology: Radiologic Imaging in Autosomal Dominant Polycystic Kidney Disease. *Clinical Journal of the American Society of Nephrology*. 2014;9(2):406-415. doi:10.2215/CJN.08940813
4. Chapman AB. Approaches to Testing New Treatments in Autosomal Dominant Polycystic Kidney Disease: Insights from the CRISP and HALT-PKD Studies. *Clinical Journal of the American Society of Nephrology*. 2008;3(4):1197-1204. doi:10.2215/CJN.00060108
5. Carone FA, Bacallao R, Kanwar Y. Role of the Matrix in Autosomal Dominant Polycystic Kidney Disease. *Ren Fail*. 1998;20(2):181-189. doi:10.3109/08860229809045101
6. Veeramuthumari P, Isabel W. Discovery of Single Nucleotide Polymorphism in Polycystic Kidney Disease among South Indian (Madurai) Population. In: *Chronic Kidney Disease - from Pathophysiology to Clinical Improvements*. InTech; 2018. doi:10.5772/intechopen.71201
7. Nobakht N, Hanna RM, Al-Baghdadi M, et al. Advances in autosomal dominant polycystic kidney disease: A clinical review. *Kidney Med*. 2020;2(2):196-208. doi:10.1016/j.xkme.2019.11.009
8. Chapin HC, Caplan MJ. The cell biology of polycystic kidney disease. *Journal of Cell Biology*. 2010;191(4):701-710. doi:10.1083/jcb.201006173
9. Ali H, Al-Mulla F, Hussain N, et al. PKD1 Duplicated regions limit clinical Utility of Whole Exome Sequencing for Genetic Diagnosis of Autosomal Dominant Polycystic Kidney Disease. *Sci Rep*. 2019;9(1):4141. doi:10.1038/s41598-019-40761-w
10. Lanktree MB, Haghghi A, di Bari I, et al. Insights into Autosomal Dominant Polycystic Kidney Disease from Genetic Studies. *Clinical Journal of the American Society of Nephrology*. 2021;16(5):790-799. doi:10.2215/CJN.02320220
11. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet*. 2014;59(1):5-15. doi:10.1038/jhg.2013.114
12. Liu J, Li L, Liu Q. Mutational analysis of PKD1 gene in a Chinese family with autosomal dominant polycystic kidney disease. *Int J Clin Exp Pathol*. 2015;8(10):13289-13292.
13. Tsuchiya K, Komeda M, Takahashi M, et al. Mutational analysis within the 3' region of the PKD1 gene in Japanese families. *Mutation Research/Mutation Research Genomics*. 2001;458(3-4):77-84. doi:10.1016/S0027-5107(01)00226-3
14. Stekrova J, Reiterova J, Svobodova S, et al. New mutations in the PKD1 gene in Czech population with autosomal dominant polycystic kidney disease. *BMC Med Genet*. 2009;10(1):78. doi:10.1186/1471-2350-10-78
15. Peltola P, Lumiaho A, Miettinen R, et al. Genetics and phenotypic characteristics of autosomal dominant polycystic kidney disease in Finns. *J Mol Med*. 2005;83(8):638-646. doi:10.1007/s00109-005-0644-6