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EXPLORATION OF MUTATION BY EXON DELETION TRHOUGH NUCLEOTIDE SEQUENCING OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

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ABSTRACT

The most prevalent inherited kidney disease is autosomal dominant polycystic kidney disease (ADPKD). Although next-generation sequencing (NGS) technology can be used to sequence tens of thousands of DNA molecules simultaneously. It captures the six PKD1 pseudogenes and GC-rich regions with poor efficiency. The technology known as multiplex ligation-dependent probe amplification (MLPA) can detect consecutive deletions of exons, but its sensitivity is lower for mutations involving only one base. However, pathogenic genes might not be detected in some patients, even when using the above methods. The clinical diagnosis of ADPKD is hampered by a significant technical issue: increasing the rate at which pathogenic genes can be detected. Other methods were used to look at four ADPKD pedigrees with mutation sites that were not found by NGS. First, MLPA was carried out. After that, multiplex polymerase chain reaction (MPCR) and targeted region sequencing were applied to pedigrees in which MLPA failed to locate pathogenic genes. Sanger sequencing was then used to confirm the mutation sites that had been discovered. The findings demonstrated that the PKD1 exonic deletion mutations PKD1-18 nt–290 nt, PKD1-up-257 nt, PKD1-up-444 nt, and PKD1-3 nt–141 nt were all found by MLPA in three pedigrees. A new mutation site was identified through targeted region sequencing in one pedigree: PKD1 NM_001009944: c.151T > C at the protein level, described as p. Cys51Arg. In conclusion, we developed a system of genetic detection and analytical techniques that included Sanger sequencing, NGS, MLPA, targeted region sequencing, and so on. For the first time in ADPKD diagnosis, we combined MPCR and targeted region sequencing, which further improved diagnosis accuracy. In addition, we discovered four new deletion mutations and one new missense mutation.

INTRODUCTION

With a prevalence of approximately 1/400–1/1,000, autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease. ADPKD manifests as multiple, progressive cysts in both kidneys that eventually destroy the structure and function, resulting in end-stage renal disease (ESRD) and a variety of extrarenal manifestations like hypertension, liver and pancreatic cysts, intracranial aneurysms, abdominal hernias, and the most prevalent cause of ADPKD is mutations in the PKD1 and PKD2 genes. In approximately 85% of patients, the pathogenic gene is PKD1, and in approximately 15%, it is PKD2. Through linkage analysis of family members, genetic testing can clearly identify mutant genes, diagnose patients early, and support prenatal testing. Improving prognosis, correcting risk factors, identifying and treating complications early, and implementing clinical intervention all benefit from genetic testing. Sanger sequencing, next-generation sequencing (NGS), and multiplex ligation-dependent probe amplification (MLPA) are three common genetic detection methods for polycystic kidney disease (PKD). Traditional Sanger sequencing is commonly employed to detect PKD, but the throughput of this method is low, and the workload is heavy. Sanger sequencing is insufficient for PKD1 mutation analysis due to its 46 exons and numerous complex repetitive regions. Whole-exome sequencing (WES), target capture sequencing (Panel), and whole-genome sequencing are the main components of NGS, a high-throughput sequencing method. Benefits include speed, accuracy, sensitivity, and coverage when sequencing tens of thousands of DNA molecules simultaneously using these techniques. The target sequence DNAs are hybridized by the probes in MLPA. After probe-specific ligation, the hybridized products are amplified by polymerase chain reaction (PCR), and the PCR product is separated by capillary electrophoresis, the data are collected, and analysed using specific analysis software. Although it has a high sensitivity for single-base mutations, MLPA can be used to detect consecutive deletions or duplicate mutations in exons. The novel PCR amplification method known as multiplex PCR (MPCR) is an improvement over conventional PCR. Two or more pairs of primers can be added to one reaction system to simultaneously amplify multiple nucleic acid fragments to increase the detection rate and identify mutations and their types. MPCR has the advantages of high efficiency, systematicity, economy, and simplicity.

In general, using MPCR to diagnose ADPKD can make it easier to analyze the PKD1 and PKD2 genes, which is crucial for early ADPKD pedigree screening and prenatal diagnosis (Deng et al., 2022). Through the use of a variety of sequencing techniques and MPCR, patients with PKD and their relatives were thoroughly identified and analyzed, revealing potential genetic causes of ADPKD.

REVIEW OF LITERATURE

1. Intracranial Aneurysms in Autosomal Dominant Polycystic Kidney Disease

List of authors Arlene B. Chapman, M.D.
Intracranial aneurysms are a feature of autosomal dominant polycystic kidney ailment; however, their incidence is unsure. We studied 90 two topics with autosomal dominant polycystic kidney illness who had no signs or signs and signs and signs and symptoms of any neurologic sickness. To determine the prevalence of intracranial aneurysms, we completed excessive-selection computed tomography (CT) in 60 subjects, four-vessel cerebral angiography in 21, and each technique in 11.

Four of the 88 topics in whom the radiologic research have been efficiently finished had intracranial aneurysms (4 percentage; 90 5 percentage self-assurance c programming language, zero.1 to 9 percentage), in comparison with the superiority of one percent counseled for an angiographic have a take a look at of the general populace. Three of the four topics had multiple aneurysms. Seven subjects for whom the consequences of CT studies were suspicious underwent cerebral angiography: two had aneurysms, and 5 had regular vascular systems that accounted for the suspicious consequences of tomography. Four topics who had everyday CT imaging research furthermore had normal angiographic examinations. Eight of the 32 topics who underwent angiography (25 percentage) had short complications, in evaluation with 22 of 220 manage topics (10 percentage) who did not have polycystic kidney ailment ($P<0.05$). We could not identify any risk factor in these subjects that was related to the occurrence of aneurysm [20].

Asymptomatic intracranial aneurysms appear to be more frequent in people with polycystic kidney disease than in the general population, although our 95 percent confidence interval includes the possibility of no difference. Because cerebral angiography is associated with increased morbidity in people with polycystic kidney disease, we recommend high-resolution CT as a screening test. (N Engl J Med 1992;327: 916–20.)

2. Von Hippel-Lindau disease masquerading as autosomal dominant polycystic kidney disease

List of authors Rupinder K. Chatha MD
The diagnostic confusion in differentiating the various causes of renal cystic diseases in adults is well documented. This confusion can include misclassifications between autosomal dominant polycystic kidney disease (ADPKD) and von Hippel-Lindau disease (VHL). We describe such a case of VHL. A review of the literature and of the patients in our database regarding typical features of each disease, mean age of onset, and frequency of these features was undertaken to provide helpful differentiating features. Pancreatic cysts are one differentiating feature. In VHL, pancreatic cysts can occur in 70% of patients, often are multiple, and rarely may cause exocrine or endocrine insufficiency. Pancreatic islet cell tumors occur. In ADPKD, pancreatic cysts are found in only 9% of patients, usually are single and asymptomatic, generally occur in conjunction with cystic liver disease, and are not found in children or unaffected family members. Pancreatic malignancies do not occur with increased frequency in ADPKD. A different pattern, especially in patients without a strong family history of ADPKD, may be a clue to VHL masquerading as ADPKD. Genetic mutation screening of the VHL gene should be used in these patients.

3. Nephrotic syndrome and autosomal dominant polycystic kidney disease

List of authors Bianca Visciano
Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder characterized by the development and growth of cysts in the kidneys and other organs. In ADPKD patients, nephrotic range proteinuria is unusual and needs to be investigated further to exclude coexisting glomerular disease. Among the anecdotal case reports of ADPKD associated with nephrotic syndrome, focal segmental glomerulosclerosis occurs most frequently.

We report the case of a 26-year-old male with ADPKD and concomitant nephrotic syndrome, in which an ultrasound (US)-guided renal biopsy showed a mesangioproliferative glomerulonephritis [21]. We treated the patient with prednisone 1 mg/kg/day, because of the failure of treatment with angiotensin-converting enzyme inhibitor/angiotensin receptor blocker association.

After 6 months of steroid treatment, we observed a stability of his GFR and a reduction of proteinuria. This case report and other cases of the literature underline the importance of a renal biopsy in patients with ADPKD and nephrotic syndrome in order to make an accurate diagnosis and an appropriate treatment/prevention of renal function deterioration.

4. A Spontaneous Extracranial Internal Carotid Artery Dissection with Autosomal Dominant Polycystic Kidney Disease

List of authors SHIRO BABA

Background and Objectives: Non-cystic manifestation of autosomal dominant polycystic kidney disease (ADPKD) is an important risk factor for cerebral aneurysms. In this report, we describe a rare spontaneous internal carotid artery (ICA) dissection in a patient with ADPKD. Observations: A 38-year-old woman with a history of ADPKD and acute myocardial infarction due to coronary artery dissection experienced severe spontaneous pain on the left side of her neck. Magnetic resonance imaging (MRI) revealed a severe left ICA stenosis localized at its origin. Carotid plaque MRI showed that the stenotic lesion was due to a subacute intramural hematoma. Close follow-up by an imaging study was performed under the diagnosis of spontaneous extracranial ICA dissection, and spontaneous regression of the intramural hematoma was observed uneventfully. Conclusions: When patients with a history of ADPKD present with severe neck pain, it is crucial to consider the possibility of a spontaneous ICA dissection. A carotid plaque MRI is beneficial in the differential diagnosis. Conservative management may benefit patients without ischemic symptoms.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary diseases characterized by bilateral renal cysts and liver cysts [22]. The non-cystic clinical manifestations of ADPKD include intracranial aneurysms, dissection of the thoracic aorta, and abnormalities of the cardiac valves [23,24]. Here, we report a case of rare spontaneous extracranial internal carotid artery (ICA) dissection in a patient with ADPKD, and her uneventful clinical course with conservative management.

5. Long-Term Outcome of Renal Transplantation in Autosomal Dominant Polycystic Kidney Disease

List of authors Peter M. Fitzpatrick MD

This study was performed to determine the long-term outcome of renal transplantation in 54 patients with end stage renal failure secondary to autosomal dominant polycystic kidney disease (ADPKD) and in 107 patients with renal diseases other than ADPKD or diabetes mellitus matched by gender, age, year of transplantation, and source of the allograft. The overall patient survival and patient survival with a functioning first renal allograft were similar in both groups. Infection and cardiovascular accidents were the leading causes of early and late death in both groups. No cause of death was greatly overrepresented in the ADPKD group. Serious complications from extrarenal manifestations of ADPKD following renal transplantation included a ruptured intracranial aneurysm in one patient, a dissection of the ascending thoracic aorta in one patient, and infected hepatic cysts in two patients. Neoplasia (other than skin or cervical) occurred in four ADPKD patients and in one control patient and included one lymphoma in each group. Two ADPKD and one control patient had monoclonal gammopathies of undetermined significance. No complications related to the retention of native kidneys were detected in 12 ADPKD patients with a mean follow-up of 3 years. Cysts were observed in the renal allografts of some patients in both groups at autopsy and in a prospective computed tomography (CT) study of the allograft. However, we failed to detect a significant difference in the occurrence and number of the cysts between ADPKD and control patients.

METHODS AND METHODOLOGY

1. Data screening and NGS Analysis

The clean reads were aligned to the reference human genome (hg19) with the help of Burrows-Wheeler aligner software (Li and Durbin, 2009), which was used to filter out low-quality variations with a quality score of less than 20. Single nucleotide polymorphisms (SNPs) and insertions or deletions (In-Dels) were identified using Genome Analysis Toolkit software, and those with a frequency ≥ 0.05 in the 1,000 Genomes Project, ESP6500, and Ex-AC databases were removed. Non-synonymous variants were evaluated using four algorithms, namely, SIFT, PolyPhen-2, Mutation-Taster, and GERP++, to predict pathogenicity (Table 1).

A. Base calling is the process by which primary analysis software transforms the raw image files into sequencing reads (FASTQ files) and gives each base a quality score.

B. Alignment and Assembly: Using instruments like BWA, reads are aligned to a reference genome for resequencing projects like variant detection. Using tools like SPAdes, reads are assembled into a contiguous sequence for de novo projects, such as new genomes.

C. Variant calling: Genetic variations like insertions and deletions (INDELs) and single nucleotide polymorphisms (SNPs) can be found in aligned data through secondary analysis.

D. Tertiary analysis establishes a connection between the identified variants or assembled sequences and biological function, the relationship between diseases, or evolutionary history.

2. MLPA

Fragment deletion was identified by MLPA. MRC-Holland, located in Amsterdam, the Netherlands, sells the SALSA MLPA kit (catalogue numbers MLPA p351-025R salsa MLPA probe mix p351 pkd1-25rxn and p352-025R salsa MLPA probe mix p352 pkd1-pkd2-25rxn). Deletion/duplication analysis of the PKD1 exons was performed according to the manufacturer's instructions. Exons were missing from the PKD1 gene as a result of MLPA testing. A fluorescence signal intensity of 0.75 to 1.3 is generally regarded as normal.

3. MPCR + targeted region sequencing

The gene primers were designed using Primer 5.0. The first primer sequence of the targeted region (C.151T upstream and downstream 100 bp) was CGGGCCCCGCCTGAGCT TGTGGCGTCCGCGGGGAT, and the second primer sequence was TATTTAGCAGGGCCGCCGTATGCCAGTCCCTCATCGC. Using the multiple PCR amplification kit KT109, genomic DNA was amplified by MPCR.

RESULT

1. MPCR + Targeted region sequencing results

Patient	Gene	Mutation site	Transcript; exon	Nucleotide changes (amino acids)	Normal/mutation (mutation ratio)
III_2	PKD1	Chr16:2185540	NM_001009944; exon1	c.151T>C (p.C51R)	128/118 (48%)
II_4	PKD1	Chr16:2185540	NM_001009944; exon1	c.151T>C (p.C51R)	31/29 (48%)
II_1	PKD1	Chr16:2185540	NM_001009944; exon1	c.151T>C (p.C51R)	29/46 (61%)

2. Variants of exon with sequences of MLPA probes

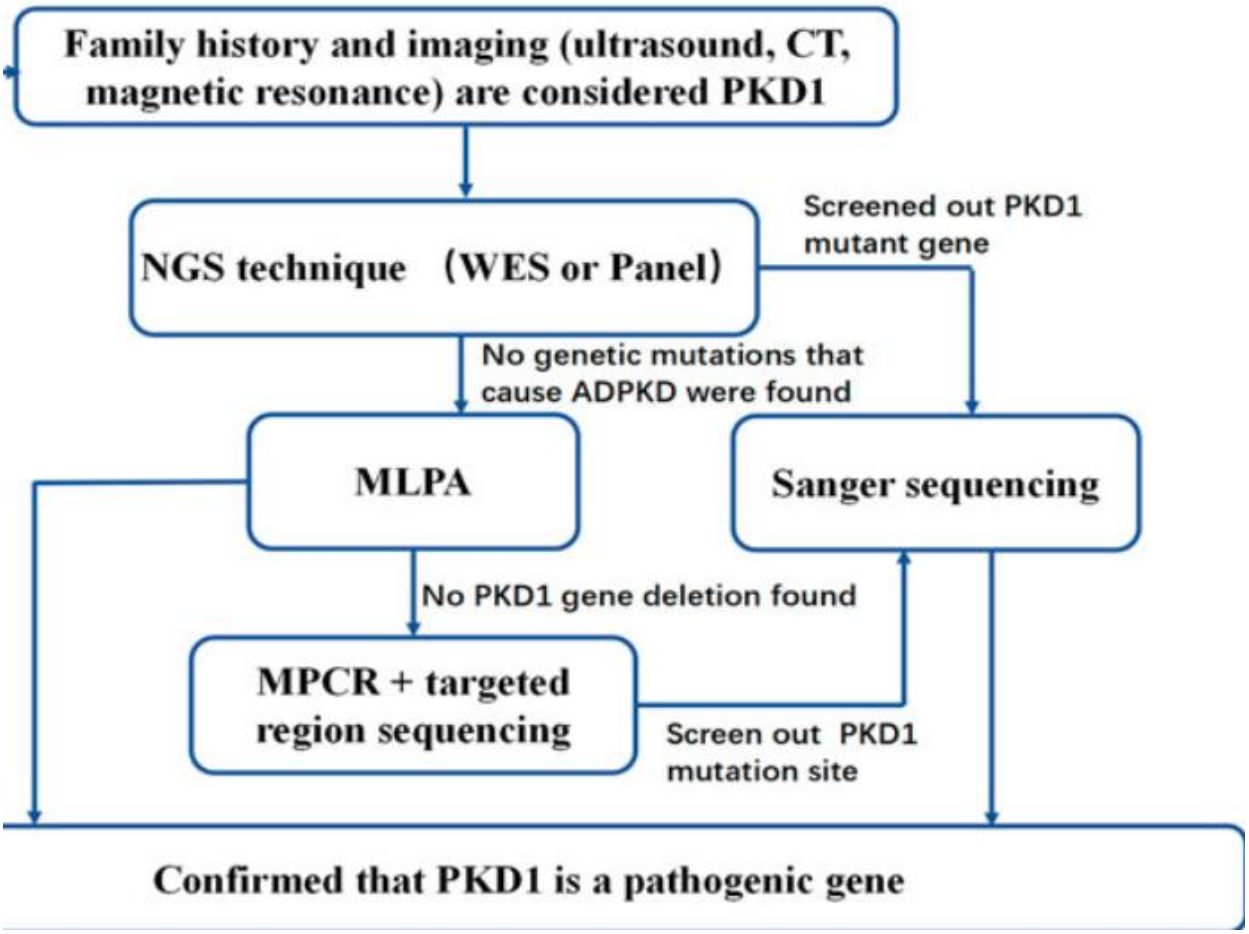
Exon	Variants	LPO	RPO
18	c.7344C>G	CGGGACGGCGAGGGATACACCTTCACGCTC	ACGGTGCTGGGCCGCTCTGGCGAGGAGG
18	c.7365C>T	CGGGACGGCGAGGGATACACCTTCACGCTC	ACGGTGCTGGGCCGCTCTGGCGAGGAGG

Note: Variants were underlined. Abbreviations: LPO, left probe oligonucleotide; RPO, right probe oligonucleotide.7Human Mutation

Specifically, the variant c.7344C>G was located at the 3′ end of the left probe oligo nucleotide (LPO) (Table 2), resulting in a mismatch at the 3′end of LPO that hindered probe amplification [26, 27].

The variant c.7365C>T was located at 8 nt from the 3′ end of the right probe oligonucleotide (RPO) (Table 2), making its effect unpredictable. Nonetheless, the presence of thec.7344C>G variant was sufficient to cause the false positive result in MLPA analysis. Thus, this case emphasized the importance of validating the MLPA method, especially for examining single-exon deletion or duplication.

Diagram of how to diagnose genetic ADPKD. Patients who have been diagnosed with ADPKD through clinical diagnosis but have not been found to have pathogenic genes that cause ADPKD through gene sequencing and MLPA are suitable for this diagnosis flowchart.



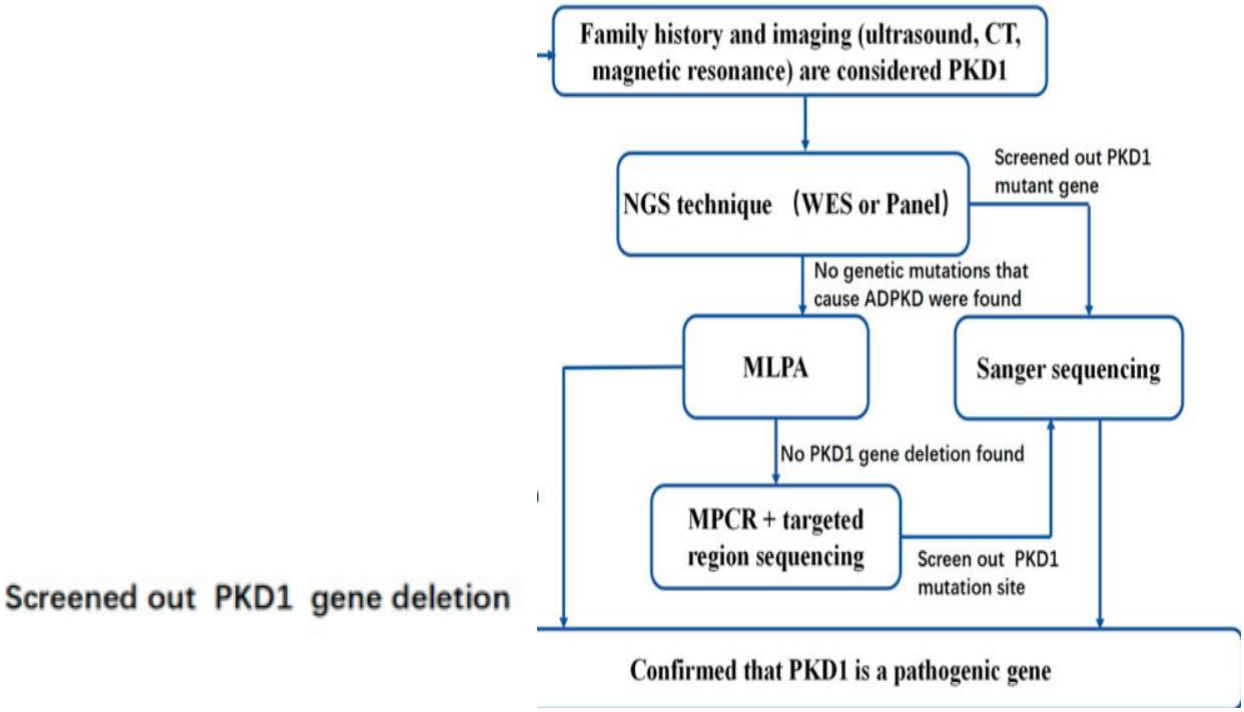


Table 1: The list of primer sequences for Sanger sequencing

The clones were randomly chosen for plasmid extraction and sequencing analysis. The primers used in this study were obtained from the literature and are listed.

Name	Forward primer ($5'\rightarrow3'$)	Reverse primer ($5'\rightarrow3'$)	Fragment size (bp)
Genomic DNA			
LR-PCR-DNA	GGCGATCACAGCGCAACTACT	ACGGAGTTGGCGGAGTTGGC	5316
Sequencing	TGGCAAACCGGATGAGTATC	TAGCTGGAGAGGCTGCC	918
cDNA			
LR-PCR-cDNA	AGCGCAACTACTTGGAGGCCC	ACCACAACGGAGTTGGCGG	2203

DISCUSSION

ADPKD is one of the most common genetic diseases affecting renal tubules. Approximately 85% of ADPKD patients have *PKD1* gene mutations. Genetic tests can identify the mutated genes in ADPKD patients, provide a basis for the diagnosis and treatment of ADPKD and be used to optimize prenatal and postnatal care and genetic blockade. In this study, patient 1 was diagnosed with PKD based on clinical features and imaging examinations. Although imaging features suggested possible *PKD1* gene mutations. Panel and WES sequencing did not detect any clear disease-related pathogenic point mutations. To further exclude the possibility of PKD caused by microdeletion or duplicate mutations of *PKD1* and/or other genes, MLPA was performed. As the examination still did not identify any relevant mutations, the possibility of a deletion or duplicate mutation was ruled out. Later, we performed pedigree linkage analysis on the raw WES data, which revealed four suspicious genes for screening. However, combining those findings with database prediction analysis of the conservation, tissue specificity, and pathogenicity of these loci indicated that these four new genes were basically excluded. Instead, we analyzed the imaging characteristics of patient 1's mother (II_4) and still considered the possibility of *PKD1* gene mutation. Overall, diagnosing ADPKD based only on clinical manifestations and imaging characteristics is uncertain, and a supporting genetic diagnosis is needed. Accordingly, we further analyzed the raw NGS data of the patient and found several suspicious sites. For example, the suspected pathogenic site NM_001009944:c.151T > C reported in the literature is considered a suspicious site. Due to poor coverage and low sequencing depth at these sites, we performed MPCR combined with 10,000 × targeted region (the target region is exons 1–10 of *PKD1*) sequencing for these suspected sites, and the results confirmed the presence of these mutations. Verification performed in II_4 and II_1 identified one point mutation. Sanger sequencing was performed in patient 1's family members, and all kidney patients in this pedigree were found to have the same mutation, while no healthy member had the mutation, which is consistent with pedigree segregation.

As for the MLPA method, the presence of PKD1 variants within the MLPA probe binding region of Exon 18 resulted in a false positive for Exon 18 deletion. It is known that the SNVs located near the ligation sites can interfere with the hybridization or ligation of the MLPA probe, impacting the accuracy of the assay [24–26], and their impact varied from the proximity to the ligation sites.

WES Identified a Heterozygous Variant and an Exon Deletion on Exon 18 of PKD1 in Proband. The WES revealed two suspected causative variants in the PKD1 gene.

In this study, we conducted a genetic analysis on a family with three ADPKD patients. The proband was initially identified to carry a heterozygous deletion of Exons 17 and 18and a heterozygous SNV in Exon 18 of PKD1 by WES and MLPA.

CONCLUSION

This study combined NGS sequencing, MLPA sequencing, and Sanger sequencing to address the difficulties in identifying *PKD1* pseudogenes and low detection rates in high-GC-content regions. This is the first study to apply MPCR combined with targeted region sequencing for genetic detection in ADPKD. We established a novel sequence of genetic detection and analytical methods that is conducive to improving the accuracy of genetic diagnosis of ADPKD patients, and it will help to guide the diagnosis and prognosis of this disease.

In conclusion, our study utilized LRS to uncover a PKD pedigree caused by gene conversion in the PKD1, a finding that was initially misidentified by WES and MLPA. Our results identified the genetic etiology of the family and pro-vided a basis for prenatal diagnosis and preimplantation genetic diagnosis. This case further highlighted the importance of validating CNVs or structural variants identified by WES through alternative methodologies, especially in complex structural regions like PKD1, where multiple approaches may be necessary. Moreover, we suggest that LRS might be more suitable than WES combined with MLPA for genetic screening of PKD, emphasizing the potential of LRS as a valuable tool in clinical diagnostics and research.

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