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Examining the Role of Polymorphisms in Exon 25 of the *PKD1* Gene in the Pathogenesis of Autosomal Dominant Polycystic Kidney Disease in Iranian Patients

Morteza Bagheri*[1, 2], Khadijeh Makhdoomi[1], Ali Taghizadeh Afshari[1], Ahmad Ali Nikibakhsh[1], Isa Abdi Rad[2]

Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a highly prevalent life-threatening monogenic disorder with high morbidity and mortality. Roughly 1:400-1000 individuals are affected with this disease worldwide. The development of ADPKD is largely attributed to mutations in the polycystic kidney disease (*PKD*)1 and *PKD*2 genes. However, the pathogenicity of the different polymorphisms in *PDK1* in the development of ADPKD remains unclear. The aim of this study was to further elucidate the role of the polymorphisms in exon 25 of the *PDK1* gene in relation to the pathogenesis of ADPKD in Iranian patients.

Methods: The genomic DNA of 36 Iranian patients with ADPKD was isolated using the standard salting out method. The PCR products were directly sequenced and analyzed.

Results: The frequencies of CAG>GAG, ATG>GTG, GTC>GTA, and GTG>ATG polymorphisms in exon 25 of the *PKD1* gene were 34 (94.44%), 33 (91.67%), 26 (72.22%), and 5 (13.89%), respectively. The most frequent polymorphism associated with ADPKD was the homozygous CAG→GAG which causes an amino acid change of Q[Gln] to E[Glu] at codon 3005.

Conclusions: Our data suggests that there is potentially a common polymorphism of *PDK1* among the Iranian population with ADPKD. This may aid in the diagnosis and genetic screening of at-risk patients for ADPKD.

Keywords: ADPKD, PKD1 Gene, Polymorphism.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disorder with a prevalence of 1:400–1000. This life-threatening genetic disease impacts nearly 12.5 million people globally (1,2). Approximately 5-10 % of adults requiring renal transplantation have ADPKD. Of the patients with ADPKD, 2 % of patients display clinical symptoms before the age of 15 (3). ADPKD is considered to be a late-onset multi-systemic disorder with high morbidity and mortality. The enlargement of cysts can occur in the kidneys, however, ADPKD can present with extra-renal manifestations and lead to the development of cysts in the liver, pancreas, seminal vesicles, and arachnoid membrane. The development of hepatic cysts can result in polycystic liver disease (PLD) and congenital hepatic fibrosis (4). In ADPKD, kidney cysts are associated with additional medical conditions such as hypertension, flank pain, and renal failure (5). In individuals with ADPKD, the progressive nature of this disease can ultimately lead to the development of end-stage renal disease (ESRD), usually occurring in the more elderly population.

1: Nephrology and Kidney Transplant Research Center, Urmia University of Medical Sciences, Urmia, Iran.
2: Cellular and Molecular Research Center, Cellular and Molecular Medicine Institute, Urmia University of Medical Sciences, Urmia, Iran.
***Corresponding author: Dr Morteza Bagheri; Tel: +98 44 32770969; Fax: +98 44 32234125; E-mail: mortezabagheri@umsu.ac.ir.**
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Hepatic cysts are the most frequent extra-renal manifestation of ADPKD (6). ADPKD is generally diagnosed by imaging the kidneys via ultrasonography (US), magnetic resonance imaging (MRI), or computerized tomography scan (CT) (7). ADPKD is genetically heterogeneous and involves two identified genes, the polycystic kidney disease (PDK)1 gene on chromosome 16p13.3 (8) and *PKD2* gene on chromosome 4q21-23 (9). The *PKD1* and *PKD2* gene mutations have a causative role in 85% and 15% of patients with ADPKD, respectively (10). Somatic mutations in the *PDK1* and *PDK2* gene within epithelial cells of the kidney promotes the growth of these cells into renal cysts (11). The *PKD1* gene consists of 46 exons and encodes for polycystin-1 (PC-1, ~460 kDa), an integral membrane protein with 4302-amino-acids. PC-1 has various domains that play an important role in the cell-cell and extracellular matrix interactions of renal epithelial cells (12,13). The N-terminal region of the PC-1 protein is extra-cytoplasmic, while the C-terminal region of the PC-1 protein is cytoplasmic and contains 9-11 transmembrane domains and 220-amino-acids (14). The function of the PC-1 protein remains to be clearly elucidated in ADPKD pathogenesis. The PC-1 protein regulates several signaling pathways, such as the JAK/STAT pathway, that result in tyrosine phosphorylation and transcriptional changes (15). The majority of the *PKD1* gene mutations are either deletions, insertions, frame-shift mutations or, nonsense mutations. Many of the *PKD1* gene mutations have been understood to hold a role in the pathogenesis of PKD however, the exact mechanism of PC-1 is not well understood. The *PKD*1 gene is highly expressed in the kidneys of patients with ADPKD. Although, PC-1 is expressed in the epithelial cells of fetal, adult and polycystic kidneys, as well as non-epithelial cell types. Over-expression of mutant PC-1 can result in the accumulation of PC-1 tail fragments in cystic tubular epithelium and can lead to the development of PKD (16). Several *PKD1* mutations have been studied in Iranian patients however these studies are often individual case studies, and thus insufficient data exists regarding large groups of Iranian ADPKD patients (17,18). Hafizi et al. (2014) has suggested that the incidence of GTG>ATG mutation (V3057M) in exon 25 of the *PKD1* gene is 60% in the Southwest of Iran. Evaluation of PKD1 mutations would assist in to accurately assessing and confirming the clinical diagnosis of ADPKD and aid in accurate genetic counseling for assessing at-risk individuals. The aim of the present study was to evaluate the polymorphisms in exon 25 of the *PKD*1 gene in Iranian patients with ADPKD.

Materials and methods

This study was approved by the ethics committee of Urmia University of Medical Sciences, permission number ir.umsu.rec.1394.381. All research participants were fully informed about the goals and procedures of the study. Written informed consent was obtained from all participants prior to beginning the study. A total of 36 patients with ADPKD were enrolled. This research study was performed at Urmia University of Medical Sciences (Urmia, Iran). All participants were evaluated by an expert nephrologist and diagnosed for ADPKD based on strict imaging criteria using US, CT, and MRI techniques (8). The imaging criteria used for the diagnosis of ADPKD and inclusion criteria for ADPKD patients in the study were as follows: the manifestation of at least three kidney cysts in at-risk patients ages 15 to 39 years old, the existence of at least 2 cysts within each kidney of individuals ages 40 to 59 years old, or the presence of at least four cysts within each kidney of individuals at or above the age of 60 (19). Three to five mL of peripheral blood was drawn into EDTA-containing tubes for DNA isolation. Blood samples were preserved at -20 ^{o}C until genomic DNA isolation. Extraction of genomic DNA was performed using the standard salting out method (20). The DNA samples were preserved at -80^{o}C until further analysis. A set of primers for PCR amplification were used, forward primer, 5'- GGT GGT TGA GCT TCC CGG -3' and reverse primer, 5'- ATG TAG GTC ACC AGG CAC AC -3'. This produced a fragment with 827 -bps. The PCR reactions were performed in a 20 µl solution containing DNA (150 ng), reaction buffer (1x), both forward and reverse primers (10 pmol), dNTPs (200 µmol),

Taq DNA polymerase (0.3 unit), and $MgCl_2$ (1.5 mmol) (Genefanavaran, Tehran, Iran). The PCR conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 35 cycles of 95 °C for 20 seconds, 59 °C for 60 seconds and, 60 seconds at 72 °C for the extension step. The PCR products were confirmed via electrophoresis on 1.5% agarose gel, then directly sequenced using the ABI 3700XL automated DNA analyzer (Applied Biosystems) via the same PCR primers. Chromas Lite version 2.1.1 (2012) was applied for chromatogram visualization of the sequenced PCR products (Chromas Lite version 2.1 (2012), Technelysium Pty Ltd, South Brisbane, Queensland, Australia). Lastly, the sequence of each sample was compared to the reported gene sequence.

Results

This study included a total of 36 patients with ADPKD from Iran with an average age of 47.92±12.54 years. A summary of our findings is presented in Table 1. Figures 1 to 4 show exon 25 polymorphism analysis of the *PKD1* gene of our patient cohort. Our results show the frequencies of CAG>GAG, ATG>GTG, GTC>GTA, and GTG>ATG polymorphisms in exon 25 of the *PKD1* gene to be, 34 (94.44%), 33 (91.67%), 26 (72.22%), and 5 (13.89%), respectively. Of the polymorphisms at exon 25 of the *PDK1* gene, the most frequent mutation was the homozygous CAG → GAG which results in an amino acid change of Q[Gln] to E[Glu] at codon 3005 (Fig. 1). The second most prevalent polymorphism found in our cohort of Iranian ADPKD patients is the homozygous ATG→GTG which causes an amino acid change of M [Met] to V[Val] at codon 3023 (Fig. 2). The third most frequent polymorphism found in the ADPKD patients was the homozygous synonymous substitution GTC→GTA which causes an amino acid change of V[Val] to V[Val] at codon 3062 (Fig. 3). The fourth most common polymorphism identified in our study was the heterozygous GTG→ATG which leads to an amino acid change of V[Val] to M[Met] at codon 3057 (Fig. 4). Figure 5 shows an ultrasound examination of the kidney in an ADPKD patient.

Table 1. Variants of the *PDK1* gene at exon 25 detected by exon sequencing

Region	Marker	Protein position	Amino Acid Change	Codon Change	Clinical Significance	Frequency (%)
EX25	rs1063401	3005	Q[Gln]/E[Glu]	CAG>GAG	Definitely Pathogenic	34 (94.44)
EX25	rs17135779	3023	M[Met]/V[Val]	ATG>GTG	Likely Neutral	33 (91.67)
EX25	rs766805672	3062	V[Val]/V[Val]	GTC>GTA	Neutral	26 (72.22)
EX25	rs778055216	3057	V[Val]/M[Met]	GTG>ATG	Likely Neutral	5 (13.89)

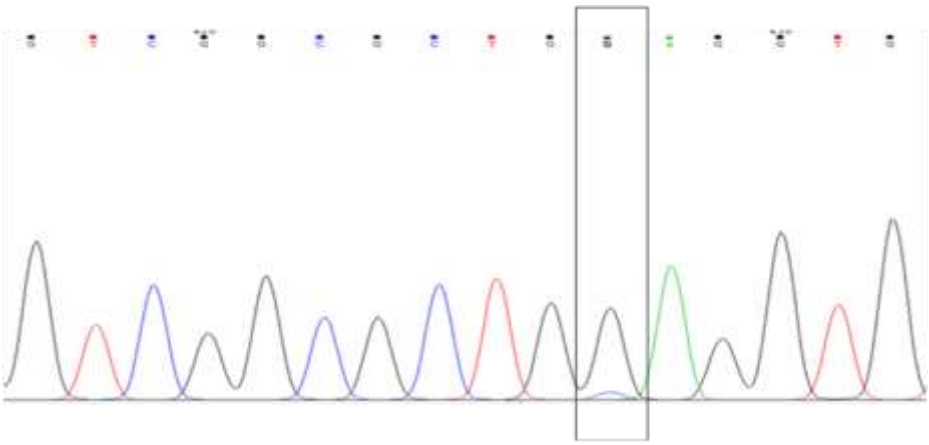

Fig. 1. The homozygous CAG → GAG substitution in patients with ADPKD at exon 25 of the *PKD1* gene, which causes an amino acid exchange of Q[Gln] to E[Glu] at codon 3005.

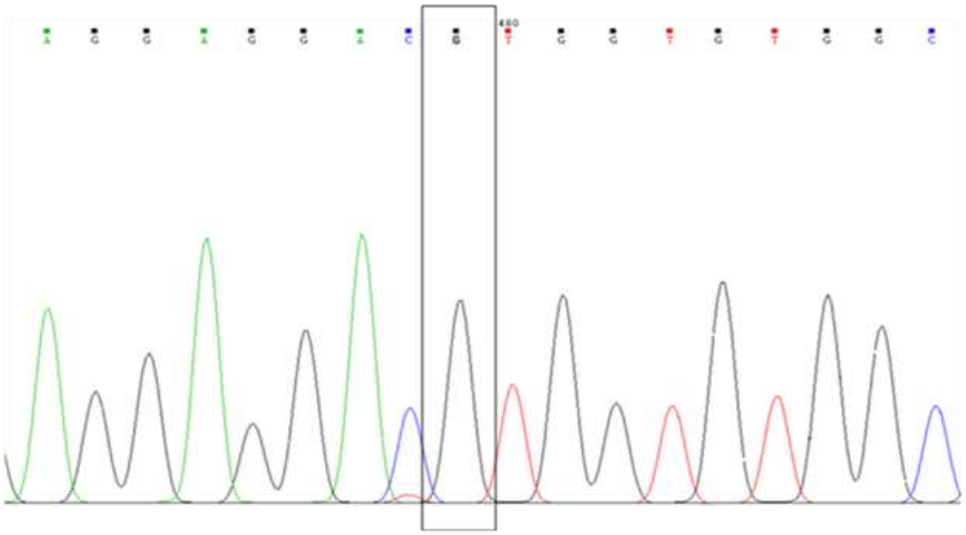

Fig. 2. The homozygous ATG→GTG substitution in patients with ADPKD at exon 25 of the *PKD1* gene, which causes an amino acid exchange of M [Met] to V [Val] at codon 3023.

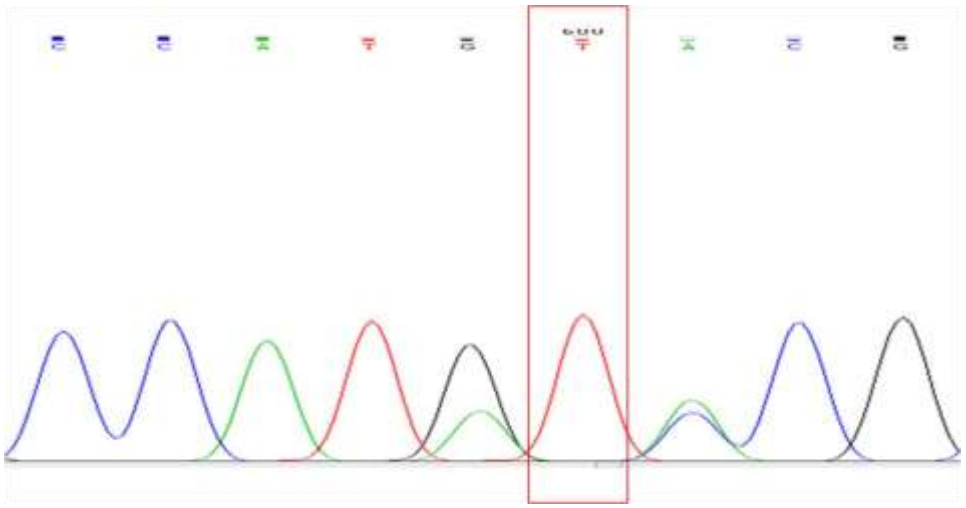

Fig. 3. The homozygous synonymous variant GTC→GTA substitution in patients with ADPKD at exon 25 of the *PKD1* gene, which causes an amino acid exchange of V[Val] to V[Val] at codon 3062.

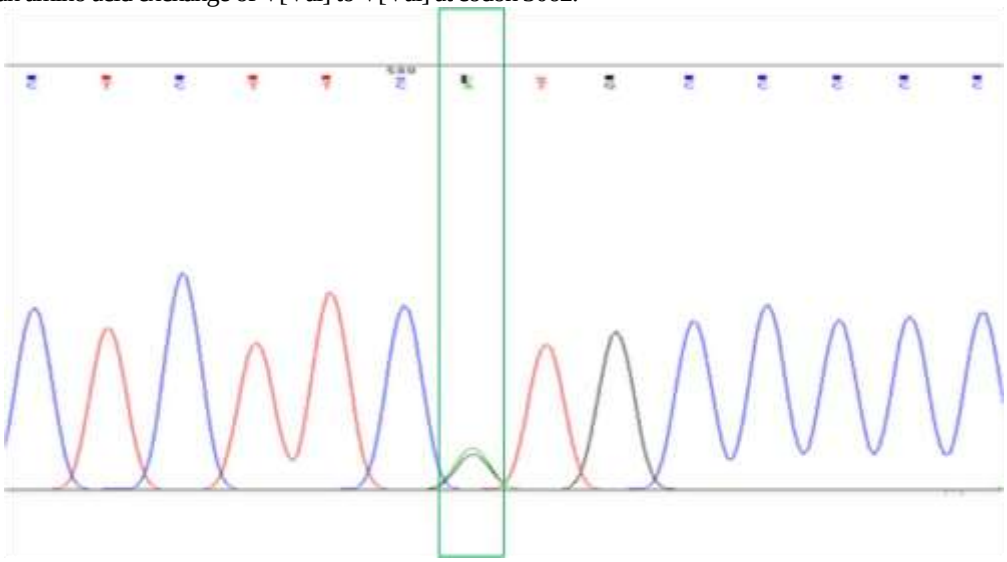

Fig. 4. The heterozygous GTG→ATG substitution in patients with ADPKD at exon 25 of the *PKD1* gene, which causes an amino acid exchange of valine to methionine at codon **3057.**

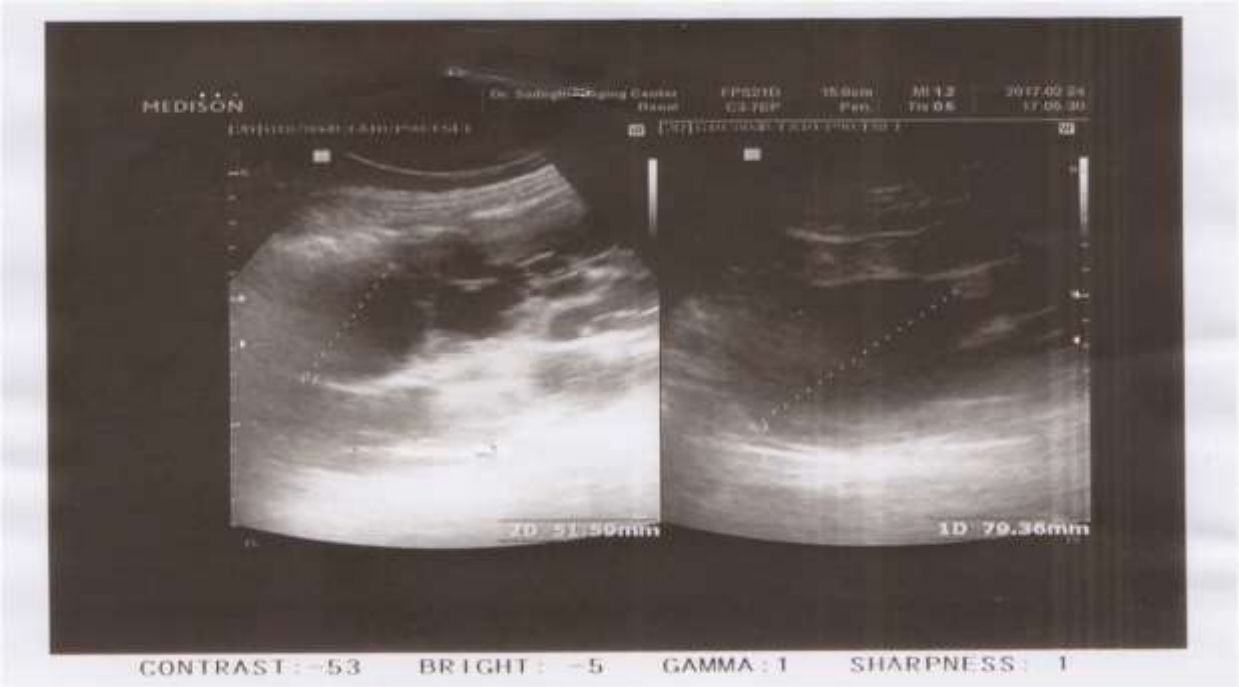

Fig. 5. Ultrasound image of kidneys. The size of both kidneys are abnormally enlarged, and in both kidneys there are a number of cystic lesions present, the largest of which are 51 mm in the right kidney and 79 mm in the left kidney.

The pathophysiology of PKD is related to defects in the primary cilia on the apical surface of the cells of the kidneys. It has been suggested that defects in PC-1, PC-2 and the cilia protein, fibrocystin, may contribute to the development of PKD (21). The exact mechanism of cyst development in PKDs is not well understood. Several studies have been conducted to investigate the pathogenesis of *PKD1* mutations and how they may influence the PKD phenotype. Sutters et al (2003) has previously reported that the presentation of the PC-1 C-terminal tail promotes abnormal epithelial cells of the kidney (22). Mutations in *PDK1* are important factors in ADPKD that result in the early onset of renal diseases and play a more integral role in the pathogenesis of ADPKD compared to *PKD2* mutations (23). Research has indicated that PC-1 can regulate STAT signaling by JAK-dependent phosphorylation which may contribute to the enlargement of renal cysts in ADPKD (24).

Several different mutations within the *PDK1* gene have been found to have a wide range of pathological consequences, therefore it is important to properly distinguish which mutations of the *PDK1* gene result in which disease phenotypes. Genetic analysis of *PKD1* is valuable for the diagnosis of ADPKD in asymptomatic persons within the population without a prior family history of ADPKD. Several different methods have been used to detect mutations in the *PKD*1 gene (25, 26). In the present study, we analyzed the *PKD1* gene using direct sequencing of PCR products that cover exon 25 with a set of newly designed primers in a cohort of 36 Iranian ADPKD patients. Four genetic variants were identified in exon 25 of *PKD1* gene. The most frequent genetic variant was a nonsense mutation with potential pathogenic clinical significance, where the amino acid changes resulted in the replacement of Glu with Gln at codon 3005. The second most frequent polymorphism includes the substitution of Val with Met at codon 3023. This polymorphism has neutral significance in ADPKD pathogenesis. The third most frequent polymorphism was a variant with neutral significance at codon 3062 and leads to codon change (GTC>GTA) without an amino acid change (Val3062Val). The next most frequent polymorphism was a substitution mutation at codon 3057 leading to amino acid change of Val 3057 Met (GTG>ATG). This mutation likely has significance with regard to the pathogenesis of ADPKD.

A previous case study by Hafizi et al. (2014) showed that a 7-year-old Iranian boy with ADPKD contained a heterozygous GTG>ATG missense mutation in exon 25 of the *PKD1* gene. This mutation causes the exchange of the valine amino acid with methionine at codon 3057 in the *PKD1* gene. Additionally, two heterozygous missense mutations were detected in the patient including, ACA→ GCA at codon 2241 in exon 15 that altered threonine to alanine, and CAC→ AAC at codon 3710 in exon 38 that altered histidine to asparagine. Hafizi et al. (2015) reported a novel CAT > GAT (H 3311R) missense mutation in exon 30 in a patient with ADPKD (27). Liu et al. (2015) analyzed the *PKD1* gene in a Chinese family with ADPKD and showed that the C10529T mutation might be a pathogenic mutation in this particular case (28). In a separate study, Li et al. (2011) studied mutations in the *PKD1* gene in a Chinese family with ADPKD and reported that c.3623-3624insGTGT might be a pathogenic mutation in this particular case (29).

Mutant PC-1 and PC-2 can result in the dysregulation of signal transduction pathways and increased concentrations of cyclic adenosine monophosphate (cAMP) that promote the development of cysts (30, 31). Regarding the dominant inheritance pattern of ADPKD, the reoccurring risk in offspring is 50%, and in 10-15% of cases the occurrence is sporadic due to the presence of *de novo* mutations (31). ADPKD is a heterogenic disorder that is caused by a wide range of mutations in the *PKD1* and *PKD2* genes. The majority of the ADPKD patients are asymptomatic, while some display clinical symptoms including, abdominal pain, hematuria and urinary tract infections (32). Analysis of the *PKD1* gene mutations can be useful in the screening for carriers and in prenatal diagnosis. The present study has some limitations such as a small sample size and poor quality of patient record keeping. Future research with a larger sample size and with more detailed clinical information is necessary to obtain more accurate results. Our study indicates that the homozygous missense mutation CAG→GAG at codon 3005 at exon 25 of the PKD1 gene is pathogenic in Iranian patients with ADPKD.

Discussion

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