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Identification of Novel Pathogenic *PKD2* Variants in Iranian Patients with Autosomal Dominant Polycystic Kidney Disease

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Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a delayed-onset renal disorder that results from a mutation in the *PKD1* or *PKD2* genes. Autosomal dominant polycystic kidney disease results in end-stage renal disease due to renal cystic dysplasia. The aim of this study was to evaluate, by exon sequencing, the disease-causing variants of *PKD2* (exons 4, 6, and 8) in Iranian ADPKD patients.

Methods: Genomic DNA was extracted from 3-5 ml of peripheral blood by the salting-out method. *PKD2* exons 4, 6, and 8 were PCR-amplified and sequenced.

Results: Three disease-causing *PKD2* variants were identified; all three were missense mutations in exon 4. The mutations were AGC → ACC (c.893G>C, cDNA.959G>C, S298T), TAC → TTC (c.1043A>T, cDNA.1109 A>T, Y348F), and GAA → GAT (c.1059A>T, cDNA.1125 A>T, E353D. These novel pathogenic variants may cause loss of the normal protein function.

Conclusions: Our results suggest that AGC → ACC (c.893G>C, cDNA.959G>C, S298T), TAC → TTC (c.1043A>T, cDNA.1109 A>T, Y348F), and GAA → GAT (c.1059A>T, cDNA.1125 A>T, E353D variants are common in Iranian ADPKD patients. These mutations modify the transmembrane domain and likely influence PC2 function.

Keywords: Pathogenic Variants, PKD2, Autosomal Dominant Polycystic Kidney Disease.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD, OMIM ID: 173900) is a delayed-onset renal disorder with a variety of pathologic features including bilateral kidney cysts, cysts in other organs including liver, seminal vesicles, pancreas, and arachnoid membranes, vascular abnormalities including intracranial aneurysms, thoracic aortic dissection, mitral valve prolapse, and abdominal wall hernia (1-3). The renal manifestations of the disease include hypertension in 50% of patients aged 20-30 years, renal pain, and renal failure. About 50% of patients with ADPKD progress to end-stage renal disease (4). ADPKD is seen in 6–10% of candidate patients for dialysis or kidney transplantation (3). The prevalence of ADPKD is not accurate and varies regarding different studies. The prevalence of ADPKD is one case/800–1000 residents in general population of Western countries (3). The phenotypes of ADPKD vary and appear in various tissues including heart, liver, bone, and the endocrine system. Hepatic cysts are the most common extra-renal finding in ADPKD (5). ADPKD is genetically heterogeneous and is caused by mutations in *PKD1* (MIM 601313) and *PKD2* (MIM 173910) (6), which encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively. *PKD1* and *PKD2* account for 85 and 15% of cases, respectively (6). Positive family histories are found in 46.4% of patients (3), while in those with no family histories, de novo *PKD2* mutations have

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been detected in 5% of patients (6). The location of mutations in *PKD2* affect clinical outcomes (7). Most *PKD2* mutation carriers are unaware of their condition (8). *PKD2* was first cloned in 1996, and to date, more than 78 different *PKD2* mutations have been identified (9). The association between the *PKD2* variants and phenotype is unclear. In young patients with ADPKD, renal cysts may not be detected by ultrasonography; therefore, identifying *PKD2* mutations in young patients is the preferred diagnostic method. The aim of this study was to evaluate, by exon sequencing, the disease-causing variants in *PKD2* exons 4, 6, and 8 in Iranian ADPKD patients.

Materials and methods

The Urmia University of Medical Sciences Ethics Committee approved all stages of this study (permit number ir.umsu.rec.1394.382). The patients and their families were informed about the contents and the study. The patients were recruited from the Imam Khomeini University Hospital in Urmia, Iran. The cases were evaluated by a nephrologist based on strict criteria regarding radiologic imaging of the kidneys; these included ultrasonography, computerized tomography (CT) scan, positron emission tomography (PET) scan, and magnetic resonance imaging (MRI) (10). These criteria included the presence of at least two unilateral/bilateral kidney cysts in patients aged <30 years, at least two cysts in both kidneys of patients aged 30–59 years, and at least four cysts in both kidney of patients aged >60 years (10). *PKD2* mutations in 40 unrelated ADPKD patients were analyzed by direct sequencing of PCR products. Linkage to other *PKD1* or *PKD2* mutations or polymorphisms were not tested due to the lack of blood samples from other family members. For genomic DNA extractions, 3-5 ml of peripheral blood were collected into blood collection tubes containing EDTA. The tubes were stored at -20 ºC until DNA isolation. The salting-out method was used to extract the DNA (11). DNA samples was stored at -80 ºC until further analysis. Primers and their characteristics, and PCR product sizes in base pairs (bp) are shown in Table 1. The PCRs were performed in 25 µl volumes containing 200 ng of DNA, 1x reaction buffer, 0.5 µl of each primer (10 pmol), 200 µmol of dNTPs, 0.3 unit of Taq DNA polymerase, and 1.5 mmol $MgCl_2$ (Genefanavaran, Tehran, Iran). The PCR program included: initial denaturation at 95 °C for 10 min, followed by 35 cycles of 95 °C for 30 sec, 58 °C for 60 sec, and extension at 72 °C for 60 sec. The PCR was completed by a 72 °C, 10-min fill-in step. The PCR products were electrophoresed on 2% agarose gels and then sequenced on an ABI 3700XL automated DNA analyzer (Applied Biosystems) using the PCR primers. Chromas Lite version 2.1.1 (2012) was used to visualize of the resulting chromatograms (Chromas Lite version 2.1 (2012), Technelysium Pty Ltd, South Brisbane, Queensland, Australia). Finally, each sample sequence was compared to the reference genome sequence.

Table 1. Primer characteristics and PCR product sizes in this study

Gene	Exon	Primer Sequence (5'->3')	Length	Tm	GC%	PCR product size (bp)
PKD2	**4**	aaccttggttcagaggaccc	20	59.23	55.00	589
		gcactgaagagtcactgaact	21	57.90	47.62	
	6	gggaagactgagcaggaagt	20	59.02	55.00	675
		acctctccacttcatactcagc	22	59.24	50.00	
	8	tgtccttttgtgtcaggctt	20	57.56	45.00	526
		gcattgtcttctttgcatggg	21	58.39	47.62	

Results

DNA samples from 40 Iranian ADPKD patients with an average age of 43.96±5.6 years were analyzed. *PKD2* mutations were found in three (7.5%) samples. The sample sequences were compared to the reference gene sequence. Figures 1, 2, and 3 show the three *PKD2*

disease-causing missense mutations in our samples, which were all found in exon 4. No disease-causing variants were found in exons 6 or 8. The three *PKD2* missense mutations were AGC → ACC (c.893G>C, cDNA.959G>C, S298T), TAC → TTC (c.1043A>T, cDNA.1109 A>T, Y348F), and AA → GAT (c.1059A>T, cDNA.1125 A>T, E353D (Table 2). These mutations are novel pathogenic variants that may cause loss of normal protein function (Fig. 4).

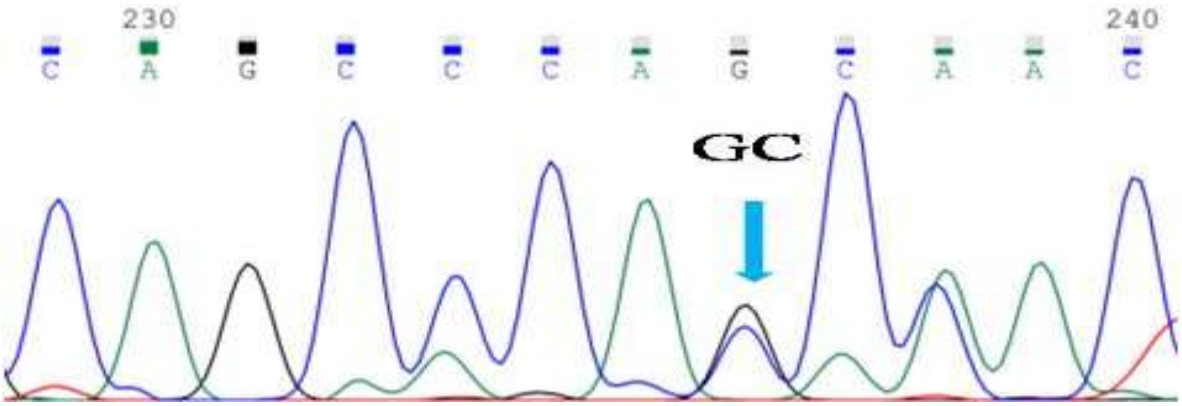

Fig. 1. The heterozygous missense mutation AGC → ACC (c.893G>C, cDNA.959G>C, g.30633 G>C, S298T) substitution in *PKD2* at codon 298 in exon 4 in one ADPKD patient.

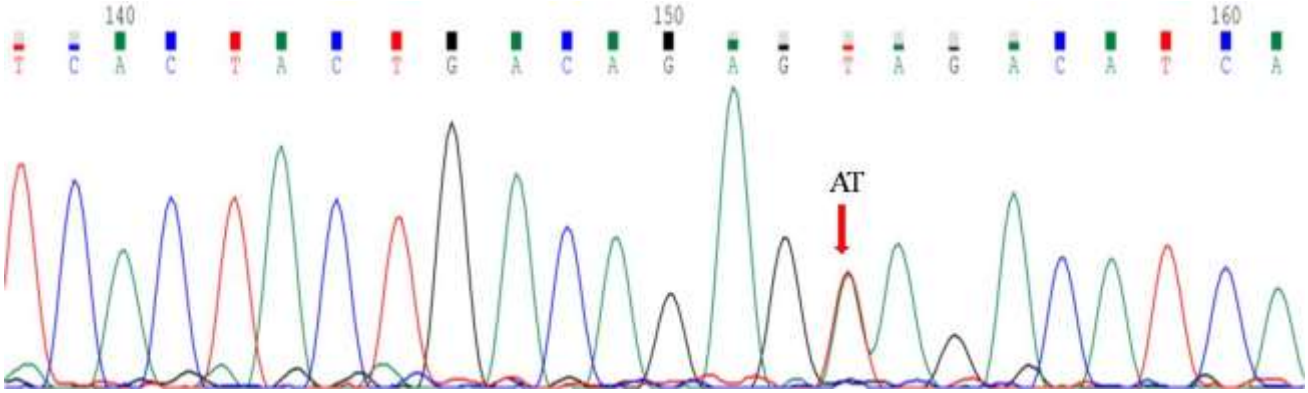

Fig. 2. The heterozygous missense mutation TAC → TTC (c.1043A>T, cDNA.1109 A>T, g.30783 A>T, Y348F) substitution in *PKD2* at codon 348 in exon 4 in one ADPKD patient.

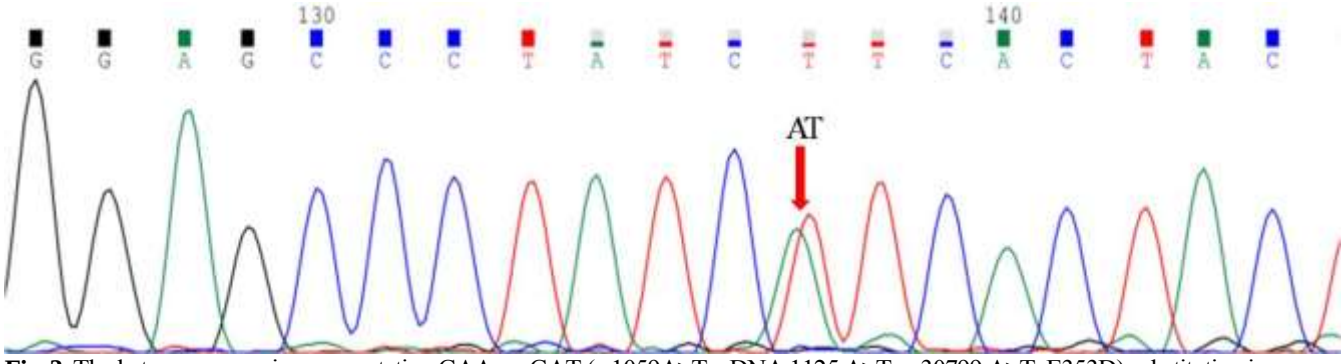

Fig. 3. The heterozygous missense mutation GAA → GAT (c.1059A>T, cDNA.1125 A>T, g.30799 A>T, E353D) substitution in *PKD2* at codon 353 in exon 4 in one ADPKD patient.

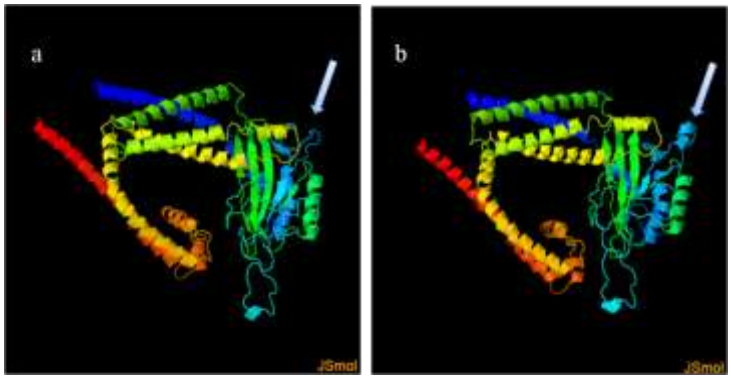

Fig. 4. Structures of normal (a) and mutated (b) polycystin-2. The differences between the two protein structures are indicated by the arrows. The images were prepared by Phyre2 software.

Table 2. Markers, codon/codon change, amino acid change, genotype, and mutation type of variants in exon 4 in this study.

Marker	Codon	Amino Acid Change	Codon Change	Genotype T/C	Mutation Type
rs200912883	298	S/T	AGC/ACC	GC	Disease causing
rs761547854	348	Y/F	TAC/TTC	AT	Disease causing
rs375164861	353	E/D	GAA/GAT	AT	Disease causing

Discussion

The diagnosis of ADPKD is usually based on patient medical histories and physical examinations (12); however, classic diagnosis may be difficult due to other disorders with overlapping manifestations. In this regard, diagnostic imaging or molecular genetic tests are necessary to confirm or rule out the other diseases. Several imaging techniques, including ultrasound, CT scan, and MRI, have been used to detect cysts in kidneys, liver, and pancreas (13). These tests are expensive (14) and generally not performed on young patients (12). Considering the limitation of these tests, genetic tests are important for definitive diagnosis of the disease. ADPKD is a heterogeneous disorder associated with disease-causing variants in *PKD1* (responsible for ADPKD-type I), *PKD2* (responsible for ADPKD-type II), and *GANAB*, which encodes the glucosidase II alpha subunit (15). *PKD1* (MIM 601313) and *PKD2* (MIM 173910 are located on chromosomes 16 (16p13.3, 46 exons) and 4 (4q21, 15 exons), respectively (16). *PKD1* and *PKD2* genetic variants have been studied in different ethnic groups by various methods; however, exon sequencing is still considered the gold standard (10). This study is the first report of *PKD2* variants in ADPKD patients living in west Azerbaijan, Iran. We performed PCR and direct sequencing of *PKD2* on patient samples and found three genetic variants in exon 4. These variants were not found in ExAC or the 1000G database. Polycystins are transmembrane proteins that participate in cell-to-cell and cell-to-extracellular matrix connections, and have roles in cilia action in nephrons and calcium channel function (17). The transmembrane domain and PC1 function are altered in mutated proteins (17). Also, it has been demonstrated that cytokines and cytokine gene polymorphisms have been associated with human diseases (18-23). In this regard, several gene polymorphisms predict the pathogenesis of kidney disease in Iranian patients. Studies with more details are necessary to evaluate the correlation between genetic polymorphisms and ADPKD risk in Iranian patients.

In conclusion, the study identified three novel *PKD2* disease-causing variants in Iranian ADPKD patients.

Acknowledgment

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