Original Article

Molecular genetic analysis of polycystic kidney disease 1 and polycystic kidney disease 2 mutations in pedigrees with autosomal dominant polycystic kidney disease

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Background: Dysfunction of polycystin-1 or polycystin-2, the proteins encoded by polycystic kidney disease 1 (*PKD1*) and *PKD2*, respectively, are the cause of autosomal dominant PKD (ADPKD). This genetically heterogeneous monogenic disorder is the most common inherited kidney disease. The disease manifests are progressive cyst growth, renal enlargement, and renal failure, due to abnormal proliferation of kidney tubular epithelium. **Materials and Methods:** In this study, mutation analysis of *PKD1* and *PKD2* genes in nine Iranian families was performed using next-generation sequencing. All patients met the diagnostic criteria of ADPKD. **Results:** Mutations were found in all 9 families in *PKD1* gene, comprising 2 novel and 7 previously reported mutations. No mutation in *PKD2* was identified. **Conclusion:** Finding more mutations and expanding the spectrum of *PKD1* and *PKD2* mutations can increase the diagnostic value of molecular testing in the screening of ADPKD patients.

Key words: Autosomal dominant polycystic kidney disease, next-generation sequencing, polycystic kidney disease 1, polycystic kidney disease 2

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequently inherited cause of renal cystics in human[1] with the prevalence ranges of 1:400 and 1:1000 worldwide.[2] ADPKD is a late-onset multisystemic disorder, characterized by massive kidney enlargement and progressive chronic renal disease, but also cysts and connective tissue abnormalities involving many other organs such as liver, seminal vesicles, pancreas, spleen, arachnoids membrane, and vascular abnormalities.[2,3] Renal symptoms include hypertension, renal pain, and renal insufficiency.[3] Approximately 50% of ADPKD patients are affected with end-stage renal disease (ESRD) in late middle age.[3,4] The disease is genetically heterogeneous, and the severity of disease varies greatly even within the same family.[5]

Mutations in at least two genes of *PKD1* and *PKD2* have been identified as the cause of ADPKD. Manifests are progressive cyst growth, renal enlargement, and renal failure, due to abnormal proliferation of kidney tubular epithelium.[1] The ~50 kb *PKD1* gene which is mapped to 16p13.3, contains 46 exons, encodes a 4302 amino acid comprising 11-pass plasma membrane glycoprotein, the polycystin-1 (PC-1). Pseudogenes *PKD1*P1-P6 (exons 1–33) are duplicated six times and located on chromosome 16, which share 97.7% sequence identity with *PKD1*, but they carry large deletions compared to *PKD1*.[6,7] The human *PKD2* gene on chromosome 4q21 contains 15 exons in a genomic

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area of ~68 kb and encodes a 968 amino acids protein, polycystin-2. Polycystin-2, a putative ion channel, which functions as a nonselective cation channel can conduct calcium ions. In approximately 85%–90% of ADPKD cases, pathogenic mutations in *PKD1* are causative whereas in approximately 10%–15% of the remaining patient's pathogenic mutations in *PKD2* are causative.[3,6] In patients with mutations in *PKD2*, milder clinical course compared to *PKD1 patient*s, fewer renal cysts and milder hypertension lead to delayed progression to end-stage kidney failure.[4]

It has been shown that the polycystin-1 and polycystin-2 as integral membrane proteins, play key roles in maintaining normal kidney tubular structure during the renal development.[6] These proteins modulate intracellular calcium homeostasis and other signal transduction pathways and mediate cell adhesion in the primary cilia of the renal epithelium cells.[6] These two proteins interact with each other through their C-terminal regions.[4] Studies suggest potential roles for polycystin-1 in the regulation of ion transport either directly or through its association with polycystin-2.[8] Dysfunctions of the PC-1 or PC-2 proteins disturb tissue morphogenesis and trigger abnormal cell proliferation and cyst formation.[4]

So far, 2323 and 278 germline variants have been reported in *PKD1* and *PKD2*, respectively, in the Autosomal Dominant PKD Mutation Database (PKDB). PKDB has also recorded 9 somatic sequence mutations of *PKD1* and 27 somatic mutations of the *PKD2* gene (http://pkdb.mayo.edu). The Human Gene Mutation Database (HGMD) has recorded 1,516 and 261 mutations of the *PKD1* and *PKD2* genes, respectively (http://www.hgmd.cf.ac.uk).

In this study, a total of nine Iranian patients from apparently unrelated families were screened for *PKD1* and *PKD2* mutations by next-generation sequencing (NGS). Nine mutations were identified throughout the *PKD1* gene which they are mainly predicted to truncate and probably inactivate the protein. No mutation in *PKD2* was identified.

MATERIALS AND METHODS

In this study, mutation analysis of *PKD1* and *PKD2* genes in nine Iranian families with a diagnosis of ADPKD was performed. Written informed consent for research was obtained from all participants. Blood samples were collected from nine families including at least one affected individual, total number of 17 patients, and 10 healthy individuals [Table 1]. The diagnosis of the disease was made by nephrologists based on a renal ultrasound finding consistent with ADPKD and enlarged cystic kidneys. Pedigrees are shown in Figure 1.

Genomic DNAs were extracted from the peripheral blood of probands and their available family members by a High Pure PCR template preparation kit (Roche; Product No, 11814770001). Genetic screening for the *PKD1* and *PKD2* genes in proband in each family was performed in BGI clinical laboratories (China) using a custom designed Nimblegen chip capturing the *PKD1* (NM_001009944) and *PKD2* (NM_000297) genes followed by NGS. In general, the test platform examined >95% of the target genes with sensitivity >99%. Point mutation, micro-insertion, deletion, and duplications (<20 bp) can be simultaneously detected. For analysis of the sequencing results, the international publicly available mutation and polymorphism databases such as 1000 genome project, Exome Aggregation Consortium (ExAC) and Exon Sequencing Projects as well as BGI self-developed local database were employed. Only variants with a frequency below 0.01 were selected. Previously reported mutations that have been described in HGMD as pathogenic were given the highest priority. Prediction of the consequence of mutations was obtained from at least three online databases namely SIFT, Polyphen2, and Mutation Taster. In addition, ConSurf (http://www.consurf.tau.ac.il) was applied to check the evolutionary conservation in the region of the mutations [Figure 2].

The identified mutations in *PKD1* gene were confirmed by direct Sanger sequencing in patients and their family members to determine whether the mutations are co-segregated with the disease in these families. The target sites amplified using primers, on request is available.

In order to confirm the identified variants, polymerase chain reaction (PCR) analysis was carried out in a total volume of 25 μL containing 0.5 μL of each forward and reverse primers, 10 μL of PCR Master mix magnesium chloride (1.5 Mm), and 1 μL of DNA (about 100 ng). The reaction was adjusted to the total volume of 25 μL by ddH2O. The PCR was performed using an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 61 for 30 s, and elongation at 72°C for 30 s. Products of PCR were examined by 2% agarose gel electrophoresis for the presence and sizes of amplicons. Consequently, DNA sequencing of the PCR products was performed on a 3130 ABI capillary electrophoresis. Sequencing chromatograms were analyzed using Codoncode aligner software version 6.0.2 (CodonCode Corporation, Centerville, MA 02632,USA).

RESULTS

This study assessed a total of 27 Iranian individuals, 9 index-cases and 18 relatives, to confirm the diagnosis of the ADPKD disease [Table 1].

Table 1: List of identified mutations

	Family member/age	Affection status	Zygosity and genotype	Chromosome and mutation location	Nucleic acid and amino acid alternation	Mutation function	Family history	Reference	PKDB
Family 1	III1/31	Affected	Heterozygote GA	Chr16:2142104 EX40/CDS40	c. 11355G>A p.Trp3785Ter	Pathogenic	Yes	[9]	Definitely pathogenic
	III2/26	Healthy	Normal GG						
	II1/60	Healthy	Normal GG						
	II2/45	Affected	Heterozygote GA						
Family 2	II3/40	Affected	Heterozygote CT	Chr16:2147225 EX34/CDS34	c. 10423C>T p.Gln3475Ter	Pathogenic	Yes	[10]	Definitely pathogenic
	I1/79	Healthy	Normal CC						
	II1/35	Affected	Heterozygote CT						
	II2/48	Healthy	Normal CC						
	I2/69	Affected	Heterozygote CT						
Family 3	III1/26	Affected	Heterozygote CG	Chr16:2166872 EX7	c. 1568C>G p.Ser523Ter	Pathogenic	No	[11]	-
Family 4	III8/36	Affected	Heterozygote N/del T	Ch16:2139962 CDS46	c. 12678 del T p.Phe4226Leufs*132	Likely pathogenic	Yes	Novel	-
Family 5	II1/36	Affected	Heterozygote GA	chr16:2142955 EX38/CDS38	c. 11156G>A p.Arg3719Gln	Pathogenic	Yes	[12]	Highly likely pathogenic
	II2/33	Affected	Heterozygote GA						
	I1/53	Affected	Heterozygote GA						
	II3/36	Healthy	Normal GG						
Family 6	II2/39	Affected	Heterozygote GA	Ch16:2160926 EX15/CDS15	c. 4242G>A p.Trp1414Ter	Likely pathogenic	No	Novel	-
Family 7	II1/33	Affected	Heterozygote CT	Ch16:2147766 EX32/CDS32	c. 10183C>T p.Gln3395Ter	Likely pathogenic	No	-	Definitely pathogenic
	I1/73	Healthy	Normal CC						
	I2/62	Healthy	Normal CC						
Family 8	III2/34	Affected	Heterozygote N/del	Ch16:2168131.2168137 EX5/CDS5	c. 856_862delTCTGGCC P.Ser286SerfsX2	Pathogenic	Yes	[13]	Definitely pathogenic
	II3/51	Affected	Heterozygote N/del						
	II4/57	Healthy	Normal N/N						
Family 9	III3/32	Affected	Heterozygote CT	Ch16:2140689 EX44/CDS44	c. 12124 C>T p.Gln4042Ter	Pathogenic	Yes	[14]	Definitely pathogenic
	III1/39	Affected	Heterozygote CT						
	II11/56	Healthy	Normal CC						
	II10/65	Affected	Heterozygote CT						
	III2/35	Healthy	Normal CC						

PKDB=Polycystic kidney disease mutation database

Figure 1: Representative pedigrees

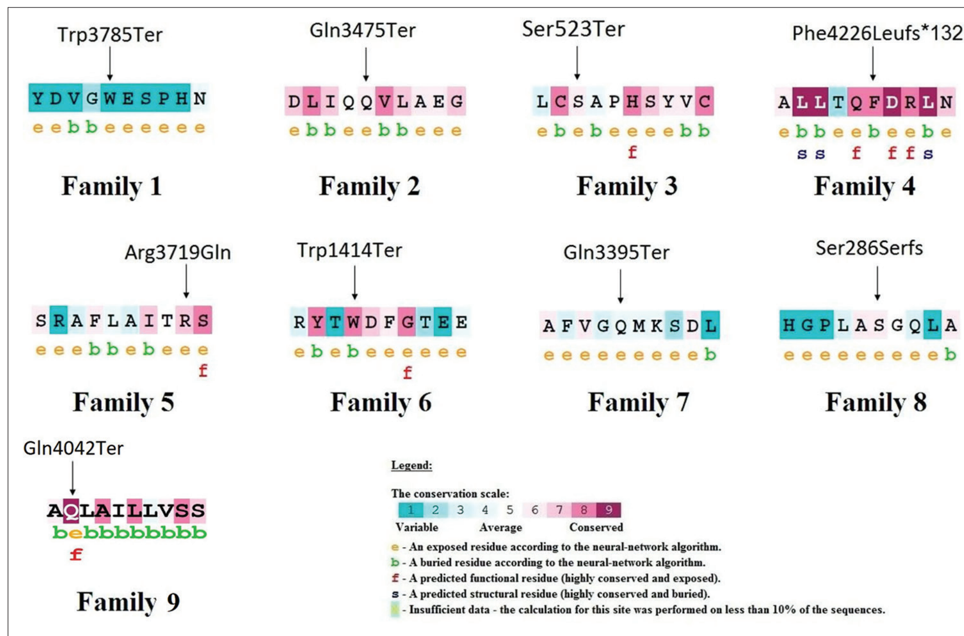

Figure 2: ConSurf results

The sequencing of the total length of *PKD1* and *PKD2* (15819 bp) with coverage ranging from 92.69% to 99.98%, average depth between 90.24X and 178.0X and minimum depth of 30X was obtained.

In total nine different mutant variants in 9 different exons, 5, 7, 15, 32, 34, 38, 40, 44, and 46, including, one missense, 2 small deletions, and 6 nonsense mutations in *PKD1* gene were found. Seven mutations in the present study have been described previously (p.Trp3785Ter, p.Gln3475Ter, p.Ser523Ter, p.Arg3719Gln, p.Gln3395Ter, P.Ser286SerfsX2, and p.Gln4042Ter) and 2 mutations were novel (p.Phe4226 Leufs*132 and p.Trp1414Ter). The two identified novel mutations were predicted to be disease-causing using prediction tools [Table 2].

Family 1

DNA from a 31-year-old symptomatic man with the possible diagnosis of ADPKD, enlarged cystic kidneys, was screened for mutations in *PKD1* and *PKD2* by NGS. A previously reported pathogenic nonsense mutation, c.11355G>A; p. Trp3785Ter, in exon 40 of *PKD1* was identified. We could show co-segregating with the disease in this family by investigating the other patient, affected mother, and two healthy individuals.

Family 2

DNA from a 40-year-old symptomatic patient with possible diagnosis of ADPKD due to enlarged cystic kidneys in abdominal ultrasonography was screened for mutations in *PKD1* and *PKD2* by NGS. A previously reported pathogenic nonsense mutation, c.10423C>T; p.Gln3475Ter, in exon 34 of *PKD1* was identified. We could show that this mutation is co-segregating with the disease in this family by investigating two additional patients and two healthy individuals.

Family 3

DNA from a 26-year-old symptomatic male with possible diagnosis of ADPKD was screened for mutations in *PKD1* and *PKD2* by NGS. A previously reported pathogenic nonsense mutation, c.1568C>G; p.Ser523Ter, in exon 7 of *PKD1* was identified.

Family 4

DNA from a 36-year-old symptomatic male with possible diagnosis of ADPKD was screened for mutations in *PKD1* and *PKD2* by NGS. A novel likely pathogenic frameshift variant, c. 12678delT; p.Phe4226 Leufs*132, in *PKD1* was identified. This variant is a single nucleotide deletion (delT) at position 12678 in exon 46 of *PKD1*, causing a frameshift at amino acid 4226 which leads to a stop codon at 132 residues

Table 2: In Silico prediction with software SIFT, Polyphen2 and mutation taster

	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9
Mutation type	Termination	Termination	Termination	Deletion T	Missense	Termination	Termination	Deletion TCTGGCC	Termination
SIFT									
dbSNP ID	CM034563: HGMD _ MUTATION	CM992200: HGMD_ MUTATION	Novel	-	-	Novel	CM0 10390: HGMD_ MUTATION	HGMD CM119057: HGMD _MUTATION	CM950939: HGMD _MUTATION
Score	N/A	N/A	N/A	-	-	N/A	N/A	N/A	N/A
Prediction	N/A	N/A	N/A	Neutral	-	N/A	N/A	Damaging	N/A
Median information content	N/A	N/A	N/A	-	-	N/A	N/A	N/A	N/A
Seqs at position	N/A	N/A	N/A	-	-	N/A	N/A	N/A	N/A
Polyphen									
Prediction	-	-	-	-	Probably damaging	-	-	-	-
Score	-	-	-	-	1.000	-	-	-	-
Sensivity	-	-	-	-	0.00	-	-	-	-
Spesivity	-	-	-	-	1.00	-	-	-	-
Mutation tasting									
Prediction	Disease causing	Disease causing	Disease causing	Disease causing	Polymorphism	Disease causing	Disease causing	Disease causing	Disease causing
1000 Genome	-	-	-	-	-	-	-	-	-
EXAC	-	-	-	-	-	-	-	-	-
HGMD	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes

EXAC=Exome aggregation consortium; HGMD=Human gene mutation database; N/A=Not available; SIFT=Sorting intolerant from tolerant

later. This mutation has not been described in 1000 Genome, ExAC and HGMD databases. This mutation was predicted to be disease-causing by mutation tasting.

Family 5

DNA from a 36-year-old symptomatic male with possible diagnosis of ADPKD was screened for mutations in *PKD1* and *PKD2* by NGS. A previously reported pathogenic missense c.11156G>A; p.Arg3719Gln mutation in *PKD1* was identified. We investigated this mutation in his affected mother and sister, and one healthy individual and could show co-segregation with the disease in this family.

Family 6

DNA from a 39-year-old symptomatic male with a possible diagnosis of ADPKD with no family history was screened for mutations in *PKD1* and *PKD2* by NGS. A novel likely pathogenic mutation, c. 4242G > A; p.Trp1414Ter, in *PKD1* gene was identified. This mutation has not been described in 1000Genome, ExAC and HGMD databases. This mutation was predicted to be disease-causing by mutation tasting.

Family 7

DNA from a 33-year-old symptomatic male with possible diagnosis of ADPKD and male infertility, was screened for mutations in *PKD1* and *PKD2* by NGS. The couple was complaining from unsuccessful conception after 3 years of unprotected sexual intercourse. A previously reported likely pathogenic nonsense mutation, c.10183C>T; p.Gln3395Ter, in *PKD1* gene was identified.

Family 8

DNA from a 34-year-old symptomatic male with possible diagnosis of ADPKD was screened for mutations in *PKD1* and *PKD2* by NGS. A previously reported pathogenic 7 bp deletion mutation of c. 856_862delTCTGGCC; p.Ser286SerfsX2 in exon 5 of *PKD1* was identified. This mutation was also found in his affected mother and was absent in his healthy Father.

Family 9

DNA from a 32-year-old symptomatic male with possible diagnosis of ADPKD and cardiovascular problem was screened for mutations in *PKD1* and *PKD2* by NGS. A previously reported nonsense mutation, c.12124 C > T; p.Gln4042Ter, in *PKD1* gene was identified. We could show co-segregation of this mutation with the disease in this family by showing its presence in the two additional patients and absence in the two healthy individuals.

The list and sequencing chromatographs of the identified mutations in the proband and another investigated individuals of each family are shown in Table 1 and Figure 3, respectively.

The in-silico pathogenicity predictions for each mutation using SIFT, polyphen2, and mutation taster software are shown in Table 2.

The identified mutations in *PKD1* gene were confirmed by direct Sanger sequencing in patients and their family members to determine whether the mutations are co-segregated with the disease in these families.

DISCUSSION

Screening for mutations has shown that mutations of *PKD1* and *PKD2* genes are affecting about 85% and 15% of ADPKD cases, respectively.[15] *PKD1* or *PKD2* has high allelic heterogeneity, and no hotspot site for mutations in these two genes has been found so far. Mutations in these genes are usually private and highly variable. Therefore, a complete mutation analysis of *PKD1* and *PKD2* is needed in ADPKD patients.[2]

Although several studies have shown that around 10%–15% of ADPKD cases are due to mutations in *PKD2* gene, here 100% of mutations found only in *PKD1* gene.

For example from the 52 mutations identified in a study in Germany, 86.7% of the mutations were in *PKD1* and 13.3% in *PKD2*.[1] A similar study in South Korea has shown 83.3% of mutations in *PKD1* and 16.7% in *PKD2*.[6] Another study in South Korea has identified a total of 76 variations (84.4%) in *PKD1* and 14 (15.6%) in *PKD2*.[16] Another study on a large cohort of 700 unrelated ADPKD patients in France has resulted in the identification of 83.8% pathogenic mutations in *PKD1* and 16.2% in *PKD2*.[17] The rate of mutations in *PKD1* and *PKD2* in the Chinese ADPKD patients has been shown to be 84.2% and 15.8% *PKD2*, respectively.[18] Mutational analysis in 18 unrelated Iranian families with ADPKD has revealed 88.9% and 11.1% of mutations in *PKD1* and *PKD2*, respectively.[19] The lack of mutation in *PKD2* in this study might be due to the fact that patients selected here had sever phenotypes and they had early onset.

Regarding the types of mutations found in this study, there were in total 8 truncating mutations (nonsense [$n = 6$] and frameshift [$n = 2$]), and 1 missense change.

Truncating mutations were the most frequent sequence changes in our patients, which is in concordance with previous studies.[15] These mutations are inactivating and cause ADPKD through a loss, or dosage reduction, of polycystin-1 protein.

The pathogenic missense mutation (p.R3719Q) is also predicted to be truncating, through its effect on the splicing. This mutation abolishes the donor splice site of intron 38 and causes integration of this exon along with

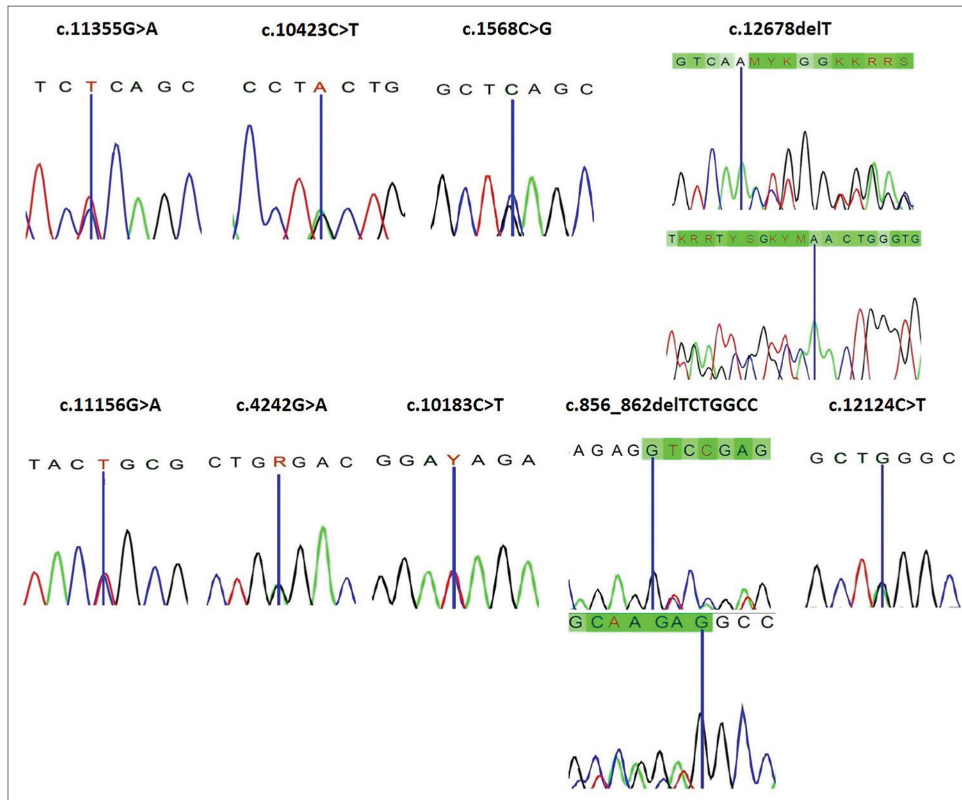

Figure 3: Sequencing result

117 bp of intron 38 and skipping of exon 39. The mRNA contains a premature stop codon which results to a smaller polycystin-1 protein lacking the last 585 C terminal amino acids and makes it susceptible to degradation by the nonsense-mediated decay.

Studies have shown that transfected cells expressing polycystin-1 lacking its carboxyl terminus do not interact with polycystin-2. On the other hand, previous studies on animal models of ADPKD have shown that the decrease of PKD1 expression is sufficient to initiate cytogenesis and vascular defects.[12]

PKD1 mutations found in this study are more frequent in the C terminal part, compared to the N terminal part.

ADPKD is a systemic disease with preferential renal involvement. The severity of renal disease and other complications of ADPKD varies among affected individuals, even within the same family.[3]

Many other organs in ADPKD patients can be affected and cause other disorders such as infertility and cardiovascular problems. ESRD in the mother of patient 5 with p.Arg3719Gln, infertility of patient 7 with p.Gln3395Ter, and vascular complications in patient 9 with p.Gln4042Ter mutations are in line with previous reports.[2,20-22] It has been shown that progressive cysts growth and renal enlargement due to *PKD1* mutations are associated with the more severe clinical course of the disease resulting in ESRD in the average age of 53.4 years.[6]

Although men with ADPKD are usually fertile, studies have presented that Polycystic is expressed in the cilia and flagella and abnormal proteins can lead to male infertility.[22]

Polycystin-1 is believed to play a role in cell–cell interactions and Polycystin-2 functions as a calcium (Ca2+) permeable ion channel with a possible role in the regulation of intracellular calcium ion concentrations. In addition, Polycystin-1 and 2 are expressed in vascular smooth muscle and endothelial, suggesting that the polycystins have a direct role in the vascular manifestations of the disease.[21]

Finding more mutations and expanding the spectrum of *PKD1* and *PKD2* mutations can increase diagnostic value of molecular testing in screening of ADPKD patients and help to reduce morbidity and mortality from renal disorders or other complications of the disease through therapeutic interventions.

CONCLUSION

In the present study, nine mutations located in nine different exons including 5, 7, 15, 32, 34, 38, 40, 44, and 46 were

detected in *PKD1* gene. Seven mutations were described previously in Chinese, French, German, Italian, and British populations. Two mutations in *PKD1* gene in this study were novel and expand the mutation spectrum of *PKD1*. Finding more mutations and expanding the spectrum of *PKD1* and *PKD2* mutations can increase the diagnostic value of molecular testing in screening of ADPKD patients.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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