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

Fatemeh Bitarafan, Masoud Garshasbi

Department of Cellular and Molecular Biology, North Tehran Branch, Islamic Azad University, 'Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Teheran, Iran

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 as 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

How to cite this article: Bitarafan F, Garshasbi M. Molecular genetic analysis of polycystic kidney disease 1 and polycystic kidney disease 2 mutations in pedigrees with autosomal dominant polycystic kidney disease. J Res Med Sci 2019;24:44.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequently inherited cause of renal cysts 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.[3,4] 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.[3]

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 PKD1P1‑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

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com
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.[3,4] In patients with mutations in \( PKD2 \), milder clinical course compared to \( PKD1 \) patients, 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.[6] Studies suggest potential roles for polycystin-1 in the regulation of ion transport either directly or through its association with polycystin-2.[6] Dysfunctions of the PC-1 or PC-2 proteins disturb tissue morphogenesis and trigger abnormal cell proliferation and cyst formation.[6]

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 \( \mu \)L containing 0.5 \( \mu \)L of each forward and reverse primers, 10 \( \mu \)L of PCR Master mix magnesium chloride (1.5 Mm), and 1 \( \mu \)L of DNA (about 100 ng). The reaction was adjusted to the total volume of 25 \( \mu \)L 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]           |
| III2/26          | Healthy          | Normal                |                                   |                                        |                  |               |               |
| II1/60           | Healthy          | Normal                |                                   |                                        |                  |               |               |
| II2/45           | Affected         | Heterozygote GA       |                                   |                                        |                  |               |               |
| **Family 2**     |                  |                       |                                  |                                        |                  |               |               |
| III1/31          | II1/35           | Affected Heterozygote CT | Chr16:2147225 EX34/CDS34         | c. 10423C>T p.Gln3475Ter             | Pathogenic       | Yes           | [10]          |
| I1/79            | Healthy          | Normal                |                                   |                                        |                  |               |               |
| II1/35           | Affected         | Heterozygote CT       |                                   |                                        |                  |               |               |
| II2/48           | Healthy          | Normal                |                                   |                                        |                  |               |               |
| I2/69            | Affected         | Heterozygote CT       |                                   |                                        |                  |               |               |
| **Family 3**     |                  |                       |                                  |                                        |                  |               |               |
| III1/26          | Affected         | Heterozygote GA       | Chr16:2166872 EX7                | c. 1568C>G p.Ser523Ter               | Pathogenic       | No            | [11]          |
| **Family 4**     |                  |                       |                                  |                                        |                  |               |               |
| III1/26          | Affected         | Heterozygote GA       | Chr16:2160926 EX15/CDS15         | c. 4242G>A p.Trp1441Ter              | Likely pathogenic | Novel         |
| **Family 5**     |                  |                       |                                  |                                        |                  |               |               |
| III1/36          | II1/53           | Affected Heterozygote GA | Chr16:2142955 EX38/CDS38         | c. 1156G>A p.Arg3719Gln              | Pathogenic       | Yes           | [12]          |
| I1/53            | Healthy          | Normal                |                                   |                                        |                  |               |               |
| I2/33            | Affected         | Heterozygote GA       |                                   |                                        |                  |               |               |
| I1/53            | Healthy          | Normal                |                                   |                                        |                  |               |               |
| II3/36           | Healthy          | Normal                |                                   |                                        |                  |               |               |
| **Family 6**     |                  |                       |                                  |                                        |                  |               |               |
| II1/33           | Affected         | Heterozygote GA       | Chr16:2147766 EX32/CDS32         | c. 10183C>T p.Gln3395Ter             | Likely pathogenic | Novel         |
| **Family 7**     |                  |                       |                                  |                                        |                  |               |               |
| II2/34           | Affected         | Heterozygote N/del T  | Chr16:2168131.2168137 EX5/CDS5   | c. 856.862delTCTGGCC p.Phe286SerfsX2 | Pathogenic       | Yes           | [13]          |
| II1/39           | Affected         | Heterozygote CT       | Chr16:2140689 EX44/CDS44         | c. 12124 C>T p.Gln4042Ter            | Pathogenic       | Yes           | [14]          |
| **Family 8**     |                  |                       |                                  |                                        |                  |               |               |
| II1/39           | II10/65          | Affected Heterozygote CT | Chr16:2140689 EX44/CDS44         | c. 12124 C>T p.Gln4042Ter            | Pathogenic       | Yes           | [14]          |
| I1/39            | Healthy          | Normal                |                                   |                                        |                  |               |               |
| I11/56           | Healthy          | Normal                |                                   |                                        |                  |               |               |
| II10/65          | Affected         | Heterozygote CT       |                                   |                                        |                  |               |               |
| II2/35           | Healthy          | Normal                |                                   |                                        |                  |               |               |

PKDB=Polycystic kidney disease mutation database
The sequencing of the total length of \textit{PKD1} and \textit{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, 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, 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 | Missense | Termination | Termination | Deletion |
| SIFT | CM034563: HGMD _ MUTATION | CM992200: HGMD_ MUTATION | Novel | - | - | Novel | CM010390: HGMD_ MUTATION | HGMD CM119057: HGMD _MUTATION | CM950939: HGMD _MUTATION |
| dbSNP ID | Score | N/A | 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 |
| | Sequs at position | N/A | N/A | N/A | - | - | N/A | N/A | N/A |
| | Polyphen Prediction | - | - | - | - | Probably damaging | - | - | - |
| | Score | - | - | - | - | 1.00 | - | - | - |
| | Sensivity | - | - | - | - | 0.00 | - | - | - |
| | Spesivity | - | - | - | - | 1.00 | - | - | - |
| Mutation tasting | Prediction | Disease causing | Disease causing | Disease causing | Disease causing | Polymorphism | Disease causing | Disease causing | Disease causing |
| | 1000 Genome | - | - | - | - | - | - | - | - |
| | EXAC | - | - | - | - | - | - | - | - |
| | HGMD | Yes | Yes | No | No | Yes | No | 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>T; p.Gln3395Ter, 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 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 and male infertility, 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 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.[6] 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
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.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Hoefele J, Mayer K, Scholz M, Klein HG. Novel PKD1 and PKD2 mutations in autosomal dominant polycystic kidney disease (ADPKD). Nephrol Dial Transplant 2011;26:2181-8.
2. Fatehi R, Khosravi S, Abedi M, Salehi R, Gheisari Y. Heterozygosity analysis of polycystic kidney disease 1 gene microsatellite markers for linkage analysis of autosomal dominant polycystic kidney disease type 1 in the Iranian population. J Res Med Sci 2017;22:102.
3. Harris P, Torres VE. Polycystic Kidney Disease, Autosomal Dominant Synonym: ADPKD. GeneReviews®. NCBI Bookshelf; 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1246/. [Last update on 2018 Jul 19].
4. Vouk K, Strmecki L, Stekrova J, Reiterova J, Bidovec M, Hudler P, et al. PKD1 and PKD2 mutations in Slovenian families with autosomal dominant polycystic kidney disease. BMC Med Genet 2006;7:6.
5. Liu B, Chen SC, Yang YM, Yan K, Qian YQ, Zhang JY, et al. Identification of novel PKD1 and PKD2 mutations in a Chinese population with autosomal dominant polycystic kidney disease. Sci Rep 2016;6:21578.
6. Choi R, Park HC, Lee K, Lee MG, Kim JW, Ki CS, et al. Identification of novel PKD1 and PKD2 mutations in Korean patients with autosomal dominant polycystic kidney disease. BMC Med Genet 2014;15:129.
7. Pirson Y, Chauveau D, Torres V. Management of cerebral aneurysms in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2002;13:269-76.
8. Arnaout MA. Kidney International. The vasculopathy of autosomal dominant polycystic kidney disease: Insights from animal models, principal discussant. Nephrol Forum 2000;58:599-610.
9. Yu C, Yang Y, Zou L, Hu Z, Li J, Liu Y, et al. Identification of novel mutations in Chinese Hans with autosomal dominant polycystic kidney disease. BMC Med Genet 2011;12:164.
10. Perrichot RA, Mercier B, Simon PM, Whebe B, Cledes J, Ferec C, et al. DGGE screening of PKD1 gene reveals novel mutations in a large cohort of 146 unrelated patients. Hum Genet 1999;105:231-9.
11. Neumann HP, Jilg C, Bacher J, Nabulsi Z, Malinoc A, Humbel B, et al. Epidemiology of autosomal-dominant polycystic kidney disease: An in-depth clinical study for south-western germany. Nephrol Dial Transplant 2013;28:1472-87.
12. Gonzalez-Paredes FJ, Ramos-Trujillo E, Claverie-Martin F. Defective pre-mRNA splicing in PKD1 due to presumed missense and synonymous mutations causing autosomal dominant polycystic disease. Gene 2014;546:243-9.
13. O’Brien K, Font-Montgomery E, Lukose L, Bryant J, Pivnica-Worms K, Edwards H, et al. Congenital hepatic fibrosis and portal hypertension in autosomal dominant polycystic kidney disease. J Pediatr Gastroenterol Nutr 2012;54:83-9.
14. Perugorria MJ, Maysuk TV, Marin JJ, Marzioni M, Bujanda L, LaRusso NF, et al. Polycystic liver diseases: Advanced insights into the molecular mechanisms. Nat Rev Gastroenterol Hepatol 2014;11:750-61.
15. Stekrova J, Reiterova J, Svobodova S, Kebrelova V, Lnenicka P, Merta M, et al. New mutations in the PKD1 gene in Czech population with autosomal dominant polycystic kidney disease. BMC Med Genet 2009;10:78.
16. Tan YC, Blumenfeld JD, Anghel R, Donahue S, Belenkaya R, Balina M, et al. Novel method for genomic analysis of PKD1 and PKD2 mutations in autosomal dominant polycystic kidney disease. Hum Mutat 2009;30:264-73.
17. Audrézet MP, Cornec-Le Gall E, Chen JM, Redon S, Quéré I, Creff J, et al. Autosomal dominant polycystic kidney disease: Comprehensive mutation analysis of PKD1 and PKD2 in 700 unrelated patients. Hum Mutat 2012;33:1239-50.
18. Zhang M, Liu S, Xia X, Cui Y, Li X. Identification of novel mutations and risk assessment of Han Chinese patients with autosomal dominant polycystic kidney disease. Nephrology (Carlton) 2018. doi: 10.1111/nep.13270.
19. Ranjzad F, Tara A, Basiri A, Aghdami N, Moghadasali R. Mutational screening of PKD1 and PKD2 genes in Iranian population diagnosed with autosomal dominant polycystic kidney disease. Clin Lab 2017;63:1261-7.
20. Nie X, Arend LJ. PKD1 is required for male reproductive tract development. Mech Dev 2013;130:567-76.
21. Rossetti S, Chauveau D, Kubly V, Slezak JM, Saggar-Malik AK, Pei Y, et al. Association of mutation position in polycystic kidney disease 1 (PKD1) gene and development of a vascular phenotype. Lancet 2003;361:2196-201.
22. Grehnaa V, Pereirab BJ, Retroza E, Coelho H, Godinhoa R, Temidoa P, et al. Mechanisms of male infertility in autosomal dominant polycystic kidney disease. Acta Urol Port 2014;31:41-4.