Identification of Novel Nonsense Mutations in Iranian patients suffering from autosomal dominant polycystic kidney disease

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Research article

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Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the predominant type of inherited kidney disorder, which occurs due to PKD1 and PKD2 gene mutations. ADPKD diagnosis is made primarily by kidney imaging; however, molecular genetic analysis needs to be implicated to confirm the diagnosis. It is critical to perform a molecular genetic analysis when the diagnosis is uncertain, particularly in simplex cases (i.e., a single occurrence in a family), in people with remarkably mild symptoms, or in individuals with atypical presentations. The main aim of this study is to determine the likelihood of PKD1 gene mutations in Iranian patients with ADPKD diagnosis.

Methods: Genomic DNA was isolated from blood samples from 26 ADPKD patients, who were referred to the Qaem Hospital in Mashhad, Iran. By using suitable primers, 16 end exons of PKD1 gene that are regional hotspots, were replicated with PCR. Then, PCR products were subjected to DNA directional Sanger sequencing.

Results: The results of DNA sequencing in the patients showed that exons 35, 36 and 37 were non-polymorphic, while most mutations had occurred in exons 44 and 45. Only in two patients, exon-intron boundary mutation had occurred in intron 44. Most of the variants were missense and non-synonymous types.

Conclusion: In this study, we present nine novel mutations/polymorphisms in PKD1. These data will contribute to an improved diagnostic and genetic counseling in clinical settings. Keywords: Autosomal dominant polycystic kidney disease; PKD1; mutational analysis; Iranian

Background

One of the most prevalent inherited kidney disorders that involves both kidneys is Autosomal dominant polycystic kidney disease (ADPKD). It leads to a progressive loss of kidney function and possible kidney failure (1). About one to two infants in 1000 live at birth are affected by this disorder and approximately 10% of people who undergo dialysis; suffer from this disease (2, 3). ADPKD occurs in two types: type I and type II caused by *PKD1* and *PKD2* mutations, respectively (4, 5).

PKD2 mutations lead to end-stage kidney or renal disease at age 74 and occur in 10-15% of cases; on the other hand, *PKD1* mutations lead in the average to end-stage kidney or renal disease at age 54.3 and occur in 80-90 % of total cases. The latter is the more severe form of the disease (1, 3, 5). Patients having end-stage kidney or renal disease should receive renal replacement therapy (RRT) support including dialysis and renal transplantation. Dialysis may be the only available modality in these patients. After all, dialysis has its limitations, including sometimes lack of vascular access, risks of vascular thrombosis and infections, diminished quality of life, and loss of biosynthetic functions of the kidney (6). ADPKD patients who have diagnosed before age 30 and hypertension and hematuria before age 35 have a worse renal outcome than those who do not have (7). ADPKD diagnosis is done typically by kidney ultrasound imaging, computed tomography scan or magnetic resonance imaging; however, considering the ADPKD

similarity to other cystic kidney disorders, conventional imaging methods do not often lead to a definite and accurate diagnosis (1, 2). In this condition, genetic methods can be effective in an accurate and careful diagnosis. Besides, molecular diagnosis methods have an important role in the confirmation of definite diagnosis, especially in young kidney donors, patients with negative family history, people who presented ADPKD with unusual symptoms in childhood and patients who have relatives suffering from this disorder (8, 9).

ADPKD is the most frequent genetic kidney pathology (frequency of about 0.1%), which results in 5%- 8% of end-stage renal diseases (ESRDs). The ailment is progressive, ending in polycystic enlarged kidneys. This typically results in ESRD in late middle age (5). Polycystin-1, large multidomain protein, is the protein encoded by *PKD1*. It has domains and regions that are homologous with a number of different proteins (10). International Polycystic Kidney Disease Consortium 1995(11,12). Polycystin- 1 may have a function in cell-cell/matrix interactions (13) instead, polycystin-2 (the PKD2 protein) is homologous to an ion-channel subunit (14,15). Most cases of ADPKD leading to ESRD, are caused by *PKD1* mutations (16). Nevertheless, the genetic determination of the locus mutation has advanced slowly, due to the fact that (1) *PKD1* contains a 12,906-bp coding sequence divided into 46 exons (2) and, the 5' region of the gene, from upstream of exon 1 to exon 33, is inserted in a complex genomic area and repeated more than 4 times, on the same chromosome (European Polycystic Kidney Disease Consortium 1994). These *PKD1*-like homologous genes (HG) have revealed a number of specific deletions and a low level of substitutions (about 2%), in comparison with *PKD1* (17). HG loci have made the analysis of *PKD1* highly difficult. Thus, the quantity of identified *PKD1* mutations is still incomplete, with 82 modifications described in the Online Human Gene Mutation Database (HGMD) (18). A number of methods have been used to screen the repeated region (19- 23), however, the 3' area has received insufficient attention, with 57.3% of all mutations found in the single-copy area covering 20% of the coding region. *PKD2* (a less-complex gene) has revealed 41 mutations with potential effects of truncating and possibly inactivating the translated protein (24). A discrete number of missense changes have also been described (19, 23- 26).

Since numerous somatic mutations are needed to explain the formation of multiple cysts and the notion of a significant rate of formation of novel germline mutations (19), it has been proposed that infrequent mechanisms promote a high rate of *PKD1* mutations. First, a long polypyrimidine region in IVS21, which could theoretically form triplex DNA structures (27, 28), has been considered as a possible cause for creating mutations in downstream exons (22). Later on, these multiple substitutions and other modifications were described to match HG sequences, possibly indicating a gene conversion with the remotely located HG loci (21, 29).

PKD1 gene (OMIM 601313) is located in the 16p13.3 chromosome region and consists of 46 exons. Exons 1-33 of *PKD1* replicate around 6 times in homologous genes, HG which has defied *PKD1* genetic analysis. Until January 2015, approximately 2322 *PKD1* sequence variants and 278 *PKD2* sequence variants were reported in ADPKD mutation databases, 1177 PKD1 sequence, and 211 *PKD2* sequences in human gene mutation (16, 17). Although mutation data for *PKD* genes of different populations are available, there are few reports for *PKD* mutations in Iranian population.

The main goal of this study was to establish the probability of mutations in *PKD1* gene in Iranian patients with ADPKD diagnosis obtained by PCR (Polymerase Chain Reaction) and DNA Sanger sequencing (30).

Methods

Patient selection

Twenty-two ADPKD patients were collected from regional city hospital; Ghaem hospital (Mashhad, Iran) between April 2012 to March 2013; they were included at diagnosis and disease characteristics of the ADPKD. The present study was approved by Mashhad University of Medical Sciences ethics committee, and before the blood sample was collected, all patients provided their informed consent.

We excluded clinically patients suffering from Von Hippel-Lindau disease and Tuberous Sclerosis. In addition, patients without symptoms of polycystic kidney disease or those who had other syndromes were also excluded in this study.

Amplification assay

Genomic DNA was isolated from 22 whole-blood samples using the standard salting-out method according to the manufacturer's instructions and it was quantified by NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA). Eight-specific primers within the area of the exon 31-46 were designed (Table1). using the Primer 3 software. and all sequences were checked for self- or inter-molecular annealing with nucleic-acid-folding software (OligoAnalyzer 3.1). We performed local-alignment analyses with the BLAST program to confirm the specificity of the designed primers (http://www-ncbi-nlm-nih-gov.acces.bibl.ulaval.ca/tools/primer-blast).Bidirectional sequence analysis was conducted for all PCR amplicons.

Isolated DNA was stored at -20 ◦C. For polymerase chain reaction (PCR), 150 ng of genomic DNA was amplified in a reaction tube containing 5 pmol of each primer, 1X PCR buffer, 2 mM MgCl2, 1 Unit Taq DNA polymerase (Genet Bio; South Korea), 0.2 mM dNTP mix, and 4 pmol of each primer. Cycling parameters were as follows: an initial denaturation at 95°C for 5 minutes, 35 cycles at 95°C for 30 s, annealing for 30 s at 52°C, 57°C, 69°C, 67°C, 54°C, 61°C and 62°C for primer#1 to#8 respectively, and a final extension step at 72°C for 35 s, ended by a last extension at 72°C for 5 minutes.

PCR products were analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide for identifying the PKD-specific products for use as a template in sequencing reactions.

Sanger sequencing

Sequencing products were run on an ABI 3130XL Genetic Analyzer (Macrogene Company South Korea), according to the manufacturer's guidelines.

Data analysis was performed with Chromas software version 2.6.5 (Technelysium, South Brisbane, Australia).

Results

Twenty-two patients with an average age of 36.69 ±7.30 years, who suffered from ADPKD, were studied. The sequencing results of the patients were reported in Table 1 and Figure 1. In patient 45.1, variations in rs10960 polymorphism in exon 44 led to the conversion of amino acid isoleucine to valine. This type of variation was considered "missense" that is recorded in the PKDB database as minor allele frequency (MAF:0.239). Moreover, a single nucleotide variant, which has also been reported in the PKDB database as almost neutral, was found as rs10960 in exon 45 of Patient 410.2. The intronic mutation was observed in intron 44 of patients 45.2 and 412.1. In four patients, including 45.3, 410.1, 417.1, and 419.1, exon 45 had a synonymous mutation (Ala4092Ala) and was reported as rs3087632 with MAF: 0.262 in the database PKDB.

Novel variants

The first variant was observed in exon 44 of Patient 45.5. This variant led to a synonymous variant in exon 44 (Gly4068Gly). rs200796474 variation was synonymous in Patient 47.1, by which serine amino acid is converted into serine (TCC / TCT). Missense mutation of rs142768096, by which glycine amino acid to valine was observed in exon 44 of Patient 48.1 (GCT / GTC). In Patient 49.4, the missense mutation converting serine to alanin (GCT/TCT) was found in exon 45. Patient 49.2 also had the missense change converting arginine amino acid to glycine in exon 45(GGG/AGG). In Patient 411.2, the missense converting arginine amino acid to leucine was observed in exon 45 and the missense variation converting arginine amino acid to glutamine was found in the same exon of the same patient (CGT/CTT). The missense change converting valine to isoleucine occurred in exon 44 of Patient 418.2 (GTG/ATG). Moreover, the nonsense mutation converting glutamine amino acid to arginine had occurred in exon 44 of the Patient 421 and was recorded as definitely pathogenic in the PKDB database. In addition, the missense variation converting threonine amino acid to alanine was found in some part of the exon 45 in the Patient 422 (ACC/GCC).

In silico functional analysis

The detected sequence variations found in our study were checked with the currently published list of *PKD* gene variants in the Human Gene Mutation Database (HGMD) (31) and Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB).

The effects of novel variations were analyzed by a web-based computational pathogenicity prediction tool, which included Mutation Taster (32) and expasy translate tools. By using expasy tool, we checked related protein products for sequence and length alteration by altered CDS (NM_001009944). For easy

communication of information about mutations, all of them were named based on CDS according to standard mutation nomenclature for molecular diagnostic aims.

The following databases have been used to retrieve polymorphism data: The UniProt database (http://www.uniprot.org) (UniProtKB ID Q8IYM9), the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/SNP/), Catalogue of Somatic Mutations in Cancer (COSMIC http://cancer.sanger.ac.uk/cosmic) and 1000 Genomes (http://www.1000genomes.org/).Variants presented in dbSNP, 1000 Genomes or COSMIC were marked as “non-novel variants”. Functional effects of nsSNPs were predicted using Polyphen-2 (http:// genetics.bwh.harvard.edu/pp2).

Discussion

To date, 2,322 pathogenic mutations for PKD1 and 278 for PKD2 have been reported in the PKDB (32) although the relative frequency of such mutations at each gene locus is unknown. In addition to these two related genes for ADPKD, Daoust et al. in the French-Canadian population, identified a family in which a classical clinical presentation of ADPKD resulted from a mutation at a locus genetically distinct from either of the previously described loci for this disease. This suggests the existence of a third genetic locus for ADPKD (5).

In our study, 16 end exons of *PKD1* gene were studied and results of sequencing in the patients showed that exons 35, 36 and 36 were non-polymorphic, without any mutations and most mutations occurred in the exons 44 and 45. In most of the patients, variants were mostly missense and same-sense types. Our results showed that there is no definite hot spot in *PKD1* and therefore, complete *PKD1* mutation analysis is needed for ADPKD Iranian patients who need a genetic diagnosis. Our new detected mutations in the Iranian population have made *PKD* mutation database richer, having high importance in the genetic consultation of ADPKD patients.

Regarding large genes involved in the ADPKD disease, screening all gene regions is expensive and time-consuming; hence, to overcome this issue a database could be generated for mutations of polycystic kidney disease among the Iranian population to determine the common mutations and characterize the mutation hotspots in Iranian population. Also, considering the clinical similarity of ADPKD with other kidney cystic diseases causing faulty clinical diagnosis in the absence of familial history, molecular study for *PKD1* and *PKD2* genes in suspected patients is recommended.

Identified pathogenic mutations in this study could be confirmed in future studies with more ADPKD families. Besides, genotype-phenotype correlation studies could be performed to determine the severity of each variant and the outcome of patients associated with a specific variant.

Conclusion

In this study, we present nine novel mutations/polymorphisms in *PKD1*. These data will contribute to an improved diagnostic and genetic counseling in clinical settings.

Abbreviations

Autosomal dominant polycystic kidney disease (ADPKD)

End-stage renal diseases (ESRDs)

Polymerase chain reaction (PCR)

Polycystic Kidney Disease Mutation Database (PKDB)

Human Gene Mutation Database (HGMD)

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Mashhad University of Medical Sciences. An informed consent was obtained from each individual participating in this study.

Consent for publication

Our study is not a case report, and identifying images or other personal or clinical detail of participants that compromise anonymity are not included. Consent to publish from the patients, or in case of minors, the patients' guardians is "Not Applicable".

Availability of data and materials

All data are included in this published article. Any additional information related to this study is available from the author for correspondence upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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The funders of this study do not have any roles in study design, data collection, analysis, result interpretation, writing and the decision to submit the manuscript for publication.

Authors' contributions

FK performed the experiments. MAK, AT, participated in the study design and scientific discussion of the data. MAK supervised the study. All authors contribute to the writing, read and approved the final manuscript.

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Tables

Table 1. Covering area of the primers

Primer	Covering exon and intron
Primer 1	Int44-exo45-int45-exo46-int46
PKD1ex45-46	Int41-exo42-int42-exo43-int43
Primer 2	Int37-exo38-int38-exo39-int39
PKD1ex42-43	Int43-exo44-int44-exo45-int45
Primer 3	Int30-exo31-int31-exo32-int32-exo33-int33-exo34-int34
PKD1ex38-39	Int 34-exo35-int35-exo36-int36-exo37-int37
Primer 4	Int39-exo40-int40-exo41-int41
PKD1ex44-45	Int40-exo41-int41-exo42-int42-exo43

Table 2. Results of DNA sequencing of Iranian patients with ADPKD

Patient	Location	Variation	Rs#	Type of variation	PKDB/HGMD database	MAF Minor allele frequency
45.1	Exon44	Ilu4045val	10960	Missense ATC/GTG	Missense ATC/GTG	0.239
45.2	Intron44.45	-	-	-	-	-
45.3	Exon44	Ala4092Ala	3087632	Synonymous GCA/GCG	Likely neutral	0.262
45.4	Exon44	Gly4068Gly	-	Synonymous GGG/GGT	Novel	-
47.1	Exon45	Ser4013ser	2007964474	Synonymous	Novel	NA
48.1	Exon44	Gly4032Val	142768096	Missense GCT/GTC	Novel	NA
49.4	Exon45	Ala4103 ser	-	Missense GCT/TCT	Novel	-
49.2	Exon45	Gly4102Arg	-	Missense GGG/AGG	Novel	-
410.1	Exon45	Ala4092Ala	rs3087632	synonymous	Likely neutral	0.262
410.2	Exon45	ilu4045val	rs10960	Missense ATC/GTC	Likely neutral	0.239
411.2	Exon45	Arg4114leu	-	Missense CGT/CTT	Novel	-
412.1	Intron44.45	-	-	-		-
412.2	Exon45	Arg4091Gln	-	Messence CGG/CAG	Novel	-
417.1	Exon45	Ala4092Ala	rs3087632	Synonymous GCA/GCG	Likely neutral	0.262
418.2	Exon44	Val4035Ile	-	Missense GTG/ATG	Novel	-
419.1	Exon45	Ala4092Ala	rs3087632	synonymous	Likely neutral	0.262
421	Exon44	Gln4005Arg	-	Nonsense CAG/CGG	Definitely Pathogenic	-
422	Exon45	Ther4073Ala	-	Missense ACC/GCC	Novel	-

Figures

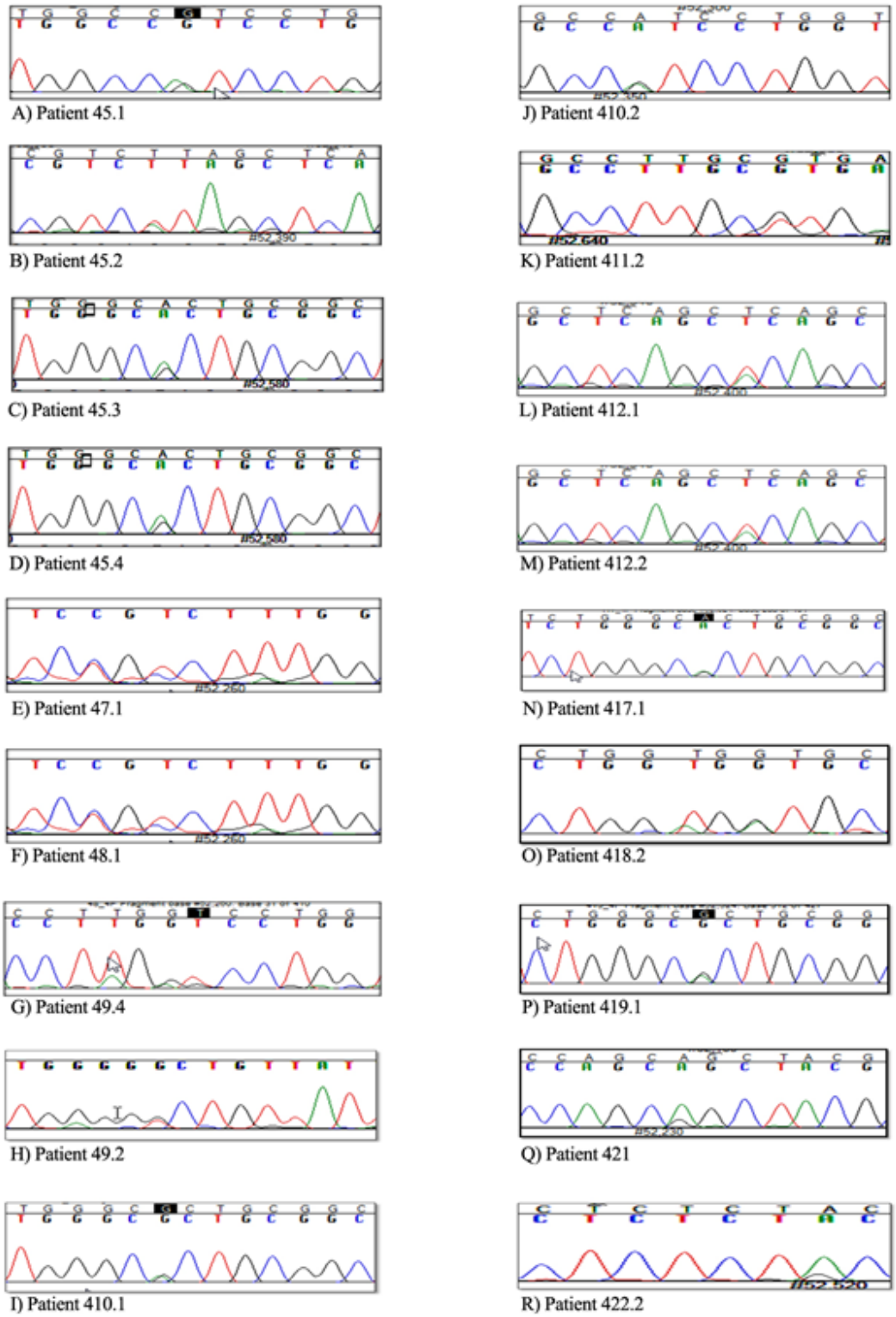

Figure 1

The sequencing results of the patients