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ARTICLE

Identification of *PKD1* and *PKD2* gene variants in a cohort of 125 Asian Indian patients of ADPKD

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) accounts for 2.6% of the patients with chronic kidney disease in India. ADPKD is caused by pathogenic variants in either *PKD1* or *PKD2* gene. There is no comprehensive genetic data from Indian subcontinent. We aimed to identify the pathogenic variants in the heterogeneous Indian population. *PKD1* and *PKD2* variants were identified by direct gene sequencing and/or multiplex ligation-dependent probe amplification (MLPA) in 125 unrelated patients of ADPKD. The pathogenic potential of the variants was evaluated computationally and were classified according to ACMG guidelines. Overall 300 variants were observed in *PKD1* and *PKD2* genes, of which 141 (47%) have been reported previously as benign. The remaining 159 variants were categorized into different classes based on their pathogenicity. Pathogenic variants were observed in 105 (84%) of 125 patients, of which 99 (94.3%) were linked to *PKD1* gene and 6 (6.1%) to *PKD2* gene. Of 159 variants, 97 were novel variants, of which 43 (44.33%) were pathogenic, and 10 (10.31%) were of uncertain significance. Our data demonstrate the diverse genotypic makeup of single gene disorders in India as compared to the West. These data would be valuable in counseling and further identification of probable donors among the relatives of patients with ADPKD.

Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by progressive enlargement of kidneys due to development of multiple fluid-filled cysts. Extra-renal manifestations include cysts in the liver, pancreas, spleen, and seminal vesicles [1]. About 10% of the patients present with intracranial aneurysms and 8% have sub-arachnoid cysts. Renal function declines in about 50% of the patients by third or fourth decade of life leading to end stage renal disease (ESRD). The prevalence of ADPKD varies from 1/500 to 1/1000 world-wide [2]. As per CKD registry of India, about 2.6% of patients with chronic kidney disease (CKD) have ADPKD [3], while in the West, ~10%

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of ESRD patients on dialysis have ADPKD [4]. Renal replacement therapies (RRT) that include dialysis or renal transplant are the only available treatments for these patients at present.

ADPKD is a monogenic disorder caused by pathogenic variant(s) in one of the two genes: *PKD1* and *PKD2*. The *PKD1* gene (*601313) is located on chromosome 16p13.3 and consists of 46 exons with an open reading frame of ~13 kb. Exons 1–33 are duplicated at least six times and located approximately 13–16 Mb proximal to *PKD1*gene on the same chromosome [5, 6]. These duplicated regions, also known as pseudogenes (PKD1P1-P6), have 98–99% homology to the actual *PKD1* gene, thus complicating the molecular analysis. The *PKD2* gene (*173910) is a relatively small gene, consisting of 15 exons with an open reading frame of ~3 kb and is localized on chromosome 4q21-22 [7]. The *PKD1* gene encodes a large, transmembrane protein called polycystin-1 (4303 amino acids), and *PKD2* gene encodes an integral membrane protein polycystin-2 (968 amino acids). The protein products of both the genes act as transient receptor potential (TRP) ion channels involved in the regulation of intracellular Ca^{2+} concentration. They interact with each other through their carboxyl terminals in renal primary cilia, forming a complex that functions as a flow-dependent mechano-sensor with a crucial role in adhesion, proliferation and differentiation of tubular epithelial cells [8].

Both *PKD1* and *PKD2* genes are highly polymorphic, and sequence variants are distributed all over the gene. The majority of disease causing variants are private to a particular family, while recurrent pathogenic variants account for 10–30% of the total [9–12]. Currently, 2323 sequence variants of the *PKD1* gene and 278 sequence variants of the *PKD2* gene have been reported in the ADPKD Mutation Database (PKDB) (http://pkdb.mayo.edu; last accessed July 2018). In general, about 85% of the patients with ADPKD have pathogenic variants in *PKD1* gene, while the rest (15%) have pathogenic variants in *PKD2* gene [12]. However, in some populations 23–25% of ADPKD patients carry pathogenic variants in *PKD2* gene [13]. About 10% of patients have *de novo* pathogenic variants [2]. Patients with pathogenic variants in *PKD1* gene have an early onset of disease with more severe phenotype and reach ESRD at a younger age, as compared to patients with pathogenic variants in *PKD2* gene (54.2 years vs 72.7 years) [14, 15].

Clinical diagnosis of ADPKD is mainly based on ultrasound findings, computed tomography or magnetic resonance imaging, but definite exclusion of the disease cannot be made in at-risk individuals until the age of 30 years, and in those linked to *PKD2* gene [16]. Molecular studies play an important role in confirming the diagnosis, especially in young kidney donors from the family, and patients with early onset or atypical presentation of the disease and in cases with no family history [8]. These are also useful for genetic counseling and for choosing reproductive options.

As there are no comprehensive reports of variants in Asian Indian subjects with ADPKD, this study was carried out to understand the pattern of pathogenic variants, and the proportion that were novel. We also aimed at identifying the recurrent variants, which would simplify molecular diagnosis in a resource poor country. We also performed genotype phenotype correlation.

Materials and methods

Study subjects

This study was approved by the Institutional Ethics Committee of Sir Ganga Ram Hospital [EC/10/10/199(A)], and written informed consent was obtained from each participant. From January 2011 to December 2015, we enrolled 125 unrelated ADPKD families through the Nephrology Department and Clinical Genetics Department of Sir Ganga Ram Hospital, New Delhi and patients referred by nephrologists from other hospitals. All probands in the study met Ravine's ultrasonographic diagnostic criteria for ADPKD [16]. Blood samples were collected from the probands and available family members, wherever possible. Majority of these patients (76%) were from North India, followed by 12% from East, 7.2% from South and 4.8% from West India.

ADPKD patients in the study were grouped into five stages of chronic kidney disease (CKD 1-5), based on the estimated glomerular filtration rate (eGFR), estimated using Modification of Diet in Renal Disease (MDRD) equation from serum creatinine values (http://www.nephromatic.com/egfr.php) [17]. The patients were re-grouped into two sub-stages: advanced stage (CKD 4 & 5) and early stage (CKD 1–3). The advanced CKD group comprised of 70 patients, while 55 patients were in early CKD stage. Overall 52 (of 125) ADPKD patients either underwent, or were in need of RRT (eGFR < 15 ml/min/1.73 m^2).

PKD1 and PKD2 gene sequencing and MLPA analysis

Genomic DNA was extracted from venous blood, using a modified salting out method [18]. The duplicated region of *PKD1* gene was amplified by Long Range-PCR (LR-PCR), using previously published gene-specific primers [19, 20]. Exon 1 was amplified using KAPA2G™ Robust PCR kit, and exons 2–33 using NEB LongAmp® Taq PCR kit. The PCR products from these LR-PCR reactions were diluted 1000 times, and used as templates for exon specific amplification by nested-PCR for exons 1–33. The exons 34–46 in the 3'unique region were directly amplified

and Sanger sequenced. All the 15 coding exons of *PKD2* gene were directly amplified and Sanger sequenced in 17 overlapping fragments as previously described [7]. The primers covered the entire coding regions of both the genes and about 50 bp flanking intronic regions. All the fragments for both the genes *PKD1* (NM_001009944.2) and *PKD2* (NM_000297.3) were sequenced on ABI 3500 DNA Analyzer (Applied Biosystems, Foster City, CA). Initially, the entire *PKD1* gene was sequenced and analyzed in each patient and if no pathogenic variants were identified, the entire *PKD2* gene was sequenced. Sequence chromatograms were analyzed using Applied Biosystems SeqScape® software and compared with the reference sequence using Genome Browsers UCSC and Ensembl. MLPA analysis was performed using SALSA MLPA kit P351-B1/P352-B1 PKD1-PKD2 kit (MRC-Holland, Amsterdam, Netherlands), according to the manufacturer's instructions. The reaction products were separated by capillary electrophoresis on an ABI 3500 DNA analyzer (Applied Biosystems, Foster City, CA) using GeneScan™ 600LIZ® size standard (Applied Biosystems) and the data were analyzed using Coffalyser.net (MRC-Holland).

Variant nomenclature and classification

All variants were annotated using the Alamut database (Alamut® Visual version 2.9.0; http://www.interactive-biosoftware.com) for prediction of pathogenicity, and cross-species conservation of nucleotides and amino acid sequences. Variants were named according to the Human Genome Variation Society guidelines [21] (http://varnomen.hgvs.org/) and reference sequence. For minor allele frequency (MAF) of the reported variants the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk), the Exome Sequencing Project (http://evs.gs.washington.edu/EVS), The Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/), 1000 genomes (http://phase3browser.1000genomes.org/ index.html), SNP database (https://www.ncbi.nlm.nih.gov/snp/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and PKDB (http://pkdb.mayo.edu) were accessed. Variants were classified into five categories based on American College of Medical Genetics (ACMG) guidelines: pathogenic, likely pathogenic, uncertain significance, likely benign and benign [22]. Novel sequence variants reported in this study were submitted to the ADPKD mutation database (http://pkdb.mayo.edu).

Statistical analysis

Comparisons between different groups were performed with SPSS software version 21 (IBM Corp) using the Chi-square test or the Fisher's exact test. P-values less than 0.05 were considered significant. Disease progression (time from birth to ESRD) was analyzed using the Kaplan–Meier method. Differences between patients with truncating and non-truncating variants were assessed using a log rank test.

Results

Diagnosis of ADPKD patients

Most of the ADPKD individuals in this cohort sought medical attention for abdominal discomfort, hematuria, and frequent urinary tract infections, cystic kidneys on imaging studies and/or incidental finding of hypertension. Very few individuals were screened before the presentation of symptoms as they had positive family history. The diagnosis of ADPKD was made incidentally in 44 (35.2%) patients during examination for other indications, 42 (33.6%) were evaluated because of abdominal pain, 12 (9.6%) were screened for renal discomfort, 12 (9.6%) for hypertension and only 13 (10.4%) were diagnosed on the basis of family history. Two patients (1.6%) had minor brain strokes that led to the diagnosis of ADPKD.

Clinical features of ADPKD patients

All the enrolled patients ($n = 125$) had multiple bilateral renal cysts, while 23 (18.4%) patients also presented with bilateral renal calculi and 48 (38.4%) patients had hepatic cysts as a major extra renal manifestation. Seventy (56%) patients had reached advanced CKD stage and 52 of these were undergoing RRT. The clinical characteristics of the patients are summarized in Table 1.

In the present study, ADPKD was more prevalent in males (71, 56.8%) than females (54, 43.2%) ($p = 0.032$). Female patients of ADPKD were diagnosed at a significantly younger age, and presented with significantly higher eGFR values compared to male patients ($p = 0.008$), hence showing a better disease prognosis. A significantly higher proportion of males (50.7%), had reached ESRD compared to 29.6% of the females. A comparative description is provided in Table 2.

Out of 125 probands enrolled, 77 had a positive family history (Group A) before/or at time of diagnosis and the remaining 48 had no known history of the disease in the family (Group B). The probands in group A presented clinical manifestations at a significantly younger age and also had a non-significant 3 years lower age at reach to ESRD, when compared to probands in group B. The probands in group B also showed a significantly higher eGFR values, suggesting a better renal survival ($p = 0.011$). Detailed clinical characteristics of patients with and without family history are shown in Table 3.

Table 1 Clinical characteristics of patients with ADPKD

Characteristic	
ADPKD probands	125
Gender: M/F	71/54
Age at the time of enrollment, years, (Mean ± SD), (Range)	41.7 ± 13.3, (15–85)
Age at the time of diagnosis, years (Mean ± SD), (Range)	35.09 ± 13.3, (10–80)
Family history of disease	77 (61.6%)
Family history diabetes mellitus	4 (3.2%)
Family history of deaths due to brain haemorrhage	2 (1.6%)
Hypertension	88 (70.4%)
Advanced CKD stage (CKD 4 and 5)	70 (56%)
Early CKD stage (CKD 1, 2 and 3)	55 (44%)
% ESRD (RRT and/or eGFR < 15 mL/min)	52 (41.6%)
Liver cysts	48 (38.4%)
Pancreatic cysts	2 (1.6%)
Ovarian cysts ($n = 54$)	1 (1.9%)
Seminal vesicle cysts with male infertility ($n = 71$)	4 (5.6%)
Intracranial aneurysms	2 (1.6%)
Renal calculi	23 (18.4%)
eGFR values (mL/min/1.73 m^2), Mean ± SD	50.6 ± 51.5

*M/F male/female, *ESRD* end stage renal disease, *RRT* renal replacement therapy-renal transplant, dialysis, *eGFR* estimated glomerular filtration rate [based on Modification of Diet in Renal Disease (MDRD)]

Table 2 Comparison between male and female ADPKD patients

Variant	Male ADPKD patients	Female ADPKD patients	*p*-value
Total	71 (56.8%)	54 (43.2%)	**0.032**
Age at diagnosis (Mean ± SD)	37.28 ± 14.9	32.13 ± 10.1	**0.031**
Hypertension	53 (74.65%)	35 (64.81%)	0.234
Liver cysts	29 (40.85%)	19 (35.19%)	0.521
Renal Calculi	8 (11.27%)	14 (25.93%)	**0.034**
Mean eGFR (Mean ± SD)	34.49 ± 44.4	58.63 ± 56.1	**0.008**
ESRD	36 (50.7%)	16 (29.63%)	**0.018**
Age at reach to ESRD	42.73 ± 13.34	44.93 ± 10.82	0.589

Values are expressed as Mean ± SD

ESRD end stage renal disease

Bold values signify statistically significant ($p < 0.05$)

Sequencing of PKD1 and PKD2 genes

PKD1 gene was sequenced in 123 ADPKD patients and 283 different variants were observed. Complete sequencing of *PKD2* gene was carried out in 97 patients and 17 different variants were observed. No deletion/duplications were detected using MLPA in *PKD1* and *PKD2* genes.

Variants in PKD1 and PKD2 genes

A total of 300 different variants (283 in *PKD1* and 17 in *PKD2*) were identified. Most of these (106, 35.33%) were missense variants. Of the variants detected, 141 (47%) were reported in various publicly available databases as benign variants, and/or were identified in more than three unrelated patients and unaffected family members (Supplementary table 1). The remaining 159 variants were classified as per ACMG guidelines (Table 4).

Pathogenic variants

Null variants (nonsense, frameshift, canonical ± 1 or 2 splice sites) were classified as pathogenic. Sixty-eight pathogenic variants were identified in 76 families; that included 38 frameshift variants, 21 nonsense variants, 1 indel substitution, 1 in frame deletion and 7 canonical splice-site variants (Supplementary Table 2).

Likely pathogenic variants

Variants that were previously reported as pathogenic and/or were reported with a very low frequency in various population databases or were reported for the first time and had multiple lines of computational evidence supporting pathogenicity were categorized as likely pathogenic variants. Twenty-eight likely pathogenic variants were identified in 29 ADPKD families consisting of 21 missense variants, 5 in frame deletions of <5 amino acid residues and 2 atypical splice site variants. A homozygous novel variant in *PKD2* exon 5: p.Ala421Thr was observed in one proband (F82) with no other probable pathogenic variants in *PKD1* or *PKD2* genes. Blood samples of the parents/other family members were not available to do segregation analysis (Supplementary Table 2).

A schematic representation of *PKD1* and *PKD2* genes and distribution of pathogenic and likely pathogenic variants is shown in figure (Figs. 1a, b). Variants were present throughout the gene, with 22% of variants identified in exon 15 of *PKD1* gene (large exon; >3000 bp). These variants were spread across the entire exon 15 and hence excluded the possibility of any hotspots.

It was noticed that the proportion of disease causing variants identified was significantly higher in ADPKD probands with a positive family history (Group A; $n = 77$), compared to those without a family history (Group B; $n = 48$) (91 vs. 73%; $\chi^2 p$-value = 0.033).

Table 3 Comparison between familial and sporadic cases of ADPKD

Variant	Familial ADPKD (Group A; $n = 77$)	Sporadic ADPKD (Group B; $n = 48$)	p-value
Gender (M/F)	42/35	29/19	0.578
Liver cysts	29 (37.66%)	19 (39.58%)	0.831
No extrarenal manifestations	35 (45.45%)	16 (33.33%)	0.182
Mean eGFR	41.55 ± 45.1	65.6 ± 57.9	**0.011**
Hypertensive	54 (70.13%)	34 (70.83%)	0.934
Advanced CKD	51 (66.23%)	19 (39.58%)	**0.004**
ESRD	41 (53.23%)	11 (22.92%)	**0.0009**
Age at diagnosis (years)	32 ± 10.9	39.8 ± 15.3	**0.0012**
Age at enrollment (years)	40.19 ± 11.96	45.04 ± 14.49	**0.044**
Age at reach to ESRD (years)	43.68 ± 12.35	46.18 ± 16.83	0.584
PKD1—Pathogenic variants	67 (87.01%)	33 (68.75%)	**0.0134**
PKD2—Pathogenic variants	2 (2.60%)	4 (8.33%)	0.147
Truncating Variants	56 (72.73%)	20 (41.67%)	**0.003**
Non truncating Variants	13 (16.88%)	17 (35.42%)	**0.003**
No pathogenic variant	9 (11.69%)	11 (22.92%)	0.06

Values are expressed as Mean ± SD

M male, *F* female, *CKD* chronic kidney disease, *eGFR* estimated glomerular filteration rate, *ESRD* end stage renal disease

Bold values signify statistically significant ($p < 0.05$)

Table 4 Variants in *PKD1* and *PKD2* genes classified as per ACMG guidelines

Type of variants	*PKD1*	*PKD2*
Pathogenic ($n = 68$)		
Deletions	25	2
Duplications	8	0
Deletion/Insertion	3	0
Inframe deletions (≥5 amino acids)	1	0
Insertions	1	0
Nonsense	19	2
Typical splicing (±1 or 2 nucleotides)	6	1
Likely pathogenic ($n = 28$)		
Missense	20	1
Inframe deletions (<5 amino acids)	5	0
Atypical splicing (±3–20 nucleotides)	2	0
Variants of unknown significance (VUS)($n = 20$)		
Missense	18	0
Atypical splicing	1	0
5′ UTR	0	1
Likely Benign ($n = 30$)		
Missense	22	1
Intronic	3	2
Untranslated region (3′ UTR and 5′ UTR)	0	2′
Benign ($n = 13$)		
Synonymous	8	0
Intronic	5	0
Total variations ($n = 159$)	147	12

Variants of uncertain significance (VUS)

Variants with moderate evidence of pathogenicity were categorized as VUS. These variants were either not reported or reported with a very low minor allele frequency (MAF < 0.1%) in the population databases. Multi-alignment based conservation of wild type residues in this category varied from moderately conserved to well conserved in the *PKD1* orthologs (human, monkey, dog, rat, mouse, opossum, chicken, *Xenopus tropicalis* and zebrafish). Twenty different variants were identified, 19 in *PKD1*gene and one in 5′ untranslated region (5′ UTR) of *PKD2* gene (Supplementary Table 3). These variants might have a direct role in disease pathogenesis in the probands where no other variant was identified, or may modify the severity of disease in families where a pathogenic variant was present.

Likely benign and benign variants

Missense variants that scored very low for pathogenicity and/or had a low MAF (<1%) in population databases were categorized as likely benign. Twenty three missense variants and seven intronic variants were classified as likely benign. The synonymous and intronic variants with no predicted effect on splicing were categorized as benign variants. Thirteen different variants in *PKD1* gene were grouped under this category. These included 8 novel synonymous variants and 5 intronic variants (Supplementary Table 3).

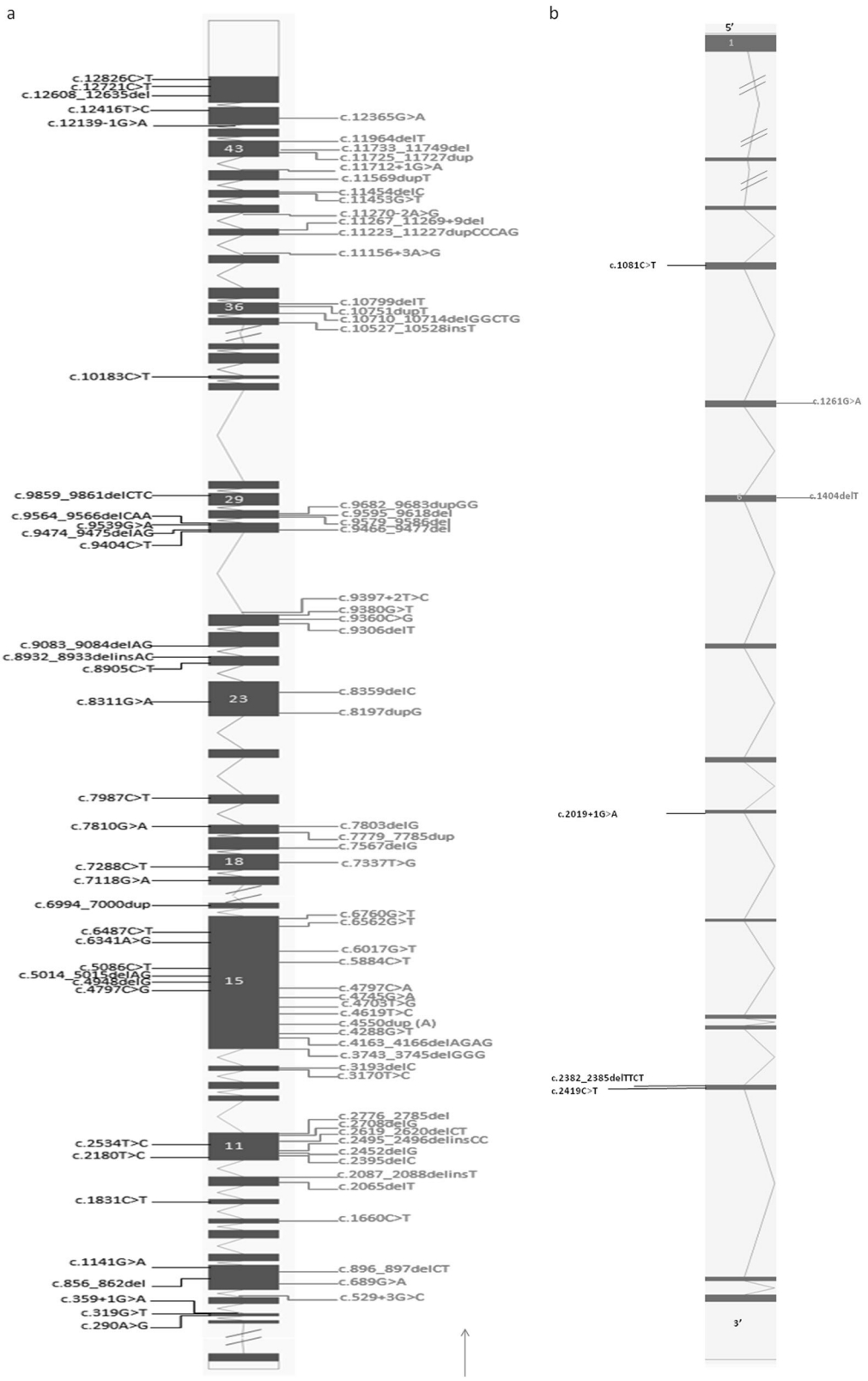

Fig. 1 Schematic representation of pathogenic and likely pathogenic variants in (**a**) *PKD1* gene and (**b**) *PKD2* gene (variants in red ink are novel variants)

Table 5 Pathogenic variants present in more than 1 unrelated family

Nucleotide change	Amino acid change	Exon	Gene	ADPKD family
c.5014_5015delAG	p.(Arg1672Glyfs*98)	15	*PKD1*	F71, F96
c.6994_7000dupGCTGGCG	p.(Val2334Glyfs*88)	16	*PKD1*	F101, F133
c.7288 C > T	p.(Arg2430*)	18	*PKD1*	F27, F29, F62, F66
c.11569dupT	p.(Tyr3857Leufs*104)	42	*PKD1*	F37, F49
c.11712 + 1 G > A		IVS42	*PKD1*	F51, F87
c.12608_12635del28	p.(Arg4203Profs*92)	46	*PKD1*	F25, F88
c.2534 T > C	p.(Leu845Ser)	11	*PKD1*	F34, F63#, F130

Nonsense variant in *PKD1* exon 8

Table 6 De novo pathogenic variants identified in the study

Family ID	Exon/ Intron	Nucleotide change	Amino acid change
PKD1			
F19	11	c.2395delC	p.(Arg799Glyfs*99)
F48	21	c.7987 C > T	p.(Gln2663*)
F110	24	c.8905 C > T	p.(Gln2969*)
F105	36	c.10751dupT	p.(Ser3585Glufs*42)
F80	3	c.290 A > G	p.(Asp97Gly)
F131	29	c.9859_9861delCTC	p.(Leu3287del)
PKD2			
F39	IVS 9	c.2019 + 1 G > A	p.(Asn674fs)

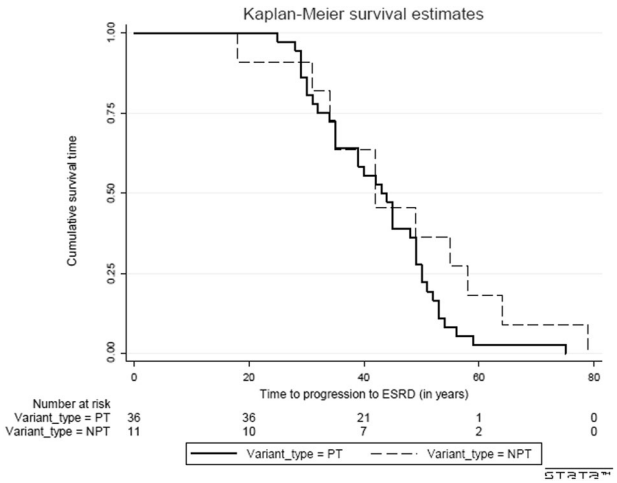

Fig. 2 Kaplan–Meier survival curves showing the time to progression to ESRD for patients with truncating variants (PT) ($n = 36$) and those with non-truncating variants (NPT) ($n = 11$) (log-rank test, p-value = 0.182)

Recurrent variants

Out of 96 pathogenic variants identified in *PKD1* and *PKD2*, six variants (6.3%) were present in more than one unrelated ADPKD family (Table 5). None of these variants were specific to any region or ethnic group, except one in exon 42 of *PKD1*: c.11569_11570insT; p.Tyr3857-Leufs*104, that was present in two unrelated patients belonging to a small endogamous Hindu community from Kashmir. The latter may represent the presence of a common ancestral allele, but more samples need to be studied to confirm this conclusion.

De novo variants

Out of 125 probands, 48 (38.4%) had no history of disease in the family (Group B). Pathogenic variants were identified in 37 (77.1%) subjects in this group; 33 in *PKD1* gene, 4 in *PKD2* gene. In seven probands the variants occurred de novo, as these were not detected in either of the parents (Table 6).

Novel and reported variants

Of 159 variants identified and classified according to ACMG guidelines, 97 (61%) were novel and 62 were reported earlier. Of the novel variants, 43 (44.33%) were classified as pathogenic, 14 (14.43%) as likely pathogenic and 10 (10.31%) as of uncertain significance. In addition to these, 30 (30.93%) likely benign & benign variants were reported for the first time in this study.

Previously reported 62 variants identified in this study comprised of 25 (40.32%) pathogenic, 14 (22.58%) likely pathogenic, 10 (16.13%) VUS and 13 (20.97%) likely benign and benign variants.

Analysis of factors for progression of disease

In the present cohort, patients with truncating variants ($n = 71$) in *PKD1* gene, manifested clinical symptoms at a significantly younger age, as compared to those with non-truncating variants ($n = 28$) (31.4 ± 9.4 years vs 38.55 ± 16.1 years; p-value = 0.006).

Out of 125 probands, 52 had already reached ESRD. No variants were detected in five probands. Kaplan–Meier survival curve analysis (Fig. 2) to investigate whether progression of the disease (age at reach to ESRD) varied in patients with truncating ($n = 36$) and non-truncating

variants ($n = 11$), did not show any significant difference between these two groups (log-rank test, $p = 0.182$).

Cox regression analysis was used to assess the effect of other factors (independent of variants) in ADPKD patients who had reached ESRD ($n = 52$), and those who had not ($n = 73$). It was observed that a positive family history (OR, 4.2; 95%CI, 0.118–0.480; $p < 0.001$) was associated with ~4 folds increased risk of progression of the disease, whereas, hypertension (OR, 2.2; 95%CI, 0.214–1.010; $p = 0.050$) were slightly associated with increased risk of progression of disease.

Discussion

The precise prevalence of ADPKD in India is not known. Vikrant et al. [23] recently evaluated renal and extrarenal manifestations of ADPKD patients from India. They observed a higher prevalence of ADPKD in males (60.6%; 126/208 total patients). Hypertension was present in 145 (69.7%) of the patients. Similar findings were observed in the present study with 56.8% of males affected and 70.4% of the patients being hypertensive. In contrast, ADPKD has been reported to be more prevalent in females in European [24] and Brazilian cohorts [25] (51.4 and 63%, respectively).

Hypertension was the most common clinical finding in the present ADPKD cohort, followed by elevated serum creatinine levels (57.6%), pain in abdomen (33.6%) and presence of renal calculi (18.4%). These observations were similar to those of Vikrant et al. [23] (54.6% with elevated serum creatinine and 46.2% presenting with pain in abdomen). However, a higher proportion of incidence of renal calculi (38.9%) was noticed in their study.

Liver cysts, the most common extrarenal manifestation, are reported to be present in more than 80% of patients with ADPKD [26, 27]. The studies from the West have documented liver cysts in 39.1% of patients in Brazilian cohort [25], 55.8% in European cohort [24] and 59.9% in a cohort from Spain [28]. These studies observed a significantly higher prevalence of liver cysts in females compared to males. In contrast to this, the studies reported from China [29], Pakistan [30] and India [23] did not observe any significant difference with respect to liver cysts in male and female ADPKD patients. The proportion of liver cysts reported were 72.1, 21.4, and 37% in Chinese [29], Pakistanis [30] and Indians [23], respectively. The present study also reports 38.4% of patients with liver cysts, with males and females equally affected.

The comprehensive molecular analysis of *PKD1* and *PKD2* genes has been extensively carried out in ADPKD patients from the Western population. A significant number of studies have also been reported from Asians. The genetic heterogeneity associated with *PKD1* and *PKD2* genes is highlighted by reports of novel pathogenic and benign variants in different patient cohorts.

In the present study pathogenic variants were detected in 105 (84%) of 125 families, which is comparable to 85.8% ($n = 127/148$) reported in the Chinese [31] and 83.9% reported ($n = 135/161$) in the Japanese [13]. Studies with large patient cohorts ($n > 100$) from the Western populations have reported pathogenic variants in the range of 62.8 to 89.9%; with 62.8 % ($n = 115/183$) in North Americans [32], 64.6% ($n = 179/277$) in south-western Germans (EKFS-ADPKD-Registry) [33], 80% ($n = 352/440$) in Italian cohort [10], 84.5% ($n = 186/220$) in Canadians (TGESP, Canada) [11], 89.1% ($n = 180/202$) in North Americans (CRISP, USA) [12] and 89.9% ($n = 629/700$) in the French (GENKYST, France) [9].

In 99 (94.3 %) families pathogenic variants were detected in *PKD1* gene, and remaining 6 (5.7%), in the *PKD2*. A recent study from India reports 27.38% ($n = 23/84$) of ADPKD patients with pathogenic variants in *PKD2* gene [34]. The proportion of ADPKD patients with variants in *PKD2* is less in this study, when compared with those reported in Caucasians, that range from 10–17%, and among Japanese (28.1%). This may be the result of having patients with more severe clinical manifestations enrolled in the present study. *PKD2* linked families have milder disease and are less likely to seek medical attention in resource poor countries like India.

Majority of the variants in the present study were confined to single families. The most frequent variant was a CpG variant, c.7288 C > T; p.Arg2430Ter in *PKD1* exon 18; present in 4 unrelated families. The variant, c.5014_5015delAG; p.Arg1672Glyfs*98 in exon 15 of *PKD1*, reported to be the most frequent pathogenic variant in Caucasians [12, 35] was present in only 2 unrelated families. This is consistent with reports in other Asian populations [13, 31].

About 10% of the ADPKD patients did not have a positive family history [36]. The high level of heterogeneity in *PKD1* and *PKD2* genes and the prevalence of private pathogenic variants in ADPKD families increase the possibility of de novo variants [9, 37, 38]. It has been suggested that *PKD1* has a higher rate of de novo germline mutation compared to *PKD2* gene [39]. In the present study, about 38.4% (48/125) did not have a positive family history. Of these 48 families, de novo variants were confirmed in 7 families, majority of these (6/7) were linked to *PKD1*.

The probands with a positive family history (Group A) had significantly higher proportion of truncating variants (73 vs. 42%; $\chi^2 p$-value = 0.0006), whereas a non-significantly higher incidence of non-truncating variants was observed in the probands with no known history of disease in family (Group B) compared to group A

(31.3 vs. 18.2%; χ^2 p-value = 0.062). Similar findings have been reported earlier in the Genkyst cohort [9].

Genotype–phenotype correlation

Genetic factors are a powerful indicator of prognosis in patients of ADPKD. The disease severity and progression to ESRD varies among families, with different pathogenic variants, as well as among the members of the same family [40]. In this study, the patients with truncating variants in *PKD1* gene had a significantly lower age at manifestation of clinical symptoms, compared to those with non-truncating variants. However using Kaplan-Meier survival curve analysis, no significant difference was observed, with respect to progression to ESRD and the type of variants (Fig. 2). The results are contrary to those previously published [11] and this may be because of the small sample size in the present study.

Co-inheritance of additional likely pathogenic variants, incompletely penetrant alleles or hypomorphic variants, in combination with truncating variants in *PKD1* and *PKD2*, can be associated with early onset (in utero or in infancy) of renal disease [41–43]. We observed two families (F7 and F9) with 'possible' hypomorphic variants resulting in ADPKD phenotype (Supplementary Information, S1). The hypomorphic alleles may cause mild disease when alone, but two such variants in trans can result in severe ADPKD phenotype [42, 44].

In a family (F82), the proband was clinically diagnosed with PKD at the age of 44 years. Proband was hypertensive and was treated for inguinal hernia. *PKD2* gene sequencing revealed a novel homozygous variant in exon 5: c.1261 G > A; p.Ala421Thr. Alanine at 421 is a highly conserved residue and lies at an invariate site 'DRGTR**A**AF' that is conserved in the polycystin cation channel domain of *PKD2* orthologs. Based on the previous reports, describing complex inheritance of ADPKD [41–43], a homozygous variant can lower the minimum threshold level of polycystins and hence result in cystogenesis.

In a family (F63), with in-utero presentation of ADPKD phenotype, the mother (25 years old) had a novel nonsense variant in *PKD1* exon 8: p.Gln554Ter coexisting with another likely pathogenic variant in *PKD1* exon 11 (p. Leu845Ser). Proband's 6 years old daughter, diagnosed prenatally on ultrasound studies, also had inherited both the variants. Blood sample of the spouse was not available but he was reported to have normal renal ultrasound study. The role of both these variants, the truncating variant in exon 8 and non-truncating variant in exon 11 causing early manifestation (in utero) of symptoms could not be confirmed as extended family members were not available for analysis [43].

In another family (F44), the 15 years old proband had bilateral renal cysts; was on dialysis and *PKD1* and *PKD2* sequencing revealed the presence of two *PKD1* variants (Intron4 c.529 + 3 G > C and exon 15 p.Val2267Gly). The affected mother, who had normal renal function, had the intronic variant, but was negative for exon 15 variant. Intron 4 variant was predicted to affect splicing and segregated with the disease. We speculate that the other missense variant (p.Val2267Gly); a highly conserved residue in *PKD1* orthologs, modified the progression of disease in the proband resulting in rapid decline in renal function.

Families with "no" pathogenic variants

Pathogenic variants were not identified in 20 (16%) of 125 probands with ADPKD in the present cohort. Majority of them had no history of renal disease in the family (12 out of 20). The results in the present study are not very different from those of CRISP [12] (10.9%) and Genkyst (10.1%) [9]. Absence of pathogenic variants in these probands may be due to the following possibilities:(i) targeting exon based gene screening may not have identified the deep intronic variants, variants in promoters and untranslated regions. (ii) VUS and suspected hypomorphic alleles that scored low pathogenicity might affect protein structure and stability; altering PC1 production below the required threshold level [45, 46] and might result in mild to severe disease. (iii) only *PKD1* and *PKD2* were screened in these patients of ADPKD. There is a possibility that these families might have variants in other genes (*HNF1β*, *PRKCSH*, *SEC63* or *PKHD1*) reported to be involved in cystic pathogenesis (cystogenesis) [47–49]. Furthermore, low level of mosaicism may influence phenotypic presentation of ADPKD but may have been missed in Sanger sequencing in the present study [50, 51].

In India, in the absence of a well curated patient database, the prevalence and incidence of ADPKD still remain elusive. Moreover there is little data on mutations in P*KD1* and *PKD2* genes in cases of ADPKD in India. This study, reports for the first time, a detailed analysis of novel and reported variants in *PKD1* and *PKD2* genes in patients of ADPKD from India. Characterization of genetic variants would help the proper classification of variants observed in Indian in future studies, help in the choice of family members for renal transplantation, and has major benefits for prenatal or preimplantation genetic diagnosis as well as for presymptomatic diagnosis of ADPKD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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