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RESEARCH ARTICLE

Mutational screening of *PKD2* gene in the north Indian polycystic kidney disease patients revealed 28 genetic variations

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Abstract. Polycystic kidney disease (PKD) is a systemic disorder which adds majority of renal patients to end stage renal disease. Autosomal dominant polycystic kidney disease (ADPKD) is more prevalent and leading cause of dialysis and kidney transplant. Linkage analysis revealed some closely linked loci, two of which are identified as *PKD1*, *PKD2* and an unidentified locus to ADPKD. This study was performed using PCR and automated DNA sequencing in 84 cases and 80 controls to test potential candidature of *PKD2* as underlying cause of PKD by *in silico* and statistical analyses. Two associated symptoms, hypertension (19%) and liver cyst (31%) have major contribution to PKD. Gender-based analysis revealed that familial female patients (27%) and familial male patients (33%) are more hypertensive. Liver cyst, the second major contributing symptom presented by large percentage of sporadic males (46%). Genetic screening of all 15 exons of *PKD2* revealed eight pathogenic (c.854_854delG, c.915C>A, c.973C>T, c.1050_1050delC, c.1604_1604delT, c.1790T>C, c.2182_2183delAG, c.2224C>T) and eight likely pathogenic (g.11732A>G, c.646T>C, c.1354A>G, g.39212G>C, c.1789C>A, c.1849C>A, c.2164G>T, c.2494A>G) DNA sequence variants. In our study, 27.38% (23/84) cases shown pathogenic / likely pathogenic variants in *PKD2* gene. Some regions of *PKD2* prone for genetic variation suggested to be linked with disease pathogenesis. This noticeable hot spot regions hold higher frequency (50%) of pathogenic / likely pathogenic genetic variants constituting single nucleotide variants than large deletion and insertion that actually represents only 41.08% of coding sequence of *PKD2*. Statistically significant association for IVS3-22AA genotype was observed with PKD, while association of IVS4+62C>T was found insignificant.

Keywords. polycystic kidney disease; autosomal dominant polycystic kidney disease; end stage renal disease; PKD2; PC2.

Introduction

Polycystic kidney is the most frequently found renal abnormality associated with several genetic and non-genetic disorders. Among all the types of polycystic kidney diseases (PKD) worldwide, prevalence of autosomal dominant polycystic kidney disease (ADPKD) is 1 : 400–1000 individuals, while autosomal recessive polycystic kidney disease (ARPKD) is 1 : 20,000 (Zerres *et al.* 1984; Torres *et al.* 2007). Among all types of cystic kidney disorders, ADPKD contributes 10% of end stage renal disease (ESRD) in humans. Cardinal symptoms of ADPKD are adult onset of bilateral multiple renal cysts of variable sizes originated from 1% of all nephrons (Fick and Gabow 1994). Dedifferentiation of nephronic epithelial cells to primitive state along with thickening, splitting and disorganization of basement membrane are initial steps of cyst generation. It is the primary cause of destruction of structural integrity of kidneys and hence, creates hindrance in normal functioning of nephron, which ultimately leads to renal failure. Because of the expansion of cyst, affected individuals show enlarged kidneys and increased abdominal mass; patients have complications of flank pain, haematuria, frequent urinary tract infection, cyst infection and cyst haemorrhage (Gardner 1988). Sometimes renal stone, extrarenal cyst such as liver cyst, prostatic cyst, cyst in testis and ovary are also present in ADPKD patients. Noncystic manifestations like cardiovascular defects, intracranial aneurysm and hypertension are also common in these patients (Grantham 2002). Diagnosis can further be confirmed by radiology (ultrasound, CT scan and MRI) and genetic procedures (De Bruyn

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and Gordon 2000). ADPKD shows genetic heterogeneity, because more than one gene is responsible for ADPKD, such as *PKD1* (localized to the 16p13.1) (Reeders *et al.* 1986), *PKD2* (localized to the 4p21) (Jeffery *et al.* 1995) and unidentified locus/loci (Daoust *et al.* 1995). Mutations in *PKD1* gene accounts for 85% ADPKD cases, while remaining 15% occurs due to mutation in *PKD2* gene (Pei 2006). The second gene *PKD2* contains 15 exons and acquires a 68 kb genomic region transcribes 5.3-kb mRNA of 2904-bp long-coding sequence, which encodes 968 amino acid long 110 kDa integral membrane protein polycystin2 (PC2). *PKD2* gene encodes two major segments transmembrane region and intracellular region with both N and C terminals. Exon 1 is 660-bp long and GC rich. Exons 2, 6, 7, 8 and 9 code for transmembrane domains, exons 1 and 2 code for N-terminal region, exons 11, 12, 13, 14 and 15 form C-terminal region and exons 3, 4 and 5 code for extracellular loop (Hayashi *et al.* 1997). PC2 shares structural feature with transient receptor potential (TRP) channel as well as voltage-activated calcium and sodium channels. It can form homomultimer and heteromultimer with the help of its C-terminal tail containing two different sites for PC2 and PC1 protein–protein interaction, respectively (Tsiokas *et al.* 1997). This protein is expressed in all segments of nephron except glomeruli. Subcellular localization of PC2 is confined to Golgi compartment, endoplasmic reticulum and plasma membrane. PC2 expressed in kidney, heart, ovary, testis, vascular smooth muscles and small intestine (Mochizuki *et al.* 1996). Molecular studies performed in animal models and human kidneys reveal that several proteins are involved in cystogenesis and among all these, two cilliary proteins PC1 and PC2 were found prominently (Yoder *et al.* 2002). Genetic screening of two candidate genes was performed in several populations using conventional and high throughput sequencing techniques (Liu *et al.* 2014). A report from Germany published that 30 sporadic cases were reported 24/30 *PKD1* and 6/30 *PKD2* mutations (Neumann *et al.* 2012). Twenty-two genetic variants were reported from screening of *PKD2* gene in 115 Czech PKD patients (Stekrova *et al.* 2003). In 56 Czech PKD patients, two likely pathogenic variants were reported in *PKD2* gene (Obeidova *et al.* 2014). Large number (643) of Italian PKD patients presented 452 novel genetic variants (Carrera *et al.* 2016). Clinical presentation of ADPKD2 (autosomal dominant polycystic kidney disease 2) is milder than ADPKD1 (autosomal dominant polycystic kidney disease 1) affected individuals. Individuals with mutation in both genes have severe clinical presentation. Polycystic kidney disease in severe cases not only affects the overall health of the individual but also leads the patient to regular dialysis and eventually to death due to complete renal failure. The treatment of patient affected by the PKD is complex, expensive and lifelong. The study was planned to test the potential candidature of *PKD2* gene as underlying cause of PKD as well as work towards identification and characterization of novel genetic variants responsible for polycystic kidney resulting chronic renal failure in adult. Identification of the novel and reported DNA sequence variants and SNPs will help in developing molecular genetic tests for diagnosis of different developmental anomalies of cystic kidney prevalent in human, defining genetic predisposition for those diseases, finding genetically compatible kidney donor in future and the risk assessment studies. The spectrum of genetic variants identified in this study includes one/two nucleotide deletions responsible for truncated protein formation and single-nucleotide substitution causing missense and silent variation at protein level. From screening of *PKD2* gene, we can draw the correlation between the genotype and clinical phenotype of cystic patients.

Materials and methods

Based on the clinical investigations, such as family history, symptoms and ultasonography, 84 polycystic patients were enrolled after informed consent from SSL Hospital, BHU (Institution Ethical Review board approval number: F.Sc./Ethics Committee/2015-16/6). Peripheral blood was collected from 84 patients and 80 healthy controls in heparinized syringe or in EDTA vials with ethical consideration. DNA was extracted using phenol–chloroform method for mutational screening of complete *PKD2* gene from polycystic patients and controls. The quantity and quality of extracted DNA was assayed by spectrophotometer (Nanodrop 2000) as well as 0.8% agarose gel electrophoresis. Patients were further categorized on the basis of gender, age and clinical symptoms to find out the specific association of *PKD2* sequence variants with disease pathophysiology. Genetic screening of candidate gene *PKD2* was performed using various molecular techniques (polymerase chain reaction (PCR) and automated DNA sequencing) followed by *in silico* and statistical analysis.

PCR

Sixteen sets of primers were used to amplify 15 exons and its flanking regions (Tsiokas *et al.* 1997; Stekrova *et al.* 2003). Some new primers were designed using Primer3 software (primer sequences are available on request). The PCR of reaction volume 25 μL (1.5 mM $MgCl_2$ and 30 ng of genomic DNA) was performed using ABI Veriti 96-well thermal cycler and the cycling parameters were as follows: initial denaturation of 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, annealing 53–55°C for 1 min, 72°C for 1 min and final extension of 72°C for 10 min with 4°C hold. PCR product was run on 2% agarose gel to check large deletion and insertion in amplified region.

DNA sequencing and analysis

PCR products were cleaned by using ExonucleaseI and rShrimp alkaline phosphatase enzyme (USB Affimetrix, USA) and subsequently cycle sequencing reaction (ABI Big Dye Terminator V3.1) was set in ABI Veriti with cycling parameter as follows: initial denaturation of 95°C for 1 min, followed by 25 cycles of 95°C for 1 min, 55°C for 5 s, 60°C for 4 min and final 4°C hold. Cycle sequencing products were cleaned using EDTA–alcohol method and run in automated sequencer ABI 3130 Genetic Analyzer. Sequencing data was analysed using sequencing analysis software ver. 5.2 (ABI, USA). Sequences were compared with reference genomic sequence available on NCBI using basic local alignment search tool (BLAST). *In silico* analysis was performed using Polyphen-2, PROVEAN, SIFT and Mutation Taster software to predict the effect of genetic changes. Genetic variants harboured by Indian patients were crosschecked with ExAC genome browser and ADPKD mutation database ver. 3.1 (http://pkdb.mayo.edu). The allelic frequencies of the SNPs examined were tested for Hardy–Weinberg equilibrium using χ^2 test ($P < 0.05$). The genetic risk among case and control groups was compared and tested by Fisher's exact test for calculating odds ratio with a 95% class interval. Two tailed P value of <0.05 was interpreted as statistical significant (using Graphpad software).

Results

Clinical symptoms screening

We have been identifying familial and sporadic cases of PKD for understanding the pathophysiology of PKD in patient cohort from northern India. Eighty-four north Indian individuals, clinically diagnosed with polycystic kidney disease at SS Hospital of Banaras Hindu University were enrolled for the present study. There is equal percentage of females to males (48.8 : 51.2) and 30.95% of enrolled PKD patients have positive family history (familial: FAM), while rest of the 69.05% have negative family history (sporadic: SPO). Four subcategories were made: two female group: females with positive family history (FFAM: 13.1%) and with negative family history (FSPO: 35.7%) and rest two are from male group: male with positive family history (MFAM: 17.86%) and negative family history (MSPO: 33.33%). Among renal and extrarenal associated clinical symptoms presented by PKD patients: extrarenal cyst, hypertension, chronic kidney disease (CKD) and stone in different organs are major contributors (figure 1a). Two other main associated symptoms like hypertension (HTN) and presence of liver cysts were found in 19 and 31% of polycystic patients, respectively.

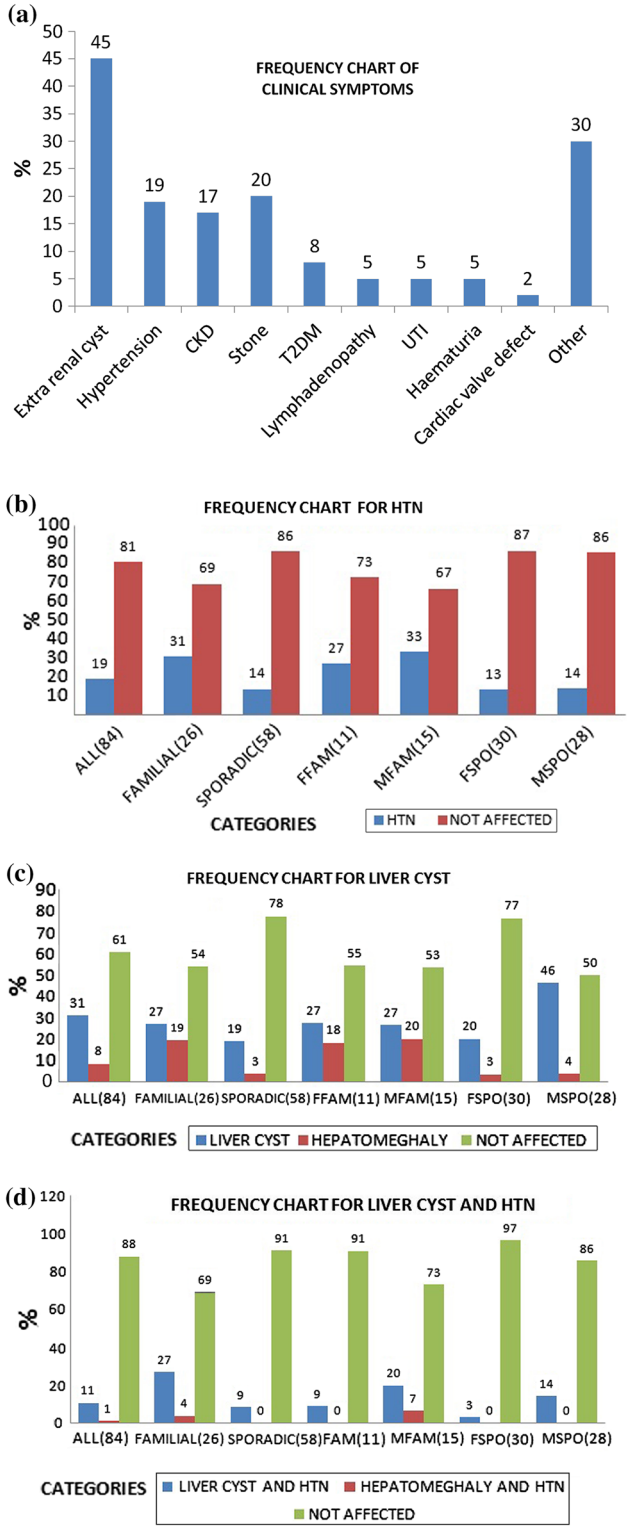

Figure 1. Frequency of clinical symptoms. (a) Frequency chart of clinical symptoms in PKD patients. (b) Frequency chart of clinical symptom, hypertension in PKD patients. (c) Frequency chart of clinical symptom, liver cyst in PKD patients. (d) Frequency chart of clinical symptoms, liver cyst and hypertension in PKD patients.

Subcategorization according to gender and family history reflects maximum frequency of HTN patients contributed by females with positive family history (FFAM: 27%) and males with positive family history (MFAM: 33%) (figure 1b). Presence of liver cyst in males with negative family history (MSPO: 46%) contributed more to another main associated symptom of PKD (figure 1c). In PKD patients, co-occurrence of liver cyst and hypertension was found in case of males with positive family history (MFAM: 20%) and negative family history (MSPO: 14%) (figure 1d).

Mutation screening

All 15 exons and their flanking regions of *PKD2* gene were screened in 84 north Indian polycystic kidney patients. As a result of PCR followed by DNA sequencing analysis of polycystic patients, we identified a total of 28 DNA sequence variants in the *PKD2* gene. Sequencing results were compared with reference genomic sequence NC_000004.12, mRNA sequence NM_000297.3 and protein sequence NP_000288.1 downloaded from NCBI. All variants were analysed *in silico* using Polyphen-2, PROVEAN, SIFT and Mutation Taster software. *In silico* results were compared with ExAC browser and ADPKD mutation database (PKDB) for marking them as novel or known (tables 1, a&b). Twenty-eight genomic changes were presented by PKD patients. Eighteen were spread in exonic region (six truncating, 10 missense and two silent) and 10 were in intronic region (two pathogenic and eight polymorphism). Ten novel (three protein truncating: c.854_854delG, c.1050_1050delC, c.1604_1604delT; five missense: c.646T>C, c.915C>A, c.1789C>A, c.1790T>C, c.1849C>A; two intronic: g.11732A>G, g.28713C>A) and eighteen reported (three protein truncating: c.973C>T, c.2182_2183delAG, c.2224C>T; five missense: c.83G>C, c.1354A>G, c.2164G>T, c.2398A>C, c.2494A>G; two silent: c.1359A>G, c.2460C>T; eight intronic: g.11761T>C, g.11775C>T, g.28743T>C, g.30562G>A, g.30896C>T, g.39212G>C, g.48386C>T, g.67319G>A) variants were spread in the entire *PKD2* gene covering exons 1, 2, 4, 6, 7, 8, 11, 13, IVS1, IVS3, IVS4, IVS6, IVS7 and IVS14. Out of 16 pathogenic / likely pathogenic variants: seven were reported and nine were novel variants presented by Indian PKD patients (figure 2, a–i). *In silico* analysis predicted that 50% of pathogenic / likely pathogenic variants (14/28) were harboured by exonic region alone. A total of 12 polymorphism / likely neutral (one novel and 11 reported) variants were found, in which two were missense and two silent variants present in exonic region, and rest eight polymorphisms were present in IVS1, IVS3, IVS4, IVS7 and IVS14. Genetic screening for novel/known variants those present in exons 4, 7, 8 and their flanking regions were absent in 80 age-related (30–70 y) healthy control individuals. One reported SNP g.48386C>T (rs372552957) was present in heterozygous state in two control individuals. Occurrence and allele frequency of DNA sequence variants (following Hardy–Weinberg equilibrium) presented by patients suggest them to be rare and unique, therefore, would have high impact on pathogenesis of PKD (table 2). Two intronic variants harboured by flanking regions of exon 4 were found in both cases and controls. Reported polymorphism IVS3-22G>A was found more frequent in controls (minor allele frequency: 0.48) than cases (minor allele frequency: 0.363). A significant association for IVS3-22AA genotype with odds ratio 0.2727 (95% CI: 0.1082–0.6876; $P = 0.0056$) was observed in recessive model. Insignificant association for IVS4+62C>T was observed in both dominant (odds ratio 0.7263, 95% CI: 0.3754–1.403; $P = 0.4318$) codominant (odds ratio 0.9739, 95% CI: 0.4868–1.948; $P = 0.9403$) model (table 3). Homozygous state of IVS4+62C>T was absent in controls, yet, heterozygous state is frequently found in cases and controls. For IVS4+62C>T, no significant difference of allelic and genotypic frequencies was observed in both dominant and codominant models (table 3).

Genotype and phenotype correlations

Among all the north Indian cystic patients, most of the *PKD2* genetic changes were presented by sporadic cases. PKD patients with hypertension did not have *PKD2* genetic variants, however, PKD patients with liver cyst had wide spectrum of *PKD2* genetic variants.

Discussion

PKD2 is less complex than *PKD1* which makes mutation detection uncomplicated. It has been well documented that *PKD2* gene contributes 15% to ADPKD cases, but in our study 27.38% (23/84) cases shown pathogenic / likely pathogenic variation in *PKD2* gene. Majority of the cases did not show pathogenic variations in *PKD2* gene suggests that genetic heterogeneity may be the cause for it. Major and minor contributors of ADPKD is *PKD1* gene and unidentified gene/s, respectively, may be responsible for those individuals which do not have *PKD2* genetic change. Most of the families have their unique genetic variants, which suggest that the interfamilial variation is common feature of *PKD2*. Four types (intronic, missense, protein truncating and silent) of variants were found in polycystic patients. Genetic variants are dispersed over the entire gene without significant clustering, however, we have found more variants in some exonic region (exons 4, 8, 11 and 13). According to ADPKD mutation database; exons 4, 8, 11 and 13 were found mutational hot spot and in our results, this hot spot has 13 (11 pathogenic / likely pathogenic and two polymorphism / likely neutral) variations. This hot spot represents only 24% of coding sequence and it has 69% pathogenic / likely pathogenic genetic variations. Novel missense variant (p.Y216H)

Table 1a. All pathogenic and likely pathogenic DNA sequence variants found in PKD patients.

EX/IVS	CDS/gene position	Protein/IVS	Type of variant	*In silico* analysis				rs ID	PKDB	Remark	Patient ID
				Polyphen-2	PROVEAN	SIFT	Mutation taster				
IVS1	g.11732A>G	IVS1-59A>G	Intronic				Pathogenic			Novel/likely pathogenic	80.1
EX2	c.646T>C	p.Y216H	Missense	Probably damaging	Deleterious	Tolerated	Pathogenic			Novel/likely pathogenic	82.1
EX4	c.854_854delG	p.G285Afs*32	Protein truncating				Pathogenic			Novel/ pathogenic	39.1
EX4	c.915C>A	p.N305K	Missense	Possibly damaging	Deleterious	Damaging	Pathogenic			Novel/ pathogenic	81.1
EX4	c.973C>T	p.R325X	Protein truncating				Pathogenic			Reported/ pathogenic	72.1
EX4	c.1050_1050delC	p.S351Vfs*24	Protein truncating				Pathogenic			Novel/ pathogenic	64.1
EX6	c.1354A>G	p.I452V	Missense	Benign	Neutral	Tolerated	Pathogenic	rs1801612	Likely neutral	Reported /likely pathogenic	38.1
IVS6	g.39212G>C	IVS6+9G>C	Intronic				Pathogenic	rs376901684		Reported /likely pathogenic	37.1, 42.1, 44.1
EX7	c.1604_1604delT	p.L535Hfs*27	Protein truncating				Pathogenic			Novel/ pathogenic	85.1
EX8	c.1789C>A	p.L597M	Missense	Probably damaging	Neutral	Damaging	Pathogenic			Novel/likely pathogenic	4.1, 5.1, 22.1
EX8	c.1790T>C	p.L597P	Missense	Probably damaging	Deleterious	Damaging	Pathogenic			Novel/ pathogenic	54.1
EX8	c.1849C>A	p.L617I	Missense	Benign	Neutral	Damaging	Pathogenic			Novel/likely pathogenic	4.1, 5.1, 22.1
EX11	c.2164G>T	p.V722L	Missense	Benign	Neutral	Tolerated	Pathogenic	rs529945469		Novel/likely pathogenic	32.1, 34.1, 81.1, 79.1
EX11	c.2182_2183delAG	p.L729Afs*10	Protein truncating				Pathogenic		Definitely pathogenic	Reported/ pathogenic	5.1, 27.1, 28.1, 43.1
EX11	c.2224C>T	p.R742X	Protein truncating				Pathogenic	rs121918040		Reported/ pathogenic	77.1
EX13	c.2494A>G	p.S832G	Missense	Benign	Neutral	Tolerated	Pathogenic	rs145574534		Reported/ likely pathogenic	60.1

Table 1b. All polymorphisms and likely neutral DNA sequence variants found in PKD patients.

EX/IVS	CDS/gene position	Protein/IVS	Type of variant	*In silico* analysis				rs ID	PKDB	Remark
				Polyphen-2	PROVEAN	SIFT	Mutation taster			
EX1	c.83G>C	p.R28P	Missense	Benign	Neutral	Damaging	Polymorphism	rs1805044	Likely neutral	Reported/polymorphism
IVS1	g.11761T>C	IVS1−32T>C	Intronic				Polymorphism	rs372764946		Reported/polymorphism
IVS1	g.11775C>T	IVS1−16C>T	Intronic				Polymorphism	rs62310565	Likely neutral	Reported/polymorphism
IVS3	g.28713C>A	IVS3+27C>A	Intronic				Polymorphism			Novel/polymorphism
IVS3	g.28743T>C	IVS3+57T>C	Intronic				Polymorphism	rs17786456		Reported/polymorphism
IVS3	g.30562G>A	IVS3−22G>A	Intronic				Polymorphism	rs2725221	Likely neutral	Reported/polymorphism
IVS4	g.30896C>T	IVS4+62C>T	Intronic				Polymorphism	rs373213485		Reported/polymorphism
EX6	c.1359A>G	p.P453P	Silent		Neutral	Tolerated	Pathogenic	rs107013754	Likely neutral	Reported/likely neutral
IVS7	g.48386C>T	IVS7−33C>T	Intronic				Polymorphism	rs372552957		Reported/polymorphism
EX13	c.2398A>C	p.M800L	Missense	Benign	Neutral	Tolerated	Polymorphism	rs2234917	Likely neutral	Reported/polymorphism
EX13	c.2460C>T	p.S820S	Silent		Neutral	Tolerated	Pathogenic	rs572822238	Likely neutral	Reported/likely neutral
IVS14	g.67319G>A	IVS14+27G>A	Intronic				Polymorphism	rs113117728		Reported/polymorphism

presented by the patient with complication of rupturing of renal cyst causing blood in urine. Exon 4 and its flanking introns were found to have those variants which are novel, pathogenic and are present in heterozygous state. Single-nucleotide variants p.N305K, p.G285Afs*32 and p.S351Vfs*24 are present in exon 4 and each variant is presented by unrelated single PKD individual. Asparagine at position 305 is highly conserved, which get replaced by lysine because of change in third nucleotide C>A. Patient with this novel pathogenic variation cause loss of glycosylation site. Another reported single-nucleotide substitution at position c.973C>T (p.R325X) leads to 325aa short protein production with the normal protein, presented by a patient having hypertension, hepatomeghaly and positive family history of PKD. Three novel deletion variants p.G285Afs*32, p.S351Vfs*24 and p.L535Hfs*27 are the result of single nucleotide deletion in one allele leading to the formation of truncated proteins. Patient having these protein-truncating variations would have two types of protein products, i.e. one is full length and second one is short, which do not have channel forming domain and several protein–protein interaction domains. Exons 4 and 7 encode the largest extracellular loop and small extracellular loop, respectively, have binding sites to different external ligands and hence, variation in this region will definitely has pathogenic effect. Two missense variants p.L597M and p.L617I, located in exon 8 seem to be linked/induced with each other are shown by three sporadic patients in heterozygous state. Both variations are result of substitution at first nucleotide of codon from pyrimidine to purine base. Leucine at both amino acid positions 597 and 617 are highly conserved which get replaced by metheonine and isoleucine, respectively. Patient carrying changes in exon 8 alone, has disease onset at 55 years. At amino acid position 597, leucine changes to proline due to change at the second nucleotide C>T. At amino acid position 597, first nucleotide substitution (CTG>ATG) found in three cases and second nucleotide substitution (CTG>CCG) found in one case suggest that this amino acid is highly prone for mutation. Other patients who harbour variation in downstream exons along with exon 8, have disease onset before 55 years. The reported pathogenic mutation c.2164G>T was also detected in the present study in three patients having known family history of PKD, while two other reported protein truncating genetic changes (1) p.L729Afs*10 shown by both familial and sporadic males and (2) p.R742X shown by familial male patients. Both variants p.L729Afs*10 and p.R742X form 738aa and 742aa long proteins with complete loss of calcium-binding domain along with PC1 and PC2 protein interacting sites. Two substitution variants p.S820S and p.S832G harboured by exon 13 are reported previously and are shown by familial and sporadic female patients, respectively. Exonic regions (exons 8, 11 and 13) prone to have genetic variations, code intracellular segment of protein, like exon 8 codes intracellular loop and exon 11 (part of EF

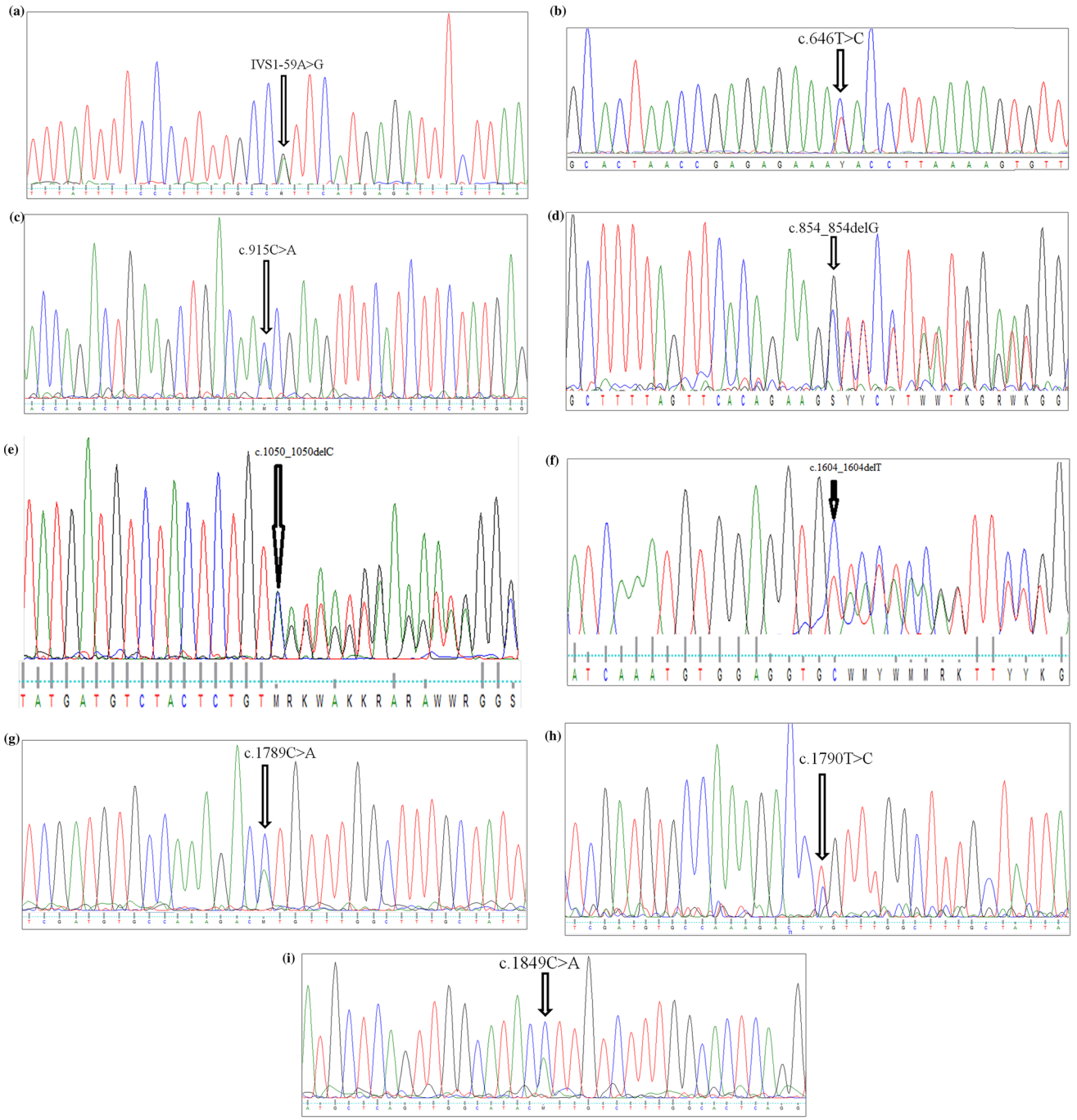

Figure 2. DNA sequence variants in PKD patients. (a) IVS1-59A>G; (b) c.646T>C; (c) c.915C>A; (d) c.854_854delG; (e) c.1050_1050delC; (f) c.1604_1604delT; (g) c.1789C>A; (h) c.1790T>C; (i) c.1849C>A.

domain) and exon 13 code C-terminal of the protein, which interact with different cytoplasmic molecules. C-terminal intracellular region is very important for homodimeric (PC2–PC2) and heterodimeric (PC2–PC1) protein–protein interaction. These regions have protein truncation and missense variations, which definitely lead to disease condition in patients. Known exonic polymorphisms shown by unrelated patients: p.R28P present in both homozygous and heterozygous state in both males and females, while p.M800L present in heterozygous state in sporadic male. DNA variants of exons 4, 6, 7, 8, 11 and 13 were presented by less number of individuals suggest that these variants are rare pathogenic variants. IVS1, IVS7, IVS14 variants were shown by less number of individuals suggest that these are rare variants, while IVS3, IVS4 and IVS6 variants were shown by large number of cases suggest that they are having deleterious impact on PKD pathogenesis when co-occurred with exonic variants. Most

Table 2. Allele frequency and *P* values of χ^2 test of *PKD2* variants in cases.

Type of variants	Homozygous (wild)	Heterozygous	Homozygous (mutant)	Total	Major allele frequency	Minor allele frequency	*P*	χ^2
R28P	67	15	2	84	0.886	0.113	0.3114	1.024
IVS1−59A>G	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS1−32T>C	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS1−16C>T	83	1	0	84	0.994	0.0059	0.9634	0.002
Y216H	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS3+27C>A	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS3+57T>C	71	13	0	84	0.922	0.0773	0.4421	0.59
IVS3−22G>A	30	47	7	84	0.636	0.363	0.0546	3.692
G285Afs*32	83	1	0	84	0.994	0.0059	0.9634	0.002
N305K	83	1	0	84	0.994	0.0059	0.9634	0.002
R325X	83	1	0	84	0.994	0.0059	0.9634	0.002
S351Vfs*24	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS4+62C>T	78	5	1	84	0.958	0.0416	0.0218	5.257
I452V	83	1	0	84	0.994	0.0059	0.9634	0.002
P453P	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS6+9G>C	81	3	0	84	0.982	0.0178	0.8665	0.028
L535Hfs*27	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS7-33C>T	83	1	0	84	0.994	0.0059	0.9634	0.002
L597P	83	1	0	84	0.994	0.0059	0.9634	0.002
L597M	81	3	0	84	0.982	0.0178	0.8665	0.028
L617I	81	3	0	84	0.982	0.0178	0.8665	0.028
V722L	80	4	0	84	0.976	0.0238	0.8186	0.052
L729Afs*10	80	4	0	84	0.976	0.0238	0.8186	0.052
R742X	83	1	0	84	0.994	0.0059	0.9634	0.002
M800L	83	1	0	84	0.994	0.0059	0.9634	0.002
S820S	83	1	0	84	0.994	0.0059	0.9634	0.002
S832G	83	1	0	84	0.994	0.0059	0.9634	0.002
IVS14+27G>A	83	1	0	84	0.994	0.0059	0.9634	0.002

P value <0.05 is not consistent with Hardy–Weinberg equilibrium.

Table 3. Association of IVS3-22G>A and IVS4-62C>T polymorphism in cases and controls.

	Cases ($n = 84$)	Controls ($n = 80$)	Odds ratio (OR)	95% Confidence interval (CI)	*P*
IVS3-22G>A					
GG	30	23	Referent		
GA	47	37	0.9739	0.4868–1.948	0.9403
AA	7	20			
G allele frequency	0.637	0.518			
A allele frequency	0.363	0.481			
Dominant model			0.7263	0.3754–1.403	0.4318
Recessive model			**0.2727**	**0.1082–0.6876**	**0.0056**
IVS4-62C>T					
CC	78	73	Referent		
CT	5	7	0.6685	0.0231–2.201	0.5604
TT	1	0			
C allele frequency	0.958	0.956			
T allele frequency	0.042	0.043			
Dominant model			0.8022	0.2575–2.499	0.7776
Recessive model			2.892	0.116–72.099	1

Significant association (indicated in bold) was observed in recessive model for IVS3-22G>A variant.

of the neutral variants present in IVS3 and IVS4 are shown by many patients and controls. IVS3-22G>A is a likely neutral variant previously reported by Stekrova *et al*. IVS3-22G>A minor allele (A allele: 0.48) is more frequent in controls than patients and recessive model suggest it as a protective allele. IVS4+62C>T is likely

neutral variant and it is equally frequent (C allele: 0.95) in cases and controls. Dominant and codominant models suggest that it may not be associated with disease. PKD patients harbour both exonic and intronic variations followed the Hardy–Weinberg equilibrium ($P < 0.05$ not consistent with HW equilibrium). These intronic changes when present with exonic change, may act as modifier for disease pathogenesis. Patients who have shown more than one likely neutral variation suggest that *PKD2* gene can tolerate more than one genetic variations, which do not have impact on protein level. Genotypic variation in *PKD2* gene was not presented by PKD patients with hypertension. Structural damage of tubules and functional damage of channel protein PC2 of tubules due to *PKD2* and *PKD2*-linked genes could be the factor for hypertension by deregulating intrarenal renin–angiotensin system. DNA variants of *PKD2* gene screened from PKD patients with liver cyst could have role in inducing liver cystogenesis in presence with the rest of *PKD2* linked genes. We could not get significant clustering of genetic variants which can correlate with disease phenotype and severity. This could be due to genetic heterogeneity, allelic heterogeneity and gene environment interaction.

In conclusion, some regions of *PKD2* gene are prone to genetic variation which suggest that location/site of the genetic variation(s) have impact on disease pathogenesis. All pathogenic variations found in north Indian PKD patients were spread in exons 2, 4, 6, 7, 8, 11 and 13, code for extracellular and intracellular loops which interact with several extracellular and intracellular proteins to regulate cell homeostasis. PC2 has many functional domains and occurrence of genetic changes in different domains will affect the domain function ultimately resulting in PKD pathogenesis in polycystic kidney patients.

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