

# Characterization of Novel Truncated Variants Identified From Indian Autosomal Dominant Polycystic Kidney Disease Cases

Sonam Raj<sup>a</sup>, Rana Gopal Singh<sup>b</sup>, Parimal Das<sup>a, c</sup>

## Abstract

**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common, late-onset and genetically heterogeneous disorder. Altered genetic background is the prime cause of ADPKD. Most of the cases showed mutations in *PKD1*, *PKD2*, *GANAB* and *DNAJB11*. Previous mutation screening studies reported that *PKD1* and *PKD2* are the major contributors to the disease. Among all types of DNA sequence variants, majority of the variants are protein truncating in nature. Loss of normal polycystin 1 (PC1) and polycystin 2 (PC2) proteins, encoded by *PKD1* and *PKD2* respectively, leads to the disease manifestation.

**Methods:** Three cases and 100 controls were selected for mutational screening of *PKD1* and *PKD2* gene using Sanger sequencing. To characterize the variants, *in silico* (mutation taster, Align-Grantham Variation Grantham Deviation (Align-GVGD), Polymorphism Phenotyping v2 (PolyPhen2), Protein Variation Effect Analyzer (PROVEAN), Sorting Intolerant From Tolerant (SIFT), Polycystic Kidney Disease Mutation Database (PKDB), ProtScale and protein Basic Local Alignment Search Tool (BLAST-p)) as well as *in vitro* (Western blot) studies were performed.

**Results:** We have identified a total of 14 variants (three truncating, three nonsynonymous, seven synonymous and one intronic). Three deletions (*PKD1*: c.445\_445delC, *PKD2*: c.854\_854delG and *PKD2*: c.1050\_1050delC) were confirmed as rare, private and novel mutations in Indian ADPKD cases. Rest 11 variants were previously reported and likely neutral in nature. Loss of conservation of amino acid, decreased hydrophobicity and decreased coil forming ability of truncated proteins were supposed to affect the protein functions. Immunoblotting verified the expression of truncated proteins in cell.

**Conclusions:** We can conclude that identified deletion variants were

truncating in nature and hence they were pathogenic mutations responsible for disease manifestation.

**Keywords:** ADPKD; *PKD1*; *PKD2*; Mutation; Truncated protein

## Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a late-onset and most common genetically heterogeneous disorder with the worldwide prevalence of one in 500 - 1,000 births [1]. It is characterized by presence of multiple cysts in both kidneys, leading to the disruption of precise architecture of the kidney. Deformed structure of the kidneys affects the renal filtration system and hence affected individuals have abnormal level (mostly higher) of serum creatine and urea. ADPKD is the fourth most common cause of end-stage renal disease eventually leading to the dialysis and kidney transplantation [2]. Most of the families showed mutation in *PKD1* and *PKD2*. The major contributor of ADPKD is *PKD1* gene encoded 4,303 amino acid (aa) long, 460kDa glycosylated integral membrane protein polycystin 1 (PC1). The second major contributor is *PKD2* gene encoded 968aa long, 110kDa channel protein polycystin 2 (PC2) [3-5]. Recently two new genes *GANAB* and *DNAJB11* were identified in a few ADPKD cases [6, 7]. Till date, ADPKD mutation database (<http://pkdb.mayo.edu>) has listed 2,331 in *PKD1* and 305 in *PKD2* DNA sequence variants [8]. PC1 and PC2 are involved in several cellular events like cell proliferation, differentiation, apoptosis, cell polarity, adhesion, maturation, tubulogenesis and extracellular matrix maintenance, which are all essential steps of kidney morphogenesis [9, 10]. Experiments on *pkd1*<sup>-/-</sup> mice indicated that PC1 played an important role in renal tubule maturation [11]. Overlapping of sub-cellular localization of PC1 and PC2 suggests their mutual function in different signaling pathways [12]. It has been reported that PC1-PC1 homomer interacted via PKD (Ig like) domains, PC2-PC2 interacted via N-terminal region and PC1-PC2 heteromeric interaction occurred via C-terminal coiled-coil domains [13]. Previous reports pointed out that *PKD1* and *PKD2* harbored high number of protein truncating mutations [14-21]. That is why both genetic testing as well as *in vitro* studies are very important to confirm and verify the cause of ADPKD. Here, we reported and confirmed novel truncating variants identified in three different

Manuscript submitted July 6, 2019, accepted January 14, 2020

<sup>a</sup>Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi, India

<sup>b</sup>Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

<sup>c</sup>Corresponding Author: Parimal Das, Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi, India.

Email: [parimal@bhu.ac.in](mailto:parimal@bhu.ac.in)

doi: <https://doi.org/10.14740/wjnu392>

Indian ADPKD cases. These variants were expected to encode the smallest truncated protein. One was discovered from this study in *PKD1* (c.445\_445delC) gene and two were reported in *PKD2* (c.854\_854delG and c.1050\_1050delC) gene from previous study [22]. These variants were expected to encode the smallest truncated protein which might be one of the causes of disease condition.

## Materials and Methods

### Patient recruitment and mutation detection

This study was approved by Institutional Ethical Committee of the Institute of Science, Banaras Hindu University, Varanasi (Institution Ethical Review board approval number: F.Sc./Ethics Committee/2015-16/6). Three clinically suspected individuals with bilateral polycystic kidney disease and 100 control individuals participated in this study with informed consent. Genomic DNA was extracted from peripheral blood samples by salting out method. The quantity and quality of extracted DNA were assayed by spectrophotometer (Nanodrop 2000) as well as 0.8% agarose gel electrophoresis. *PKD1* and *PKD2* gene specific primers [5, 17, 18] were used for screening of three patients. *PKD2* gene screening of two cases was previously performed and reported [21]. Long range-polymerase chain reaction (LR-PCR) followed by second generation nested PCR (nPCR) was performed for *PKD1* gene screening. LR-PCR product was run on 1% agarose gel and DNA was extracted according to prescribed manufacture protocol using RBC DNA elution kit (Real Genomics). Eluted DNA products were diluted in 1:1,000 deionized water and then used as a template to perform nPCR following previous protocol [5, 17, 18]. PCR products were cleaned by using Exonuclease I and rShrimp alkaline phosphatase enzyme (USB Affimetrix, Santa Clara, CA, USA). Sequencing of PCR product with forward and/or reverse primer was achieved following ABI Big Dye Terminator V3.1 cycle sequencing kit (Applied Biosystem, Foster City, CA, USA). Sequencing results were compared with reference genomic sequence NC\_000016.10, mRNA sequence NM\_001009944.2 and protein sequence NP\_001009944.2 for *PKD1* and NC\_000004.12, mRNA sequence NM\_000297.4 and protein sequence NP\_000288.1 for *PKD2* available on NCBI using basic local alignment search tool (BLAST). Discovered pathogenic variants were also screened in 100 controls.

### In silico analysis

*In silico* analysis was performed using online software like mutation taster, Align-Grantham Variation Grantham Deviation (Align-GVGD), Polymorphism Phenotyping v2 (PolyPhen2), Protein Variation Effect Analyzer (PROVEAN), and Sorting Intolerant From Tolerant (SIFT), and was also compared with ADPKD mutation database (Table 1). Amino acid conservation of novel PC1 and PC2 variants was analyzed using protein BLAST (BLAST-p). Hydrophobicity of wild and mutant proteins was compared by ProtScale (Hopp & Woods). Secondary

structure forming capability of wild and mutant proteins was analyzed using ProtScale (Chou & Fasman).

### Plasmid preparation

Two plasmids with *PKD1* and *PKD2* genes were procured from Addgene (<https://www.addgene.org>) for *in vitro* studies. Due to difficulty in site-directed mutagenesis (SDM) and transfection of big-size wild-type green fluorescence protein (GFP-WT) (AF-41) plasmid, sub-cloning of 2kb region of *PKD1* in pcDNA3.1 plasmid was performed. GFP-WT (AF-41) was used as a template to amplify wild-type 2kb *PKD1* gene fragment which covered exon1-5, tagged with GFP at N-terminal. Sub-cloning of amplified 2kb fragment into pcDNA3.1 mammalian expression vector was completed using forward primer with *BamHI* and reverse primer with *XhoI* cut site (Table 2). This plasmid worked as a template in SDM experiment. SDM primers were designed using NEB mutagenesis primer designing web server (Table 2). SDM primer introduced the deletion of G nucleotide during the SDM-PCR using Q5 site directed mutagenesis kit (NEB, Ipswich, MA, USA). The SDM-PCR was performed in ABI thermal cycler (ABI, Foster City, CA, USA), programmed with initial denaturation at 98 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 30 s and annealing-extension at 72 °C for 2 min and one final extension of 72 °C for 5 min. NEB10 beta (NEB10B) cells were transformed with wild and mutant plasmids. Positive colonies were picked, grown in LB<sup>+</sup> ampicillin culture and then plasmids were extracted using Qiagen plasmid extraction kit. SDM created mutant plasmid DNA (pcDNA 3.1+2kb ex4delC *PKD1*) was confirmed by digestion, by colony PCR amplification of the insert and Sanger sequencing. Similarly, two variants from *PKD2* (c.854\_854delG and c.1050\_1050delC) were selected for SDM. M-PKD2 (OF2-3) plasmid having wild-type *PKD2* was used as a template to introduce the deletion using SDM primers and Q5 site directed mutagenesis kit (Table 2). NEB10B competent cells were transformed with the wild M-PKD2 (OF2-3) plasmid as well as SDM made mutant plasmids (pcDNA 3.1+2kb ex4delC *PKD1*, M-PKD2-c.854delG and M-PKD2-c.1050delC). Positive clones were confirmed by PCR amplification of the *PKD2* insert and by Sanger sequencing.

### Transfection of COS7 cells, protein isolation and Western blotting

Transfection was carried in 6-wells plate seeded with  $3 \times 10^5$  COS7 cells/well. At around 90% of confluency, Dulbecco's minimum essential medium (DMEM) was replaced with the Opti-MEM (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and then cells were transfected with the wild and mutant plasmids using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer protocol. For *PKD1* gene variant study, COS7 cells were transfected with the wild (pcDNA 3.1+2kb wild *PKD1*) and mutant (pcDNA 3.1+2kb ex4delG *PKD1*) plasmids of *PKD1* gene. Similarly, for *PKD2* gene variant study,

**Table 1.** List of Variants Identified in Three Patients

Sample ID	PKD1/PKD2	rs ID	Protein	Domain	Mutation taster	Align-GVGD	Poly-Phen2	In silico			Remark
								PROVEAN	SIFT	PKDB	
PKD16.1	c.445_445delC	NA	p.Q149Sfs*141	LRRCT	Pathogenic	NA	NA	NA	NA	NA	Disease causing
	c.7165T>C	rs2457533	p.L2389L	REJ	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	c.7441C>T	rs2003782	p.L2481L	REJ	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	c.12133A>G	rs10906	p.L4045V	TM10	Polymorphism	Class C25	Benign	Neutral	Tolerated	Likely neutral	Likely neutral
	c.12176C>T	rs3209986	p.A4059V	ND	Polymorphism	Class C55	Benign	Neutral	Tolerated	Likely neutral	Likely neutral
	c.12276A>G	rs3087632	p.A4092A	TM11	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	c.12409C>T	rs79899502	p.L4137L	ND	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	c.12630T>C	rs7203729	p.P4210P	ND	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	IVS3-22G>A	rs2725221			Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	PKD39.1	PKD2: c.854_854delG	Reference	p.G285Afs*32	First extracellular loop	Pathogenic	NA	NA	NA	NA	NA
PKD64.1	c.1119C>T	rs199685642	p.L373L	ND	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	PKD2: c.1050_1050delC	Reference	p.S351Vfs*24	First extracellular loop	Pathogenic	NA	NA	NA	NA	NA	Disease causing
	c.1119C>T	rs199685642	p.L373L	ND	Polymorphism	NA	NA	NA	NA	NA	Likely neutral
	c.10529C>T	rs45478794	p.T3510M	ND	Polymorphism	Class C65	Possibly damaging	Neutral	Damaging	Likely neutral	Damaging

ND: not defined; NA: not possible to analyze; Align-GVGD: Align-Grantham Variation Grantham Deviation; PolyPhen2: Polymorphism Phenotyping v2; PROVEAN: Protein Variation Effect Analyzer; SIFT: Sorting Intolerant From Tolerant; PKDB: Polycystic Kidney Disease Mutation Database.

**Table 2.** List of Primers Used in Sub-Cloning and SDM

Primer name	Sequence 5'-3'
<i>PKD1_F_BamHI</i> <sup>a</sup>	TAATATGGATCCGGCATGGTGAGCAAGGG
<i>PKD1_R_XhoI</i> <sup>a</sup>	TAATATCTCGAGGTAGCAGTGCCCCGTTGCC
<i>PKD1_EX4_C.445delC_PF</i> <sup>b</sup>	°AGCCCGAGGCAGCCACGT
<i>PKD1_EX4_C.445delC_PR</i> <sup>b</sup>	CACCACCCGCACCTGCTG
<i>PKD2_EX4_C.854delC_PF</i> <sup>b</sup>	°CTCCTTATTGGATGGGCTG
<i>PKD2_EX4_C.854delC_PR</i> <sup>b</sup>	CTTCTGTGAACTTCCAGAAG
<i>PKD2_EX4_C.1050delC_PF</i> <sup>b</sup>	°AGTAGTGAAGATAGGGCTC
<i>PKD2_EX4_C.1050delC_PR</i> <sup>b</sup>	ACAGAGTAGACATCATAGC

<sup>a</sup>Primers used in sub-cloning; <sup>b</sup>SDM primers; <sup>c</sup>Site of nucleotide deletion.

COS7 cells were transfected with the wild (M-PKD2 (OF2-3)) and mutant (M-PKD2-c.854delG and M-PKD2-c.1050delC) plasmids. After 40 h of transfection, total cellular protein was isolated and protein concentration was estimated by using Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer protocol. A total of 10% SDS-PAGE was used to resolve the proteins. A total of 50 µg of denatured protein was loaded into each well and electrophoresed at 100 volts (constant) for 4 h at 4 °C. Proteins were transferred from gel to polyvinylidene fluoride (PVDF) membrane. After transfer, membrane was treated with blocking buffer (having 5% non-fat milk) at room temperature for 2 h. Blocking membrane was incubated with primary antibody at 4 °C overnight. For PC1 protein detection, membrane was incubated with 1:1,000 dilution of primary antibody anti-PC1 raised in mouse (Santa Cruz, CA, USA) and anti-GFP raised in mouse (Santa Cruz, CA, USA). For PC2 protein detection, membrane was incubated with 1:1,000 dilution of primary antibody anti-Myc raised in mouse (Sigma-Aldrich, St. Louis, MO, USA). The 1:1000 dilution of anti-beta actin raised in mouse (Sigma-Aldrich, St. Louis, MO, USA) primary antibody was used to detect internal loading control beta actin protein. After incubation, membrane was washed in Tris-buffered saline (TBS) three times for 10 min and incubated at room temperature for 2 h with secondary antibody. Two secondary antibodies (1:1,000 dilution), goat anti-mouse IgG-ALP (Bangalore Genei, Bangalore, India) and goat anti-mouse IgG-HRP (Bangalore Genei, Bangalore, India) were used separately to process the membrane. Membrane was washed two times with TBS for 10 min and developed by either nitro-blue-tetrazolium-5-bromo-4-chloro-3-indolyl-phosphate (NBT-BCIP) or electrochemiluminescence (ECL) technique. Protein isolated from PC1 wild and mutant plasmid transfected cells displayed bands of 72KDa and 54KDa. Protein isolated from PC2 wild and mutant plasmid transfected cells displayed bands of 110KDa, 46 KDa and 42KDa.

## Results

### *PKD1* variant

A 42-year-old female patient (sample ID: PKD16.1) with neg-

ative family history of ADPKD had bilateral cysts for the past 9 years. She has grown an enlarged kidney of size 13 cm × 5.28 cm of the right kidney and 15.8 cm × 8.3 cm of the left kidney. Serum analysis revealed the increased level of urea (22 mg/dL) and creatinine (1.2 mg/dL). She has other complications like flank pain, loss of appetite, weakness, painful urination and urinary tract infection. Mutation screening of *PKD1* and *PKD2* genes revealed nine DNA sequence variants in the patient genome (Table 1). She has heterozygous c.445\_445delC in *PKD1* (Fig. 1) variant specifically located in N-terminal extracellular leucine rich repeat c-type lectin (LRRCT) domain of PC1. Along with the c.445\_445delC pathogenic variant, there were seven in *PKD1* (c.7165T>C, c.7441C>T, c.12133A>G, c.12176C>T, c.12276A>G, c.12409C>T and c.12630T>C) and one in *PKD2* (IVS3-22G>A) variants discovered in patient. Absence of c.445\_445delC in 100 controls and in other populations determined it as a rare and novel variant. *In silico* analysis revealed that c.445\_445delC encoded 288aa truncated protein (p.Q149Sfs\*141). Rest of the variants were not damaging in nature. Multiple sequence alignment of wild-PC1 and mutant (p.Q149Sfs\*141) proteins revealed 149aa sequence similarity and after that 139aa altered sequence ultimately ending with the premature stop codon. This mutant protein was able to form the wild-type structure upto first transmembrane domain and rest of the downstream domains were either compromised or lost (Fig. 2). ProtScale web server suggested drop in the hydrophobic microenvironment around the mutant PC1: p.Q149Sfs\*141 compared to wild-PC1 (Fig. 3a, b). Secondary structure forming capacity analyzed by ProtScale revealed decreased coil forming capacity of mutant PC1: p.Q149Sfs\*141 compared to the wild-PC1 (Fig. 3c, d). It was expected that pcDNA 3.1+2kb wild *PKD1* plasmid will encode the 72kDa protein and the SDM created mutant pcDNA 3.1+2kb ex4delG *PKD1* plasmid will encode the 54kDa truncated protein in transfected cells. GFP and PC1 specific primary antibody detected the specific 72kDa and 54kDa protein band and verified that c.445\_445delC is protein truncating in nature (Fig. 4a-f).

### *PKD2* variant

A 68-year-old female patient (sample ID: PKD39.1) with

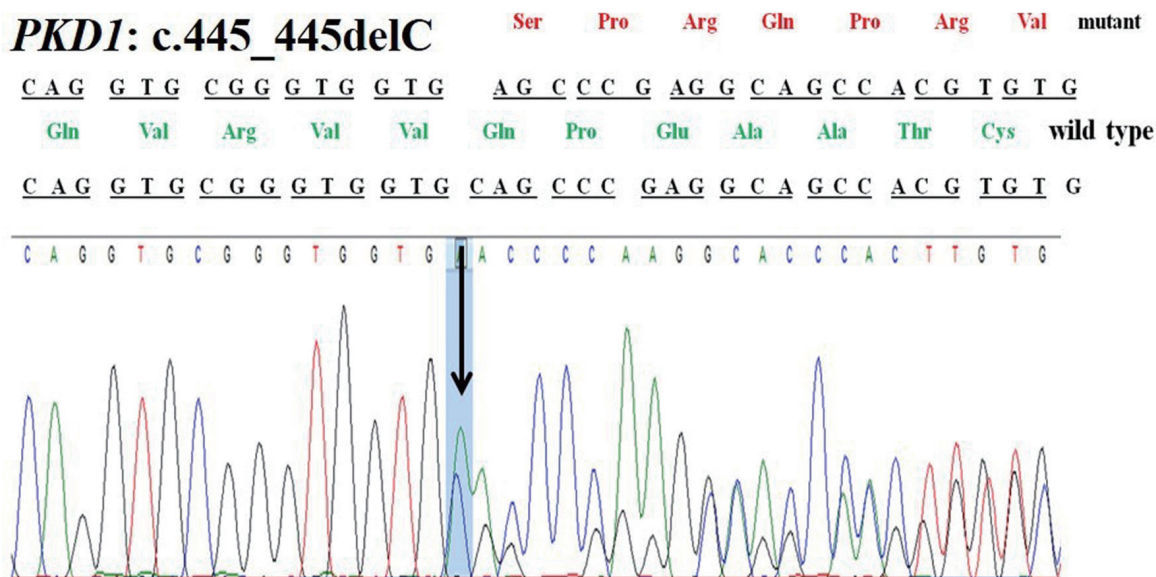


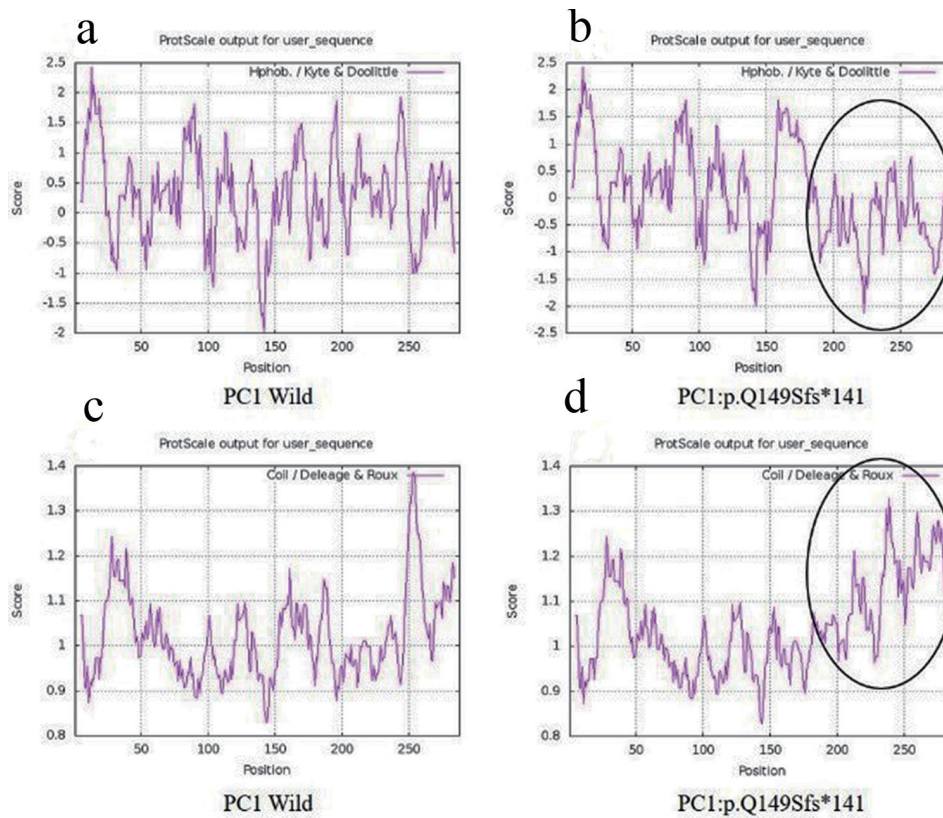
Figure 1. Chromatogram of *PKD1*: c.445\_445delC deletion in patient 16.1.

negative family history of ADPKD had complications of bilateral cysts and enlarged kidney for the past 7 years. Serum analysis showed the increased level of urea 40 mg/dL and creatinine 1.59 mg/dL. She had other clinical complications like flank pain, loss of appetite and weakness. Mutation screening revealed two variants: one in *PKD1* (c.1119C>T) and another

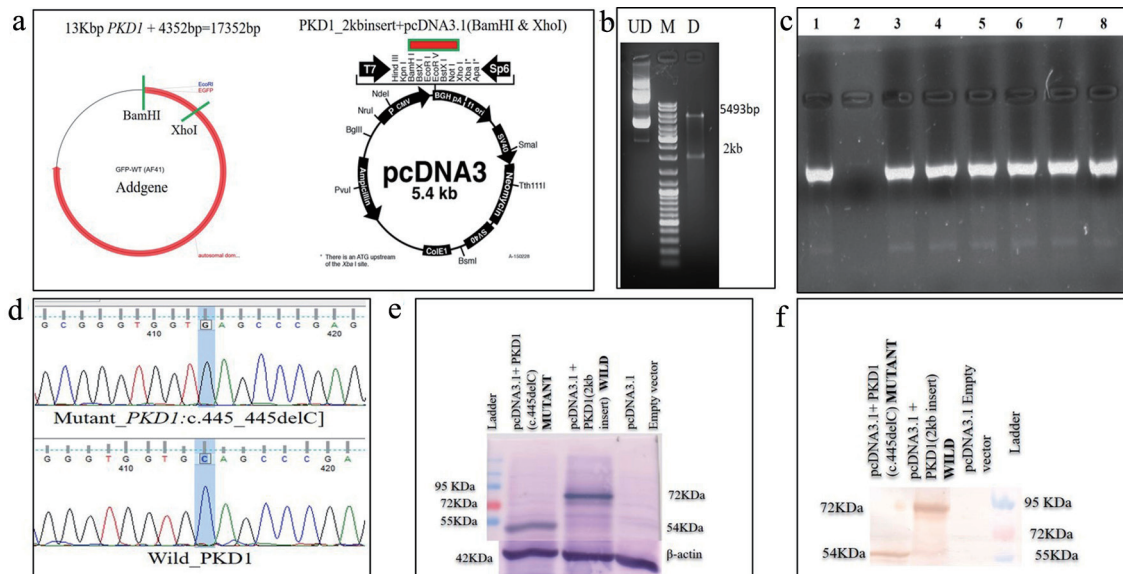
one in *PKD2* (c.854\_854delG) (Table 1). Heterozygous c.854\_854delG variant was absent in 100 controls and other population suggested it as rare and novel. *In silico* analysis revealed that c.854\_854delG variant was present in N-terminal first extracellular loop which was supposed to encode 317aa truncated protein p.G285Afs\*32 and the c.1119C>T was likely



Figure 2. Multiple sequence alignment of wild PC1 and predicted mutant c.445\_445delC-PC1 protein. PC1: polycystin 1.



**Figure 3.** Hydrophobicity and helix forming capacity calculation of wild and mutant proteins using ProtScale. (a, b) Hydrophobicity of PC1-wild and PC1: p.Q149Sfs\*141; (c, d) helix forming capacity around PC1-wild and PC1:p.Q149Sfs\*141. PC1: polycystin 1.



**Figure 4.** Schematic diagram of (a) excision of GFP-tagged wild *PKD1* 2kb region using *Bam*HI and *Xho*I and sub-cloned into pcDNA3.1 mammalian expression vector; (b) confirmation of sub-cloned plasmid using restriction enzyme; (c) colony PCR to confirm SDM after transformation using *PKD1* specific primer; (d) Sanger sequencing of plasmids, mutant pcDNA 3.1+2kb ex4deIC *PKD1*, and wild pcDNA 3.1+2kb wild *PKD1*; (e) expression of wild and mutant proteins in transfected COS7 cells, lane 1 displaying 54kDa mutant and lane 2 displaying 72kDa mutant protein using anti-PC1 antibody, and lane 3 displaying empty vector; (f) lane 1 displayed 54kDa mutant, lane 2 displayed 72kDa mutant protein and lane 3 displayed empty vector using anti-GFP antibody. GFP: green fluorescence protein; PCR: polymerase chain reaction; SDM: site-directed mutagenesis.

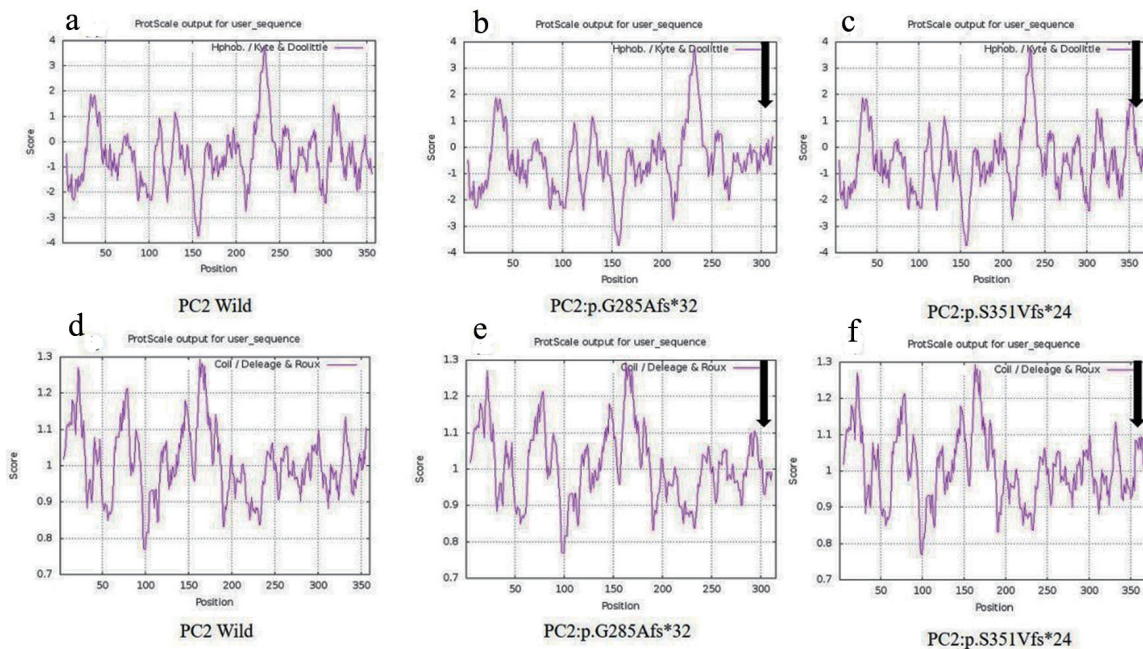


**Figure 5.** Multiple sequence alignment of wild PC2 and predicted mutants c.854\_854delG-PC2 and c.1050\_1050delC-PC2 proteins.

neutral in nature.

Another 30-year-old male patient (sample ID: PKD64.1) with positive family history of ADPKD had complications of bilateral cysts for the past 4 years with enlarged kidney of size 10.8 cm × 4.6 cm of the right kidney and 14.9 cm × 7.4 cm of the left kidney. Serum analysis indicated the increased level of urea 21 mg/dL and creatinine 1.0 mg/dL. He has complications like flank pain, loss of appetite, weakness, swelling in body and hematuria. Mutation screening of *PKD1* and *PKD2* gene revealed three variants in the patient (Table 1). Heterozygous c.1050\_1050delC variant was discovered in N-terminal first extracellular loop. Along with the c.1050\_1050delC, two other *PKD1* (c.1119C>T and c.10529C>T) variants were also harbored by the patient 64.1.

*In silico* analysis revealed that c.1050\_1050delC encoded 373aa truncated protein p.S351Vfs\*24 and rest two variants c.1119C>T and c.10529C>T were likely neutral in nature. Multiple sequence alignment analysis of both truncated proteins (p.G285Afs\*32 and p.S351Vfs\*24) revealed 285aa sequence similarity with wild-PC2. The 250aa sequence was supposed to encode upto the first transmembrane domain and the remaining downstream domains were suspected to be missing in both mutant variants (Fig. 5). ProtScale web server suggested drop in the hydrophobic microenvironment around the PC2: p.G285Afs\*32, PC2: p.G285Afs\*32 compared to wild-PC2. Secondary structure forming capacity analyzed by ProtScale revealed decreased coil forming capacity of PC2: p.G285Afs\*32, PC2: p.G285Afs\*32 compared to the wild-



**Figure 6.** Hydrophobicity and helix forming capacity calculation of wild and mutant proteins using ProtScale. (a-c) Hydrophobicity of PC2-wild, PC2: p.G285Afs\*32 and PC2: p.S351Vfs\*24, and (d-f) helix forming capacity of PC2-wild, PC2: p.G285Afs\*32 and PC2: p.S351Vfs\*24.

PC2 (Fig. 6). To study the frameshift-induced protein truncation, COS7 cells were transfected with wild and mutant variant containing plasmids. It was expected that the wild PC2 will encode 110kDa PC2, c.854\_854delG will encode 46kDa truncated PC2 and c.1050\_1050delC will encode 42kDa truncated PC2. Primary antibody against Myc detected the 42kDa, 46kDa and 110kDa molecular weight protein band and verified both variants c.854\_854delG and c.1050\_1050delC as protein truncating mutations (Fig. 7a-g).

## Discussion

Previous reports published from American [15-17, 19-21], Asian [18], Indian [22] and Iranian [23] populations suggested that protein truncation is one of the prime causes of ADPKD. A total of 14 DNA sequences were identified and three deletion variants were verified to be mutation. Absence of deletion variants *PKD1*: c.445\_445delC, *PKD2*: c.854\_854delG and *PKD2*: c.1050\_1050delC in controls and other populations suggested that these are rare and novel. Change in hydrophobicity and coil forming ability of truncated protein might affect the structure of the protein. *In silico* analysis determined all novel variants (*PKD1*: c.445\_445delC, *PKD2*: c.854\_854delG and *PKD2*: c.1050\_1050delC) as damaging and rest of the 11 variants as not damaging. *In silico* studies revealed that *PKD1* (c.445\_445delC) and *PKD2* (c.854\_854delG and c.1050\_1050delC) encoded truncated protein (p.Q149Sfs\*141 of *PKD1*, p.G285Afs\*32 and p.S351Vfs\*24 of *PKD2*) respectively. 3D and 2D cell cultures are suitable models to study the functional impact of mutations responsible for formation

and growth of cyst [24]. Immunoblotting confirmed expression of truncated mutations *in vitro* using 2D cell culture. The loss of one functional allele might disrupt the critical window of normal protein availability in the cell to perform the normal cellular functions like channel property and the protein-protein interaction and hence prove their pathogenicity for disease. Occurrence of pathogenic truncating variation in the patients is the cause of disease. Although there is no cure for ADPKD, specific treatment can not only ease the symptom, but can even prolong the life span. Better understanding of this disease may help to develop novel strategy for early diagnosis and treatment.

## Acknowledgments

We are very thankful for participation of patients and healthy control individuals. SR is thankful to UGC, New Delhi, India for providing SRF.

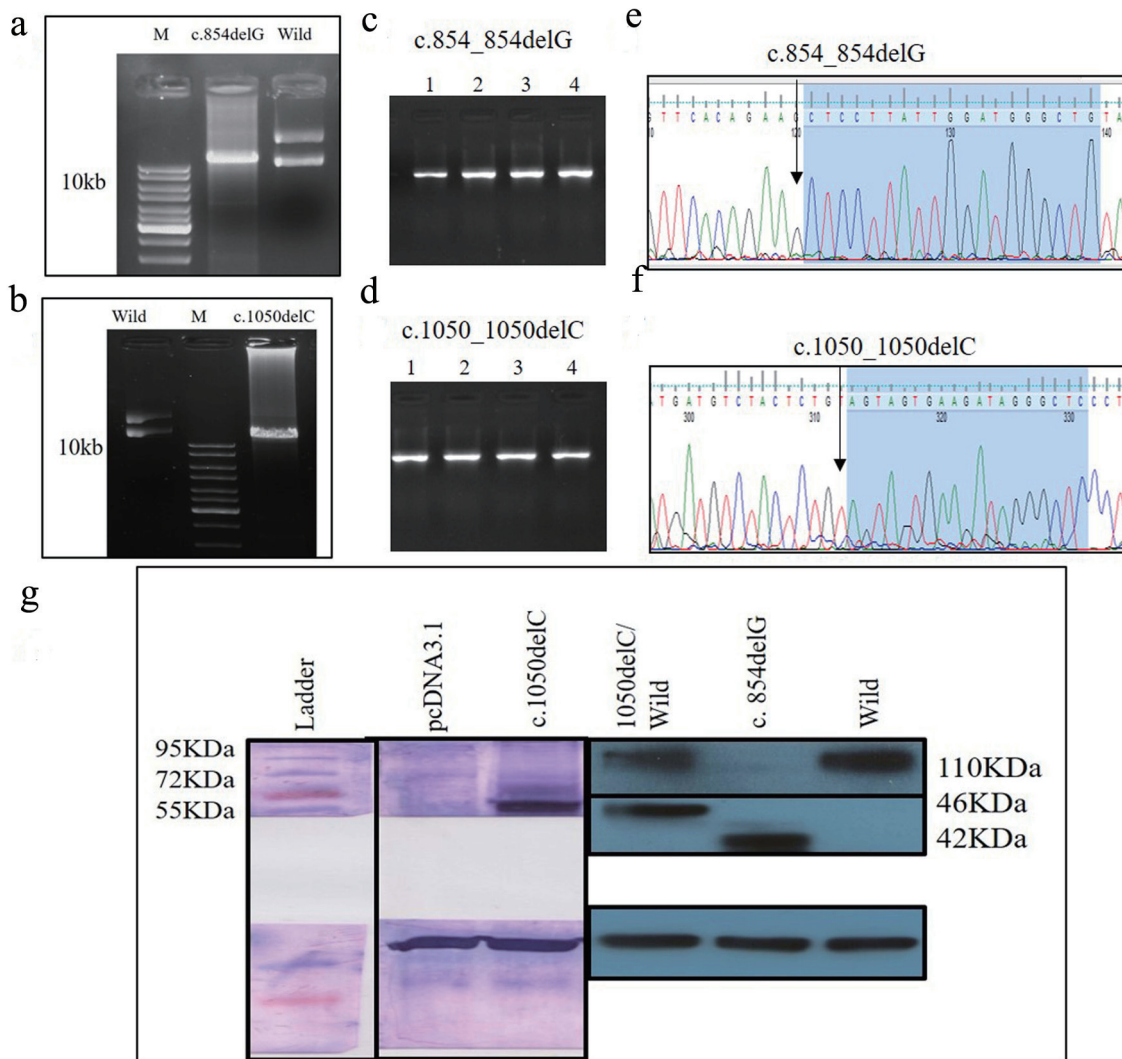
## Financial Disclosure

This work was supported by UGC-UPE focus area-II, Banaras Hindu University, India.

## Conflict of Interest

We certify that the research findings presented here are original and free of conflict of interest.





**Figure 7.** (a) PCR product of c.854\_854delG SDM, (b) PCR product of c.1050\_1050delC SDM, (a, d) colony PCR to confirm the transformation using *PKD2* specific primer, (e, f) Sanger sequencing of plasmids mutants c.854\_854delG, c.1050\_1050delC, and (g) identification of wild and mutant PC2 proteins using Myc-tag antibody. PCR: polymerase chain reaction; SDM: site-directed mutagenesis; PC2: polycystin 2.

## Informed Consent

Individuals participated in this study provided informed consent.

## Author Contributions

This study was designed by PD. Experiments were designed and performed by SR. Clinical diagnosis was carried out by RGS. Manuscript was drafted by SR and PD.

## References

- Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet*. 2007;369(9569):1287-1301.
- Wilson PD. Polycystin: new aspects of structure, function, and regulation. *J Am Soc Nephrol*. 2001;12(4):834-845.
- Polycystic kidney disease: the complete structure of the PKD1 gene and its protein. The international polycystic kidney disease consortium. *Cell*. 1995;81(2):289-298.
- Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science*. 1996;272(5266):1339-1342.
- Hayashi T, Mochizuki T, Reynolds DM, Wu G, Cai Y, Somlo S. Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). *Genomics*. 1997;44(1):131-136.
- Porath B, Gainullin VG, Cornec-Le Gall E, Dillinger EK,

- Heyer CM, Hopp K, Edwards ME, et al. Mutations in GANAB, encoding the glucosidase IIalpha subunit, cause autosomal-dominant polycystic kidney and liver disease. *Am J Hum Genet.* 2016;98(6):1193-1207.
7. Cornec-Le Gall E, Torres VE, Harris PC. Genetic complexity of autosomal dominant polycystic kidney and liver diseases. *J Am Soc Nephrol.* 2018;29(1):13-23.
  8. ADPKD mutation database (<http://pkdb.mayo.edu/>).
  9. Ward CJ, Turley H, Ong AC, Comley M, Biddolph S, Chetty R, Ratcliffe PJ, et al. Polycystin, the polycystic kidney disease 1 protein, is expressed by epithelial cells in fetal, adult, and polycystic kidney. *Proc Natl Acad Sci U S A.* 1996;93(4):1524-1528.
  10. van Adelsberg JS. The role of the polycystins in kidney development. *Pediatr Nephrol.* 1999;13(5):454-459.
  11. Lantinga-van Leeuwen IS, Dauwerse JG, Baelde HJ, Leonhard WN, van de Wal A, Ward CJ, Verbeek S, et al. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum Mol Genet.* 2004;13(24):3069-3077.
  12. Cai Y, Maeda Y, Cedzich A, Torres VE, Wu G, Hayashi T, Mochizuki T, et al. Identification and characterization of polycystin-2, the PKD2 gene product. *J Biol Chem.* 1999;274(40):28557-28565.
  13. Newby LJ, Streets AJ, Zhao Y, Harris PC, Ward CJ, Ong AC. Identification, characterization, and localization of a novel kidney polycystin-1-polycystin-2 complex. *J Biol Chem.* 2002;277(23):20763-20773.
  14. Tsiokas L, Kim E, Arnould T, Sukhatme VP, Walz G. Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc Natl Acad Sci U S A.* 1997;94(13):6965-6970.
  15. Watnick TJ, Piontek KB, Cordal TM, Weber H, Gandolph MA, Qian F, Lens XM, et al. An unusual pattern of mutation in the duplicated portion of PKD1 is revealed by use of a novel strategy for mutation detection. *Hum Mol Genet.* 1997;6(9):1473-1481.
  16. Watnick T, Phakdeekitcharoen B, Johnson A, Gandolph M, Wang M, Briefel G, Klinger KW, et al. Mutation detection of PKD1 identifies a novel mutation common to three families with aneurysms and/or very-early-onset disease. *Am J Hum Genet.* 1999;65(6):1561-1571.
  17. Phakdeekitcharoen B, Watnick TJ, Germino GG. Mutation analysis of the entire replicated portion of PKD1 using genomic DNA samples. *J Am Soc Nephrol.* 2001;12(5):955-963.
  18. Phakdeekitcharoen B, Watnick TJ, Ahn C, Whang DY, Burkhart B, Germino GG. Thirteen novel mutations of the replicated region of PKD1 in an Asian population. *Kidney Int.* 2000;58(4):1400-1412.
  19. Neumann HP, Bacher J, Nabulsi Z, Ortiz Bruchle N, Hoffmann MM, Schaeffner E, Nurnberger J, et al. Adult patients with sporadic polycystic kidney disease: the importance of screening for mutations in the PKD1 and PKD2 genes. *Int Urol Nephrol.* 2012;44(6):1753-1762.
  20. Roelfsema JH, Spruit L, Saris JJ, Chang P, Pirson Y, van Ommen GJ, Peters DJ, et al. Mutation detection in the repeated part of the PKD1 gene. *Am J Hum Genet.* 1997;61(5):1044-1052.
  21. Rossetti S, Strmecki L, Gamble V, Burton S, Sneddon V, Peral B, Roy S, et al. Mutation analysis of the entire PKD1 gene: genetic and diagnostic implications. *Am J Hum Genet.* 2001;68(1):46-63.
  22. Raj S, Singh RG, Das P. Mutational screening of PKD2 gene in the north Indian polycystic kidney disease patients revealed 28 genetic variations. *J Genet.* 2017;96(6):885-893.
  23. 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.
  24. Sharma M, Reif GA, Wallace DP. In vitro cyst formation of ADPKD cells. *Methods Cell Biol.* 2019;153(93-111).