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Presence of De Novo Mutations in Autosomal Dominant Polycystic Kidney Disease Patients Without Family History

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Abstract

Background—At the University of Colorado Health Sciences Center, approximately 10% of patients with autosomal dominant polycystic kidney disease (ADPKD) on detailed questioning gave no family history of ADPKD. There are several explanations for this observation including occurrence of a de novo pathogenic sequence variant or extreme phenotypic variability. In order to confirm de novo sequence variants we have undertaken a clinical and genetic screening of affected offspring and their parents.

Study Design—Case series.

Setting and Participants—Twenty-four patients with a well-documented ADPKD phenotype and no family history of PKD and both parents of each patients.

Outcome—Presence or absence of *PKD1* or *PKD2* pathogenic sequence variants in parents of affected offspring.

Measurements—Abdominal ultrasound of affected offspring and their parents for ADPKD diagnosis. Parentage testing by genotyping. Complete screening of *PKD1* and *PKD2* genes by using

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genomic DNA from affected offspring; analysis of genomic DNA from both parents to confirm absence or presence of all DNA variants found.

Results—A positive diagnosis of ADPKD by ultrasound or genetic screening was made in one parent of four patients (17%). No *PKD1* or *PKD2* pathogenic sequence variants were identified in 10 patients (42%) while possible pathological DNA variants were identified in 4 patients (17%) and one of their respective parents. Parentage was confirmed in the remaining 6 patients (25%) and de novo mutations sequence variants were documented.

Limitations—Size of patient group. No direct examination of RNA.

Conclusion—Causes other than de novo pathogenic sequence variants may explain the negative family history of ADPKD in certain families.

Index Words

Polycystic kidney disease; Genetics; Sequence Variant; Gene

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a very common inherited disease accounting for 5% of end-stage renal disease (stage 5 chronic kidney disease) within the United States. Phenotypic variability between families is a common finding and has been ascribed to involvement of 2 causative genes, *PKD1* located on chromosome 16 and *PKD2* located on chromosome 4.[1,2] Moreover, the expressed effects of different pathogenic sequence variants within the respective genes also contributes to disease heterogeneity.[3–5]

A high de novo mutation rate of the involved genes was previously suggested based on the high percentage of ADPKD patients who have an apparent negative family history of the disorder.[6] At the University of Colorado ADPKD Research Center, approximately 10% of ADPKD patients do not report a family history of the disease. There are several potential explanations for this observation. For instance, substantial phenotypic variability has been shown to occur within the same family and may be attributed to the effects of modifer genes, environmental and other factors.[7–9] Thus, the affected parent of the proband could be asymptomatic and undiagnosed. This might be more common in patients with PKD2, in which the onset of clinically apparent disease occurs much later in life than PKD1.[10] Different parentage could also explain the absence of family history. Similarly occurrence of a de novo pathogenic sequence variant in the proband would also result in the absence of positive family history for ADPKD.

In order to identify a de novo sequence variant in an ADPKD patient, several criteria must be met. The ADPKD phenotype and pathogenic sequence variant must be established in the ADPKD proband, and both of the proband's parents, as documented by parentage testing, must not be shown to have the ADPKD phenotype and the specific pathogenic sequence variant. To our knowledge, such definitive evidence has not heretofore been documented.

Methods

Patient recruitment and clinical evaluation

All patients in this study were either participants in natural history or genetic studies of ADPKD at the University of Colorado Denver (UCD) or were recruited from our ADPKD mailing list. Patients were considered eligible for the study if they had no family history of ADPKD, and both of their parents were available and willing to undergo an abdominal ultrasound and donate a blood sample for genetic screening. Two hundred ninety-six families were reviewed from

our clinical database; among these, 8 families met the eligibility criteria and all 8 families consented to participate in the study. Sixteen additional families were recruited from our ADPKD mailing list.

The Colorado Multiple Institution Review Board approved the study protocol and informed consent was obtained from all participants. Individuals were studied on the General Clinical Research Unit at the University of Colorado Hospital or on the Pediatric Clinical Research Unit at the Childrens Hospital in Denver, CO, as previously described.[11] A patient visit included a detailed family history and physical examination, blood collection for routine chemistries, CBC, genetic testing, a urinalysis and 2 separate 24-hour urine collections. Parents were either evaluated by abdominal ultrasound in Denver or, if they were unwilling or unable to travel, arrangements were made for the ultrasounds to be performed in their local area. Our staff radiologist devised a detailed ultrasound protocol for use as a guideline by outside radiology facilities and all films were returned to UCD to eliminate inter-reader variability. A diagnosis of ADPKD was based on the number of kidney cysts detected by ultrasonography after application of the diagnostic criteria described by Ravine et al 1994.[12] All patients included in the study met the diagnostic criteria for ADPKD.

Genetic Analyses

Parentage testing, and DNA screening of the *PKD1* and *PKD2* genes were performed on all families included in the study. Genomic DNA samples were prepared from whole blood or mouthwash samples using a PUREGene extraction kit (Gentra Biosystems, Minneapolis, MN). The following microsatellite markers were used to confirm parentage: D1S2655, D4S402, D6S1567, D9S1791, D10S220, D11S1751, D12S1648, D14S77, D17S1840, D20S109, DXS7107, DYS19. DNA from both parents and offspring was amplified in 3 multiplexed polymerase chain reactions (PCR) using one fluorescently labeled primer and analyzed by electrophoresis on an automated DNA sequence analyzer (ABI 377; Applied Biosystems, Foster City, CA). Results were analyzed to confirm Mendelian inheritance in the affected offspring.

Screening of *PKD1* was performed as follows: long-range PCR was used to generate primary amplicons for exons 1–34. Exons 1, 2–7 and 8–12 were amplified using primers and conditions described by Zhang et al.[13] Advantage-GC polymerase mix (Clontech, Mountain View, CA) was used for amplifications. Primary amplicons containing exons 13–15, 16–21 and 22–34 were generated using the previously described primers and amplification conditions[14] with Master AMP Long range PCR kits (Epicenter, Madison, WI.). Individual amplicons were amplified by nested PCR from the longer fragments with amplicon-specific primers[14] using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). Exons 35–45 were amplified directly from genomic DNA using AmpliTaq Gold enzyme.[14] Exon 1 was analyzed by DNA sequence analysis on an ABI 3730 automated sequence analyzer using BigDye terminator sequencing kits (Applied Biosystems). All other amplicons were initially screened by DHPLC on a Transgenomics WAVE model 7000 with Wavemaker 4.0.32 software (Transgenomics, Omaha, NE).[14] Variant samples were sequenced in both directions and the sequence compared to the normal (wild type) NCBI GenBank nucleotide database reference sequence NM_000296.2 to confirm sequence variants. 150 normal non-ADPKD chromosomes were also screened for all newly identified sequence variants in order to distinguish common sequence variants (present in non-ADPKD individuals) from potential pathogenic sequence variants.

Mutation screening of the *PKD2* gene was undertaken using primers and conditions as previously described.[14] All amplicons were initially screened by DHPLC analysis and all samples with a variant pattern were selected for DNA sequence analysis as described for *PKD1*. The genomic reference sequence for *PKD2* was GenBank NM_000297.

Analysis of the Effects of Missense and Potential Splice DNA Variants

All protein alterations reported were deduced based on cDNA sequence. Several prediction programs were used to test the effects of DNA nucleotide substitutions resulting in missense amino acid changes or potential splice site alterations. Polyphen was utilized to predict the effects of deduced amino acid substitutions on protein structure.[15] ConSeq was used to calculate conservation scores as a relative measure of evolutionary conservation of affected amino acids (based on a range of 1–9, where a score of 1 is variable, 9 is evolutionarily conserved and 5 average).[16] Changes in splice score associated with a DNA base substitution within splice donor or acceptor sites were calculated using the Cold Spring Harbor Laboratory splice score analysis website.[17] The returned splice score compares how closely the splice site DNA sequence fits the consensus splice site sequence. The splice score for a perfect match for a 5′ site is 12.6 and the mean splice score for consecutive exons is 8.1. Lower values reflect a poorer match between the variant sequence and consensus splice site.

Statistical Analysis

Although sample sizes were small in the comparison groups, ANOVA with age and sex as covariates was used to compare each group to the control group made up of patients with a positive family history of ADPKD for continuous variables. Chi-Square test of independence or Fisher's Exact was used to analyze categorical variables between each group and the control group. Descriptive statistics are expressed as Mean ± SE or percents. In addition the median value is reported for serum creatinine, creatinine clearance and kidney volume. Results were considered significant at $p < 0.05$.

Results

Twenty-four patients with a well-documented ADPKD phenotype and parents willing to undergo abdominal ultrasonography and provide blood for genetic evaluation were included in the study.

De Novo ADPKD Mutations

Six patients were found with documented parentage in which the ADPKD sequence variant was not found in either biological parent. The demographic characteristics of these six patients are listed in Table 1. Their mean kidney volumes were increased approximately three-fold and kidney function was mildly diminished (stage 2 chronic kidney disease). There were significantly more patients in this group with severe liver cystic disease (>15 cysts of varying size with 1–24% reduction in normal parenchyma) compared to the control group of 688 patients with a positive family history of ADPKD (83 vs. 25% $p < 0.05$). Five of the patients with de novo *PKD1* sequence variants had children; among these children there was only one case of clinically confirmed ADPKD. The *PKD1* sequence variants found in these patients are shown in Table 2. Five of the six sequence variants are first reported in the present paper, and the sixth pathogenic variant was previously reported by Rossetti et al.[14] A G to T nucleotide change occurring in intron 31 at a position 1 base beyond coding nucleotide 10,217 (c.10217 +1G>T) (the reference sequence used was NM_000296.2, but all numbering of variants is based on the +1 position being the A of the ATG translation initiation codon). This substitution is predicted to destroy the splice site, with a decrement of −10.8 (difference between splice score with G; 8.4 T; −2.4) in the strength of the splice score. A C to T nucleotide change at coding nucleotide 11,173 (c.11173C>T) is predicted to have a damaging effect on protein structure based on Polyphen analysis, supporting the classification of these DNA variants as probably pathogenic (Table 2).

ADPKD Patients without Detectable Pathogenic Sequence Variants in either PKD1 or PKD2

In 10 ADPKD patients, several previously reported common sequence variants (found also in normal non-ADPKD samples) were identified; however, no known or potential pathogenic sequence variants in *PKD1* or *PKD2* were discovered. The demographic characteristics of these 10 ADPKD patients are shown in Table 1. These patients had a significantly later age at PKD diagnosis compared to the 688 patients with a positive family history of ADPKD (37 vs 25 years, $p < 0.05$).

Patients with ADPKD Phenotype and the Same Possible Pathogenic Sequence Variant in Both Proband and One Parent

In four patients a previously undescribed *PKD1* sequence variant was identified. The same sequence variant was also identified in genomic DNA from one of each patient's respective parents. These sequence variants resulted in either a missense change or had a potential effect on splicing as shown in Table 3. These sequence variants were not detected in 150 normal chromosomes. Three of the sequence variants detected in the probands from families 2712, 2365 and 1193 resulted in a missense change. The effect of these amino acid changes on protein structure was predicted to be benign in the case of c.3502C>T, which results in a deduced proline to serine change at amino acid position 1,168 (p.Pro1168Ser; protein sequence numbering includes a signal peptide) (proband 2365) and c.4546G>A, which is associated with a deduced alanine to threonine substitution at amino acid 1,516 (p.Ala1516Thr) (proband 1193). No prediction was made for c.107C>A, resulting in a deduced proline to histidine amino acid change at amino acid position 36 (p.Pro36His) detected in the proband from family 2712 (Table 3). However, an average conservation score of 5 associated with amino acid Pro36 is indicative of some evolutionary variability for this residue. A slight decrease of −1.7 (difference between splice score with C; 7.7 and T; 6.0) in the strength of the splice score was predicted for a C to T nucleotide substitution occurring in intron 37, at a position 3 bases before coding nucleotide 11,014 (c.11014–3C>T) (proband 2272) (Table 3). No other definitive *PKD1* or *PKD2* pathogenic sequence variants were identified in these patients. The demographics of these patients are also shown in Table 1. Overall patients in this category had less severe kidney disease (stage 1 chronic kidney disease) and significantly higher incidence of severe liver cystic disease compared to 688 patients with a positive family history of ADPKD (50 vs 25%, $p < 0.001$). Two women probands from this group from families 2365 and 1193 presented with very severe liver disease (grossly enlarged liver with > 15 cysts and >25% reduction in normal parenchyma).

Patients with Subsequent Clinical or Genetic Diagnosis of ADPKD in a Parent or Close Relative

In this category there were 4 patients with either genetic and/or clinical confirmation of ADPKD within their respective families. The same *PKD1* sequence variant identified in the proband was found in one parent in two study families (families 2688 and 818) as depicted in Table 4. In family 2688 a previously described *PKD1* splice site sequence variant was identified in the proband and the same sequence variant also occurred in her mother.[18] While the mother remained asymptomatic, a single kidney cyst was revealed on abdominal ultrasound examination performed at age 77. Ultrasound examination of the probands' father in family 2688 was negative. In family 818 the proband was a woman aged 41 years at time of diagnosis. The proband had biopsy-confirmed congenital hepatic fibrosis. Ultrasound examination of the presumed unaffected parents resulted in a clinical diagnosis of ADPKD in her 76-year-old father. The clinical diagnosis was confirmed by identification of the same *PKD1* pathogenic sequence variant, a deletion of coding nucleotide 12,742 (c.12742delC) in the proband and in her father.

In two additional families (2727 and 2968), a clinical diagnosis of ADPKD was made by abdominal ultrasound screening. The proband in family 2727 was a fetus who was diagnosed during a routine prenatal ultrasound examination. Subsequent abdominal ultrasound analysis of both parents resulted in a diagnosis of ADPKD in the proband's 31-year-old father. No pathogenic sequence variants were identified in the proband from family 2727. The boy proband in family 2968 presented with hematuria and was diagnosed with bilateral kidney cysts at age 13. No cysts were detected in either parent by ultrasonography at the time of his evaluation. An update of family history after a 6-year time lapse revealed a subsequent diagnosis (by ultrasound imaging) of ADPKD in a paternal aunt and cousin of the proband. However, the father remained asymptomatic and repeated abdominal ultrasound revealed no kidney cysts. A potential *PKD1* pathological sequence variant, c.10526C>T, resulting in a deduced amino acid change of threonine to methionine at protein position 3,509 (p.Thr3509Met), was identified in the proband and his father.[19,20] Analysis of the potential effect of this missense change on protein structure by PolyPhen predicts a possibly damaging effect of the resultant amino acid substitution.[15]

Discussion

There are several reasons for ADPKD patients not to provide a family history of the disease. In the present study two possibilities could be reasonably excluded. Parentage screening demonstrated that all 48 identified parents of the 24 probands were indeed the biological parents. The overall efficiency of DNA sequence screening of *PKD2* has been estimated at approximately 70% using published methods.[14] None of the 26 ADPKD patients demonstrated pathogenic sequence variants in the *PKD2* gene. A confirmed *PKD1* pathogenic sequence variant was identified in 2 of the 24 ADPKD patients whose parents were asymptomatic but had the same sequence variant as their affected child. In two additional patients a confirmed diagnosis of ADPKD was made in the parent of one patient and in an aunt and cousin of the second patient.

In 10 of the 24 patients a pathogenic sequence variant could not be found even though the phenotype supported the ADPKD diagnosis. Because of the large size and complex structure of the *PKD1* gene, pathogenic sequence variant detection is estimated at between 64–76% in all patients. Thus, these 10 patients may fall into the 36–24% category in which a pathogenic sequence variant cannot be found. The possibility of recessive PKD (ARPKD) in some of these patients should also be considered. Recent studies have demonstrated a broadened spectrum of ARPKD presentation, notably with diagnosis in adulthood and a predominant liver phenotype in these later presenting cases.[21] The milder presentation of ARPKD is notably associated with missense sequence variants in the *PKDH1* gene.[21–23] While 6 out of the 10 patients in whom no *PKD1* or *PKD2* pathogenic sequence variant was detected presented with predominant kidney symptoms including kidney cysts and hypertension, severe liver disease including hepatomegaly was present in the other 4 patients. Cholelithiasis was present in 3 patients and kidney stones in one other patient with no detectable mutation. No liver biopsy was available from these patients for histological diagnosis of hepatic fibrosis. Lastly a third, rare PKD locus has been proposed but not yet found.[24–26] Thus such a *PKD3* pathogenic sequence variant cannot be excluded in these 10 patients.

There were 4 of the 24 patients who had a parent with the same possible pathogenic *PKD1* DNA variant as the proband. Without a functional assay it is difficult to state unequivocally whether or not the patient's parent has the same ADPKD pathogenic DNA change as the child or whether these sequence variants represent rare variants unrelated to ADPKD. Three of these DNA variants resulted in an amino acid change. However, a benign effect of the amino acid change was predicted in 2 of these cases while no prediction was returned for c.107C>A (p.Pro36His) (Table 3) An average conservation score based on Conseq analysis was associated

with amino acid Pro36. This is indicative of some evolutionary variability at this residue and is not supportive of the pathogenicity of this variant. As two patients, probands 2365 and 1193, with missense DNA variants c.3502C>T (p.Pro1168Ser) and c.4577G>A (p.Ala11516Thr), respectively, had very severe liver disease (grossly enlarged liver with >15 liver cysts, and > 25% reduction in normal liver parenchyma) a possible diagnosis of adult onset ARPKD should be considered in these patients. A small decrease in the strength of the splice score associated with the potential splice variant c.11014–3C>T found in the proband from family 2272 affords modest support for the pathogenic nature of this DNA variant. However, it should be noted that as mRNA was not directly examined in the patients, so with potential splice site sequence variants the effect of these variants on splicing cannot be confirmed. It is also possible that a second pathogenic sequence variant is present in the proband from these families that was not identified in the current screening.

Thus in 18 of the 24 patients without a family history, a de novo pathogenic sequence variant could not be established. In the remaining 6 ADPKD patients a de novo pathogenic sequence variant was identified and parentage was established.

In two ADPKD patients (probands 2688 and 2968) with kidney cysts, one with the same confirmed pathogenic sequence variant in one biological parent and one with a confirmed ADPKD diagnosis in his paternal aunt and cousin, the parents had not yet presented with their phenotype as assessed by abdominal ultrasonography. Because magnetic resonance imaging (MRI) is more sensitive than ultrasonography,[27] this imaging procedure may have identified cysts in these parents with potential pathogenic ADPKD sequence variants. The phenotype variability within the same family may be due to environmental influences or genetic factors including modifier genes,[7–9] sequence variant location within the gene,[3–5] or anticipation.[28] The evidence for anticipation, however, has recently been considered to be weak.[29–31]

In summary, de novo pathogenic sequence variants have definitively been shown to occur in ADPKD patients. As other causes may explain absence of a family history in PKD patients, molecular analysis is necessary to document a de novo ADPKD pathogenic sequence variant. From the present paper it is difficult to estimate the incidence of de novo ADPKD sequence variants, but it appears to be less common than previous estimates of 10–15%. However, it is clear that there are several reasons to explain ADPKD patients without a family history other than de novo pathogenic sequence variants.

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Table 1

Comparison of demographic characteristics of patients with various sequence variant categories and patients with a positive family history of ADPKD.

	De novo Sequence variant	Unknown sequence variant	Possible pathologic DNA variant	ADPKD diagnosis in parental generation	Positive family history of ADPKD
No.	6	10	4	4	688
Women	67%	50%	75%	50%	61%
Age (years) at clinical evaluation	39 ± 1	43 ± 8	37 ± 5	26 ± 11	34 ± 16
Paternal age range (years) at clinical evaluation	65–74	50–87	58–80	31–81	
Age at diagnosis (y)	24 ± 5	37 ± 4*	36 ± 7	21 ± 7	25 ± 1
Hypertension	67%	90%	75%	50%	69%
Age at hypertension diagnosis (years)	25 ± 6	32 ± 4	39 ± 7	3[a]	31 ± 1
Serum creatinine (mg/dl) [b] (median)	1.7 ± 0.4 (1.3)	2.3 ± 0.3 (1.7)	1.3 ± 0.5 (1.3)	1.0 ± 0.5(0.7)	1.5 ± 0.1 (1.0)
Creatinine clearance (ml/min/1.73m^2) [c] (median)	80 ± 12 (69)	72 ± 9 (61)	99 ± 17 (97)	103 ± 17 (106)	83 ± 1 (86)
Renal volume (cm^3) [b] (median)	702 ± 219 (695)	698 ± 169 (918)	249 ± 310 (321)	413 ± 268 (237)	684 ± 21 (449)
Liver cysts	100%	60%	75%	25%	50%
Men[d]	100%	60%	0%	0%	44%
Women[d]	100%	60%	100%	50%	55%
Severe liver cystic disease[e] (> 15 liver cysts)	83%*	40%	50%**	25%	25%
Men[f]	50%	40%	0%	0%	18%
Women[f]	100%	40%	67%	50%	30%
Ruptured intracranial aneurysm	0%	10%	0%	0%	2.4%

Note: To convert serum creatinine in mg/dl to μmol/L, multiply by 88.4; to convert creatinine clearance in ml/min/1.73 m2 to ml/s/1.73 m2, multiply by 0.01667.

*P < 0.05

**P <0.001

[a] Age at hypertension diagnosis only available for 1 patient.

[b] Age and sex adjusted

[c] Age adjusted.

[d] % Total number of patients with liver cysts

[e] >15 cysts of varying size with evidence of 1–25% reduction in normal liver parenchyma

[f] % Total number of men or women patients with liver cysts who have severe liver cystic disease

Table 2

De novo DNA variants of presumed pathogenicity in the PKD1 gene identified in 6 patients during screening of 24 ADPKD patients without family history of ADPKD.

Sequence variant	Predicted codon change	Mutation type	Splice score	Amino acid conservation score (Conseq)	Predicted effect of amino acid change (Polyphen)	Family	Present in parent	Reference
c.323_325delAAG	p.Glu108del	In-frame deletion				1866	No	This report
c.4069delC	p.Leu1357TrpfsX9	Truncating				1203	No	This report
c.10217+1G>T		Splice	8.4[a]			2594	No	This report
c.11173T>C	p.Trp3725Arg	Missense		5[b]	Probably damaging	2820	No	This report
c.12395_12403del9	p.Leu4133_Arg4135del	In-frame deletion				1118	No	14
c.12528_12529insA	p.Pro4177ThrfsX32	Truncating				2358	No	This report

The reference sequence used in describing these variants is NM_000296.2; the numbering begins with the first nucleotide of the coding sequence (i.e. A of ATG codon is +1). Accordingly, c. 323_325delAAG describes deletion of the bases AAG starting at coding position 323 and c.10217+1G>T describes a G to T nucleotide substitution occurring in intron 31, 1 base beyond coding nucleotide 10,217. All protein alterations were deduced based on changes in DNA sequence. Protein change p.Leu1357TrpfsX9 refers to a leucine to tryptophan frameshift occurring at amino acid 1,357 resulting in a premature stop codon at the following 9th codon caused by deletion of coding nucleotide 4,069 (c.4069delC). In like manner, p.Pro4177ThrfsX32 refers to a proline to threonine frameshift occurring at amino acid 4,177 resulting in a premature stop codon at the following 32nd codon caused by insertion of A between coding nucleotides 12,528 and 12,529. (c.12528_12529insA).

[a]The splice score compares how closely the splice site DNA sequence fits the consensus splice site sequence. The splice score for a perfect match for a 5′ site is 12.6 and the mean score for consecutive exons is 8.1. Lower values reflect a poorer match between the variant sequence and consensus splice site.[17]

[b]The amino acid conservation scale reported for Conseq analysis ranges from 1–9, where a score of 1 is variable, 9 is evolutionarily conserved and 5 average.[16]

Table 3

PKD1 variants of possible pathogenicity identified in patients with no clinical diagnosis of ADPKD in either parent.

Sequence Variant	Predicted codon change	Mutation type	Splice score	Amino acid conservation score(Conseq)	Predicted effect of amino acid change (Polyphen)	Family	Present in parent	Reference
c.107C>A	p.Pro36His	Missense		1.902[b]	No prediction	2712	Yes	This report
c.3502C>T	p.Pro1168Ser	Missense		5[b]	Benign	2365	Yes	This report
c.4546G>A	p.Ala1516Thr	Missense		5[b]	Benign	1193	Yes	This report
c.11014–3C>T		Splice	6.0[a]			2272	Yes	This report

The reference sequence used in describing these variants is NM_000296.2; the numbering begins with the first nucleotide of the coding sequence (i.e. A of ATG codon is +1). Accordingly, c.107C>A describes a C to A nucleotide substitution occurring at position 107 in the coding sequence that results in substitution of the amino acid histidine for proline at protein position 36 (p.Pro36His). All protein alterations were deduced based on changes in DNA sequence.

[a]The splice score compares how closely the splice site DNA sequence fits the consensus splice site sequence. The splice score for a perfect match for a 3′ site is 14.2 and the mean score for consecutive exons is 7.9. Lower values reflect a poorer match between the variant sequence and consensus splice site.[17]

[b]The amino acid conservation scale reported for Conseq analysis ranges from 1–9, where a score of 1 is variable, 9 is evolutionarily conserved, and 5 average.[16]

Table 4

PKD1 variants identified in patients with subsequent clinical or genetic diagnosis of ADPKD in a parent or close relative.

cDNA Position	Codon Change	Mutation Type	Splice score	Amino acid conservation score (Conseq)	Predicted effect of amino acid change (Polyphen)	Family	Present in Parent	Reference
c.10526C>T	p.Thr3509Met	Missense		5[b]	Possibly damaging	2968	Yes	19,20
c.11534+2_11534+3insGGG		Splice	7.7[a]			2688	Yes	18
c.12742delC	p.Arg4248AlafsX56	Truncating				818	Yes	This report

The reference sequence used in describing these variants is NM_000296.2; the numbering begins with the first nucleotide of the coding sequence (i.e. A of ATG codon is +1). Accordingly, c.11534+2_11534+3insGGG describes an insertion of nucleotides GGG occurring in intron 41, between 3 and 4 bases beyond coding nucleotide 11,534. All protein alterations were deduced based on changes in DNA sequence. Protein change p.Arg4248AlafsX56 refers to an arginine to alanine frameshift occurring at amino acid 4,248 resulting in a premature stop codon at the following 56th codon caused by deletion of coding nucleotide 12,742 (c.12742delC).

[a]The splice score compares how closely the splice site DNA sequence fits the consensus splice site sequence. The splice score for a perfect match for a 5′ site is 12.6 and the mean score for consecutive exons is 8.1. Lower values reflect a poorer match between the variant sequence and consensus splice site.[17]

[b]The amino acid conservation scale reported for Conseq analysis ranges from 1–9, where a score of 1 is variable, 9 is evolutionarily conserved and 5 average.[16]