Evidence of a third ADPKD locus is not supported by reanalysis of designated PKD3 families

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Abstract

Mutations to PKD1 and PKD2 are associated with autosomal dominant polycystic kidney disease (ADPKD). The absence of apparent PKD1/PKD2 linkage in five published European or North American families with ADPKD suggested a third locus, designated PKD3. Here we re-evaluated these families by updating clinical information, re-sampling where possible, and mutation screening for PKD1/PKD2. In the French-Canadian family we identified PKD1: p.D3782_V3783insD, with misdiagnoses in two individuals and sample contamination explaining the lack of linkage. In the Portuguese family, PKD1: p.G3818A segregated with the disease in 10 individuals in three generations with likely misdiagnosis in one individual, sample contamination, and use of distant microsatellite markers explaining the linkage discrepancy. The mutation, PKD2: c.213delC, was found in the Bulgarian family, with linkage failure attributed to false positive diagnoses in two individuals. An affected son but not the mother, in the Italian family had the nonsense mutation, PKD1: p.R4228X, which appeared de novo in the son; with simple cysts probably explaining the mother’s phenotype. No likely mutation was found in the Spanish family, but the phenotype was atypical with kidney atrophy in one case. Thus, re-analysis does not support
the existence of a PKD3 in ADPKD. False positive diagnoses by ultrasound in all resolved families shows the value of mutation screening, but not linkage, to understand families with discrepant data.

**Keywords**

ADPKD; PKD3

**Introduction**

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary disorders with a frequency of 1:500 - 1:1000. It is characterized by the development of fluid-filled cysts in the kidneys and several extrarenal manifestations (1, 2). ADPKD is genetically heterogeneous with mutations in two genes causing the disease. PKD1, localized to 16p13.3 (3), is a large gene with 46 exons and a 12909 bp coding sequence (CDS) (4-6). The locus is complex since exons 1 - 33 lie in a region genomially reiterated as six pseudogenes, with ~98% similarity to PKD1, ~20 Mb more proximal on 16p (4, 7). The second causative gene, PKD2, is localized to 4q21 (8, 9), and has 15 exons and a CDS of 2904bp (10). PKD1 and PKD2 encode polycystin-1 (PC-1) and -2 (PC-2), respectively. PC-2 is a TRP-like calcium channel and PC-1, a cleaved, large receptor-like protein; the polycystins are thought to complex via their C-terminal tails (11, 12). The site of localization of this complex related to its role in maintaining normal renal tubular differentiation appears to be on the sensory, primary cilium, and, hence, PKD is considered a ciliopathy (13).

In screens of large clinically defined ADPKD populations, mutations have been detected in ~90% of families, and of these ~85% are PKD1 and ~15% PKD2 (14, 15). PKD1 patients have more severe disease with end-stage renal disease (ESRD) occurring on average 20yrs earlier than in PKD2 (~54yrs vs. ~74yrs) (16). Soon after PKD1 and PKD2 were mapped, a number of ADPKD families unlinked to either of these loci were described, suggesting a third locus, PKD3. These reports involved families of French-Canadian (17), Portuguese (18), Bulgarian (19), Italian (20) and Spanish (21) origin. The Italian, Spanish and Bulgarian families were described to have milder ADPKD, whereas the French-Canadian and Portuguese families had more aggressive, PKD1-like disease (22).

More recently, further complexity of ADPKD genetics has been noted. An apparently unlinked multi-generational family was revealed to have bilineal inheritance of a PKD1 and a PKD2 mutation (23). Interestingly, the two gene groups were phenotypically similar due to the PKD1 mutation being hypomorphic (24). Hypomorphic PKD1 alleles have also been found in homozygosity, associated with typical to severe ADPKD, with heterozygotes having very mild disease; an inheritance pattern that confounds linkage studies (25). Mosaicism associated with de novo mutation can also confuse linkage analysis in ADPKD (26), while mutations at other loci, notably HNF1B, can mimic an ADPKD phenotype (27). Despite the possible mechanisms described above, evidence of unlinked families and the consistent level of ~10% of ADPKD families where no mutation is identified (14, 15),
suggest further locus heterogeneity. To explore that option, we rigorously re-evaluated the previously described “PKD3” families to determine if they still supported the presence of an additional ADPKD locus.

**Results**

The described “PKD3” families were reanalyzed by linkage studies, reassessment of clinical data, collection of new samples where possible, and mutation screening of *PKD1* and *PKD2*. The locations of genetic markers within and flanking the *PKD1* gene used for linkage analysis in the original papers and additional markers analyzed here are shown (Figure 1A).

**The French-Canadian family**

The described three-generation “PKD3” family of French-Canadian origin had six affected members and linkage analysis initially excluded *PKD1* and *PKD2* (17). However, our direct sequencing of the ADPKD genes in II-3 showed a novel *PKD1* variant in exon 40: c. 11347_11348insACG, resulting in p.D3782_V3783insD (Figure 1B). This is only a moderately conserved region in the large extracellular loop of PC-1, but one not displaying indel variants between orthologs (Figure 1C). Description of a similar insertion of glutamic acid at this position was scored as Indeterminate previously (28), but has been classified as Likely Pathogenic by the ADPKD Mutation Database (http://pkdb.mayo.edu) (29).

Segregation analysis with the original DNA samples showed that III-1, III-2, III-3 and III-6 also had this variant. Previously, III-6 was defined as unaffected, whereas III-4 and III-5, who did not inherit this variant, were defined as affected. While this was consistent with the unlinked status of this family, and the questionable significance of the *PKD1* insertion, repeat ultrasounds of III-4 (at 28yrs) and III-5 (at 24yrs) showed that they were misdiagnosed as having PKD when previously analyzed at 16 and 12yrs, respectively (Figure 1D, E). Repeated ultrasound analysis of III-6 at 32yrs confirmed the negative diagnosis, but analysis of a freshly collected DNA sample did not show the p.D3782_V3783insD variant. Microsatellite marker analysis of the original III-6 DNA sample showed it was likely contaminated by the III-1 (affected) sample, explaining the aberrant result. Patient II-4, who was not originally studied, was recently diagnosed with PKD and reached ESRD at 45yrs; sequencing showed the presence of p.D3782_V3783insD (Figure 1D). Consequently, analysis of new DNA samples and the most recent clinical information showed that *PKD1*: p.D3782_V3783insD, along with a haplotype of variants, completely segregated with the disease and so was likely pathogenic (Figure 1D).

p.D3782_V3783insD first appearing in Generation II, coincident with the onset of PKD, emphasized this point. Haplotype analysis indicates that the mutation originated from the grandfather (I-2), who has the affected haplotype but not the mutation or cysts at 70yrs. It is likely that the grandfather is a germline mosaic for p.D3782_V3783insD (hence two affected offspring), but no sign of the variant in blood DNA (or the phenotype) indicates it is not in somatic tissue, although low-level somatic mosaicism cannot be completely ruled out.

**The Portuguese family- 7001PKD**

A four-generation Portuguese family with more than 20 affected members was described as apparently unlinked to *PKD1* or *PKD2* (18, 22). Subsequently, genotyping errors and/or
sample mix-ups were suggested as a large number of apparent recombinants were found in a short genetic distance (30). The authors of the original paper reconciled their findings to the misinterpretation of one marker. However, rescoring this marker and generating a new haplotype still did not show positive linkage to PKD1 or PKD2 (31).

Limitations of the original study were linkage analysis employing markers distant from the genes, plus the question of sample integrity. To circumvent these limitations, we repeated this analysis on freshly collected samples with markers intragenic or closely flanking PKD1/ PKD2 (Figure 1A). This analysis showed segregation of a PKD1 haplotype in available affected individuals (Figure 2A), except III-14, who had one cyst in each kidney and a serum creatinine of 1.0 mg/dl at 31yrs. Direct sequencing of both genes in II-9 revealed, PKD1: c.11453G>C in exon 41, resulting in a novel missense change p.G3818A (Figure 2B). Although this is a rather conservative change located between the described PC-A and PC-B domains (32) (in the same extracellular loop mutated in the French-Canadian family), glycine at this position is invariant in a wide range of PC-1 orthologs (Figure 2D) and homologs (Figure 2E). In silico analysis of this substitution employing SIFT and AlignGVGD supported its likely pathogenicity (Figure 2C) and a restriction site assay confirmed segregation in all available affected family members, except III-14 (Figure 2F). The latest available ultrasound of this patient at 36yrs still showed just two renal cysts, which probably represent simple cysts. Hence, this is likely a PKD1 family with the mutation p.G3818A, with a misdiagnosis in III-14. Many of the affected members had a mild to moderate decline in renal function by the late 30s, similar to typical PKD1 disease progression (Figure 2G), consistent with this being a fully inactivating allele.

The Bulgarian family- 7865

Linkage analysis in 22 Bulgarian families showed absence of linkage to PKD1 (Lod = −7.75) and PKD2 (Lod = −2.69) in Family 7865 (19). However, direct sequencing of II-1 showed a frame-shifting deletion, PKD2, exon 1: c.213delC resulting in p.A71fs45X (Figure 3A). This mutation segregated in II-3 and III-5 but not in III-1 and III-3 (Figure 3B), who were originally diagnosed with ADPKD based on the apparent appearance of a small number of cysts at 33 and 30yrs, respectively. Ultrasound data shows clear cysts in III-5, but no definite evidence of cysts in III-3 (Figure 3C). Both of these subjects had normal renal function when last analyzed (Figure 3D), but they have been lost to follow-up and so no new imaging or renal function reanayizes have been possible. In this case, ADPKD is due to a PKD2 truncating mutation, consistent with the milder disease within the family (Figure 3D), with the unlinked designation due to misdiagnoses by ultrasound in two sisters.

The Italian family

I-2 (mother) and II-2 (son) in the Italian “PKD3” family had multiple bilateral renal cysts on ultrasound examination at 46 and 21yrs, respectively (20). Subsequently, II-2 had nephrolithiasis and hypertension at 34yrs, whereas the mother did not show any of these symptoms by 58yrs. Linkage analysis showed both PKD1 and PKD2 haplotypes shared by the affected subjects but also an unaffected individual, II-3, excluding linkage to either of these genes (20). Recent clinical analysis of the mother at 75yrs showed a normal creatinine
level and no additional renal cysts. In contrast, her affected son, II-2, progressed to ESRD at 51yrs.

Direct sequencing revealed a pathogenic mutation, PKD1, exon 46: c.12682C>T, resulting in p.R4228X in II-2 (Figure 4A); however, this mutation was not found in the mother or other family members. Relationship testing confirmed the pedigree as shown (see Methods for details) and analysis of PKD1 intragenic SNPs was consistent with the original linkage data (Figure 4B). Suspecting mosaicism in I-2, we analyzed urine and buccal cell DNA samples by sequencing; however, we did not find the mutation. In addition, allele-specific PCR developed to specifically amplify the c.12682C>T (p.R4228X) allele amplified a 250bp PCR product only from II-2 and not I-2 (Figure 4C). This evidence suggests a de novo mutation in II-2 (with the origin of the haplotype that is mutated not determined) and simple cysts in I-2.

The significance of the rare PKD1 variant p.T2250M found in I-2 and II-1 was also explored. This variant (Figure 4D, E) was originally described as pathogenic (33) but more recent evidence found it with a pathogenic mutation in several ADPKD patients, suggesting it is not a fully penetrant mutation (http://pkdb.mayo.edu). However, Irazabal et al (34) described this change in an ADPKD patient in association with a second, weak PKD1 variant, p.S1619F, suggesting that it might be a weak hypomorphic allele. The p.T2250M variant is present in the REJ domain of PC-1 and cleavage of PC-1 at the GPS site can be affected by variants in this domain (12, 35), however, our analysis did not show that p.T2250M influences this cleavage (Figure 4E).

The Spanish family

This Spanish family was first diagnosed with possible ADPKD when II-5 presented with congenital posthydronephrotic atrophy in the left kidney (length 91mm), multiple small cysts in the right kidney (Figure 5A) and a few liver cysts at 36yrs; hypertension was diagnosed at 27yrs (Figure 5C) (21). The sister (II-1) had five and three small cysts in the right and left kidney, respectively, without hypertension at 42yrs, and the father (I-1) was diagnosed with hypertension at 67yrs with multiple small cysts in both kidneys. Original linkage analysis excluded linkage to PKD1 and PKD2 (21).

Direct sequencing of PKD1 and PKD2 in I-1 and II-5 identified no likely pathogenic mutations and analysis of intragenic SNPs confirmed lack of linkage to PKD1/PKD2 (Figure 5B). The atrophic kidney observed in II-5 prompted us to also screen HNF1B for mutations, which causes the Renal Cysts and Diabetes Syndrome (RCAD) (36). However, no likely pathogenic HNF1B variants were detected by direct sequencing or Multiple Ligation-Dependent Probe Amplification (MLPA) to detect larger rearrangements (37, 38). This mutation negative and apparently unlinked family remains unresolved, but is notable because of the mild disease and atypical presentation in II-5, questioning the ADPKD diagnosis.
Discussion

Here we have screened the five previously described unlinked ADPKD (“PKD3”) families (17-21) and showed by mutation analysis that three have PKD1, one PKD2, while one remains unresolved. As Paterson et al (30) highlighted, there are several confounders that can prevent the detection of linkage, including a single incorrect diagnosis or sample mix-up. We could add to that list the need to employ markers close to and flanking the gene, and that ~10% of ADPKD families can be traced to a de novo mutation (39, 40), and that some of these cases are mosaics (26, 41). These confounders can fully explain the mistaken classification of the families in this study. Complex inheritance of hypomorphic alleles (see Introduction and Pei et al (42)), can also manifest as unlinked ADPKD.

Of note, all four misclassified families had at least one false positive diagnosis (two had two such cases), all via ultrasound analysis. These included cysts detected during childhood that were not confirmed in adulthood (Figure 1) (17), or cases with a small number of cysts that were confirmed later but did not progress (Figures 2 and 4) (20, 22). The inability to rescreen the Bulgarian family means that the reasons for the two false positives diagnoses are unknown (Figure 3) (19). There is no doubt that ultrasound technology has developed greatly since the 1990s and that CT and MRI are now used more widely, especially to help diagnose equivocal imaging cases. Nevertheless, even with the new ultrasound diagnostic criteria (43) there are cases with one or two cysts where a definitive diagnosis cannot be made. MRI and CT can help in these cases, but the greater resolution means that even unaffected individuals display more small “simple” cysts (44-46); still leaving some cases in diagnostic limbo. Mutation screening can help, as illustrated here, and can be the gold standard if a definite mutation is detected.

Two families were traced to a new mutation within the studied pedigree. The natural inclination in a dominant disorder is to consider one of the parents as affected, even if the disease is much milder in the parent; a confounder of linkage in one family (Figure 4). It is intriguing in the French-Canadian pedigree that two individuals in the second generation had the disease and the mutation while the parents were clinically and molecularly negative (Figure 1). Germline mosaicism seems the only likely explanation for this inheritance pattern, and germline/somatic mosaicism also cannot be completely ruled out in the Italian family (Figure 4). Greater awareness about the level of de novo mutation in ADPKD, and that this probably much more often involves mosaicism than presently recognized, would be valuable when making diagnoses and determining the risk of recurrence in families with an apparent negative family history. Mosaicism is probably under-recognized because low-level alleles are difficult to identify by sequence analysis. Also, due to variability in levels between tissues/organs, the mutant allele may often be underrepresented or completely absent in leukocyte/buccal cell derived DNA (26, 41).

Sample mix-ups are difficult to completely eliminate, although good diagnostic laboratory practices should reduce them to a minimum. The advantage of mutation screening over linkage to obtain a definite diagnosis in families with contradictory data is illustrated here, where analysis of just one individual with a definite diagnosis (with no sample confusion)
can provide the diagnosis. A detected mutation can then be segregated in all family members and further imaging and resampling completed to resolve any inconsistencies.

The Spanish family is the only “PKD3” family where we did not identify a PKD1 or PKD2 mutation and we confirmed that it was unlinked to these loci; screening of HNF1B also did not reveal a mutation. This leaves open the possibility of further genetic heterogeneity in ADPKD, although the disease is rather atypical in II-5 (with kidney atrophy) and a milder course with small cysts in the other affected family members (Figure 5). Further genetic heterogeneity is also suggested by the study of large ADPKD cohorts that have consistently shown that PKD1/PKD2 mutations are not detected in ~10% of patients (14, 15). It is possible that these patients have atypical mutations to the existing genes that have not been detected or recognized as pathogenic. This could include low level mosaicism, unrecognized pathogenic missense changes at poorly conserved sites, or intronic variants beyond the regions screened by conventional screening. Additionally, allele drop out may cloud mutation identification due to the genomic duplication of PKD1 and the consequent locus-specific long-range PCR mutation screening methods, plus nested PCR. Also, gene conversion events shown to cause mutations at this locus (47, 48) may be more common than presently described but missed because they extend over one or more PKD1-specific primer. Next generation sequencing (NGS), employing fewer PKD1 specific primers, longer products covering all introns, and a single round of amplification may help to identify some of these missing mutations (48). Further characterization of the clinical, imaging and family history details of mutation negative families would be valuable to see if they represent an atypical subgroup. Such analysis would also identify multiplex families suitable for whole exome sequencing to identify further ADPKD genes. The possibility of a PKD3 gene cannot be dismissed until these cases are resolved.

Methods

Sample and data collection

The relevant institutional review boards or ethics committee approved all studies and participants gave informed consent. All of these families were published previously, with the geographic designations used here identifying their origin. New DNA samples and updated imaging and clinical data were collected as available from all the families after the original publication except from family 7865 that was lost to follow up. Urine and buccal smear samples were collected from I-2 in the Italian family. New members were added to the previously published pedigree of 7001PKD and employed for the analysis of the phenotype.

Haplotype analysis

The Portuguese family, 7001PKD was analyzed using four previously published microsatellite markers, KG8, 16AC2.5 (D16S291), CW2 (D16S663) SM6 (D16S665) (49), and a novel marker telomeric to PKD1 (Figure 1A), MC1786 (Primers MC1786-F 5′-ACGGTCTGCTGCTTAGC-3′ and MC1786-R 5′-GCCATTGTGAAAAGTGAAATAG-3′), as described previously (50).
Mutation screening and variant classification

Exonic and intragenic flanking regions of *PKD1* and *PKD2* were screened in each family by direct sequencing (14). Exons 1-9 of *HNF1B* were amplified as 9 exonic fragments (PCR primers and conditions available upon request). An MLPA protocol to look for large gene arrangements in the *HNF1B* gene was completed using the SALSA MLPA kit P241 (MRC-Holland, Amsterdam, The Netherlands) (38). Segregation analysis in each family was completed by sequencing the exonic fragment that contained the mutation. Two web-based programs, SIFT and AlignGVGD that predict the pathogenicity of amino acid variations based on the degree of conservation of the amino acid in multiple sequence alignments were used to further characterize the novel mutation found in the Portuguese family (51).

Restriction Fragment Length Polymorphism (RFLP) analysis in the Portuguese family, 7001PKD

A RFLP method exploiting the generation of a new restriction site for *Eco*O109I in the mutant sequence was developed to test the segregation of c.11453G>C change in 7001PKD. Briefly, exon 41 of the *PKD1* gene was amplified from the family members and restriction digested with *Eco*O109I for 37°C for ~ 2 hours and visualized on a 2.5-3.0% agarose gel.

Confirmation of biological relationship in the Italian family

A custom-made panel of short tandem repeats (STR) markers identical to the 13 core loci of the Combined DNA Index System (CODIS) used in forensic investigations was used to test the biological relationships within the Italian family at the Mayo Genotyping Core Facility. Briefly, a set of 14 microsatellite markers were amplified using ABI Taq Gold using 2μl multiplexes per sample. These were then run on the ABI 3730 DNA Analyzer and analyzed using the ABI GeneMapper version 4 (Applied Biosystems, USA).

Allele-specific PCR to test R4228X mosaicism and cleavage assay to determine mutagenicity of the variant T2250M in the Italian family

We developed an allele-specific PCR method to specifically amplify the c.12682C>T mutation in the Italian family as previously described (52). Briefly, a reverse primer (5′-GTAGACGTCCCTGCGCCCTGGAATGTTG-3′) was designed with its 3′-end nucleotide complimentary to the mutation (A). A mismatched nucleotide (G) was also added to the penultimate position in this primer in order to increase the specificity of this PCR. A forward primer (5′-TCCGCTTTGAAGGGATGGAGCCGCTGCCT-3′) was designed to match the wildtype sequence. The specificity of the reverse primer in this primer set only permitted amplification of the mutant allele. Western blotting and quantification for the cleavage assay was performed as described previously (35).

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Figure 1. The French-Canadian Family

A: Map of the short arm of chromosome 16 from telomere to centromere with the genetic markers(49) analyzed in the original reports (black) and those employed here (blue) indicated. The location of \textit{PKD1} is shown in red. Green scale bar shows the genomic distance. B: Top panel shows the wildtype chromatogram and bottom panel shows the c.11347_11348insACG (p.D3782_V3783insD) variant identified in affected family members. C: Multiple sequence alignment of PC-1 orthologs showing the position of insertion of the aspartic acid residue. D: Revised pedigree and haplotype data of the French-Canadian family. II-4, who was not studied in the original report, was diagnosed as affected and also found to carry the mutation. III-4 and III-5, who were originally described as affected but shown as unaffected with a repeat ultrasound, are shown in grey. Haplotype data with the segregation of microsatellite markers (KG8 and SM6), polymorphic PC-1 amino acid
changes (p.A4059V, p.I4045V and p.A3512V) and the mutation p.D3782_V3783insD are shown. E: Initial and the latest clinical and molecular diagnoses of the French-Canadian family. Negative ultrasound (-ve US), Hypertensive (HTN), mutation not detected (ND).
Figure 2. The Portuguese family
A: Partial pedigree of the four-generation Portuguese family, 7001PKD redrawn from two previously published pedigrees.(18, 22) II-15, II-20, and II-21 are drawn as described in de Almeida S. et al.(18) New members were added and some original members were taken out of the fourth generation from previous publications. III-14, who was originally reported as affected, but only had two cysts and did not inherit the p.G3818A mutation is shown in grey. The haplotype shaded in blue with the microsatellite markers MC1786, KG8, SM6, 16AC2.5 and CW2 segregates with the disease. B: Direct sequencing showing the wildtype
chromatogram and the PKD1: c.11453G>C nucleotide change and the corresponding p.G3818A amino acid change found in affected family members. C: Analysis of the likely pathogenicity of p.G3818A using SIFT and Align GVGD. VS: Variation score; MG: Mutation group. p.G3818A has a SIFT score of 0.000 and Align GVGD score of C55 which correspond to the Highly Likely Pathogenic mutation designation(25). D: Multiple sequence alignment of PC-1 orthologs showing the well-conserved glycine at position 3818 (red arrow) across a wide-variety of species. E: Multiple sequence alignment of PC-1-like proteins in humans, sea urchin and C. elegans compared with human PC-1, further showing the conservation of glycine at position 3818 (red arrow). F: Restriction digest analysis with EcoO109I confirms the c.11453G>C change only in affected individuals. Absence of an EcoO109I site in unaffected individuals (yellow fonts) results in a 288bp band after the restriction digestion of the PKD1-exon 41 PCR product, whereas affected individuals (red fonts) have two bands of 174 and 114bp. G: Estimated glomerular filtration rate (eGFR) in affected individuals calculated using the latest available serum creatinine measurements showing a decline in eGFR in 7001PKD family members by the late third or early fourth decade (blue squares). A reference trend line showing typical PKD1 progression was generated by analyzing 106 ADPKD patients with definitely pathogenic PKD1 mutations.
Figure 3. The Bulgarian Family
A: Top panel shows the wildtype PKD2 chromatogram and the bottom panel the frameshifting mutation c.213delC, resulting in p.A71fs45X. B: Revised pedigree and haplotype of the previously described Bulgarian family including the PKD2 SNP p.R28P. III-1 and III-3 who were previously described as affected but do not carry the mutation are shown in grey. C: Ultrasound images of the right kidney from III-3 (misdiagnosed with ADPKD) and the right kidney of III-5 (confirmed diagnosis of ADPKD), showing a few cysts (blue arrows). D: Table showing the age at renal function analysis by serum creatinine measurement and expressed as eGFR, plus the mutation status of family members. Mutation not detected (ND).
Figure 4. The Italian family

A: Top panel shows the wildtype sequence found in I-2. Bottom panel shows the c.12682C>T nucleotide change and the corresponding p.R4228X amino acid change found in II-2. B: Revised pedigree and haplotype data of the two-generation Italian family. I-2, who was originally reported as affected is shown in grey. Segregation of the genetic markers KG8, 16AC2.5, SM7 and 26.6, as previously reported, PKD1 intragenic variants (c.12570C>T and p.T2250M), and the p.R4228X mutation, are also shown. C: Allele-specific PCR to amplify the mutant p.R4228X allele amplified a 250bp product only from II-2 and not from blood, urine or buccal cell samples of I-2. D: Multiple sequence alignment of PC-1 orthologs showing position 2250, where there is a T>M change in the mother (I-2) with a few cysts and II-1. E: Cleavage analysis at the GPS domain of PC-1 in wildtype (WT), the variant found in I-2 and II-1 (T2250M) a the previously described (35) hypomorphic allele (R2220W) and the completely cleavage mutant (E2771K) (n=12 transfections). FL= full length PC-1 and CTP= C-terminal product. Statistical analysis were done using Student’s t test (***P<0.001). Error bars represent ± SD.
Figure 5. The Spanish family
A: Ultrasound image of the right kidney in II-5 showing a few cysts consistent with a diagnosis of ADPKD (blue arrows). B: Pedigree of the Spanish family with the intragenic PKD1 and PKD2 SNPs identified through direct sequencing. C: Initial and latest diagnoses in the Spanish family.