Nephrol Dial Transplant (2004) 1 of 7
DOI: 10.1093/ndt/gfh083

Nephrology Dialysis Transplantation

Original Article

PKD2 mutations in a Czech population with autosomal dominant polycystic kidney disease

Jitka Štekrová[1], Jana Reiterová[2], Miroslav Merta[2], Jirt Damborský[3], Jana Židovská[1], Vera Kebrdlová[1] and Milada Kohoutová[1]

[1]Department of Biology and Medical Genetics and [2]Department of Nephrology, Charles University, Prague and [3]National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic

Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) is genetically heterogeneous and caused by mutations in at least three different loci. Based on linkage analysis, mutations in the PKD2 gene are responsible for ~15% of the cases. PKD2-linked ADPKD is supposed to be a milder form of the disease, its mean age of end-stage renal failure (ESRF) ~20 years later than PKD1.

Methods. We screened all coding sequences of the PKD2 gene in 115 Czech patients. From dialysis centres in the Czech Republic and from the Department of Nephrology of the General Hospital in Prague, we selected 52 patients (29 males, 23 females), who reached ESRF after the age of 63, and 10 patients (three males, seven females) who were not on renal replacement therapy at that age. The age of 63 was used as the cut-off because it is between the recently published ages of onset of ESRF for PKD1 and PKD2. From PKD families we also selected 53 patients (26 males, 27 females) who could be linked to either the PKD1 or PKD2 genes by linkage analysis. An affected member from each family was analysed by heteroduplex analysis (HA) for all 15 coding regions. Samples exhibiting shifted bands on gels were sequenced.

Results. We detected 22 mutations (six new mutations)—14 mutations in 62 patients (23%) with mild clinical manifestations, eight in 53 families (15%) with possible linkage to both PKD genes. As the detection rate of HA is ~70–80%, we estimate the prevalence of PKD2 cases in the Czech ADPKD population to be 18–20%. We identified nonsense mutations in eight patients (36.5%), frameshifting mutations in 12 patients (54.5%) and missense mutations in two patients (9%).

Conclusion. In this study in the Czech population we identified 22 mutations (six of which were new mutations). The prevalence of PKD2 cases was 18–20% and the mean age of ESRF was 68.3 years. An at-least weak hot spot in exon 1 of the PKD2 gene was found.

Keywords: autosomal dominant polycystic kidney disease; germline mutations; heteroduplex analysis; PKD2 gene

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited renal cystic disorder, with a prevalence of 1:500–1:1000. It is a systemic disorder, with cysts and connective tissue abnormalities involving many organs. The progressive formation and enlargement of renal cysts causes decline of renal function. The disease is genetically heterogeneous. At least three different genes are involved. The PKD1 locus (MIM 601313), is linked to the short arm of chromosome 16, at 16p13.3, codes the protein polycystin-1. Later, a second locus, PKD2 (MIM 173910), coding the protein polycystin-2, was localized to 4q13-23. The PKD3 gene has not yet been identified. Two independent teams recently cloned the PKDH1 gene (polycystic kidney and hepatic disease), located in the critical region on chromosome 6p21.1-p12 [1]. The gene is the cause of autosomal recessive polycystic kidney disease (MIM 263200). By now, more than 60 mutations have been identified in the PKDH1 gene. The gene encodes the protein named polyductin (or fibrocystin), which is composed of 4074 amino acids that form a large extracellular domain, a single transmembrane segment and a short C-terminal tail. Recently, truncating mutations in the PRKCSH gene, which encodes the already described protein kinase C substrate, 80K-H (or the non-catalytic

Correspondence and offprint requests to: Jitka Štekrová, Institute of Biology and Medical Genetics, General Hospital, Prague, Czech Republic. Email: jstek@lf1.cuni.cz

β-subunit of glucosidase II), have been identified as causes of autosomal dominant polycystic liver disease in three large, independent families [2].

The PKD2 gene consists of 15 exons. So far, more than 60 different germline mutations have been identified in it. Mutation screening of the PKD2 gene has usually been accomplished by single-strand conformation polymorphism (SSCP), heteroduplex analysis (HA) or by direct sequencing. Denaturating, high-performance liquid chromatography was recently used as a reliable detection method for both PKD genes. Mutations are dispersed over the entire PKD2 gene with no particular hot spots. Considered hot spots are CpG dinucleotides (predominantly in exon 1), which lead through cytidine methylation and subsequent deamination to thymidine.The 5.4 kb PKD2 transcript encodes the protein polycystin 2 (PL-2), which consists of 968 amino acids. The bulk of PL-2 is located in the endoplasmatic reticulum. PL-2 is a non-selective cation channel that can conduct calcium ions; it also shares structural features with transient receptor potential channels. The C-terminal domain of PL-2 interacts with PL-2 (homodimerization) and with PL-1. Deletions in the C-termini of either proteins prevent this interaction. In addition, the interaction of PL-2 with PL-1 inhibits G-protein signalling [3]. The C-terminus domains contain a motif, known as EF hands, which can bind calcium. The polycystin L (product of the PKD2 homologue PD2L gene), PRKSCH protein and PKHD1 gene in mice contain EF hands. Due to the truncating mutation (R742X), the mutant PL-2 lacks EF hands, resulting in the deficient sensitivity to changes of intracellular Ca^{2+} concentration of the Ca^{2+} permeable cation channel that is formed by the mutant PL-2 [4]. Loop 5 of PL-2 was found to interact with the protein Hax-1, which associates with the F-actin-binding protein, cortactin, in a yeast hybrid [5]. The C-terminal domain of PL-2 interacts directly with the tropomyosin-1 and with the cardiac troponin I. These associations suggest a link between the PL-2 and the actin cytoskeleton. One of the few missense mutations that corresponds to the S3 membrane-span, D511V in exon 6, resulted in the complete loss of the K^+ channel activity [6]. Since the mutations in exons 1–5 in the first large extracellular loop result in the most severe phenotype [7], we suspect that this part of PL-2 has an important role in interactions with other proteins. Truncating mutations in this region probably result in an insufficient amount of functional protein, or missense variants that could preferentially influence the interactions with other proteins and impair folding of the PL-2.

Of the cases of ADPKD, 85% are caused by the mutation of the PKD1 gene on chromosome 16; in ~15% of cases the PKD2 gene on chromosome 4 is mutated, according to linkage analysis. The prevalence of PKD2 mutations was 39.1% among selected ADPKD patients who reached end-stage renal failure (ESRF) at the age of 63 or later [8]. PKD2 patients compared with PKD1 patients seem to have milder clinical courses. The cumulative probability of survival is significantly shorter for PKD1 than PKD2 patients (median ages: for PKD1, 53.0 years; for PKD2, 69.1 years). In addition, it had been shown that PKD2 mutations are responsible for a 10 year reduction in median survival (death defined as death or onset of ESRF) compared with the survival of persons without ADPKD [9].

The aim of our study was to establish the prevalence of ADPKD caused by PKD2 mutations in the Czech population. The goal was to find the real prevalence of PKD2 cases. We screened a large ADPKD group without clinical manifestations for possible linkage to both PKD genes. A second group of patients with mild clinical courses were chosen because of the expected higher prevalence of PKD2 cases among them, to search for new and more highly probable PKD2 mutations or hot spots of mutation. Furthermore, we tried to observe the clinical courses of patients with a defined PKD2 mutation.

Subjects and methods

We screened all coding sequences of the PKD2 gene in 115 Czech patients. Informed consent was obtained from all individuals involved. We selected, from dialysis centres in the Czech Republic, 40 ADPKD patients (HD 1–40), 12 ADPKD patients (HD 41–52) from the Transplantation Center in Prague, and 10 patients with mild renal insufficiency (HD 53–62) from the Department of Nephrology in Prague. This group together (HD 1–62) included 52 patients (29 males, 23 females) who reached ESRF after 63 years of age and 10 patients who were not on renal replacement therapy at that age (three males, seven females). The age of 63 years was used as the cut-off value because it is between the ages of onset of ESRF for PKD1 and PKD2 published in recent studies. The patients were not related, to our knowledge, and were Caucasian. From PKD families we selected 53 patients (26 males, 27 females) who could be linked to either the PKD1 or PKD2 genes by linkage analysis. We did not have any clear information about the clinical status of those individuals or their families. These families were first analysed by linkage analysis using four CA-repeat markers for the PKD1 gene (16AC2.5, CW2, CW3D, KG8) and four CA-repeat markers for the PKD2 gene (D4S231, D4S414, D4S1534, D4S1563, AICA1, AICA4, JSTG3, JSTG4). Asymptomatic at-risk individuals were examined by ultrasonography, according to the criteria described by Ravine. In most patients, the onset of arterial hypertension was established retrospectively. Hypertension was defined as blood pressure higher than 140/90 mmHg, repeatedly measured, or normal blood pressure maintained by the use of antihypertensive drugs.

Blood samples were obtained from all participants, and genomic DNA was extracted from peripheral blood lymphocytes following standard procedures. The 15 coding exons and adjacent splice junction sites of the PKD2 gene of one affected member from each family were amplified by the polymerase chain reaction using a set of 17 primers described previously [11]. One affected member from each family was analysed by HA [12].

DNA sequencing

Samples that exhibited shifted bands on MDE gel were amplified and after purification sequenced in both directions on an automatic fluorescent sequencer, ABI Prism™ 310 Genetic Analyzer (PE Applied Biosystems), according to the manufacturer's instructions.

One other affected member from each family was also sequenced. The segregation of the mutation with the disease in each family was tested by either HA or by sequencing.

Results

Mobility shifts were detected in 22 probands. Three new mutations in a Czech population (families 185, 37, 195) have been described already [12]. We identified six new mutations. The mutations are spread throughout the gene. We found 14 mutation in 62 patients (23%) who either had reached ESRF after the age of 63 or who had not reached ESRF at that age. We identified eight mutations in 53 families (15%) with probable linkage to both PKD genes. The mutations/polymorphisms are summarized in Table 1 and Figure 1. The ages at onset of ESRF, or serum creatinine levels and the presence of hypertension are given in Table 2.

Nonsense mutations

We identified nonsense mutations in eight patients (36.5%). The nonsense mutation (145 C > T) in exon 1 was identified in two patients with mild renal insufficiency—65 and 74 years old. So far as we could determine, their families were not related. But, common haplotypes were present, suggesting an ancestral relationship.

The nonsense mutation (478 C > T) in exon 1 had already been found in Czech family 185 [12]. We established the presence of this mutation in three new probands from the dialysis centres. The comparison of haplotypes does not exclude a common ancestor.

The nonsense mutation (916 C > T) in exon 4 that substitutes an arginine for a stop codon at codon 306 in dialysed patient 71 has been described already in three Bulgarian families [13] and in one Czech family with mild clinical courses [12]. We found common haplotypes in two Czech families; the Bulgarian families were not available for this analysis.

The last identified nonsense mutation (1249 C > T) in exon 5 was established in a family with mild clinical manifestation. The oldest grandmother had a creatinine of 260 μmol/l at 73 years.

Mutations causing frameshift

We identified frameshifting mutations in 12 patients (54.5%). We identified the insertion of a cytosine (198–230 ins C) in seven non-related Czech patients; two patients came from families with mild clinical courses, the rest were patients from dialysis and transplantion programmes. These families did not share common haplotypes.

In exon 4, we identified a new deletion of one cytosine (1078–1081 del C) in the stretch of three cytosines producing a stop after codon 373, which induces 13 other amino acids.

The insertion of seven bases (1339–1345 ins GCAACAG) in exon 6 was detected in one dialysed patient. This mutation was already desribed in a Czech family clearly linked to the PKD2 gene [12]. These two families share the common haplotypes.

The new deletion of one thymidine (1946–1947 del T) in exon 9, causing a stop after codon 671, which induces 24 other amino acids, was identified in a family with mild clinical manifestations.

Table 1. Mutations identified in the PKD2 gene

Family	Exon	Sequence change	Codon change	Reference
HD 54	1	145 C > T	Q49X	New mutation
HD 62	1	145 C > T	Q49X	New mutation
Family 233	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
Family 241	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
HD 10	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
HD 22	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
HD 58	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
HD 52	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
HD 47	1	198–203 ins C	Frameshifting after codon 68	Torra *et al.* [15]
HD 18	1	478 C > T	Q160X	Reiterová *et al.* [12]
HD 19	1	478 C > T	Q160X	Reiterová *et al.* [12]
HD 53	1	478 C > T	Q160X	Reiterová *et al.* [12]
Family 185	1	478 C > T	Q160X	Reiterová *et al.* [12]
Family 37	4	916 C > T	R306X	Veldhuisen *et al.* [13]
Family 71	4	916 C > T	R306X	Veldhuisen *et al.* [13]
HD 2	4	917 G > A	R306Q	New mutation
Family 172	4	1078–1081 del C	Frameshifting after codon 373	New mutation
HD 55	5	1258 A > G	R420G	New mutation
HD 26	6	1339–1345 ins 7 bp	Frameshifting after codon 471	Reiterová *et al.* [12]
Family 122	9	1946–1947 del T	Frameshifting after codon 672	New mutation
Family 195	10	2050–2053 del TACT	Frameshifting after codon 685	Reiterová *et al.* [12]
HD 31	11	2208–2213 del AAACTT	Deletion of codon 736, 737	New mutation

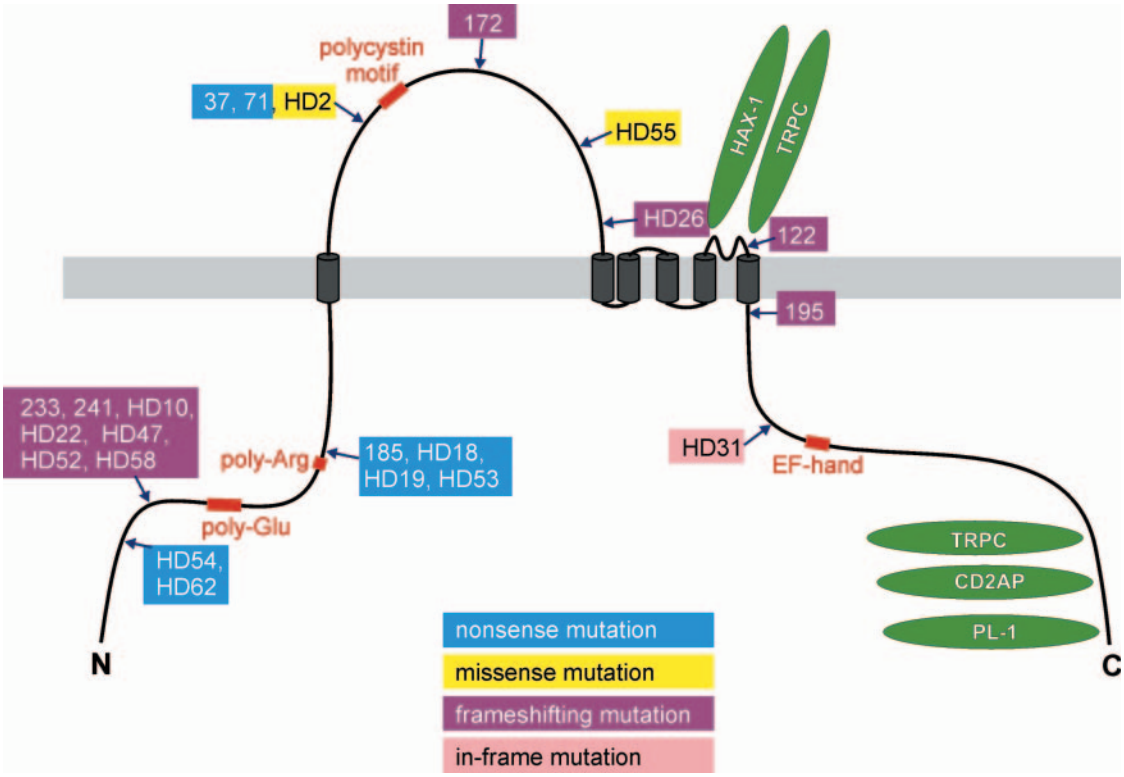

Fig. 1. PL-2 protein topology with identified mutations. Protein is in black (cylinders represent transmembrane helices), membrane is in grey, conserved motifs are in red, mutations are in frames in different colours and interacting proteins are in green (PL-1, TRPC, CD2AP and HAX-1). Assigment and description of mutations is presented in Table 1.

In-frame deletion

The last new deletion of six bases (2208–2213 del AAACTT) leading to the deletion of two amino acids without frameshifting was found in a dialysed patient. The analysis of the PL-2 protein sequence indicates the presence of an EF-hand domain in position 763–774. This type of domain consists of a 12 residue loop flanked on both sides by a 12 residue α-helical element. The mutation HD 31 results in the deletion of residues 736 and 737, located in a helix preceeding both the EF-hand loop and the flanking helices. The helix, truncated due to deletions, in our view is too far from the EF-hand loop to exert a direct effect on its structure and function. Secondary effects due to large-scale conformational changes, modified helix to helix interactions, or changes in the dynamics of the protein backbone, however, cannot be ruled out.

The deletion segregated with the disease in family HD 31. The clinical course in the family was mild. The female proband reached ESRF at age 66. She had been on two antihypertensive drugs since she was 60, when renal insufficiency was already present. Her diagnosis was incidentally established by ultrasound before cholecystectomy at the age of 60. Other renal complications (haematuria, proteinuria, urolithiasis) were not present. As for extrarenal complications, three hepatic cysts were detected under ultrasound examination, with normal liver function. On echocardigraphy, there was only mild hypertrophy of the left ventricle without valvular defect. Because of cerebral aneurysms, magnetic resonance imaging was not performed. Her 40-year-old daughter has normal renal function, blood pressure and echocardiography, and no hepatic cysts. An ultrasonogram showed multiple cysts smaller than 3 cm in both of her kidneys.

Missense mutations

We found two new missense mutations (9%). The missense mutation (917 G > A) in exon 4, leading to the change from positively charged arginine to the polar, uncharged amino acid glutamine, was identified in one dialysed patient. This substitution segregated with the disease in the family (three affected persons). No other similar change was observed in 100 unrelated individuals tested by HA.

The last missense mutation (1258 A > G) in exon 5 was identified in a dialysed patient. This substitution leads to the change from a positively charged arginine to the special amino acid glycine. Glycine may adopt different conformations, which can result in a new folding of this loop followed by the impaired function of the protein. This substitution segregated with the disease in the family (four affected persons). In the 100 unrelated individuals tested by HA, no similar change was observed. Arginine in exons 4 and 5 is the conservative amino acid in the murine model of PL-2.

Table 2. Clinical features of affected family members

Family	Age of affected member (years)	Sex	Renal function, serum creatinine (μmol/l)	Blood pressure
HD 54	74	F	200	HT at 50
HD 62	64	F	N	HT at 58
	28, 30	M, F	N	N
Family 233	64	M	ESRF	HT at 60
	40	M	N	HT at 35
	15, 9	F, F	N	N
Family 241	58	M	N	HT at 35
	60	M	N	HT at 40
	28	M	ESRF	HT at 18
	24	M	N	HT at 24
HD 10	67	F	ESRF	HT at 65
HD 22	65	F	ESRF	HT at 65
HD 58	72	M	270	HT at 58
HD 52	65	M	ESRF	HT at 65
HD 47	68	M	ESRF	HT at 60
HD 18	70	F	ESRF	HT at 60
HD 19	80	M	ESRF	HT at 60
HD 53	70	F	ESRF	HT at 54
	52	F	N	N
	33	F	N	N
Family 185	69	F	91	HT at 63
	40	M	N	HT at 44
Family 37	64	F	340	HT at 48
	40	M	N	N
Family 71	70	M	ESRF	N
	48	M	124	HT at 44
	4	F	N	N
HD 2	67	F	ESRF	HT at 65
Family 172	65	F	448	HT at 55
	41	M	N	HT at 28
	36	M	N	N
HD 55	66	M	N	HT at 60
	29	M	ESRF	N
HD 26	68	M	ESRF	HT at 68
	68	F	ESRF	HT at 68
Family 122	60	F	N	HT at 45
	38	M	N	HT at 38
Family 195	50	F	N	HT at 50
	31	M	N	N
	24	M	N	N
HD 31	66	F	ESRF	HT at 60
	40	F	N	N

HT, hypertension; N, normal blood pressure and normal renal function (defined as serum creatinine under 110 μmol/l); F, female; M, male.

Polymorphisms

We observed polymorphisms in exon 4 in IVS3 (844–22 G > A) and in exon 1, the R28P.

Persons with early ESRF and PKD2 mutation

We found one person with ESRF at the age of 28. He is from family 241 with a mutation in exon 1 (198–203 ins C). He has severe hypertension (~200/110 mmHg without therapy) controlled by five antihypertensive drugs.

His renal angiography excluded stenosis of the renal artery, and the adrenal glands examined by CT scan were normal. His urinalysis was normal, without proteinuria or microalbuminuria. His father and his uncle have normal renal functions at the ages of 58 and 60; both have hypertension, sufficiently treated since young adulthood. His unaffected mother suffers from severe hypertension controlled with five antihypertensive drugs, her renal function is normal at the age of 55. His younger affected brother (24 years), with normal renal function, has hypertension treated by two antihypertensive drugs. Severe, insufficiently treated hypertension eventually contributed significantly to ESRF in the 28-year-old individual.

In addition, we have identified a patient with the missense mutation (1258 A > G) in exon 5 and ESRF at the age of 28. His father, aged 66, has only mild renal insufficiency, and has the same missense mutation in exon 5 as his son. The patient with early ESRF did not suffer from hypertension, and his urinalysis was normal. His mother died suddenly at age 45, probably because of unrecognized uraemia. During her autopsy, enlarged polycystic kidneys were found. So, her severe clinical course probably was caused by inherited

mutations in both the PKD1 (the mutation in PKD1 was not analysed) and PKD2 genes.

In eight patients with proven PKD2 mutations, echocardiography found no significant valvular or septal abnormalities. Diastolic dysfunction was present in five of them, who were hypertensive.

Discussion

We identified 22 mutations, six of them new mutations, in a group of 115 patients. We found 14 mutations in 62 patients (23%) who had onset of ESRF after the age of 63 or who, at that age, had not reached ESRF. We identified eight mutations in 53 families (15%) with possible linkage to both PKD genes (without known clinical manifestations). The number of PKD2 patients may be underestimated because the mutation could lie in the promoter region or within intronic parts of the gene, resulting in splicing impairments, or the mutation could be due to a large rearrangement. The detection rate of SSCP is ~70–80%. In a recent study, we used both methods to detect PKD2 mutations, and found the same detection rates [12]. As no detection method is 100% reliable, we estimate an overall 18–20% prevalence of PKD2 cases in the general Czech ADPKD population. This prevalence is greater than the 15% previously proposed based on linkage studies—probably underestimated because many families were not clearly linked to the PKD1 or PKD2 gene, and perhaps also because some PKD2 patients may be unaware of their disease, because of milder clinical manifestations. On the other hand, the prevalence, ~23%, of PKD2 patients in the selected group of patients with onset of ESRF after the age of 63, or with mild clinical courses, is much lower than the 39.1% in the study by Torra *et al.* [8]. We found no clear explanation for these discordant findings, but fewer patients (48 *vs* 62) could partly explain the differences. Moreover, the mean age of ESRF in the Czech population was slightly lower (68.3 ± 4 years) than the 74 years published by Hateboer *et al.* [9]. We probably should have used a slightly lower cut-off value than 63 years for ESRF.

The mean age of ESRF in our group was 68.3 ± 4 years (64–80 years). In addition, seven patients did not achieve dialysis parameters at age 63 years or later (mean age 69.2 ± 4.3 years). We could speculate about the influence of the predialysis care of ADPKD patients in the Czech Republic, about antihypertensive therapy, and about environmental factors. In any event, all our subjects with defined mutations had sufficient antihypertensive therapy, with blood pressures repeatedly under 130/80 mmHg. All our PKD2 patients had mild clinical manifestations (considering their ages at onset of ESRF) of their diseases, without any differences related to the type and localization of mutations within the PKD2 gene.

We found two patients with defined PKD2 mutations and early onset of ESRF. The rest of both of their families had milder clinical courses. One patient with onset of ESRF at the age of 28 suffered from severe hypertension. We speculate that that may have contributed to his severe clinical course, but other factors causing intra-familial variability must be further studied. The severe clinical course of a second patient with onset of ESRF at 29 is probably caused by inherited mutations in both the PKD1 (the mutation in PKD1 was not analysed) and PKD2 genes. Two individuals, trans-heterozygous for both mutations and with more severe renal cystic diseases (with ESRF at ages 48 and 58) have already been described [9]. Recently, somatic mutations in the PKD2 gene have been described in cysts with a PKD1 germline mutation. This finding suggests that the PKD genes can also be modifying genes. It is probable that, in the presence of the reduced dosage of the second polycystin protein, a critical threshold of activity is reached earlier, resulting in cyst formation.

We identified nonsense mutations in seven patients (36.5%), frameshifting mutations in 12 patients (54.5%) and missense mutations in two patients (9%). All nonsense mutations were caused by the substitution of cytosine by thymine. Cytosine is prone to methylation followed by deamination, leading to thymine. We identified the insertion of a cytosine in exon 1 in six non-related Czech patients. (This mutation was also found as a germinal mutation in a British patient.) In addition, this insertion in the stretch of five cytosines was established as a somatic mutation in seven cysts in an inherited healthy allele [15]. The probable mechanism of this is DNA slipping during replication in the cytosine stretch. This seems to be a hot spot for mutations, and was not found in more patients probably because, in some cases, the analysis of exon 1 was not systematic. The deletion of thymidine, causing frameshifting after codon 672 in exon 9, in a family with mild clinical manifestations (normal glomerular filtration rates at age 60), is the first mutation defined in exon 9. The exons 4, 5 and 6, encoding for a region representing 24.2% of the PKD2 coding sequence, harbour 35% (7/20) of germline mutations. Two missense mutations were also found in exons 4 and 5, in the first extracellular loop of polycystin 2, which is very large and seems to be important for interactions with other molecules and for the folding of the protein in the membrane [7].

PKD2 homozygous mice died *in utero* and as well as renal and pancreatic cysts, they had defects in cardiac septation [16]. Mitral valve prolapse is a characteristic finding in ADPKD patients. Other valvular or structural cardiac defects were not found significantly more frequently in the ADPKD population. We did not observe severe valvular disease or defects in cardiac septation in eight patients with the PKD2 mutation.

The types of mutations identified in this study can be discussed in terms of structure–function relationships. Since the three-dimensional structure of PL-2 is unknown, and cannot be reliably predicted using available modelling tools, its possible effects can only be related to protein topology. Figure 1 presents the

putative topology of the PL-2 protein, localization of mutations within the protein sequence and interactions of PL-2 with other proteins. Our literature search revealed that PL-2 interacts with at least four different proteins: polycystic kidney disease type I protein (PL-1) [17]; transient receptor potential channel (TRPC) [18]; CD2-asociated protein (CD2AP) [19]; and the protein associated with the actin cytoskeleton (HAX-1) [5]. HAX-1 protein interacts with PL-2 in the region of the calcium channel, located between transmembrane helices 5 and 6, while the TRPC protein interacts both in the region of the calcium channel and in the C-terminal domain. PKD1 and CD2AP interact in the C-terminal domain only. None of the point mutations reported in this study seem to occur in the regions important for protein-to-protein interactions; therefore, mainly frameshifting mutations will affect this important function of PL-2. All but the mutation in family 195 will result in truncated proteins unable to realize any of the four intermolecular interactions. The mutation in family 195 might result in protein influencing the interaction with HAX-1, but not with any of the other three proteins. Furthermore, all truncated proteins produced by the translation of PKD2 genes carrying frameshifting mutations will be unable to bind Ca^{2+} due to the absence of the calcium-binding domain known as the EF hand.

In conclusion, we established an 18–20% prevalence of PKD2 in the Czech Republic among ADPKD cases without known clinical manifestations and a 23% prevalence in a group of ADPKD patients with relatively milder clinical courses. The identification of new mutations could help in the future to more clearly characterize the functional areas of PL-2. The cytosine insertion in the cytosine stretch of exon 1, identified in seven non-related Czechs, could be an at-least weak hot spot of mutation in the PKD2 gene. The mean age at onset of ESRF in PKD2 patients in the Czech population was 68.3 ± 4 years. We also described two cases with severe forms of ADPKD and PKD2 mutations. The course of the disease may have been influenced by severe hypertension in one of them, but establishing the proof is complicated because of difficulties with the interpretation of the renal angiogram and because it is virtually impossible to do renal biopsies in ADPKD patients.

Acknowledgements. We are grateful to the families and their physicians, and to clinical geneticists for their cooperation. This work was supported by grant IGA MZ ČR NE 7633.

Conflict of interest statement. None declared.

References

1. Onuchic LF, Furu L, Nagasawa Y *et al*. PKDH1, the polycystic and hepatic kidney disease 1 gene encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats. *Am J Hum Genet* 2002; 70: 1305–1317
2. Li A, Davila S, Furu L *et al*. Mutations in PRKCSH cause isolated autosomal dominant polycystic liver disease. *Am J Hum Genet* 2003; 72: 691–703
3. Parnell SC, Magenheimer BS, Maser RL *et al*. The polycystic kidney disease-1 protein, polycystin-1, binds and activates heterotrimeric G-proteins in vitro. *Biochem Biophys Res Commun* 1998; 251: 625–631
4. Vassilev PM, Guo L, Chen XZ *et al*. Polycystin-2 is a novel cation channel implicated in defective intracellular Ca 2+ homeostasis in polycystic kidney disease. *Biochem Biophys Res Commun* 2001; 282: 341–350
5. Gallagher AR, Cedzich A, Gretz N, Somlo S, Witzgall R. The polycystic kidney disease protein PKD2 interacts with Hax-1, a protein associated with the actin cytoskeleton. *Proc Natl Acad Sci USA* 2000; 97: 4017–4022
6. Reynolds DM, Hayashi T, Cai Y *et al*. *J Am Soc Nephrol* 1999; 10: 2342–2351
7. Hateboer N, Veldhuisen B, Peters D *et al*. Location of mutations within the PKD2 gene inluences clinical outcome. *Kidney Int* 2000; 57: 1444–1451
8. Torra R, Badenas C, Pérez-Oller L *et al*. Increased prevalence of polycystic kidney disease type 2 among elderly polycystic patients. *Am J Kidney Dis* 2000; 36: 728–734
9. Hateboer N, van Dijk MA, Bogdanova A *et al*. Comparison of phenotypes of polycystic kidney disease types 1 and 2. *Lancet* 1999; 353: 103–107
10. Ravine D, Gibson RN, Walker RG *et al*. Evaluation of ultrasonographic diagnostic criteria for autosomal dominant polycystic kidney disease 1. *Lancet* 1994; 343: 824–827
11. Hayashi T, Mochizuki T, Reynolds DM, Wu GQ, Cai Y, Somlo S. Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). *Genomics* 1997; 44: 131–136
12. Reiterová J, Štekrová J, Peters DJM *et al*. Four novel mutations of the PKD2 gene in Czech families with autosomal dominant polycystic kidney disease. *Human Mutat MIB* 2002; 506 (Online)
13. Veldhuisen B, Saris JJ, de Haij S *et al*. A spectrum of mutations in the second gene for autosomal dominant polycystic kidney disease (PKD2). *Am J Hum Genet* 1997; 61: 547–555
14. Torra R, Viribay M, Telleria D *et al*. Seven novel mutations of the PKD2 gene in families with autosomal dominant polycystic kidney disease. *Kidney Int* 1999; 56: 28–33
15. Koptides M, Hadjimichael C, Koupepidou P, Pierides A, Constantinou Deltas C. Germinal and somatic mutations in the PKD2 gene of renal cysts in autosomal dominant polycystic kidney disease. *Hum Mol Genet* 1999; 8: 509–513
16. Wu G, Markowitz GS, Li L *et al*. Cardiac defects and renal failure in mice with targeted mutation in Pkd2. *Nat Genet* 2000; 25: 75–78
17. Qian F, Germino FJ, Cai Y, Zhang X, Somlo S, Germino GG. PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat Genet* 1997; 16: 179–183
18. Tsiokas L, Arnould T, Zhu C, Kim E, Walz G, Sukhatme VP. Specific association of the gene product of PKD2 with the TRPC1 channel. *Proc Natl Acad Sci USA* 1999; 96: 3934–3939
19. Lehtonen S, Ora A, Olkkonen VM *et al*. *In vivo* interaction of the adapter protein CD2-associated protein with the type 2 polycystic kidney disease protein, polycystin-2. *J Biol Chem* 2000; 275: 32888–32893

Received for publication: 4.2.03
Accepted in revised form: 5.11.03