® Postepy Hig Med Dosw (online), 2013; 67: 157-163
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2013.01.09
Accepted: 2013.02.13
Published: 2013.03.07

Novel PKD1 mutations – the effect on clinical phenotype of ADPKD patients in Lower Silesia

Mutacje w genie PKD1 - wpływ na obraz kliniczny pacjentów ze zwyrodnieniem wielotorbielowatym nerek z Dolnego Śląska

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development and progressive enlargement of cysts in the kidneys. The diagnosis of ADPKD is usually determined by criteria of renal ultrasound imaging of the development and number of cysts. However, in atypical cystic disease, for the recognition of ADPKD, DNA-based assays may be required.

Materials and methods: In the present study PCR amplified fragments of the *PKD1* gene (covering exons 15 and 43- 44) from genomic DNA of 134 Lower Silesia patients were analyzed for mutations and polymorphisms. Among them, the clinical significance of different PKD1 mutations was investigated in 81 persons.

Results: Eight new, previously undescribed, and 2 recurrent mutations were discovered. The presence of 3 known polymorphisms was confirmed. Seven of the 8 new discovered mutations were heterozygous.

Discussion: The results of the present study demonstrated that the frequency of genetic abnormalities in the analyzed fragments of the PKD1 gene in the Lower Silesian population is smaller than previously reported. Moreover, we could not detect deletions and insertions, which are often present is these regions of the PKD1 gene, which may be due to the limited number of screened patients. We conclude that none of the discovered changes in the PKD1 gene had any effect on clinical phenotype of the disease.

Keywords: **ADPKD • phenotype • PKD1 mutation**

Streszczenie

Wprowadzenie: Autosomalna, dominująca postać zwyrodnienia torbielowatego nerek (ADPKD) jest jedną z najczęstszych schorzeń genetycznych, charakteryzująca się rozwojem niezliczonej liczby, różnej wielkości torbieli w korze i rdzeniu nerek. Rozpoznanie choroby opiera się głównie na podstawie badań obrazowych, przede wszystkim badaniu ultrasonograficznym jamy brzusznej. W przypadkach nietypowych pomocne mogą być badania genetyczne.

Metody: W pracy analizowano mutacje i polimorfizmy fragmentów genu PKD1 obejmujących eksony 43-44 oraz ekson 15. Fragmenty namnożono za pomocą metody PCR z genomowego DNA 134 dolnośląskich pacjentów. U 81 osób analizowano kliniczne znaczenie różnych mutacji w genie PKD1.

Wyniki: Wykryto osiem nowych, poprzednio nieopisanych mutacji oraz dwie już wcześniej znane. Potwierdzono występowanie trzech znanych polimorfizmów. Większość (7 na 8) nowych mutacji miała charakter heterozygotyczny.

Dyskusja: W pracy wykazano, że częstotliwość mutacji w analizowanych fragmentach genu PKD1 w populacji dolnośląskiej jest mniejsza, niż publikowane dane literaturowe dotyczące populacji innych obszarów geograficznych. Nie wykryto delecji i insercji, które są często obecne w analizowanych wyżej opisanych rejonach genu PKD1. Może być to związane z ograniczoną liczbą analizowanych pacjentów. Uzyskane rezultaty wskazują, że wszystkie odkryte przez nas mutacje w genie PKD1 nie wywierają istotnego wpływu na obraz kliniczny choroby.

Słowa kluczowe: **zwyrodnienie wielotorbielowate · fenotyp · mutacja w genie PKD1**

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=XXXXXX

Word count: 4144
Tables: 3
Figures: –
References: 14

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Abbreviations: **ACE** – enzym konwertujący angiotensynę, **ADPKD** – autosomalna dominująca postać zwyrodnienia torbielowatego nerek, **dNTP**– deoksynukleozydo-5'-trifosforan, **ESRD** – schyłkowa niewydolność nerek, **MSSCP** – wielotemperaturowy polimorfizm konformacji pojedynczej nici DNA, **SSCP** – polimorfizm konformacji pojedynczej nici DNA, **PCR** – reakcja łańcuchowa polimerazy, **PKD1** – gen odpowiedzialny za zwyrodnienie torbielowate nerek 1, **PKD2**– gen odpowiedzialny za zwyrodnienie torbielowate nerek 2.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disorder, with an incidence of 1 in 400 to 1000 [7]. It is characterized by the development and progressive enlargement of cysts in the kidney, with end-stage renal disease (ESRD) typically occurring in mid-adulthood (between 40 and 75 years of age). ADPKD is caused by mutation in one of two genes: PKD1 (85% of cases), and PKD2 (15%); the PKD1 gene is located on chromosome 16 (position: 16q13.3) and PKD2 on chromosome 4 (position: 4q21-22) [2,5].

The diagnosis of ADPKD is usually determined by criteria of renal ultrasound imaging of the development and number of cysts. However, there are a number of scenarios in which clinicians may encounter patients with atypical cystic disease in whom the diagnosis may not be obvious. In such cases, DNA-based assays for the recognition of ADPKD may be required.

Clinically, mutations in the PKD1 gene indicate more severe disease than PKD2 mutations, with medium ages of ESRD development of 54.3 vs. 74 years [7]. Linkage analyses for ADPKD are of limited utility, because of genetic heterogeneity as well as the method, which requires the participation of more affected family members. Sequencing analysis of the PKD1 gene appears challenging, because of the large amount of gene duplication, which is designated as allele heterogeneity, as well as the fact that most mutations are unique to a single family [7,9]. Genetic heterogeneity of PKD1 mutations results in a significant number of ADPKD patients without recognized genetic defects. The number of mutations in the PKD1 sequence varies depending on the particular population and geographic region. Various mutations in the PKD1 gene probably have different effects on the clinical course of the disease.

The goal of the present study was to evaluate the utility and potential relationship between mutations discovered in exons 43-44 and 15 of the PKD1 gene in a Lower Silesian population and the clinical course of ADPKD.

Materials and methods

Subject selection

Patients were recruited from the Outpatient Nephrology Department of the Medical University Hospital in Wroclaw, Poland. The study was approved by the Medical University Ethics Committee. The genetic analysis was performed among 134 patients from Lower Silesia. The genetic investigation and complete set of clinical data were available from 81 patients (46 female and 35 male, mean age: 42.3± 13.1 years). The control pool consisted of 49 persons.

Clinical examination

The diagnosis of ADPKD was based on clinical history, symptoms, and ultrasound analyses.

DNA isolation

Genomic DNA was extracted from peripheral blood by a salting-out procedure using proteinase K [4]. In some cases, DNA was additionally purified with a Qiaex II Gel Extraction Kit (Qiagen).

Identification of mutations in the PKD1 gene

Polymerase chain reactions (PCRs) were used to amplify fragments of the 3' and 5' regions of the PKD1 gene.

The 256-bp fragment amplified with primers C1 (5'CGCCGCTTCACTAGCTTCGAC3') and C3 (5'CCAAGGTGACCCCCAGGAG3') and the 174-bp fragment, which derived from the previous one, belonged to the 3' PKD1 gene region. In the case of the 5' region, a 4.4 kb long-range PCR product, amplified by the primers F13 (5'TGGAGGGAGGGACGCCAATC3') and R27 (5'GTCAACGTGGGCCTCCAAGT 3'), which covered part of exon 15, served as the template for nested PCR to obtain the fragments of 568 bp and 417 bp. The 568-bp fragment was amplified with primers 568U (5 GGAGGAGGGGCTGAGCTGGGAGAC3') and 568L (5'GACGCGGGGGAAGCTGTGGGAGAA3'). The 417-bp fragment was amplified with primers 417U (5'AGTCACCGCGTCCAACAACATCTC3') and 417L (5'CACCCAGGAATAGCGCACATCACT3'). The PCR assays were carried out on a BIOMETRA (Personal Cycler 3,01 Biotron 1995) thermocycler. The PCR mixture (depending on the amplified fragment) contained in a 20-50 ml total volume 20-50 ng of genomic DNA (in the case of long-range PCR, the amount of DNA was doubled), 0.25 mM of each deoxyribonucleotide (dNTP), 0.45 µM of each forward and reverse primer (indicated above), 1 x conc. DyNAzyme DNA (or 1 x DyNAzyme EXT DNA for the long-range PCR assay) polymerase buffer and 0.5-1 unit of DyNAzyme DNA (or 2 units of DyNAzyme EXT DNA) polymerase (Finnzymes). The thermal profile was as follows: initial denaturation at 96°C for 5 min and then 35 cycles (denaturation at 94°C for 1 min, annealing at 60-63°C for 45 s to 1 min, elongation at 72°C for 2-3 min, depending on the primer pair), and final elongation at 72°C for 10 min.

In the case of the 256 bp fragment all patients were first screened by heteroduplex analysis then single-stranded conformational polymorphism (SSCP), multitemperature single-stranded conformational polymorphism (MSSCP), and then submitted to sequencing.

PCR products were separated by electrophoresis on 1.5% agarose gel and stained with ethidium bromide. After subsequent purification (using Gel Out kit, A&A Biotechnology) according to the manufacturer's protocol, they were bi-directionally sequenced employing the Big Dye Tv 3.1 sequencing reaction kit (Applied Biosystems) with the appropriate primer and run on the au-

tomatic capillary sequencing system (ABI Prism 31.30 XL, Applied Biosystems). All sequencing analyses were performed at least in 3 biological replicates at the local facility (Faculty of Biology, Adam Mickiewicz University in Poznań). For the identification of mutations within analyzed fragments of the PKD1 gene, genomic and cDNA reference sequences (Ref_Seqs) with accession numbers NC_000016.9 and NM_001009944.2, respectively, were used.

Angiotensin-converting enzyme (ACE) polymorphism was determined by the method of Cambien et al. [1] with the modification of Zak et al. [14] and verified by additional PCR with primers described by Lindpaintner et al. [3].

Statistical analysis

The significance of all clinical parameters as well as their correlation with the analyzed genotypes was statistically analyzed using the STATISTICA software package v. 8.00. The ANOVA test, Wilcoxon's test, chi-squared test, Pearson' correlation, and Spearman's correlation were applied where appropriate.

Table 1. *PKD1* gene mutations and polymorphic changes identified in the Silesian population

Patient ID	Region	Size of the analyzed gene fragment	Alterations in the genomic Refseq (flanking regions are also displayed)	Alterations in the mRNA Refseq	Remarks
77	3' region, EX42	174	g.44418 T > G GCG T>G CCG	c.11863T > G	novel, heterozygous, change in restriction sites, aminoacid substitution (p.V3884G)
77	3' region, EX43	256	g.44991G > C CTC G>C CTG	c.12188G > C (p.(=))	novel, heterozygous, change in restriction sites
116			g.44976T > G CCG T>G GGC	c.12173T > G (p.(=))	novel, heterozygous
118	3' region, EX44		g.45092C > A AGG C>A TGC	c.12214C > A	novel, heterozygous, change in restriction sites, aminoacid substitution (p.A4001N)
47	5' region, EX15	568	g.26180C > T CTT C>T GTG	c.5657C > T (p.(=))	novel, heterozygous
47			g.26361G > C TCA G>C CCA	c.5838G > C	novel, heterozygous, change in restriction sites, aminoacid substitution (p.A1877P)
77			g.26359C > A TCT C>A AGC	c.5836C > A	novel, create premature stop codon (p.S1876*)
23			g.26456G > A TCA G>A ATC	c.5933G > A (p.(=))	novel, heterozygous, change in restriction sites
78			g.26495G > A CCT G>A CAG	c.5972G > A (p.(=))	recurrent, heterozygous, change in restriction sites, likely neutral polymorphism (PKDB), synonymous (dbSNP)
17			g.26495G > A CCT G>A CAG	c.5972G > A (p.(=))	recurrent, change in restriction sites, likely neutral polymorphism (PKDB) , synonymous (dbSNP)
17		417	g.25397A > C TGC A>C AGC	c.4874A > C (p.(=))	recurrent, heterozygous, rare polymorphism, likely neutral polymorphism (PKDB), synonymous (dbSNP)
22			g.25397 A > C TGC A>C AGC	c.4874A > C (p.(=))	recurrent, heterozygous, rare polymorphism, likely neutral polymorphism (PKDB), synonymous (dbSNP)

Accession numbers for the genomic and cDNA reference sequences (Refseqs): genomic sequence [GenBank: NC_000016.9]; cDNA sequence [GenBank: NM_001009944.2]

Table 2. Clinical characterization of patients showing genetic changes

Family no.	Patient no.	Sex	Age at diagnosis	Age at study entry	eGFR	ICS	Hypertension	Kidney size	Liver cysts	ACE	Mutation/ polymorphisms	Other symptoms
16	47N	M	41	55	71,6	7.5	severe	medium	no	DD	C→T G→C	Myocardial hypertrophy
	77N	K	-	24	82.8	-	-	-	-	DD	C→A T→G G→C	2 cysts in left kidney
	78N	M	-	19	134.3	-	-	-	-	DD	G→A	Renal colic
10	116N	F	43	55	82,7	6.7	moderate	medium	yes	II	T→G	Leucopenia
	118N	M	53	61	43,8	13.7	severe	normal	no	DD	C→A	LA hypertrophy, mitral valve insufficiency
15	1N	M	40	56	89,3	6.2	moderate	medium	no	ID		Myocardial hypertrophy
	21N	K	-	30	124	-	-	-	-	II		Cardiac pulse disorder
	22N	K	-		119.3	-	?	?	?	DD	A→C	Kidney stones
-	23N	K	20	22	94	2.3	no	Middle	no	II	G→A	-
-	17N	K	37	-	-	-	-	-	?	DD	A→C G→A	Colonic diverticulosis

Results

In the present study we tried to evaluate whether the discovered novel mutations in the PKD1 gene are associated with the clinical phenotype of the disease. The data of all the sequenced fragments were compared with the sequences of corresponding fragments of 49 healthy persons.

In total, four novel mutations were found in the 3' PKD1 region and four in the 5' region. One mutation (A→C) at position 25397 represented recurrent change – according to the newest data of the Mayo database [6]. Two others at position 26495 are considered as likely polymorphisms according to the NCBI/SNP Database [10]. However, in our case, this change represents a rare polymorphism. We consider three of the polymorphic changes detected in the 5' end of the PKD1 gene region as rare in the Lower Silesian population; however, the Mayo database describes them as polymorphisms (Table 1). One mutation (C→A at nucleotide position 26359) created the stop codon TAA, making polycystin 1 shorter (Table 1). Five mutations caused changes in the restriction site.

It is worth noting that in one family, one member displayed three mutations and another displayed two. Apart from members of three families displaying mainly all the detected novel mutations and known polymorphic changes (Table 1 and 2) other ADPKD patients were also screened for mutations and recurrent polymorphisms.

The clinical phenotypes of these patients are shown in Table 2. The clinical significance of different PKD1 mutations was investigated in 81 persons. Apart from patients shown in Table 2, in the remaining group derived from 81 patients, 8 had severe hypertension, 5 had large and 16 had moderate kidney size, and 6 of them represented DD ACE genotype. None of them had genetic changes. It has to be added that 2 members of family no. 16 had not yet shown typical clinical ADPKD phenotype. Interestingly, all the analyzed members of this family displayed the DD ACE genotype.

The clinical severity of ADPKD was determined based on two criteria: the rate of estimated glomerular filtration rate (eGFR) decline and the level of the hypertension score. To determine eGFR decline rate, the index of clinical severity (ICS) was calculated according to the formula: (age at entry into the study)/eGFR (abbreviated MDRD formula) × 10. The severity of hypertension (HS) was scored based on the number antihypertensive drugs taken. A score of 1 was given to patients without hypertension, 2 to persons receiving one or two antihypertensive medications, and 3 to patients treated with three or more drugs.

ACE polymorphism of the analyzed patients (46 women, 35 men) did not reveal a predominance of the DD genotype or correlation with the severity of the disease.

Table 3. Correlations between genetic changes and clinical course of the disease

Patients with revealed genetic defect ICS (±SD)		Patients with unrevealed genetic defect ICS (±SD)	
Mean ICS	**Hypertension, no. of patients**	**Mean ICS**	**Hypertension, no. of patients**
6.5 ± 3.89	None, 2 Moderate, 3 Severe, 2	9.98 ±13.82	None, 16 Moderate, 36 Severe, 24

Discussion

PKD1 mutations are considered an important factor causing ADPKD; however, some observations suggest that genetic susceptibility might contribute to the varied disease phenotype.

We expected that finding recurrent mutations may have diagnostic implications and simplify further molecular analyses. Indeed, in one case we confirmed the A→C substitution at nucleotide position 25397 of PKD1 as well as another, i.e. G→A at position 26495. The latter is considered as a likely polymorphism [6] (Table 1). Generally, we could not detect many genetic abnormalities (deletions and insertions), which are often present in these regions of the PKD1 gene [9,12]. This may be due to the limited number of patients used in the research [9].

The latter seems to be especially important in determining the influence of newly discovered mutations and polymorphisms on the clinical phenotype.

The latest data [11,13] concerning mutations in the 5'- and 3'-end region of *PKD1* in Czech and Chinese populations showed few novel mutations. In the 3'-end region of the PKD1 gene in a Czech population Stekrova et al. showed six new mutations, displaying only substitutions [11]. Interestingly, in the Lower Silesian population we also detected only substitutions (eight) that were not previously reported.

Recently, Yu et al. [13] described a mutation that resulted in an amino acid substitution at codon 1908 (CAG) of the polycystin amino acid sequence. In contrast, in our case in the third position of the same codon a silent change occurred only (c.5933G>A). The obtained results confirmed that some mutations were present in particular families living in a particular geographical region.

The newly detected mutations were discovered in three families (Table 1); this may suggest significant selection eliminating mutations [9] or reflect different predispositions to mutation of the wild allele of PKD1, as well as indicating the interaction of modified genes and environmental conditions. A major question arising from this study is whether the detected mutations influence the expression product of the PKD1 gene. The characteristic feature of the detected mutations is that most of them are heterozygous (7/8). Recently, Rossetti et al. [8] identified alleles of the PKD1 gene with no or incomplete penetration, which may cause different severity of disease depending on whether they are inherited in the heterozygous or homozygous state or in trans with another mutation. This may account for the considerable phenotypic variability seen in ADPKD families. In the analyzed population, none of the discovered changes in the PKD1 gene had any effect on clinical phenotype of the disease (i.e. rate of eGFR decline, kidney size, hypertension severity). No correlation between ACE genotype and disease severity, hypertension, or vascular complication was found. All these data support the view that genetic defects of the PKD1 gene are extremely heterogeneous and at present it is difficult to discover a direct correlation between genetic abnormalities and the clinical shape of the disease. It has to be noted that we did not perform any PKD2 analysis.

References

[1] Cambien F., Poirier O., Evans A., Cambou J.P., Arveiler D., Luc G., Bard J.M., Bara L., Ricard S., Tiret L., Amouyel P., Alhenc-Gelas F., Soubrier F.: Deletion polymorphism in the gene for angiotensin--converting enzyme is a potent risk factor for myocardial infarction. Nature, 1992; 359: 641-644

[2] European Polycystic Kidney Disease Consortium: The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. Cell, 1994; 77: 881-894

[3] Lindpaintner K., Pfeffer M.A., Kreutz R., Stampfer M.J., Grodstein F., LaMotte F., Buring J., Hennekens C.H.: A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N. Engl. J. Med., 1995; 332: 706-711

[4] Miller S.A., Dykes D.D., Polesky H.F.: A simple salting out procedure for extracting DNA from human nucleated cells. Nucl. Acids Res., 1988; 16: 1215

[5] Mochizuki T., Wu G., Hayashi T., Xenophontos S.L., Veldhuisen B., Saris J.J., Reynolds D.M., Cai Y., Gabow P.A., Pierides A., Kimberling W.J., Breuning M.H., Deltas C.C., Peters D.J., Somlo S.: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science, 1996; 272: 1339-1342

[6] Polycystic kidney disease mutation database. http://pkd.mayo.edu (15.05.2012)

[7] Rossetti S., Consugar M.B., Chapman A.B., Torres V.E., Guay-Woodford L.M., Grantham J.J., Bennett W.M., Meyers C.M., Walker D.L., Bae K., Zhang Q.J., Thompson P.A., Miller J.P., Harris P.C., CRISP Consortium: Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. J. Am. Soc. Nephrol., 2007; 18: 2143-2160

[8] Rossetti S., Kubly V.J., Consugar M.B., Hopp K., Roy S., Horsley S.W., Chauveau D., Rees L., Barratt T.M., van't Hoff W.G., Niaudet P., Torres V.E., Harris P.C.: Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. Kidney Int., 2009; 75: 848-855

[9] Rossetti S., Strmecki L., Gamble V., Burton S., Sneddon V., Peral B., Roy S., Bakkaloglu A., Komel R., Winearls C.G., Harris P.C.: Mutation analysis of the entire PKD1 gene: genetic and diagnostic implications. Am. J. Hum. Genet., 2001; 68: 46-63

[10] Single nucleotide polymorphism database. http://www.ncbi.nlm.nih.gov/SNP (15.05.2012)

[11] Stekrova J., Reiterova J., Svobodova S., Kebrdlova V., Lnenicka P., Merta M., Viklicky O., Kohoutova M.: New mutations in the PKD1 gene in Czech population with autosomal dominant polycystic kidney disease. BMC Med. Genet., 2009; 10: 78-88

[12] Torra R., Badenas C., Peral B., Darnell A., Serra E., Gamble V., Turco A.E., Harris P.C., Estivill X.: Recurrence of the PKD1 nonsense mutation Q4041 X in Spanish, Italian and British families. Hum. Mutat., 1998; Suppl.1: S117-S120

[13] Yu C., Yang Y., Zou L., Hu Z., Li J., Liu Y., Ma Y., Ma M., Su D., Zhang S.: Identification of novel mutations in Chinese Hans with autosomal dominant polycystic kidney disease. BMC Med. Genet., 2011; 12: 164

[14] Żak I., Niemiec P., Sarecka B., Balcerzyk A., Ciemniewski Z., Rudowska E., Dylag S.: Carrier-state of D allele in ACE gene insertion/deletion polymorphism is associated with coronary artery disease, in contrast to the C677 T transition in the MTHFR gene. Acta Biochim. Polon., 2003; 50: 527-534

The authors have no potential conflicts of interest to declare.