Original Article

Pilot Study of the Occurrence of Somatic Mutations in Ciliary Signalling Pathways as a Contribution Factor to Autosomal Dominant Polycystic Kidney Development

(polycystic kidney disease / signalling pathways / next-generation sequencing / genetic variants)

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Abstract. Autosomal-dominant polycystic kidney disease (ADPKD) is an inherited disease that results in multiple kidney cysts, and it is a common cause of end-stage renal disease. Recent studies have shown that disease progression can be slowed by simultaneous disruption of the primary cilium and polycystins. The exact genetic mechanism of this process is still unknown. The aim of the present study was to characterize the mutation profile of ciliary signalling pathways in the renal epithelial cells of ADPKD patients. In our study, we performed an analysis of 110 genes encoding the components of Sonic Hedgehog, Hippo, Notch, Wnt and planar cell polarity signalling (PCP) by targeted next-generation sequencing. We analysed 10 formalin-fixed, paraffin-embedded (FFPE) tissue samples of patients with ADPKD. We identified a unique mutation profile in each of the analysed ADPKD samples, which was characterized by the presence of pathogenic variants in eight to 11 genes involved in different signalling pathways. Despite the significant genetic heterogeneity of ADPKD, we detected five genes whose genetic variants affected most ADPKD samples. The pathogenic variants in *NCOR2* and *LRP2* genes were present in all analysed samples of ADPKD. In addition, eight out of 10 samples showed a pathogenic variant in the *MAML2* and *FAT4* genes, and six out of 10 samples in the *CELSR1* gene. In our study, we identified the signalling molecules that may contribute to the cystogenesis and may represent potential targets for the development of new ADPKD treatments.

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Abbreviations: ADPKD – autosomal-dominant polycystic kidney disease, cAMP – cyclic adenosine monophosphate, CELSR1 – cadherin EGF LAG seven-pass G-type receptor 1, COSMIC – Catalogue of Somatic Mutations in Cancer, dbSNP – single-nucleotide polymorphism database, ESRD – end-stage renal disease, FAT4 – FAT atypical cadherin 4, FFPE – formalin-fixed, paraffin-embedded, GANAB – glucosidase II α subunit, HGVS – Human Genome Variant Society, LRP2 – low-density lipoprotein-related protein 2, MAF – minor allele frequency, MAML2 – Mastermind-like transcriptional coactivator 2, MAPK/ERK – mitogen-activated protein kinase/extracellular-signal-regulated kinase, mTOR – mammalian target of rapamycin, NCOR2 – nuclear receptor corepressor 2, NUMB – Numb, endocytic adaptor protein, PCP – planar cell polarity, PKD1 – polycystin 1, PKD2 – polycystin 2, SHH – sonic hedgehog, TAZ – telomere length regulator taz1, YAP1 – Yes-associated protein 1.

Introduction

Autosomal-dominant polycystic kidney disease (ADPKD) is the most frequent hereditary kidney disease characterized by bilateral formation and progressive expansion of renal cysts that lead to end-stage kidney disease. ADPKD affects nearly 12.5 million people in the world and despite intensive research, targeted therapy is not yet available (Spithoven et al., 2014). The reason is that there are gaps in the understanding of the exact mechanism of pathogenesis of the disease at the molecular level.

Mutations in genes for polycystin 1 (*PKD1*), polycystin 2 (*PKD2*) or glucosidase II α subunit (*GANAB*) have been identified as the cause of ADPKD (Bastos et al., 2011, Porath et al., 2016). Although a germline mutation

is necessary to induce the cyst formation in ADPKD, cysts occur in only a part of kidney tubules and hepatic bile duct. However, in adult tissues, both copies of the mutated polycystin gene undergo recessive loss of function resulting in cyst formation to be accelerated in a subset of tubular epithelial cells. This condition is caused by the occurrence of an additional somatic mutation (Kim and Park, 2016). Although the mechanism of somatic second hit is generally applicable to human ADPKD, additional factors contribute to determining the extent of the cyst formation. More evidence has accumulated in recent years showing that the primary cilium plays an important role in the development of ADPKD.

The primary cilium is a sensor organelle, which protrudes into the extracellular environment and ensures regulation of many signalling components that pass through it (Nachury, 2014). Recent studies have revealed a relationship between cilia and polycystins, which regulates the severity of ADPKD. According to this model, the progression of cysts is regulated by the duration of the time interval between the initial loss of polycystins and the subsequent ablation of cilia (Ma et al., 2013; Lee and Somlo, 2014). This cilia-dependent activating mechanism of cystogenesis was found to be independent of several known cystogenic pathways, including mitogen-activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK), mammalian target of rapamycin (mTOR) and cyclic adenosine monophosphate (cAMP) signalling. This means there is a new unknown mechanism for the progression of cystic kidney disease regulated by the primary cilium.

In recent years, the signalling pathways regulated by the primary cilium have been described, involving Sonic Hedgehog (SHH), Hippo, Notch, canonical Wnt and planar cell polarity (PCP) signalling (Basten and Giles, 2013). In order to maintain efficiency of these signalling pathways, it is important to preserve intact cilia and prevent ciliary localization without affecting functions of the trafficked signalling protein (Mukhopadhyay et al., 2017). Many studies have shown that aberrant activation of these pathways in the renal cells may represent an early event in the cystogenic transformation (Happé et al., 2011; Tran et al., 2014; Wang et al., 2015). There are many genetic causes for aberrant activation of the signalling pathways, such as mutations or aberrant expression of signalling components. While expression studies of the signalling pathways have recently been carried out, their mutation profile is unknown (de Almeida et al., 2016).

The aim of our study was to identify the mutation profile of ciliary pathways whose aberrant activation may contribute to the process of cystogenesis in ADPKD. In this study, we used targeted next-generation sequencing of 110 genes encoding key signalling components Notch (25 genes), Wnt (17 genes), Hippo (22 genes), PCP (21 genes), and SHH (25 genes). We used 10 formalin-fixed and paraffin-embedded (FFPE) tissue samples from patients with ADPKD obtained by nephrectomy. The results of our study revealed genetic changes in the key signalling pathways and introduced potential targets for the development of new treatments for ADPKD.

Material and Methods

Statements

All the procedures performed in our study complied with ethical guidelines corresponding to the relevant laws that were implemented by European Union member states and were included in institutional ethical policies**.**

Sample collection

In our study, we obtained archived FFPE samples from 10 non-relative ADPKD patients (four women and six men), which were subsequently subjected to histopathological examination. The clinical course of the disease in individual patients is shown in Table 1. Samples with more than 50% content of cystic cells were considered suitable for genetic analysis. The genetic testing of *PKD1* and *PKD2* genes was carried out previously in our laboratory. All of the patients had a confirmed mutation in the *PKD1* gene. The identified types of *PKD1* mutations are shown in Table 2. Our study was approved by an ethics committee and informed consent was provided by all patients at the inception of the study.

DNA extraction and sample quality control

Genomic DNA was extracted from FFPE tissues with a blackPREP FFPE DNA kit (Analytik Jena, Germany) and from peripheral blood using a QIAamp DNA Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The extracted DNA specimens from FFPE were further quantified using a Qubit dsDNA HS assay kit (Life Technologies/Fisher Scientific, Waltham, MA). DNA quality and quantity of these samples were assessed using an Agilent NGS FFPE QC Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's instructions.

Library preparation and quality control

Sequencing libraries were prepared using Agilent SureSelect^{XT} Custom 0.5 Mb up to 2.9 Mb according to the manufacturer's instructions. Two hundred ng of DNA from each sample was fragmented into 150–200 base pairs by Covaris S220 sonication prior to the library construction. The quality of DNA fragments was assessed by a High sensitivity DNA kit (Agilent Technologies) with a 2100 Bioanalyzer instrument. The concentration of each index tagged captured library was determined using an Agilent QPCR NGS Library Quantification Kit (for Illumina).

Sequencing and data analysis

The libraries were pooled at equimolar concentrations and loaded into flow cells with a HiSeq PE Cluster Kit v2 (Illumina, San Diego, CA). The flow cells were transferred to HiSeq 2500 sequencers, and sequencing

Table 1. Clinical course of the disease in the examined patients

	Sex	Age at the time of diagnosis	Age at the onset of ESRD	Complications of PKD	Reason for nephrectomy
1	M	31	46	arterial hypertension, recurrent urinary infection, lumbar and abdominal pain	lack of space in the abdomen
2	F	30	50	arterial hypertension	lack of space in the abdomen
3	M	27	56	arterial hypertension, haematuria, renal colic	massively enlarged polycystic kidneys
4	F	45	57	arterial hypertension, hepatic cystic involvement	massively enlarged polycystic kidneys
5	M	41	54	arterial hypertension, recurrent infection of urinary tract	massively enlarged polycystic kidneys
6	F	26	52	arterial hypertension, recurrent infection of urinary tract	massively enlarged polycystic kidneys
7	F	33	45	arterial hypertension, hepatic cystic involvement	infection
8	M	34	50	arterial hypertension, recurrent infection of urinary tract, nephrolithiasis, haematuria	infection
9	M	40	62	arterial hypertension	infection
10	M	34	46	arterial hypertension	lack of space in the abdomen

Table 2. Types and localization of identified mutations in the PKD1 gene

	Mutation at the DNA level	Mutation at the protein level	Site of exon
1	c.5358_5359insCC	p.Leu1787Argfs*17	15
2	c.6752_6754delTGG	p.Val2251del	15
3	c.566C>G	p.Ser189*	5
4	c.6653_6655delTGC	p.Leu2218del	15
5	c.1213delC	p.Leu405Serfs*60	6
6	c.1692delC	p.Asp5646Glufs*20	8
7	c.5358_5359insCC	p.Leu1787Argfs*17	15
8	c.4685delT	p.Leu1562Argfs*5	15
9	c.5976_5978delCAC	p.Phe1992_Thr1993del	15
10	c.9485C>T	p.Arg3162Cys	27

was done with a Hiseq Rapid SBS kit v2 (Illumina) in a 2 × 100 bp mode. Data were analysed using a software package that was commercially available – NextGENe™ (SoftGenetics, State College, PA). Raw data from the 10 samples were analysed in NextGENe™ according to the manufacturer's instructions. Variants characterized by a coverage depth < 10, allele ratio < 0.10 or strand bias were excluded from further analysis on the basis of recommendations (Rehm et al., 2013). Evaluation of the functional impact of genetic variants was facilitated using Geneticist Assistant software (SoftGenetics). This software provides a unique tool for the management, control, visualization and functional interpretation with the purpose of identifying pathogenic variants. The software analyses and determines the presence of variants in various databases such as dbSNP for known polymorphisms and their population allele frequency (1000 Genomes, ExAC and Exome Variant Server), or in a disease-related variant database such as ClinVar, OMIM, HGMD, and Catalogue of Somatic Mutations in Cancer (COSMIC). Potential functional consequences of the variants are assessed using methods SHIFT, Polyphen, LRT, Mutation Taster, FATHMM and CADD & Mutation Assessor.

Classification of identified variants

In our study, we classified sequence variants according to the recommendations issued by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richard et al., 2015). In these recommendations, the variants are classified according to the results of combination of the scoring rules. In our study, all variants were classified into four groups: pathogenic, likely pathogenic, uncertain significance, and benign. The fifth recommended class likely benign was not used due to the absence of these variants in our study. The benign variants represent known synonymous variants, variants with allele

frequency > 5 % in the population databases and with a clearly benign status.

The variants that were included into the pathogenic group represent known pathogenic variants and frameshift or nonsense variants with expected loss of function. Likely pathogenic variants included missense variants with the same amino acid change as a previously established pathogenic variant regardless of the nucleotide change, located in a functional domain of proteins and with a predicted deleterious effect in more than five prediction tools. The group of variants "uncertain significance" represents unknown missense variants with unclear results of prediction tools and variants with minor allele frequency (MAF) < 1 % in dbSNP. The presence of the identified pathogenic variants in ADPKD tissues was verified by the standard Sanger sequencing method. The somatic origin of these variants was confirmed by analysis of DNA extracted from peripheral blood using the standard Sanger sequencing method.

Results

Data analysis and selection of variants

Analysis of the sequencing data showed high read depth and target coverage in all examined samples. Overall, 99.46 % of the targeted region was covered with > 30% coverage ranging from 92–96 % within the different sequences. The mean depth of coverage was 462× reads.

A total of 2,446 variants were detected in the samples of ADPKD. The most common variants were included in the group of benign variants, which accounted for 94 % of cases (2,302/2,446). They were followed by 100 pathogenic variants, which consisted of 64 variants included in the pathogenic group and 36 variants in the likely pathogenic group. In all cases, the pathogenic variants were classified as somatic because they did not appear in the peripheral blood samples. The third group represented 44 variants with uncertain significance. This group included 38 missense, two frameshift and four splice variants.

Variants in signalling pathways

The most frequent genetic changes were identified in the genes belonging to the Notch signalling pathway. We identified 56 variants in 13 of 25 analysed genes. Overall, we detected 31 pathogenic, five likely pathogenic and 20 variants of uncertain significance. The most common pathogenic variants were found in the nuclear receptor corepressor 2 (*NCOR2*) gene, where we identified a total of 10 variants. The second most frequently mutated gene was the Mastermind-like transcriptional coactivator 2 (*MAML2*) gene with five pathogenic and two likely pathogenic variants. They were followed by *NOTCH4* and Numb, endocytic adaptor protein (*NUMB*) genes with four pathogenic variants and *NOTCH3* with three pathogenic variants. In the other eight genes of the Notch signalling pathway, we identified only one pathogenic variant.

The SHH pathway was the second most frequently mutated pathway in ADPKD samples. We identified 21 pathogenic, seven likely pathogenic and eight variants of uncertain significance in 12 out of 25 genes. The most frequently mutated gene in this group was low-density lipoprotein-related protein 2 (*LRP2*). In this gene, we detected 18 variants: eight pathogenic, two likely pathogenic and eight missense variants of uncertain significance. In contrast, in the other 11 genes, only one to three genetic variants were detected.

In the Hippo signalling pathway, genetic variants were identified in 14 out of 22 genes. The genes were predominantly affected by the likely pathogenic missense variants, which were detected in 17 cases. They were followed by the variants of uncertain significance in eight cases and pathogenic variants in six cases. The most frequently mutated gene was FAT atypical cadherin 4 (*FAT4*) with five likely pathogenic and one pathogenic variants. In the other genes, we detected one to four genetic variants belonging to different groups.

In our study, we analysed 17 genes of the canonical Wnt signalling pathway and 22 genes of the PCP pathway. Despite the large number of detected variants, most of them were benign variants. Overall, we detected only 11 non-benign variants in five genes of the PCP and five non-benign variants in three genes of the Wnt signalling pathway. In the PCP signalling, the most frequently mutated gene was cadherin EGF LAG seven-pass G-type receptor 1 (*CELSR1*) with four pathogenic and two likely pathogenic variants.

Of all identified variants, only 11 pathogenic variants in four genes (*NCOR2*, *FAT4*, *LRP2* and *CELSR1*) were listed in the known databases of somatic mutations. The number of genetic variants in the individual genes involved in signalling pathways is shown in Table 3. An overview of the identified pathogenic variants in the most frequently mutated genes is shown in Table 4.

Variants in individual samples of ADPKD

Each analysed sample of ADPKD had a specific mutation profile. We detected the presence of a unique group of eight to 11 genes affected by the pathogenic variants in each analysed ADPKD sample. The different mutation profiles indicate the genetic heterogeneity of the disease. However, we identified genes whose variants affected most of the analysed samples. The pathogenic variants in *NCOR2* and *LRP2* genes were present in all analysed samples. The frequent occurrence of variants was also noted in *MAML2* (eight samples), *FAT4* (eight samples) and *CELSR1* (six samples) genes. An overview of genes affected by the pathogenic variants in individual samples of ADPKD is shown in Table 5.

Discussion

In the present study, we assessed the mutation profile of 110 genes of the major ciliary signalling pathways in renal cystic tissues of ADPKD. Despite the analysis of a large number of genes, cystic cells did not show a high

Table 3. Number of identified genetic variants in individual genes of the signalling pathways

		Number of genetic variants per gene			
Signalling pathway	**Gene**	**Pathogenic**	**Likely pathogenic**	**Variant of unknown significance**	**Total**
Notch	*NCOR2*	10		8	18
	MAML2	5	2	4	11
	NOTCH4	2	2	3	7
	NUMB	4		3	7
	NOTCH3	2	1		3
	NOTCH2	1		2	3
	DDL3	1			1
	DDL4	1			1
	NCSTN	1			1
	NOTCH1	1			1
	DTX1	1			1
	JAG1	1			1
	JAG2	1			1
SHH	*LRP2*	8	2	8	18
	BOC	2	1		3
	HHIP	2			2
	IHH	2			2
	PTCHD1	2			2
	PTCHD2	1			1
	PTCHD3	2	1		3
	Gli2	1			1
	SHH	1			1
	Gli3		1		1
	STK36		1		1
	PTCH2		1		1
Hippo	*FAT4*	1	5		6
	FAT1	1	2		3
	FAT2		2	2	4
	FAT3		2	2	4
	LATS1	1	2		3
	MO1B1	1			1
	NF2	1			1
	YAP1	1			1
	FMRD1		2		2
	WWC2		2		2
	TEA D4			1	1
	FMR6			1	1
	LATS2			1	1
	DCHS1			1	1
PCP	*CELSR1*	4	2		6
	CELSR2			2	2
	CELSR3			1	1
	PRINCKLE1			1	1
	VANGL1			1	1
WNT	*BTRC*	1		1	2
	EP300		1	1	2
	DVL1			1	1

Table 4. Overview of identified pathogenic variants in the most frequently mutated genes

NCOR2	***MAML2***	***LRP2***	***FAT4***	***CELSR1***
p.Ile976fs*94	p.Gln612Serfs*69	p.Arg2095*	p.Pro4543Argfs*27	p.Gly614fs*64
p.Gly1839_Gly1840insSerSerGly	p.Thr121Serfs*20	p.Glu4639Gln	p.Phe1107Leu	p.Glu525*
p.Leu1412fs*1	p.Gln648Hisfs*33	p.Asn4240Asp	p.Phe1107Ser	p.Ala269Ser
p.Gln1006*	p.Gln606Hisfs*75	p.Arg938*	p.Gly3524Cys	p.His319*
p.Met1157Leu	p.Gln640Hisfs*41	p.Leu152Pro	p.Gly3524Asp	p.Ala761Thr
p.Met512Cysfs*98	p.Ser634Alafs*47	p.Asp1198fs*102	p.Arg4708Gly	p.Glu1147Val
p.Ser1817_Gly1839dup	p.Gln639Leu	p.Trp3422*		
p.Pro815Hisfs*35	p.Leu23Thr	p.Gly3188Glu		
c.4007C>T, p.Pro1336Cys	p.Gly24Gln	p.His1542Asn		
p.Gly1846_Ser1847insAlaAlaAla	p.Gln658Glu	p.Ile584Met		
	p.Gln646Arg			

Sequence changes at the protein level described according to HGVS (Human Genome Variation Society) recommendations for protein sequence variants. Variants labelled with grey are known somatic mutations recorded in the COSMIC (Catalogue of Somatic Mutation In Cancer) database.

Table 5. Overview of genes affected by pathogenic variants in individual samples of ADPKD

	NOTCH	**SHH**	**HIPPO**	**PCP**	**WNT**
1	***NCOR2***, ***MAML2***, *NOTCH4, DDL3*	***LRP2***, *BOC, PTCHD3*	***FAT4***, *FAT1, YAP1*	***CELSR1***	
2	***NCOR2***, ***MAML2***, *NOTCH3*	***LRP2***, *HHIP, GLI2*	***FAT4***, *LATS1, FMRD1*		
3	***NCOR2***, ***MAML2***, *NUMB, NOTCH2*	***LRP2***, *BOC, SHH*	***FAT4***, *FAT2*	***CELSR1***	
4	***NCOR2***, ***MAML2***, *NUMB, JAG2*	***LRP2***, *IHH, PTCHD3*	***FAT4***, *FAT2, WWC2*	***CELSR1***	
5	***NCOR2, MAML2***, *NOTCH3, DDL4*	***LRP2***, *PTCHD1*	*FAT1, LATS1, FMRD1*	***CELSR1***	
6	***NCOR2, MAML2***, *NUMB, JAG1*	***LRP2***, *HHIP, PTCH2*	***FAT4***, *FAT3*		*DVL1*
7	***NCOR2, MAML2***, *NUMB, NCSTN*	***LRP2***, *PTCHD1, STK36*	***FAT4***, *FAT3*	***CELSR1***	
8	***NCOR2, MAML2***, *NOTCH4, NOTCH1*	***LRP2***, *IHH, GLI3*	*FAT1, MO1B1*		
9	***NCOR2***, *NOTCH4, DTX1*	***LRP2***, *PTHD2*	***FAT4***, *NF2*		*BTRC*
10	***NCOR2***, *NOTCH4, NOTCH3*	***LRP2***, *BOC, PTCHD3*	***FAT4***, *LATS1, WWC2*	***CELSR1***	*EP300*

degree of mutagenicity. The pathogenic variants were identified in only eight to 11 genes. Although each sample had a unique mutation profile, we identified a group of five genes whose variants dominated in most of the analysed samples. The most frequently mutated genes of the Notch signalling pathways were *NCOR2* and *MALM2*. The pathogenic variants in the *NCOR2* gene were present in all ADPKD samples and in the *MAML2* gene in eight out of 10 samples. The mutations of these genes can lead to deregulation of Notch signalling. *NCOR2* is a transcriptional corepressor, which binds to nuclear receptors in the absence of ligand and thus prevents their activation (Heck et al., 2012). In contrast, *MAML2* act as a transcriptional coactivator of the Notch signalling. This pathway is important for kidney development and repair of kidney damage (Sirin and Susztak, 2012). Many studies have confirmed that disturbances of the Notch signalling may lead to cystic renal disease (Surendran et al., 2010; de Almeida et al., 2016). The effect of loss of function of NCOR2 and MALM2 on aberrant activation of Notch and their contribution to the cystic process in ADPKD will require further studies.

We also confirmed the frequent presence of somatic pathogenic variants in the *LRP2* gene, which were represented in all analysed ADPKD samples. This gene is included in the SHH signalling pathway and encodes megalin, transmembrane glycoprotein that serves as endocytic receptor. This receptor is predominantly localized in the primary cilium and is responsible for the clearance of low-molecular weight plasma proteins from the glomerular filtrate in the renal proximal tubules (Christ et al,, 2016). The role of *LRP2* in ADPKD has not yet been investigated. To clarify the impact of *LRP2* mutations on the cystogenesis and processes related to deregulation of SHH, further studies will also be required.

A fourth gene whose pathogenic mutations were present in eight out of 10 ADPKD samples was the *FAT4* gene. The influence of genetic alterations of the *FAT4* gene on ADPKD cystogenesis is relatively well known. The studies in animal models have confirmed that the loss of *FAT4* disrupts oriented cell divisions and tubule elongation during the kidney development, leading to cystic kidney disease (Saburi et al., 2008). In addition,

this gene is included in the Hippo signalling pathway, whose possible impact on the development of ADPKD has been shown in current studies. The major function of the Hippo pathway is the control of organ size by restriction of the tissue growth and modulation of cell proliferation, differentiation and migration (Zhipeng et al., 2016). The studies have shown that deregulation of transcriptional coactivators Yes-associated protein 1 (YAP1) or telomere length regulator taz1 (TAZ) resulted in formation of cysts in animal models (Plouffe et al., 2015; Wong et al. 2016). In our study, we detected a clearly pathogenic variant in the *YAP1* gene only in one sample of ADPKD. In contrast, no genetic variant was confirmed in the *TAZ* gene.

Frequent pathogenic variants were also present in the *CELSR1* gene, which is involved in PCP signalling. These variants affected six out of 10 ADPKD samples. The *CELSR1* gene encodes the transmembrane receptor involved in the regulation of establishment and maintenance of planar cell polarity (Brzóska et al., 2016). The PCP genes play an important role in kidney differentiation, especially controlling tubular diameter, partly through orienting cell division. A recent study has confirmed that mutations in the *CELSR1* gene are the cause of many renal malformations (Brzóska et al., 2016). Based on the results of our study, we can assume their influence in renal cystogenesis of ADPKD.

Many studies in model organisms and cultured cells have shown that activation of the WNT/β-catenin signalling may constitute a common causative event in the formation of cysts (Lal et al., 2008; Kim et al., 2016). In our study, we examined the occurrence of mutations in 22 key genes of this pathway. However, the presence of pathogenic variants in the genes of the Wnt signalling pathway was very rare in the ADPKD samples. Therefore, the aberrant activation of this pathway may contribute to cystogenesis, but based on a different genetic mechanism.

The results of our study may be limited by a small number of analysed samples. However, this is the first mutation analysis utilizing human ADPKD tissues. The reason is the fact that the majority of ADPKD patients do not require native nephrectomy, and cystic kidneys are not generally biopsied for technical and ethical reasons. Consequently, the most often used materials for many studies are the existing cell lines and the rodent model of ADPKD. However, these models do not completely mimic the human disease (Happé and Peters, 2014). In human, ADPKD is a genetically heterozyous disease with a slowly progressive course. By contrast, the rodent models of ADPKD require homozygous deletion of the gene immediately after birth, which leads to accelerated progression of the disease with numerous cysts developing contemporaneously (Grantham et al., 2012). The results of our study thus reliably reflect the actual molecular condition of the renal cystic cells in ADPKD.

In conclusion, our study brought new information about the mutation status of the key signalling pathways in ADPKD. We identified major genes encoding signalling molecules that were predominantly mutated in ADPKD samples. These genes may represent likely causative factors for aberrant activation of ciliary signalling pathways and potential molecular targets for the development of new therapies for ADPKD. To unambiguously confirm these hypotheses, the results must be verified in a larger number of samples and matched tissue controls. Our study is an important and necessary first step for starting further analyses focused on clarifying the relationships between mutated signalling molecules, and revealing their impact on the initiation of cystogenesis and progression of disease.

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