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Basic Study

## Genetic defects in ciliary genes in autosomal dominant polycystic kidney disease

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## Abstract

### AIM

To evaluate the genetic defects of ciliary genes causing the loss of primary cilium in autosomal dominant polycystic kidney disease (ADPKD).

### METHODS

We analyzed 191 structural and functional genes of the primary cilium using next-generation sequencing analysis. We analyzed the kidney samples, which were obtained from 7 patients with ADPKD who underwent nephrectomy. Each sample contained polycystic kidney tissue and matched normal kidney tissue.

### RESULTS

In our study, we identified genetic defects in the 5 to 15 genes in each ADPKD sample. The most frequently identified defects were found in genes encoding centrosomal proteins (*PCM1*, *ODF2*, *HTT* and *CEP89*) and kinesin family member 19 (*KIF19*), which are important for ciliogenesis. In addition, pathogenic mutations in the *PCM1* and *KIF19* genes were found

in all ADPKD samples. Interestingly, mutations in the genes encoding the intraflagellar transport proteins, which are the basis of animal models of ADPKD, were only rarely detected.

## CONCLUSION

The results of our study revealed the actual state of structural ciliary genes in human ADPKD tissues and provided valuable indications for further research.

**Key words:** Polycystic kidney disease; Primary cilium; Ciliary genes; Next-generation sequencing; Genetic variants

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**Core tip:** Many studies have confirmed that the loss of primary cilia promotes renal cyst formation in autosomal dominant polycystic kidney disease (ADPKD). However, these studies are based on mouse models by the inactivation of various ciliary genes, and the actual status of these genes in human ADPKD tissues is unknown. In our study, we analyzed genetic defects in ciliary genes in the human polycystic kidney tissues and matched normal kidney tissues by next-generation sequencing. We found that the loss of the primary cilia in the human ADPKD tissues may be predominantly caused by defects of centrosomal proteins and KIF19 protein.

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## INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a multisystem disorder characterized by the formation of cysts in the kidneys and other organs. ADPKD affects more than 12.5 million people worldwide<sup>[1]</sup>. Approximately 50% of patients with ADPKD have end-stage renal disease (ESRD) by 60 years of age. Dialysis and kidney transplantation are the only treatment options for patients with ESRD<sup>[2]</sup>. The reason for the absence of targeted treatment is insufficient understanding of the molecular mechanism of cystogenesis.

The molecular nature of the disease includes germline mutation of either the *PKD1* (polycystin-1), *PKD2* (polycystin-2) or *GANAB* gene<sup>[3,4]</sup>. Although the germline mutation is present in every cell of the body, formation of cysts is limited to a small number of nephrons. This means that the germline mutation is not in itself sufficient to produce cysts; a second somatic event is also required<sup>[5]</sup>. 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 signaling organelle that extends from the surface of the plasma membrane of most mammalian cells. Assembly of cilia depends on cell cycle progression, because the centrioles regulating the cell cycle are essential components in the formation of the basal body of the cilia<sup>[6]</sup>. The maintenance and elongation of cilia is ensured by a process called intraflagellar transport (IFT). In this process, protein complexes known as IFT trains carry cargo along tracks that run along the length of the cilia. Different motor proteins power the IFT trains in different directions. Kinesin moves IFT trains from the base to the tip, while dynein moves them back in the opposite direction<sup>[7]</sup>.

Many studies have shown that the loss of primary cilia promotes renal cyst formation *in vivo*<sup>[8,9]</sup>. However, the epithelial cells of renal cysts are characterized not only by an absence of cilia but also by excessively long cilia<sup>[10-12]</sup>. Recent studies have confirmed that kidney cysts occurred following inactivation of polycystins in otherwise intact cilia or following complete removal of cilia by inactivation of IFT proteins. In addition, a relationship was shown between cilia and polycystins that regulates the severity of ADPKD. According this model, the progression of cysts is regulated by the duration of the interval time between the initial loss of polycystins and the subsequent ablation of cilia. These studies identified a new mechanism of cystogenesis based on the evidence that the loss of renal cilia inhibits cyst growth if the cilia are disrupted at the same time as the polycystins<sup>[13,14]</sup>. In addition, it was shown that defective primary cilia or the inactivation of ciliary genes induces aberrant signaling pathways associated with proliferation, differentiation and development in various PKD mouse models<sup>[13-16]</sup>. The structural deformity or absence of primary cilia is thus a key driving force in the development of ADPKD.

The results of these studies were achieved in mouse models by targeted inactivation of various ciliary genes, especially genes encoding IFT proteins. However, the actual mutation profile of ciliary genes in human ADPKD tissues is unknown. The aim of our study was to identify the mutation state of genes encoding the structural ciliary components in tissues from patients with ADPKD obtained by nephrectomy through targeted next-generation sequencing analysis.

## MATERIALS AND METHODS

### Sample collection

In our study, we obtained archived FFPE samples of polycystic kidney tissues and matched normal controls from 7 patients with ADPKD (four women and three men) who had undergone radical nephrectomy. Four patients had nephrectomy for the occurrence of complications associated with the enlarged kidney involved the recurrent infection of the urinary tract, arterial hypertension, and chronic pain. Three patients required nephrectomy to provide the space for the



kidney allograft. The FFPE samples were subjected to histopathological examination. The samples containing at least 50% epithelial cysts were considered suitable for genetic analysis. The genetic testing of the *PKD1* and *PKD2* genes had been carried out previously in our laboratory. The mutation in the *PKD1* gene was confirmed for all of the patients. 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**

DNA was extracted from the FFPE tissues using the commercially available blackPREP FFPE DNA kit (Analytik Jena, Germany) and from peripheral blood using the QIAamp DNA Mini kit (Qiagen, Valencia, California, United States) according to the manufacturer's instructions. The extracted DNA specimens from FFPE were further quantified using the Qubit dsDNA HS assay kit (Life Technologies/Fisher Scientific, United States) and the Agilent NGS FFPE QC Kit according to the manufacturer's instructions.

#### **Design of the gene panel**

Candidate genes were selected using a gene list in the SysCilia Gold Standard (SCGC) Version 1 database<sup>[17]</sup>. A panel of 191 genes was designed using the web-based tool SureDesign (Agilent Technologies, United States). The regions of interest included coding regions with 10 base pair (bp) upstream and 10 bp downstream for capture of splicing donor and acceptor sites. Overall, we analyzed genes encoding the proteins of basal bodies (31 genes), centrioles (25 genes), centrosomes (22 genes), IFT (24 genes), transition zone (17 genes), axoneme (9 genes), ciliary membrane (8), cilioplasm (5 genes), ciliary proteins located in the nucleus (5 genes), plasma membrane proteins (5 genes), ciliary tip (4 genes), regulatory ciliary proteins in the Trans-Golgi network (3 genes), ciliary root (2 genes) and ciliary proteins with non-specific localization (31 genes).

#### **Library preparation and variant calling**

Sequencing libraries were prepared using the Agilent SureSelect<sup>XT</sup> Custom 0.5 Mb up to 2.9 Mb according to the manufacturer's instructions. The sequencing analyses were performed on the HiSeq 2500 sequencing system (Illumina, United States). Data were analyzed using a software package that was commercially available - NextGENe<sup>™</sup> (SoftGenetics, United States). Only variants with a minimum of 50-fold coverage for at least 80% of the targeted bases were included into analysis. Variants were marked as potential errors if they exhibited strong strand bias ( $< 0.10$ ), low depth of coverage ( $< 10$ ), low-quality score ( $< 30$ ) or low average quality ( $< 2.0$ ). The functional analysis of variants was performed by using Geneticist Assistant software (SoftGenetics, United States). This software used combined computational prediction methods (SIFT, Polyphen2, LRT, MutationTaster, ANNOVAR, FATHMM, CADD and Mutation Assessor) to calculate

the mean pathogenicity score of identified variants. The pathogenic variants detected by NGS were verified by using the Sanger sequencing method. The germline origin of the pathogenic variants was excluded by analysis of DNA extracted from peripheral blood.

## **RESULTS**

#### **Analysis of sequencing data**

In our study, we achieved a high quality of sequenced data. The mean total reads generated per sample was approximately 5000000, with more than 98% of reads aligned with the reference genome and more than 80% of reads mapping to targeted regions. In addition, 93% of the targeted regions were covered by more than 30 reads. Overall, 1440 variants were identified in 7 ADPKD samples. These variants represent disease-specific genetic changes without occurrence in matched normal kidney tissues. Based on the results of the functional analysis, 908 variants were classifying as benign. These variants were reported in known databases of somatic mutations and single nucleotide polymorphisms. Of these, 732 variants were classified as clearly benign. The remaining 176 variants were of unknown significance, but with high value of minor allele frequency, on the basis of which they were determined as polymorphisms.

The other 532 identified variants represented novel genetic changes without record in databases. The results of the functional prediction determined 52 variants as clearly pathogenic. For the other 480 variants, benign status was clearly confirmed in all predictive software. Identified pathogenic variants were present in 39 genes encoding the various structural components of the primary cilium. An overview of these genes is shown in Table 1. The most common pathogenic variants affected the proteins of basal bodies (10 genes), centrosomes (7 genes), centrioles (6 genes) and ciliary proteins with non-specific localization (5 genes). The proteins in other parts of the cilium were rarely mutated.

#### **Analysis of disease-related pathogenic variants**

The pathogenic variants were further analyzed based on their occurrence in individual ADPKD samples. The mutation profile of each analyzed sample was unique. Each sample had identified pathogenic variants in 5 to 15 genes, which varied from each other. However, the pathogenic variants in 5 genes were present in the vast majority of the ADPKD samples. Of these, 4 included genes encoded the centrosomal protein HTT (Huntingtin), the subdistal appendage of centriole ODF2 (outer dense fiber protein 2), the distal appendage of centriole CEP89 (centrosomal protein of 89 kDa) and a component of centriolar satellite PCM1 (pericentriolar material 1). The most affected gene was *PCM1*, in which pathogenic variants were present in all samples of ADPKD. The pathogenic variants in the *HTT*, *ODF2* and *CEP89* genes were identified in 5 of the 7 ADPKD samples. Another gene whose pathogenic variants affected all of the ADPKD samples was the *KIF19* (Kinesin

**Table 1** Summary of genes affected by pathogenic variants and localization of their coding proteins

Localization	Genes affected by pathogenic variants
Basal body	<i>ODF2, AZI1, BBS2, BBS2, C21orf2, FLNA, LZTFL1, OFD1, PDYD7, TRAF3IP1</i>
Centrosome	<i>CEP89, HTT, CEP135, CEP290, MDM1, NINL, TRAPPC9</i>
Centriole	<i>PCM1, PIBF1, CBY1, FBF1, NEK1, SCLT1</i>
Unspecific localization	<i>STX3, PKD1L1, TTLL3, WDR60, CCDC35</i>
Ciliary tip	<i>KIF19, GLIS2</i>
Intraflagellar transport	<i>IFT172, IFT80</i>
Transition zone	<i>NUP37, NUP62</i>
Regulatory proteins	<i>RAB11FIP3, TRIP11</i>
Ciliary membrane	<i>CRB3</i>
Ciliary root	<i>CROCC</i>
Axoneme	<i>TMEM67</i>

**Table 2** Description of the most common pathogenic variants identified in autosomal dominant polycystic kidney disease samples

Gene	DNA-level	Protein-level	Exon	Number of samples with mutation
<i>PCM1</i>	c.3423dupC	p.Ser1142Glnfs*7	22	7
<i>KIF19</i>	c.49dupC	p.Arg17Profs*20	2	7
<i>CEP89</i>	c.412_413delAA	p.Lys138glyfs*16	4	5
<i>HTT</i>	c.108_110delGCA	p.Gln2643del	1	5
<i>ODF2</i>	c.1118delT	p.Leu373Tyrfs*80	10	5

Family Member 19) gene. This gene encodes a key regulator of ciliary length located on the very top of the primary cilia in the ciliary tip included in anterograde IFT. In addition, we identified only one type of the pathogenic variant in these 5 genes (Table 2). In most cases, it was a frameshift mutation resulting in a premature stop codon and truncation of protein. According to the results of the predictive software, all detected variants negatively affected the function of the encoded proteins.

Interestingly, the pathogenic variants in genes encoding proteins of retrograde IFT and other structural ciliary genes were detected in only one of the ADPKD samples. The coverage of protein-coding regions and the flanking regions of all analyzed IFT genes showed high values in each of the ADPKD samples.

## DISCUSSION

In the present study, we identified genetic changes in the structural ciliary genes in human ADPKD tissues. The most frequently affected genes encoded the centriolar and centrosomal proteins *PCM1*, *ODF2*, *HTT* and *CEP89*, which are essential for ciliogenesis. Recent studies have confirmed that the loss of these proteins specifically blocks ciliogenesis at the step of centriole-to-membrane docking. Undocked centrioles lose the signs of cilia assembly, even when they were previously under the influence of signals that support ciliogenesis<sup>[18,19]</sup>. Thus, disruption of the function of these genes may be the cause of cilia loss in the epithelial cells of renal cysts. The most commonly affected gene in this group was *PCM1*, which is also essential for the correct localization of several centrosomal proteins and for anchoring microtubules to the centrosome. It is generally known that centrosome dysfunction is linked to aneuploidy and chromosomal instability<sup>[20]</sup>. Many studies have

shown increased incidence of chromosome imbalances and abnormal chromosome segregation in ADPKD tissues<sup>[21,22]</sup>. Loss of function of the *PCM1* gene may therefore be a key factor in these processes.

Another pathogenic variant, which was present in all of the analyzed ADPKD samples, was identified in the *KIF19* gene. This gene encodes a member of the kinesin superfamily protein included in anterograde IFT, which is localized to ciliary tips. The results of a recent study have provided evidence that *KIF19* is a key determinant of the optimal length of cilia. The length of cilia was abnormally extended by knockdown *Kif19* in a mouse model<sup>[23]</sup>. Many studies have revealed that the change in the length of the primary cilium is an important trigger of various pathological processes in ADPKD<sup>[24,25]</sup>. The epithelial cells of renal cysts usually show an absence or shortening of the primary cilium. However, stages of interstitial fibrosis and end-stage renal disease are associated with the elongation of the primary cilia<sup>[26,27]</sup>. In our study, we analyzed the mutation profile in the nephrectomized ADPKD tissues withdrawn at the end stage of renal disease. Given that we identified the pathogenic variant of the *KIF19* gene in all analyzed ADPKD tissues, it may represent a significant event in the progression of the disease. However, these claims will require further analysis.

An interesting result of our study was the low frequency of mutations in the genes encoding IFT proteins. Kidney cysts arise in most mouse models following the disruption of cilia by targeted inactivation of genes encoding IFT components such as the heterotrimeric kinesin components *KIF3a* and the IFT proteins *IFT20* and *IFT88*. However, genetic changes in these genes were not present in any analyzed ADPKD sample. We confirmed the presence of the pathogenic variants in only two genes (*IFT172* and *IFT80*) of the total number

of analyzed IFT genes. In addition, the pathogenic variants in these genes were detected in only one ADPKD sample. The results of our study showed that the loss of the primary cilia in the human ADPKD tissues may be predominantly caused by defects of centrosomal proteins and KIF19 protein. However, this claim requires confirmation by functional analysis of the use of animal models. It is also necessary to verify the results by analyzing a larger number of samples.

In our study, we identified the simultaneous occurrence of genetic changes in various ciliary genes. Each ADPKD tissue had pathogenic variants in 5 to 15 genes. The occurrence of multiple structural defects of the primary cilium may be due to several factors. Firstly, we analyzed the tissues at an advanced stage of the disease, in which genetic changes could have been accumulated. However, disturbances of centrosomal proteins may also be the cause of the multiple defects in the ciliary structure. An interesting finding was the presence of only one pathogenic variant in each individual gene. To determine whether they are “hotspot” mutations representing secondary somatic events in the development of the disease will require further analysis.

The results of our study may be limited by the relatively small number of analyzed samples. The reason for this small number 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. However, this is the first study examining the complex mutational status of the structural and functional ciliary genes in human ADPKD tissues.

In conclusion, our study revealed genetic defects of ciliary genes that can lead to loss of the primary cilium in human ADPKD tissues. In addition, we identified unique genetic findings associated with the disease, which may play a significant role in the pathogenesis of the disease. However, other functional analyses are necessary to confirm this hypothesis. The results of our work thus provided valuable indications for the direction of further research in the area of molecular pathogenesis of ADPKD.

## ARTICLE HIGHLIGHTS

### Research background

The primary cilia and polycystins play an important role in the regulating the severity of autosomal dominant polycystic kidney disease (ADPKD). While the loss of cilia or polycystins alone results in the development and progression of renal cysts, renal cilia involution reduces the progression of cyst growth induced by the inactivation of polycystins. The epithelial cells of renal cysts usually show various structural deformities of the primary cilium involve an absence, shortening or elongation. These structural changes can be caused by genetic defects of ciliary proteins. Mutation profile of ciliary genes in human ADPKD tissues is unknown. Revealing a genetic basis for ciliogenesis defects may identify causative factors of disease progression and the potential molecular targets for the development of new therapies of ADPKD.

### Research motivation

Genetic defects of various ciliary genes whose inactivation leads to the development and progression of ADPKD have been identified in mouse models.

However, recent studies have confirmed that the animal models of ADPKD incompletely mimic the human disease. Therefore, it is important to detect genetic abnormalities that can affect ciliogenesis directly in ADPKD human tissues.

### Research objectives

The main objectives of this study is to identify the genetic defects of ciliary genes causing the loss of primary cilium in ADPKD human tissues. The results of our study are important indicators for directing further analysis.

### Research methods

In our study, we analyzed 191 structural and functional ciliary genes using next-generation sequencing analysis. The tissue samples used in this study were obtained from 7 patients with ADPKD who underwent nephrectomy. Each sample contained polycystic kidney tissue and matched normal kidney tissue. All analyzed samples were formalin-fixed and paraffin-embedded. The germline origin of the identified variants was excluded by analysis of DNA extracted from peripheral blood.

### Research results

We identified unique of mutation profile in each of analyzed ADPKD samples, which was characterized by the presence of pathogenic variants in 5 to 15 ciliary genes. The most frequently identified defects were found in genes encoding centrosomal proteins and kinesin family member 19, which are important for ciliogenesis. In addition, pathogenic variants in the *PCM1* and *KIF19* genes were found in all ADPKD samples.

### Research conclusions

Our study had found that the human ADPKD tissues are characterized by the presence of several genetically altered ciliary proteins that plays an important role in ciliogenesis. The structural and functional disturbance of the primary cilium can be induced by mutations of these proteins. An interesting finding of our study was that the mutations in genes encoding the proteins of intraflagellar transport (IFT) were rarely mutated. These genes are considered candidate genes related to ADPKD in mouse models. Centrosomal proteins and kinesin family member 19 are the most commonly mutated ciliary proteins in renal epithelial cells derived from human ADPKD cysts. Consistent with finding of recent studies, we can confirm that animal models not completely mimic the human disease. Mouse models induce polycystic kidney disease by genetic inactivation of one ciliary gene especially genes encoding IFT proteins. However, the genes encoding the proteins of IFT are rarely mutated in the human renal cystic cells. This study offered new insight into comprehensive mutation profile of ciliary genes in human ADPKD tissues. The results of our study suggested that the loss of the primary cilia in the human ADPKD tissues may be predominantly caused by defects of centrosomal proteins and kinesin family member 19. The defects of centrosomal proteins may be also the cause of chromosome imbalances, which are often present in human ADPKD tissues. The genetic defects of the *KIF19* gene may be cause of the primary cilium elongation, which is a characteristic feature of the end-stage renal disease. This is the first study used of archived formalin-fixed and paraffin embedded tissues (FFPE) of ADPKD in order to determine mutation profile of ciliary genes by next-generation sequencing methods. An interesting finding was the presence of only one somatic pathogenic variant in each individual ciliary gene. To determine whether they are “hotspot” mutations representing secondary somatic events in the development of the disease will require further analysis. Somatic mutations in genes encoding centrosomal proteins and KIF19 were present in all analyzed ADPKD samples. These mutations were present exclusively in polycystic kidney tissues and did not occur in matched control so we can assume their effect on cystogenesis. If our hypotheses will be confirmed by further studies, the identified ciliary proteins may represent potential molecular targets for the development of new treatments.

### Research perspectives

Mutation profile of ciliary genes can be analyzed directly from archived FFPE tissues of ADPKD by NGS. The first step, it is necessary to verify the results on a larger number of samples and matched tissues controls. Further research should be focused on the functional analysis of identified genetic variants. Consequently, it will be necessary to determine whether the inactivation of



these genes will lead to a change in the structure of renal cilia and affect the development or progression of ADPKD.

## REFERENCES

- 1 **Chebib FT**, Torres VE. Autosomal Dominant Polycystic Kidney Disease: Core Curriculum 2016. *Am J Kidney Dis* 2016; **67**: 792-810 [PMID: 26530876 DOI: 10.1053/j.ajkd.2015.07.037]
- 2 **Cornec-Le Gall E**, Audrézet MP, Rousseau A, Hourmant M, Renaudineau E, Charasse C, Morin MP, Moal MC, Dantal J, Wehbe B, Perrichot R, Frouget T, Vigneau C, Potier J, Jousset P, Guillolo MP, Siohan P, Terki N, Sawadogo T, Legrand D, Menoyo-Calonge V, Benarbia S, Besnier D, Longuet H, Férec C, Le Meur Y. The PROPKD Score: A New Algorithm to Predict Renal Survival in Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol* 2016; **27**: 942-951 [PMID: 26150605 DOI: 10.1681/ASN.2015010016]
- 3 **Bastos AP**, Onuchic LF. Molecular and cellular pathogenesis of autosomal dominant polycystic kidney disease. *Braz J Med Biol Res* 2011; **44**: 606-617 [PMID: 21625823]
- 4 **Porath B**, Gainullin VG, Cornec-Le Gall E, Dillinger EK, Heyer CM, Hopp K, Edwards ME, Madsen CD, Mauritz SR, Banks CJ, Baheti S, Reddy B, Herrero JI, Bañales JM, Hogan MC, Tasic V, Watnick TJ, Chapman AB, Vigneau C, Lavainne F, Audrézet MP, Férec C, Le Meur Y, Torres VE; Genkyst Study Group, HALT Progression of Polycystic Kidney Disease Group; Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, Harris PC. Mutations in GANAB, Encoding the Glucosidase IIa Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. *Am J Hum Genet* 2016; **98**: 1193-1207 [PMID: 27259053 DOI: 10.1016/j.ajhg.2016.05.004]
- 5 **Harris PC**. What is the role of somatic mutation in autosomal dominant polycystic kidney disease? *J Am Soc Nephrol* 2010; **21**: 1073-1076 [PMID: 20488953 DOI: 10.1681/ASN.2010030328]
- 6 **Paridaen JT**, Wilsch-Bräuninger M, Huttner WB. Asymmetric inheritance of centrosome-associated primary cilium membrane directs ciliogenesis after cell division. *Cell* 2013; **155**: 333-344 [PMID: 24120134 DOI: 10.1016/j.cell.2013.08.060]
- 7 **Chien A**, Shih SM, Bower R, Tritschler D, Porter ME, Yildiz A. Dynamics of the IFT machinery at the ciliary tip. *Elife* 2017; **6**: pii: e28606 [PMID: 28930071 DOI: 10.7554/eLife.28606]
- 8 **Pazour GJ**, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, Witman GB, Cole DG. Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol* 2000; **151**: 709-718 [PMID: 11062270]
- 9 **Lin F**, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, Somlo S, Igarashi P. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci USA* 2003; **100**: 5286-5291 [PMID: 12672950 DOI: 10.1073/pnas.0836980100]
- 10 **Mokrzan EM**, Lewis JS, Mykityn K. Differences in renal tubule primary cilia length in a mouse model of Bardet-Biedl syndrome. *Nephron Exp Nephrol* 2007; **106**: e88-e96 [PMID: 17519557 DOI: 10.1159/000103021]
- 11 **Besschetnova TY**, Kolpakova-Hart E, Guan Y, Zhou J, Olsen BR, Shah JV. Identification of signaling pathways regulating primary cilium length and flow-mediated adaptation. *Curr Biol* 2010; **20**: 182-187 [PMID: 20096584 DOI: 10.1016/j.cub.2009.11.072]
- 12 **Hildebrandt F**, Bezing T, Katsanis N. Ciliopathies. *N Eng J Med* 2011; **364**: 1533-1543 [DOI: 10.1056/NEJMr1010172]
- 13 **Ma M**, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nat Genet* 2013; **45**: 1004-1012 [PMID: 23892607 DOI: 10.1038/ng.2715]
- 14 **Lee SH**, Somlo S. Cyst growth, polycystins, and primary cilia in autosomal dominant polycystic kidney disease. *Kidney Res Clin Pract* 2014; **33**: 73-78 [PMID: 26877954 DOI: 10.1016/j.krcp.2014.05.002]
- 15 **Ibraghimov-Beskrovnya O**, Natoli TA. mTOR signaling in polycystic kidney disease. *Trends Mol Med* 2011; **17**: 625-633 [PMID: 21775207 DOI: 10.1016/j.molmed.2011.06.003]
- 16 **Eguether T**, San Agustin JT, Keady BT, Jonassen JA, Liang Y, Francis R, Tobita K, Johnson CA, Abdelhamed ZA, Lo CW, Pazour GJ. IFT27 links the BBSome to IFT for maintenance of the ciliary signaling compartment. *Dev Cell* 2014; **31**: 279-290 [PMID: 25446516 DOI: 10.1016/j.devcel.2014.09.011]
- 17 **van Dam TJ**, Wheway G, Slaats GG; SYSCILIA Study Group, Huynen MA, Giles RH. The SYSCILIA gold standard (SCGSv1) of known ciliary components and its applications within a systems biology consortium. *Cilia* 2013; **2**: 7 [PMID: 23725226 DOI: 10.1186/2046-2530-2-7]
- 18 **Tanos BE**, Yang HJ, Soni R, Wang WJ, Macaluso FP, Asara JM, Tsou MF. Centriole distal appendages promote membrane docking, leading to cilia initiation. *Genes Dev* 2013; **27**: 163-168 [PMID: 23348840 DOI: 10.1101/gad.207043.112]
- 19 **Wang L**, Lee K, Malonis R, Sanchez I, Dynlacht BD. Tethering of an E3 ligase by PCM1 regulates the abundance of centrosomal KIAA0586/Talpid3 and promotes ciliogenesis. *Elife* 2016; **5**: 5 [PMID: 27146717 DOI: 10.7554/eLife.12950]
- 20 **Pihan GA**. Centrosome dysfunction contributes to chromosome instability, chromoanagenesis, and genome reprogramming in cancer. *Front Oncol* 2013; **3**: 277 [PMID: 24282781 DOI: 10.3389/fonc.2013.00277]
- 21 **AbouAlaiwi WA**, Rodriguez I, Nauli SM. Spectral karyotyping to study chromosome abnormalities in humans and mice with polycystic kidney disease. *J Vis Exp* 2012; **3**: pii: 3887 [PMID: 22330078 DOI: 10.3791/3887]
- 22 **de Almeida RM**, Clendenen SG, Richards WG, Boedigheimer M, Damore M, Rossetti S, Harris PC, Herbert BS, Xu WM, Wandinger-Ness A, Ward HH, Glazier JA, Bacallao RL. Transcriptome analysis reveals manifold mechanisms of cyst development in ADPKD. *Hum Genomics* 2016; **10**: 37 [PMID: 27871310 DOI: 10.1186/s40246-016-0095-x]
- 23 **Niwa S**, Nakajima K, Miki H, Minato Y, Wang D, Hirokawa N. KIF19A is a microtubule-depolymerizing kinesin for ciliary length control. *Dev Cell* 2012; **23**: 1167-1175 [PMID: 23168168 DOI: 10.1016/j.devcel.2012.10.016]
- 24 **Verghese E**, Zhuang J, Saito D, Ricardo SD, Deane JA. In vitro investigation of renal epithelial injury suggests that primary cilium length is regulated by hypoxia-inducible mechanisms. *Cell Biol Int* 2011; **35**: 909-913 [PMID: 21241248 DOI: 10.1042/CBI20090154]
- 25 **Ong AC**. Primary cilia and renal cysts: does length matter? *Nephrol Dial Transplant* 2013; **28**: 2661-2663 [PMID: 23935132 DOI: 10.1093/ndt/gft354]
- 26 **Saito S**, Tampe B, Müller GA, Zeisberg M. Primary cilia modulate balance of canonical and non-canonical Wnt signaling responses in the injured kidney. *Fibrogenesis Tissue Repair* 2015; **8**: 6 [PMID: 25901180 DOI: 10.1186/s13069-015-0024-y]
- 27 **Han SJ**, Jang HS, Kim JI, Lipschutz JH, Park KM. Unilateral nephrectomy elongates primary cilia in the remaining kidney via reactive oxygen species. *Sci Rep* 2016; **6**: 22281 [PMID: 26923764 DOI: 10.1038/srep22281]

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