**SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)**

# Examination of genetic variations underlying kidney diseases

**by Petronella Éva Orosz**

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UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF LAKI KÁLMÁN

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| Head of the **Examination Committee**: | József Balla, DSc |
| Members of the Examination Committee: | András Tislér, PhD |
| | Tibor Flaskó, PhD |

The Examination takes place at the Library of Building "A" Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 22.03.2024. 12:00

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| Head of the **Defense Committee**: | József Balla, DSc |
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The PhD Defense takes place at the Lecture Hall of Emergency Department, Faculty of Medicine, University of Debrecen, 22.03.2024. 13:30

## Introduction

A large group of inherited, genetically determined kidney diseases affects many people around the world. In adult patients with chronic kidney failure, the diagnosis of "chronic kidney disease of unknown etiology" is common, which in many cases may reveal an unknown genetic background. The deterioration of kidney function takes place insidiously, without symptoms, until renovascular hypertension caused by moderately reduced kidney function or the symptoms of end-stage renal failure or uremia draw attention to it. The factors that complicated the diagnosis are the atypical form of the disease, the absence or negativity of family history (also in case of de novo mutations), the lack of knowledge about genotype-phenotype correlations, and inadequate evaluations of detected symptoms. Because of all this, paying attention to alarming symptoms in childhood is essential.

With genetic testing methods, pathological mutation can be identified more precisely. These are expensive testing procedures, so they should be used judiciously and targeted as far as possible. Therefore, it is vital to observe the clinical symptoms of the phenotype and periodically reassess it as necessary.

Polycystic kidney diseases are perhaps the most significant of the hereditary kidney diseases. The diseases listed here belong to the large group of ciliopathies, which can be explained by mutations of the proteins that build and are related to primary cilia. More than 950 genes have been identified in the background of ciliopathies, including the genes responsible for polycystic kidney diseases. Cysts that develop in both kidneys and increase in number and size even lifelong are characteristic of autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). Still, cystic lesions of the kidney also occur in other ciliopathies. In terms of differential diagnosis, nephronophthisis (NPHP), autosomal dominant tubulointerstitial kidney diseases (ADTKD), and tuberous sclerosis (TSC) may also arise. The time of discovery of renal involvement, the differences described during the imaging examination (hyperreflectivity, presence of cysts, location, size), the longitudinal (cranio-caudal) diameter of the kidneys, the appearance of the cavity system, and changes in renal function can help the differentiation. The appearance of renal hypertension is a general symptom; it often correlates with the degree of damage to kidney function, but in the case of certain pathologies, it can even be the first symptom. It is essential to look for and monitor extrarenal symptoms, the cysts in the liver, spleen, or pancreas, identification of intracranial aneurysms, liver fibrosis, nervous system involvement, mental retardation, retinal changes, or signs of diabetes, as well as skin symptoms.

## Objectives

My thesis focused on examining genotype-phenotype correlations between the clinical symptoms of childhood polycystic kidney disease and the genetic differences underlying the symptoms. This can form the basis of differential diagnostic clues in everyday pediatric nephrology practice. Another goal of mine was the complex way of thinking required in cases with atypical appearance, the search for extended examination systems, the development of a targeted examination strategy, and the indication of when it is necessary to perform a comprehensive examination in each clinical picture.

Despite the principal role of the *PKHD1* gene in ARPKD, no *PKHD1* mutations are identified in 13–20% of the cases by sequencing. During my work, I searched for the answer to whether mutations in undetectable positions or copy number changes are in the background in such cases or the appropriate next step is researching "second locus" mutations and phenocopies? In this way, the further goal was to develop an algorithm that can be used well in examining children born with autosomal recessive polycystic kidney disease. Furthermore, I searched for the typical phenotypic features that explicitly reinforce the diagnosis of ADPKD.

Another focus of my research was the group of patients living with tuberous sclerosis, including the tuberous sclerosis-polycystic kidney disease *TSC2-PKD1* contiguous gene deletion syndrome (CGS) patients. Both tuberous sclerosis and autosomal dominant polycystic kidney disease can be diagnosed based on clinical symptoms, official criteria, and family tree analysis. The suspicion of the large deletion syndrome involving two genes may also arise based on the clinical examination. Still, only a genetic test can provide full confirmation of this. According to the Human Genetics Act, human genetic testing cannot be performed under 14 unless there are therapeutic or strategic consequences for care. However, in the case of *TSC2-PKD1* continuous gene deletion syndrome, there is a rightful need for children and their families to be informed about the disease with end-stage renal failure that develops up to 3 decades earlier. Considering that there is no curative procedure available for the disease (the only solution is a kidney transplant), I was looking for an answer to the question, when is it recommended to propose a genetic test? Determining the primary examination method and, based on the findings in the examined patient material, the indication of the extension of the targeted genetic examination is also an essential question.

In summary, my objectives were to find answers to these questions:

1. What are the phenotypic signs that make the diagnosis of ARPKD highly probable?
2. If sequencing of the *PKHD1* gene does not identify a mutation in children phenotypically diagnosed with ARPKD, is the examination of copy number variations or the search for phenocopies the next recommended procedure?
3. When and which genetic testing method is primarily recommended in case of suspicious *TSC2-PKD1* contiguous gene deletion syndrome?
4. When is it recommended to extend the genetic testing for *TSC2-PKD1* contiguous gene deletion syndrome?

## Patients and Methods

## Examination of ARPKD patients

36 unrelated children were included based on the following criteria:

1. hyperreflective kidneys with microcysts
2. cysts < 2 cm in diameter on ultrasound
3. a kidney length above the 50th percentile (http://radiology-universe.org/calculator/pediatric-kidney-sizes/calculator.php) on at least one side
4. a transmission compatible with autosomal recessive inheritance
5. no urinary tract malformation
6. no extra-renal and hepatic involvement suggestive of other ciliopathies

The patients were selected from the four large Hungarian pediatric nephrology centers.

The DNA samples extracted from the patient's blood samples were analyzed in the Clinical Genetics Laboratory of the Laboratory Medicine Institute of the University of Debrecen. First, the Sanger sequencing of the *PKHD1* gene. If the result of sequencing confirmed a pathogenic mutation on both alleles, a targeted test was performed on the parents' blood samples to confirm trans-heterozygosity. If Sanger sequencing could not confirm a biallelic mutation, copy number variations were screened by MLPA testing of the *PKHD1* gene. In the event of a negative result, the phenotypic characteristics of the patients were re-evaluated, and the second gene was examined (*NPHP1, HNF1B, TMEM67, PKD1, PKD2, TSC2*) corresponding to other emerging ciliopathies. These tests were carried out partly in the Clinical Genetics Laboratory of the Laboratory Medicine Institute of the University of Debrecen, partly in MTA-SE Lendület Nephrogenetic Laboratory in Budapest, and partly in the Genetic Diagnostics Laboratory of the

Szent-Györgyi Albert Clinical Center of the University of Szeged. The obtained genetic results were made available to me for the present scientific investigation after informing the affected patients and their relatives.

## Examination of TSC2-PKD1-contiguous gene deletion syndrome patients

Four children were included based on the following criteria:

1. The clinical diagnosis of tuberous sclerosis can be made according to the criteria based on the guidelines of the International Tuberous Sclerosis Complex Consensus Working Group (2 major or 1 major+2 minor criteria)
2. Cysts are more significant in the kidneys, and the number of AMLs are low.
3. The increase in size or number of cysts in the first three years of life is increased compared to the classic tuberous sclerosis (>10 cysts at least 1 cm in diameter before the age of 3 years)
4. The craniocaudal length of the kidneys exceeds the 50th percentile or the +2SD for age.

The genetic tests were performed in the laboratory of Medical Genetics, Medical School, Clinical Centre, University of Pécs. In two cases, it started with the sequencing of the *TSC1* and *TSC2* genes, after which no pathogenic mutation was detected and considering the progressing clinical symptoms, an MLPA test was performed in the first round to examine the same genes and then the large deletion affecting the *PKD1* gene as well. The targeted MLPA testing of the *TSC2* and *PKD1* genes was performed in two cases. In the case of one patient, further extension of the MLPA test was considered, and the *NTHL1* gene was also examined. The targeted genetic testing of the parents was realized in the case of two children. The obtained genetic results were made available to me for the present scientific investigation after informing the affected patients and their parents.

Both ARPKD and *TSC2-PKD1*-contiguous gene deletion syndrome patients regularly underwent the following examinations routine during follow-up:

- Imaging study: abdominal ultrasound and MRI, the latter in case of need during short general anesthesia
- Blood pressure measuring with an upper arm blood pressure cuff adapted to the children's size. Blood pressure values above the 95th percentile, representing hypertension, were determined using the Hungarian Hypertension Society's online

calculator for medical professionals and pediatricians. https://www.merckmanuals.com/medical-calculators/BloodPressurePercentBoys.htm and https://www.merckmanuals.com/medical-calculators/BloodPressurePercentGirls.htm

- Laboratory tests, among others, to monitor kidney function. GFR was calculated with the Schwartz formula based on the measured creatinine level. In the case of two patients, everolimus drug level measuring was performed regularly.
- Urine sample examination to monitor proteinuria and possible urinary tract infection.

## Genetic testing

## Genetic testing of ARPKD patients

Genomic DNA was isolated from peripheral blood by standard methods. The Sanger sequencing of the *PKHD1* gene was first performed. Patients without biallelic point mutations were subsequently screened by MLPA for copy number variations. Parental samples were screened for the identified mutations to confirm segregation and trans-heterozygosity. Patients without biallelic *PKHD1* mutations were reevaluated based on their most recent phenotype and were screened for second locus mutations accordingly. The "second locus" mutations in the *NPHP1*, *HNF1B*, *TMEM67*, *PKD1*, and *TSC2* genes were examined using different methods.

## Genetic testing of TSC2-PKD1 contiguous gene deletion syndrome patients

In the case of two patients, a multiplex PCR test of the *TSC1* and *TSC2* genes was performed for the first time, which did not identify any mutations. After that, an MLPA test was performed, which was also extended to test the *PKD1* gene. In the case of two patients, targeted MLPA testing of the *TSC2* and *PKD1* genes was performed. Based on the clinical symptoms, one patient's genetic examination was extended to the examination of the *NTHL1* gene.

## Results

## Results of ARPKD patients

Of the 36 unrelated patients with a clinical diagnosis of ARPKD, 27 (75%) were found to carry biallelic *PKHD1* mutations. Among them, 25 patients carried biallelic point mutations, and two were compound heterozygous for a point mutation and either a duplication of exons 33– 35 or a large deletion encompassing exons 1 to 55. Furthermore, one patient was found to carry a single heterozygous frameshift mutation. No *PKHD1* mutation was found in 8 families. Two mutations, p.Thr36Met and p.Ser2639*, were found frequently in 15/54 (28%) and 8/54 (15%) of the mutated alleles, respectively. Seven mutations were novel: besides the duplication of exons 33–35 and the deletion of exons 1–55, three truncating mutations (c.5_8delCTGC, p.Ala3Glyfs*2; c.5088delTG, p.Gly1696fs*1; c.12036delA, p.Gly4013Alafs*24) and two missense mutations (c.4328G > A, p.Cys1443Tyr and c.10621A > T, p.Asn3541Tyr). We considered these two latter also pathogenic because both affect amino acids conserved in mammals. All eight patients without *PKHD1* mutations were found to carry second locus mutations. Three children diagnosed with hyperechogenic, normal-sized kidneys in utero or infancy carried a de novo deletion of *HNF1B*. The renal morphology of two children became suggestive of ADPKD between 2 and 4 years of age. They both harbored de novo *PKD1* mutations. One patient was diagnosed with tuberous sclerosis at the age of 4 years and carried a de novo *TSC2*/*PKD1* deletion. Finally, the phenotype of a patient diagnosed at the age of 11 years with end-stage renal disease was suggestive of juvenile nephronophthisis. He was compound heterozygous for a complete and partial deletion of *NPHP1*, as described recently. A sibling pair, diagnosed with hyperechogenic kidneys and hepatic fibrosis, was homozygous for the frequent *TMEM67* missense mutation, p.Cys615Arg. They had no neurological involvement. The patient with a heterozygous *PKHD1* mutation was not found to carry a second locus mutation, even by clinical exome sequencing.

Of the 27 patients with *PKHD1* mutations, 19 children (70%) developed perinatal respiratory failure, and nine (33%) died perinatally (< 3 months of age). In contrast, only one of the eight patients with second locus mutations had a transient perinatal respiratory failure secondary to an infection. Among the perinatal cases who survived beyond that period, all but one of the ten children with *PKHD1* mutations developed hypertension by the age of 1 year, compared with none of the six patients with second locus mutations. A mean kidney length above + 4 SD at diagnosis was also specific for *PKHD1*-associated ARPKD: 19 of 27 patients (70%) with biallelic *PKHD1* mutations, but none of the eight patients with second locus mutations had such an enlarged kidney. There was no difference

in renal survival between patients with *PKHD1* mutations who survived the perinatal period and the heterogeneous group of patients with second locus mutations.

## Results of TSC2-PKD1 contiguous gene deletion syndrome patients

A brief description of the clinical symptoms and phenotypic characteristics of the four patients is essential to interpret genetic results. The first patient is, at present, a 15-year-old boy. Postnatal ultrasound revealed small cysts in both of his kidneys, leading to a diagnosis of polycystic kidney disease. The ultrasonography and MRI follow-up revealed an abnormal growth in the kidneys with moderately increasing cysts. At the latest ultrasound, numerous tiny angiomyolipoma suspects echogenic foci were detected in both renal parenchyma. His skin lesions, developed at the age of five (hypopigmented spots, angiofibroma, and Shagreen patch), are typical of tuberous sclerosis, which appears in his case in an asymptomatic, less severe form. Genetic analysis by sequencing the TSC genes did not identify pathogenic mutations. A multiplex ligation-dependent probe amplification (MLPA) confirmed a large deletion of exons 17–42 of the *TSC2* gene and the complete deletion of the *PKD1* gene in heterozygous form.

In the case of the second and third patients, the symptoms of tuberous sclerosis showed a typical appearance; the association of polycystic kidney disease appeared later. The second patient is, at present, a five-year-old girl. An intrauterine ultrasound examination revealed multiplex rhabdomyomas in her heart. During a postnatal abdominal ultrasound, numerous cysts (3–14 mm in size) were discovered in both kidneys. A close follow-up detected fast-growing cysts. At the age of two, suspected angiomyolipoma lesions appeared. After the start of everolimus therapy at the age of two, the growth rate of cysts slowed, and the number and size of detected AMLs remained unchanged. Still, this effect seems to be temporary. The sequencing of the *TSC1* and *TSC2* genes confirmed no genetic abnormalities. The MLPA analysis of the *TSC2* gene revealed that a heterozygous deletion affects exons 14–42 of the *TSC2* gene and continues to exons 11–46 of the *PKD1* gene.

The third patient is, at present, a 13-year-old boy. The postpartum cardiology ultrasound examination confirmed large rhabdomyomas in his heart, which is a “red flag” sign of tuberous sclerosis. A neonatal abdominal ultrasound examination described a cyst 7 mm in diameter in the right kidney. At six months, 1.5–3.5 cm cysts were revealed in both kidneys, which showed continuous growth in number and size. Until now, angiomyolipoma could

not be confirmed with an MRI or ultrasound, perhaps because he has been receiving everolimus therapy since the age of 2.5 years with a neurological indication. A targeted genetic examination with MLPA verified the heterozygous deletion of exons 22–42 of the *TSC2* gene and the total deletion of the *PKD1* gene.

The fourth patient is, at present, a 17-year-old boy. He was born from a complicated pregnancy with multiple cardiac rhabdomyomas diagnosed prenatally. His polycystic kidneys were also recognized perinatally. All this raised the possibility of contiguous gene deletion syndrome early on. At the age of eight, in the lower pole of the right kidney, an echogenic, progressively increasing mass with a diameter of 35 mm was visible on abdominal ultrasound, which was not angiomyolipoma. He underwent tumor removal surgery. The histology showed nephroblastoma. After chemotherapy, radiotherapy, and surgical intervention, he was in remission. His renal function remained in the normal range, though modest proteinuria (1 g/day) appeared at 14. Unfortunately, one year later, his eGFR dropped to 64 mL/min/1.73 m2, his proteinuria progressed (2.5 g/day), and hypertension developed, requiring ACE-inhibitor therapy. At 16, a common control abdominal MRI presented, aside from numerous cysts, a lesion previously thought to be angiomyolipoma, and it showed a nonspecific appearance and growth. A close follow-up revealed a slow-growing renal cell carcinoma and a metastatic tumor in his liver. Further therapy is in progress. Now, at 17, he is in stage CKD3b (his GFR is 30 mL/min/1.73 m2). Genetic examinations by MLPA revealed that a large heterozygous deletion affected the whole *PKD1* gene, the entire *TSC2* gene, and, surprisingly, exon one of the *NTHL1* genes.

## Discussion

## ARPKD patients

Our *PKHD1*-positive rate of 78% corresponds well with previous reports. We found no biallelic copy number variations in the *PKHD1* negative cases. We only found a three-exon duplication and a large deletion in two out of three patients with a single heterozygous *PKHD1* point mutation. This finding was due to the low prevalence of *PKHD1* copy number variations in other cohorts. These results emphasize that screening with MLPA is primarily crucial in patients with a heterozygous *PKHD1* point mutation. If no point mutation is found on any allele, the next rational step is to reevaluate the phenotypic features and look for

phenocopies. We found all patients without *PKHD1* mutations to carry causal mutations in second loci by re-evaluating the phenotype and targeted mutation screening, indicating that 22% of the initial clinical ARPKD diagnoses were false. This emphasizes that regular reevaluation of clinical symptoms and phenotype is the cornerstone of targeted genetic testing. We failed to identify a second locus mutation in only one patient. Her case thus points to the difficulty in identifying some PKHD1 mutations even by the combined approach of sequencing and MLPA. It suggests a potential role of an intronic or a regulatory *PKHD1* mutation.

Our data suggest that perinatal respiratory failure, a kidney length > + 4 SD, and early-onset hypertension increase the likelihood of *PKHD1*-associated ARPKD. It is consistent with the literature data that the development of early hypertension was described in about 2/3 of the affected patients. I had no way to consistently examine hyponatremia in this group of patients, which can be associated with this by some authors.

Following the literature, the phenotype of patients with PKHD1 mutations strongly correlated with the causal mutations; no patient survived the perinatal period with biallelic loss-of-function mutations. Interestingly, a patient, compound heterozygous for a second and a last exon truncating mutation, p.Gly4013Alafs*24, presented with a moderate phenotype, indicating that the loss of the C-terminal 62 amino acids of fibrocystin does not cause complete loss of function. The intracellular C-terminal part of fibrocystin consists of 192 amino acids and is known to modulate the mTOR pathway. It also contains the ciliary targeting sequence (p.3876_3893CLVCCWLKRSKSRKTKPE) that remains unaffected in the Gly4013Alafs*24 fibrocystin.

Among ARPKD patients, respiratory difficulties in the newborn period are typical for severe forms, the background of which is primarily pulmonary hypoplasia caused secondarily by enormously enlarged kidneys. Based on literature data, approximately 31-41% of patients require respiratory support. In our cohort, invasive or non-invasive ventilation became necessary in the perinatal period in 70% of the patients, and almost half of the affected patients (9 out of 19 patients) died perinatally.

## TSC2-PKD1 contiguous gene deletion syndrome patients

*TSC2-PKD1* contiguous gene deletion syndrome was first described in 1994 by Brook-Carter et al. In his report, all six presented cases had typical polycystic kidney disease and epileptic seizures in infancy. In addition, five of the six patients had hypertension, and all

six had characteristic skin lesions. The initial renal presentation was similar in our cases as the number and diameter of renal cysts were typical (more than ten cysts with a diameter > 2 cm). In all but the first case, fetal rhabdomyoma was the first typical sign of the disease. For this reason, patient one received a diagnosis of polycystic kidney disease at first, while the other three patients received a diagnosis of tuberous sclerosis in infancy. In patient one, the possibility of TSC arose only after the appearance of hypomelanotic macules. In the other three cases, *TSC2-PKD1* contiguous gene deletion syndrome was diagnosed as secondary, based on the rapidly growing cysts.

Although renal cysts are only a minor criterion in diagnosing tuberous sclerosis, their presence can be helpful in the differential diagnosis. The diagnosis of *TSC2-PKD1*-CGS is often based on the typical appearance of renal cysts (diameter exceeding 2 cm at an early stage) and variable coexistence with angiomyolipoma. When comparing TSC patients with TSC/ADPKD patients, the renal phenotype is more severe in the latter, where large cysts predominate over AMLs, and there is a progressive enlargement of the kidneys. Symptomatic kidney cysts are seen in 30–50% of TSC patients with renal manifestations. The rupture of the cysts might cause macroscopic hematuria; however, it is usually not as severe as the bleeding of AMLs. In patients one and three, AMLs have not been present in the kidneys for a long. The typical clinical phenotype of large *TSC2* gene deletions was seen in our second case. However, the observed renal symptoms were presumably modified thanks to the everolimus therapy, which started with a neurological indication. According to current recommendations, the mTOR inhibitor everolimus or sirolimus is the first-choice treatment for AMLs measuring 3 cm or more or rapidly growing AMLs. The mTOR inhibitors significantly reduces the size and growth rate of AMLs and helps maintain kidney function. In our second patient, everolimus slowed the growth rate of AMLs and cysts. The third patient still has no detectable AML, possibly due to everolimus therapy. After discontinuation of treatment, AMLs begin to grow rapidly, indicating a reversible effect of the mTOR inhibitor. In addition, the number, sum diameter, and volume of kidney cysts decreased with the use of the mTOR inhibitor. mTOR-inhibiting therapy has also been safely used in polycystic kidney patients, but its effect on slowing down progression was not pronounced. Newer therapeutic options are still in the experimental phase.

The correlation between hypertension and kidney volume has been demonstrated in many studies. Hypertension was verified in patients one and three despite their retained kidney function. In the fourth case, hypertension developed in parallel with the deterioration of kidney function, but in his case, the enlargement of the solitary kidney, possibly

associated with secondary FSGS (suspected based on the appearance of proteinuria), could also have influenced all of this.

Extrarenal symptoms of ADPKD can also occur in *TSC2-PKD1* contiguous gene deletion syndrome but in a more severe form. So far, we have seen only a mild manifestation of this among our patients.

The reported average onset time of malignancies in TSC is 36 years; still, several case reports show that children have an increased risk for malignant tumors. Patient four had an atypical occurrence of malignancies. Nephroblastoma was never written with TSC or ADPKD, though it can also occur in the case of *WT1* germline and somatic mutations. Unfortunately, we have no data on whether he has a *WT1* mutation. However, the coincidence with *TSC2-PKD1*-CGS would probably be a literary rarity. Some authors assume a relationship between mTOR-pathway activation and Wilms tumor, though the details remain unclear. The second tumor of patient four was renal cell carcinoma, which can be associated with both TSC and ADPKD. However, renal cell carcinoma may occur in patients with ADPKD, usually in end-stage renal disease, and it can often be multifocal. This unusual early oncology symptom has highlighted the need for an extension of genetic testing that verified the deletion of exon one in the *NTHL1* gene. The *NTHL1* gene lies immediately adjacent to *TSC2*, head-to-head, and encodes a DNA glycosylase protein. An increased risk for colorectal and breast cancer typically characterizes biallelic mutations in *NTHL1*. Additionally, urothelial, and mesothelial carcinomas can occur. Heterozygous mutations in *NTHL1* can usually cause benign tumors. A second hit, the “loss of heterozygosity” mutation, could probably explain his tumor, though further examinations are needed. To the best of our knowledge, our case report is the second in the literature suggesting that the mutations of these three genes provoked clinical symptoms, and it is the first describing a large deletion affecting all the *NTHL1-TSC2-PKD1* genes.

Initiating genetic tests in both ADPKD and tuberous sclerosis can be a dilemma. In everyday clinical practice, the diagnosis of ADPKD can determined with the knowledge of a positive family history, the autosomal dominant inheritance pattern, the bilateral enlarged kidneys with several centimeters big cysts, and the extrarenal symptoms. In the typical phenotypic cases, the genetic testing of *PKD1* and *PKD2* genes won't perform, which could also be a technical challenge due to their large size. However, the molecular genetic examination can explore the exact defect, determining the remaining protein dose, and this result can predict the prognosis and help in therapeutic approaches. Nevertheless, there is no consensus in the literature on whether the mutation's type or location is more

critical in the genotype-phenotype relationship. But based on the type of mutation, it is possible to predict whether the development of the rapidly progressive form is expected according to the amount of residual protein product. This is especially important to make decisions about new therapeutic options. Current guidelines consider using tolvaptan, a vasopressin 2 receptor antagonist that regulates cAMP levels. Studies have demonstrated the role of arginine vasopressin-mediated cAMP production as a driver of cyst formation and fluid secretion in ADPKD. Consequently, tolvaptan, primarily used at the early stages of the disease, may help preserve kidney function proportionally to the total kidney volume (TKV). In children with ADPKD, the TKV significantly increases more than in adults. In several countries, tolvaptan is currently approved for the treatment of rapidly progressive disease in adult patients with ADPKD. However, limited results are available about drugs' safety and efficacy in children with ADPKD. Side effects, such as aquaresis with potentially severe dehydration, reversible liver injury, or headache, could be observed during pharmacological studies. However, the use of tolvaptan in children is not excluded in severe cases, with obviously intensified monitoring.

The diagnosis of tuberous sclerosis can be made according to the criteria based on the guidelines of the International Tuberous Sclerosis Complex Consensus Working Group; 2 major or 1 major+2 minor criteria is enough. However, genetic tests are also easily available in Hungary. Sanger sequencing of the *TSC1* and *TSC2* genes is suitable for testing point mutations; if the sequencing does not identify a mutation but clinical symptoms correspond to tuberous sclerosis, an MLPA test or next-generation sequencing (NGS) is recommended to detect larger deletions. In cases when the coexistence of tuberous sclerosis and polycystic kidney disease is suspected, only genetic testing can confirm the diagnosis. The targeted MLPA or NGS testing of *TSC2* and *PKD1* genes is suggested in patients with the diagnosis of tuberous sclerosis based on the criteria, but in whom the renal phenotype is characterized by cysts that develop early and rapidly grow to several centimeters and an early increase in the total kidney volume can be observed. Differing opinions and doubts regarding the ethical aspect naturally necessitate a personalized investigation plan. However, adequate information about the potentially more severe form of the disease is essential for children and their parents. With this awareness, they can give informed consent to genetic testing or refuse to carry it out. Nevertheless, in the atypical form of *TSC2-PKD1*-CGS, the genetic examination can help the work of nephrologists when the more progressive form of the disease is confirmed or excluded. Obviously, the genetic diagnosis of *TSC2-PKD1*-CGS

predicts the appearance of a more severe phenotype, and affected patients need improved attention with assistance to prepare for earlier occurring end-stage renal disease.

**Conclusion**

Based on the observations made during my work, I can draw the following conclusions:

1. The diagnosis of ARPKD can be assumed. A targeted examination of the *PKHD1* gene can be recommended if the following phenotypic signs are observed in the polycystic kidney patient: the craniocaudal length of the kidneys is significantly greater than the +4 SD of age average, but the diameter of the most giant cysts does not exceed 2 cm, if there was a need for respiratory support in the perinatal period if severe hypertension develops before the age of 1 year.
2. If the sequencing of the *PKHD1* gene does not reveal a pathological mutation on any allele, the next step is to reconsider the suspected diagnosis of ARPKD, reevaluate the phenotypic features, and look for phenocopies. The possibility of biallelic copy number variation is low, so MLPA testing is only recommended if heterozygous pathogenic point mutation is confirmed, and the examination of deletions and duplications occurring with low prevalence on the other allele is necessary.
3. Among the renal criterion symptoms of tuberous sclerosis are usually smaller cysts and angiomyolipomas that grow to several centimeters during age. Suppose this does not appear typically but is dominated by rapidly growing cysts in number and size (>10 cysts before the age of 3, their diameter exceeds 2 cm), in addition to smaller, often almost imperceptible AMLs. The coexistence of tuberous sclerosis and autosomal dominant polycystic kidney disease is suspected in that case. To identify this, targeted MLPA testing of the *TSC2* and *PKD1* genes is recommended to detect contiguous gene deletion. The genetic test is indicated because a closer follow-up strategy is necessary if the diagnosis is confirmed, and due to the progressive decline of kidney function, the use of available pharmacotherapy options may be appreciated. After receiving appropriate information, patients can decide whether they want to know about the possibility of decades-earlier end-stage renal failure.
4. Recognizing the *TSC2-PKD1* contiguous gene deletion syndrome is also based on the constant re-evaluation of the phenotypic features, which can be atypical even within the rare disease. The extent of the deletion affecting the two genes is variable; very rarely, a third gene, a section of the adjacent *NTHL1*, can be deleted. The MLPA examination

of this gene may become necessary if the suspected or confirmed *TSC2-PKD1*-CGS patient develops unusual, repeated malignant tumors at a young age.

## List of own publications

DEBRECENI EGYETEM
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Nyilvántartási szám: DEENK/403/2023.PL
Tárgy: PhD Publikációs Lista

Jelölt: Orosz Petronella
Doktori Iskola: Laki Kálmán Doktori Iskola
MTMT azonosító: 10065565

### A PhD értekezés alapjául szolgáló közlemények

1. **Orosz, P.**, Kollák, Z., Pethő, Á. G., Fogarasi, A., Reusz, G., Hadzsiev, K., Szabó, T.: The Importance of Genetic Testing in the Differential Diagnosis of Atypical TSC2-PKD1 Contiguous Gene Syndrome: Case Series.
*Children-Basel. 10* (3), 1-9, 2023.
DOI: http://dx.doi.org/10.3390/children10030420
IF: 2.4 (2022)

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**A közlő folyóiratok összesített impakt faktora: 15,625**
**A közlő folyóiratok összesített impakt faktora (az értekezés alapjául szolgáló közleményekre): 5,216**

A DEENK a Jelölt által az iDEa Tudóstérbe feltöltött adatok bibliográfiai és tudománymetriai ellenőrzését a tudományos adatbázisok és a Journal Citation Reports Impact Factor lista alapján elvégezte.

Debrecen, 2023.09.01.

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