NEW TECHNOLOGIES IN DNA ANALYSIS OF POLYCYSTIC KIDNEY DISEASES

Jana Reiterová[1], Lena Obeidová[2], Veronika Elisakova[2], Tomas Seeman[3], and Jitka Stekrova[2]

[1] Department of Nephrology, 1st Faculty of Medicine, Charles University in Prague and General University Hospital in Prague
[2] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague
[3] Department of Paediatrics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital

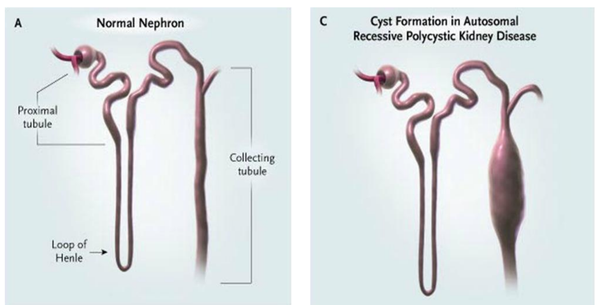

Fig. 1: Dilatations of renal collecting ducts in ARPKD patients
Wilson, 2004

1. INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD, OMIM #263200) is a severe form of polycystic kidney disease with histological features comprising fusiform dilatations of renal collecting ducts (**Figure 1**) and ductal plate malformations. The majority of patients are identified in utero or at birth. The most severely affected fetuses display enlarged echogenic kidneys and oligohydramnios that can result in critical degree of pulmonary hypoplasia. Arterial hypertension affects up to 80% of children with ARPKD and usually develops within the first months of life.

ARPKD is primarily caused by mutations in the ***PKHD1*** gene, however the phenotype of polycystic kidneys can be also manifested as a part of other syndromes, such as renal cyst and diabetes syndrome (RCAD) caused by mutations in the ***HNF1β*** gene, adult form of PKD with mutations in ***PKD1*** a ***PKD2***, nephronophthisis (mutations in ***NPHP*** genes) and others.

2. MOLECULAR ANALYSIS

The molecular analysis of the *PKHD1* and *HNF1β* genes was carried out using next-generation sequencing (NGS) method on GS Junior (Roche). In patients without 2 causal mutations of the *PKHD1* gene and without 1 mutation in *HNF1β* gene, the subsequent MLPA (multiplex ligation-dependent probe amplification) analysis of *PKHD1* and *HNF1β* gene was performed. Nowadays, new methods are established:

1. amplicon-based sequencing of the *PKHD1*, *HNF1β*, *PKD1* and *PKD2* genes using Nextera XT kits on MiSeq (Illumina),
2. target enrichment by NimbleGen on MiSeq (Illumina). This method enables us to analyze 120 genes responsible for nephropathies especially for cystic kidney diseases.

Cohort of 58 families with clinically suspected ARPKD was divided into 2 groups (A and B) on the basis of their fulfillment of clinical criteria of ARPKD, which are:

1. Presence of renal cysts
2. Typical liver involvement
3. No sign of kidney damage on US of both parents of affected child

Patients fulfilling all the clinical criteria were placed in group A, which consisted of 25 patients. Group B consisted of 35 patients who did not meet all of the presented criteria, including 14 families with severe polycystosis of fetus leading to termination of pregnancy.

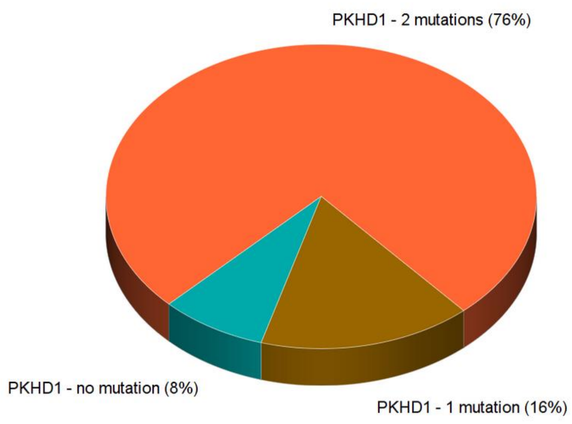

Fig. 2: Mutations within **Group A**

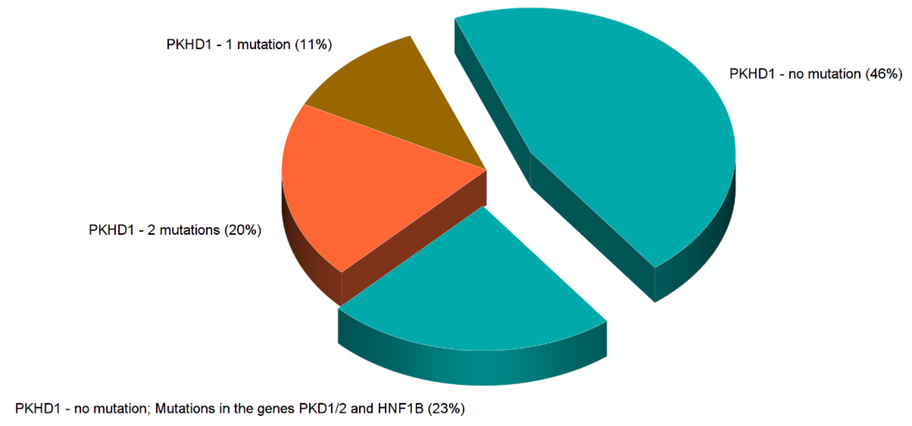

Fig. 3: Mutations within **Group B**

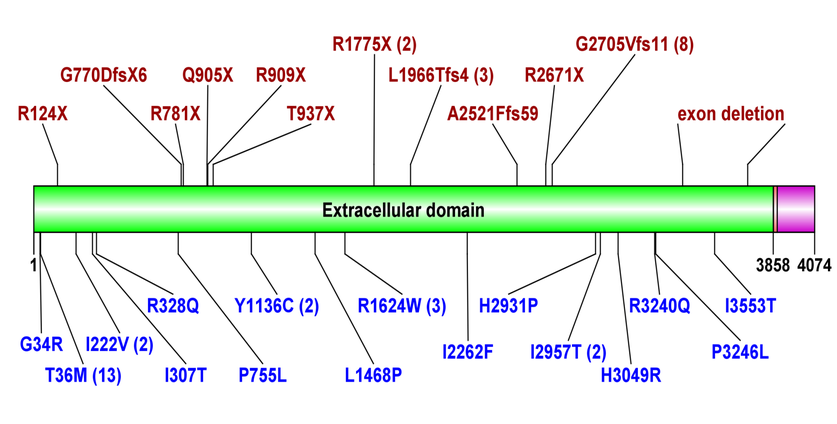

Fig. 4: Location of mutations along the protein fibrocystin

3. RESULTS

In Group A, two underlying mutations in *PKHD1* were detected in 19 out of 25 families (76%), one mutation in 4 families, and 2 families had no mutation found. Thus, the overall detection rate amounts to 84% in Group A (**Figure 2**). In Group B, two underlying mutations were detected in 7 out of 35 patients, one mutation in 4 patients, and no mutations could be detected in 24 patients. Thus, the overall detection rate is 26% in Group B. Moreover, 8 families within Group B without mutation in the *PKHD1* harbored mutations in other genes: 5 families had mutation in the *HNF1β* gene, 2 families in the *PKD1* gene and 1 family in the *PKD2* gene (**Figure 3**). The T36M missense mutation of the *PKHD1* gene was found repeatedly in severe cases.

The lowest detection mutation rate was found in fetuses with renal cysts from terminated pregnancies. 14 terminated fetuses were analyzed, mutations on both alleles of the *PKHD1* gene were confirmed in 2 cases, the *PKHD1* mutation on one allele in 1 family. No *HNF1β* mutation was found in terminated pregnancies. Subsequently, other syndromes than ARPKD with renal cysts were established during autopsy which surely contributed to the low mutation detection rate.

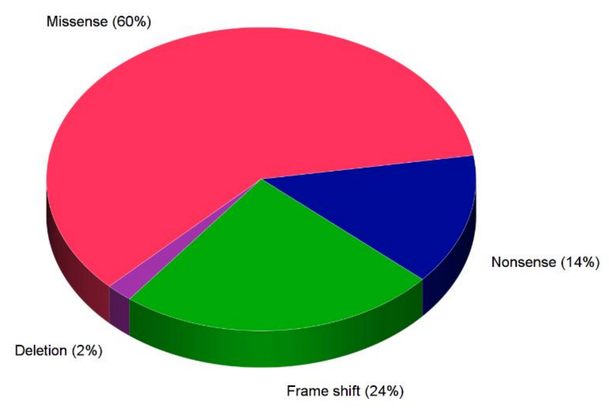

Fig. 5: Types of *PKHD1* mutations found within our group of patients

4. CONCLUSIONS

The detection rate of *PKHD1* mutations in children who fulfilled all three of the clinically diagnostic criteria of ARPKD is high, reaching 76%. Because of the etiologic heterogeneity of polycystic kidney disease phenotype, the complex diagnosis including mutational analysis of several genes is needed for reliable differential diagnosis. Sequence Capture method analyzing more than 100 gene responsible for different nephropathies will be especially useful in affected fetuses with cysts accompanied by other anomalies.

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Corresponding author: Lena.Obeidova@gmail.com
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