Refining genotype–phenotype correlations in 304 patients with autosomal recessive polycystic kidney disease and *PKHD1* gene variants

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Autosomal recessive polycystic kidney disease (ARPKD) is a severe disease of early childhood that is clinically characterized by fibrocystic changes of the kidneys and the liver. The main cause of ARPKD are variants in the *PKHD1* gene encoding the large transmembrane protein fibrocystin. The mechanisms underlying the observed clinical heterogeneity in ARPKD remain incompletely understood, partly due to the fact that genotype-phenotype correlations have been limited to the association of biallelic null variants in *PKHD1* with the most severe phenotypes. In this observational study we analyzed a deep clinical dataset of 304 patients with ARPKD from two independent cohorts and identified novel genotype-phenotype correlations during childhood and adolescence. Biallelic null variants frequently show severe courses. Additionally, our data suggest that the affected region in *PKHD1* is important in determining the phenotype. Patients with two missense variants affecting amino acids 709-1837 of fibrocystin or a missense variant in this region and a null variant less frequently developed chronic kidney failure, and patients with missense variants affecting amino acids

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1838-2624 showed better hepatic outcome. Variants affecting amino acids 2625-4074 of fibrocystin were associated with poorer hepatic outcome. Thus, our data expand the understanding of genotype-phenotype correlations in pediatric ARPKD patients and can lay the foundation for more precise and personalized counselling and treatment approaches.

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Autosomal recessive polycystic kidney disease (ARPKD) is a hepatorenal disorder with tremendous burden for affected children and their families. It represents one of the most common indications for kidney replacement therapy (KRT) and combined or sequential liver and kidney transplantation (CLKTx, SLKTx) in childhood. Variants in the *PKHD1* gene have been identified as the main genetic basis of the disease.[1,2] Additionally, variants in *DZIP1L* have been reported in 7 patients from 4 families so far with a moderate renal phenotype.[3] *PKHD1*, with the longest open reading frame of 67 exons, encodes the cilia-associated protein fibrocystin. Fibrocystin's cellular function is very poorly understood.[2,4] Fibrocystin consists of 4074 amino acids with a large extracellular part containing various domain structures, a single transmembrane part, and a short cytoplasmic tail.[1]

Genotype–phenotype association studies in ARPKD so far have been restricted largely to the type of *PKHD1* variants. Severe affection with perinatal demise was shown in patients with 2 truncating variants,[5–7] and it was concluded that at least 1 missense variant was required for survival beyond the neonatal period. Recently, however, first case reports and data collections have described patients with biallelic null variants surviving the perinatal period.[8–10] In addition, 2 missense variants do not preclude a severe perinatal course with demise.[5,6] Utmost caution is therefore needed in the setting of prenatal and/or genetic counselling with the intention of deducing or predicting the clinical course based on the type of variants.[11,12]

With respect to the localization of variants, Furu *et al.* reported 9 of 17 missense variants, observed exclusively in the patients surviving the neonatal period, residing between the amino acids (AAs) 1000 and 2000, with 6 of these between AAs 1600 and 2000.[5] Bergmann *et al.* reported a cluster of variants around AAs 2831-2840 and AAs 3051-3209 in 15 patients with a liver-predominant phenotype.[6] Further descriptions of associations between variant localizations and phenotype were hampered by a large number of detected, often private, variants in *PKHD1* and the challenges of merging patient cohorts from multiple sources into a representative and substantial patient cohort of this rare disease. To address these challenges, we recently established the mainly European clinical ARPKD registry study ARegPKD[13,14] to complement the existing work of the ARPKD mutation database.[15] In the current analysis, we evaluated the hepatorenal disease courses in ARPKD to identify potential genotype–phenotype correlations.

METHODS

The final cohort of 304 patients analyzed here is derived from 2 sources: the ARegPKD registry study cohort (209 patients included in analysis) and a large collection of ARPKD patients submitted for genetic testing to the Department of Human Genetics, RWTH Aachen University, Germany (95 patients included in analysis; Supplementary Figure S1). In the international cohort study, ARegPKD patients with the clinical diagnosis of ARPKD are followed according to the previously described protocol.[13,14] In summary,

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basic data and regular follow-up datasets including the hepatorenal aspects analyzed in ARegPKD are collected by the participating sites prospectively and retrospectively and are subject to regular data quality control. For the Aachen cohort, clinical data were obtained mainly from available clinical reports at the time of genetic analysis, resulting in more retrospective data and a shorter follow-up period. Details of both subcohorts can be found in Supplementary Table S1. The ARegPKD study protocol was approved by the Ethics Committee of the Faculty of Medicine of Cologne University and the institutional review boards of participating sites. Regarding the patients' data deriving from Aachen, the study falls under previous local institutional review board approval of genotype/phenotype studies in cystic kidney disease by the Ethics Committee of the University Hospital Aachen. Both projects are in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Data of both subcohorts were checked for potential double reporting of specific patients. Due to an assumed reporting bias of early deceased and/or palliatively managed neonates, we restricted the analysis to survivors of the neonatal period (surviving the first 30 days of life).

For genetic analyses, all reported *PKHD1* variants were classified according to the revised criteria of the American College of Medical Genetics (ACMG).[16] Patients with ≥1 *PKHD1* variant classified as a variant of unknown clinical significance, likely pathogenic or pathogenic (ACMG classes 3 to 5), were included in further analyses. Functional consequences of variants were categorized as truncating (nonsense and frameshift variants), missense, splicing, exon deletion or duplication, in-frame, or synonymous, based on the predicted cDNA and amino acid changes (Figure 1). Variants with an *a priori* assumed missense effect were classified as splice variants if functional evidence exists to prove a significant splice effect, or if they affect the first/last exonic nucleotide, and a splice site loss is strongly predicted (≥75% loss of MaxEntScan splice site prediction score[17]). Synonymous

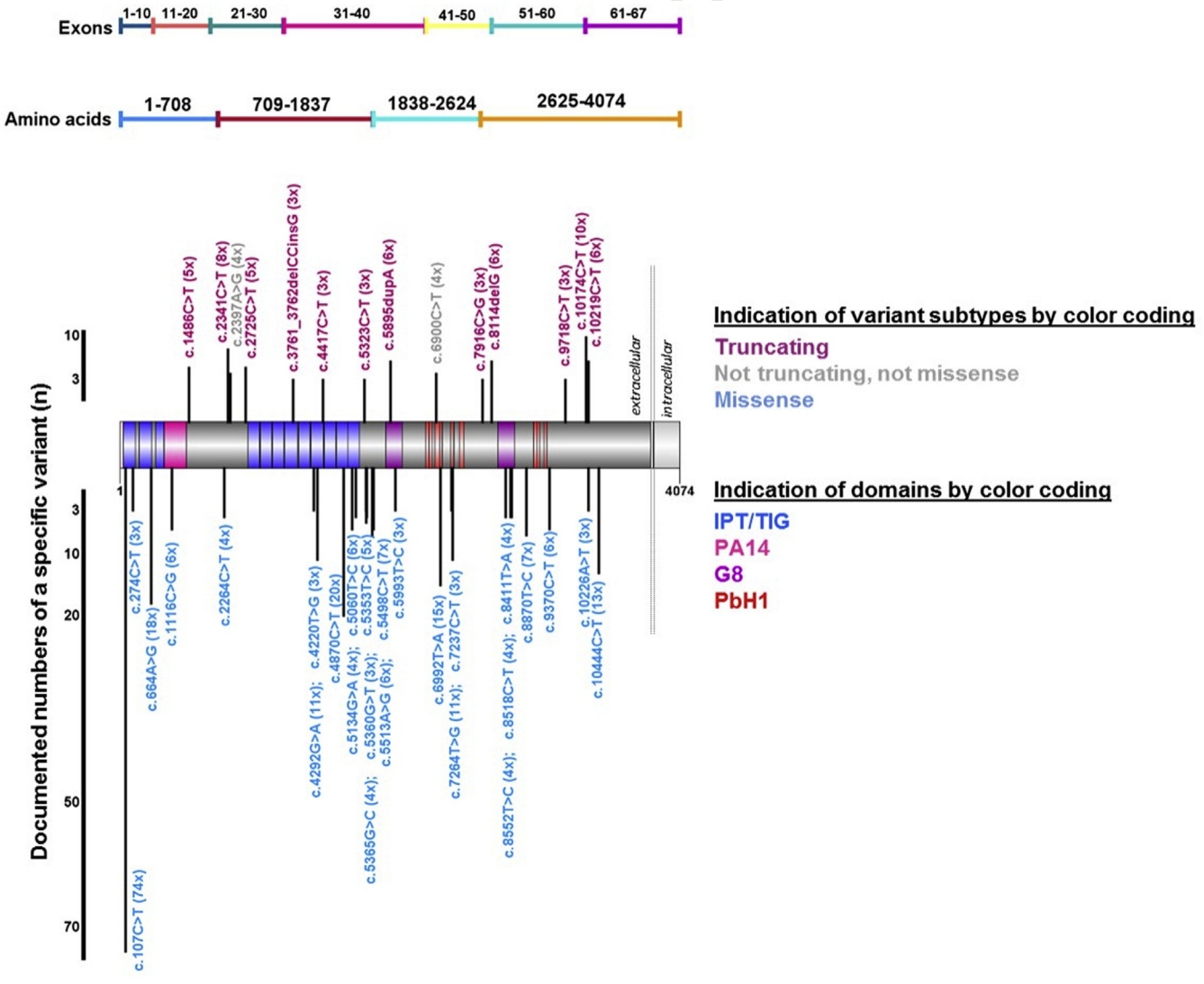

Figure 1 | Scheme of fibrocystin/polyductin with depiction of corresponding exons (1–67), functional domains, and categorization into 4 amino acid domains (1–708, 709–1837, 1838–2624, 2625–4074). Variants occurring ≥3 times in the studied cohorts are indicated as bars, with the bar length representing the number of observations of a specific variant in this study. G8, named for 8 conserved glycines; IPT, Ig-like, plexins, transcription factors; PA14, PbH1, parallel beta-helix repeats; TIG, transcription factor immunoglobin. Q27

variants were classified as variants of unknown significance in case of positive prediction of splice site involvement or for highly conserved nucleotides. All variants were checked by Alamut Visual software. The genotypes of each patient were additionally assigned to functional classes termed 2 null variants (Null/Null), 2 missense variants (Mis/Mis), 1 null and 1 missense (Null/Mis), only 1 variant (Single variant) and 1 group containing all other combinations (Others; this group contains multiple subgroups, e.g., truncating and 2 missense variants, truncating and in-frame variant, missense and in-frame variant, 2 in-frame variants, missense and synonymous variants, noncanonical splice-site variants). For the purposes of this genotype-based grouping, canonical splice-site variants (±1–2 intronic bases from the exon/intron boundary) were included in the "null variant" group in accordance with ACMG criteria. Furthermore, nonsense variants, frameshift variants, and whole exon or gene deletions were also included in the "null variant" group. Patients were additionally subgrouped according to molecular genetic diagnostic certainty, that is, "Confirmed" (≥2 *PKHD1* variants detected, with ≥2 classified as likely pathogenic or pathogenic), "Probable" (≥2 *PKHD1* variants detected, with only 1 classified as likely pathogenic or pathogenic), and "Unknown" (≥2 *PKHD1* variants of unknown clinical significance detected or only 1 *PKHD1* variant detected, classified as a variant of unknown clinical significance, likely pathogenic or pathogenic; Figure 2). Localization of missense variants was categorized according to exons (*PKHD1* transcript NM_138694.3, segmentation via integrative genomics viewer Broad institute, IGV) and functional domains and repeats (segmentation via www.uniprot.org). Provided domains and repeats encompassed the Ig-like, plexins, transcription factors/transcription factor immunoglobin (IPT/TIG), PA14, G8 (named for 8 conserved glycines), and PbH1 (parallel beta-helix repeats) domains in the extracellular part of fibrocystin. The distribution of the exons and domains, as well as previously published data on the low frequency of variants in the transmembrane and cytoplasmic domains, served as the basis for our categorization of variants into 4 protein regions (amino acids (AAs) 1–708, 709–1837, 1838–2624, 2625–4074; Figure 1; Supplementary Figures S2 and S3). Categorization into 1 of the 4 groups AA1–708, AA709–1837, AA1838–2624, or AA2625–4074, or into the groups of exon segments as well as amino acid functional domains and repeats, took place if the patient carried either 2 missense variants in this specific area of the protein or a single missense variant in this specific area and a null variant (Figure 3; Supplementary Figures S4 and S5).

The start of KRT was defined by the documentation of any type of dialysis or kidney transplantation (either isolated kidney transplantation or CLKTx), whichever occurred first. Sonographic splenomegaly was diagnosed according to the upper limits of normal as defined in pediatric and adult reference studies,[18–20] using a uniform definition of splenomegaly (spleen length >mean + 2SD in pediatric patients, and ≥13.0 cm in adult patients). Portal hypertension was diagnosed in cases of documentation of thrombocytopenia (platelet count <150.000/µl), sonographic splenomegaly, collateral blood flow (varices, variceal bleeding), portosystemic shunt, or liver transplantation (isolated liver transplantation [LTx] or CLKTx). Substantial hepatic complication was defined as occurrence of variceal bleeding, portosystemic shunt, or LTx/CLKTx.

Statistics

All statistical analyses were performed using SPSS 25 (IBM Corp., Armonk, NY). Data analysis was performed on the ARegPKD dataset available in April 2019; the Aachen dataset was accessed in December 2017. Individual genetic findings were added during the revision of

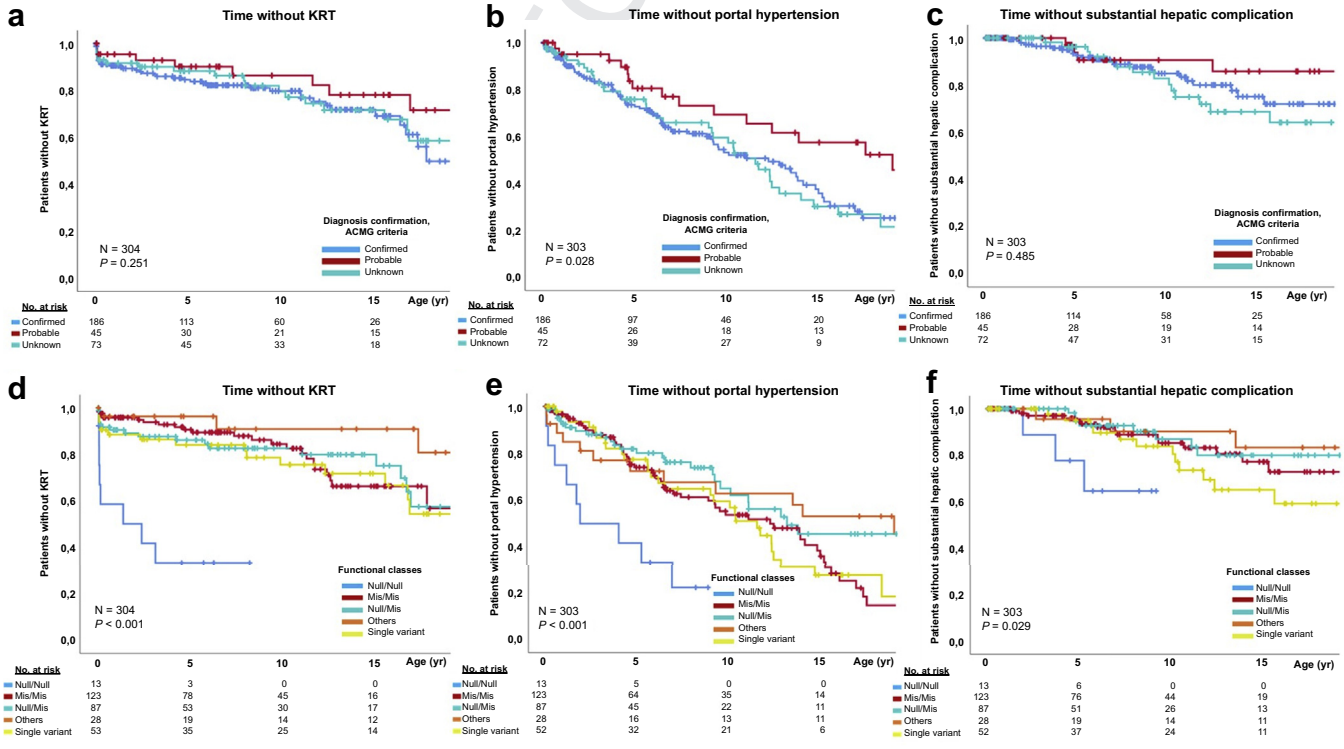

Figure 2 | Kaplan–Meier survival without (a,d) kidney replacement therapy (KRT), (b,e) portal hypertension, and (c,f) substantial hepatic complication, by molecular (a–c) genetic diagnostic certainty groups and (d–f) functional classes. ACMG, American College of Medical Genetics; Mis, missense.

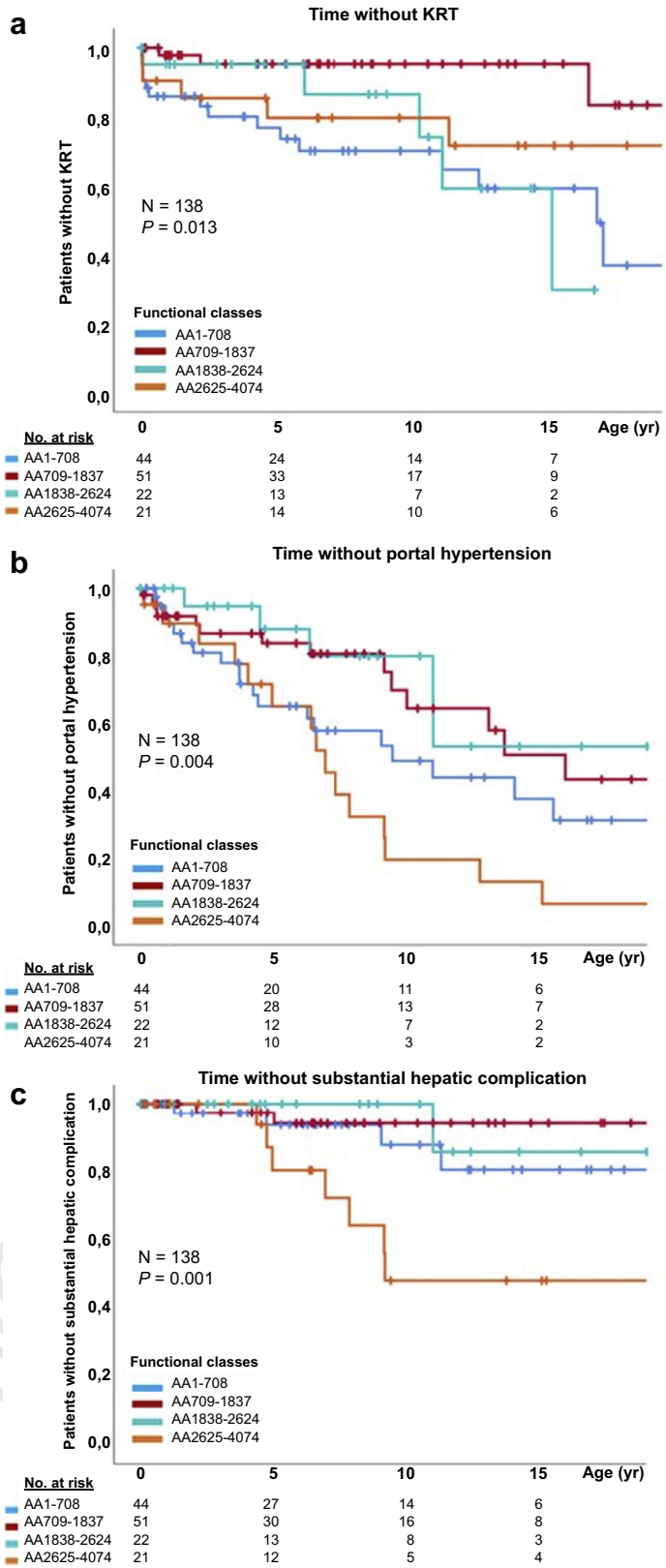

Figure 3 | Kaplan–Meier survival without (a) kidney replacement therapy (KRT), (b) portal hypertension, and (c) substantial hepatic complication, by functional classes categorized according to the localization of affected amino acids (AAs) of the corresponding missense variants in patients with Null/Missense or Missense/Missense variants. In the case of Null/Missense variants, localization of affected amino acids of the missense variant is decisive; in the case of Missense/Missense variants, localizations of the affected amino acids have to be within the same localization group for both variants. No., number.

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the article. Data completeness varied by variable. Continuous variables were described using the number of non-missing values, mean, and SD, as well as median and interquartile range (IQR). For binary or categorical variables, absolute and relative frequencies were provided. Event-free survival rates were estimated by the Kaplan–Meier method and compared using the log-rank test over the entire follow-up period. The statistical tests did not adjust formally for multiplicity due to the exploratory nature of the analysis. No imputation was performed. All analyses are exploratory, and P values of <0.05 were considered significant in a descriptive manner in distinguishing between the groups.

RESULTS

Patients

Patients' characteristics are displayed in Table 1. We restricted the analysis to survivors of the neonatal period and patients with one or more *PKHD1* variant of unknown significance or a likely pathogenic or pathogenic (LP/P) variant, resulting in a cohort of 209 ARegPKD patients with sufficient clinical data for further analysis of renal and hepatic phenotypes. In the Aachen cohort, 95 subjects had sufficient clinical documentation available for further analysis. Comparative characteristics of the 2 subcohorts are presented in Supplementary Table S1. Adult patients were not excluded per se, and 36 patients were followed-up into adulthood (≥18.0 years of age). Yet, pediatric patients were by far more common, and suitable follow-up visits of most patients were mainly available for childhood and adolescence.

Deaths

Six patients of the reported 304 neonatal survivors died at a median (IQR) age of 0.32 (0.14–0.98) years. All patients started peritoneal dialysis (PD) prior to their death (Supplementary Table S2).

PKHD1 variants

PKHD1 variants classified as ACMG classes 3 to 5 were detected in 304 patients from 277 families (Figure 1; Supplementary Table S3). A total of 53 patients carried a single variant, 243 patients carried 2 variants, and 8 patients carried 3 variants (total variant number: 563). Data on documented parental segregation of *PKHD1* variants were available for 113 patients (Supplementary Table S3). We identified 98 *PKHD1* variants that to our knowledge had not been published previously (Supplementary Table S4).

Kidney phenotype

KRT-free survival did not differ by either sex (15-year survival: male 75%, female 70%; data not shown) or molecular genetic diagnostic certainty—that is, "confirmed," "probable," and "unknown" genetic ARPKD disease status (Figure 2a). However, renal outcome was significantly inferior in patients with Null/Null variants compared to all other groups of variant type combinations (Null/Null vs. all other groups by pairwise comparison, $P < 0.001$; Figure 2d). Furthermore, patients with 2 variants classified as "Others" showed better renal survival (Others vs. Mis/Mis $P = 0.034$; Others vs. Single variant $P = 0.075$ by pairwise comparison each).

Table 1 | Characteristics of 304 analyzed patients with ≥1 *PKHD1* variant that is either pathogenic, likely pathogenic, or of unknown clinical significance

	Total (n = 304)
Sex	
Male	166 (54.6)
Female	138 (45.4)
Age at last visit, yr	8.21 (4.12–14.87)
Age at last visit, yr, mean (SD)	9.82 (8.21)
Follow-up time, yr	2.92 (0.00–7.25)
Follow-up time, yr, mean (SD)	4.74 (5.72)
Geographic region of parental origin	
Europe	201 (66.1)
Asia	29 (9.5)
Africa	8 (2.6)
North America	2 (0.7)
South America	1 (0.3)
Europe/North America	3 (1.0)
Europe/Asia	2 (0.7)
Unknown	58 (19.1)
Genetic testing and information	
Date of *PKHD1* testing; min–max	12/2010 (09/2004–12/2015); 11/2002–12/2018
Molecular genetic diagnostic certainty	
Confirmed	186 (61.2)
Probable	45 (14.8)
Unknown	73 (24.0)
Functional genotype groups	
Null/Null variant	13 (4.3)
Missense/Missense variant	123 (40.5)
Null/Missense variant	87 (28.6)
Others	28 (9.2)
Single variant	53 (17.4)
Localization of affected amino acid in Null/Missense or Missense/Missense variants	n = 138
AA 1–708	44
AA 709–1837	51
AA 1838–2624	22
AA 2625–4074	21
Specification of variants	**Total variant number: 563**
ACMG classification	
Pathogenic	340 (60.4)
Likely pathogenic	120 (21.3)
Variant of unknown significance	103 (18.3)
Predicted translation impact	
Truncating	120 (21.3)
Missense	389 (69.1)
Splicing	33 (5.9)
Exon deletion/duplication	8 (1.4)
In-frame	8 (1.4)
Synonymous	5 (0.9)
Five most-frequent variants	
c.107C>T (Thr36Met)	74 (13.1)
c.4870C>T (Arg1624Trp)	20 (3.6)
c.664A>G (Ile222Val)	18 (3.2)
c.6992T>A (Ile2331Lys)	15 (2.7)
c.10444C>T (Arg3482Cys)	13 (2.3)

Values are n (%), or median (interquartile range), unless otherwise indicated.
AA, amino acid; ACMG, American College of Medical Genetics; max, maximum/latest; min, minimum/earliest.

In a next step, patients were grouped into functional classes of Mis/Mis and Null/Mis variants according to the localization of the affected amino acids (AA; 1–708, 709–1837, 1838–2624, 2625–4074). We chose 4 sections to allow applicability in daily clinical work. We speculated that in

patients with a Null/Mis combination, the missense variant might determine the clinical phenotype. Little is known about the cellular function of the ARPKD protein fibrocystin, but we hypothesized that a hypomorphic allele might result in impaired function, as for example, by altered trafficking, with residual activity. We observed better kidney survival in patients with variant combinations affecting AAs 709–1837 (pairwise comparison: AAs 709–1837 vs. AAs 1–708, $P = 0.001$; AAs 709–1837 vs. AAs 1838–2624, $P = 0.012$; AAs 709–1837 vs. AAs 2625–4074, $P = 0.080$; Figure 3a). The Kaplan–Meier survival curves and corresponding numbers of all observed subgroups of Mis/Mis and Null/Mis allelic variant combinations, including small subgroups, are shown in Supplementary Figure S2 and Supplementary Table S5. Particular additional subgroups of allelic combinations did not show an event of KRT during the period of up to 18 years, but numbers were small (Supplementary Figure S2A and B; Supplementary Table S5). The categorization into exon segments or AA domain groups did not show relevant differences between the groups (Supplementary Figure S4, A and D). Analyses of subgroups suggest a possible association of variants in specific regions, with more rapid deterioration in renal phenotype, such as in IPT/TIG 1 (Supplementary Figure S5, A and D).

Liver phenotype

For the hepatic phenotype, we subclassified the clinical presentation and describe survival without any "signs of portal hypertension" (thrombocytopenia, splenomegaly, collateral blood flow [varices, variceal bleeding], portosystemic shunt, or LTx/CLKTx) and survival without signs of "substantial hepatic complication" (variceal bleeding, portosystemic shunt, or LTx/CLKTx).

Portal hypertension-free survival did not differ by sex (15-year survival male 40%, female 35%; not shown), but it was slightly better in patients with the diagnosis confirmation status of Probable (pairwise comparison: Probable vs. Confirmed, $P = 0.016$; Probable vs. Unknown, $P = 0.014$; Figure 2b). Portal hypertension-free survival was significantly lower in patients with Null/Null variants compared to all other variant type combinations (pairwise comparison: Null/Null vs. Mis/Mis, $P < 0.001$; Null/Null vs. Null/Mis, $P < 0.001$; Null/Null vs. Others, $P = 0.01$; Null/Null vs. Single *PKHD1* variant, $P = 0.001$; Figure 2e). Patients with 2 missense variants showed poorer hepatic survival than the groups Null/Mis and Others (Mis/Mis vs. Null/Mis, $P = 0.032$; Mis/Mis vs. Others, $P = 0.022$, by pairwise comparison for each). Differentiating the functional classes of Mis/Mis and Null/Mis combinations according to AA groups as described above revealed inferior outcomes for patients with missense variant combinations affecting AAs 2625–4074 (AAs 2625–4074 vs. AAs 1–708, $P = 0.074$; AAs 2625–4074 vs. AAs 709–1837, $P = 0.001$; AAs 2625–4074 vs. AAs 1838–2624, $P = 0.006$; Figure 3b).

The patient subgroups Mis1–708/Mis2625–4074 (n = 17), Mis2625–4074/Mis2625–4074 (n = 12), and Null/Mis2625–4074 (n = 9) showed 10-year portal hypertension-free survival rates that appeared comparable to the course of Null/Null patients. In contrast, patients in the group Mis1838–2624/Mis2625–4074 (n = 8) did not show an event of portal hypertension during the period of up to 18 years (Supplementary Figure S3, A and C; Supplementary Table S5). When categorizing into exon segments, portal hypertension-free survival was best with missense variant combinations affecting exons 41–50 and was poorest in patients with missense variant combinations affecting exons 51–60. Combinations of missense variants affecting G8 domains and the IPT/TIG1 and IPT/TIG9 domains were associated with a substantially lower survival free of portal hypertension (Supplementary Figures S4E and S5, B and E).

Survival without substantial hepatic complication was similar in the 3 groups of molecular genetic diagnostic certainty and lower in the Null/Null group compared to all other functional classes (Null/Null vs. Mis/Mis, $P = 0.015$; Null/Null vs. Null/Mis, $P = 0.015$; Null/Null vs. Others, $P = 0.056$; Null/Null vs. Single *PKHD1* variant, $P = 0.076$ in pairwise comparison for each; Figure 2c and f). Again, there was no relevant difference between male and female patients (15-year substantial hepatic complication-free survival: male 80%, female 70%; not shown). The trend toward a difference between the functional AA groups concerning portal hypertension-free survival was even more pronounced with regard to the survival time without substantial hepatic complication: Null/Mis or Mis/Mis variant combinations affecting AAs 2625–4074 showed the poorest outcome (AAs 2625–4074 vs. AAs 1–708, $P = 0.025$; AAs 2625–4074 vs. AAs 709–1837, $P = 0.001$; AAs 2625–4074 vs. AAs 1838–2624, $P = 0.019$ in pairwise comparison for each; Figure 3c). Again, patients classified into Mis1-708/Mis2625–4074 (n = 17) and Mis2625–4074/Mis2625–4074 (n = 12) showed 10-year substantial hepatic complication-free survival, appearing comparable to Null/Null patients. Patients with missense variants corresponding to Null/Mis2625–4074 (n = 9) appeared to have the poorest hepatic outcome.

In contrast, patients with various combinations of 2 independent missense variants did not show any events of substantial hepatic complication during up to 18 years of observation (Supplementary Figure S3B and D; Supplementary Table S5). Strikingly, this included the subgroups with 2 missense variants involving at least 1 variant in AAs 1838–2624, although overall numbers in these subgroups remained small. The categorization into exon segments showed worst survival in patients whose missense variants corresponded to exons 51–60 and 61–67 (Supplementary Figure S4C). Grouping by affected amino acid domains highlights the hypothesis of a relevant role of variants affecting the second G8 domain for development of liver disease (Supplementary Figures S4F and S5, C and F).

Patients with late diagnosis

A subanalysis of the data evaluated a patient subcohort of 29 patients with late presentation, which we defined as diagnosis

of ARPKD at the age of ≥5.0 years. In this subcohort, no patient with biallelic null variants was identified. Nine patients had a Null/Mis combination, 11 showed a Mis/Mis combination, 5 were in the category "Others," and 4 had a single *PKHD1* variant (Supplementary Figure S6A). Variants were distributed all over the gene, but 6 of 11 truncating variant alleles showed a localization of this variant in the 3' part of *PKHD1* corresponding to AAs 2625–4074 (Supplementary Figure S6B).

Data on modifying factors

A subset of patients had available data on the analysis of additional known PKD genes, including, for example, *PKD1*, *PKD2*, *HNF1B*, and *DZIP1L*. We identified information on *PKD1* testing in 55 patients, *PKD2* testing in 54 patients, *HNF1B* testing in 56 patients, *GANAB* testing in 15 patients, and *DZIP1L* testing in 15 patients. Only a single additional *PKD1* ACMG class 3 variant was identified. Details are presented in Supplementary Table S3. Multiple sibling pairs are included in this study, with mostly comparable disease courses. Examples of partly discordant phenotypes include the families F60 or F82 (Supplementary Table S3).

DISCUSSION

We present data on clinical outcomes during childhood and adolescence of a genotyped cohort of 304 ARPKD patients surviving the neonatal period. To our knowledge, this is the largest cohort with detailed clinical and genetic data analyzed to date. We focused on the 2 main affected organs in ARPKD—the kidney and the liver—and on hard endpoints that may also become relevant for future clinical trials.

The criterion of diagnostic confirmation of ARPKD based on the ACMG criteria is not suitable to deduce the severity of the further course of the disease. Instead, categorization according to variant types yielded a clearer picture: patients with 2 variants leading to chain termination, so-called null variants, showed the poorest overall, renal, and hepatic outcomes. Nonetheless, 13 patients with Null/Null variants survived the perinatal period, adding to a published small case series[9] and a case report[8] that previously described 1 of these patients in detail. Two of these 13 patients died within the further disease course, but 11 patients with Null/Null variants survived even during the median (IQR) observation period of 6.17 (4.42–9.08) years. Furthermore, 2 patients with Null/Null variants died postnatally, due to respiratory failure, but they were not included in the presented analysis of neonatal survivors. It appears that the number of patients in our datasets with Null/Null variants surviving the neonatal period is more frequently reported in recent years. One might speculate that advances in neonatal and pediatric intensive care medicine, as well as pediatric nephrology, over the past 2 decades have a relevant positive impact on the survival of the most severely affected infants. However, as patients with severe disease manifestations dying in the neonatal period and prenatally, severely affected fetuses from terminated pregnancies were not included; these numbers are not representative for all patients with Null/Null genotypes.

Endpoints of both kidney and liver phenotype exhibited overall similar courses of functional survival in patients with Mis/Mis and Null/Mis variants, respectively. Carrying 1 null variant did not generally result in a poorer outcome than carrying no null variant. This may suggest that, in cases with Null/Mis variants, the respective missense variant determines the phenotype. The null variants detected in our patients were distributed throughout the part of the gene encoding the extracellular regions of the protein. We did not see differences in our patients in comparison to previously reported patients with null variants. In accordance with previous findings,[6] we speculate that null variants in the sequence encoding extracellular parts of the protein will result in complete and uniform loss-of-function. Missense variants, however, may act as a hypomorphic allele and show more clinical variability. Two missense variants also can be associated with severe phenotypes. We therefore chose to apply an additional categorization based on the localization of Missense variants. Such categorization will to a certain extent always remain arbitrary, but it may still be very helpful for daily clinical life. Our data on the affected regions of *PKHD1* are in accordance with previous findings from smaller cohorts.[5,6]

For the primary endpoint of renal survival, we identified 3 subgroups of patients with 2 missense variants that did not require KRT during up to 18 years of observation. The largest subgroup consists of 16 patients with 2 missense variants corresponding to AAs 709–1837, suggesting that combined variants in this region are associated with a slower loss of kidney function. Further straightforward genotype–phenotype correlations could not be established for the renal phenotype.

For the hepatic phenotype, especially for the primary endpoint of survival without substantial hepatic complications, we were able to identify enhancing and mitigating *PKHD1* variants. Despite a low patient number in the subgroup (n = 9), patients with variants corresponding to Null/AA2625–4074 showed even poorer hepatic outcomes than patients in the Null/Null subgroup. Furthermore, patients in the Mis1–708/Mis2625–4074 group showed poorer hepatic 5- and 10-year event-free survival than Null/Mis1–708 patients. We cannot exclude a selection bias, but the data may also point to at least partial dominant negative effects of variants in this area on the liver phenotype. Mis/Mis variant combinations in this region were also associated with very poor hepatic survival curves. In contrast, missense variants affecting AAs 1838–2624 seem to "protect" from substantial hepatic complications, as patients with Mis/Mis variant combinations carrying at least 1 variant affecting this region did not show any hepatic event during up to 18 years of follow-up. The beneficial effect of variants affecting AAs 1838–2624 seemed to outweigh the heightened hepatic complication risk associated with variants in AAs 2625–4074, at least in our small subgroup of 8 patients. However, the genotype group most commonly associated with the absence of substantial hepatic complications during childhood was Null/AA709–1837 (n = 35).

We saw the same tendency for the endpoint of portal hypertension, which includes all criteria of substantial hepatic complications plus less severe criteria such as documentation of thrombocytopenia (platelet count <150.000/μl), sonographic splenomegaly, or collateral blood flow (detection of varices). As a consequence, survival without signs of portal hypertension in general is lower than survival without substantial hepatic complications.

Although the categorization of missense variants according to exon segment or amino acid functional domains and repeats yielded some interesting insights regarding hepatic outcomes, these categorizations proved inferior to amino acid localization–based grouping in predicting clinical outcomes. The data suggest a functional relevance for variants in the 3' area of *PKHD1* and partly within the G8 domains for hepatic complications. Little is known about the function of this part of the protein and this domain that contains 8 conserved glycines. Direct reliable inferences from the localization of the defect to specific functions of the fibrocystin protein do not yet appear feasible. More functional work is required.

Previous work discussed potential correlations between the kidney and liver phenotypes versus potential organ-specific courses in ARPKD.[6,21] A study on fetuses and severely affected neonates found evidence for parallel severity of ARPKD.[22] Our data may shed some light on this area. Biallelic null variants seem to be associated with severe phenotypes in both organs, whereas the localization of the variants may influence the phenotype in patients with at least 1 missense variant. The role of modifier genes, which has been discussed previously,[4] cannot be elucidated from our data.

Some limitations of our study deserve mention. The majority of the patients studied are participants in the ARegPKD registry study, where some degree of inclusion bias due to the limited availability of very severely ill patients or those who died early, and of patients with late, atypical, or liver-predominant disease presentation, cannot be excluded. Furthermore, it is mainly pediatric nephrology centers that are contributing to ARegPKD, which may lead to a bias toward more complete description of the renal as compared to the hepatic phenotype and may have enhanced the expected predominance of pediatric findings. Most of the centers are in Europe, and data from other parts of the world are needed to confirm the findings. Due to the structure of the registry, *PKHD1* testing was performed in multiple international genetic laboratories between 2002 and 2018. One-third of patients derive from the patient pool of the Department of Human Genetics, RWTH Aachen University (Aachen, Germany) with *PKHD1* testing performed between 2003 and 2015. Although structured clinical data were acquired, follow-up time was substantially shorter than that for ARegPKD patients, and for almost half of the cohort, only cross-sectional clinical information was available. Therefore, the detection of hepatic complications especially, which typically occur later than the decline of kidney function, may have been incomplete in this subcohort. We therefore also analyzed the ARegPKD dataset separately, confirming the main findings on the liver and kidney genotype–phenotype correlations (data not shown). A substantial proportion of the total cohort (53 of 304; 17%) carried only a single detectable *PKHD1* variant, meaning that these patients' respective disease status could not be genetically confirmed. It should be noted that the mutation detection rate in ARPKD, even in clinically diagnosed cases, is not complete and is usually in the range of 75%–85%.[23,24] As such, the non-detection of a putative second *PKHD1* variant in 17% of patients in this cohort would not be unexpected. A subcohort of patients who received genetic analysis in the years 2002–2006 showed the highest percentage of patients with a single detectable variant, suggesting that technical aspects may be relevant in this cohort (Supplementary Figure S7).

We cannot exclude the possibility that there has been insufficient coverage of intronic hotspots including regulatory regions in these patients. Furthermore, deleterious variants were not uniformly distributed throughout the *PKHD1* gene. It is to be expected that common disease-causing missense variants in our cohort (such as c.107C>T (T36M); 13% of all variants) more strongly affected the analysis results than rare disease-causing missense variants. Given that this preponderance of certain variants is a result of the *de facto* wildly unequal frequencies of deleterious *PKHD1* variants in the general population, however, this does not invalidate our observations or their generalizability. The same holds true for rare variants that were subsumed in the group "Others."

Further limitations are as follows: potential modifier genes or variants in other PKD genes were not systematically analyzed; segregation analysis and hence the proof of biallelism was only available for ~37% of patients; our study does not include individuals with terminated pregnancy or patients that did not survive the first 30 days of life; and the number of patients followed into adulthood remains small. We have previously reported descriptive findings of an ARegPKD subcohort of young adult ARPKD patients.[25]

In summary, we are able to give important descriptive insights into the genotype–phenotype correlations of ARPKD patients with *PKHD1* variants during childhood and adolescence. Although Null/Null variants are associated with the poorest renal and hepatic outcome, they are not predictive of neonatal demise in all cases. Biallelic missense variants affecting amino acids 709–1837 seem to be associated with a milder renal phenotype, and missense variants affecting amino acids 2625–4074 of fibrocystin were associated with a higher risk of substantial hepatic complications.

APPENDIX

List of additional ARegPKD Consortium collaborators (ordered according to countries and centers in alphabetical order)

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DISCLOSURE

All the authors declared no competing interests.

DATA SHARING STATEMENT

Original data are available from the authors upon reasonable request.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Characteristics of 304 analyzed patients according to databases from ARegPKD and the Department of Human Genetics, RWTH Aachen University.

Table S2. Characteristics of 6 deceased patients in the current analysis of patients with ≥1 *PKHD1* variant classified as pathogenic, likely pathogenic, or of unknown clinical significance.

Table S3. Characteristics of 304 analyzed patients with respect to family history, phenotype, and genotype. Variants in brackets were interpreted as least relevant for the phenotype and therefore not primarily included for categorization of functional classes. bro, brother; c, confirmed; f, female; M, male; na, not available; nd, not done; p, probable; sis, sister; u, unknown; –, no/negative; +, yes.

Table S4. Unpublished *PKHD1* variants detected in this study with number of observations and ClinVar annotation, if available.

Table S5. Fifteen-year-survival without kidney replacement therapy (KRT), portal hypertension, and substantial hepatic complication by functional classes categorized according to the localization of affected amino acids of the corresponding missense variants. The numbers derive from the Kaplan–Meier survival analysis shown in Supplementary Figures S2 and S3. If there are no cases at risk at 15 years within a specific group, "–" is indicated.

Figure S1. Flowchart of patient inclusion.

Figure S2. Kaplan–Meier survival without KRT by functional classes categorized according to the localization of affected amino acids of the corresponding missense variants. Groups with (**A**) n ≥ 10 and (**B**) n < 10 in specific groups.

Figure S3. Kaplan-Meier survival without (**A,C**) portal hypertension and (**B,D**) substantial hepatic complication by functional classes categorized according to the localization of affected amino acids of the corresponding missense variants. Group sizes with (**A,B**) n ≥ 10 and (**C,D**) n < 10.

Figure S4. Kaplan–Meier survival without (**A,D**) KRT, (**B,E**) portal hypertension, and (**C,F**) substantial hepatic complication by (**A–C**) exon segments and (**D–F**) amino acid functional domains and repeats. Distribution to a specific group was possible if both missense variants corresponded to the same specific subgroup, in the case of Missense/Missense variants, or if a single missense variant corresponded to the subgroup, in the case of Null/Missense variants.

Figure S5. Kaplan–Meier survival without (**A,D**) KRT, (**B,E**) portal hypertension, and (**C,F**) substantial hepatic complication by individual functional domains and repeats. The group IPT/TIG indicates all IPT/TIG domains not localized in the same specific domain. Only groups with n > 2 are indicated.

Figure S6. Genetic findings in patients with late presentation: bar graph of (**A**) functional classes and (**B**) scheme of fibrocystin/polyductin with depiction of corresponding exons (1–67), functional domains, and categorization into 4 amino acid domains (1–708, 709–1837, 1838–2624, 2625–4074). Fifty-six variants occurring in 29 patients with presentation at the age of ≥5.0 years are indicated as bars, with the bar length indicating the number of observations of a specific variant.

Figure S7. Included patients with a single detectable variant according to periods of first analysis.

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