Modelling polycystic liver disease progression using age-adjusted liver volumes and targeted mutational analysis

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Graphical abstract

Study design

| Methods                      | Results                                                                 |
|------------------------------|-------------------------------------------------------------------------|
| Genetic analysis + age-adjusted total liver volume provides predictive information with regard to PLD-related hospitalization |

Highlights

- ADPKD-related PLD and isolated ADPLD may lead to equally severe hepatomegaly.
- Age at first PLD-related hospitalization may serve as a novel clinically applicable endpoint to assess PLD severity.
- Genetic confirmation is predictive for risk of hospitalization in both isolated and non-isolated PLD.
- Age-adjusted liver volumetry may improve disease prognostication at early stages.

Lay summary

Polycystic liver disease (PLD) is a highly variable condition that can be asymptomatic or severe. However, it is currently difficult to predict clinical outcomes such as hospitalization, symptom burden, and need for transplantation in individual patients. In the current study, we aimed to investigate the clinical value of genetic confirmation and an age-adjusted total liver volume classification for individual disease prediction. While genetic confirmation generally pointed to more severe disease, estimated age-adjusted increases in liver volume could be useful for predicting clinical outcomes.

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Modelling polycystic liver disease progression using age-adjusted liver volumes and targeted mutational analysis

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Background & Aims: Polycystic liver disease (PLD) manifests as numerous fluid-filled cysts scattered throughout the liver parenchyma. PLD most commonly develops in females, either as an extra-renal manifestation of autosomal-dominant polycystic kidney disease (ADPKD) or as isolated autosomal-dominant polycystic liver disease (ADPLD). Despite known genetic causes, clinical variability challenges patient counselling and timely risk prediction is hampered by a lack of genotype-phenotype correlations and prognostic imaging classifications.

Methods: We performed targeted next-generation sequencing and multiplex ligation-dependent probe amplification to identify the underlying genetic defect in a cohort of 80 deeply characterized patients with PLD. Identified genotypes were correlated with total liver and kidney volume (assessed by CT or MRI), organ function, co-morbidities, and clinical endpoints.

Results: Monoallelic diagnostic variants were identified in 60 (75%) patients, 38 (48%) of which pertained to ADPKD-gene variants (PKD1, PKD2, GANAB) and 22 (27%) to ADPLD-gene variants (PRKCSH, SEC63). Disease severity defined by age at waitlisting for liver transplantation and first PLD-related hospitalization was significantly more pronounced in mutation carriers compared to patients without genetic diagnoses. While current imaging classifications proved unable to differentiate between severe and moderate courses, grouping by estimated age-adjusted total liver volume progression yielded significant risk discrimination.

Conclusion: This study underlines the predictive value of providing a molecular diagnosis for patients with PLD. In addition, we propose a novel risk-classification model based on age- and height-adjusted total liver volume that could improve individual prognostication and personalized clinical management.

Keywords: polycystic disease; polycystic kidney disease; ADPLD; ADPKD; PCLD; PLD; PRKCSH; SEC63; PKD1; PKD2; GANAB; hepatomegaly; total liver volume.

Introduction

Polycystic liver disease (PLD) is characterized by progressively growing liver cysts originating from the biliary epithelium. Clinically and etiologically, PLD can be differentiated into isolated and non-isolated forms typically with familial background as a common denominator. Most commonly, PLD presents as an extra-renal manifestation of autosomal-dominant polycystic kidney disease (ADPKD), which is based on monoallelic genetic variation in two main disease genes PKD1 (encoding polycystin 1) and PKD2 (encoding polycystin 2). ADPKD shows almost obligatory but highly variable liver involvement. More rarely, isolated familial PLD occurs without significant cystic kidney involvement. This condition, which is termed autosomal-dominant polycystic liver disease (ADPLD), is mostly based on monoallelic genetic aberration in another two disease genes, PRKCSH and SEC63. Unlike ADPKD, clinically significant ADPLD is a rare disorder with an estimated prevalence of less than 1:10,000 live births. Morphologically, ADPKD- and ADPLD-associated liver cysts cannot be differentiated from one another and clinical signs and symptoms broadly overlap, ranging from asymptomatic courses to severe impairment due to mass effects. Known PLD risk factors include female sex and...
premenopausal estrogen exposure; therefore, women are more likely to endure severe courses. Interestingly, a genetic interaction network involved in N-glycosylation and quality control in the endoplasmic reticulum mechanistically links both entities via polycystin 1 and seems to govern the degree of cystic liver and kidney involvement. Thereby, typical ADPKD and ADPLD represent both ends of a cystic disease spectrum, overwhelmingly associated with either pathogenic variants in PKD1/ PKD2 (ADPKD), or pathogenic PRKCSH/SEC63 variation (ADPLD). Additional disease genes (LRP5, SEC61B, ALG8, ALG9, GANAB, DNAJB11, PKHD1) have been primarily linked to atypical forms or hybrids further reflecting the phenotypic continuum of cystic liver and kidney disease. As a molecular diagnosis is not possible in about 50% of patients with ADPLD, additional causative genes remain to be discovered. However, genotype-phenotype correlations are poorly defined. While the odds of renal survival correlate with the ADPKD genotype, in terms of PKD1-truncation/non-truncation and PKD2, a similar association was not shown for liver volume progression. Also, previous genetic studies on PLD either investigated PKD1/PKD2 or PRKCSH/SEC63 mutually exclusive but found mutation carriers to exhibit more severe courses than non-mutation carriers, at least in the latter. Furthermore, predictive imaging modalities, such as the ADPKD-Mayo classification, are only established for renal survival. For liver outcomes, however, currently available imaging categories (Quian criteria, Kim classification) are non-predictive. Moreover, unlike definite renal endpoints such as end-stage kidney disease (ESKD), clinically significant liver endpoints are more challenging to define. While wait-listing for liver transplantation (LTx) may represent such a clinical endpoint, the use of the model for end-stage liver disease (MELD) score is not fully applicable in PLD. Standard and non-standard exception MELD scores are not being used beyond the Eurotransplant region and have raised concerns regarding outcome disparities. Hence, applicable liver endpoints and predictive classifications are urgently needed to allow for more accurate disease prognostication. With this study, we aimed to combine imaging and genetic modalities to comparatively assess patients with PLD and propose ways to overcome current limitations.

**Patients and methods**

**Study population**

After written informed consent (institutional review board protocols at University of Leipzig. Ethics vote 289/20-ek), 132 adult patients with clinically diagnosed PLD since 2009 were formally enrolled at the Leipzig University Medical Center between May 2018 and May 2021. After revision of electronic health records and imaging data, we included 80 patients with PLD from 68 families eligible for continued follow-up from the outpatients’ clinics for hepatobiliary diseases, nephrogenetic diseases, and transplantation. Fifty-two cases were excluded because of loss to follow-up or there being less than three liver cysts upon imaging (ultrasound/CT/MRI) (Fig. 1A).

**Genetic analyses**

Pathogenic alterations were assessed by targeted next-generation sequencing (tNGS) and multiplex ligation-dependent probe amplification (MLPA) at the Institutes of Human Genetics Bio-scienza and Medical Genetics Mainz. The customized gene panel covered all exon-intron boundaries and coding regions of PKD1, PKD2, GANAB, PRKCSH, SEC63, PKHD1, HNF1B, ALG8, ALG9, DNAJB11, and SEC61B. Furthermore, whole exome sequencing was carried out on tNGS- and MLPA-negative cases. Segregation analysis was performed by direct sequencing methods upon sample availability. Nonsense, frameshift, large deletion/insertions, and (canonical) splice site variants were categorized as truncating, while small in-frame deletions/insertions (delins) and missense variants were grouped as non-truncating. Variants were classified according to diagnostic criteria of the American College of Medical genetics and Genomics (ACMG). Class III variants (alias variants of uncertain significance, VUS) were only included if “tepid”, “warm” or “hot” according to published Association for Clinical Genomic Sciences (ACGS) guidelines (https://www.acsg.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf).

**Radiological assessment**

CT and MRI studies were used to determine total liver volume (TLV) and total kidney volume (TKV). If more than one scan was available, most recent imaging before any type of surgical volume reduction was used for TLV, TKV assessment (index image). Pre-surgery liver imaging was available in 65 patients and kidney imaging in 67 patients. Intellispace Portal 9.0 software (Philips, The Netherlands) was used to perform 3D reconstruction through both manual and semi-automatic segmentation. Regions of interest were manually highlighted and extrapolated between slices, facilitating 3D volume calculation.

**Symptom questionnaire**

Clinical questionnaires were sent to all patients to complement electronic health record data and to assess current symptom burden (e.g. pain, fullness, fatigue, reflux, limited mobility). We divided symptom count into three sections: 0–4 were considered mild symptom burden, while 5–9 were regarded as moderate and above 10 as severe. Response rate was 79% (n = 34/43) among non-transplanted patients.

**Study endpoints and classifications**

Height-adjusted TLVs (hTLVs) were categorized according to the severity classification proposed by Kim H et al. into mild (hTLV <1,600 ml/m), moderate (hTLV 1,600–3,200 ml/m), and severe (hTLV >3,200 ml/m). We defined survival without a PLD-related hospitalization (alias liver event) as the primary endpoint. For accurate definition of hepatic events, we scrutinized medical histories for the age at first PLD-related hospitalization (e.g. treatment either conservatively or by surgical intervention, such as cyst aspiration/fenestration, resection or even liver transplantation). Secondary endpoints included survival without LTx and renal survival defined by absence of ESKD. TKVs were used to estimate renal disease severity and progression as proposed by the Mayo classification. Laboratory MELD scores as well as standard exception MELD scores were compiled for selected cases.

**Statistical analyses**

All statistical analyses were carried out using SPSS software, version 17 (IBM Corp., USA) and GraphPad Prism version 9.2.0 (GraphPad Software, USA). Statistical testing utilized a p value of <0.05 as the significance threshold. For normally distributed data we used Student’s t test and ANOVA; for non-normal distributions we used Mann-Whitney U test and Kruskal–Wallis test. Multiple comparisons were corrected by Tukey’s and Dunn’s test. Categorical variables were analyzed using Chi-square or Fisher’s exact
test. By regression and correlation analyses, we investigated the relationship between potential confounder variables with hTLV as a dependent variable. Consecutive logistic regression aimed to assess the likelihood of experiencing a PLD-related event, adjusting for predictor variables (e.g., hTLV, genetic etiology). Survival analyses were performed by the Kaplan-Meier method and differences between the curves were compared by log-rank testing. Cox proportional hazard regression was used to investigate the effect of aforementioned predictor variables on survival.

**Results**

**Baseline cohort characteristics**

Baseline cohort characteristics of all patients are displayed in Table 1. The cohort included 65 (81%) females and 15 (19%) males, with a mean age of 62 ± 1 years. Upon volumetry, median hTLV was 3,116 ml/m² [IQR 1,567–1,998 ml/m²] and median hTKV was 4,723 ml/m² [IQR 4,125–5,250 ml/m²] (Fig. 1B). Thirty-six (45%) patients had either undergone LTx or been registered on the LTx waitlist (mean age of 51 ± 2 years), whilst 21 (26%) had reached ESKD at a mean age of 56 ± 7 years.

**Genetic analysis**

Mutation analysis revealed diagnostic variants (ACMG class IV-V) in 60 (75%) patients from 49 families (n = 50 unique monoallelic variants, 16 [32%] novel variants), 38 (48%) of which pertained to ADPKD-associated genes (PKD1, PKD2, GANAB, PRKCSH, and SEC63) according to ACMG classification (class IV-V).

**Fig. 1. Study design and cohort stratification by genetic analyses and liver/kidney volumetry.** (A) Recruitment strategy of PLD patients and genetic interaction network between ADPKD and ADPLD. (B) Liver and kidney volumetry and resulting distribution according to current imaging classification (Kim and Mayo) of the cohort. (C) Genetic analysis by gene panel and MLPA yielding molecular diagnoses in PKD1, PKD2, GANAB, PRKCSH, and SEC63 according to ACMG classification (class IV-V).
| Genetic stratification | Total | ADPKD genes | ADPLD genes | Unknown | Statistical testing |
|------------------------|-------|--------------|-------------|---------|---------------------|
|                        | n (%) | Mean ± SEM   | Median IQR1 | IQR3    | n (%) | Mean ± SEM | Median IQR1 | IQR3 | n (%) | Mean ± SEM | Median IQR1 | IQR3 | Omnibus p values |
| Sex [female]           | 80 (100) | 43 (54) | 61.0 ± 1.3 | 32 (40) | 22 (28) | 15 (19) | 67.1 ± 2.8 | 16 (20) | 77.0 ± 1.9 | 24 (29) | 81.0 ± 1.7 | 0.125                 |
| Current age [yr]       | 80 (100) | 61.9 ± 1.3 | 43 (54) | 60.1 ± 2.8 | 22 (28) | 69.2 ± 2.8 | 15 (19) | 77.0 ± 1.9 | 24 (29) | 81.0 ± 1.7 | 0.125                 |
| BMI [kg/m²]            | 79 (99) | 26.0 ± 0.5 | 21 (26) | 26.5 ± 1.3 | 15 (19) | 26.4 ± 1.4 | 0.764                 |
| BSA [kg/m²]            | 79 (99) | 1.8 ± 0.03 | 21 (26) | 1.8 ± 0.1 | 15 (19) | 1.8 ± 0.1 | 0.719                 |
| Age at diagnosis [yr]  | 69 (86) | 41.2 ± 1.5 | 20 (25) | 40.9 ± 2.7 | 15 (19) | 53.8 ± 3.1 | 0.002                 |
| Age at LTx waitlisting [yr] | 36 (44) | 51.0 ± 1.5 | 9 (11) | 52.3 ± 4.7 | 1 (1) | 49.7 ± 0.8 | 0.887                 |
| LabMELD [6-40]         | 62 (78) | 32 (40) | 20 (25) | 16 (20) | 7 (9) | 14 (18) | 0.013                 |
| seMELD [11-40]         | 14 (18) | 29 (37) | 29 (37) | 5 (5) | 29 (37) | 14 (18) | 6 (7) | 6 (7) | 8 (10) | 0.946                 |
| Age at first PLD related event [yr] | 57 (71) | 51.5 ± 1.4 | 15 (18) | 50.6 ± 3.7 | 6 (7) | 46.0 ± 4.6 | 0.013                 |
| Age at first PLD complication [yr] | 35 (44) | 52.8 ± 2.0 | 14 (17) | 51.2 ± 3.9 | 5 (6) | 46.0 ± 4.6 | 0.026                 |
| Imaging analysis       | 65 (81) | 34 (41) | 30.0 ± 1.9 | 22 (27) | 17 (21) | 14 (18) | 40.0 ± 2.0 | 12 (15) | 40.0 ± 2.7 | 0.764                 |
| hTLV [ml/m]            | 65 (81) | 3,116 | 3,797 | 5,408 | 18 (22) | 3,112 | 4,809 | 0.013                 |
| Log hTLV               | 65 (81) | 3.4 ± 0.04 | 3.6 ± 0.04 | 3.5 ± 0.1 | 3.5 ± 0.1 | 14 (18) | 3.0 ± 0.1 | 0.013                 |
| Maximum cyst diameter [cm] | 65 (81) | 8.4 | 8.1 | 10.9 | 8 (11) | 8.4 | 12.4 | 0.013                 |
| Age at kidney segmentation [yr] | 67 (84) | 52.4 ± 1.3 | 35 (41) | 50.0 ± 1.4 | 14 (18) | 60.5 ± 2.2 | 0.003                 |
| hTKV [ml/m]            | 67 (84) | 383 | 616 | 127 | 18 (22) | 206 | 246 | 0.003                 |
| Renal function         | 74 (93) | 50.5 ± 3.8 | 19 (24) | 54.7 ± 1.6 | 19 (24) | 57.5 ± 3.3 | 0.001                 |
| eGFR [ml/min/1.73 cm²] | 74 (93) | 40 (50) | 19 (24) | 70.0 ± 2.5 | 15 (19) | 75.4 ± 2.8 | 0.001                 |
| Laboratory data        | 74 (93) | 1.5 | 1.5 | 3.5 | 0.6 | 3.1 | 20 (91) | 0.7 | 2.0 | 0.035 | 0.450 | 0.306 | 0.029 |
| GGT [xULN]             | 72 (90) | 0.8 | 0.8 | 1.0 | 0.6 | 1.0 | 18 (22) | 0.7 | 0.8 | 0.038 |
| ALP [xULN]             | 71 (89) | 8.6 | 8.3 | 11.6 | 6.3 | 10.9 | 18 (22) | 8.4 | 6.6 | 10.9 | 10.7 | 7.1 | 13.8 | 0.363 |
| Bilirubin total [µmol/L] | 67 (84) | 44.4 | 43.6 | 45.4 | 42.3 | 45.4 | 16 (73) | 44.8 | 41.8 | 48.0 | 14 (18) | 45.2 | 44.1 | 47.3 | 0.069 |
| Albumin [g/L]          | 60 (75) | 111.4 | 101.1 | 120.2 | 94.9 | 88.8 | 14 (18) | 112.7 | 95.2 | 129.0 | 13 (18) | 131.8 | 117.9 | 143.9 | 0.002 |
| Cholinesterase [kat/L] | 60 (75) | 33 (41) | 10 (13) | 19 (25) | 22 (29) | 22 (29) | 0.002 |

Table 1. Baseline characteristics of total cohort and genotype-based subgroup.
Fig. 2. Clinical comparison by genotype-based subgroups. (A) Patients without molecular diagnoses (unsolved - grey) were significantly older at index imaging used for liver/kidney volumetry as compared to genetically confirmed ADPKD (red) and genetically confirmed ADPLD (blue) cases (One-Way Anova; Tukey). (B) Mean height-adjusted total liver volumes (hTLVs) were similar in ADPKD and ADPLD cases, but significantly greater than in unsolved cases (grey). Of note, Kim-imaging classes are illustrated by green (mild), yellow (moderate), and red (severe) background (Kruskall-Wallis; Dunn’s). (C) Mean height-adjusted total kidney volumes (hTKVs) were significantly increased in ADPKD patients when compared to both patients with ADPLD (blue) and unsolved (grey) (Kruskall-Wallis; Dunn’s). (D) Illustration of the relationship between hTLVs (y-axis) and hTKVs (x-axis) for individual patients shows majorly normal kidney volumes in non-ADPKD patients and large heterogeneity of liver-kidney enlargement in ADPKD cases (discordant and non-discordant organ growth). (E) Maximum cyst diameters (MCD) were non-significant between groups; however, ADPLD patients exhibit overall largest MCDs (blue) (Mann–Whitney). (F) MCDs (y-axis) correlated significantly with hTLVs (x-axis) in all three groups (simple linear regression). (G) Exemplary imaging (CT-scans), representative for cystic kidney and liver enlargement in each group (ADPKD – red, ADPLD – blue, and unsolved – yellowish-grey). (H) Correlation of normalized TLVs (nTLV; y-axis) with patient age (x-axis). (I) Correlation of normalized TKVs (nTKV; y-axis) with patient age (x-axis). (J) Individual nTLVs and nTKVs in chronological order (by age at imaging) for each group showing liver-predominance in ADPLD, but heterogeneity in ADPKD patients. Levels of significance p = 0.0332 (*); 0.0021 (**); 0.0002 (***); <0.0001(****)
PKD1-VUS in addition to diagnostic PRKCSH variants, while the other two cases concerned VUS in LRP5 and GANAB on top of diagnostic SEC63 variants. Among patients with ADPKD, six cases also comprised more than one (likely) pathogenic variant; five individuals with additional PKD1-VUS, and in one case an added GANAB-VUS (Table S1).

Genotype-phenotype correlations
With the underlying molecular diagnoses, we divided patients into the following three subgroups, ADPKD-associated PLD (PKD1, PKD2, GANAB), isolated PLD (ADPLD) (PRKCSH, SEC63), and genetically unsolved PLD. In all subgroups, female sex was predominant (84% in ADPKD vs. 77% in ADPLD). Patients with a genetically confirmed diagnosis were significantly younger at diagnosis than those without genetic confirmation ($p < 0.0001$) (Table 1). Also, family history of PLD was significantly more frequent in the genetically confirmed subgroup ($p < 0.0001$) (Table 1). Among patients eligible for LTx, standard exception MELD score did not differ between ADPKD and ADPLD ($p = 0.946$), while laboratory MELD scores were significantly higher for ADPKD cases ($p = 0.013$), mirroring impaired renal function (mean estimated glomerular filtration rate 48 ml/min/cm$^3$ vs.

**Fig. 3. Clinical endpoints by genotype-based subgroups.** (A) Median age at waitlisting for liver transplantation (LTx) was non-significant across groups. Of note, only one patient in the genetically unsolved group (grey) fell into this category (One-Way Anova; Tukey); (B) Comparison of height-adjusted liver volumes (hTLVs) between patients waitlisted for LTx vs. patients not-waitlisted for LTx only showing significant differences in-between the ADPKD group (red) (Kruskall–Wallis; Dunn’s). (C) Univariate logistic regression showing significant association between probability of LTx-waitlisting (y-axis) and hTLVs (x-axis) (simple logistic regression; 95% CI). (D) Kaplan-Meier Analysis of LTx-free survival between patients with ADPKD-related PLD (red) and ADPLD (blue) did not reach statistical significance (mean 58 years vs. 65 years) (Log-rank (Mantel-Cox)). (E) Median age at first liver event presented non-significant differences between ADPKD (red) and ADPLD (blue) but occurred significantly later in patients without molecular diagnoses (unsolved – grey) (One-Way Anova; Tukey). (F) Comparison of height-adjusted liver volumes (hTLVs) in patients with and without liver event showing non-significant differences (Kruskall–Wallis; Dunn’s). (G) Univariate logistic regression showing significant association between probability of liver event (y-axis) and hTLVs (x-axis) for all groups (simple logistic regression; 95% CI). (H) Liver event-free survival was similar in patients with ADPKD-related PLD (red) and ADPLD (blue) (mean 50 years vs. 58 years) but significantly superior in genetically unsolved (grey) patients (mean 71 years). (Log-rank (Mantel-Cox)). Levels of significance $p = 0.0332$ (*); 0.0021 (**); 0.0002 (**); <0.0001(****)

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76 ml/min/cm³, respectively, \( p = 0.003 \). Ultimately, ESKD was reached at a mean age of 55 ± 2 years in 44% of ADPKD probands. While laboratory liver function parameters (e.g., albumin, cholinesterase) showed only subtle alterations throughout all groups, gamma-glutamyl transferase (GGT) was elevated for both ADPKD and ADPLD cases in contrast to unsolved PLD \( (p = 0.029) \). Median hTLV for patients with ADPKD and ADPLD was equivalently severe \((3,797 \text{ ml/m} \text{ [IQR 2,570–5,408 ml/m]} \) vs. \(3,112 \text{ ml/m} \text{ [IQR 1,763–4,809 ml/m]}\), \( p > 0.99 \)) and significantly larger than median hTLV in those without molecular diagnoses (994 ml/m [IQR 794–1,820 ml/m], \( p < 0.001 \)) (Fig. 2A, B). As expected, hTKVs differed significantly between ADPKD and ADPLD subgroups (median hTKV 616 ml/m [IQR 126–417 ml/m] vs. 206 ml/m [IQR 174–246 ml/m], \( p < 0.0001 \)) (Fig. 2C). Clinically, unsolved cases did not show renal involvement except in two cases. Hence, their median hTKV was similar to the ADPLD group (221 ml/m [IQR 174–507 ml/m], \( p = 0.924 \)) (Fig. 2C, D). Liver cyst size clinically appeared to be larger in patients with ADPLD than in those with ADPKD, but investigation of the maximum cyst diameter (MCD) among subgroups revealed only a statistical
trend ($p=0.068$) (Fig. 2E). However, MCD correlated with hTLV in the overall cohort, indicating that this parameter could potentially be useful in clinical evaluation ($r=0.61$; 95% CI 0.42–0.74; $p<0.0001$) (Fig. 2F).

When comparing intra-individual organ enlargement, liver and kidney growth did not correlate, for individuals displayed discordant cystic organ involvement (Fig. 2G). Of note, 65% (n = 13) of ADPLD cases exhibited kidney cysts, however, none had more than 10 cysts. Based on a standard baseline liver volume of 850 ml/m$^3$ at the age of 20 years, we normalized the rate of liver enlargement (nTLV) (Fig. 2H, I). For normalization of kidney enlargement, we used a baseline hTKV of 150 ml/m (nTKV).$^{21}$ The relationship between normalized kidney and liver enlargements did not correlate with age for either genetically confirmed or unsolved cases (Fig. 2J). Nonetheless, genetic confirmation was 3.4-fold (95% CI 1.8–8.5) more likely with every doubling of TLV in our cohort ($p<0.0001$) (Fig. S2).

The mean number of PLD-related symptoms was similar for both genetic subgroups ($p>0.99$) but was significantly lower among unsolved cases ($p=0.009$ vs. ADPKD) (Fig. S3A). While patients with genetically unsolved cases more often suffered from pain, tiredness, and limited mobility, loss of appetite, sarcopenia or shortness of breath were associated with moderate to severe courses in genetically confirmed cases (Fig. S3B).

In terms of co-morbidities, arterial hypertension was most prevalent throughout all subgroups (Fig. 3A, Fig. S4A-B). Overall, hTLV was significantly higher in patients eligible for LTx than in those who were ineligible (median 4,292 ml/m [IQR 3,169–6,151 ml/m] vs. 1,702 ml/m [IQR 994–3,157 ml/m]; $p<0.001$), independent of genetic background and age; 49% of patients with ADPKD (median hTLV = 4,835 ml/m [IQR 3,306–6,244 ml/m]) and 41% with ADPLD (median hTLV of 4,202 ml/m [IQR 2,613–6,411 ml/m]) were eligible for LTx (Fig. 3B). In order to evaluate the likelihood of LTx, logistic regression was conducted with hTLV as a predictor variable. Log-likelihood ratio test (LRT) was statistically significant for both genetic subgroups ($p=0.0017$ and $p=0.024$, AUC 0.79 and 0.83) and hTLV contributed to the models with an odds ratio (OR) of 2.3 (95% CI 1.3–4.2) for ADPLD and of 2.1 (95% CI 1.1–7.1) for ADPKD (Fig. 3C). LTx-free survival differed between patients with genetically confirmed and unsolved disease, but not between genetic subgroups (57 years for ADPKD vs. 65.3 years for ADPLD, $p=0.104$) (Fig. 3D). As an alternative clinical endpoint for disease severity, we analyzed the age at first PLD-related hospitalization (alias liver event). The most common event was liver cyst fenestration (39%), followed by liver cyst rupture (13%) and hemi-hepatectomy (10%) (Fig. 3C). Using this primary endpoint, patients with genetic diagnoses were more likely to exhibit a liver event (79% for genetically confirmed vs. 40% for unsolved, $p=0.009$) and were significantly younger at first liver event compared to patients with genetically unsolved disease (Fig. 3E). As patients with liver events showed significantly higher hTLVs compared to those without an event (Fig. 3F), we further investigated the relationship of hTLV with regard to the risk of events using logistic regression. This analysis rendered significant model fit (LRT $p=0.0004$) with an AUC of 0.80 and an OR of 2.1 (95% CI 1.3–4.0) for every liter increase in hTLV (Fig. 3G). Genetic subgroup comparison showed only a significant effect for the ADPLD subgroup ($p=0.019$, OR 5.3; 95% CI 1.2–13.09). Upon survival analysis, median age at first liver event was non-significant between ADPKD and ADPLD (50.3 years vs. 57.7 years, $p=0.136$), whereas patients in the unsolved group presented significantly later on average (median age of 71.1 years) ($p<0.0001$ vs. ADPKD). Furthermore, each centimeter increase in MCD was associated with a 38% risk elevation for experiencing an event in the total cohort (LRT $p=0.0005$, OR 1.38; 95% CI 1.13–1.81) (Fig. S5).

**Classification of total liver volumes**

We next categorized hTLVs according to the current imaging classification by Kim (Kim classes I, II, III – Table S2), separately for genetic subgroups as well as for both sexes without yielding significant differences (ADPKD vs. ADPLD $p=0.498$, male vs. female $p=0.741$) (Fig. 4A,B). While none of the patients with Kim class I was eligible for LTx, 39% of the moderately enlarged group (Kim class II) and 74% of the severest Kim class III were registered for LTx (at mean age 53 ± 3 and 51 ± 2 years) (Fig. 4C). Furthermore, 31% of Kim class I, 83% of Kim class II, and 90% of Kim class III patients experienced liver events (Fig. 4D). Survival analyses using both LTx and liver events as clinical endpoints could not statistically discern between patients with moderately (Kim II) and severely (Kim III) enlarged livers (median survival 58.2 vs. 55.6 years, $p=0.080$) (Fig. 4E-F). Comparison of liver event-free survival reached statistical significance between mild and severe classes, but non-significance between moderate and severe classes (1 vs. III $p=0.002$, II vs. III $p=0.099$) (Fig. 4F).

Of note, female sex was not associated with an overall higher risk for LTx, but median LTx-free survival was 12 years lower than in men (60.6 years vs. 73.3 years, $p=0.282$) (Fig. 4G, Table S3). Also, analysis of onset of liver events yielded a significant difference in survival of 13 years in favor of male sex (52.2 years vs. 65 years, $p=0.014$) (Fig. 4H).

Using cox proportional hazards regression analysis, we investigated the hazard ratios for experiencing a liver event based on Kim-imaging classes and genetic etiology (sex-adjusted). As a result, severe Kim III classes were associated with a 5-fold increase in the risk of any liver event compared to mild Kim I classes (hazard ratio 4.9; 95% CI 1.6–15.1) and the likelihood for any liver event was 10-fold higher in females (hazard ratio 10.4; 95% CI 2.8–38.9) (Fig. 4I). Genetic confirmation yielded a significant effect on overall survival compared to inconclusive genetic testing (Fig. 4J).

**Model of age-adjusted progression groups**

To assess individual disease progression, liver volumes are best interpreted in the light of patient age. As the Kim-classification does not consider patient age at index imaging, we sought to adjust for age by extrapolating liver growth rates under an exponential growth assumption relative to the standard liver volume (Fig. 5A, Fig. 56). To further define three age-adjusted progression groups (PG I-III), we delineated the following thresholds: less than 3.3% growth rate/year (green/mild), greater than 3.3% but less than 6.6% growth rate/year (red/moderate), and exceeding 6.6% growth rate/year (black/severe) (Fig. 5A). Subsequent survival analyses with aforementioned clinical endpoints (LTx and liver events) yielded significant differences for all three progression groups (Fig. 5B-C). Hence, categorization into predefined progression groups enabled discrimination regarding
the probability of event-free survival in terms of transplantation and PLD-related hospitalization (events) \((p = 0.0002\) for PG I vs. II, \(p < 0.0001\) PG I vs. III, \(p = 0.0006\) PG II vs. III) (Fig. 5B–C).

**Discussion**

Despite the given limitations of a retrospective single-center analysis, the rationale of our study was to improve PLD risk prediction by means of genetic determination, deep-phenotyping, and probabilistic radiological assessment.

To date, there are several co-existing PLD classification systems (Gigot,26 Qian,22 Kim,6 Schnelldorfer27). However, none of the existing systems is truly predictive, as they are designed to categorize current disease severity descriptively. As a matter of fact, present PLD classifications do not consider underlying molecular diagnoses and do not adjust for patient age at imaging. As PLD is often clinically silent, these limitations call for optimization in order to adequately counsel patients at early stages; a situation that is notably warranted in dominant disorders for subsequent generations. Likewise, patients affected by an autosomal recessive disorder can be relieved with regard to the negligible risk their own offspring bears for the disease.

Key findings of our study are that genetic confirmation is associated with (i) higher TLV, (ii) younger age at diagnosis, (iii) higher symptom burden, (iv) earlier onset of liver-related events, and (v) higher probability of registration for LTx when compared to patients with negative genetic testing. Without drawing firm conclusions, our study points to the predictive value of providing a molecular diagnosis for all patients with PLD, both isolated and non-isolated. For isolated PLD (ADPLD), this is in line with previous reports on PRKCSH/SEC63 nonsense variants. However, for non-isolated PLD (ADPKD), we are not aware of similar analyses comparing PLD severity of PKD1/PKD2 carriers with genetically unsolved cases. In contrast to renal outcomes, PKD1/PKD2 mutational status did not correlate with PLD severity in previous analyses,28 corroborating the notion of liver disease modification by environmental (extrinsic) factors and genetic background.

Another major finding is that age-adjusted \(hTLV\)-categories based on extrapolated liver growth rates correlated with clinically relevant endpoints such as hospitalization and transplantation. Therefore, these age-adjusted progression groups warrant validation in larger PLD populations. Ideally, a clinically predictive classification should hence comprise both genetics and age-adjusted \(hTLV\) classes to allow for timely risk-stratification.

For evaluating PLD severity, we further suggest the use of age at first PLD-related event as a clinical endpoint. We believe that PLD-related events are highly relevant to both patients and physicians and seem more applicable to the entirety of PLD population than LTx, which only applies to the most severe cases, further depending on non-medical conditions, such as national transplant-regulations.

To date, the clinical diagnosis of PLD is poorly defined and in contrast to PKD,26 cyst count is not adjusted for age at index imaging. Therefore, our recruitment criterion (\(\geq 3\) cysts at any given age) was chosen inclusively to also detect low impact variants. In line with previous data reporting on hypomorphic PRKCSH/SEC63 missense variants, we also found genetic involvement in 57% (\(n = 9/16\)) of clinically mild PLD cases, with less than 10 cysts.20 Otherwise, identified PRKCSH/SEC63 variants were predominantly \((n = 12, 8\%)\) truncating. Possibly, non-truncating PRKCSH/SEC63 alterations remain clinically undiagnosed more often, and thus would bypass tertiary referral.

In total, we obtained a diagnostic yield of 75% in our cohort, leaving only 25% genetically unsolved. Amongst the latter, there are likely new PLD disease genes to be discovered but most of these PLD cases may rather be due to complex genetics and environmental factors.

Interestingly, median \(hTLV\)s were similar in both ADPKD and ADPLD. In addition, no significant differences were identified between liver cyst morphology in ADPLD and ADPKD. In accordance with previous observations,31 however, ADPLD patients displayed an overall tendency for larger cyst size in our cohort. Recently, maximum cyst diameter has been proposed as a prognostic parameter for ADPKD-associated PLD.31 Indeed, the correlation of MCD with \(hTLV\) and clinical outcomes supports utilization of MCD as a prognostic marker in ADPLD as well. Still, we did not find significant prognostic differences in patients with genetically confirmed ADPKD and ADPLD in terms of clinical endpoints such as LTx and PLD-related hospitalization.

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**Fig. 5. Age-adjusted progression groups for risk-assignment of LTx and first liver event.** (A) Model of age adjusted progression groups (PG) defined by differential yearly growth rates (<3.3%/year – mild PG I in green; >3.3–6.6%/year – moderate PG II in red; >6.6%/year – severe PG III in grey/black). (B) LTx-free survival showing significant discrimination for all groups (median age at registration for LTx 42.9 yrs (PG III) vs. 59.5 yrs (PG II) vs. not-applicable in PG I) (Log-rank (Mantel-Cox)). (C) Liver event-free survival showing significant discrimination for all groups (median age at first liver event 43.4 yrs (PG III) vs. 56.2 yrs (PG II) vs. 67.9 yrs (PG I). (Log-rank (Mantel-Cox)). Levels of significance \(p = 0.0332\) (*); 0.0021 (**); 0.0002 (**); <0.0001(****)
Therefore, the major clinical difference remains preserved kidney function in ADPLD, demonstrated by normal TKV and absence of ESKD. Moreover, it is unclear whether additional growth of cystic kidneys would further worsen the PLD phenotype. With less abdominal space, one could expect an earlier onset of PLD-related symptoms and events in ADPKD. This hypothesis, however, was not confirmed by our data.

This study has several limitations. First, inclusion bias has to be considered, as patients were recruited exclusively from the time of PLD-related symptoms and events in ADPKD. This growth of cystic kidneys would further worsen the PLD phenotype, demonstrated by normal TKV and kidney function in ADPLD, illustrated by our data.

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Conflict of interest
The authors declare no conflict of interest concerning the content of this manuscript. Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions
J.H. and T.B. designed the study; D.S., A.H., J.F., J.d.E., and N.L. carried out phenotypic analyses; C.B., A.F., R.S. and E.H. conducted genetic analyses; D.S. and R.S. analyzed the data and created the Figs; D.S., R.S. and J.H. drafted the paper; all authors approved the final version of the manuscript.

Data availability statement
All data is available from the corresponding authors upon special request.

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Abbreviations
ACGS, Association for Clinical Genomic Sciences; ACMG, American College of Medical genetics and Genomics; ADPKD, autosomal-dominant polycystic kidney disease; ADPLD, autosomal-dominant polycystic liver disease; ESKD, end-stage kidney disease; hTKV, height-adjusted total kidney volume; hTLV, height-adjusted total liver volume; LRT, log-likelihood ratio test; MCD, maximum cyst diameter; LTx, liver transplantation; MELL, model for end-stage liver disease; MLPA, multiplex ligation-dependent probe amplification; nTKV, normalized total kidney volume; nTLV, normalized total liver volume; PG, progression groups; PKD1, polycystin 1; PKD2, polycystin 2; PLD, polycystic liver disease; OR, odds ratio; tNGS, targeted next-generation sequencing; VUS, variants of uncertain significance.

Supplementary data
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