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Sex, Genotype, and Liver Volume Progression as Risk of Hospitalization Determinants in Autosomal Dominant Polycystic Liver Disease

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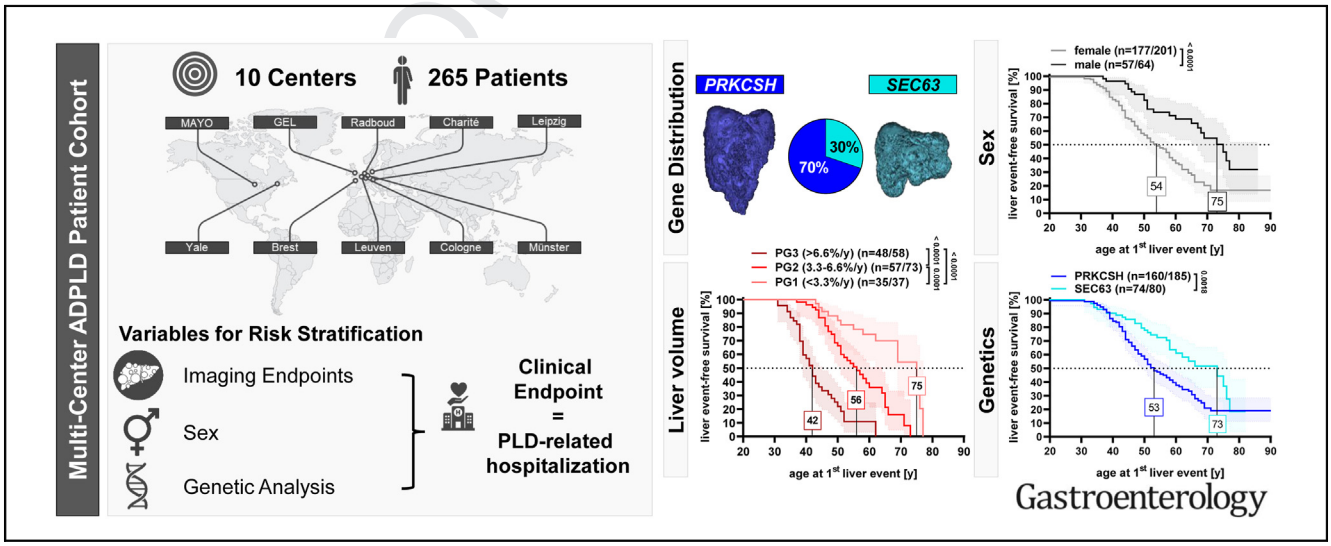

BACKGROUND & AIMS: Autosomal dominant polycystic liver disease is a rare condition with a female preponderance, based mainly on pathogenic variants in 2 genes, *PRKCSH* and *SEC63*. Clinically, autosomal dominant polycystic liver disease is characterized by vast heterogeneity, ranging from asymptomatic to highly symptomatic hepatomegaly. To date, little is known about the prediction of disease progression at early stages, hindering clinical management, genetic counseling, and the design of randomized controlled trials. To improve disease prognostication, we built a consortium of European and US centers to recruit the largest cohort of patients with *PRKCSH* and *SEC63* liver disease. **METHODS:** We analyzed an international multicenter cohort of 265 patients with autosomal dominant polycystic liver disease harboring pathogenic variants in *PRKCSH* or *SEC63* for genotype–phenotype correlations, including normalized age-adjusted total liver volumes and polycystic liver disease–related hospitalization (liver event) as primary clinical end points. **RESULTS:** Classifying individual total liver volumes into predefined progression groups yielded predictive risk discrimination for future liver events independent of sex and underlying genetic defects. In addition, disease severity, defined by age at first liver event, was considerably more pronounced in female patients and patients with *PRKCSH* variants than in those with *SEC63* variants. A newly developed sex-gene score was effective in distinguishing mild, moderate, and severe disease, in addition to imaging-based prognostication. **CONCLUSIONS:** Both imaging and clinical genetic scoring have the potential to inform patients about the risk of developing symptomatic disease throughout their lives. The combination of female sex, germline *PRKCSH* alteration, and rapid total liver volume progression is associated with the greatest odds of polycystic liver disease–related hospitalization.

Keywords: ADPLD; PCLD; PRKCSH; SEC63; TLV.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Despite discovery of the main disease genes of polycystic liver disease 20 years ago, little is known about how to use genetic and clinical information for disease prognostication at early stages.

NEW FINDINGS

With this multicenter study, we introduce novel clinical end points—normalized, age-adjusted total liver volume and polycystic liver disease–related hospitalization—for prognostic risk stratification. As a result, the risk was greatest in female patients with PRKCSH-mediated disease.

LIMITATIONS

Although large for rare diseases, the cohort size limits generalizability.

CLINICAL RESEARCH RELEVANCE

Both the sex-gene score and the novel imaging classification have the potential to inform decision making in patients with polycystic liver disease, when applied in a consecutive manner.

BASIC RESEARCH RELEVANCE

This work provides new hypotheses for basic research, as the molecular mechanism of differential disease severity in both female patients and PRKCSH-mediated disease is poorly understood.

Autosomal dominant polycystic liver disease (ADPLD) is a genetic cholangiopathy characterized by numerous fluid-filled cysts arising from intrahepatic biliary epithelia.[1] Unlike autosomal dominant polycystic kidney disease (ADPKD), symptomatic ADPLD is a bona fide rare condition with an estimated prevalence of 1:10,000.[2] Clinically, ADPLD is further distinguished from ADPKD by little or no kidney involvement, and distinct underlying genetic alterations different from *PKD1/2*. In ADPLD, the 2 disease genes accounting for most of the symptomatic cases are *PRKCSH*[3,4] (MIM #174050), and *SEC63*[5] (MIM #617004). Additional ADPLD genes (LRP5,[6] *SEC61B*,[7] SEC61A1,[8] ALG6,[9] ALG8,[7] ALG9,[10] *GANAB*,[7] and *PKHD1*[11]) play relatively minor roles and have been linked primarily to hybrid forms, reflecting the continuum of cystic liver and kidney diseases. Etiologically, both *PRKCSH* and *SEC63*—but also *SEC61B*, *ALG6*, *ALG8*, *ALG9*, *GANAB*, and *DNAJB11*—encode proteins in the endoplasmic reticulum that are involved in endoplasmic reticulum quality control and maturation machinery. During embryogenesis, monoallelic genetic defects were associated with ductal plate malformation, and a second somatic mutation is thought to result in cellular loss of heterozygosity and consequent hepatic cyst formation.[12,13]

Approximately 50% of patients with ADPLD remain without a genetic diagnosis after screening, although these unresolved cases are associated with attenuated and overall mild disease.[14,15] Moreover, clinical hepatic differences among genetically diagnosed forms of ADPLD have not been established, and no differentiation has been identified for *PRKCSH* and *SEC63* in terms of liver survival or other liver outcome parameters; therefore, there are questions about the prognostic value of genetic testing in this disorder. This is in contrast to ADPKD, where *PKD1* and *PKD2* are separated by a 20-year difference in kidney survival and clinical-genetic prediction scores are available.[16] Because ADPLD rarely leads to liver failure, and the indications for liver transplantation are not harmonized among centers and countries, we previously introduced the following new end point, which we deemed clinically relevant: PLD-related hospitalization (liver event).[14] We also suggested a predictive PLD-imaging classification, similar to the Mayo imaging classification for kidney survival in ADPKD.[14,17–19] This PLD-imaging classification is based on extrapolated growth rates derived from fold over normal total liver volume

Abbreviations used in this paper: **ADPKD, autosomal dominant polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; CT, computed tomography; HR, hazard ratio; MRI, magnetic resonance imaging; PG, progression group; nTLV, normalized total liver volume.**

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(normalized total liver volume [nTLV]) at the age of index imaging vs a nonenlarged standard volume of 850 mL at age 20 years. By annual growth rates, the classification discerns the following 3 so-called progression groups (PGs): mild (<3.3% increase per year), moderate (3.3%–6.6% increase per year), and severe (>6.6% increase per year) (Supplementary Figure 1). In a small single-center cohort with isolated and nonisolated PLD, PGs correlated with age at first liver event.[14] This finding prompted us to investigate larger, independent PLD cohorts.

Given the paucity of applicable data, predictive classifications and clinical scores are needed urgently to improve prognostication and well-informed decision making in ADPLD. In this study, we aimed to conduct a multicenter replication of the novel clinical end point liver event as a potential surrogate outcome parameter in future clinical trials. Therefore, we built an international collaboration to collect the largest study cohort of genetically diagnosed ADPLD for comparative assessment of differences between PRKCSH- and SEC63-mediated liver disease.

Methods

Study Population

Written informed consent was obtained from all patients included in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in *a priori* approval by the following participating institutions and human research committees: Institutional Review Board protocols at the University of Leipzig (ethics code 289/20-ek), Charité Berlin (EA4/066/21), University of Cologne (NCT02497521), Genomics England HRA Committee East of England Cambridge South (Research Ethics Committee ref. 14/EE/1112), CMO Arnhem-Nijmegen for Radboudumc (protocol ID2020-6326), Mayo Clinic (476-95 and 13-003971), and Yale University (HIC#0003010983, and others). After consenting, 265 adult patients from 10 tertiary centers (Radboud, Mayo, Brest, Leipzig, Münster, Yale, Leuven, GEL, Charité, and Cologne) were formally included on the basis of a clinical diagnosis and genetically confirmed as ADPLD. Diagnostic variants of either *PRKCSH* or *SEC63* were available for all study participants, and those with variants in other disease genes were excluded. Clinical diagnosis required multiple cysts on imaging (ie, ultrasonography, computed tomography [CT], and magnetic resonance imaging [MRI]). Clinical assessment was based on electronic health records, which were screened for predefined variables, including imaging end points (ie, MRI or CT–based liver volumetry) and clinical end points (ie, PLD-related hospitalization due to interventional, surgical, or nonsurgical reasons) (Figure 1).

Genetic Analyses

Patients were investigated for pathogenic alterations using targeted next-generation sequencing and multiplex ligation-dependent probe amplification upon availability. For patients from Leipzig, Berlin, and Münster, genetic testing was performed at the Institute of Human Genetics Bioscientia and Medical Genetics Mainz. The customized gene panel covered all the exon–intron boundaries and coding regions of *PKD1*, *PKD2*, *GANAB*, *PRKCSH*, *SEC63*, *PKHD1*, *HNF1B*, *ALG8*, *ALG9*, *DNAJB11*, and *SEC61B*. Segregation analysis was performed using direct sequencing based on sample availability. The Mayo cohort was genetically screened by means of targeted next-generation sequencing or Sanger analysis.[20] For Nijmegen and Leuven, targeted next-generation sequencing for *PRKCSH* and *SEC63* was performed at the Institute of Human Genetics Radboudumc. In Brest, a custom gene panel (Nimblegen, Roche) was used to capture the coding regions and approximately 50-bp flanking regions of 25 genes known to be associated with either ADPKD or ADPLD or other inherited nephropathies associated with kidney cysts or ADTKD.[21] At Yale University, whole-exome sequencing and analysis of the established ADPKD and ADPLD genes were performed. Using the Genomics England 100,000 Genomes Project data set, genomic and clinical data of the rare disease cohort of patients (71,991 participants) in the main program data release, version 9 (dated April 2, 2020) were reviewed. By analyzing tiering data within the rare disease cohort, we specifically examined deleterious variants in PRKCSH and SEC63 with clinical phenotypes, including cystic liver or kidney disease. Variants from identified patients were annotated using the Ensembl variant effect predictor, confirming variants in the canonical transcript; variants were selected for further analysis if they had a potentially high impact, as defined by ClinVar. Recruiting physicians were contacted using the Genomics England portal to assign more detailed clinical phenotypes to identified patients. Nonsense, frameshift, large deletion/insertions, and (canonical) splice site variants were categorized as truncating and small in-frame deletions/insertions, and missense variants were grouped as nontruncating. Variants were classified according to diagnostic criteria of the American College of Medical Genetics and Genomics.[22] Class III variants (alias variants of uncertain significance) were only included if "tepid," "warm," or "hot," according to Association for Clinical Genomic Science guidelines.[23]

Radiologic Assessment

CT and MRI were used to determine the TLV. If more than 1 scan was available, the most recent imaging before any type of surgical volume reduction was used for the TLV assessment (index image). Preoperative liver imaging data were available for 168 patients. *Intellispace* Portal, version 9.0; *Intellispace* Discovery, Analyze, version 11.0; 3-dimensional slicer, version 4.11.2; and ITK-snap software were used to perform 3-dimensional reconstruction through manual and semi-automatic segmentation, as described previously.[24,25]

Study End Points and Classifications

We defined survival without PLD-related hospitalization (ie, liver event) as the primary clinical end point. For an accurate definition of hepatic events, we scrutinized medical histories for the age at first PLD-related hospitalization (eg, treatment—interventional, nonsurgical, or surgical—such as cyst aspiration/fenestration, resection, or liver transplantation). The primary imaging end point was defined as nTLV. On the basis of a standard baseline liver volume of 850 mL/m^2[26] at the age of 20 years, we normalized the rate of liver enlargement (nTLV = fold over standard baseline TLV at age 20 years), as described previously (Supplementary Figure 1).[14] The "PLD-Progression

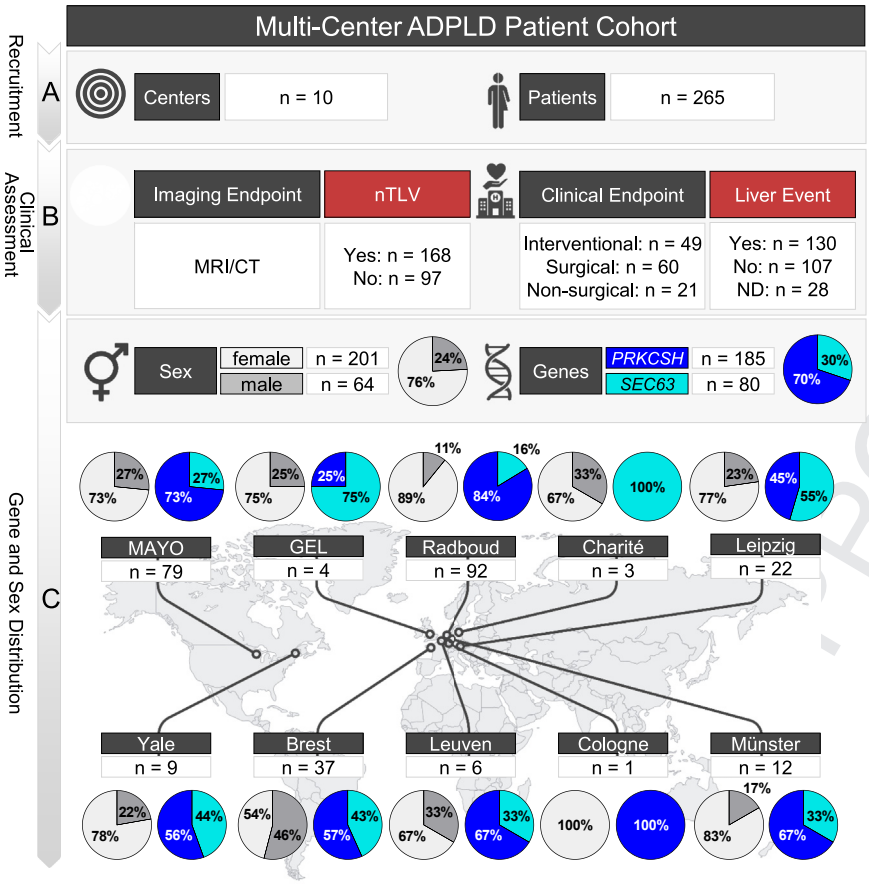

Figure 1. Study design and cohort stratification by genotype and sex in each of the participating centers. (*A*) Recruitment strategy of patients with ADPLD. (*B*) Cohort composition referring to imaging-derived nTLV and cyst-associated hospitalization (liver event), subdivided into interventional, surgical, and nonsurgical indication. (*C*) Location of participating centers, as well as genotype (*PRKCSH*/*SEC63*) and sex distribution for the total cohort and each center, respectively.

Grouper" web application was created to aid in the visualization and prognostic assessment of PLD progression. Vue.js and Chart.js were used to create an interactive platform for entering patient-specific data (eg, age and TLV) and visualizing nTLV. Users enter pseudonymized identifiers, age, and liver volume metrics, which the app uses to calculate nTLV and assign PGs on the basis of liver growth rates. These data points are displayed on a responsive chart to classify disease progression as mild, moderate, or severe. Calculated nTLVs and PGs are color-coded for clarity. For data documentation and sharing, the app allows printing the page, downloading the image, and exporting data in JSON or Excel formats. The web-app (http://pld-progression-grouper.org/) and its source code (https://github.com/halbritter-lab/pld-progression-grouper) are freely available.

Statistical Analyses

All statistical analyses were performed using SPSS software, version 25 (IBM Corp) and GraphPad Prism, version 9.2.0 (GraphPad Software). Statistical testing used $P < .05$ as the significance threshold. For normally distributed data, we used Student *t* test and analysis of variance; for non-normal distributions, we used the Mann-Whitney U test and Kruskal-Wallis test. Multiple comparisons were performed using Tukey and Dunn tests. Categorical variables were analyzed using χ^2 test or Fisher exact test. For regression and correlation analyses, we investigated the relationship between potential confounding variables (eg, sex and age) with nTLV as the dependent variable. Survival analyses were performed using the Kaplan-Meier method, and differences between the curves were compared using log-rank testing. Cox proportional hazard regression was used to investigate the effects of the aforementioned variables on survival.

Results

Baseline Cohort Characteristics

The study cohort (n = 265) was recruited from 10 tertiary centers comprising 8 European and 2 major US institutions (Figure 1*A*). In total, 201 women (76%) and 64 men (24%), with a mean (SD) age of 61 (12) years at last follow-up, were included on the basis of clinical PLD and a diagnostic gene variant in either *PRKCSH* (n = 185 [70%]) or *SEC63* (n = 80 [30%]) (Table 1 and Figure 1). The total cohort was divided into 4 subgroups according to sex (female/male) and underlying disease genes (*PRKCSH*/*SEC63*) (Table 1). Imaging data for the calculation of liver volume, including nTLV, for defining the primary imaging end point, were available for 168 patients (63%). In total, the median height-adjusted TLV was 2485 mL/m (interquartile range, 1561–3508 mL/m). The primary clinical end point (first PLD-related hospitalization as liver event) was reached in 129 patients (49%) (Figure 1*B*). Most frequently, surgical

Table 1. Baseline Characteristics of Study Cohort

Characteristic	Total cohort	Sex		Statistical test, P value	Genotype		Statistical test, P value
		Female	Male		*PRKCSH*	*SEC63*	
Total, n (%)	265 (100)	201 (76)	64 (24)	ND	185 (70)	80 (30)	ND
Age at imaging, *y*, median (95% CI); n	48 (46–51); 168	47 (45–49); 136	54 (49–67); 32	MW, <.0001****	47.5 (45–50); 114	50 (46–55); 54	MW, .6012; NS
hTLV, *L/m*, median (95% CI); n	2.5 (2.0–2.7); 139	2.5 (2.1–2.8) 113	1.9 (1.4–3.6); 26	MW, .5703; NS	2.5 (2.1–2.8); 97	2.1 (1.7–2.8); 42	MW, .1846; NS
nTLV, *au*, median (95% CI); n	4.6 (3.9–5.0); 168	4.6 (3.9–5.0); 136	4.1 (3.0–7.4); 32	MW, .6958; NS	4.8 (4.1–5.3); 114	3.7 (3.0–5.0); 54	MW, .0484*
Liver growth rate per year, % (n)	ND	5.1 (136)	4.4 (32)	*F* test, .0114*	5.2 (114)	4.3 (54)	*F* test, .0015**
Age at first liver event, *y*, median (95% CI); n	47 (44–50); 130	46 (44–49); 107	51 (47–67); 23	MW, .0013**	46 (44–49); 100	50.5 (40–58); 30	MW, .2203; NS
Liver event–free survival, *y*, median (95% CI); n	58 (234)	54 (177)	75 (57)	LR MC, <.0001****	53 (160)	73 (74)	LR MC, .0018**
Positive family history, % (n)	67.8 (199)	69.2 (146)	64.2 (53)	χ^2, .502; NS	73.9 (136)	55.4 (65)	χ^2, .009*

hTLV, height-adjusted total liver volume; LR MC, log-rank (Mantel-Cox); MW, Mann-Whitney; ND, not determined; NS, not significant.

and interventional treatments (83%) led to PLD-related hospitalization (Figure 1*B*). Sex and gene distribution were similar across recruiting centers, with a female preponderance in 9 of 10 centers and predominance of *PRKCSH*-mediated disease in 7 of 10 centers (Figure 1*C*). Notably, a family history of PLD was reported in 68% (134 of 199) of cases. Interestingly, a family history of PLD was significantly more frequent in patients with PRKCSH than in patients with SEC63 (χ^2, $P = .009$) (Table 1).

Genetic Landscape of ADPLD-PRKCSH *and ADPLD*-SEC63

Forty-seven different diagnostic *PRKCSH* variants (American College of Medical Genetics and Genomics classes IV and V) and 55 diagnostic *SEC63* variants (American College of Medical Genetics and Genomics classes IV and V) were identified in 265 patients from 209 families. A total of 102 unique monoallelic variants were included in the analysis, 56 (55%) of which were novel, and 46 variants (45%) were reported previously in ClinVar and/or gnomAD, with a minor allele frequency of <0.1%, except for the *SEC63* variant Glu568del (Figure 2*A*, Supplementary Table 1). This specific variant has been reported previously in multiple families with ADPLD, including the initial gene discovery publication.[5] However, its high minor allele frequency (0.37%) suggests incomplete penetrance. Of note, on the basis of clinical and molecular expert assessment, we deemed 3 nontruncating *SEC63* variants (ie, Ala373_Gln375del, Glu568del, and Thr676Ala) as diagnostic variants that were listed as variants of uncertain significance in ClinVar (Supplementary Table 1). Overall, most of the diagnostic variants were predicted to lead to protein truncation (n = 74; n = 33 in *PRKCSH* (70%) and n = 41 in *SEC63* (74%)). Another 22 variants constituted splice site alterations (n = 10 in *PRKCSH* and n = 12 in *SEC63*) (Figure 2*A*, Supplementary Table 1). Only 3 diagnostic variants represented missense alleles, all of which were related to *PRKCSH* (mean Combined Annotation Dependent Depletion Phred score, 26.8). A fraction of 19% of diagnostic variants was present at low minor allele frequency in the population databases (gnomAD, version 2.1.1). Interestingly, 2 previously reported *PRKCSH* splice site variants (NM_001379608: c.292+1G>C and c.1341-2A>G) accounted for a substantial number of patients from the Netherlands and northwestern Germany (n = 68 [37%]), indicating a founder effect (Figure 2*BI* and Supplementary Table 1). Interestingly, these splice sites concern either the N-terminal G2B domain, which is crucial for interaction with the enzymatic protein product of *GANAB* to build the functional enzyme glucosidase II (GlucII),[27] or the C-terminal MRH (mannose 6-phosphate receptor homology) domain, which is required for N-glycan recognition of GlucII substrates, including glycans on polycystin 1 (PC1) (Figure 2*BI*). In contrast, no such variant accumulation was observed for *SEC63* (Figure 2*CI* and *II*). 3-Dimensional protein modeling yielded an equal distribution of deduced stop codons in both protein structures, with loss of the endoplasmic reticulum retention signal (HDEL), and thus, a

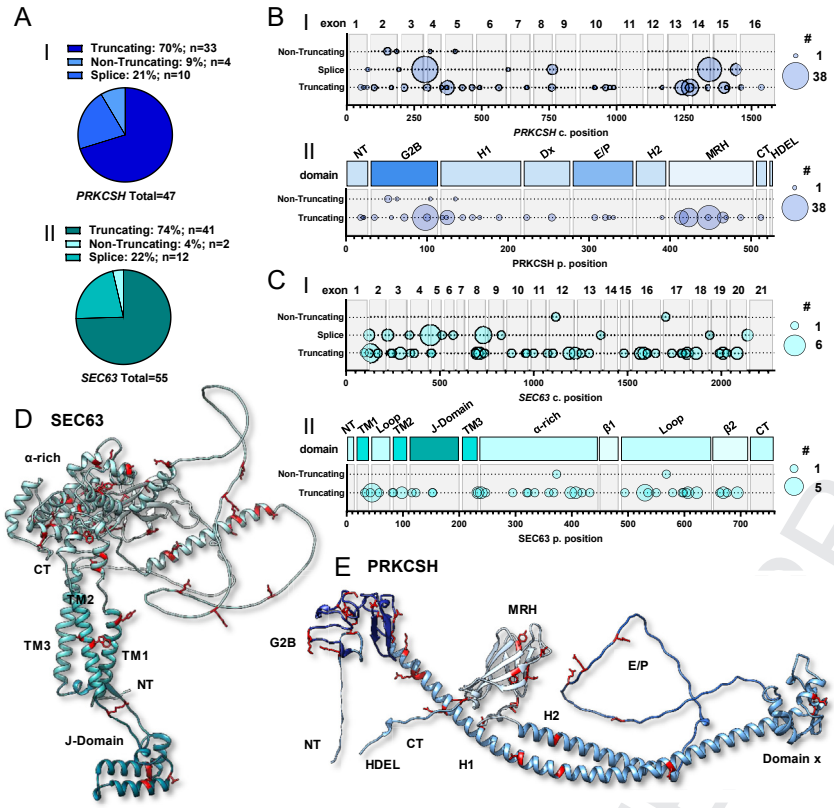

Figure 2. *PRKCSH* and *SEC63* protein structure and cohort variants. (*A*) Distribution of mutation types for unique mutations in *PRKCSH* (*I*) and *SEC63* (*II*) respectively, subdivided into truncating, nontruncating and splice site variants. (*B*) Number of affected patients and mutation type for each variant aligned according to coding (*I*) and protein (*II*) positions on the *PRKCSH* gene. (*C*) Number of affected patients and mutation type for each variant aligned according to coding (*I*) and protein (*II*) positions on the *SEC63* gene. (*D*) 3-Dimensional (3D) model of the *SEC3* protein with domain labels; patient variants marked in *red*. (*E*) 3D model of the *PRKCSH* protein product (β subunit of GlucII) with domain labels; patient variants marked in *red*.

loss of function, as the common denominator on PRKCSH truncation (Figure 2*D* and *E*). Furthermore, the low proportion of missense variations in our cohort suggests that there could be a threshold for cystogenesis initiation that is not reached by minor changes in protein function. Interestingly, the 3 missense variants in *PRKCSH* are located within or close to the G2B region. In particular, Asp63 and Asp104, which were mutated in subjects from our cohort, are thought to be directly involved in Ca^{2+} complexation and, thus, required for correct folding of the G2B domain and efficient subunit interaction.[28]

Imaging-, Sex-, and Genotype-Based End-Point Analyses

With the available nTLV data set, we sought to corroborate previously suggested PGs (http://pld-progression-grouper.org/).[14] By assigning patients to 1 of 3 PGs (Supplementary Figure 2*A*), we were able to see significant differences in both the age at first PLD-related hospitalization (liver event) and the probability of experiencing a liver event at a given age; median age was 42 years in PG3 vs 56 years in PG2 vs 75 years in PG1 (Figure 3*A* and *B*). Statistical significance was observed when removing the 22 ADPLD cases from the previous discovery cohort (Supplementary Figure 2*B*).[14] Notably, patients with an estimated yearly liver growth rate of >6.6% (PG3, n = 58) were significantly younger at initial imaging (Supplementary Figure 2*C*) and first liver event, independent of sex and underlying disease gene, as shown by subgroup analyses (female/male/PRKCSH/SEC63) (Supplementary Figure 2*D*–*G*). Nevertheless, PG3 individuals were enriched in female patients with PRKCSH, whereas PG1 individuals harbored most instances of male patients with SEC63 (Figure 3*C*). The proportion of female PRKCSH cases gradually increased from PG1 to PG3 at the expense of both male and female SEC63 patients (Figure 3*C*). Looking at the type of liver event in more detail, inpatient interventional treatments (eg, aspiration sclerotherapy) were reported most frequently, followed by surgical (fenestration > resection > liver transplantation > umbilical hernia) and conservative therapies that required hospitalization (anti-pain > anti-infectious > anti-bleeding/rupture > ascites) (Figure 3*D*, Supplementary Table 2). Among patients who underwent these interventional treatments (aspiration sclerotherapy), female sex, PG3, and ADPLD-*PRKCSH* were predominant (Figure 3*D*). As an example of highly discrepant disease severity, we highlight the comparison of 2 patients presenting with identical nTLVs at different ages (Figure 3*E*): a male patient with ADPLD-*SEC63* and an nTLV of 8.5 at age 72 years (PG1), in contrast to a female patient with ADPLD-*PRKCSH* and the same nTLV at age 42 years (PG3). Although histologically, no distinct patterns correlating with the mutations were found in a blinded investigation of n = 3 per gene group, the clinical relevance of a 30-year time span in developing severe hepatomegaly becomes evident (Figure 3*E*).

To further decipher the clinical variables driving the observed differences, we separately investigated the

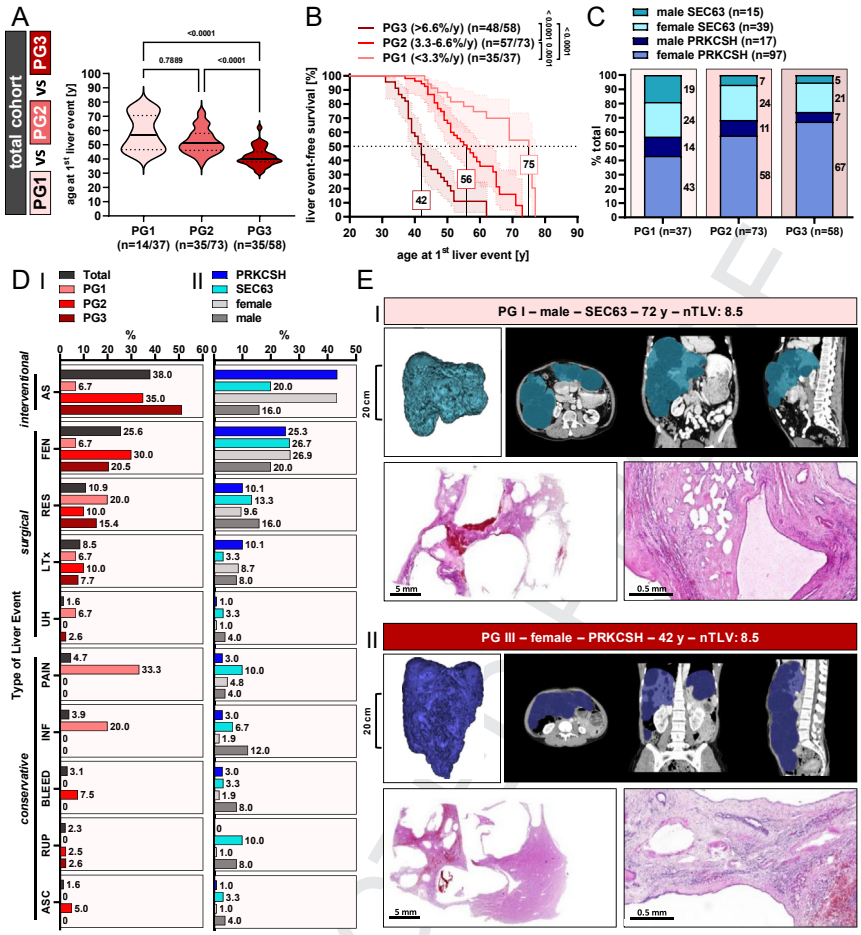

Figure 3. Age-adjusted progression groups serve to determine the risk for first hospitalization. Model of age-adjusted PGs defined by differential yearly growth rates (<3.3% per year is mild PG1 in *light red*; >3.3%–6.6%/year is moderate PG2 in *red*; >6.6% per year is severe PG3 in *dark red*) and characterization of indications for hospitalization. (*A*) Median age at first liver event presented nonsignificant between PG1 and PG2 (56.5 years vs 51 years; $P = .7889$), but occurred significantly earlier in PG3 (40 years; $P < .0001$ both compared with PG1 and PG2) (Kruskal-Wallis and Dunn tests). (*B*). Liver event–free survival showing significant discrimination for all groups (median age at first liver event, 42 years (PG3) vs 56 years (PG2) vs 76 years (PG1) (log-rank [Mantel-Cox]). (*C*) Distribution of sex within genotype subgroups for each progression group. (*D*) Fraction of interventional, surgical, and nonsurgical event types leading to hospitalization within the total cohort and according to PGs (*I*), as well as sex and genotype (*II*). Conservative-treated events include ascites (ASC), cyst rupture (RUP), hemorrhage (BLEED), infection (INF), and abdominal pain (PAIN). Surgical therapy comprises umbilical hernia repair (UH), liver transplantation (LTx), partial liver resection (RES), and cyst fenestration (FEN). Percutaneous aspiration sclerotherapy (AS) refers to an interventional treatment of selected cysts, the most common indication for hospitalization in the total cohort. (*E*) Illustrative 3-dimensional model and volumetry of polycystic liver in multiplanar reconstruction along with exemplary histopathologic microscopy *images* of patients' tissue samples at 0.5× and 7.5× magnification, stained with H&E. (*I*) Example of PG1: imaging from 72-year-old man with a diagnostic *SEC63* variant, liver tissue marked in *turquoise*, nTLV = 8.5. (*II*) Example of PG3: imaging from a 42-year-old woman carrying a *PRKCSH* truncating variant, liver tissue marked in *purple*, nTLV = 8.5. Q22 Q23

influence of sex and gene on nTLV (imaging end point) and liver event (clinical end point), respectively (Figure 4). As expected, female sex was independently associated with liver growth (nTLV at age) and a higher likelihood of PLD events at a lower age (median age of 54 years vs 75 years; $P < .0001$) (Figure 4*AI–III*). In addition, women were statistically younger at first imaging, as a proxy for age at diagnosis (Supplementary Figure 3*A*). Furthermore, *PRKCSH* carriers had significantly higher nTLVs (Supplementary Figure 3*B*) and were more likely to be hospitalized at a younger age than *SEC63* carriers, independent of their sex (median 53 vs 73 years; $P = .0018$) (Figure 4*BI–III*). When investigating women and men separately, stratified by disease gene only, we observed the same trend of increased disease severity for *PRKCSH*-mediated ADPLD in terms of both imaging and clinical end points (Figure 4*CI–DII*, Supplementary Figure 3*C–D*). However, for low sample sizes in subgroup analyses, these differences remained statistically significant for male liver event-free survival only (median age at first liver event: 65 years in *PRKCSH* vs 76 years in *SEC63* disease; $P = .0427$) (Figure 4*DIII*).

In line with the notion of an aggregate risk model, the rate of hospitalized patients in the cohort increased with each additional risk factor (eg, sex, gene, and PG) from

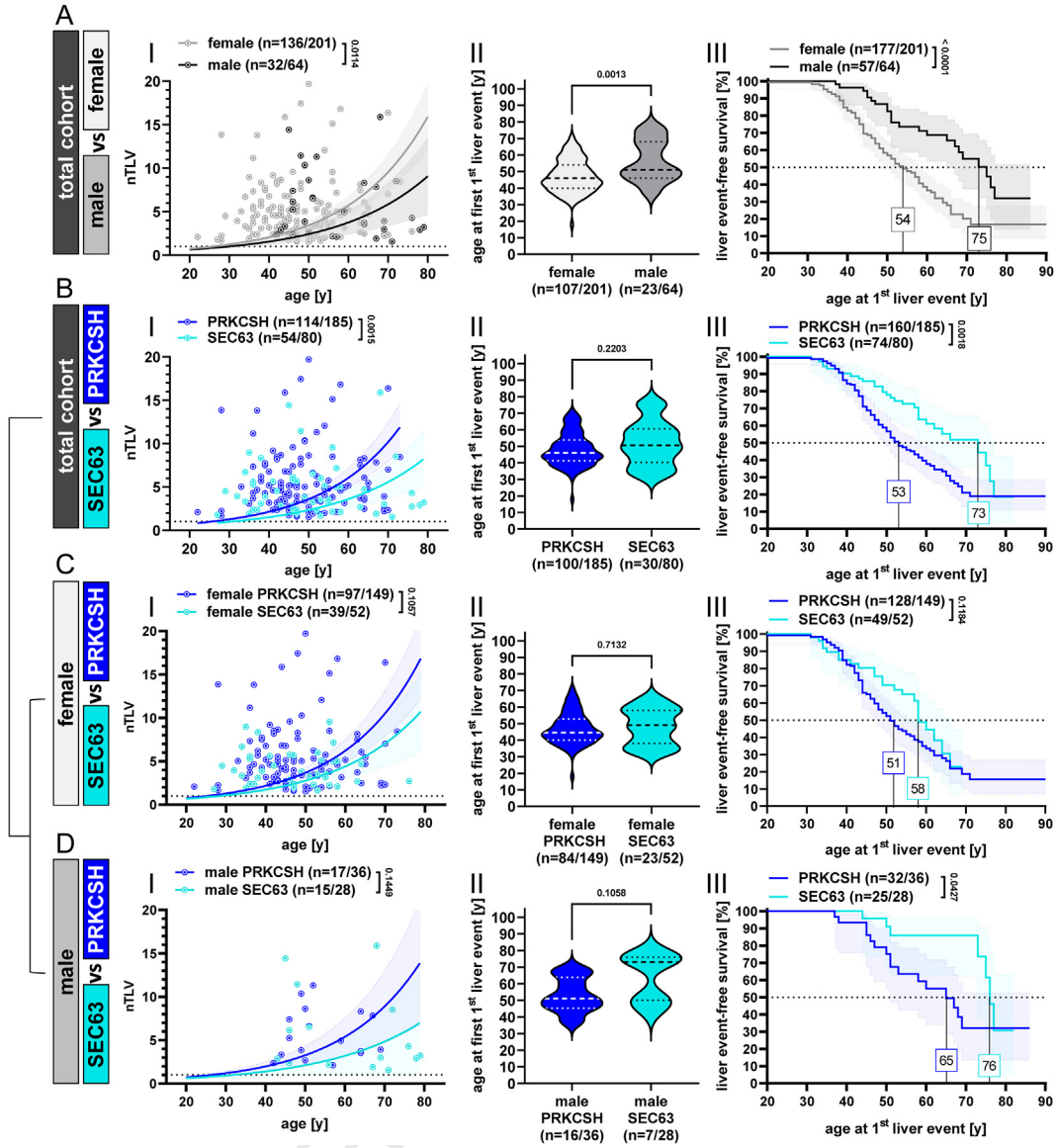

Figure 4. Female patients and *PRKCSH*-mutation carriers are at higher risk for severe courses by use of imaging and clinical end points. Subanalyses comprising (*I*) correlation of nTLV (*y-axis*) with patient age (*x-axis*) (nonlinear regression analysis, growth rate comparison with *F* test), (*II*) comparison of median age at first liver event among subgroups (Mann-Whitney), and (*III*) Kaplan-Meier analysis of liver event–free survival (log-rank [Mantel-Cox]). (*A*) Sex comparison revealed a significantly (*AI*) higher liver growth rate, (*AII*), younger age at first liver event, and (*AIII*) increased risk of experiencing liver events at young age in female compared with male patients (median age 54 vs 75 years; $P < .0001$; log-rank [Mantel-Cox]). (*B*) Genotype comparison revealed (*BI*) a significantly higher liver growth rate for patients with *PRKCSH*, (*BII*) but although symptomatic patients were aged similarly at first liver event, (*BIII*) *PRKCSH* alteration correlated with an increased risk of experiencing liver events in mid-life, showing a 20-year difference for median age at event between patients with *PRCKSH* and *SEC63* (median age, 53 years vs 73 years; $P = .0018$; log-rank [Mantel-Cox]). (*C*) Genotype stratified by sex showed nonsignificant differences in growth rate (*CI*), age at first liver event (*CII*), and risk of liver events (*CIII*) among female patients (median age, 51 years vs 58 years; $P = .1184$; log-rank [Mantel-Cox]). (*D*) Although both growth rate (*DI*) and age at events (*DII*) yielded nonsignificant differences between male patients with *PRKCSH* and male patients with *SEC63*, the risk of experiencing liver events until the 7th decade of life was higher in male patients carrying diagnostic *PRKCSH* variants (median age, 65 years vs 76 years; $P = .0427$; log-rank [Mantel-Cox]).

42.9% (no risk factor) to 45.5% (1 of 3 risk factors) to 54.5% (2 of 3 risk factors) to 75.7% (presence of all 3 risk factors) in a nonweighted approach (Supplementary Table 3).

Next, we sought to deduce a clinically applicable score for enhanced disease prognostication. In multivariate analyses using Cox regression, we tested several models with all 3 risk factors in a binary (PG1/2 vs PG3 and PG1 vs PG2/3) and nonbinary fashion (PG1 vs PG2 and vs PG3) (Supplementary Figure 4). Thereby, the hazard ratio (HR) of PG3 exceeded all other variables when compared with PG1 (HR, 10.01) or PG1/2 (HR, 4.69) as a reference

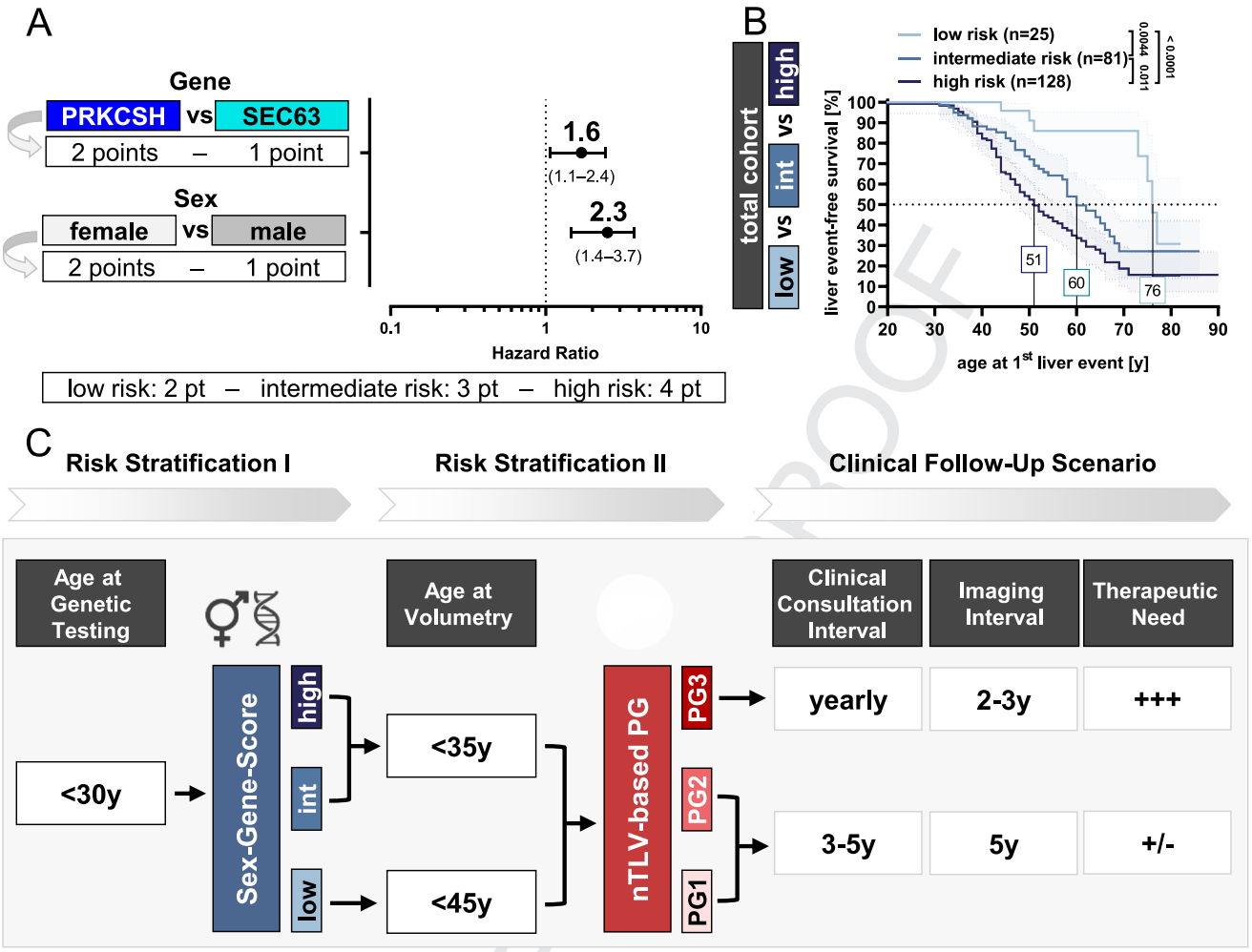

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Figure 5. Clinical score based on genotype and sex discriminated among mild, moderate, and severe courses. (*A*) *Forest plot* showing HRs for PRCKSH vs SEC63, as well as female vs male with regard to experiencing liver events using Cox proportional hazard regression. Thereafter, the following 3 risk classes were defined according to HRs: low risk (male SEC63), intermediate (int) risk (male PRKCSH plus female SEC63), and high risk (female PRKCSH). (*B*) Consequently, liver event–free survival showed significant discrimination for all clinical score groups (median age at first liver event 76 years [low risk] vs 60 years [intermediate risk] vs 51 years [high risk]) (log-rank [Mantel-Cox]). (*C*) Proposed work flow for clinical implementation using the sex-gene score in absence of liver volumetry and young age (younger than 30 years) as an initial risk stratification tool. As a next step for more detailed risk stratification, liver volumetry is needed for assignment into 1 of 3 PGs (PG1–3). Depending on the initial assessment as high or intermediate risk and presence of any clinical signs and symptoms, liver volumetry should be performed immediately (younger than 35 years). In the absence of signs and symptoms and a low risk score, liver volumetry may be postponed, but is indicated at least once before the age of 45 years. In a clinical follow-up scenario, patients with PG3 may benefit from yearly consultations and repeated imaging after 2–3 years. In asymptomatic patients with PG1–2, a reconsultation after 3–5 years and repeated imaging after 5 years may be sufficient.

(Supplementary Figure 4). However, gene and sex remained statistically significant risk factors in the binary PG model (Supplementary Figure 4). We therefore decided to generate a separate sex-gene score with the 2 age-independent risk factors, in addition to the risk stratification by PGs, representing the strongest predictor, notably when hepatomegaly allows for sufficient growth discrimination. By doing so, *PRKCSH* was associated with an HR of 1.6 (95% CI, 1.1–2.4) compared with *SEC63* in terms of reaching the primary clinical end point. In addition, female sex yielded an HR of 2.3 (95% CI, 1.4–3.7) compared with male sex (Figure 5*A*). Taking into account previous HRs, we weighted the inclusion of both variables (sex and gene) into the clinical score and defined the following 3 risk classes: low risk (male-*SEC63*, 2 points), intermediate risk (male-*PRKCSH* plus female-*SEC63*, 3 points), and high risk (female-*PRKCSH*, 4 points) (Figure 5*A*). As proof of concept, we ran previous clinical end point analyses with the newly defined risk classes and obtained discriminative values in terms of liver event–free survival and age at first liver event (median age, 51 years at high risk vs 60 years at intermediate risk vs 76 years at low risk) (Figure 5*B*). In an effort to facilitate translation into clinical practice, we suggest the following stepwise risk stratification: the sex-gene score is most informative in the absence of liver volumetry and during the earliest stages of the disease (before 30 years of age)

(Figure 5*C*). Depending on this initial step, liver volumetry will be prioritized (younger than 35 years or younger than 45 years). As a clinical follow-up scenario, we propose yearly consultation and repeated imaging for high-risk patients based on an PG3 assignment (Figure 5*C*). Conversely, for asymptomatic patients in PG1–2, the interval of specialized consultation and repeated imaging may be prolonged to 3–5 years.

Discussion

In 2003 (*PRKCSH*) and 2004 (*SEC63*), the major molecular mechanisms of inherited liver cystogenesis were first discovered through linkage analysis and direct sequencing of large ADPLD pedigrees.[3–5] Nevertheless, despite identification of the 2 primary ADPLD genes 2 decades ago, the clinical implications of genetic testing remained limited. Beyond genetics, physicians and clinical geneticists lack predictive tools for offering specific counseling and tailored surveillance for affected individuals and their families. As PLD is often and clinically silent, false counseling may lead to either overestimating or underestimating the risks, resulting in unwarranted anxiety on the one hand, or lack of consideration on the other. This situation calls for urgent optimization to provide clinicians with the right tools for giving patient advice at an early stage.

By introducing a new risk-based imaging classification and establishing a clinical-genetic score (sex-gene score), we hope to inform decision making by means of individualizing ADPLD management starting from the time of the first diagnosis. Despite the known limitations of retrospective cohort analyses, the suggested tools offer enhanced disease prognostication and counter a nihilistic attitude toward ADPLD patient care.

To the best of our knowledge, this is the first study to demonstrate phenotype–genotype correlations in ADPLD. Owing to the rarity of ADPLD, previous study cohorts were either underpowered or incompletely genotyped, which hindered statistical correlation analyses. In this study, we compiled the largest data set of genetically confirmed and phenotypically characterized ADPLD over an extensive observation period. The sample size was achieved through broadly collaborative efforts, enabling a critical number of patients to test the hypothesis that age-adjusted liver imaging and genotype–phenotype traits matter in terms of disease severity.

The rationale of our study was to assess the risk of PLD-related hospitalization by means of considering hospitalization as an indicator of disease burden. To date, a major challenge is the lack of an established clinical end point in ADPLD, as patients commonly show preserved organ function and do not experience liver failure. Consequently, indications for liver transplantation are not handled uniformly across countries and centers. To overcome this limitation, we recently found that hospitalization represented a promising new clinical end point.[14] Hospitalization is highly relevant to both patients and health care systems and seems more applicable to the entirety of patients with ADPLD when compared with liver transplantation, which only applies to a subset of the most severe cases, further depending on national transplant regulations. In the current study, we validated PLD-related hospitalization as a significant end point. Thus, we suggest using PGs and PLD-related hospitalization as potential end points in future clinical trials.

In this study, we also validated the concept of an age-adjusted nTLV classification, which aimed to translate the well-established and widely used kidney imaging classification (ADPKD-Mayo)[19] to the liver. As several co-existing PLD imaging classifications did not adjust for age at imaging (eg, Gigot et al,[29] Qian et al,[30] Kim et al,[26] and Schnelldorfer et al[31]), we sought to incorporate "age-adjustment" into risk-class assignment to warrant predictive value. Independently, Bae et al[18] also introduced an age-adjusted imaging classification based on liver cystic volume. However, due to the lack of a clinical end point, there was no predictive correlation of the assigned growth rate classes. In contrast, by using the clinical end point of hospitalization, we found that independent of sex and gene, deduced PG constituted the strongest predictor. However, liver volumetry is time-consuming, incompletely automated, and often unavailable to patients from nontertiary centers. Therefore, we sought alternatives to imaging-based risk stratification. Similarly, a clinical-genetic score used to assess the odds of kidney survival in ADPKD (PROPKD score) served as a template to discern rapid from slow disease progression by means of weighing genic, allelic, and clinical information.[16] Although in ADPKD, male patients are at increased risk of kidney failure in both ADPLD- and ADPKD-associated PLD, female patients have a greater risk of developing pronounced hepatomegaly. These results are in line with a previous study on female sex being a risk factor in ADPLD.[32] Here, we found that genetic information can further enrich clinical risk assessment, leading to the conclusion that women with *PRKCSH* alterations have the poorest prognosis, in contrast to men with *SEC63* mutations that harbor the most favorable prognosis. The fact that *PRKCSH* was identified first, ahead of *SEC63*, fits well with the hypothesis that gene discovery takes place in a chronological order ranging from more severe to less severe phenotypes, a commonly observed phenomenon in genetic disorders (eg, ADPKD, where *PKD1*[33] was discovered well before *PKD2*,[34] which is associated with milder disease).

The key findings of our study are that the considerable risk of symptomatic hepatomegaly is associated with higher nTLV and deduced PG class, female sex, and *PRKCSH* carrier status. Our study corroborates the predictive value of genetic testing for all patients with ADPLD. Apart from prognostic differences in *PRKCSH*- vs *SEC63*-mediated disease, previous studies have found that the lowest risk concerns patients without a molecular diagnosis (ie, no mutation identified through appropriate testing).[14,15]

Despite the strength of a multicenter approach and a relatively large study population, given the rarity of ADPLD, this study has several limitations. First, the retrospective collection of data from multiple sources is prone to incomplete clinical information. Next, patients were recruited exclusively from tertiary referral, and previous publications using height-adjusted TLV as the primary outcome variable reported lower median TLVs,[32,35] indicating inclusion bias

toward the most severe fraction of patients with PLD; milder cases were underrepresented. This limits our conclusions regarding the prevalence and prognosis of clinically unrecognized cases. In addition, most patients in this cohort underwent single imaging only, but no longitudinal MRI/CT scan data allowed intraindividual liver growth assessment. Therefore, we used simplified liver growth assumptions, which are likely associated with inaccuracy in both ways (potential under- and overestimation). Unlike in prospective studies, our retrospective design did not allow consistent observation periods, yet such an assessment may be critically important, as in longitudinal imaging studies of ADPKD-associated PLD, liver growth rate changed or even regressed in some cases, particularly in women older than 48 years.[18,34]

Independent of sex and underlying genetic defects, patients with an estimated liver growth rate of more than 6.6% per year (PG3) are at the highest risk of being hospitalized for symptomatic ADPLD in their 40s. In the absence of MR/CT-based liver volumetry and before onset of hepatomegaly (younger than 30 years of age), genetic testing is most informative, as the combination of female sex and *PRKCSH* alteration is associated with the highest odds of symptomatic ADPLD in mid-life, in contrast to the low-risk profile in male patients with *SEC63* variation (Figure 5*C*). Thus, we propose to use the PG-imaging classification and the sex-gene score as a complementary but consecutive 2-tier prognostication model to guide clinical follow-up strategies, including intervals for consultation, repeated imaging, and therapeutic intervention (Figure 5*C*). As a next step, it will be crucially important to follow the natural history of this disease with and without intervention to validate the prediction models for individual patients. Selecting the most progressive courses in the early stages will be key for a randomized controlled trial design. Our proposed prognostication model will prove helpful in identifying patients who may benefit most from tight monitoring, avoidance of extrinsic progression factors (eg, estrogens), and inclusion in future clinical trials, eventually improving the therapeutic armamentarium for high-risk patients.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://doi.org/10.1053/j.gastro.2023.12.007

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Melissa Boerrigter, PhD (Data curation: Supporting; Formal analysis: Supporting).
Tabinda Jawaid, PhD (Data curation: Supporting; Formal analysis: Supporting; Writing – review & editing: Supporting)
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Conflicts of interest

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Data Availability Statement

All data are available from the corresponding authors upon special request. Q8