Received: 28 June 2021 | Revised: 31 March 2022 | Accepted: 13 May 2022

DOI: 10.1002/mgg3.1998

Molecular Genetics & Genomic Medicine Open Access WILEY

CLINICAL REPORT

Rare variants in *PKHD1* associated with Caroli syndrome: Two case reports

Carola Giacobbe[1] | **Fabiola Di Dato**[2] | **Daniela Palma**[1] | **Michele Amitrano**[3] | **Raffaele Iorio**[2] | **Giuliana Fortunato**[1]

[1]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II and CEINGE S.C.a r.l. Advanced Biotechnology, Naples, Italy

[2]Department of Translational Medical Science, Section of Pediatrics, University of Naples Federico II, Naples, Italy

[3]Department of Advanced Biomedical Science, University of Naples Federico II, Naples, Italy

Correspondence
Giuliana Fortunato, Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II and CEINGE S.C.a r.l. Advanced Biotechnology, Naples, Italy.
Email: fortunat@unina.it

Abstract

Background: Caroli disease (CD, OMIM #600643) is a rare autosomal recessive disorder characterized by polycystic segmental dilatation of the intrahepatic bile ducts and extreme variability in age of onset and clinical manifestations. When congenital hepatic fibrosis is associated with the polycystic dilatation of the biliary tract, the condition is referred as Caroli syndrome. The disease is thought to be caused by pathogenic variants in the *PKHD1* gene (OMIM *606702).

Method: We report the clinical, biochemical, and molecular characterization of three patients with a clinical suspicion of CS belonging to two different families. The genetic screening was performed using a target custom panel and sequencing was performed on Illumina platform.

Results: Genetic analysis revealed the presence of rare variants in the *PKHD1* gene of the analyzed patients. In the first case, and his younger sister, two pathogenic variants (c.2702A>C and c.4870C>T) were found to be associated with a hepatic phenotype at clinical onset, followed by renal disease probably age-related; while in the second case, one pathogenic variant (c.5879C>G) and a complex allele with uncertain clinical significance [c.3407A>G; c.8345G>C; c.8606C>A] were found to be associated with a severe hepatic phenotype.

Conclusion: The identification of the genetic causes of the disease and their relationship with the clinical phenotype could have a favorable impact on clinical management and complication prevention.

KEYWORDS

Caroli disease, genetic screening, *PKHD1* gene, uncertain significance variants

1 | INTRODUCTION

Caroli disease (CD, OMIM #600643) is a rare autosomal recessive disorder, firstly reported in 1958 by Caroli et al. (1958), characterized by polycystic segmental dilatation of the intrahepatic bile ducts (Cabral Correia & Morgado, 2017; Issar & Issar, 2014). The age of onset and its clinical manifestations including recurrent cholangitis,

Carola Giacobbe and Fabiola Di Dato contributed equally to this work.

jaundice, and portal hypertension complications are extremely variable (Sgro et al., 2004). When the malformation of the biliary tract is associated with congenital hepatic fibrosis, the condition is called Caroli Syndrome (CS) (Gunay-Aygun, 2009; Umar et al., 2021). CD and CS are probably two different presentations of a continuum of pathology (Gunay-Aygun, 2009). Hepatic involvement can be isolated or associated with polycystic renal disease (Sgro et al., 2004). CS/CD may be included in the group of hepatic ciliopathies, a heterogeneous group of disorders due to cilia abnormalities comprising congenital hepatic fibrosis, Caroli syndrome, Caroli disease, and other fibrocystic liver diseases (Drenth et al., 2010; Gunay-Aygun, 2009; Umar et al., 2021). The exact incidence and prevalence of the disease are not known, but it is estimated to occur in approximately 1 out of 1,000,000 live births (Jarry et al., 2010).

It has been reported that rare pathogenic variants in the *PKHD1* gene (OMIM *606702), which encodes a large single-pass transmembrane protein fibrocystin/polyductin (Obeidova et al., 2015), are involved in the disease (Umar et al., 2021). Although the exact function of fibrocystin in the clinical phenotype remains unclear, it has been hypothesized that localization in bile duct primary cilia (Courcet et al., 2015) may play a significant role in maintaining tubular architecture (Obeidova et al., 2015), and genetic abnormalities in this protein result in fibrocystic changes in the kidney and liver (Umar et al., 2021).

To date 520 pathogenic variants in the *PKHD1* gene have been reported in the Human Gene Mutation Database HGMD [http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PKHD1], 4 of which are described as causative of CD, while in the PKHD1 database [http://www.humgen.rwth-aachen.de/] more than 700 variants are reported. Causative variants are identified throughout the *PKHD1* gene; extensive genetic heterogeneity renders genotype–phenotype correlation difficult; so the molecular diagnosis is further complicated by the large spectrum of phenotypes observed in affected patients (Yonem & Bayraktar, 2007).

Herein, we report the clinical, biochemical, and molecular characterization of three patients with a clinical suspicion of CS.

2 | MATERIALS AND METHODS

2.1 | Clinical presentation

2.1.1 | Case 1

We describe a 31-month-old boy with itching and enlarged liver and spleen. Abdominal ultrasound showed several cystic lesions with mild splenomegaly. Laboratory evaluation showed mild signs of cholestasis and iron deficiency anemia. Cholangio-Pancreato Magnetic Resonance (CPRM) confirmed the diagnosis of CS (Figure 1a). During follow-up, he had only one episode of cholangitis. At the last observation he was 9.7 years old and presented chronic liver disease complicated by portal hypertension with hypersplenism and esophageal varices and mild impairment of liver function; high blood pressure for age; stage 3 chronic renal failure; osteopenia. He was being treated with iron and fat-soluble vitamin supplements, diuretics, and ursodeoxycholic acid (UDCA).

His younger sister was 17-month-old when she had her first cholangitis. Her diagnosis of CS was based on: family history, liver enlargement with cystic images in the right hepatic lobe at ultrasound and CPRM (Figure 1b) and splenomegaly. At the last observation she was 5 years old

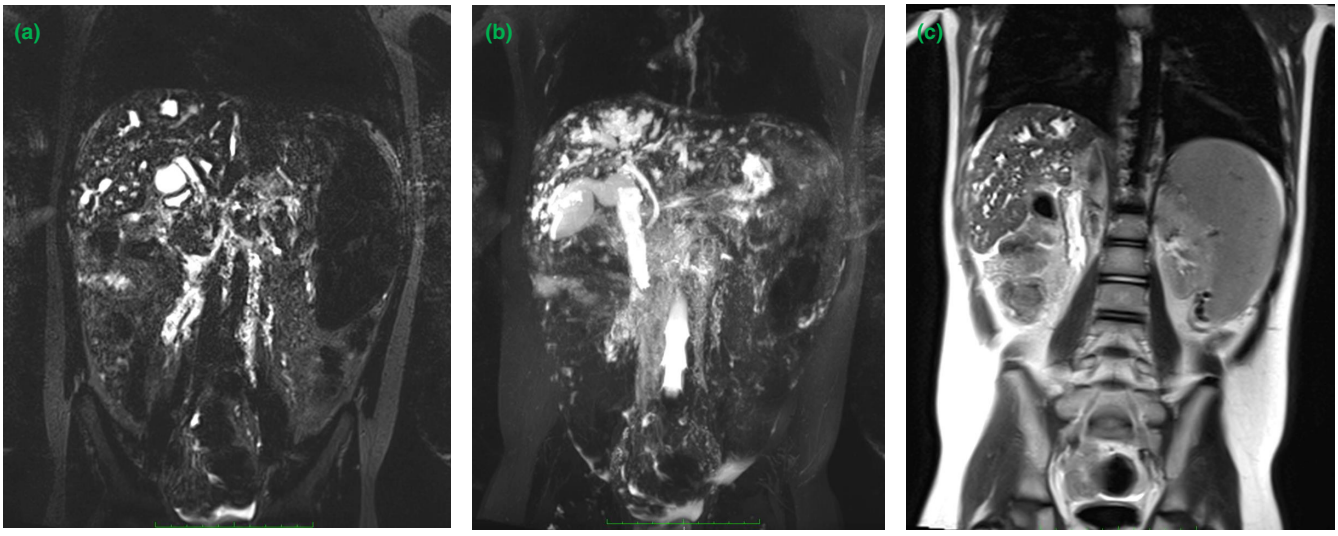

FIGURE 1 CPRM scan of three patients with CS. (a) Case 1, index case; (b) case 1, index cases's sister; (c) case 2

with liver enlargement but normal spleen diameter, normal liver, and kidney functions and osteopenia. She was under UDCA treatment and was taking fat-soluble vitamin supplementation.

The parents and a third brother did not show any clinical and laboratory signs of liver and kidney disease. Ultrasound examination did not reveal hepatic and renal cysts in any of them.

2.1.2 | Case 2

A 14-year-old girl was referred for CS. Her history was characterized by several findings of high liver enzyme values for which she underwent an abdominal ultrasound and CPRM (Figure 1c) at the age of 13 years. Imaging showed diffusely ectasis biliary tree complicated by portal hypertension with hypersplenism and F1 esophageal varices. At the age of 14 years, she had her first episode of cholangitis and 7 months later the girl underwent liver transplantation. Liver histology showed diffuse fibrosis with enlarged intrahepatic biliary ducts (mainly in the right lobe) and multiple multilobular cysts. Regarding her kidney function, after starting immunosuppressive treatment she showed moderate renal impairment, and 3 months after transplantation she was switched from tacrolimus to sirolimus with the improvement of renal function. At the last evaluation, the girl was 15.7-year-old and presented normal liver function and good renal function. The father and brother did not show any clinical and laboratory signs of hepatic and renal disease, while the mother presented with gallbladder stones associated with a slight increase in aminotransferases with normal indices of cholestasis.

2.2 | Genetic screening and data analysis

Informed consent for the study was obtained from at least one parent of each patient. Genomic DNA was extracted from peripheral blood by using ReliaPrep™ Blood gDNA Miniprep System, according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA). A custom panel of 94 liver disease-related genes, including *PKHD1* (NG_008753.1), was used to screen *Case 1* and *Case 2*. For each gene we included the coding regions, 25 bp in each of the intronic boundaries, the 5′UTR and the 3′UTR regions. Samples preparation was performed by the SureSelect QXT Target Enrichment kit for Illumina Sequencing (Agilent Technologies, Santa Clara, CA, USA). High-throughput sequencing was performed on the Illumina MiSeq platform (llumina Inc., SanDiego, CA, USA).

The obtained Fastq files were aligned against the hg38 genome assembly; variant alignment and calling were performed by Alissa Align and Call (Alissa v5.2.6—Agilent Technologies, Santa Clara, CA, USA) whereas variant filtering was performed by Alissa Interpret software (Alissa v5.2.6—Agilent, Santa Clara, CA, USA).

Agilent SureCall software (Agilent Technologies, Santa Clara, CA, USA) was used to search for copy number variations (CNVs).

Variants were firstly filtered on their minor allele frequency (MAF) and the pathogenicity of rare variants (with a MAF < 1%) was assessed according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015). All the prioritized variants were confirmed by Sanger sequencing.

3 | RESULTS

NGS analysis revealed that the number of reads in the target regions was about 70% with an average read depth of 160X for both patients.

Genetic analysis revealed the presence of rare variants in the *PKHD1* gene; the cascade screening was carried out in the relatives of the probands to verify variants segregation, showing that both index cases presented at least one rare variant in both alleles (Figure 2).

CNV analysis was negative in all three cases.

Table 1 shows the biochemical features of three CS patients, while the complete list of variants detected is shown in Table 2.

Case 1 was a compound heterozygous for two missense variants (c.2702A>C and c.4870C>T), both previously reported in literature and classified as likely pathogenic; after genetic cascade screening, the same genotype was observed in his younger sister, diagnosed with CS too.

Case 2 showed a complex genotype; in fact, she was compound heterozygous for a missense variant (c.5879C>G), inherited by her mother, and for a complex allele, consisting of three different missense variants [c.3407A>G; c.8345G]>C; c.8606C>A], two of them (c.3407A>G and c.8606C>A) were reported in the GnomAD database with a MAF of almost 1%.

4 | DISCUSSION

Since the very first description of CS, following the introduction of more advanced molecular biology techniques, several familial cases have been reported even before molecular defects at the level of the *PKHD1* gene had been identified (Hoglund et al., 1989; Teufel & Farack, 1987; Wu et al., 2002). Herein, we report the

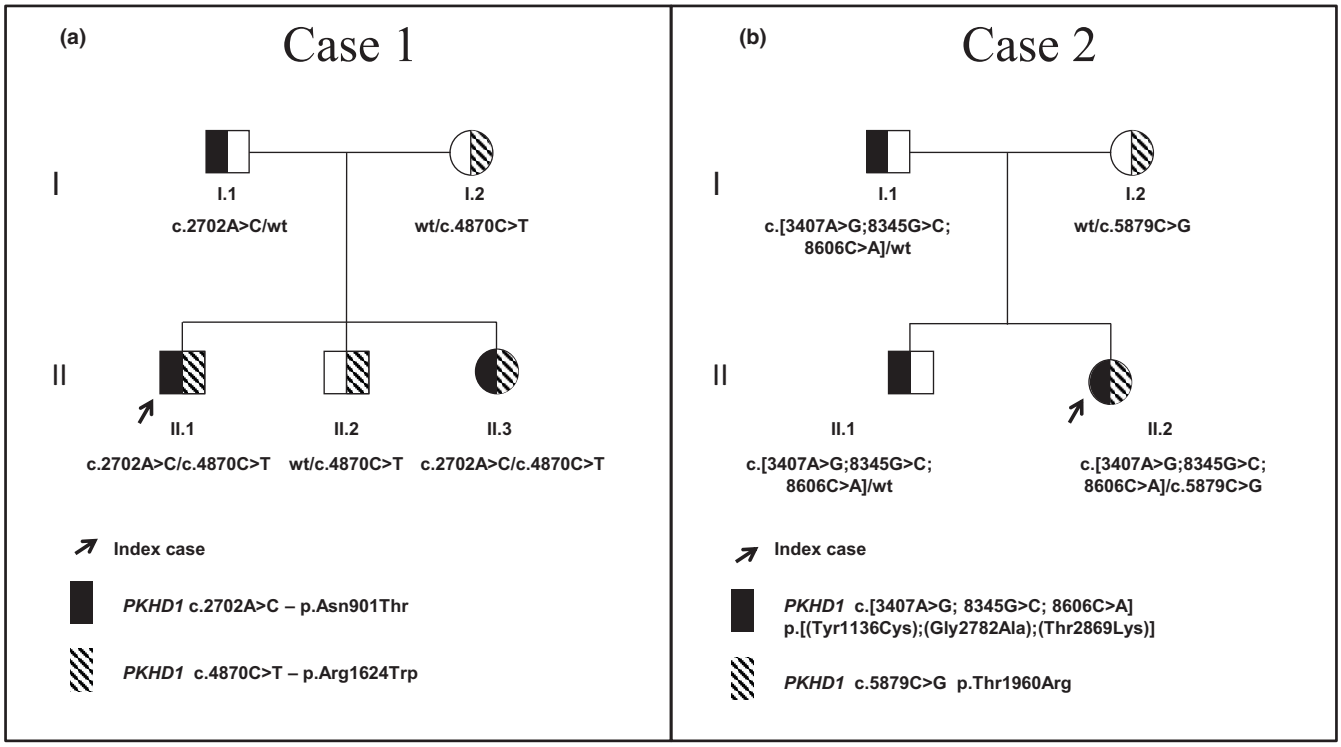

FIGURE 2 Pedigree of the case 1 family (a) pedigree of the case 2 family (b) with segregation analysis of the variants. Both index case were compound heterozygote for different *PKHD1* variants

TABLE 1 Biochemical features of 3 CD patients

	Case 1		Case 1's sister		Case 2	
	At entry	At last observation	At entry	At last observation	At entry	At last observation
Sex	M		F		F	
Age (years)	2.6	9.7	1.4	5	14.6	15.7
AST (UI/L)	62	68	60	57	35	34
ALT (UI/L)	22	27	21	24	29	40
GGT (UI/L)	50	35	27	23	94	55
TB (mg/dl)	0.4	1.95	0.31	0.64	0.73	1.55
DB (mg/dl)	NA	1.08	0.12	0.28	0.52	0.57
Albumin (g/dl)	4.6	3	4.4	4.5	3.2	4.8
Creatinine (mg/dl)	0.5	1.22	0.4	0.5	0.62	0.77
Urea (mg/dl)	46	67	43	36	20	44
Leukocytes	6600	8570	10,050	6390	11,970	4330
Hb (g/dl)	9.7	13.2	7.7	11.9	9.3	12.9
Platelets	209,000	81,000	451,000	130,000	229,000	102,000

Abbreviations: F, female; DB, direct bilirubin; M, male; TB, total bilirubin.

clinical, biochemical, and molecular characterization of three CS patients from two different families. In the first family, of Moroccan origin, two siblings were compound heterozygotes for two likely pathogenic variants (c.2702A>C and c.4870C>T) in the *PKHD1* gene associated with liver and kidney progressive disease, probably age-related. Both variants have been reported in the HGMD database as causative of polycystic kidney disease and not as causative of CS. The variant c.2702A>C has been previously reported in a large cohort of pediatric patients with autosomal recessive polycystic kidney disease (ARPKD), at compound heterozygous status (Melchionda et al., 2016) and more recently the same variant has been reported in literature as a compound

TABLE 2 PKHD1 rare variants identified in patients

Patient	HGVS (coding)[a]	HGVS (protein)[b]	Status	Inheritance	RefSeq ID	MAF (GnomAD)	Prediction SIFT (score)	HGMD	Reported phenotype HGMD	ARPKD	AGMG classification
Case1 and his sister	c.4870C>T	p.Arg1624Trp	het	M	rs200391019	T = 0.000151	Damaging (0.002)	CM020959-DM	Polycystic kidney disease	#320	Likely pathogenic
	c.2702A>C	p.Asn901Thr	het	F	rs764696718	C = 0.00002	Damaging (0.009)	CM194575-DM	Polycystic kidney disease	#225	Likely pathogenic
Case2	c.8345G>C	p.Gly2782Ala	het	F	rs147222255	C = 0.00223	Tolerated (0.367)	Not reported	na	#535	Uncertain Significance
	c.8606C>A	p.Thr2869Lys	het	F	rs142522748	A = 0.009274	Damaging (0.004)	CM32328-DM?	Polycystic kidney disease	#556	Benig/likely benign
	c.3407A>G	p.Tyr1136Cys	het	F	rs41273726	G = 0.007639	Tolerated (0.107)	CM051142-DM?	Polycystic kidney disease	#259	Uncertain Significance
	c.5879C>G	p.Thr1960Arg	het	M	rs534831346	G = 0.000004	Damaging (0)	Not reported	na	na	Likely pathogenic

Note: SIFT (sorts intolerant from tolerant) is an in silico prediction tool for nonsynonymous variants based on sequence homology derived from closely-related sequences collected through PSI-BLAST. Range 0–1 with values <0.05 usually considered intolerant. Forty percent of the values in this database are below 0.01 (dbNSFP version 4.2).

Abbreviations: ACMG classification, classification to American College of Medical Genetics and Genomics guidelines (ACMG; Richards et al., 2015); ARPKD, Mutation Database Autosomal Recessive Polycystic Kidney Disease (ARPKD/PKHD1); DM, disease mutation; F, father; Het, heterozygous; HGMD, Human Gene Mutation Database (HGMD Professional 2021.1); M, mother; MAF, minor allele frequency; na, not available.

[a]Transcript NM_138694.4.

[b]Protein NP_619639.3.

heterozygote with other missense variants, in an adult patient with chronic kidney disease (CKD) of unknown etiology, who also presented a congenital hepatic fibrosis (Connaughton et al., 2019). The second variant c.4870C>T, found in the family, has been already previously detected in patients affected by ARPKD with other clinical manifestations such as developmental and speech delay, hepatomegaly, gallbladder stones, dilated segmental intrahepatic biliary radicles (Alfares et al., 2017); as this variant has been observed with high frequency in individuals living in the Middle East region, it is supposed to have a possible founder effect (Al Alawi et al., 2020).

In the first family, our findings are consistent with literature data as to the pathogenicity of the variants examined. To date, this is the first case in which these variants were found in a patient with hepatic phenotype at clinical onset, followed by renal disease, probably age-related. This hypothesis is confirmed by the fact that the younger sister, in whom we observed the same genotype, did not manifest any renal phenotype.

In the second family, of Italian origin, the presence of a complex allele with uncertain significance [c.3407A>G; c.8345G>C; c.8606C>A] in the index case was associated with a probably pathogenic variant (c.5879C>G), leading to liver transplantation because of recurrent cholangitis. The variant c.5879C>G was classified as likely pathogenic according to the ACMG and was of matrilineal inheritance. It seems that to date, although this nucleotide can be found in the dbSNP database, it has never been reported in literature. However, another nucleotide change, causing the same amino acid change has been reported, at compound heterozygous state, in a 6-month-old German child affected by ARPKD (Bergmann et al., 2005). The pathogenicity of this variant is also evidenced by the presence of gallbladder stones and of transaminase slight increases in the mother.

The complex allele consists of three missense variants [c.3407A>G; c.8345G>C; c.8606C>A], found on the same paternal chromosome; the variant c.3407A>G was reported several times *in cis* with other rare variants; Maylikeev et al. described it in cis with c.8606C>A variant in a 37-year-old male suffering from CS with cystic dilatation of the intrahepatic biliary ducts, liver cirrhosis, and portal hyperthension (Mavlikeev et al., 2019); in this work, the authors considered the c.3407A>G variant as pathogenic and the c.8606C>A variant as benign. Gunay-Aygun et al reported the variant c.3407A>G *in cis* with a nonsense mutation and considered the variant as unlikely pathogenic, at least in the families examined (Gunay-Aygun et al., 2010).

The c.3407A>G variant has been reported in literature also in a compound heterozygous state, in patients

suffering from CD and considered as probably pathogenic (Eisenberger et al., 2015). The c.8606C>A variant has been described as benign variant in one study (Furu et al., 2003) and as a possible disease-causing variant in another (Rossetti et al., 2003).

The last variant of the complex allele was the c.8345G>C; this variant, at best of our knowledge, has been reported two times and considered as benign (Bergmann et al., 2005; Sharp et al., 2005).

Due to conflicting literature data, ithas not been possible to confer a clear pathogenic role to this complex allele but, given the severe phenotype observed in our patient, it may be inferred that at least one of these three variants, or maybe their combination, could affect protein function. This is in agreement with the presence, on the other allele, of a likely pathogenic variant in a disease with recessive transmission.

The cases described herein confirm that pathogenic variants in the *PKHD1* gene can be associated with a large spectrum of phenotypes from asymptomatic cases to patients with severe liver and/or kidney involvement (Serra et al., 2020; Wang et al., 2021). Szabo et al showed how biallelic loss-of-function mutations in a cohort of ARPKD patients were associated with fatal outcome in the perinatal period (Szabó et al., 2018). The genotype–phenotype correlation was recently emphasized by Burgmaier et al. who suggested the involvement of the *PKHD1* affected region in determining both phenotype and outcome (Burgmaier et al., 2021). These findings clearly demonstrate the importance of molecular characterization in clinically affected patients, also in order to carry out prenatal diagnosis and predict possible complications in future generations (Maruotti et al., 2013).

In conclusion, genetic characterization followed by cascade screening and genotype–phenotype correlation is particularly beneficial for the follow-up and clinical management of CS patients.

AUTHOR CONTRIBUTIONS

Carola Giacobbe and Giuliana Fortunato: Conceptualization; Fabiola Di Dato, Daniela Palma and Michele Amitrano: Methodology; Giuliana Fortunato e Raffaele Iorio: Validation; Carola Giacobbe and Fabiola Di Dato: Writing, original draft preparation; Giuliana Fortunato and Raffaele Iorio: Writing, review and editing.

ACKNOWLEDGMENT

None.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable.

ETHICAL COMPLIANCE

The study was performed according to the Declaration of Helsinki. Written informed consent was obtained from participants recruited in this study. All methods were performed in accordance with the relevant guidelines and regulations.

ORCID

Carola Giacobbe https://orcid.org/0000-0002-6422-3989
Giuliana Fortunato https://orcid.org/0000-0002-2209-383X

REFERENCES

Al Alawi, I., Molinari, E., Al Salmi, I., Al Rahbi, F., Al Mawali, A., & Sayer, J. A. (2020). Clinical and genetic characteristics of autosomal recessive polycystic kidney disease in Oman [research support, non-U.S. Gov't]. *BMC Nephrology*, *21*(1), 347. https://doi.org/10.1186/s12882-020-02013-2

Alfares, A., Alfadhel, M., Wani, T., Alsahli, S., Alluhaydan, I., Al Mutairi, F., Alothaim, A., Albalwi, M., al Subaie, L., Alturki, S., Al-Twaijri, W., Alrifai, M., Al-Rumayya, A., Alameer, S., Faqeeh, E., Alasmari, A., Alsamman, A., Tashkandia, S., Alghamdi, A., … Zada, A. A. P. (2017). A multicenter clinical exome study in unselected cohorts from a consanguineous population of Saudi Arabia demonstrated a high diagnostic yield [multicenter study]. *Molecular Genetics and Metabolism*, *121*(2), 91–95. https://doi.org/10.1016/j.ymgme.2017.04.002

Bergmann, C., Senderek, J., Windelen, E., Küpper, F., Middeldorf, I., Schneider, F., Dornia, C., Rudnik-Schöneborn, S., Konrad, M., Schmitt, C. P., Seeman, T., Neuhaus, T. J., Vester, U., Kirfel, J., Büttner, R., Zerres, K., & APN (Arbeitsgemeinschaft für Pädiatrische Nephrologie). (2005). Clinical consequences of PKHD1 mutations in 164 patients with autosomal-recessive polycystic kidney disease (ARPKD) [research support, non-U.S. Gov't]. *Kidney International*, *67*(3), 829–848. https://doi.org/10.1111/j.1523-1755.2005.00148.x

Burgmaier, K., Brinker, L., Erger, F., Beck, B. B., Benz, M. R., Bergmann, C., Boyer, O., Collard, L., Dafinger, C., Fila, M., Kowalewska, C., Lange-Sperandio, B., Massella, L., Mastrangelo, A., Mekahli, D., Miklaszewska, M., Ortiz-Bruechle, N., Patzer, L., Prikhodina, L., … ARegPKD Consortium. (2021). Refining genotype-phenotype correlations in 304 patients with autosomal recessive polycystic kidney disease and PKHD1 gene variants. *Kidney International*, *100*(3), 650–659. https://doi.org/10.1016/j.kint.2021.04.019

Cabral Correia, P., & Morgado, B. (2017). Caroli's disease as a cause of chronic epigastric abdominal pain: Two case reports and a brief review of the literature [Review]. *Cureus*, *9*(9), e1701. https://doi.org/10.7759/cureus.1701

Caroli, J., Soupault, R., Kossakowski, J., Plocker, L., & Paradowska, M. M. E. (1958). Congenital polycystic dilation of the intrahepatic bile ducts; attempt at classification. *La Semaine des Hôpitaux*, *34*(8/2), 488–495.

Connaughton, D. M., Kennedy, C., Shril, S., Mann, N., Murray, S. L., Williams, P. A., Conlon, E., Nakayama, M., van der Ven, A. T., Ityel, H., Kause, F., Kolvenbach, C. M., Dai, R., Vivante, A., Braun, D. A., Schneider, R., Kitzler, T. M., Moloney, B., Moran, C. P., ... Hildebrandt, F. (2019). Monogenic causes of chronic kidney disease in adults [evaluation study; multicenter study; research support, N.I.H., extramural; research support, non--U.S. Gov't]. *Kidney International*, *95*(4), 914–928. https://doi.org/10.1016/j.kint.2018.10.031

Courcet, J. B., Minello, A., Prieur, F., Morisse, L., Phelip, J. M., Beurdeley, A., Meynard, D., Massenet, D., Lacassin, F., Duffourd, Y., Gigot, N., St-Onge, J., Hillon, P., Vanlemmens, C., Mousson, C., Cerceuil, J. P., Guiu, B., Thevenon, J., Thauvin-Robinet, C., ... Faivre, L. (2015). Compound heterozygous PKHD1 variants cause a wide spectrum of ductal plate malformations. *American Journal of Medical Genetics: Part A*, *167A*(12), 3046–3053. https://doi.org/10.1002/ajmg.a.37352

Drenth, J. P. H., Chrispijn, M., & Bergmann, C. (2010). Congenital fibrocystic liver diseases. *Best Practice & Research Clinical Gastroenterology*, *24*(5), 573–584. https://doi.org/10.1016/j.bpg.2010.08.007

Eisenberger, T., Decker, C., Hiersche, M., Hamann, R. C., Decker, E., Neuber, S., Frank, V., Bolz, H. J., Fehrenbach, H., Pape, L., Toenshoff, B., Mache, C., Latta, K., & Bergmann, C. (2015). An efficient and comprehensive strategy for genetic diagnostics of polycystic kidney disease. *PLoS ONE*, *10*(2), e0116680. https://doi.org/10.1371/journal.pone.0116680

Furu, L., Onuchic, L. F., Gharavi, A., Hou, X., Esquivel, E. L., Nagasawa, Y., Bergmann, C., Senderek, J., Avner, E., Zerres, K., Germino, G. G., Guay-Woodford, L. M., & Somlo, S. (2003). Milder presentation of recessive polycystic kidney disease requires presence of amino acid substitution mutations [research support, U.S. Gov't, P.H.S.]. *Journal of the American Society of Nephrology*, *14*(8), 2004–2014. https://doi.org/10.1097/01.asn.0000078805.87038.05

Gunay-Aygun, M. (2009). Liver and kidney disease in ciliopathies. *American Journal of Medical Genetics Part C-Seminars in Medical Genetics*, *151C*(4), 296–306. https://doi.org/10.1002/ajmg.c.30225

Gunay-Aygun, M., Tuchman, M., Font-Montgomery, E., Lukose, L., Edwards, H., Garcia, A., Ausavarat, S., Ziegler, S. G., Piwnica-Worms, K., Bryant, J., Bernardini, I., Fischer, R., Huizing, M., Guay-Woodford, L., & Gahl, W. A. (2010). PKHD1 sequence variations in 78 children and adults with autosomal recessive polycystic kidney disease and congenital hepatic fibrosis [research support, N.I.H., extramural; research support, N.I.H., intramural]. *Molecular Genetics and Metabolism*, *99*(2), 160–173. https://doi.org/10.1016/j.ymgme.2009.10.010

Hoglund, M., Muren, C., & Schmidt, D. (1989). Carolis-disease in 2 sisters—diagnosis by ultrasonography and computed-tomography. *Acta Radiologica*, *30*(5), 459–462. https://doi.org/10.1177/028418518903000502

Issar, P., & Issar, S. K. (2014). Caroli's disease. *Indian Journal of Gastroenterology*, *33*(5), 500. https://doi.org/10.1007/s12664-013-0403-5

Jarry, J., Leblanc, F., & Saric, J. (2010). Monolobar Caroli disease. *Presse Médicale*, *39*(7–8), 847–848. https://doi.org/10.1016/j.lpm.2009.10.018

Maruotti, G. M., Sarno, L., Simioli, S., Castaldo, G., & Martinelli, P. (2013). Prenatal screening and counseling for genetic disorders. *The Journal of Maternal-Fetal & Neonatal Medicine*, *26*(Suppl. 2), 68–71. https://doi.org/10.3109/14767058.2013.829701

Mavlikeev, M., Titova, A., Saitburkhanova, R., Abyzova, M., Sayfutdinov, I., Gizzatullina, N., Kotov, I., Plaksa, I., Isaev, A., Abdulkhakov, S., Kiyasov, A., & Deev, R. (2019). Caroli syndrome: A clinical case with detailed histopathological analysis [case reports]. *Journal of Gastroenterology*, *12*(2), 106–111. https://doi.org/10.1007/s12328-018-0917-6

Melchionda, S., Palladino, T., Castellana, S., Giordano, M., Benetti, E., de Bonis, P., Zelante, L., & Bisceglia, L. (2016). Expanding the mutation spectrum in 130 probands with ARPKD: Identification of 62 novel PKHD1 mutations by sanger sequencing and MLPA analysis. *Journal of Human Genetics*, *61*(9), 811–821. https://doi.org/10.1038/jhg.2016.58

Obeidova, L., Seeman, T., Elisakova, V., Reiterova, J., Puchmajerova, A., & Stekrova, J. (2015). Molecular genetic analysis of PKHD1 by next-generation sequencing in Czech families with autosomal recessive polycystic kidney disease [research support, non-U.S. Gov't]. *BMC Medical Genetics*, *16*, 116. https://doi.org/10.1186/s12881-015-0261-3

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [consensus development conference guideline; research support, N.I.H., extramural]. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Rossetti, S., Torra, R., Coto, E., Consugar, M., Kubly, V., Málaga, S., Navarro, M., el-Youssef, M., Torres, V. E., & Harris, P. C. (2003). A complete mutation screen of PKHD1 in autosomal-recessive polycystic kidney disease (ARPKD) pedigrees [research support, non-U.S. Gov't; research support, U.S. Gov't, P.H.S.]. *Kidney International*, *64*(2), 391–403. https://doi.org/10.1046/j.1523-1755.2003.00111.x

Serra, G., Corsello, G., Antona, V., D'Alessandro, M. M., Cassata, N., Cimador, M., Giuffrè, M., Schierz, I. A. M., & Piro, E. (2020). Autosomal recessive polycystic kidney disease: Case report of a newborn with rare PKHD1 mutation, rapid renal enlargement and early fatal outcome [case reports]. *Italian Journal of Pediatrics*, *46*(1), 154. https://doi.org/10.1186/s13052-020-00922-4

Sgro, M., Rossetti, S., Barozzino, T., Toi, A., Langer, J., Harris, P. C., Harvey, E., & Chitayat, D. (2004). Caroli's disease: Prenatal diagnosis, postnatal outcome and genetic analysis. *Ultrasound in Obstetrics & Gynecology*, *23*(1), 73–76. https://doi.org/10.1002/uog.943

Sharp, A. M., Messiaen, L. M., Page, G., Antignac, C., Gubler, M. C., Onuchic, L. F., Somlo, S., Germino, G. G., & Guay-Woodford, L. M. (2005). Comprehensive genomic analysis of PKHD1 mutations in ARPKD cohorts [case reports; letter; research support, non-U.S. Gov't; research support, U.S. Gov't, P.H.S.]. *Journal of Medical Genetics*, *42*(4), 336–349. https://doi.org/10.1136/jmg.2004.024489

Szabó, T., Orosz, P., Balogh, E., Jávorszky, E., Máttyus, I., Bereczki, C., Maróti, Z., Kalmár, T., Szabó, A. J., Reusz, G., Várkonyi, I., Marián, E., Gombos, É., Orosz, O., Madar, L., Balla, G., Kappelmayer, J., Tory, K., & Balogh, I. (2018). Comprehensive

genetic testing in children with a clinical diagnosis of ARPKD identifies phenocopies. *Pediatric Nephrology*, *33*(10), 1713–1721. https://doi.org/10.1007/s00467-018-3992-5

Teufel, J., & Farack, U. M. (1987). Hepatobiliary fibropolycystic diseases—2 cases of Carolis-disease. *Scandinavian Journal of Gastroenterology*, *22*, 76–80. https://doi.org/10.3109/00365528709089778

Umar, J., Kudaravalli, P., & John, S. (2021). *Caroli disease*. StatPearls.

Wang, J., Yang, H., Guo, R., Sang, X., & Mao, Y. (2021). Association of a novel PKHD1 mutation in a family with autosomal dominant polycystic liver disease. *Annals of Translational Medicine*, *9*(2), 120. https://doi.org/10.21037/atm-20-3318

Wu, K. L., Changchien, C. S., Kuo, C. M., Chuah, S. K., Chiu, Y. C., & Kuo, C. H. (2002). Caroli's disease—A report of two siblings. *European Journal of Gastroenterology & Hepatology*, *14*(12), 1397–1399. https://doi.org/10.1097/00042737-200212000-00019

Yonem, O., & Bayraktar, Y. (2007). Clinical characteristics of Caroli's disease [review]. *World Journal of Gastroenterology*, *13*(13), 1930–1933. https://doi.org/10.3748/wjg.v13.i13.1930

How to cite this article: Giacobbe, C., Di Dato, F., Palma, D., Amitrano, M., Iorio, R., & Fortunato, G. (2022). Rare variants in *PKHD1* associated with Caroli syndrome: Two case reports. *Molecular Genetics & Genomic Medicine*, *10*, e1998. https://doi.org/10.1002/mgg3.1998