Genes & Diseases (2024) 11, 72–75

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

RAPID COMMUNICATION

A *BBS4* mutation causes autosomal dominant polycystic liver disease

Autosomal dominant polycystic liver disease (ADPLD) refers to a condition characterized by the presence of numerous cholangiocytes-lined and fluid-filled cysts in the liver and the absence of polycystic kidney disease.[1] Although patients with ADPLD may be asymptomatic, some patients suffer from abdominal pain, gastroesophageal reflux, and nausea, because of hepatomegaly. These symptoms compromise the patient's quality of life. In severe cases, complications such as intracystic hemorrhage, infection, and rupture may occur. This disease exhibits high genetic heterogeneity.[1] Six genes, including *PRKCSH*, *SEC63*, *ALG8*, *SEC61B*, *GANAB* and *LRP5*, have been identified to have pathogenic variants causing ADPLD.[1] However, about 55%–70% of cases cannot be explained by the known loci.[1] Hepatic cysts in ADPLD are derived from cholangiocytes.[2] The development and progressive expansion of cysts are associated with a process called ductal plate malformation (DPM), which manifests as hyperproliferation and perturbed polarization of cholangiocytes as well as the abnormal structure and function of primary cilia in cholangiocytes.[2] Most of the proteins encoded by genes associated with ADPLD are localized in the endoplasmic reticulum.[1] So far, the role of ciliary-associated genes' mutations in the pathogenesis of ADPLD has not been reported. Bardet-Biedl syndrome 4 (*BBS4*) is associated with the formation of the primary cilium and its mutation causes Bardet-Biedl syndrome, a rare autosomal recessive disorder.[3] However, the role of BBS4 in ADPLD has not yet been reported.

To identify genetic factors underlying ADPLD, 5 Chinese multiplex ADPLD-affected families were screened by whole-exome sequencing (Fig. 1A; Fig. S1), and the subsequent Sanger sequencing was performed to validate the mutated genes segregated with the disease. A novel *SEC63* mutation (c. 733+1G > A) (Fig. 1A; Fig. S2) was identified in one family (family 1), while no mutations of the other known pathogenic genes were observed in these five families. In family 1 with six ADPLD patients, both of the proband and her husband were diagnosed with ADPLD. The *SEC63* mutation was identified only in three patients, including the husband of the proband. When looking at mutations of cilium-associated genes for the other three patients without the *SEC63* mutation, including the proband, a heterozygous 2-bp deletion (c. 1548_1549delAA) in *BBS4* gene was identified, which segregated with the disease (Fig. S2). The variant in *BBS4* resulted in an in-frame change of amino acids in the C-terminal (p. 516fsX7), a highly conserved region among mammals (Fig. S2). Furthermore, careful clinical examination ruled out the diagnosis of Bardet-Biedl syndrome in this pedigree. Interestingly, the proband's grandson carried both the *SEC63* mutation and the *BBS4* mutation and was diagnosed with ADPLD at 20 years old, which is much younger than his father's age of onset. To further examine the role of *BBS4* in the pathogenesis of ADPLD, we constructed a liver-specific *BBS4* knockout mice strain ($BBS4^{LKO}$) by crossing Alb-Cre mice with $BBS4^{f/f}$ mice (Fig. S3). Both gross observation and micro-CT scanning showed that the $BBS4^{LKO}$ mice spontaneously developed hepatic cysts at the age of 10 months, while no cysts were observed in $BBS4^{f/f}$ mice up to 18 months old (Fig. 1B; Fig. S3). The number and size of cysts increase with the age in $BBS4^{LKO}$ mice (Fig. S3). Pathologically, hematoxylin and eosin staining and immunohistochemistry staining for mature ductal cell marker CK19 revealed progressive abnormal bile duct dilation in $BBS4^{LKO}$ mice (Fig. S3).

The abnormal remodeling of the ductal plate during the morphogenesis of the bile duct leads to hepatic cystogenesis.[2] Towards the periphery of the liver, at E18.5 and P1, $BBS4^{LKO}$ mice showed decreased asymmetric primitive ductular structures, detected by immunostaining for the hepatocyte marker (HNF4α) on the parenchymal side and the biliary-specific marker (SOX9) on the portal side, compared with $BBS4^{f/f}$ mice (Fig. S4). These findings suggest that the absence of *BBS4* leads to deficient maturation of primitive ductular structures and impairs the process of morphogenesis of the bile duct. Bile duct profile could be classified as well-formed (1 layer of biliary cells, a round

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.02.042

2352-3042/© 2023 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

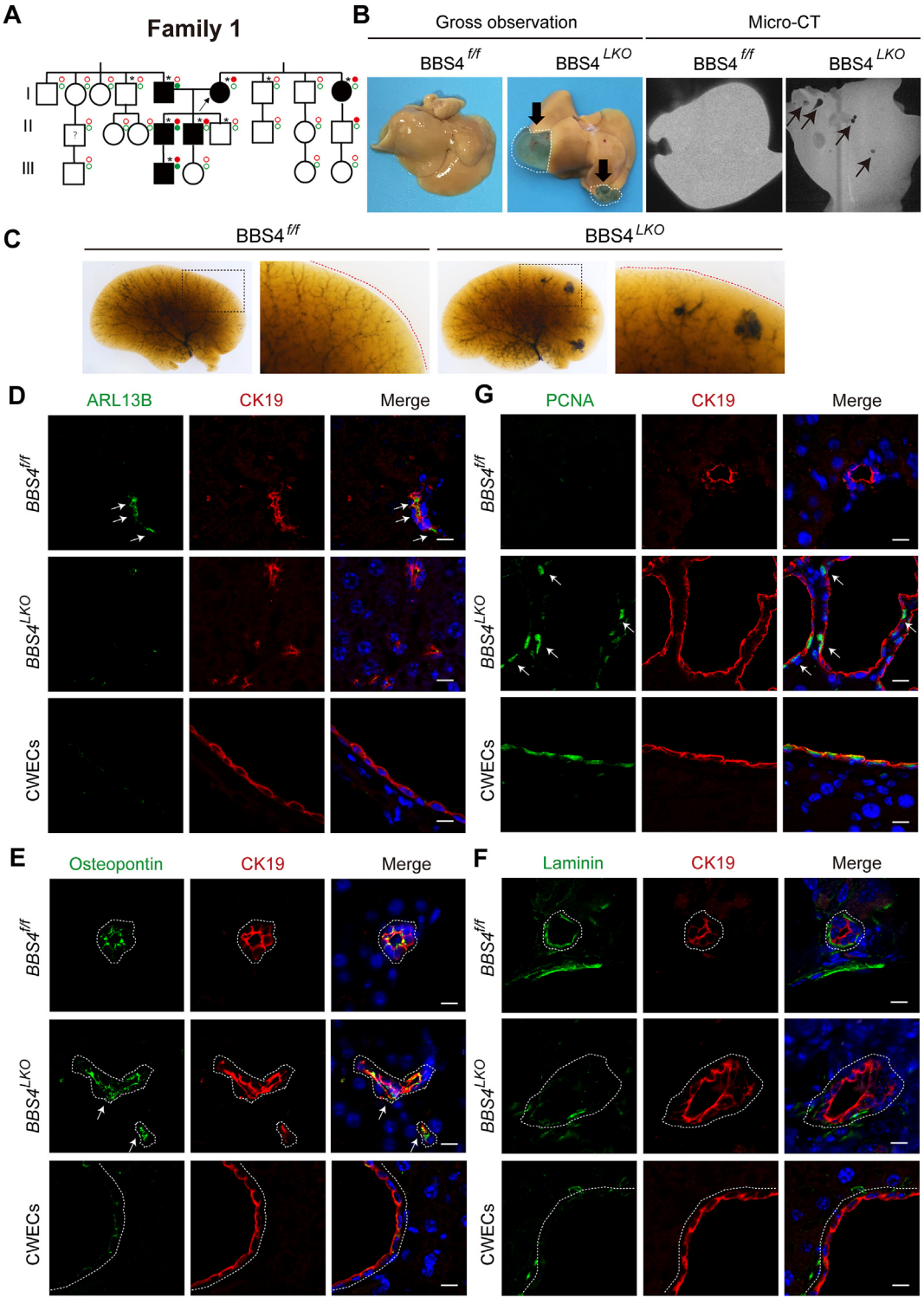

Figure 1 Clinical data and functional research of this study. **(A)** Pedigree of family 1. The asterisks indicated individuals for which whole-exome sequencing was performed. Red hollow circles mark individuals with wide-type *BBS4*, while solid red circles indicate individuals carrying the *BBS4* mutation. Green hollow circles mark individuals with wide type *SEC63*, and solid green circles indicate individuals carrying the *SEC63* mutation. **(B)** Representative images of gross observation and high-resolution X-ray micro-CT scanning for the external and internal structure of the livers of 18-month BBS4$^{f/f}$ mice and BBS4^{LKO} mice. **(C)** Biliary tree visualized by retrograde ink injection into the common bile duct of BBS4$^{f/f}$ mice and BBS4^{LKO} mice. The left lobes are shown. Red lines mark the liver edge. **(D)** Immunofluorescence staining of the primary cilium marker ARL13B identified a reduction in the primary cilia of the cholangiocytes in 18-month BBS4^{LKO} mice. Arrows indicate the cholangiocytes with primary cilia. CWECs, cystic wall endothelial cells. **(E)** Immunofluorescent staining of PCNA and CK19 in the liver tissues of 18-month BBS4$^{f/f}$ mice and 18-month BBS4^{LKO} mice. **(F)** Immunofluorescence showed diffusely distributed osteopontin (OPN) in the cytoplasm of cholangiocytes in 18-month BBS4^{LKO} mice. Arrows indicate the cholangiocytes with disturbed OPN. **(G)** Immunofluorescence indicated down-regulation of laminin in 18-month BBS4^{LKO} mice. Arrows indicate cholangiocytes with down-regulated laminin. Scale bars = 10 μm.

lumen), functional (1 or more layers of biliary cells, a discernible lumen), or clustered (clusters of biliary cells, no discernible lumen). Adult *BBS4*^{LKO} mice counted more bile ducts of functional profile and clusters of biliary cells, and less well-formed ducts (Fig. S4), indicating the morphogenesis of the bile duct in *BBS4*^{LKO} mice was disrupted. In addition, retrograde ink injection into the common bile duct showed that the biliary tree and cholestasis are impaired in *BBS4*^{LKO} mice (Fig. 1C).

The BBS4 protein locates in the centriolar satellites of centrosomes and the basal bodies of primary cilia.[3] Therefore, we sought to investigate the effect of *BBS4* depletion on the formation of primary cilia in cholangiocytes. As expected, two important markers of primary cilia Ac-tubulin- and ARL13B-positive cholangiocytes significantly decreased in *BBS4*^{LKO} mice compared with *BBS4*$^{f/f}$ mice (Fig. 1D; Fig. S5). In particular, the primary cilium was absent in the cystic wall endothelial cells of *BBS4*^{LKO} mice (Fig. S5). The abnormal polarity of cholangiocytes represents an important hallmark of DPM.[4] The polarity of cholangiocytes in *BBS4*^{LKO} mice was evaluated by immunofluorescent staining of osteopontin (OPN, apical marker) and laminin (basal pole marker). OPN in some cholangiocytes was not expressed at the apical pole of CK19-positive cells and was instead diffusely distributed in the cytoplasm of those cells in *BBS4*^{LKO} mice (Fig. 1E). In addition, laminin was absent in some CK19-positive cells in *BBS4*^{LKO} mice (Fig. 1F). In a healthy individual, there is no proliferation of normal cholangiocytes, while cystic cholangiocytes show an increase in proliferation.[2] We detected the expression of proliferation markers PCNA and Ki67 by immunofluorescent staining. Enhanced expression of PCNA and Ki67 was observed in the CK19-positive cells of *BBS4*^{LKO} mice including the cystic wall endothelial cells, indicating that loss of *BBS4* activated the proliferation of cholangiocytes (Fig. 1G; Fig. S6).

So far, nearly all the pathogenic variants of ADPLD were reported in European and American populations. The studies on the Chinese population are still at preliminary status. Recently, Wang et al[5] found that the frequencies of *PRKCSH* and *SEC63* mutations were lower in the Chinese population than in European and American populations, indicating that the genetic profile of ADPLD in the Chinese population may be different from that in European and American populations. Here, we also showed that only one of the five families that we studied had patients carrying a *SEC63* mutation, and none of the known variants were observed in the other families. ADPLD has already been identified as a disease with high heterogeneity, in which the six genes known to be involved in the pathogenesis only explain approximately 35% of the cases. In the present study, the five families enrolled did not share any variant, further confirming the complex genetic background of ADPLD.

The ADPLD phenotype has not been observed in the previous *BBS4* knock-out mouse studies and patients with Bardet-Biedl syndrome. Only one study reports that the livers of *BBS4* knock-out mice are enlarged, most likely due to the increase in fat deposits.[3] In the present study, we performed a thorough evaluation of the abnormality of cholangiocytes and bile ducts in the *BBS4* knock-out mice. The anomalies of cholangiocytes included abnormal bile duct morphogenesis, a decrease of primary cilia formation, disturbance of the polarity, and hyperproliferation of cholangiocytes. These results provide new evidence of the involvement of *BBS4* in DPM, to some extent clarifying the cellular mechanism of the *BBS4* depletion in the development of ADPLD.

In conclusion, we were able for the first time to connect the pathogenic variant of *BBS4* to the development of ADPLD. Evidence from both a family with *BBS4* deficiency and a mouse model demonstrates that a lack of *BBS4* leads to the development of ADPLD. The analysis of the mouse mutants shows the phenotypes and specific manifestations of DPM. Our findings expand the spectrum of genes whose variants are known to be possible causes of ADPLD and describe novel genetic characteristics of the previously rarely studied Chinese ADPLD population.

Ethics declaration

The institutional ethical committees of Nantong University and Naval Medical University have approved permission to perform this study. Signed informed consent was obtained from each participant of this study for obtaining blood samples and publication of the identifying materials in the journal. All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Scientific Investigation Board of Naval Medical University.

Author contributions

Y.L.C. and W.P.X. designed and performed the experiments, wrote the manuscript, and analyzed data. J.P.L., S.Q.L., W.H., and S.Y.H. assisted with performing experiments and analyzing data. X.Z., C.H.L., and W.F.X. coordinated the project, revised the manuscript, and assisted with designing the experiments and analyzing data.

Conflict of interests

All authors declare that they have no conflict of interests.

Funding

This work was supported by the Medical Discipine Construction Project of Pudong Health Committee of Shanghai (No. PWYgf2021-08 to S.Y.H, the National Natural Science Foundation of China (No. 82030021 to W.F.X., 82070624 to C.H.L., and 82000581 to J.P.L.) and the Deep Blue Talent Project of Naval Medical University to W.P.X.

Acknowledgements

We would like to thank the families enrolled in the study for their participation and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.042.

References

1. Lee-Law PY, van de Laarschot LFM, Banales JM, et al. Genetics of polycystic liver diseases. *Curr Opin Gastroenterol*. 2019; 35(2):65—72.
2. Wills ES, Roepman R, Drenth JPH. Polycystic liver disease: ductal plate malformation and the primary cilium. *Trends Mol Med*. 2014;20(5):261—270.
3. Swiderski RE, Nishimura DY, Mullins RF, et al. Gene expression analysis of photoreceptor cell loss in bbs4-knockout mice reveals an early stress gene response and photoreceptor cell damage. *Invest Ophthalmol Vis Sci*. 2007;48(7):3329—3340.
4. Xu WP, Cui YL, Chen LL, et al. Deletion of Sox 9 in the liver leads to hepatic cystogenesis in mice by transcriptionally down-regulating Sec63. *J Pathol*. 2021;254(1):57—69.
5. Wang J, Yang H, Guo R, et al. Association of a novel *PKHD1* mutation in a family with autosomal dominant polycystic liver disease. *Ann Transl Med*. 2021;9(2):120.

Yalu Cui [a,1], Wenping Xu [a,1], Jinpei Liu [b,1], Shuqing Liu [a], Wei Huang [c], Yihai Shi [b,1], Xin Zhang [a], Cuihua Lu [c,*], Weifen Xie [a,**]

[a] *Department of Gastroenterology, Changzheng Hospital, Naval Medical University, Shanghai 200003, China*
[b] *Department of Gastroenterology, Gongli Hospital of Shanghai Pudong New Area, Shanghai 200135, China*
[c] *Department of Gastroenterology, Affiliated Hospital of Nantong University, Nantong University, Nantong, Jiangsu 226001, China*

*Corresponding author. Department of Gastroenterology, Affiliated Hospital of Nantong University, Nantong University, 20 Xisi Road, Nantong, Jiangsu 226001, China.

**Corresponding author. Department of Gastroenterology, Changzheng Hospital, Naval Medical University, 415 Fengyang Road, Shanghai 200003, China.
E-mail addresses: lch670608@sina.com (C. Lu), weifenxie@medmail.com.cn (W. Xie)

10 November 2022
Available online 5 April 2023

[1] These authors contributed equally to this work.