Proposal of a liver histology-based scoring system for bile salt export pump deficiency

Yoh Zen[1,*], Hiroki Kondou[2], Atsuko Nakazawa[3], Ken Tanikawa[4], Yasuhiro Hasegawa[5], Kazuhiko Bessho[5], Kazuo Imagawa[6], Takashi Ishige[7], Ayano Inui[8], Mitsuyoshi Suzuki[9], Mureo Kasahara[10], Kouji Yamamoto[11], Takako Yoshioka[12], Masayoshi Kage[13], Hisamitsu Hayashi[14,*]

[1] Department of Diagnostic Pathology, Kobe University, Hyogo, Japan

[2] Department of Pediatrics, Kindai University Nara Hospital, Nara, Japan

[3] Department of Clinical Research, Saitama Children's Medical Center, Saitama, Japan

[4] Department of Diagnostic Pathology, Kurume University Hospital, Fukuoka, Japan

[5] Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan

[6] Department of Pediatrics, University of Tsukuba Hospital, Ibaraki, Japan

[7] Department of Pediatrics, Gunma University Graduate School of Medicine, Gunma, Japan

[8] Department of Pediatric Hepatology and Gastroenterology, Saiseikai Yokohama City Tobu Hospital, Kanagawa, Japan

[9] Department of Pediatrics, Juntendo University Graduate School of Medicine, Tokyo, Japan

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/hepr.13494

[10] Organ Transplantation Center, National Center for Child Health and Development, Tokyo, Japan

[11] Department of Biostatistics, School of Medicine, Yokohama City University, Yokohama, Japan

[12] Department of Pathology, National Center for Child Health and Development, Tokyo, Japan.

[13] Kurume University Research Center for Innovative Cancer Therapy, Fukuoka, Japan

[14] Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Science, The University of Tokyo, Tokyo, Japan

Contact information:

Yoh Zen M.D., Ph.D., FRCPath. Institute of Liver Studies, King's College Hospital & King's College London, Denmark Hill, London SE5 9RS, UK. Tel: (+44) 20 3299 4627, Fax: (+44) 20 3299 3125, E-mail: yoh.1.zen@kcl.ac.uk

Hisamitsu Hayashi, Ph.D., Laboratory of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel: (+81) 3 5841 4771, Fax: (+81) 3 5841 4766, E-mail: hayapi@mol.f.u-tokyo.ac.jp

Running title: Histology-based scoring for BSEP deficiency

Conflict of interest: Dr. Hiroki Kondou has received NaPB as a gift from OrphanPacific, Inc. for an ongoing clinical trial (UMIN000024753). The other authors declare no conflict of interest.

Financial support: This work was supported by grants JP17ak0101036 and JP19ek0109298 from Japan Agency for Medical Research and Development to H.H.

Abbreviations: BSEP, bile salt export pump; GGT, γ-glutamyltransferase; MDR3, multidrug resistance protein 3; NaPB, sodium 4-phenylbutyrate; PFIC, progressive familial intrahepatic cholestasis.

ABSTRACT

Aim: Bile salt export pump (BSEP) deficiency manifests a form of progressive intrahepatic cholestasis. This study aimed to establish a scoring system of liver histology for the uncommon genetic condition.

Methods: After a roundtable discussion and a histology review, a scoring system for BSEP deficiency was established. Eleven tissue samples were independently evaluated by three pathologists based on the proposed standard for an interobserver agreement analysis. In four cases with serial tissue samples available, correlation between changes in histology scores and clinical outcome was examined.

Results: Of 14 initially listed histopathological findings, 12 were selected for scoring and grouped into the following four categories: cholestasis, parenchymal changes, portal tract

changes, and fibrosis. Each category consisted of two to four microscopic findings that were further divided into three to six scores; therefore, each category had a maximum score of 8 to 11. Interobserver agreement was highest for pericellular fibrosis (κ value: 0.849) and lowest for hepatocellular cholestasis (κ value: 0.241) with the mean and median κ values of the 12 parameters being 0.561 and 0.602, respectively. For two patients whose clinical features worsened, score changes between two time points were interpreted as deteriorated. In two patients, who showed a good clinical response to preprandial treatment with sodium 4-phenylbutyrate, histological changes were evaluated as improved or unchanged.

Conclusions: The proposed histology-based scoring system for BSEP deficiency with moderate interobserver agreement may be useful not only for monitoring microscopic changes in the clinical practice but also for a surrogate endpoint in clinical trials.

Keywords: BSEP, intrahepatic cholestasis, PFIC, liver biopsy, NaPB, clinical trial

Introduction

Progressive familial intrahepatic cholestasis (PFIC) is caused by molecular alterations of genes that encode canalicular transporters such as familial intrahepatic cholestasis protein 1, bile salt export pump (BSEP), multidrug resistance protein 3 (MDR3), or tight junction protein 2.[1-4] MDR3 deficiency presents with jaundice and an elevation in serum γ-glutamyltransferase (GGT) levels, while the remaining three forms are characterized by cholestasis with normal levels of serum GGT.[5] Recently, children with homozygous mutations in *NR1H4*, which encodes the farnesoid X receptor, a bile acid-activated nuclear hormone receptor that regulates bile acid metabolism, were also found to present with low-GGT neonatal cholestasis.[6]

ABCB11 encodes BSEP, an ATP-binding cassette transporter responsible for the secretion of bile salts from hepatocytes to the bile canaliculi. Biallelic mutations of *ABCB11* lead to diverse clinical manifestations. The most severe form is PFIC, which eventually leads to cirrhosis. Children with that progressive disease present with the neonatal hepatitis syndrome in infancy. A liver biopsy typically shows extensive giant cell transformation of hepatocytes, lobular cholestasis, lobular inflammation, and the loss of BSEP expression using immunostaining.[7] A milder form is characterized by recurrent episodes of low-GGT cholestasis and minimal liver fibrosis. This form is called benign recurrent intrahepatic cholestasis. The severity of the clinical manifestations is determined by the residual activity of BSEP. Patients' mothers may also have a history of cholestasis during pregnancy, which is

another associated manifestation of the hereditary liver disease. BSEP deficiency is known to increase the risk of hepatocellular carcinoma, particularly in the cirrhotic state.[8, 9]

Currently, a liver transplantation is the only cure for BSEP deficiency. We have published experimental evidence that sodium 4-phenylbutyrate (NaPB), a drug approved for treating urea cycle disorders, has a newly identified pharmacological effect that increases the expression and function of BSEP.[10] Pre-trial observational studies using NaPB also suggested that this drug partially restores the expression of BSEP on the canalicular membrane of hepatocytes, and therefore, improves the clinical abnormalities of patients with BSEP deficiency.[11-14] These promising results may warrant future clinical trials. However, the evaluation of the treatment efficacy by solid clinical endpoints such as death or liver transplantation is hindered by the chronic, diverse disease manifestations and the low prevalence of this condition. Therefore, surrogate endpoints including biochemical biomarkers and liver histology need to be considered. A beneficial aspect of a liver biopsy is that the histological changes directly measure the degree of disease severity; however, it is a subjective evaluation with inconsistent interobserver reproducibility. To overcome that limitation, several studies tested the reproducibility and prognostic values of histology-based scoring systems for chronic liver diseases such as primary sclerosing cholangitis and non-alcoholic steatohepatitis, and suggested a potential usefulness of a liver biopsy scoring system for the evaluation of treatment efficacy.[15-18]

The present study aimed to establish a scoring system for liver histology of patients with BSEP deficiency, that reflects disease progression and is potentially useful for future clinical trials.

Methods

This study was approved by the institutional review boards and performed in accordance with the amended Declaration of Helsinki. We obtained informed consent in written form from patients' parents or provided opportunities for refusal to participate in this study by opt-out. The participating medical institutions disclosed the opt-out document approved by the ethics committee.

Outline of the study

Four pathologists and eight pediatric hepatologists met twice in 2016 in order to discuss the liver biopsy findings of patients with BSEP deficiency. Histology slides of liver biopsies and explants obtained from nine patients with BSEP deficiency were prepared. For each case, hematoxylin and eosin staining and reticulin or Masson's trichrome staining were available. After a roundtable discussion and a histology review using a multihead microscope, a histology scoring system was established and evaluated through the following four-step process.

Step 1. Listing microscopic findings potentially useful for the scoring system

Step 2. Establishing the scoring system using shortlisted parameters

Step 3. Conducting an interobserver agreement study of the proposed system

Step 4. Correlating the histology scores and the clinical outcomes

Interobserver agreement analysis

Eleven tissue samples obtained from seven patients with BSEP deficiency were independently scored by three pathologists. Eight samples were liver biopsies, while the remaining three were liver explants. The diagnosis of BSEP deficiency in those patients was established by low-GGT cholestasis, histology findings, and genetic analysis. Immunohistochemical signal of BSEP was below the detection limit in five patients and was not tested in the other patients, who carried the reported PFIC2-type mutations in both alleles. Two patients were males and five were females. Tissue samples were obtained at the age of 3 to 34 months (median: 22 months). Pathologists were blinded to any clinical information including patient's name, age, symptoms, treatment history, and serological data. A CD-ROM containing virtual slides of the hematoxylin and eosin staining and reticulin or Masson's trichrome staining was sent to the pathologists. Interobserver agreement was evaluated by calculation of κ values for individual microscopic findings. κ values were interpreted as previously described:[19] 0.00–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–1.00, almost perfect agreement.

Correlation analysis of histology scores and clinical outcomes

Using paired biopsy and explant samples available in two patients, changes in the histology scores were determined. One of the two transplant patients had the initial biopsy at the age of 11 months and a liver transplantation 12 months later, while the other underwent biopsy at the age of five months and had a liver transplantation three months later. No immunohistochemical signal of BSEP was detectable in the biopsy and explant samples of both patients. In addition, three biopsy samples (taken before and during NaPB therapy) were available in two other patients. The period between each biopsy was 12 months. Their detailed clinical courses have been published elsewhere.[20] One patient showed a good clinical response to NaPB with improved serological findings including bilirubin, while, the other showed no obvious improvement in 12 months after starting postprandial oral administration of NaPB. Therefore, the therapy was switched to a preprandial regimen, and the patient's clinical symptoms and biochemical liver function tests gradually improved thereafter. In both patients, immunohistochemical signal of BSEP was below the detection limit before the NaPB therapy and partially restored 12 months after onset of its posprandial regimen.

Results

Establishing the scoring system

Liver tissue biopsy findings of patients with BSEP deficiency vary and depend on when they are taken.[21] One example is the giant cell transformation of hepatocytes, which is

extensive in neonates but becomes less pronouced later on. Therefore, we agreed that multiple microscopic findings were required to establish the scoring system. We also agreed that the scoring scheme should consist of several groups of related microscopic findings similar to scoring systems for other liver diseases. In the non-alcoholic fatty liver disease activity score system, three independent findings (steatosis, lobular inflammation, and ballooning) are used to estimate activity.[22] The proposed scoring standard also needed to be applicable to early and advanced stages of the disease.

The following 14 histopathological findings were initially listed: hepatocellular cholestasis, bile plugs, cholestatic rosette of hepatocytes, lobular necroinflammation, giant cell change of hepatocytes, hepatocyte swelling, confluent or submassive necrosis, cobblestone appearance of the parenchyma, distortion of the sinus trabecular pattern, portal inflammation, interface hepatitis, ductular reaction, periportal fibrosis, and pericellular fibrosis. Cobblestone appearance of the parenchyma and distortion of the sinus trabecular pattern were eventually excluded, because the biological nature of the former is unclear, and the latter is thought to overlap with swelling or the giant cell change of hepatocytes.

In addition, the histological review of nine cases using a multihead microscope led to the conclusion that the degrees of cholestasis, fibrosis, and lobular inflammation were not always correlated. Although portal tract changes of BSEP deficiency were not well described in the literature, some cases, particularly cases of advanced disease, showed conspicuous portal inflammation and ductular reaction. Therefore, the microscopic findings were grouped

into four categories (cholestasis, parenchymal change, portal tract change, and fibrosis). Finally, a scoring system consisting of 12 microscopic findings was established.

Proposed scoring system

The proposed histology-based scoring scheme is described in Table 1, and the scoring standards of the individual findings are summarized in Table 2. Representative pictures of the microscopic findings are available in Figures 1–4. There are several caveats for scoring. Firstly, for scoring bile plugs, only bile casts outside the hepatocytes need to be counted, in order to reduce the risk of double counting. Intracellular bile plugs are analyzed separately as a part of hepatocellular cholestasis. Secondly, score 2 is given to confluent or submassive necrosis because confluent or submassive necrosis suggests a more aggressive nature than other microscopic findings. Thirdly, a giant cell change is defined as enlarged hepatocytes with three or more nuclei, while hepatocyte swelling requires cell enlargement 2-times larger in diameter than “normal” hepatocytes for the scoring purpose, in order to increase reproducibility (Figure 2). Finally, unlike other scoring systems, pericellular fibrosis needs to be evaluated separately from the overall fibrosis stage, because some cases showed widespread pericellular fibrosis but limited portal or septal scaring (Figure 4B).

In clinical trials, two or more liver needle biopsies are usually evaluated before and after treatment, with histology scores being compared at multiple points. Standardization is also required for the evaluation of the score changes. Given sampling variation of the needle biopsies, we agreed that a 1-score change in a single category is not enough to be qualified

as either improvement or deterioration. Table 3 summarizes the evaluation standards agreed on for the interpretation of score changes between two time points.

Interobserver agreement study

Eleven tissue samples were independently evaluated by three board-certified pathologists. Fleiss' K (kappa) values for individual findings are summarized in Table 4. The interobserver agreement was highest for pericellular fibrosis with a κ value of 0.849, followed by the ductular reaction (κ value = 0.776) and fibrosis (κ value = 0.744). Three parameters in the cholestasis category showed slightly lower κ values ranging from 0.241 to 0.350 indicating fair agreement. Weaker agreement was also observed for interface hepatitis (κ value = 0.288). Interobserver concordance for the remaining eight microscopic findings was moderate or higher, with κ values greater than 0.400.

Correlation between histology scores and clinical outcomes

This correlation study was performed using four cases. Some samples were included in the interobserver agreement study, and the others were separately scored by the same three pathologists. Pathologists were blinded to which samples belonged to which patients and to which cases were going to be used for the correlation analysis. As for the two transplant cases, most categorical scores increased during the period from liver biopsy to transplantation. Based on the interpretation standard described above (Table 3), evaluations of all pathologists indicated deterioration of microscopic findings (Supplementary Tables 1

and 2). One pathologist could not score the overall fibrosis in the liver explants, because extensive pericellular fibrosis restricted the evaluation of the degree of bridging or septal fibrosis. At the discussion afterward, we agreed that score 5 (cirrhosis) should be given to those cases, similarly to alcoholic cirrhosis which often showed widespread pericellular fibrosis without broad fibrous septa.

For a biopsy case which showed a good clinical response to NaPB therapy,[20] liver histological changes in the first 12 months were evaluated as “improved” by two pathologists and “unchanged” by the other. Microscopic changes in the following 12 months were evaluated as “unchanged” by two pathologists and “improved” by the other, respectively (Supplementary Table 3). The last patient showed no obvious response to NaPB therapy in the first 12 months. Therefore, the regimen was switched to preprandial oral administration, and beneficial effects of the NaPB therapy were observed.[20] The change in the liver histology of this patient in the first 12 months after the beginning of NaPB therapy was determined as “deteriorated” by all pathologists, but in the next 12 months it was evaluated as “unchanged” by two pathologists and “improved” by the other (Supplementary Table 4).

Discussion

Histological scoring systems for some other conditions consist of two categories, one representing fibrosis, while the other is designed to grade inflammatory activity.[23, 24] However, we propose a more complex system consisting of four categories for BSEP deficiency, in order to evaluate multiple microscopic findings in a systematic manner, similar to the Ishak

system for chronic hepatitis.[25] Unlike other chronic liver diseases, in which fibrosis is a major denominator of disease progression, BSEP deficiency may manifest in fibrosis-poor refractory jaundice, which meets the criteria of liver transplantation. Furthermore, fibrosis may already be extensive even in the initial biopsy samples taken in the first several months of life. The case shown in Supplementary Table 2 showed a fibrosis score of 7-8 at the age of five months. In those cases, microscopic parameters other than fibrosis are required for the longitudinal histological evaluation. In addition, multiple categories will also help to determine which microscopic changes are improved or deteriorated by treatments in future clinical trials.

In this report, we also propose a standard for the evaluation of score changes between paired tissue samples (Table 3). Although each clinical trial sets up a different definition for histological improvement, the proposed standard seems to be reasonable and stringent enough. To determine histological change as “improved” in our scoring system, no score increase in any of the four categories is allowed. One might argue that a significant decrease in fibrosis is essential for histological improvement. However, it may not be observed during a short period of time. Observational periods vary among clinical trials, and posttreatment biopsies may be conducted shortly after the treatment is completed. Multiple categories will also be useful to cover diverse clinical and morphological manifestations ranging from recurrent cholestasis to rapidly progressing liver injury.[21, 26-28]

The proposed system is designed for BSEP deficiency, but may also be applicable to other forms of PFIC, which warrants further studies. Although the pathophysiology differs, microscopic changes such as cholestasis, lobular injury, portal inflammation, and fibrosis are

observed in any types.[29-31] Given that NaPB treatment partially restores the expression levels of BSEP proteins,[11-14] immunostaining for BSEP may also be useful for the assessment of its efficacy. However, that analysis should be done independently of the results of the histology scoring system, given that staining patterns of BSEP using immunohistochemistry vary among patients.[21] In some cases, the antibody reacts with functionally defective transporters, while other cases may show entirely negative staining despite a residual activity of the transporter. Therefore, staining results do not always correlate with the residual function of the transporter.

The interobserver agreement study indicated a moderate concordance for most histological parameters. One might argue that the κ values are too low, but the mean and median values were 0.561 and 0.602, which are higher than those for scoring systems of primary biliary cholangitis, nonalcoholic steatohepatitis, and biliary intraepithelial neoplasia.[32-34] We tried to objectively define some microscopic findings such as giant cell change or swelling of hepatocytes, which might have contributed to the relatively high agreement. Special staining may also improve the histological evaluation. On Perls' staining, bile is demonstrated as greenish granules which are more easily distinguishable from blue-stained hemosiderin and yellow-brown lipofuscin. Van Gieson staining also stains bilirubin green. The continuous usage of the proposed criteria will also improve the diagnostic concordance.

We also investigated the correlation between the scoring system and the clinical outcome using four cases. For the two cases whose clinical features worsened, most histological parameters indicated deterioration (Supplementary Tables 1 and 2). In the

remaining two cases,[20] clinical improvement by NaPB therapy was associated with an "improved" or "unchanged" liver histology score (Supplementary Tables 3 and 4). Since the data are still preliminary, future studies involving more patients will be required in order to prove the correlation of this scoring system and clinical outcome.

In conclusion, we proposed a histology-based scoring system of BSEP deficiency, and discussed its potential value as a surrogate marker. This standard showed a moderate interobserver agreement and a good correlation with clinical outcome. It may be used not only for monitoring microscopic changes in the clinical practice but also for serving as a surrogate endpoint in clinical trials.

Acknowledgments

This work was supported by grants JP17ak0101036 and JP19ek0109298 of the Japan Agency for Medical Research and Development to H.H.

References

1 Bull LN, van Eijk MJ, Pawlikowska L, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet. 1998 Mar;18: 219-24.

2 de Vree JM, Jacquemin E, Sturm E, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci U S A. 1998 Jan 6;95: 282-7.

3 Sambrotta M, Strautnieks S, Papouli E, et al. Mutations in TJP2 cause progressive cholestatic liver disease. Nat Genet. 2014 Apr;46: 326-8.

4 Strautnieks SS, Bull LN, Knisely AS, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet. 1998 Nov;20: 233-8.

5 Jacquemin E. Progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol. 2012 Sep;36 Suppl 1: S26-35.

6 Gomez-Ospina N, Potter CJ, Xiao R, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun. 2016 Feb 18;7: 10713.

7 Morotti RA, Suchy FJ, Magid MS. Progressive familial intrahepatic cholestasis (PFIC) type 1, 2, and 3: a review of the liver pathology findings. Semin Liver Dis. 2011 Feb;31: 3-10.

8 Knisely AS, Strautnieks SS, Meier Y, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. Hepatology. 2006 Aug;44: 478-86.

9 Zen Y, Vara R, Portmann B, Hadzic N. Childhood hepatocellular carcinoma: a clinicopathological study of 12 cases with special reference to EpCAM. Histopathology. 2014

Apr;64: 671-82.

10 Hayashi H, Sugiyama Y. 4-phenylbutyrate enhances the cell surface expression and the transport capacity of wild-type and mutated bile salt export pumps. Hepatology. 2007 Jun;45: 1506-16.

11 Gonzales E, Grosse B, Cassio D, Davit-Spraul A, Fabre M, Jacquemin E. Successful mutation-specific chaperone therapy with 4-phenylbutyrate in a child with progressive familial intrahepatic cholestasis type 2. J Hepatol. 2012 Sep;57: 695-8.

12 Gonzales E, Grosse B, Schuller B, et al. Targeted pharmacotherapy in progressive familial intrahepatic cholestasis type 2: Evidence for improvement of cholestasis with 4-phenylbutyrate. Hepatology. 2015 Aug;62: 558-66.

13 Hayashi H, Naoi S, Hirose Y, et al. Successful treatment with 4-phenylbutyrate in a patient with benign recurrent intrahepatic cholestasis type 2 refractory to biliary drainage and bilirubin absorption. Hepatol Res. 2016 Feb;46: 192-200.

14 Naoi S, Hayashi H, Inoue T, et al. Improved liver function and relieved pruritus after 4-phenylbutyrate therapy in a patient with progressive familial intrahepatic cholestasis type 2. J Pediatr. 2014 May;164: 1219-27 e3.

15 Bell LN, Wang J, Muralidharan S, et al. Relationship between adipose tissue insulin resistance and liver histology in nonalcoholic steatohepatitis: a pioglitazone versus vitamin E versus placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis trial follow-up study. Hepatology. 2012 Oct;56: 1311-8.

16 Carter-Kent C, Yerian LM, Brunt EM, et al. Nonalcoholic steatohepatitis in children: a

multicenter clinicopathological study. Hepatology. 2009 Oct;50: 1113-20.

17 de Vries EM, de Krijger M, Farkkila M, et al. Validation of the prognostic value of histologic scoring systems in primary sclerosing cholangitis: An international cohort study. Hepatology. 2017 Mar;65: 907-19.

18 de Vries EM, Verheij J, Hubscher SG, et al. Applicability and prognostic value of histologic scoring systems in primary sclerosing cholangitis. J Hepatol. 2015 Nov;63: 1212-9.

19 Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977 Mar;33: 159-74.

20 Nakano S, Osaka S, Sabu Y, et al. Effect of food on the pharmacokinetics and therapeutic efficacy of 4-phenylbutyrate in progressive familial intrahepatic cholestasis. Sci Rep. 2019 Nov 19;9: 17075.

21 Evason K, Bove KE, Finegold MJ, et al. Morphologic findings in progressive familial intrahepatic cholestasis 2 (PFIC2): correlation with genetic and immunohistochemical studies. Am J Surg Pathol. 2011 May;35: 687-96.

22 Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005 Jun;41: 1313-21.

23 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 1996 Aug;24: 289-93.

24 Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol. 1999 Sep;94: 2467-74.

25 Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995 Jun;22: 696-9.

26 Davit-Spraul A, Fabre M, Branchereau S, et al. ATP8B1 and ABCB11 analysis in 62 children with normal gamma-glutamyl transferase progressive familial intrahepatic cholestasis (PFIC): phenotypic differences between PFIC1 and PFIC2 and natural history. Hepatology. 2010 May;51: 1645-55.

27 van Mil SW, van der Woerd WL, van der Brugge G, et al. Benign recurrent intrahepatic cholestasis type 2 is caused by mutations in ABCB11. Gastroenterology. 2004 Aug;127: 379-84.

28 Waisbourd-Zinman O, Surrey LF, Schwartz AE, Russo PA, Wen J. A Rare BSEP Mutation Associated with a Mild Form of Progressive Familial Intrahepatic Cholestasis Type 2. Ann Hepatol. 2017 May - Jun;16: 465-8.

29 Bull LN, Carlton VE, Stricker NL, et al. Genetic and morphological findings in progressive familial intrahepatic cholestasis (Byler disease [PFIC-1] and Byler syndrome): evidence for heterogeneity. Hepatology. 1997 Jul;26: 155-64.

30 Jacquemin E, De Vree JM, Cresteil D, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. Gastroenterology. 2001 May;120: 1448-58.

31 Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010 Jul;53: 170-8.

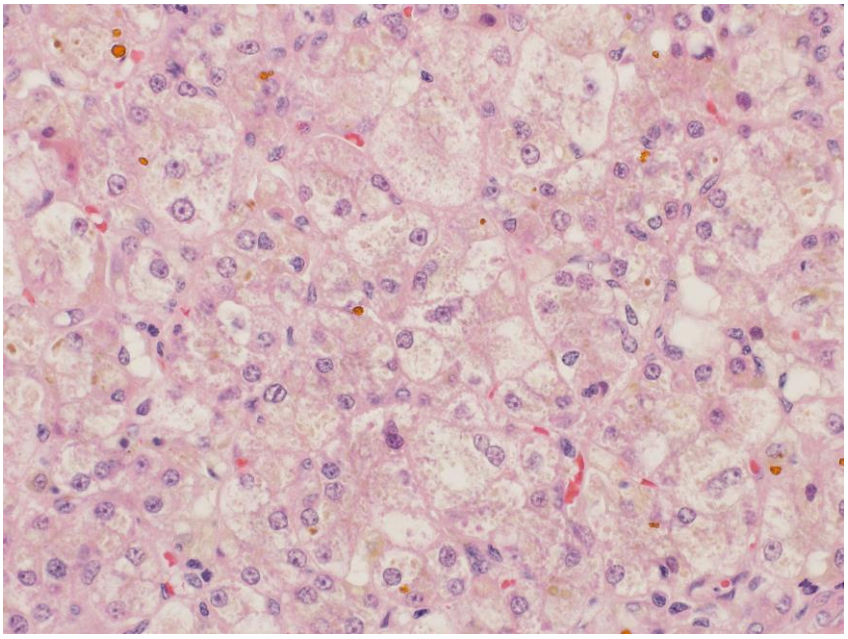
32 Juluri R, Vuppalanchi R, Olson J, et al. Generalizability of the nonalcoholic

steatohepatitis Clinical Research Network histologic scoring system for nonalcoholic fatty liver disease. J Clin Gastroenterol. 2011 Jan;45: 55-8.

33 Nakanuma Y, Zen Y, Harada K, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. Pathol Int. 2010 Mar;60: 167-74.

34 Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. Mod Pathol. 2007 Jun;20: 701-9.

(A)



(B)

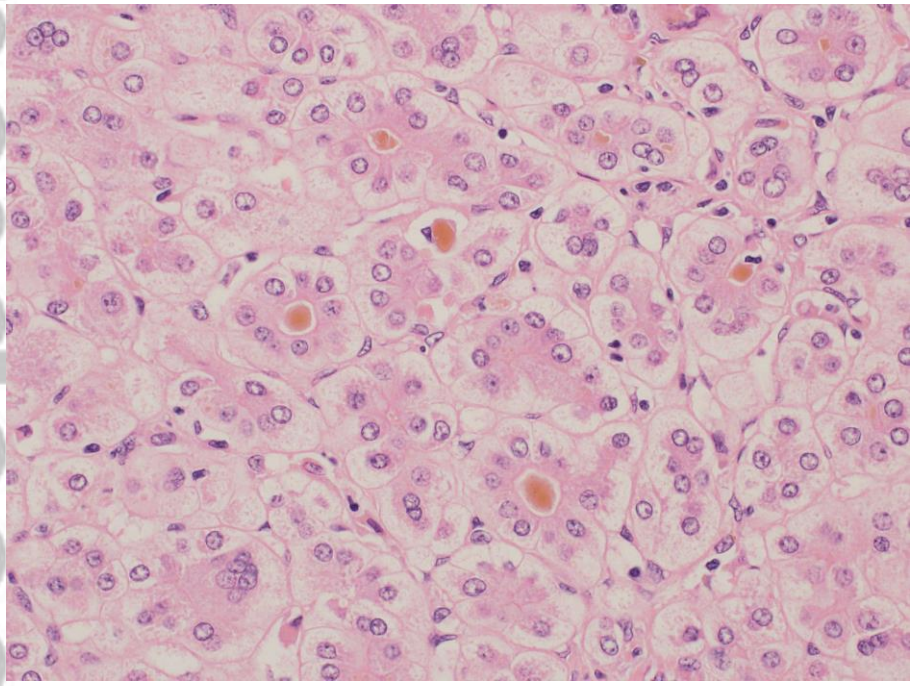
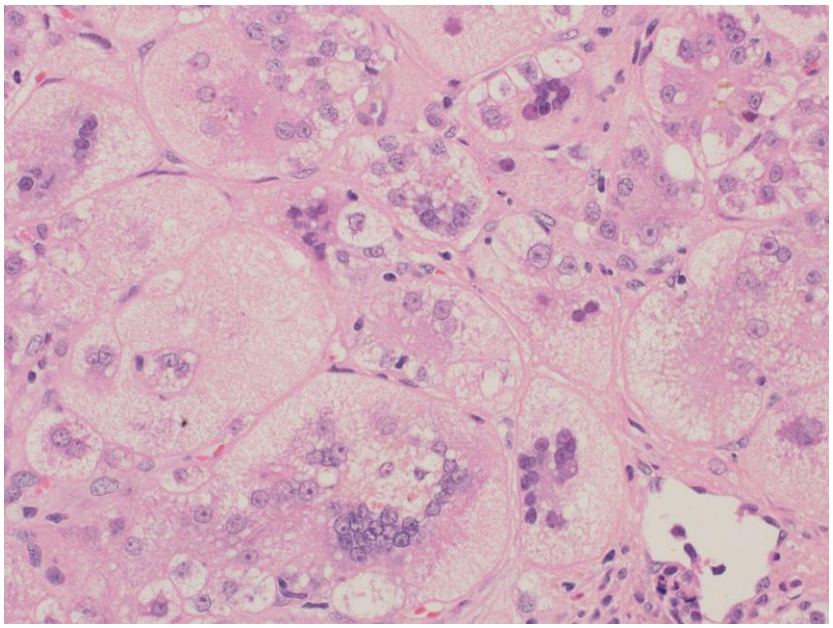


Figure 1. Microscopic findings of cholestasis.

(A) Hepatocellular cholestasis: bile pigments are observed in the cytoplasm of hepatocytes.
(B) Cholestatic rosettes of hepatocytes and canalicular bile casts.

(A)



(B)

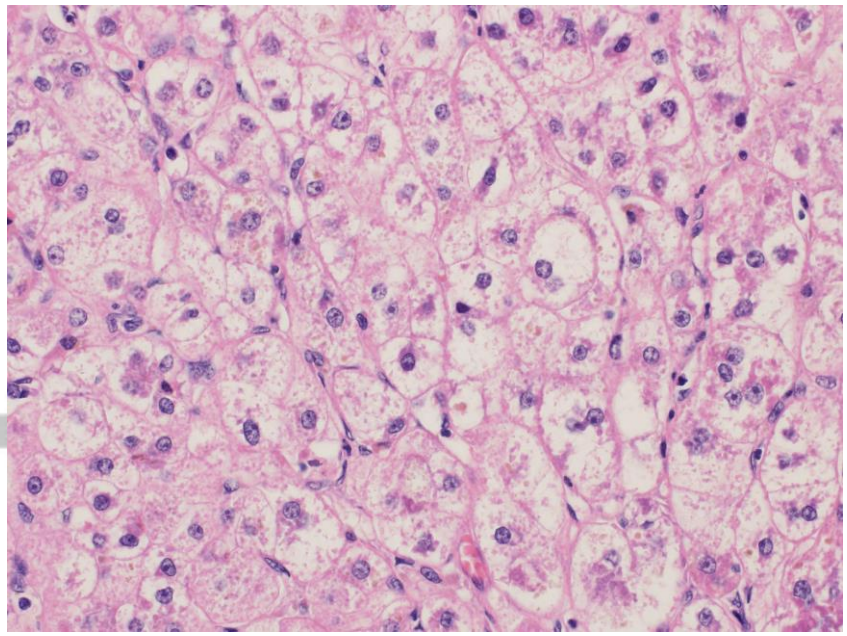


Figure 2. Microscopic findings of parenchymal changes.

(A) Giant cell change of hepatocytes: some enlarged hepatocytes with three or more nuclei are present. (B) Hepatocyte swelling: several hepatocytes are enlarged with clear cytoplasm.

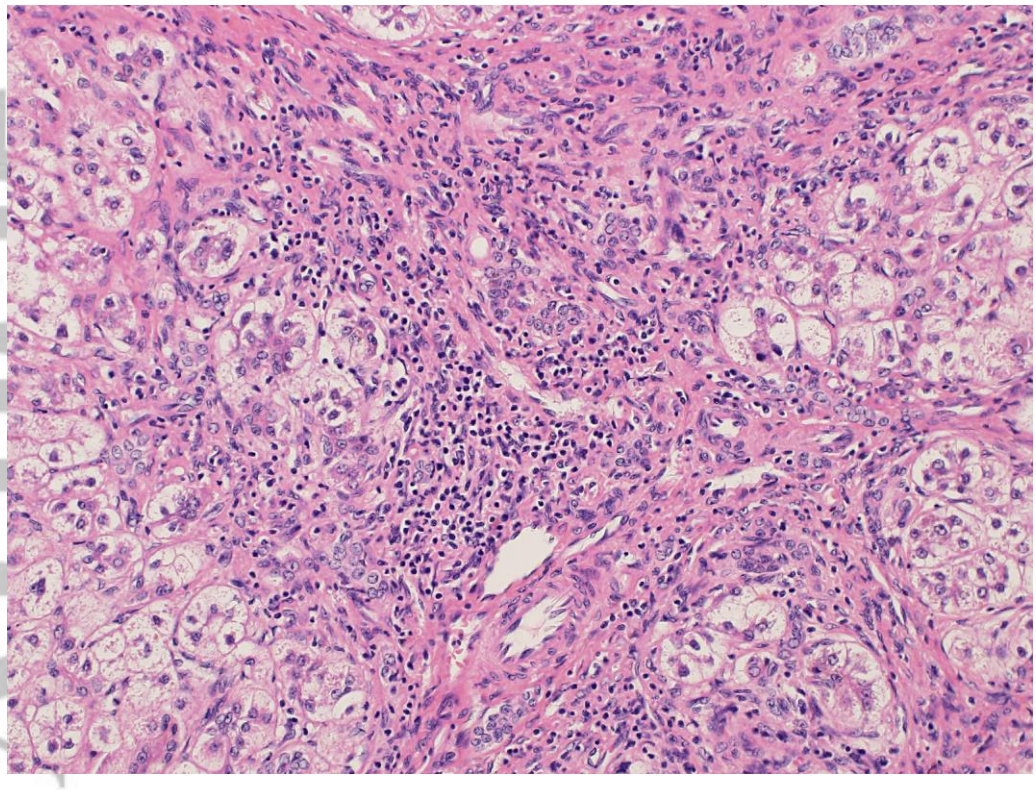
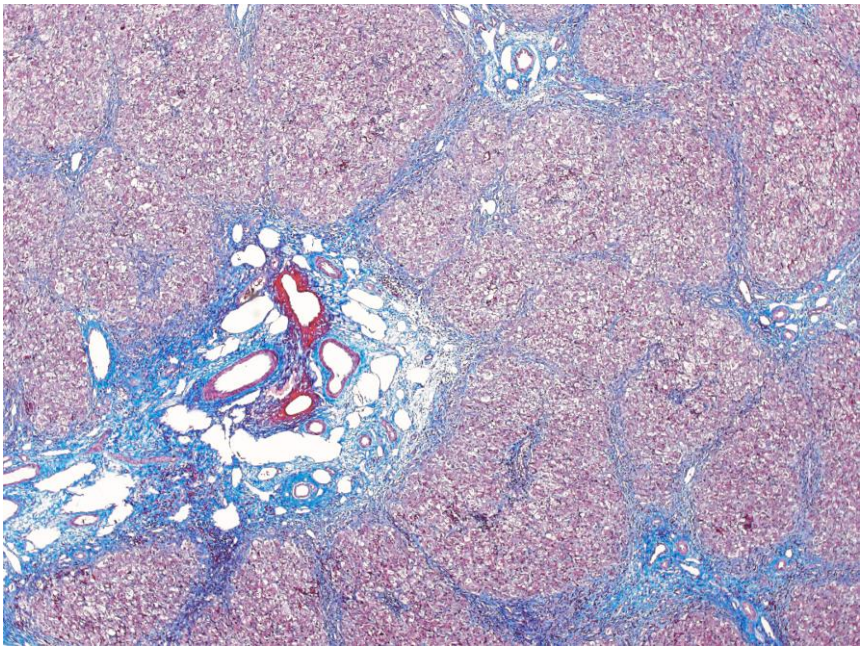


Figure 3. Microscopic findings of portal tract changes.
Portal tracts are expanded with conspicuous ductular reaction and mild inflammation.

(A)



(B)

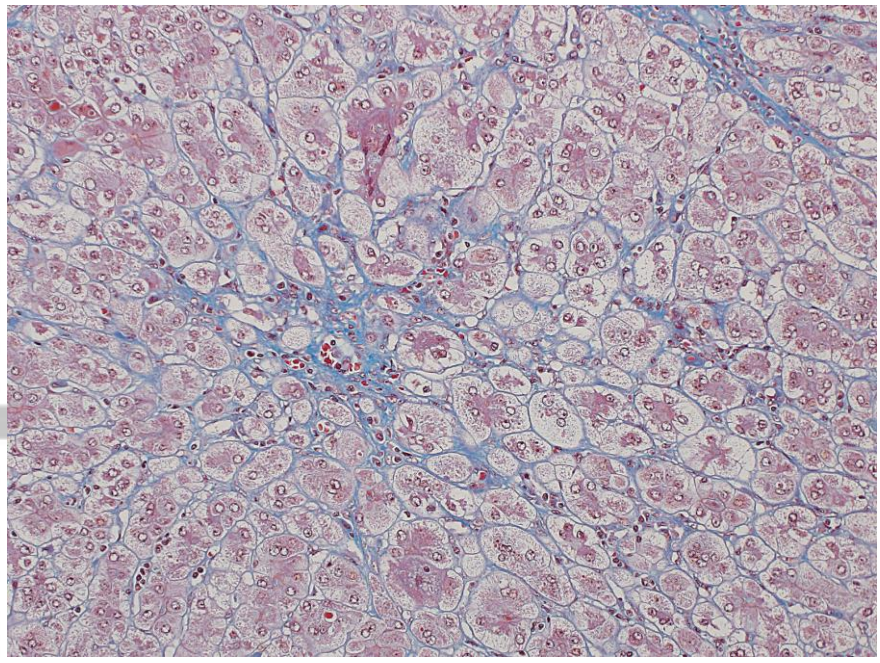


Figure 4. Microscopic findings of fibrosis.

(A) Fibrosis: multiple bridging fibrosis is observed. (B) Pericellular fibrosis: collagen fibers surround hepatocytes. Both: Masson's trichrome staining.

Table 1. A proposed scoring system for the liver histology of patients with BSEP deficiency.

CHOLESTASIS
Hepatocellular cholestasis (score 0–3)
Bile plugs (score 0–3)
Cholestatic rosette of hepatocytes (score 0–3)
PARENCHYMAL CHANGE
Lobular necroinflammation (score 0–3)
Confluent or submassive necrosis (score 0 or 2)
Giant cell change of hepatocytes (score 0–3)
Hepatocyte swelling (score 0–3)
PORTAL TRACT CHANGE
Portal inflammation (score 0–3)
Interface hepatitis (score 0–3)
Ductular reaction (score 0–3)
FIBROSIS
Fibrosis (score 0–5)
Pericellular fibrosis (score 0–3)

Table 2. Grading scheme of microscopic findings of the scoring system.

Hepatocellular cholestasis	
0	Absent
1	Present in <10% of hepatocytes
2	Present in ≥10% and <50% of hepatocytes
3	Present in ≥50% of hepatocytes
Bile plugs*	
0	Absent
1	1–10 bile plugs per 10 hpf.
2	11–19 bile plugs per 10 hpf.
3	≥20 bile plugs per 10 hpf.
Cholestatic rosette of hepatocytes	
0	Absent
1	1–3 rosettes per 10 hpf.
2	4–9 rosettes per 10 hpf.
3	≥10 rosettes per 10 hpf.
Lobular necroinflammation	
0	Absent
1	1 or 2 foci of foal/spotty necrosis or acidophilic bodies per hpf
2	4–5 foci of focal/spotty necrosis or acidophilic bodies per hpf
3	≥6 foci of focal/spotty necrosis or acidophilic bodies per hpf
Confluent or submassive necrosis	
0	Absent
2	Present
Giant cell change of hepatocytes†	
0	Absent
1	1–3 giant hepatocytes per 10 hpf.

2 4–9 giant hepatocytes per 10 hpf.

3 ≥10 giant hepatocytes per 10 hpf.

Hepatocyte swelling‡

0 Absent

1 Present in <10% of hepatocytes

2 Present in ≥10% and <50% of hepatocytes

3 Present in ≥50% of hepatocytes

Portal inflammation

0 Absent

1 Mild

2 Moderate

3 Severe

Interface hepatitis

0 Absent

1 Affecting <10% of interface

2 Affecting ≥10 and <50% of interface

3 Affecting ≥50% of interface

Ductular reaction

0 Absent

1 Focal

2 Between 1 and 3

3 Extensive

Fibrosis

0 No fibrosis

1 Periportal fibrosis

2 Elongation of periportal septa but no complete bridging

3 Bridging fibrosis present in <50% of liver tissue

4	Bridging fibrosis present in ≥50% of liver tissue
5	Cirrhosis
Pericellular fibrosis	
0	Absent
1	Present in <1/3 of the parenchyma
2	Present in ≥1/3 and <2/3 of the parenchyma
3	Present in ≥2/3 of the parenchyma

*, bile casts outside hepatocytes to be evaluated; †, defined as an enlarged hepatocyte with three or more nuclei; ‡, defined as hepatocyte enlargement >2 times larger in diameter than “normal” hepatocytes; hpf, high power field.

Table 3. A proposed standard for the interpretation of score changes between two time points.

Interpretation	Definition
Improved	One-point improvement in two categories with no score increase in the other two OR two-point improvement in one category with no score increase in the other three
Deteriorated	One-point deterioration in two categories OR two-point deterioration in one category.
Unchanged	Neither of the above.

Table 4. Results of the interobserver agreement study.

	Agreement (%)	κ value
Hepatocellular cholestasis	33.3	0.241
Bile plugs	42.4	0.346
Cholestatic rosettes of hepatocytes	51.5	0.350
Lobular necroinflammation	36.4	0.438
Confluent or submassive necrosis	87.9	0.614
Giant cell change of hepatocytes	51.5	0.776
Hepatocyte swelling	36.4	0.602
Portal inflammation	57.6	0.505
Interface hepatitis	54.5	0.288
Ductular reaction	57.6	0.767
Fibrosis	71.0	0.744
Pericellular fibrosis	69.7	0.849