Hepatic symptoms and histology in 13 patients with a Zellweger spectrum disorder

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Abstract

Patients with a Zellweger spectrum disorder (ZSD) have a defect in the assembly or maintenance of peroxisomes, leading to a multisystem disease with variable outcome. Liver disease is an important feature in patients with severe and milder phenotypes and a frequent cause of death. However, the course and histology of liver disease in ZSD patients are ill-defined. We reviewed the hepatic symptoms and histological findings of 13 patients with a ZSD in which one or several liver biopsies have been performed (patient age 0.2-39 years). All patients had at least some histological liver abnormalities, ranging from minor fibrosis to cirrhosis. Five patients demonstrated significant disease progression with liver failure and early death. In others, liver related symptoms were absent, although some still silently developed cirrhosis. Patients with peroxisomal mosaicism had a better prognosis. In addition, we show that patients are at risk to develop a hepatocellular carcinoma (HCC), as one patient developed a HCC at the age of 36 years and one patient a precancerous lesion at the age of 18 years. Thus, regular examination to detect fibrosis or cirrhosis should be included in the standard care of ZSD patients. In case of advanced fibrosis/cirrhosis expert consultation and HCC screening should be initiated. This study further delineates the spectrum and significance of liver involvement in ZSDs.
Take-home message: Liver fibrosis and cirrhosis with or without clinical symptoms of liver disease is already present at a very young age in patients with a Zellweger spectrum disorder and they are at increased risk to develop a hepatocellular carcinoma.
General Rules

Details of the contributions of individual authors: KB, BGPK; conception and design, data acquisition, analysis, and interpretation, manuscript draft and revision. FCCK; data acquisition, manuscript draft. ME; data acquisition, manuscript draft. FR; data acquisition, analysis, interpretation and manuscript revision. MML; data acquisition, analysis, manuscript draft and revision. PGJN; data acquisition, manuscript revision. JV; data acquisition, analysis, interpretation and manuscript revision. BTPT; conception and design, data acquisition, analysis, and interpretation, manuscript draft and revision.

The name of the corresponding author: Bwee Tien Poll-The

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Details of ethics approval: For this project the IRB issued a waiver since the study is retrospective and only anonymised data was used. Permission for autopsy studies for scientific purposes was obtained from the parents of patient 7 and 13

A list of approximately six keywords: Zellweger spectrum disorder, peroxisome biogenesis disorders, liver disease, hepatocellular carcinoma, bile acid intermediates, liver biopsy, autopsy
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DHCA</td>
<td>Dihydroxycholestanoic acid</td>
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<td>HCC</td>
<td>Hepatocellular carcinoma</td>
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<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
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<tr>
<td>PBD</td>
<td>Peroxisome biogenesis disorder</td>
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<tr>
<td>THCA</td>
<td>Trihydroxycholestanoic acid</td>
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<tr>
<td>VLCFA</td>
<td>Very long-chain fatty acids</td>
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<tr>
<td>ZSD</td>
<td>Zellweger spectrum disorders</td>
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1. Introduction

Zellweger spectrum disorders (ZSDs) are autosomal recessive disorders with a deficiency of functional peroxisomes caused by mutations in one of the peroxisome assembly (PEX) genes (Waterham and Ebberink 2012). Biochemically, the most prominent features are the accumulation of bile acid intermediates (i.e. dihydroxycholestanolic acid (DHCA) and trihydroxycholestanolic acid (THCA)), pristanic- and phytanic acid, pipecolic acid and very long-chain fatty acids (VLCFA) (Waterham et al. 2016). These abnormalities contribute to a variety of symptoms with a spectrum of severity, including developmental delay, hearing and vision deficits.

In addition to neurological symptoms, liver disease is a prominent feature (Heubi et al. 2017) (Heubi et al. 2018) and a frequent cause of death in patients with ZSDs (Wanders and Ferdinandusse 2012) (Poll-The et al. 2004). Liver disease develops in all ZSD patients with a severe phenotype at an early age, with disease progression in the majority (Roels et al. 1991). Hepatic symptoms, such as hepatomegaly, cholestasis, elevated transaminases and liver dysfunction, usually become apparent within the first year of life. Despite clinical and pathological evidence of liver involvement since the very first reports of ZSDs, literature of histological characteristics and course of liver disease is scarce. Only five case reports of ZSD post-mortem studies, with the oldest patient being 12 years of age, highlight liver disease as one of the main ZSD features (Powers et al. 1985) (Gilchrist et al. 1976) (Aubourg P et al. 1986) (Chow et al. 1992)
(Torvik et al. 1988). Earlier and more recent studies mentioned, in addition to absence of peroxisomes, other ultrastructural abnormalities such as large angulate lysosomes containing trilamellar inclusions (Mooi et al. 1983) (Depreter et al. 2003) (Warren et al. 2018).

Here, we describe the largest reported histological series of liver biopsies and/or reports of autopsy from 13 patients (age range from 0.2 to 39 years) to further delineate the spectrum and significance of liver involvement in ZSDs.
2. Methods

Material was collected at the Amsterdam University Medical Centre (Amsterdam UMC) and University Medical Centre Utrecht (UMCU), The Netherlands. The Amsterdam UMC is the centre of expertise for peroxisomal disorders in The Netherlands. For this study the institutional review board issued a waiver. Patients data were retrospectively collected by reviewing the medical records both at the Amsterdam UMC and UMCU. The liver biopsies were obtained between 1993 and 2004 according to in-house protocols. For this study, biopsies were re-examined by light microscopy using hematoxylin and eosin (H&E), copper staining, iron staining and collagen stainings (i.e. sirius red, Azan, Elastica von Giesson). Liver tissue was evaluated for the presence of steatosis. In addition, portal inflammation, interface activity and lobular inflammation were classified as: none, mild, moderate, or severe. Presence of siderosis was classified as grade 0: none/not observed, grade 1: \( \leq 25\% \) of hepatocytes, grade 2: 26–50\% of hepatocytes, grade 3: 51–75\% of hepatocytes or grade 4: >75\% of hepatocytes. Presence of bilirubinostasis and copper was evaluated. Finally, grade of fibrosis was descriptively reported, independent of underlying disease: (a) none, (b) perisinusoidal or periportal, (c) perisinusoidal and portal/periportal, or (d) bridging fibrosis, involving less than 50\% of the portal tracts and/or central veins (e) bridging involving more than 50\% of the portal tracts and/or central veins and (f) cirrhosis. Miscellaneous findings in biopsies, not being part of the initial scoring system, were also noted. All biopsies were examined by two
different experienced liver pathologists (JV and MML). Electron microscopy studies and localisation of peroxisomal enzymes and proteins were performed as previously described by Roels et al (Espeel and Van Limbergen 1995) (Roels et al. 1995). Permission for autopsy studies for scientific purposes was obtained from the parents of patient 7 and 13.

3. Results

We searched our ZSD database and found reports on liver histology in 13 patients (9 females and 4 males). Diagnosis was based on laboratory investigations to assess peroxisomal functions, and confirmation by enzymatic analysis in fibroblasts, and/or PEX gene mutation studies (Table 1). In the 13 patients with liver histology, age ranged from 0.2 to 39 years. Five patients are still alive. The histology includes 16 liver biopsies from 12 patients and 3 reports of autopsy, 2 from patients whom previously had undergone liver biopsies. Overviewing the neurological phenotype of these 13 patients, all patients had the well-known symptoms of ZSD patients, such as developmental delay, hearing and visual impairment and enamel hypoplasia. Zellweger severity scores (Klouwer et al. 2018b) and liver related symptoms are depicted in Table 1. Based on symptoms of liver disease we noticed two broad categories of liver phenotypes. The first group consisted of patients having predominant neurological symptoms, such as epilepsy and leukodystrophy with relatively mild and often transient symptoms and signs of liver
disease, such as hepatomegaly and intermittent mildly elevated liver enzymes. Disease progression in this group is dominated by progression of neurological symptoms (Berendse et al. 2016a). Symptoms in the second group of patients are dominated by more severe liver related signs and symptoms, such as progressive cholestasis, severe coagulation defects and cirrhosis. To compare the results of hepatic histology, we divided our cohort in two groups based on the severity of liver related symptoms; group 1 (patients 1-8) and group 2 (patients 9-13). Patients in group 1 presented with mild liver related symptoms in 5 out of 8 patients, these included prolonged neonatal jaundice (n=2), hepatomegaly (n=4) and hepatosplenomegaly (n=1). Three out of 8 patients had no liver related symptoms at time of liver biopsy. Laboratory tests in group 1 showed normal or only minor elevated liver enzymes. Coagulation parameters (i.e. abnormal PT, Factor V and VII) were mildly disturbed in all those with available data. None suffered from bleeding complications. Despite this mild liver phenotype, severe liver disease did develop in 2 patients of this group during long term follow-up. Patient 2, currently 14 years of age, presented with transient neonatal jaundice and mainly suffered from severe intellectual disability since. However, at age 10 years, she developed liver cirrhosis with multiple regenerative nodules on imaging. Patient 7 died at the age of 36 years of pneumonia and respiratory failure. She never showed signs or symptoms of liver disease on physical, radiological and laboratory examination. However, on autopsy, the liver showed cirrhosis with a prominent 4 cm nodule on the external surface. Morphology
showed a poorly differentiated hepatocellular carcinoma (HCC, Glypican 3 positive (Wang et al. 2008)).

Patients in group 2 suffered from a more pronounced liver phenotype. Three out of 5 patients presented with prolonged neonatal jaundice, 5/5 had hepatomegaly and splenomegaly was reported in 4/5. Except patient 13, all died before the age of 2 years. Patient 13 had symptoms of liver disease at the age of 8 years and died due to end-stage liver disease with encephalopathy, hepatorenal and hepatopulmonary syndrome at the age of 18 years. Autopsy of the liver showed extensive micro- and macronodular cirrhosis, with prominent nodules on the external surface. Negative immunohistochemical stainings (glutamine synthetase, beta-catenine and glypican 3) together with morphology suggested a dysplastic nodule and not (yet) a HCC.

Biochemically, all patients in both groups had elevated concentrations of VLCFA (C26:0), DHCA and THCA in plasma. Unfortunately, phytanic- and pristanic acid levels in plasma and plasmalogens in erythrocytes are missing in the majority.

**Histological characteristics**

Findings of liver biopsy and/or autopsy are described in Table 2. Although not visible on light microscopy, patient 5 and 6 showed steatosis on electron microscopy. Bilirubinostasis was only observed in biopsies from three patients in group 2. Siderosis was detected in 4 biopsies (3 patients), accumulation of copper in one and portal/lobular
inflammation in 13/18 samples (Figure 1 and 2). Interestingly, 7/18 biopsies showed remarkable presence of glycogenated nuclei and 5/18 biopsies showed abnormal bile duct epithelium with reactive epithelial changes. All patients, except patient 8, showed liver fibrosis or cirrhosis on liver biopsy/autopsy, albeit patients in group 2 had a younger age. In patient 5, the degree of fibrosis could not be determined due to poor quality of one biopsy. Overall, the fibrosis was located in perisinusoidal, periportal and/or pericellular space. Bridging fibrosis was seen in both groups (11/16 biopsies, Figure 1 and 2). The bridging fibrosis involved more than 50% of the portal tracts and/or central veins in 3/11 biopsies. There was no differences in localization of fibrosis between the two groups. Follow-up biopsies showed similar degree of inflammation and fibrosis compared to the first biopsies (Figure 2). Sclerosis of portal areas in the absence of cirrhosis (i.e. hepatoportal sclerosis) was present in the biopsy of patient 8 and 11 (both groups). In addition to cirrhosis, we found a HCC in patient 7 and a dysplastic nodule in patient 13 at autopsy (Figure 1). Cytochemical localisation of peroxisomal enzymes (electron and light microscopy) in the liver revealed either a total absence of peroxisomes or peroxisomal mosaicism (i.e. a mixed population of cells with or without import competent peroxisomes, described in ZSD patients with a mild phenotype (Waterham and Ebberink 2012)). Peroxisomal enzymes can localized in organelles with the morphology of lysosomes (Figure 3). Catalase staining on frozen sections after formaldehyde prefixation can reveal the presence of mosaics (Additional Figure 1). Data of peroxisomes on
electron microscopy was not available for patient 7. Peroxisomal mosaicism was seen in 5 patients, of whom 4 belong to group 1. This mosaicism is associated with longer survival and more benign clinical course (Roels et al. 2003). Patient 4 had, in addition to deficient hepatic peroxisomes, functional peroxisomes in the duodenum epithelium. Angulate lysosomes (with trilamellar inclusions), abundant in patients with a ZSD (Mooi et al. 1983), were seen in 15/18 biopsies (Figure 3). In two patients such lysosomes could not be found. Both died at an early age, reflecting that the typical trilamellar inclusions result from a storage phenomenon.

4. Discussion

Liver involvement is already reported since the very first description of ZSDs (i.e. cerebro-hepato-renal syndrome) but the liver histology features are underreported. Here, we present the largest reported series of liver biopsies and reports of autopsy from 13 patients and show that histological abnormalities range from mild fibrosis to severe cirrhosis in both patients with a mild and severe phenotype (group 1 and 2). Our results emphasize the relevance of liver disease in ZSDs with a broad spectrum of histomorphological findings, not only in severely affected patients (defined as patients with signs and symptoms of liver disease, group 2) but also in patients with mainly neurological symptoms that survive into adulthood (group 1). In addition to Heubi et al (Heubi and Bishop 2018), this study suggests that ZSD patients are at increased risk to
develop a HCC based on the finding of a pre-malignant dysplastic nodule and a HCC at 18 (patient 13) and 36 years of age (patient 7), respectively. Similar to patients with other inborn errors of metabolism with prolonged survival (Erez et al. 2011).

Bridging fibrosis was seen in 8 out of 13 patients, indicating advanced fibrosis. Hepatoportal sclerosis is characterized by the presence of sclerosis of portal areas in the absence of cirrhosis (Cantez et al. 2013). In our cohort, patients 8 and 11 suffered from hepatoporal sclerosis, previously described as a cause of noncirrhotic portal hypertension in patients with Zellweger syndrome (Schouten et al. 2011). The majority of patients had at least some portal and/or lobular inflammation. Glycogenated nuclei were observed in 7/18 samples of which the clinical significance is uncertain; these nuclei can be found in non-alcoholic steatohepatitis, Wilsons disease, represent early hepatic injury and can be seen in many other disorders like diabetes mellitus, glycogen storage disorders and in alcohol abuse. However they also have been observed in biopsies of healthy children (Burt AD, Portmann BC, Ferrell LD 2006). Accumulation of copper and/or siderosis was found in 4 patients and is associated with chronic liver disease.

Liver fibrosis is potentially reversible, but when progressed to cirrhosis the disease is mostly irreversible (Tsochatzis et al. 2014). The most severe, and possibly fatal, complications of liver cirrhosis are oesophageal variceal bleeding due to portal hypertension, hepatopulmonary syndrome, hepatorenal syndrome, hepatic encephalopathy and coagulation defects due to liver failure (Tsochatzis et al. 2014).
(Fattovich et al. 2004) (Amitrano et al. 2002). Patients are also at risk to develop a HCC (Fattovich et al. 2004). Hepatoportal sclerosis, present in 2 patients, is also a risk factor for portal hypertension and oesophageal variceal bleeding. These findings illustrate the importance of early intervention to prevent the development of fibrosis or the progression of fibrosis to cirrhosis and associated complications. Cholic acid was shown to lower toxic bile acid intermediates in plasma in patients with a mild phenotype (Berendse et al. 2016b). It was hypothesis that cholic acid therapy could prevent disease progression and progression of liver disease. However, short- and long-term follow-up showed, besides significant increase in weight, no improvement of clinical symptoms or liver phenotype (Klouwer et al. 2018a)(Heubi et al. 2018). Long-term follow-up did show stabilization of liver function/enzymes (Heubi et al. 2018). However, if the stabilization of liver function was caused by cholic acid supplementation and not by natural course of disease is unknown due to missing data of natural history of liver phenotype in ZSDs. Liver transplantation could be a therapeutic option in end stage liver failure. Only few case reports of liver transplantation in ZSDs are published. These studies showed normalisation of phytanic acid, pristanic acid and pipecolic acid in plasma after liver transplantation. Unfortunately, detailed data of C_{27}-bile acid intermediates is missing (Matsunami et al. 2016) (Demaret et al. 2018).

A possible cause of the liver abnormalities are the C_{27}-bile acid intermediates (i.e. DHCA and THCA), which were increased in all patients (in group 1 and 2). Due to
impaired bile acid synthesis, these intermediates accumulate in plasma and organs of ZSD patients. They are considerably more toxic than physiological C\textsubscript{24}-bile acids, cholic- and chenodeoxycholic acid (Ferdinandusse et al. 2009a). Furthermore, they are less efficiently excreted from the hepatocytes into the bile ducts leading to cholestasis (Ferdinandusse et al. 2009b). Unfortunately, because detailed data of these intermediates are missing in several patients of this study, a correlation between the levels of intermediates and the severity of histological abnormalities in both groups could not be investigated and has to be studied in a prospective natural history study. Supporting the hypothesis of a hepatotoxic effect of C\textsubscript{27}-bile acid intermediates, is the finding of liver fibrosis in patients with single peroxisomal enzyme deficiencies, other disorders in which these C\textsubscript{27}-bile acid intermediates accumulate. These diseases include D-bifunctional protein deficiency (DBP) deficiency (Ferdinandusse et al. 2006), acyl-CoA oxidase 2 (ACOX2) (Vilarinho et al. 2016), ATP-binding cassette transporters 3 (ABCD3) (Ferdinandusse et al. 2015) and alpha-methylacyl-CoA racemase (AMACR) deficiency (Setchell et al. 2003).

Besides patients, mouse models also give insight into the pathophysiology and potential oncogenesis of liver disease in ZSD. An interesting model is the AMACR mouse, in which C\textsubscript{27}-bile acid intermediates accumulate. Surprisingly, knockout mice have normal survival and no hepatic phenotype (i.e. normal histology) (Savolainen et al. 2004). When such mice are fed phytol (a precursor of phytanic acid) elevated levels of
phytanic and pristanic acid can be found and mice develop a liver phenotype with inflammation and fibrosis (Selkälä et al. 2015). Suggesting that accumulation of bile acid intermediates alone is not sufficient to cause liver injury and a liver phenotype. In addition, isolated phytanic acid accumulation is not sufficient to develop a liver disease either, as illustrated by patients with Refsum disease who have no liver phenotype (Wanders and Ferdinandusse 2012). In contrast to the AMACR model, mouse models for peroxisomal biogenesis disorders are associated with a liver phenotype including hepatomegaly, cholestasis and liver fibrosis (for a comprehensive review see (Baes and Van Veldhoven 2016)). In a mouse model with complete knock-out of functional peroxisomes and accumulation of peroxisomal metabolites (i.e. PEX5), an upregulation of C-myc in liver has been reported (Peeters et al. 2015). C-myc is a known proto-oncogene involved in cancer metabolism that also plays a crucial role in the pathogenesis of HCCs (Lin et al. 2010). C-myc can be induced by activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARα) (Gonzalez and Shah 2008). In humans, bile acids have been shown to activate PPARα (Pineda Torra et al. 2003) and can thus potentially also activate C-myc (Li and Chiang 2009). It would be interesting to measure expression of C-myc in liver biopsies of our patients.

Limitations of our study include the possible sample error associated with needle liver biopsies. All biopsies were examined by the same liver pathologists to exclude and minimize the inter- and intra-observer variation. Furthermore, because of the
retrospective character of the study with some patients living in the early 90’s, detailed clinical features (including use of medication) and biochemical parameters are missing.

In view of the high risk for ZSD patients to develop liver cirrhosis, even in the absence of liver related symptoms, and increased risk of HCC monitoring of and counselling on liver disease is warranted. To provide comprehensive supportive management for ZSD patients and other peroxisomal diseases associated with liver disease, we advise to include yearly screening for liver fibrosis by either ultrasound, preferably combined with elastography given its superior accuracy to diagnose fibrosis. When signs of significant liver fibrosis or HCC develop, a paediatric gastroenterologist should be consulted. When cirrhosis is diagnosed, patients should be counselled on the risk of decompensation of cirrhosis and the development of a HCC. Most importantly, the risk of variceal bleeding and the pros and cons of oesophageal varices screening should be discussed not only in patient with cirrhosis but also in patients with signs of portal hypertension in the absence of cirrhosis (e.g.due to hepatoporal sclerosis). Although the exact risk of HCC in Zellweger is unknown, it is presumably high based on two HCC cases (Heubi and Bishop 2018 and this study) in this rare disorder. In view of this risk, HCC surveillance by performing an ultrasound should be considered in ZSD patients with cirrhosis at the discretion of a consulting hepatologist. However, the decision to screen for HCC can only be made on individual basis and in consultation with the guardians and patient. Aspects that should be weighted are the burden of performing
ultrasounds and additional testing if suspicion lesions are found, the anxiety screening can cause and the limited treatment options in these severely affected patients with an often limited life expectancy. It remains to be established whether ZSD patients without cirrhosis are at increased risk of developing HCC and whether screening in this group should also be considered.

In conclusion, we show that liver fibrosis and cirrhosis is already present at a very young age. In mild patients with prominent neurological features, liver disease develops more slowly and insidious. This study also suggests that ZSD patients are at increased risk to develop a HCC. These findings support rigorous monitoring for liver disease in all patients with a ZSD.

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acid synthesis with transaminase elevation, liver fibrosis, ataxia, and cognitive impairment. Proc Natl Acad Sci U S A 113:11289–11293


Figure legends

Figure 1, liver histology:
A; Liver cirrhosis (Sirius Red Stain for collagen) in patient 7 at autopsy. B; Hepatocellular carcinoma (H&E staining) in patient 7 at autopsy. C; Liver cirrhosis (Sirius Red Stain for collagen) in patient 13 at autopsy. D; Portal inflammation (H&E staining) in patient 13 at autopsy. E; Hepatocytes with many glycogenated nuclei (H&E staining) in patient 13 at autopsy.

Figure 2, liver histology:
A and B from patient 4 at the age of 1.5 years and C and D same patient at the age of 4.5 year. A; Azan staining with periportal and pericellular fibrosis (blue). B; H&E staining with portal sclerosis and minimal portal inflammation (arrow). Some hepatocytes demonstrated glycogenated nuclei (circles). asterisk (*) shows ballooning. C; Azan staining with periportal and pericellular fibrosis (blue). D; H&E staining with lobular inflammation (arrow) and several hepatocytes with glycogenated nuclei (circles).

Figure 3, Liver electron microscopy:
A; Angulate lysosome of patient 5 at the age of 6 years. B; Import of labeled peroxisomal thiolase of patient 5 at the age of 4 years, i.e. functional peroxisomes (arrow) that are absent in many other hepatocytes. Import of labeled peroxisomal alanine glyoxylate
aminotransferase (C) and thiolase (D) in patient 6 at the age of 7.5 years. Gold particles are seen in organelles with the morphology of lysosomes (arrows), not within peroxisomes. Catalase in these livers is in the cytoplasm, not in peroxisomes.

Peroxisomal enzymes in lysosomal structures were also reported in patient 8 ((Roels et al. 2003). Other larger organelles are mitochondria showing their cristae but no gold label (courtesy of Marc Espeel, Ghent University).

Additional Figure 1, liver light microscopy:
A; (patient 4 at 4.5 years) Immunostaining of alanine glyoxylate aminotransferase. Positive in cytoplasm and some nuclei. There is no catalase immunoreactivity in the peroxisomes. Crystal of cytoplasmic AGT is seen as a small rod (arrow). B; (patient 4 at 4.5 years) Immunostaining of catalase. Positive in all hepatocyte nuclei and cytoplasm but not within peroxisomes. C, D; (patient 5 at 4 years) Immunostaining for catalase. Positive in cytoplasm and few peroxisomes (arrow), i.e peroxisomal mosaicism.
Table 1: Liver related symptoms at time of liver biopsy/autopsy of 13 patients with a Zellweger spectrum disorder

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<tr>
<th>Patient</th>
<th>Gender</th>
<th>PEX mutation</th>
<th>Zellweger severity score</th>
<th>Age at biopsy, yr</th>
<th>Current age or age at death, yr</th>
<th>Cause of death</th>
<th>Presence of cirrhosis/HCC</th>
<th>Prolonged neonatal jaundice</th>
<th>Hepato-megaly</th>
<th>Spleno-megaly</th>
<th>Portal hypertension</th>
<th>Ascites</th>
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<td>3</td>
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<td>--</td>
<td>+</td>
<td>+</td>
<td>nm</td>
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<td>+</td>
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<td>PEX1 p.G843D + p.G843D</td>
<td>0.25</td>
<td>1.5</td>
<td>#</td>
<td>RF</td>
<td>+</td>
<td>--</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>f</td>
<td>PEX1***</td>
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<td>0.6</td>
<td>0.6 #</td>
<td>RF</td>
<td>--</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>nm</td>
<td>nm</td>
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<tr>
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<td>RF</td>
<td>--</td>
<td>+</td>
<td>nm +</td>
<td>+</td>
<td>nm</td>
<td>nm</td>
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<td>f</td>
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<td>7.5</td>
<td>18.5</td>
<td>18.5 #</td>
<td>LF</td>
<td>+</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>+</td>
<td>+</td>
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</tbody>
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# deceased, -- = absent, + = present, *autopsy, **intracranial hemorrhage, ***complementation group (mutation unknown)

1Because of the retrospective character of the study, we were unable to determine the severity score in all patients. Maximum score is 30 (most severe phenotype)

f=female, LF= liver failure, m=male, nm=not mentioned, RF= respiratoiry failure, yr=year

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<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age at biopsy, yr</th>
<th>Current age/age at death, yr</th>
<th>Medication use</th>
<th>Steatosis</th>
<th>Inflammation</th>
<th>Bilirubinostasis</th>
<th>Siderosis</th>
<th>Copper accumulation</th>
<th>Glycogenated nuclei</th>
<th>Abnormal bile duct epithelium</th>
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<tr>
<td>1</td>
<td>m</td>
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<td>7</td>
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<td>-</td>
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<td>-</td>
<td>+ (grade I)</td>
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<td>2</td>
<td>f</td>
<td>0.5</td>
<td>14</td>
<td>vit D/K</td>
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<td>lobular (mild)</td>
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<tr>
<td>3</td>
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<td>1</td>
<td>6</td>
<td>vit E, DHA/AA</td>
<td>-</td>
<td>-</td>
<td>+ (grade I)</td>
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<td>4</td>
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<td>-</td>
<td>portal (moderate)</td>
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<td>-</td>
<td>+</td>
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<td>mozaik</td>
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<td>36*</td>
<td>36*</td>
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<td>+ (canalicular)</td>
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<td>+</td>
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<tr>
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<td>m</td>
<td>0.25</td>
<td>1</td>
<td>-</td>
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<td>lobular (mild)</td>
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<td>lobular (mild)</td>
<td>+ (canalicular)</td>
<td>+ (grade I)</td>
<td>-</td>
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<tr>
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<td>f</td>
<td>1.5</td>
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<td>portal (mild)</td>
<td>-</td>
<td>+</td>
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<tr>
<td>13</td>
<td>f</td>
<td>7.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>lobular (mild), portal (severe)</td>
<td>+ (canalicular and ductular)</td>
<td>+ (grade I)</td>
<td>-</td>
<td>-</td>
<td>cirrhosis, HCC</td>
</tr>
</tbody>
</table>

* deceased, # deceased; autopsyped = absent; + = present; DHA = docosahexaenoic acid; AA = arachidonic acid; EM = electron microscopy; f = female; ferro = ferrous fumarate; HCC = hepatocellular carcinoma; nd = not determined; urso = ursodeoxycholic acid.
Figure 2

(A) [Image of histological section with a scale bar of 100 µm]

(B) [Image of histological section with a scale bar of 50 µm]

(C) [Image of histological section with a scale bar of 50 µm]

(D) [Image of histological section with a scale bar of 50 µm]