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Revision of P. Ellinger et al. "Partial External Biliary Diversion in BSEP deficiency: association between outcome and mutation"

Review 1 (Reviewer 02954069):

I wonder whether the authors can acknowledge the readers about the differences between BRIC-2 and PFIC-2 with respect to mutations of BSEP.

Answer: A short paragraph is now included with respect to the differences between PFIC-2 and BRIC-2 (Page 5 of the revised manuscript)

Review 2 (Reviewer 03668426):

Methods: why did you choose six non-cholestatic patients only operated for oncological reasons? I'm guessing if there's any difference in bile characteristics between healthy patients?

Answer: The aim was to compare the bile samples of the two patients with bile from the main bile duct of non-cholestatic patients. Patients who underwent endoscopic retrograde cholangiography (ERC) were not suitable, because ERC was hardly ever performed in non-cholestatic patients (We have added a statement concerning the bile sampling from cholestatic patients via ERC in the Methods section, page 6).
Gallbladder bile (e.g. collected during standard cholecystectomy) again would not have been the appropriate reference. Therefore, patients with normal liver function, who underwent liver resection for oncological reasons, offered easy access to bile of the main bile duct. These patients were considered being closest to the desired controls.

Review 3 (Reviewer 03476246):

Major points: - What are the other factors studied as determinants for the outcome of PEBD? - What about the clinical, laboratory, and histological status of the 2 patients?

Leading symptom in both patients was intractable, disabling pruritus, which was the clinical parameter for indication of PEBD and for assessing the success of PEBD (this is mentioned in the text).
For the female patient, determination of liver stiffness by Fibroscan at the age of 17 is now mentioned (9) which revealed some liver damage.
We now included more detailed information about the outcome of PEBD in terms of bile salt concentrations in the male patient. Despite some decrease of serum bile salt levels in patient 2 disabling pruritus persisted, which was eventually the reason for LTX.

For how long both patients received UDCA and other therapies before undergoing to surgical intervention by PEBD? It is known that UDCA will affect the BSEP expression and BS synthesis and excretion.

The girl receives UDCA life-long (now mentioned on page 9). The boy initially received UDCA, which was discontinued due to lack of an effect. Afterwards he

received rifampicin without sufficient relief of pruritus. We agree with the reviewer and now mentioned, that UDCA affects BSEP expression (page 10).

Selection of controls is not appropriate.

We believe that the bile samples of the two patients rather reflect bile from the main duct and not from the gallbladder due to the constant outflow of bile from the liver through the gallbladder in the setting of PEBD. Assuming a residual concentrative effect of the gallbladder in PEBD bile salts in patient's bile duct bile would be lower. Consequently the bile to serum ratio of bile acids (as a measure of hepatocellular concentration capacity) would be even overestimated (see table 1).
The most appropriate control would be bile from the main duct of healthy children. However, it is almost impossible to obtain such samples.
Nevertheless, the statement that the investigated mutations have a significant effect on bile acid transport remains consistent.

It is premature to conclude this from 2 case studies, especially with some differences as sex, among others. It is better to be reported as a limitation in this work.

We agree and have included a note into the concluding remark of the discussion according to the reviewer suggestion (page 14).

Minor points: Abstract: - Aim: it is better to say "to investigate the relation of two different mutations to the outcome of partial external biliary diversion", as no significant other causes were studied.

We agree and have adopted the abstract according to the reviewer suggestion (page 3).

How you classify the disease status as severe and others? Please explain.

The severity of cholestasis was classified by clinical judgement by hepatology paediatricians. The clinical diagnosis PFIC was confirmed years later by genetic analysis.

Some typing mistakes: e.g. mistakes regarding usage of abbreviations; PEBD was not abbreviated at its first appearance in the abstract; on the other hand BSEP was presented in an abbreviated form in their 1st appearance. Also, abbreviation should follow the spelled out name and not the reverse (e.g. UPLC- MS/MS). In results: HEK293 was abbreviated before in the methods section of the abstract.

All these points are now corrected.

Results: line 5; "while total BS were reduced to <3% of controls". It is known that biliary BS is low in BSEP deficiency disease. So, what about the pre-PEBD biliary BS measure to say reduced to < 3%. The same for the other patient.

We regret that our phrasing is misleading: "reduced to <3%" is not related to a pre-PEBD measurement (which was not done due to lack of a sample), but it is related to the control values. We have rephrased this part of the abstract.

Introduction: - The last paragraph: don't mention the results in this section.

We agree and the sentences with aspects of the results are now omitted.

Review 4 (Reviewer 03546970):

For the article references it suggests to update some recent studies (5 years).

We have now added several more recent papers.