Peer Review File

Article Information: http://dx.doi.org/10.21037/atm-20-3747

Reviewer A:

Comment 1: The study is about PFIC3, I don't quite understand what is the point in the title to refer to the Chinese nationality of the patient, if this is the case, it is not at all addressed in the manuscript.

Reply 1: Thanks for your careful review and comments on our manuscript. We have removed "Chinese" from the title.

Changes in the text: We have modified our text as advised (see Page 1, line 4).

Comment 2: The term proband is not well understood and should be replaced by "patient" in the manuscript.

Reply 2: The term "proband" has been replaced by "patient".

Changes in the text: We have modified our text as advised (see Page 7, line 137; Page 8, line 165, line 179; Page 10 line 217; Page 11, line 233, line 237; Page 12, line 264; and the title of Table 1, Figure 4).

Comment 3: Concerning the name of the gene, from now on we use ABCB4 and not MDR3, the name must be corrected in the manuscript.

Reply 3: The gene name has been corrected to "ABCB4".

Changes in the text: We have modified our text as advised (see Page 9, line 192, line 194; and the title of Figure 6).

Comment 4: For ease of reading the manuscript, authors may wish to retain only the protein numbering of the mutated amino acids L852P and V1051A rather than the full designation c. T2525C.

Reply 4: Thanks. We used the full designation of the mutations when it first appeared in the manuscript and present it with amino acid changes elsewhere.

Changes in the text: We have modified our text as advised (see Page 4, line 87; Page 9, line 186, line 187; Page 10, line 203, line 204, line 214-215; Page 11 line 236-237; Page 12, line 254, line 256; Page 13, line 290; Page 14, line 303).

Comment 5: Page 4/ line 75, authors used the term "correlate" but PFIC3 is caused by ABCB4 variation.

Reply 5: We changed the term "correlate with" to "cause".

Changes in the text: We have modified our text as advised (see Page 4, line 75).

Comment 6: Page 5/ line 102 authors should add the presence of cholesterol crystals that induce intrahepatic cholestasis in the effects related to the absence of PC in bile.
Reply 6: Thank you. We added the presence of cholesterol crystals that induce intrahepatic cholestasis in the effects related to the absence of PC in bile.
Changes in the text: We have modified our text as advised (see Page 5, line 109).

Comment 7: Page 5/ line 107, PFIC3 is characterized by a rise in GGT, it allows by biochemical analysis to quickly orientate on this type 3 of PFIC.
Reply 7: We modified the sentence as" Elevated gamma-glutamyl transferase (γ-GT) level is regarded as a serological feature in patients with PFIC3".
Changes in the text: We have modified our text as advised (see Page 5, line 114; Page 6, line 115).

Comment 8: Page 5/ line 110-111 authors must add a reference for the sentence "To date.....mutations".
Reply 8: We added a reference for the sentence "To date.....mutations".
Changes in the text: We have modified our text as advised (see reference 9).

Comment 9: Page7 /line 138, the authors announce a portal vein stenosis with reference to figure1, CT should be more implicit and bear the full name of the technique used: Contrast-enhanced computed tomography.
Reply 9: We have modified "CT" to "Contrast-enhanced computed tomography".
Changes in the text: We have modified our text as advised (see Page 7, line 154).

Comment 10: Page 7/ line 151, the authors state that the patient's mother underwent cholecystectomy, did she have ICP in all three pregnancies?
Reply 10: Thanks. We contacted the patient's mother and she did not have ICP in all three pregnancies.
Changes in the text: We added it to the manuscript (see Page 8, line 176).

Comment 11: Page 8/ line 159, the authors identify ABCB4 mutations, it is necessary to specify which ABCB4 isoform is used as a reference for the analysis.
Reply 11: We used *ABCB4* isoform A for analysis.
Changes in the text: We added it to the manuscript (see Page 9, line 183).

Comment 12: Page 8/ line 167-169, the authors must specify on which abcb4 PDB structure they have worked on as well as the publication reference and which modeling program is used.
Reply 12: The structure of ABCB4 was obtained from Protein Data Bank (Primary Citation of Related Structures: 6S7P). And the mutant structures of ABCB4 were constructed by SWISS-

MODEL (https://swissmodel.expasy.org/interactive). Molecular graphics were analyzed by Swiss-Pdb Viewer 4.1.0 software.
Changes in the text: We added these to the manuscript (see Page 9, line 193-198).

Comment 13: Page 9/ line 195, the authors announce that the mutations N168N, L57L and R652G are related to PFIC3 with reference 22, this announcement must be tempered as it is only a susceptibility to the disease in the article.
Reply 13: As you mentioned, Carola et al. showed that the mutations N168N, L57L and R652G increase susceptibility to cholestasis. So, we modified the term “are related to PFIC3” to “may contribute to cholestasis”.
Changes in the text: We have modified our text as advised (see Page 11, line 230).

Comment 14: Page 11/
It is intriguing that the panel of 289 genes used by the authors to perform the analysis does not give any other variations on the other genes involved in biliary pathologies except for ABCB4. Can the authors confirm that for all the patients tested, patient and family, there are no mutations in the genes ABCB11, ABCG5, ABCG8? This could explain the differences in siblings on the appearance or not of biliary symptoms from a clinical point of view.
The fact that there are no mutations in the other genes is imperatively to be reported in the manuscript.
If only patient II-1 was tested for all 289 genes and the family only for ABCB4, this should be stated in the manuscript.
Reply 14: We tested the liver metabolism gene panel for the patient (II-1), but for his family members, only the *ABCB4* gene was tested. As you said, This could explain the differences in siblings on the appearance or not of biliary symptoms. However, considering the cost and the willingness of the patient's family, we did not further test other genes.
Changes in the text: We added these in the Result and Discussion (see Page 9, line 201-202; Page 14, line 299-301).

Comment 15: Do the authors confirm that the consents of all family members were obtained for this study?
Reply 15: We obtained the consent of all family members and got informed consent.
Changes in the text: We uploaded the informed consent.

Comment 16: There is a major problem with the figure number in the manuscript. Figures 4 and 6 are not present in the text, so it is essential to check and correct these errors.
Reply 16: Sorry, there is an error in the figure number in the manuscript.
Changes in the text: We revised the figure number in the manuscript (see Page8, line 169; Page 9, line 185; Page 10, line 205).

Comment 17: Table 1

The addition of a reference column of individuals with the standards usually found for these different parameters is essential to enhance the value of the analyses.

Reply 17: We added the reference range of the indicators in Table 1.

Changes in the text: see Table 1.

Comment 18: Table 2

The authors should test the mutations using the commonly used mutpred2 algorithm and present the results in Table 2.

Reply 18: OK. We have used mutpred2 to predict the pathogenicity of mutations.

Changes in the text: see Page 9, line 85; and Table 2.

Comment 19: Figure 3

The C-image is blurred, is it possible to find better image for the figure 3-C?

The authors measure ductopenia and ductular proliferation by K7, the correct name is CK-7 and must be corrected.

There are no materials and methods in the article, for this figure it is necessary to add a team reference for the manipulations of figure 3.

Reply 19: Thanks. We uploaded a clearer image for the figure 3-C and modified "K7" to "CK-7". We have added a reference to the handling and staining of liver specimens.

Changes in the text: see Page 7, line 158; Page 8, line 159; Figure 3 and reference 12.

Comment 20: Figure 4

There is a figure number error in two places in the text, page 8/ line 172-174, it is figure 6 and not 2 which refers to gastroscopy, it must be corrected.

Two individuals II:4 and III:1 are presented in the figure but absent from the text of the manuscript, is it planned to perform the sequencing? and to perform a follow-up of the patient III:1?

Reply 20: Sorry, there is an error in the figure number in the manuscript. We revised the figure number in the manuscript. As you said, we plan to sequence the *ABCB4* gene for II:4 and III:1 and follow up III:1. However, given the cost and willingness of the family members, we have not yet implemented it.

Changes in the text: We revised the figure number in the manuscript and added that "The patient and his family need continued follow-up." (see Page 8, line 169; Page 14, line 299-302).

Comment 21: Figure 5

It is imperative to specify in the legend the pdb number of the structure as well as the modeling program used.

Reply 21: The structure of ABCB4 was obtained from Protein Data Bank (Primary Citation of Related Structures: 6S7P). And the mutant structures of ABCB4 were constructed by SWISS-MODEL (https://swissmodel.expasy.org/interactive). Molecular graphics were analyzed by

Swiss-Pdb Viewer 4.1.0 software.

Changes in the text: We added these in the legend of Figure 6.

Comment 22: Figure 6

There is an error in the figure number, page 8/ line 160, it is figure 4 and not 2 which refers to gastroscopy, it must be corrected.

Reply 22: Sorry, there is an error in the figure number in the manuscript.

Changes in the text: We revised the figure number (see Figure 5).

Reviewer B

Comment 1: Have these specific mutations been reported elsewhere in other patients and if so what findings have been reported? If not, better context could be provided on how these two fit within the range of pathologies of different commonly studied mutations.

Reply 1: Thanks for your careful review and comments on our manuscript. The mutation L842P is located in TMD, while V1051A is located in NBD. At present, the pathogenesis of these two mutations remains unclear. We speculated that L842P, similarly to the reported mutations P726L, S346I and A286V in TMD, may interact with residues in the core of ABCB4, resulting in impaired function. Likewise, variation V1051A may cause ATP binding disorder, which is essential for ABCB4-mediated PC extrusion, in a manner similar to mutation S1076C located in NBD.

Changes in the text: We added these in the Discussion (See Page 12, line 255-261).

Comment 2: Sufficient explanation of the software analysis is lacking. The conclusion of the high conservation of the two amino acids could be better explained, as could a hypothesis for the contradicting result from InterVar.

Reply 2: Thanks. We further discussed the analysis results of the software, including the conservation of amino acids and the contradictory results of InterVar. InterVar is a semi-automatic bioinformatics tool. It is helpful for clinical interpretation of genetic variants by algorithms different from other software. This may account for, at least in part, the difference in predictive results. In addition, PhastCons and PhyloP indicated the high conservation of these two amino acids, suggesting that these two mutations have a great impact on the function of proteins.

Changes in the text: See Page 11, line 241-247.

Comment 3: Were the genetics of any other family members analyzed besides the parents and 2 sisters? Possibly with a heterozygous mutation or homozygous wildtype ABCB4?

Reply 3: We tested the *ABCB4* mutation of the patient's parents and two sisters. Given the cost and willingness of the family, we did not sequence the *ABCB4* gene for II:4 and III:1.

Comment 4: Blood analysis following treatment with UDCA would be of value.

Reply 4: Thanks. After therapy, the boy stayed in a relatively stable state with mild itching, and elevated γ-GT exhibited a remarkable decrease (Table 1).

Changes in the text: We added these in the text (see Page 8, line 163-164).

Comment 5: Be careful about the sex of pronouns (use "his" instead of "hers" when referring to the boy).

Reply 5: Sorry, there is an error in the sex of pronouns in the manuscript.

Changes in the text: We revised the sex of pronouns (see Page 4, line 80; Page 10, line 204).