Author's response to reviews

Title: Localization of ABCG5 and ABCG8 proteins in human liver, gall bladder and intestine

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Author's response to reviews: see over
The reviewers’ comments are in italics and a detailed response to each concern is addressed below it.

**Response to concerns of Dr. Victor Ling**

We would like to thank Dr. Ling for his careful and thoughtful review of our manuscript.

**Major Essential Revisions**

1) The authors should provide more detail on page 6 as to how the peptides used for raising antibodies to G5 and G8 were chosen…? What evidence is there that these antisera do not cross-react with each other…? The authors also need to assure the reader that the antisera do not cross react with other proteins in the western blots…

More detail has been added as to the selection of the immunogen peptides in the results section. The immunogen peptides were not selected to be human specific but rather they were selected based upon being outside of any predicted transmembrane domain. The peptide for ABCG5 is a conserved sequence between human, mouse and rat. We cannot at this time explain why there is low immunoreactivity in the mouse and none in the rat. This may be due to differences in post-translation modification of this protein between species. The peptide for ABCG8 is specific to the human and thus explains the lack of immunoreactivity seen in the mouse and rat.

To address the issue of cross reactivity between ABCG5 and ABCG8, the two respective peptide sequences are completely different. Additionally, in tissue culture experiments they do not cross-react with heterologously expressed protein. Based upon the predicted sequences of the immunogen peptides, the differences seen by western blotting/fractionation and immunohistochemistry, we are fairly confident that there is little if any cross reactivity between anti-ABCG5 and anti-ABCG8.

To address the issue of cross reactivity with other proteins, specifically higher molecular weight proteins, we have not detected any thus far. The western blots shown are cropped (secondary to Publishers requests) but we can assure the reviewer that the bands shown are the only bands detected. There are no bands above the 150 kDa marker. Below the reviewer will find a representative uncropped image (top to bottom of gel) from the first two western blots from figure 1 panel A, for inspection.

![Western Blot Images](image)

2) The conclusion that G5 and G8 co-localized with each other and function as heterodimers (bottom of page 10) is not as strongly supported by the data as it could be. The conclusion in Figure …. In Figure 1A, G5 "peaked" in fraction 10 while G8 "peaked" in fraction 9 and, in Figure 1B, …. Thus, statements such as those on bottom of page 10 need to be modified. While the immunohistochemical staining data are convincing that different staining patterns are observed for G5 and G8 (as shown in Figure 3…. The discussion needs to be revised in light of these comments.
The text has been altered in the results section to reflect that the overlap of ABCG5 and ABCG8 in the fractionation experiments suggest co-localization. Additionally, the discussion has been altered to reflect this.

3) The blot for MDR1 in Figure 2 is poor. If C219 antibody is used...

We agree that the blot provided is not the best quality. Unfortunately, we have been unsuccessful in obtaining good signals/detectable bands with either anti-human BSEP (provided by Bruno Stieger) or anti-MRP2 antibodies (from either Chemicon or Alexis) on our blots. Since the anti-MDR1 (C219) antibody is the only antibody that “works” it was provided as part of the data. The methods section has been altered to reflect that this antibody also detects MDR2/3. Additionally, in the discussion the text has been altered to indicate that the antibody used is an apical marker instead of a bile canalicular marker.

Response to concerns of Dr. Folkert Kuipers
We would like to thank Dr. Kuipers for his review of our manuscript.

Minor Essential Revisions
page 12, line 10: “In the liver, cells lining the hepatobiliary tracks (should be "tracts"). Sentence apparently not yet finished ... 

We thank the reviewer for picking up this error. The text has been edited to read “tracts” instead of “tracks.” Additionally, the following sentence has been edited for completion.

Discretionary Revisions

Presence of ABCG8 alone in bile ductular cells is surprising but not further commented on-do the authors have any suggestion for a physiological function?

At the present time we do not have an explanation for this, but based upon the current rodent models of sitosterolemia there may be a functional difference for Abcg5 and Abcg8 in the liver and intestine. At this stage there is insufficient evidence to be categorical about this.