Author’s response to reviews

Title: The rat STSL locus: characterization, chromosomal assignment, genetic variations in sitosterolemic hypertensive rats

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We thank the reviewers for their inciteful and helpful comments.

Response to Dr. Aitman’s review
We thank Dr. Aitman for his critical comments.

Major comments

1. There is no linkage data that links sitosteroleemia to chromosome 6....

We completely agree that this is the best evidence that we could have put forward to make our case that the mutation in Abcg5 is responsible for the sitosteroleemia in the mutant rat strains. To accomplish this within a reasonable space of time, we would need to carry out extensive breeding and backcross analyses, almost to the point of generating congenic lines, a simple process that requires a large amount of time and resources. Congenics are clearly important and it will be necessary to isolate such animals, if we are to study the pathophysiology (as there are no 'strain' controls). However, given the premise of this manuscript, the considerable data presented that support the genetic characterization, biochemical characterization and the various strains analyzed, we feel we have made a sufficiently strong case to demonstrate that the mutation in Abcg5 is likely responsible for the sitosteroleemia. Additionally, the extensive characterization of the human disease, STSL mutations and now a published mouse KO model would all support the notion that the STSL locus regulates the plasma sitosterol level. We completely agree that both segregation and mapping data will prove this case. These studies are underway, but will take time to complete and should be part of future studies.

2. There is no biochemical data to indicate missense mutation in codon 583 alters function...

Again, we completely agree. We had discussed this in our Discussion and in several places and were deliberate about our lack of a functional assay. But we will point out that despite the extensive literature on role of the STSL locus on both causing sitosteroleemia in human and how this locus
regulates sterol absorption and secretion, there is no direct assay for the functions of these proteins. It is not for the lack of trying. Many research groups are currently actively pursuing this problem, with little progress. We have no direct biochemical assay for any of the humans mutations we have extensively reported on. Given the information presented in this manuscript, we believe we have made a strong case. Had we direct biochemical evidence, we would jumping with joy and our hearts would soar like eagles. As it is, our struggle to develop an assay continues.

3. The possibility the mutation is an epiphenomenon.....

This criticism, though a little harsh, is unfortunately also true and is, in part, related to the first point that the reviewer makes. We agree that linkage would have allowed us to address this point and as stated earlier, we hope that our future expts. will address this. However, we respectfully suggest that these studies constitute a separate study and our current study to highlight an important observation in a widely utilized rat is of considerable importance. Furthermore, although the reviewer asks us to discuss the potential effect of the missense mutation on protein structure or activity, we can not address the latter. However, we now show new immunofluorescence data (Figure 9) to suggest that the missense protein is expressed and its apical targetting in the intestine is not different to that seer in SD rats. The Methods and the text have been appropriately altered.

Minor Criticisms

1. in qPCR, use of GAPDH as a control

Dr. Aitman makes a very valid point. However, even though GAPDH is likely to be variable, so is almost any single gene target chosen. In our experience, the commonly used 18S RNA as standard is inappropriate due to abundance issues. We have shown (and this in part relates to the Reviewer 2 point) that the liver protein expression patterns for SD, SHR etc. are very different. This emphasizes the need for congenics (again a long process that is underway). We examined the Ct cycles for GAPDH and this is shown below.

Among strains (Sprague-Dawley, SHR, WKY and Wistar) the GAPDH expression level is the same +/- 0.5 Average Ct. Mean is 20.1

Strain Average Ct GAPDH (n=3)
SD 20
Wistar 19.5
WKY 20.5
SHR 20.2

This is about as good as one may be able to attain, until congenics are available.

2. The Commassie stain......

We deliberately and purposefully stressed the differences we observed in the manuscript and drew the readers' attention to this. It is important to recognize that we need to be cautious in comparing SD, Wistar, SHR liver protein expression based upon total protein amounts as the PAGE shows the pattern of protein is not comparable. We chose transferin as a control as we needed a membrane protein that theoretically should be affected. We could delete these data, but then the reader would not be exposed to the very important point that the protein expression patterns are also different. We prefer that the RNA expression data not be mis-interpreted. Again, congenics will help.

However, we also hope that the lack of congenics and 1 cM linkage data do not preclude publishing our very important manuscript.
Response to Dr. Pravenec's review
We thank Dr. Pravenec for his critical comments.

Major comments

None, and we thank him for this.

Minor Criticisms

All of the typographical errors, as well as the textual changes listed, have been corrected and we do not list these. We thank him for improving our manuscript and educating us about the very complex strain origins.

However, we have deviated a little from point 1, that the WKY rat is 'not hypertensive'. We agree that WKY does not exhibit similar levels of blood pressure that SHR/SHRSP/GH do. However, in comparison to SD and Wistar, the BP readings are consistently higher. Since BP is a continuum, whether any given level of BP is classified as hypertension or not depends upon the cut-off. We would draw Dr. Pravenec's attention to the paper by some of us (Ikeda I, Nakagiri H, Sugano M, Ohara S, Hamada T, Nonaka M, Imaizumi K: Mechanisms of phytosterolemia in stroke-prone spontaneously hypertensive and WKY rats. Metabolism 2001, 50:1361-1368) where WKY shows a consistently higher BP level upon aging. We maintain that WKY is hypertensive, but does not have severe hypertension.