Dear Editor,

Thank you for providing us with the opportunity of revising this manuscript. I appreciated the reviewers' careful analysis of our manuscript and their critiques. In response to reviewers' comments and suggestions, I have revised the manuscript. Major revisions made (shown in BLUE in the manuscript) are as follows:

1) Addition of "effects of CaSR-/-" paragraph discussing the phenotype of the absence of the CaSR gene.
2) Addition of Table 2 summarizing the known CaSR activators as antidiarrheals.

In addition, the following documents are attached:

1) Signed Conflict of Interest statement is provided in a PDF format, is also mentioned as a footnote in the manuscript text.
2) Figure 1 in a ppt format is also provided.
3) Audio core tip.
4) First pages of refs 3 and 73.
5) Copyright assignment
6) Google Scholar

Enclosed below are my responses to reviewers' specific comments and questions.

Yours sincerely,

Sam Cheng, MD, PhD

Reviewers' Comments:
Reviewer #02821664

"This review by S. Cheng focuses on the role of calcium-sensing receptors (CaSR) in diarrhea. Clearly, diarrhea is a leading cause of mortality and morbidity, especially in developing countries, and the cost and effect of diarrhea deserve urgent attention. The need for additional therapies is highlighted in this review, given limited efficacy and feasibility of current approaches, especially in children, who are most vulnerable to acute diarrhea. The review does an excellent job of explaining the pathophysiology of diarrhea and how different pathways, mechanisms, and

treatments affect this. Most of the review focuses on CaSR. First, the roles of the protein are described, with specific focus on effects on intestinal transport. Detailed studies focused on the role of CaSR in secretion, absorption, specific ion channels, and the enteric nervous system are then presented. Effects on gut inflammation and barrier functions as described as well. Finally, clinical experience using Ca to treat diarrhea in mice and humans is described, together with discussing the translational potential of this pathway. The review is very timely, well written, and provides recent data on CaSR, which I'm sure many of the journal's readers know little about. However, there are a few points that require further attention.

General comments:

1. CaSR certainly seems to have an impressive array of effects on many intestinal aspects leading to diarrhea. The review includes detailed descriptions of various channels and pathways affected by CaSR but most of these studies are based on observations from cell lines or use of Ussing chambers. There is a need to highlight these limitations and the fact that studying these effects in isolation is artificial. A few mouse studies are mentioned, but these are mainly related to the inflammatory interactions of CaSR. It would be useful to have a description of the phenotype of CaSR-/- mice in a separate section, in order to better define the net effect of the absence of this gene. Do these mice spontaneously develop diarrhea?"

Author's response: Thank you for your careful review and comments on this manuscript. In response to your suggestion, I have now added additional section "Effects of CaSR-/-" in page 17 of the revised manuscript highlighting the limitations of currently available studies in vitro in isolated tissues and summarizing the known phenotype of CaSR-/-. Clearly, more in vivo studies at the organismal and systemic levels are needed in order to better define the net effect of the absence of the gene. These studies are under way.

"2. Are there any reports associating SNPs in CaSR for diarrheal conditions? Most of the reports I could find just showed correlation with Ca serum levels and kidney stones. One would expect that polymorphisms or mutations in such a profoundly involved molecule would lead to clinical conditions. This suggests redundancy with other pathways, which needs to be discussed."

Author's response: To my best knowledge, there has been no report associating SNPs or mutations in CaSR for diarrheal conditions. While this suggests redundancy with other pathways, it may also imply that the multiple confounding factors that occur as a result of CaSR mutations or polymorphisms in these individuals may

counteract the action of CaSR and prevent spontaneously developing diarrhea symptoms at steady state. In this regard, it is believed that use of conditional CaSR, rather than global, knockouts would be more helpful. We are performing studies to characterize these animals both under basal and challenged conditions with and without CaSR activators and inhibitors. This has now been discussed in pages 18-19 of the revised manuscript.

"3. Page 18 – Figures 2 & 3 are mentioned but they do now appear anywhere in the review."

Author's response: This was a typo error, which has not been corrected.

"4. Page 22 - use of calcium in animal and human studies is indeed well-established to protect against diarrhea but this ion has diverse physiologic roles that need to be considered. How would one go about confirming the specificity of Ca effects to CaSR?"

Author's response: Thank you for pointing it out, which has now been discussed in the revised manuscript (page 16; also in pages 18-19).

"Specific comments:

1. Page numbering starts at page 5."

Author's response: Page numbers are corrected.

2. Page 9 (of the author's order), last paragraph: the sentence 'Consequently, ORS does not offer a rapid relief of diarrhea symptom' should be toned down; the author makes a good case for the limitations of ORS but it still does have an important role.

Author's response: I agree that ORS still does an important role in the management of acute diarrhea. This fact has been emphasized in the section "The success and failure of ORS". To be consistent, I have now followed your suggestion and toned down the sentence (page 5).

3. Page 11, last paragraph: 'While anti-microbial is useful in some cases' – either change to 'anti-microbials are…' or to 'anti-microbial therapy is…'

Author's response: It is corrected. Thanks.

4. Page 20: there are many ways of classifying mechanisms of diarrhea. The author proposes secretory and diarrhea, but there are also osmotic and mixed causes that should be mentioned.

Author's response: Thanks for having pointed it out, which has now been mentioned in page 16 and discussed in Figure 1 legend.

5. Page 21, bottom of page: I would not say that intestinal CaSR is a mechanism; rephrase.

Author's response: This sentence has been rephrased as follows: "intestinal CaSR is an antidiarrheal GPCR in the gut" (page 19).

Reviewer #01559576

"The author discusses modulations of CaSR as anti-diarrheal therapies. Since these modulators seem promising agent, the summary table(s) informing names of agents, efficacy and adverse events (if any) should be provided."

Author's response: Thank you for your review of this manuscript. In response to your suggestion, I have added additional Table 2 in the revised manuscript summarizing the CaSR-activating substances used in the literature, their efficacy and adverse events.