

March 18, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 8927-revsed.doc).

**Title:** Association of caveolin-3 and cholecystokinin A receptor with cholesterol gallstone disease in mice

**Author:** Guo-qiang Xu, Cheng-fu Xu, Hong-tan Chen, Shan Liu, Xiao-dong Teng, Gen-yun Xu, Chao-hui Yu

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 8927

The manuscript has been improved according to suggestions of the reviewers and editors. The following are our responses to the reviewers' comments.

**Replies to Reviewer No. 00053888:**

**Comment 1:** This is an interesting and well written manuscript addressing some known and some postulated causes for the production of cholesterol gallstones in an animal model. This is an interesting piece of work but we wait to see whether this is relevant to humans and secondly whether there is any therapeutic intervention that can prevent these effects.

**Reply:** Thanks very much for your positive comments and valuable suggestions!

We agree that it would be very interesting to further investigate whether knocking down caveolin-3 or CCKAR expression aggregates lithogenic diet-induced cholesterol gallstone formation, and whether overexpression of caveolin-3 or CCKAR

expression inhibits the formation of cholesterol gallstone. We will take this into consideration in our future studies. We have added this information in the Discussion section on page 9, line 21-23.

Page 9, line 21-23: Further study is also needed to clarify whether our findings are relevant to humans, and whether there is any therapeutic intervention that can prevent these effects.

**Comment 2: There are a small number of grammatical errors that requiring addressing e.g. paragraph 1 - prevalent not prevent & para 3 residence time is meaningless. There are others but these are examples.**

Reply: Thanks very much for this comment! The English expression has been improved by a native English speaker recommended by Medjaden Bioscience Limited.

**Replies to Reviewer No. 00053417:**

**Comment 1: The roles of caveolin-3 and cholecystokinin A receptor (CCKAR) in cholesterol gallstone (CGD) have been documented in animal and human studies in literature. In this manuscript, the authors investigated the potential involvements of caveolin-3 and CCKAR in CGD animal model. The scientific contents (design and methods of the study) are good. As the related work has been published, it is needed to introduce the innovation of the research. It is suggested to update the information in the sections of introduction, discussion and references to find the points of innovation.**

Reply: Thanks very much for your positive comments and valuable suggestions!

We have added some information to explain the innovation of this study in the Introduction and Discussion section of revised manuscript. Related references are also added.

Page 5, line 3-10: A recent *in vitro* study observed that CAV3 was involved in the regulation of gallbladder muscle hypomotility [18]. Knockdown of CAV3, mediated via small interfering RNA, decreased contraction of gallbladder muscle cells isolated from guinea pigs, and increased cholecystokinin A receptor (CCKAR) in the caveolae [18]. CCKAR is a major physiologic mediator of smooth muscle contraction of the gallbladder. Lack of CCKAR may deteriorate gallbladder contraction and enhance gallstone formation [19]. Polymorphism of *Cckar* gene was associated with gallstone in human patients [20, 21].

Page 5, line 11-13: To date, the *in vivo* association between CAV3 and CGD has not been fully elucidated, and whether CCKAR is differentially expressed during the process of cholesterol gallstone formation remains uncertain.

Page 9, line 8-10: Besides its role in cholesterol metabolism, CAV3 was also observed involved in regulation of gallbladder muscle hypomotility *in vitro* [18]. However, the *in vivo* association between CAV3 and CGD is not fully understood.

Page 9, line 14-17: One-third of CCKAR(-/-) mice spontaneously developed gallstone disease at 12 and 24 months of age [19]. Polymorphism of *Cckar* gene in patients was also an independent risk factor for gallstone disease [21]. However, whether CCKAR is differentially expressed during the process of CGD formation remains unclear.

**Comment 2:** Now, the references are old, only two (No. 17, 18) were published within the last 5 years. These two new references are not directly relevant to the topic of this manuscript.

**Reply:** Thanks very much for this comment! We have updated the references and added some more recent related publications in the revised manuscript.

**Replies to the editor:**

**Comment 1: Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B). For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies mentioned in ‘The Revision Policies of BPG for Article’.**

**Reply:** We have attached a language certificate provided by the professional English language editing company that assisted with the language editing of this manuscript.

**Comment 2: It’s too short. Please add it to 80 words. Thank you!**

**Reply:** We have rewritten the Methods section of the Abstract (page 3).

**Comment 3: RESULTS (no less than 120 words): You should present P value where necessary and must provide relevant data to illustrate how it is obtained, e.g.  $6.92 \pm 3.86$  vs  $3.61 \pm 1.67$ ,  $P < 0.001$ .**

**Reply:** We have rewritten the Results section of the Abstract (page 3).

**Comment 4: Please provide the “Highlighted contents” here, which is a necessary content.**

**Reply:** We have provided the “Highlighted contents” in the revised manuscript.

**Comment 5: At least 26 references should be included.**

**Reply:** Thanks very much for this comment! We have updated the references and added some more recent publications in the revised manuscript.

**Comment 6: The coordinate graphs supplied should be decomposable (each part of your figure could be moved so as to easily edit). You can send it as excel, word or powerpoint format so that I can edit them easily.**

**Reply:** We have modified the Figures accordingly, and uploaded as a single PPT file.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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