Response to reviewer's comment

Modernising autism spectrum disorder model engineering and treatment via CRISPR-Cas9: A gene reprogramming approach.

Manuscript number: 82809

We want to thank the reviewers for their suggestions and comments, and after a detailed analysis of the comments, all of them were carefully reviewed, answered, and highlighted in red colourvin the main manuscript.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors: This manuscript has reviewed the establishment and treatment of autism spectrum disorder (ASD) model by CRISPR-Cas9 system.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Very interesting and informative review manuscript on an emerging topic in ASD research. The manuscript is well, concisely, and coherently organized and presented. Figures help in understanding the text.

Reviewer #3:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: I have minor edits in the article (see attached)

Company editor-in-chief

Please prepare and arrange the figures using PowerPoint to ensure that all graphs orarrows or text portions can be reprocessed by the editor. In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if

the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the reference source and copyrights. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023

All responses have been marked in red color.

Query 1:The major problem is that these two subject presentation in the title is not proper, and author should show their inner connections in this manuscript. Maybe author can demonstrate the activation and inhibition functions of CRISPR-Cas9 system for the same object via different aspects. In addition, author need provide the similar genes including SHANK, CHD8, MECP2, et al between the establishment and treatment fields of ASD.

Response: First of all**,** we have tried to explain the use of CRISPR-Cas9 technology in establishing various ASD models by making changes in their DNA structure either by deleting or adding base pairs, changing sequences and ultimately bringing mutations. The inhibition function of CRISPR could be explained through the models used in the manuscript such as KNOCKOUT models in which firstly, particular gene has been recognized using sequencing and then knocked out using CRISPR-Cas9 technology. We have summarized the use of CRISPR-Cas9 in modeling of ASD as well as for the therapeutic purpose in Conclusion section on page no. 19 and 20. Also, activation function of CRISPR could be explained through studies showing methods like CRISPR activation (CRISPRa), which have been added in therapeutic part on page no. 16 and 17.

In the therapeutic section, we have tried to include genes based on the available literature. Unfortunately, only few studies targeting genes such as *MECP2, SHANK2, UBE3A, FMR1, ITGB3, dip2a and dip2c, MEF2C, CNTNAP2, and SCN2A* have been documented so far for the therapeutic purpose.

Query 2: The structure of this manuscript seems difficult to read. The introduction is too short to hard achieve the content of full manuscript. But the descriptions of Genetic architecture of ASD as well as structure and function of CRISPR-Cas9 are too long to mislead the theme of this manuscript.

Response: We have tried to tried to improve the manuscript based on your valuable comments. The introduction part has been elaborated on page no. 5 and 6 with the addition of some valuable information. Also, we have reduced the content from the section “genetic structure of ASD” on page no. 6 and 7 as well as from “structure and function of CRISPR-Cas9” present on page no. 7,8 and 9.

Query 3:The related genes of figure 1 should be simplified because they are repeated in table1. And CRISPR-Cas9-based therapeutic strategies and potential targets genes should be added in the other table.

Response: We have tried to simplify the figure 1 on page no. 30. Only potential novel therapeutic targets have been added in the figure 1. Also, table presenting CRISPR-Cas9-based therapeutic target genes (Table 2) has been added on page no. 33 and 34.

Query 4: The grammar of this manuscript should be corrected, especially in the presentation of singular and plural, use of conjunctions.

Response: We have tried to improve the manuscript according to your suggestions regarding the grammar.

Query 5: In the paragraph "CRISPR-Cas9 engineered organoids of ASD", authors could better address the lack of suitable ASD models.

Response: We have added “ASD” in the paragraph "CRISPR-Cas9 engineered organoids of ASD" on page no. 12

Query 6: In the Conclusions, more limitations on the use of CRISPR technology in clinical trials could be added.

Response: In the conclusion section, we have added more limitations on the use of CRISPR technology in clinical trials on page no. 19 and 20, as advised by you.

Query 7: Check acronymusfrist time used. - Check English spelling.

Response: We have revised the manuscript accordingly.

Query 8: If the picture is ‘original’, the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2023

Response: Thank you for your feedback. We have made the necessary changes to the picture by adding the copyright information to the bottom right-hand side of the picture in PowerPoint (PPT). The information now reads ©Sandhu et al. 2023. I believe this meets the requirement for original content and proper attribution

Round 2

Specific Comments To Authors: The revision manuscript of title "Modernising autism spectrum disorder model engineering and treatment via CRISPR-Cas9: A gene reprogramming approach" has been progressed better than previous manuscript. But the main problem of two subject presentation in the title always exists. The best solution is suggested to change the title into "Modernising autism spectrum disorder model with engineered applications via CRISPR-Cas9: A gene reprogramming approach" of this revised manuscript. Some sentences must be corrected as followings: 1 Plethora of studies is being conducted worldwide using several targets in cultured cells or in animal models, however, their extrapolation to the patients is yet to achieve.(page 15) 2 Among these gnomic editing tools, CRISPR-Cas9 is being considered the most extensive and effective, with the advantages of low mutation rate, high target efficiency and moreover, the cost of its development is very low. (page 16) 3 CRISP has enabled the creation of models that reproduce exactly the same causal mutations identified in patients, which has made it possible to determine an appropriate and disease-specific drug therapy. (page 16) 4 Thus, creating a reliable model, establishing a causal factor and representing all the characteristics of the disease is difficult. (page 16)

Reply to Comments Specific Comments to Authors: The revision manuscript of title "Modernising autism spectrum disorder model engineering and treatment via CRISPR-Cas9: A gene reprogramming approach" has been progressed better than previous manuscript. But the main problem of two subject presentation in the title always exists. The best solution is suggested to change the title into "Modernising autism spectrum disorder model with engineered applications via CRISPR-Cas9: A gene reprogramming approach" of this revised manuscript. Reply: The title has been changed accordingly. Some sentences must be corrected as followings: 1 Plethora of studies is being conducted worldwide using several targets in cultured cells or in animal models, however, their extrapolation to the patients is yet to achieve.(page 15) 2 Among these gnomic editing tools, CRISPR-Cas9 is being considered the most extensive and effective, with the advantages of low mutation rate, high target efficiency and moreover, the cost of its development is very low. (page 16) 3 CRISP has enabled the creation of models that reproduce exactly the same causal mutations identified in patients, which has made it possible to determine an appropriate and disease-specific drug therapy. (page 16) 4 Thus, creating a reliable model, establishing a causal factor and representing all the characteristics of the disease is difficult. (page 16) Reply: All the sentences have been corrected (red colour)