Peer-review Comments and Author Responses

Reviewer 1

1. *Abstract: Please review the wording. The authors should not mention precision medicine here because this term encompasses many different aspects that this Mini-Review does not cover.*

We agree with your comment that a review of the wording is necessary to strengthen the scope of this mini-review. We accepted your suggested changes and further improved the structure and content of the abstract.

1. *Background: indicate here this is the reason why you have chosen to review only monogenetic obesity. Or are there studies on polygenic obesity that the authors did not review?*

In this paragraph, we want to address the challenges of polygenic obesity treatment. The scope of the review is not limited to monogenic obesity. However, most of the studies focus on a single target strategy. We specified this and added the part from the Discussion section, as suggested in the comment.

1. *Since this is a Review, please delete "extensive literature search". In addition, the Review does not address either feasibility or safety. Of note. At least safety should have been included. A review addressing only efficacy without addressing safety is considered incomplete or biased.*

We agree that the wording does not describe the scope of the review. We therefore accept your changes. You addressed your reasonable concerns regarding the safety evaluation. We added available information on the included studies in Table 1. We have added a small section in the results discussion summarizing safety data. This mini-view analyzes the safety and efficacy of the current developments in genetic obesity treatment over the last 5 years, including current preclinical approaches.

Results section: Moreover, all included studies do not report limitations due to severe toxic reactions (Table 1), independent of the different target approaches used in the studies.

Importantly, all studies had a small sample size that is reasonable for the early stages of development. However, all included studies had appropriate control groups that increased the validity of the data.

1. *Please rewrite the sentence highlighted in yellow – were there non-important treatment approaches not included? How did the authors decide? In blue: it is not clear why a table is mentioned here. Just inform that the results were cross-checked. In green: correct to WAS.*

Thank you for your advice on this part. We rewrote this part and added your suggestions in this section. The search procedure was verified and performed by different authors. We used the search terms for PubMed and the ClinicalTrials register. We identified some studies and topic-related search results cross-checked to fulfill the eligibility criteria defined. Bias was assessed using the SYRCLE risk of bias tool for animal studies (Hooijmans et al., 2014). Moreover, relevant safety information was searched. Each paper was double-checked by at least two authors. Any disagreement with the inclusion of a specific paper in the analysis was solved by discussion with a third author.

5. *Rewrite the sentence highlighted in yellow – it is not clear. It is expected that all studies will be extracted (blue). The sentence written in green is a result and should not be included here.*

The sentences were rewritten to improve the understanding of this paragraph:

The search procedure was verified and performed by different authors using the search terms for PubMed and the ClinicalTrials register. Identified studies and topics-related search results were cross-checked to fulfill the eligibility criteria defined. Bias was assessed using the SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014).

6. Since CRISP/CAS (please correct spelling) is a major genetic therapy, authors should explain why it should be considered different from other genetic ones. Also, it has been covered by several reviews – were they addressing obesity? It is not clear why they were excluded.

We corrected the spelling. We agree that it is a major therapy. However, it can be attributed to gene editing methods. Therefore, the search term would not cover the whole number of publications with CRISPR/CAS which would limit the systematic literature search. We think – due to the importance of this technology – and the varieties of gene editing technologies developed in recent years, CRISPR/CAS (or gene editing) in obesity treatment should be discussed in a separate mini-review. Including CRISPR/CAS would go beyond the scope of this review, identifying current target strategies for gene therapy in obesity. The cited reviews in the text are related to CRISPR/CAS gene therapy treatment in obesity. We specified our reasons for this decision as explained above. We hope you agree with our position. We adjusted the sentence in the method section and added additional information about this in the study selection results section.

To strengthen the scope of this mini-review, CRISPR/CAS-related gene editing therapies were excluded from the search results. This was based on the complexity and variety of methods that need to be addressed in a separate review. Limited by our search terms, gene editing was not covered completely which would limit the systematic search approach. Moreover, an overview of CRISPR/CAS-based treatments for obesity has already been covered in recently published reviews (Franco-Tormo et al., 2018; Jayachandran et al., 2023).

7. Delete "meticulous" (yellow). In blue: This mini-review is supposed to address only treatment, not the investigation of obesity-related genes. Unless authors explicitly indicate how this would result in treatment strategies.

"Meticulous" was deleted. We modified the sentence.

The resulting references encompass different gene therapy treatment approaches in different obesity animal models to study efficacy and safety.

8. Here, the authors should sort the different strategies into groups (according to the next paragraph). Are all these strategies aimed at reducing obesity? Are there studies only investigating genes associated with obesity that could be of interest in the future?

All these strategies address different targets. We grouped these approaches into I) Single gene targeting II) Multiple gene targeting and III) Enzyme targeting to provide a better overview of the currently identified strategies. The corresponding text was changed accordingly.

9. This part seems to be out of the correct place. Since CRISPR/CAS studies were excluded, they should not be mentioned here (maybe in former sections)

We removed CRISPR/CAS from the scope of our review and removed the part from the results section.

10. If there are no human studies, delete the sentence highlighted in yellow.

All these evaluations were considered in the SYRCLE risk of bias tool we used for assessment. However, we changed the order of the sentence to improve the understanding of this sentence. The risk of bias in each selected study was assessed to ensure the quality and validity of the findings. Since the search strategy included animal experiments, SYRCLE's risk of bias tool for animal studies (Hooijmans et al., 2014) was utilized for risk assessments. The evaluation considered factors such as study design, sample size, blinding, randomization, and potential sources of bias. The results of the risk assessment are presented in Figure 2.

(Moreover, the PRISM flow diagram was added as Figure 1 in the text)

11. *Indicate the number of the figure mentioned here.*

The respective Figure number was added. Moreover, the Figure order was changed and the new Figure 1 (PRISM-Flow-Diagram) mentioned in the text
The results of the risk assessment are presented in Figure 2. The evaluation of the bias was limited by the reporting of information regarding the in vivo study design

12. Discussion and Conclusion adjustments.

 Since many different small comments were suggested, we have rewritten the whole discussion part. Please see the changes marked in the text that addressed all of your comments and suggestions.

Reviewer 2

13. *It will help the reader if you clarify whether you are referring to the existing gene therapy approach or the future gene therapy approach.*
Besides environmental and lifestyle factors, obesity is also influenced by a genetic predisposition(Loos & Yeo, 2022), which is the basis for developing novel gene therapeutic approaches.
14. *This paragraph can be edited to avoid redundancy.*
 We agree that this paragraph is redundant. Therefore, it was edited and shortened.
With the increasing clinical experiences of gene therapy-based treatments in recent years and the improvement and advances in genetic evaluation and analysis. A large number of obesity-related genes have already been identified and preclinical evaluated.
15. *Ideally, the inclusion criteria should be defined a priori to reduce the risk of bias. Since the inclusion criteria were changed to increase the number of accessible papers, a clear and justified reason should be given. For example: - the initial criteria were too restrictive and you realized it excluded papers important to your research question. Or maybe a broader inclusion criteria is needed to provide a comprehensive review.*
We changed this sentence as you suggested and agree with the potential risk of bias. Preclinical in vivo animal and human studies, clinical trials, prospective and retrospective studies, and case reports were eligible for inclusion if they included gene therapy as a single or combined interventional treatment against obesity. Therefore, initial criteria were extended for preclinical studies to provide a comprehensive review. Only studies published in peer-reviewed journals published in the English language were included.

16. *Already mentioned directly under methods.*
This paragraph was deleted to avoid redundancy.

17. *This gives us more idea of why the study was extended. However, a concise sentence justifying the reason, will make it clear to the reader and justify your decision to extend the search.*
In question 2, we specified it to justify our motivation: Due to the current absence of ongoing, planned, or finished clinical trials, this minireview includes preclinical data from animal experiments. The search revealed a variety of in vivo studies, commentaries, reviews, and treatments of obesity-related diseases that were excluded according to the predefined eligibility criteria
18. *Exclusion reasons are not clear from this sentence, it can help the reader's comprehension if you clarify this a bit more.*

Following exclusion criteria were selected for the literature screening: Non-peer-reviewed articles, preprints, editorial commentaries, conference abstracts, reviews, and in vitro studies were excluded. CRISPR/CAS-related gene editing therapies were excluded. Moreover, genetic treatment approaches for obesity-related diseases/indications were excluded.

The search on the MEDLINE-Database identified 26 publications. Based on the selected inclusion and exclusion criteria, most of the studies needed to be excluded for different reasons (other indications (n=11), reviews/perspective (n=6), Preprints (n=2) only in vitro experiments (n=1), and CRISPR/CAS gene editing (n=1)).

19. Which figure? Figure 1 or Figure 2?
We changed it to Figure 2.

Results section: The results of the risk assessment are presented in Figure 2. The evaluation of the bias was limited by the reporting of information regarding the in vivo study design.

20. At the beginning pandemic was mentioned, and in the discussion you use epidemic. It can confuse the readers.
The sentence was changed based on the suggestions of reviewer one. We changed the word "epidemic" to "pandemic" in the in the sentence.

21. Is type 2 diabetes associated with polygenic obesity? If so, can it be mentioned in the introduction, or if mentioned in the conclusion, adding some context will help not catch the reader by surprise. Nicely written. However, to leave readers with a concise conclusion of your mini-review, mentioning how your mini-review shows the need or proves the need for further investigation can help bring forward the value and significance of your review.
Thanks for this helpful advice. According to MI McCarthy et al., there is a multifactorial relation between obesity and type 2 diabetes. Based on the suggestion of Reviewer 1, the conclusion was rewritten.

Reviewer 3

22. There is no need to include the PICO on the title page.
We agree and delete the PICO from the title page.

23: References should be in APA style.
We edited all the references and the citation style, including all newly used references.

24. I would include an explanation of why you restricted the search to the last five years.
Thank you for the advice to specify it in the manuscript. To address the newest developments in a mini-review, we had to restrict the time based on the high number of publications within the last decades. We inserted an extra paragraph in the results sections explaining selecting this restriction. The search was limited to the last five years to identify the newest target strategies in the rapidly growing and developing field of gene therapy. And to extend already existing reviews from the last years.

25. The last paragraph from the search strategy has information that belongs in the results section
Following reviewer 1, we moved this section to the results section.

26. In the study selection section, I would eliminate the first five sentences as they don't explain how you arrived at the final sample of articles.
We agree to modify and specify this part of the method section to avoid redundancy and to improve the structure of the method section.

27. *Figure 2 is not referenced in the text.*
As already addressed by the other reviewers, the citation of the figures was added in the main text and specified. The order of the Figures was changed accordingly. Results section: The results of the risk assessment are presented in Figure 2. The evaluation of the bias was limited by the reporting of information regarding the in vivo study design

28. *When presenting the study characteristics you should talk about the included articles. CRISPR/CAS was beyond the scope of your review, so I would exclude that. I would also expand a little more on the included studies' findings and explain a bit more about their interventions.* Following reviewer 2, we specified the reason for excluding CRISPR/CAS in the methods and considered it in the results section. Moreover, the addition of the safety information from the studies was added to the results and discussion section to improve the reporting of the included studies (see results section). To strengthen the scope of this mini-review, CRISPR/CAS-related gene editing therapies were excluded from the search results. This was based on the complexity and variety of methods that need to be addressed in a separate review. Limited by our search terms, gene editing was not covered completely which would limit the systematic search approach. Moreover, an overview of CRISPR/CAS-based treatments for obesity has already been covered in recently published reviews(Franco-Tormo et al., 2018; Jayachandran et al., 2023).