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# PEER-REVIEW REPORT

**Name of journal:** World Journal of Hepatology
**Manuscript NO:** 37312
**Title:** Homologous recombination mediates stable transgene integration and phenotypic correction in tyrosinemia mouse-model
**Reviewer's code:** 02741591
**Reviewer's country:** Egypt
**Science editor:** Fang-Fang Ji
**Date sent for review:** 2017-12-11
**Date reviewed:** 2017-12-12
**Review time:** 1 Day

| CLASSIFICATION | LANGUAGE EVALUATION | SCIENTIFIC MISCONDUCT | CONCLUSION |
|---|---|---|---|
| [ ] Grade A: Excellent | [ ] Grade A: Priority publishing | Google Search: | [ ] Accept |
| [ ] Grade B: Very good | [Y] Grade B: Minor language polishing | [ ] The same title | [ ] High priority for publication |
| [Y] Grade C: Good | [ ] Grade C: A great deal of language polishing | [ ] Duplicate publication | [ ] Rejection |
| [ ] Grade D: Fair | [ ] Grade D: Rejected | [ ] Plagiarism | [Y] Minor revision |
| [ ] Grade E: Poor | | [Y] No | [ ] Major revision |
| | | BPG Search: | |
| | | [ ] The same title | |
| | | [ ] Duplicate publication | |
| | | [ ] Plagiarism | |
| | | [Y] No | |

## COMMENTS TO AUTHORS

The title could be refined to to refer to the precise Fah gene The first 4 lines in the abstract results could better move to the background of the abstract as they do not include results found in this work. The abstract results is superficially presented and values of signficance much be presented. The introduction is nicely written The methodology is adequate but could better be wrapped up. The results should be revised and written in a way to present the reached findings values. Many parts within the results would be more sutiable for the methodology or the discussion. Discussion: In many in vitro 18 and some in vivo 19 studies.... COMMENT: Using the words 'many' and 'some' 'studies' implies that more than 2 references are cited. Rephrase the sentence to best reflect the single references cited. ....with a point mutation for FAH..... COMMENT: Unify using the abbreviation through out the article 'Fah'. Almost all the

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second paragraph of the discussion (17 lines) is not cited by appropriate references. In our serial transplantation experiments, ..... whereas only 1/5 mice injected with rAAV8-R26.Fah had FAH-positive clusters. COMMENT: The third paragraph is not cited by the required references although it refers to serial studies of the authors that should also have been cited and references presented. We could not find any tumour formation in any of our mice, ..... of cell doubling for the hepatocytes 24. COMMENT: It is unclear from this paragraph if the statements in this short paragraph refer to the present study or that of the reference cited [24].

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# PEER-REVIEW REPORT

**Name of journal:** World Journal of Hepatology
**Manuscript NO:** 37312
**Title:** Homologous recombination mediates stable transgene integration and phenotypic correction in tyrosinemia mouse-model
**Reviewer's code:** 00291404
**Reviewer's country:** United States
**Science editor:** Fang-Fang Ji
**Date sent for review:** 2017-12-11
**Date reviewed:** 2017-12-14
**Review time:** 3 Days

| CLASSIFICATION | LANGUAGE EVALUATION | SCIENTIFIC MISCONDUCT | CONCLUSION |
|---|---|---|---|
| [ Y] Grade A: Excellent | [ ] Grade A: Priority publishing | Google Search: | [ Y] Accept |
| [ ] Grade B: Very good | [ Y] Grade B: Minor language polishing | [ ] The same title | [ ] High priority for publication |
| [ ] Grade C: Good | [ ] Grade C: A great deal of language polishing | [ ] Duplicate publication | [ ] Rejection |
| [ ] Grade D: Fair | [ ] Grade D: Rejected | [ ] Plagiarism | [ ] Minor revision |
| [ ] Grade E: Poor | | [ Y ] No | [ ] Major revision |
| | | BPG Search: | |
| | | [ ] The same title | |
| | | [ ] Duplicate publication | |
| | | [ ] Plagiarism | |
| | | [ Y ] No | |

## COMMENTS TO AUTHORS

This is a very good study, well thought and controlled. The manuscript is also well written.

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# PEER-REVIEW REPORT

**Name of journal:** World Journal of Hepatology
**Manuscript NO:** 37312
**Title:** Homologous recombination mediates stable transgene integration and phenotypic correction in tyrosinemia mouse-model
**Reviewer's code:** 01806391
**Reviewer's country:** Spain
**Science editor:** Fang-Fang Ji
**Date sent for review:** 2017-12-11
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**Review time:** 3 Days

| CLASSIFICATION | LANGUAGE EVALUATION | SCIENTIFIC MISCONDUCT | CONCLUSION |
|---|---|---|---|
| [ ] Grade A: Excellent | [ ] Grade A: Priority publishing | Google Search: | [ ] Accept |
| [ ] Grade B: Very good | [ Y] Grade B: Minor language polishing | [ ] The same title | [ ] High priority for publication |
| [ Y] Grade C: Good | [ ] Grade C: A great deal of language polishing | [ ] Duplicate publication | [ ] Rejection |
| [ ] Grade D: Fair | [ ] Grade D: Rejected | [ ] Plagiarism | [ ] Minor revision |
| [ ] Grade E: Poor | | [ Y ] No | [ Y] Major revision |
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| | | [ ] Duplicate publication | |
| | | [ ] Plagiarism | |
| | | [ Y ] No | |

## COMMENTS TO AUTHORS

In this study the authors have tried to demonstrate that in a state of extensive hepatocyte proliferation, targeted integration by homologous recombination would be superior to gene therapy based on episomal AAV gene therapy. Both the promoter and the recombination strategy and locus have been already used by this and other groups. The study is interesting but of limited originality. COMMENTS In the primary recipient mice of both experimental groups (rAAV8-TTR.Fah and rAAV8-ROSA26.HAL-TTR.Fah-ROSA26HAR) survival and phenotypic rescue that would be derived by clonal expansion of corrected hepatocytes (which implies vector integration) were found. The authors explain these findings by a selection advantage for corrected hepatocytes and random integration or another mechanism of integration when using rAAV8-TTR.Fah. To demonstrate the advantage of using AAV gene therapy

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with homologous recombination they included another set of experiments of serial transplantation, in which they observe an advantage of homologous recombination vs AAV without ROSA26 seq. 1. Why if in primary recipient mice rAAV8-TTR.Fah showed similar long-term efficacy to rAAV8-ROSA26.HAL-TTR.Fah-ROSA26HAR the authors concluded that there was an advantage of the later in the second recipient mice? Could this be explained by a marked difference in the number of Fah positive hepatocytes isolated in each group of primary recipient animals? Fah immunohistochemistry would be required to calculate the percentage of positive Fah hepatocytes in each case. 2. To demonstrate that homologous recombination improves the survival and phenotypic rescue in the second recipient mice a selection of isolated hepatocytes should by develop to inject the same number of Fah positive hepatocytes (from rAAV8-ROSA26.HAL-TTR.Fah-ROSA26HAR and rAAV8-TTR.Fah mice) in the second recipient mice. If authors wanted to justify that the episomal expression was lost after the implant in the second group of mice, they should have made sure that they have used the same number of Fah-positive hepatocytes in one case and another. 3. In the last part of paper, the authors used specific primers to demonstrate successful targeted integration of Fah in the ROSA26 locus using rAAV8-ROSA26.HAL-TTR.Fah-ROSA26HAR. As expected no PCR product was observed in rAAV8-TTR.Fah using these primers. However, it would be interesting to analyze the expression of TTR.Fah in samples of both groups of animals in order to compare the level of transgene expression in both cases. 4. Figures 2a, 2d, 3a and 3d are not clear enough and it should be improved. An explanation of the abbreviations (PH, HcTx) used in the figures should be included. 5. In Figures 2d and 3d an arrow (or another indicator) should be used to indicate the end of the body weight line of the mouse used to carry out partial hepatectomy and FAH staining. 6. Untreated controls (injected with sodium chloride) in Figure 2a and 3a are the same animals? Figure 2a includes three control mice and figure 3a includes only two. 7. In results section the title of the paragraph "Absence of long-term in vivo correction of Fah in the absence of homologous sequences" must be corrected because primary recipient mice injected with rAAV8-TTR.Fah survived and showed phenotypic rescue after >280 days of NTBC withdrawal. 8. Figure 4 should be simplified or any explanation about the numbers of each line must be included. Since the number of animals in each group was very low it would be better if samples of all the animals were shown in the PCR gel electrophoresis (the results could be reinforced if a sample of all the animals was included in the gel, not only two). In addition, there is no information regarding whether the samples used in the PCR were from primary or secondary recipient mice, and in what moment these samples were collected.