Reviewer's report

Title: The expression of GLI3 regulated by HOXD13 may play a role in idiopathic congenital talipes equinovarus

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Reviewer: Marian Ros

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In my opinion this paper requires a Major Compulsory Revision. The reasons for this decision are the following:

1.- The writing is not acceptable in its present form as it includes multiple grammatical errors. It is difficult to read.

2.- There are multiple mistakes in the Introduction indicating that the understanding of limb development by the authors is nor sufficient. For example, it is unclear the meaning of “segregated expression of transcription factors” and even more in relation to the ZPA. Also, for Drosophila should be hh (not Shh) and the explanation of Gli3 Fl versus truncated form is confused.

3.- The methods are not well described:
- There is no enough information about the experimental and control group of patients. The number of controls is unknown as well as how they were selected and how they match the experimental group regarding age, sex, etc…
- The number of ICTEV animal used is unknown, neither the percentage that developed ICTEV after RA treatment. A picture of the morphology of the induced ICTEV in the rats should be shown to appreciate the similarity with the human ICTEV
- It is unclear what the authors refer as “ankle muscle” because there is no muscle in the ankle, mostly tendons. They should precisely indicate from which muscle or muscles was the biopsy obtained and why the muscle tissue was selected.
- There is no information about how the lung tissue was obtained.
- For the Gli3 western blot there is some contradictory information as whether the cytoplasmic or nuclear fraction or both were analyzed. More important, in the western blot (figure 3) only the FL is shown while both the FL and the short truncated form are required.
- The immunos in Fig. 4 are referred either as ankle muscle of ICTEV animals or as hindlimbs?

4. - The results section is clearly deficient.
- It is unclear whether the authors set to analyze Gli3 expression in the “ankle
muscle” of patients of unknown age. There is no rational for this.

- If they are thinking in a genetic implication of Gli3 in ICTEV, it is unclear whether they set up to study the non-genetic effects of RA. Furthermore, they don’t take into account that RA, at least during limb development, is able of inducing Hoxd13 expression. The relation between the model they use and the human ICTEV is unclear. It is of some interest that they don’t find genetic mutations in Gli3 in patients with ICTEV.

- The results of the Hoxd13 binding sites in the 5’ region of the rat Gli3 are of potential interest but preliminary and the connection with the pathogenesis of the ICTEV unclear.

5.- There is almost no discussion or interpretation of the data.

**Level of interest**: An article of limited interest

**Quality of written English**: Not suitable for publication unless extensively edited

**Statistical review**: Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests**:

I declare that I have no competing interests