Review of: "Combinatorial effects on gene expression at the Lbx1/Fgf8 locus resolve Split-Hand/Foot Malformation type 3"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The manuscript by Giulia Cova and coauthos "Combinatorial effects on gene expression at the Lbx1/Fgf8 locus resolves Split-Hand/Foot malformation type 3" is a remarkable work that clarifies the structure and consequence of duplications and inversions at the Lbx-FGF8 multigenic chromosome region, mainly at the molecular level. The experiments are well done and nicely illustrated. The altered TAD status following Dup. and Inv. are quite convincing.

At the end of this beautiful work, however, the actual pathogenic mechanism remains elusive, and I am not sure that SHFM3 is now resolved. I would not over-emphasize this in the title and in the manuscript.

In the Discussion the authors attempts some explanation, however the real question is: why should the misespression of Lbx and Btrc in the AER lead to a developmental malformation, in particular SHFM ? in developmental terms. Are the AER cells misfunctional ? What are the altered or lost signalling properties the authors mention ? What if the signalling properties are not significantly changed – considering that Fgf8 ins normally expressed in the Dupl. – but something else ? stratification, epithelial-mesenchymal transition, tension or mechanical forces, others ….

I would prefer that the discussion be limited to the observed results, avoiding for instance the concluding sentence that seems quite an overstatement. At least until the real pathogenic event leading to SHFM3, at the cell and developmental level, be clarified.

Are we really sure that the Dactyaplasia mouse strain is not an adequate animal model ? Cleary is not a human and this needs to be accounted for, but this is true for every mouse model. After all this mouse presents with the expected phenotype, similar – not identical – of that in human. This cannot be a coincidence

Specific Questions / Suggestions

- How reliable are the CTCF sites, based on CTCF motifs ? Since their position is key to the definition of the TADs, possibly one or two pairs of them should be valided experimentally
- Likewise, how reliable is "vitrual 4C" ? Again one or two of them, the most significant ones, might need to be validated experimentally.
- For completedness, I would suggest that the authors report also the presence and location of TF binding sites for p63 and for Distall-less, two well-known transcriprion factors directly implicated in other forms of SHFM in human, with corresponding mouse models. The relocation of these sites following a duplication or an insertion at the SHFM3 locus at large, is likely to alter the TAD in a way that FGF8, LBX and BTRC mRNAs might be misexpresssed. This should be included in the discussion.