Reviewer’s report

Title: Genetic Polymorphisms in Bone Morphogenetic Protein Receptor Type IA Gene predisposes individuals to Ossification of the Posterior Longitudinal Ligament of the Cervical Spine via the Smad signaling pathway

Version: 1 Date: 18 Nov 2017

Reviewer: Chikenji Takako

Reviewer’s report:

GENERAL COMMENTS

In this study, the authors carried out SNPs in BMPR-IA genotyping in 356 OPLL patients and 617 non-OPLL patients, and 4A>C and -349C>T polymorphisms of BMPR-IA was significantly higher in the OPLL patients than those in control group. Transfection of this BMPR-IA in the C3H10T1/2 cells were performed and phosphorylated Samd1/5/8, and Smad4 expression of the transfected cells were examined by western blotting, and alkaline phosphatase and osteocalcin activity were also examined. Although other investigations have reported the association between BMP SNPs and OPLL (Liang Yan et al. Aging Dis. 2017, doi: 10.14336/AD.2017.0201), this study have weakness in Vitro experiment and statistical analysis, which would be difficult to suggest "the Smad signaling plays a major role in the pathological process of OPLL induced by SNPs in BMPR-IA gene" as in their conclusion.

SPECIFIC COMMENTS

1. Please address sample size of all In Vitro experiment. In addition, the authors used ANOVA followed by LSD posthoc multiple comparison in their in vitro study. Fisher's LSD is restricted to 3 groups comparison. The LSD method does not control family-wise α error level. Therefore it is inappropriate for multiple comparison procedure where the control of family-wise α error level is necessary (PMID: 25984481), and the LSD method can not be applied in the case of 4 groups or more. Please reconsider the statistics methods and indicate statistical software which you used.

2. Although the levels of phosphorylated Smad1/5/8 and ALP activity were significantly increased in pcDNA3.1/BMPR-IA (MT -349C>T) vector-transfected C3H10T1/2 cells than the WT vector-transfected cells, the results would be overreaching to suggest "Smad signaling pathway plays a major role in the pathological process of OPLL induced by SNPs in BMPR-IA gene" in their conclusion.

3. In their In Vitro experiments, they used only normal media condition for the transfected cells. If they use some stimulation factors which would affect BMPR-IA during the cell culture, the transfected BMPR-IA function would be more understandable.
Figures

Fig. 5

1. Please indicate sample size of this experiment in the Figure or Manuscript body.

2. In the (B), please add the explanation of "*" in the Lane 4 in the Figure Legends.

Fig. 6

1. Please indicate sample size of this experiment in the Figure or Manuscript body.

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Fig. 8

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Fig. 9

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Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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