Reviewer's report

Title: Various parts of human skeleton differ in expression of genes for some isoforms of bone morphogenetic proteins (BMPs)

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Reviewer: Julia Kuliwaba

Reviewer's report:

General
The authors used real-time RT-PCR analysis to investigate mRNA expression levels of BMP-2, BMP-4, and BMP-6 in bone tissue sampled from multiple skeletal sites of the human skeleton. They show differential expression of BMP-2 and BMP-4 mRNA between various skeletal sites sampled from six young male cadaveric cases. They do not detect BMP-6 mRNA expression in any of the seven skeletal sites examined. The study attempts to further understand the potential influence of BMP expression on differing bone healing rates throughout the human skeleton, by assessing basal mRNA expression levels of BMP isoforms implicated in bone formation and healing. The results presented for BMP-2 and BMP-4 show small differences in the magnitude of mRNA expression between trabecular and cortical bone sites. The data presented for BMP-6 expression are not convincing based on the melting curve plots showing non-specific PCR products, and lack of a single identifiable peak. The results section should be expanded to describe all of the data presented in tables and figures.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. Title should be altered to be more specific about study findings.
2. Statements made in the first paragraph of the background section should be referenced.
3. Is it known whether the ‘young healthy men’ had been on any medications or had any medical condition which may have affected bone turnover?
4. How were the bone tissue samples taken from the cadavers? Using a bone saw? Were sterile conditions used?
5. What was the approximate size of the bone biopsies?
6. Was the marrow from trabecular bone samples included in the powderised bone used for RNA extraction?
7. What is the time interval between death and bone sampling? i.e., the postmortem interval for the six cases? Does the postmortem interval differ between the six cases? RNA is known to degrade with increasing postmortem
interval. Have the authors taken this into account? Have the authors investigated whether beta-actin, BMP-2, BMP-4, and BMP-6 mRNA degrades as a function of postmortem interval? It is mentioned that the quality of the extracted RNA was verified by gel electrophoresis. Can the authors provide further justification of the quality of the RNA obtained? The authors should consider showing some of these results in the manuscript.

8. The authors have cited a rat study (37) for the BMP-6 primer sequences they list in Table 1. Are all of the primers, including BMP-6, listed in Table 1 specific for human? Were the primers designed to be mRNA-specific? It would be helpful to list NCBI/genbank accession numbers for the human mRNA sequences the primers have been designed for. Did the authors check for primer-dimer formation and for specificity of each of their primer pairs (in addition to Table 5)?

9. At what level was statistical significance set at?

10. Table 5 shows some variability in expression of the housekeeping gene beta-actin between different skeletal sites and between different cadaveric cases. Do the authors know whether postmortem interval differs between cases? Did the authors consider using an additional housekeeping gene, such as GAPDH, to rule out skeletal site variation in housekeeping gene expression?

11. Tables 3, 4, and 5 could be deleted, and replaced with an example of an amplification curve, melting curve, and gel photo for each mRNA species.

12. The melting curves (Table 4) show 2 peaks for BMP-4 mRNA expression. How did the authors determine specificity of their primers for BMP-4? What do the 2 peaks represent in the melting curves for BMP-4?

13. It appears that the primers used for BMP-6 are not specific since the melting curves show multiple peaks, and lack of a single identifiable PCR product peak. How did the authors check specificity of their primers for BMP-6?

14. Table 6, The use of ANOVA to compare expression levels of BMP-2 and BMP-4 in the groups of examined bones is not clear. Why were t-tests not used?

15. Discussion section, first line, the study did not detect differences in expression of BMP-6.

16. It would be helpful if the authors stated which skeletal sites comprise solely trabecular bone and which are cortical bone.

17. In the discussion section the authors should expand upon how their data may aid in understanding differences in healing rates throughout the skeleton, i.e., the authors should discuss what is different about the skeletal sites examined – are rates of bone turnover known to be different? (due to differences in mechanical loading, trabecular bone vs. cortical bone differences).

18. Which cell types from the bone sample are likely to contribute to the expression of BMPs measured in this study? Would this differ between trabecular and cortical bone samples?

19. Conclusion 1, state that the expression level differed specifically for BMP-2 and BMP-4, rather than ‘particular BMP isoforms’.

20. Conclusion 6, can this statement be made based on the above questions re:
specificity of BMP-6 primers?

21. The final few sentences regarding future applications could be expanded upon to discuss and emphasise the important role of BMPs in fracture healing.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The manuscript should be edited by someone fluent in English, as there are problems with sentence structure.

2. Figures 1 and 2 should have titles, label y-axes in Figures 1 and 2. The error bars for bones 4 and 5 do not appear in the reproduced figures.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.