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# Comment to Ruiyu Liu et al.: Comparative analysis of gene expression profiles in normal hip human cartilage and cartilage from patients with necrosis of the femoral head

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See related research by Liu et al., <http://arthritis-research.biomedcentral.com/articles/10.1186/s13075-016-0991-4>

It was with great interest that we read the study entitled “Comparative analysis of gene expression profiles in normal hip human cartilage and cartilage from patients with necrosis of the femoral head (NFH)” by Ruiyu Liu and colleagues published in *Arthritis Research & Therapy* in May 2016 [1].

The authors identified a set of differentially expressed genes which may contribute to the destruction of articular cartilage in patients with necrosis of the femoral head (NFH). Specimens were collected from 18 patients with non-traumatic NFH, classified by the Ficat system as grade III, and 18 healthy control subjects. However, the paper did not provide sufficient information about the main causes of NFH. Thus, the conclusion that the cartilage damage in NFH is related to the differential expression of these genes is debatable. The causes of non-traumatic NFH are complicated. A nationally representative survey of 30,030 people demonstrated that blood levels of triglycerides, total cholesterol, LDL-cholesterol, non-HDL-cholesterol, male gender, urban residence, family history of osteonecrosis of the femoral head, heavy smoking, alcohol abuse and glucocorticoid intake, overweight, and obesity were all significantly associated with an increased risk of non-traumatic NFH [2]. Some of these factors may also result in differential gene expression in articular cartilage. A study by Sniekers et al. [3] showed that estradiol affects the expression of anabolic and catabolic genes (e.g., those encoding aggrecan (AGC)1, matrix metalloproteinase (MMP)2, MMP14, and tissue inhibitor of metalloproteinase (TIMP)2, transforming growth

factor (TGF)B2, and TGFB3) in bovine cartilage explants. Another study showed that leptin, a hormone associated with obesity, induces ADAMTS-4, ADAMTS-5, and ADAMTS-9 gene expression through mitogen-activated protein kinase and NF- $\kappa$ B signaling pathways in human chondrocytes [4]. Considering these issues might help to avoid irrelevant variables. The control group should be in accord with the NFH group and the established causes of NFH, such as estrogen in-take history, alcohol abuse, obesity, and so on. In addition, it would be better to provide basic information like height, habits, or history of drug use.

Another concern is that the methods for both selecting genes for quantitative RT-PCR validation and the immunohistochemical analysis might not been explained clearly. The nine genes were not the most significantly differentially expressed according to the gene expression ratios in Table 2 showing the significance analysis of microarrays (SAM) results of the patients with NFH. We would suggest selecting the genes with extreme gene expression ratio values from the nine functional groups mentioned in Table 2. Moreover, the microarray analysis may be more convincing if the sample size could be increased.

In this study, it remains unclear whether the changes in gene expression lead to the cartilage damage in NFH. To clarify their role, we suggest an additional animal experiment to study the dynamic change in gene expression and loss of cartilage in models at different stages of NFH, which may help to clarify the mechanisms by which the identified genes affect the development of NFH.

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## Authors' response

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We thank Long and colleagues for their interest in our recent publication [1]. We would like to respond to their comments.

First, we totally agree with the comment that non-traumatic necrosis of the femoral head (NFH) is a complex disease, mainly caused by excessive steroid use, alcohol drinking, as well as idiopathic NFH without certain etiological factors [5]. Additionally, heavy smoking, overweight, and hyperlipemia were reported to contribute to the development of NFH. To control the potential impacts of steroids, alcohol, and other confounding factors on our study results, all case samples were idiopathic NFH patients without histories of steroid and alcohol abuse, heavy smoking, hyperlipemia, and obesity. The healthy control samples were matched to case samples for age and sex and also did not have histories of steroid and alcohol abuse, heavy smoking, hyperlipemia, and obesity.

Second, Long and colleagues suggest selecting the genes with extreme expression ratios for quantitative real-time PCR (qRT-PCR) validation. We think that it was better to select genes by considering both expression ratios and biological relevance to NFH. The selected nine qRT-PCR validation genes were not only significantly differentially expressed between NFH and controls but also generally had evidence supporting their roles in the normal growth, homeostatic maintenance, or damage of cartilage. Additionally, we agree with their comment that using more cartilage samples could increase the accuracy of the microarray studies. However, it is usually difficult to collect suitable cartilage samples in practice, especially healthy cartilage samples. The same sampling design has been widely used in previous microarray studies of cartilage [6].

Third, Long and colleagues advise us to conduct animal experiments to clarify the differences between gene expression patterns at different stages of NFH. This is a good suggestion and will be performed in our future studies.

### Abbreviations

NFH: necrosis of the femoral head; qRT-PCR: quantitative real-time PCR

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

ZL carried out the sequence alignment and drafted the manuscript, TH participated in the design of the letter and performed the statistical analysis. WW participated in its design and coordination. All authors read and approved the final manuscript.

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