Correspondence on 'Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells'

We read with interest the paper by Zhu *et al*¹ on DNA methylation, mRNA expression and their role in the pathology of rheumatoid arthritis (RA). We found that their findings highlighted the importance of *PARP9* gene DNA methylation in RA. However, we found that the study had some limitations that necessitate a cautious interpretation of the findings and merit further attention.

First, 43 subjects (RA:healthy controls=25:18) were included during the discovery stage, 52 (RA:healthy controls=25:27) for DNA methylation and 70 (RA:healthy controls=35:35) for mRNA expression. However, we found that the ratio of patients to healthy controls in the first two phases was not suitable. The number of healthy controls should be greater than that of patients (patients vs healthy controls=1:R, where R is equal to or less than 4) in case-control studies.

Second, patients with RA were separated into three sample sets, and the consistency of the results needs to be interpreted with caution. The main reasons for this are as follows: (1) patients with RA may have different disease status (eg, disease activity and age) across the three stages; (2) the three assays had different levels of sensitivity (ie, microarray analysis, bisulfate sequencing and real-time quantitative-PCR). However, the authors did not take these matters into account or adjust for them accordingly.

Third, we do not know why the authors chose the *PARP9* gene instead of other genes (eg, *IFI44L* and *MX1*) for their in vitro study. Is the *PARP9* gene the most representative gene available with respect to diagnostic value?

Fourth, Jurkat cells, as the authors said, are an immortal line of human T lymphocytes. They are frequently used as a cell model in studies of immune-related diseases.² The authors used Jurkat cells to investigate the functional effects of the *PARP9* gene. Are the Jurkat cells specific or RA-related? If they are not specific, we can say that these effects are also suitable for other autoimmune diseases including systemic lupus erythematosus.

Lastly, the authors used T cells from patients with active RA to explore the correlation between methylation and mRNA, which may mean that their results are applicable to many situations. Because the disease activities and inflammation levels of active

RA are more intense than those of stable patients, it may be more appropriate to use T cells from newly detected and newly diagnosed cases.

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