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Differential methylation within the major histocompatibility complex region in rheumatoid arthritis: a replication study

SIR, Within a single decade the research on complex diseases such as RA has changed from hypothesis-driven genetic studies to discovery-driven genome-wide association studies, and presently the field is moving further to the evaluation of dysfunctions in multiple systems and changes at different levels, such as epigenetics, transcriptomics, proteomics and metabolomics. A key issue in discovery-driven approaches is the replication of findings. For the genome-wide SNP studies, the risk of false-positive findings due to multiple testing was dealt with by the requirement to replicate findings in independent datasets. For epigenetic studies, the design, analysis and interpretation is significantly more complex, and although the need for replication is well established, criteria remain to be developed [1].

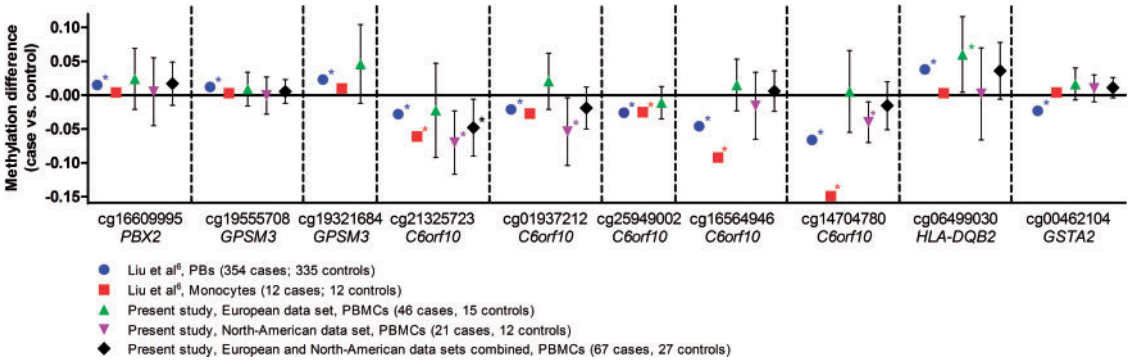
Changes in DNA methylation presumably contribute to RA development [2]. Few genome-wide methylation studies have been performed thus far. Two studies compared fibroblast-like synoviocytes of RA patients and OA controls. Both studies comprised six RA and six OA samples [3, 4] and observed several differentially methylated loci. Different loci were found in these studies and validation of the results in independent populations was not included. The largest epigenome-wide study in RA was published recently by Liu *et al*. [5]. Using peripheral blood samples of 354 Swedish RA patients and 335 healthy controls as the identification set and monocytes of 12 patients and 12 controls as the validation set, they reported on 10 differentially methylated positions (DMPs) within and outside the MHC region at chromosome 6 in RA. The authors also used methodology to address confounding influences on DNA methylation from cell heterogeneity and the consequences of the disease itself [5]. Because of the relevance of independent replication, we aimed to evaluate the methylation levels at these 10 positions in peripheral blood mononuclear cells (PBMCs) of RA patients and healthy controls in two independent datasets of different continents.

The first replication dataset comprised PBMCs from 46 European RA patients (mean age 55.6 years, 63.0% female, 45.5% ACPA positive) and 15 healthy controls [6]. The PBMCs were collected at time of diagnosis {median symptom duration until diagnosis 4.7 months [interquartile range (IQR) 2.2–7.1]}, reducing the risk that methylation was influenced by effects of long-standing inflammation or treatment. The second replication dataset included PBMCs of 21 North American RA patients (mean age 53.1 years, 53.1% female) and 12 healthy controls. Seven patients were untreated at the time of sample collection and 14 were treated with biologics ($n = 8$), and/or MTX ($n = 7$) and/or corticosteroids ($n = 4$). All subjects in both datasets were white. Epigenome-wide methylation assessment was done using the 450K methylation array (Illumina, San Diego, CA, USA). Methylation analysis of the European PBMC samples was initially done with the aim of identifying DMPs associating with DMARD-free sustained remission in RA; no epigenome-wide significant results were obtained here (data not shown). To evaluate methylation differences between the RA patients and healthy controls in the European dataset, methylation levels of the positions reported as differentially methylated by Liu *et al*. [5] were extracted. Methylation of the North American samples was obtained similarly, using the Illumina 450K methylation array. All analyses were adjusted for age and gender. All patients gave informed consent and approval was obtained from the medical ethics committee of the Leiden University Medical Center and the institutional review boards of the University of California, San Diego and BioMed (San Diego, CA, USA).

Fig. 1 presents the results; for direct comparisons, the methylation differences in cases *vs* controls as observed by Liu *et al.* [5] are also depicted. Although the methylation changes reported by Liu *et al*. were relatively small (1.2–6.6% difference in methylation), differences in methylation in the same direction were seen in both replication datasets for several positions. We first evaluated the DMPs within the MHC region. Cg21325723 was significantly associated with RA in the analysis combining both replication datasets (4.8% difference in methylation, $P = 0.026$). For cg16609995, cg19555708, cg19321684 and cg25949002 the effect sizes in our replication cohorts were comparable to the effect sizes observed by Liu *et al*. (Fig. 1), although statistical significance was not obtained. The fact that our number of samples was smaller than that of Liu *et al.* might have played a role. Liu *et al*. also reported on cg00462104 in *GSTA2* outside the MHC region at chromosome 6; this association was not replicated in our two datasets (Fig. 1).

In conclusion, the present data support the finding that at least one DMP in the MHC region (cg21325723) is associated with RA, a region that also harbours the most important genetic risk factor for RA. A major limitation is that Liu *et al*. [5] used full blood and monocytes and we studied PBMCs. Four of the 10 DMPs identified by Liu *et al*. in full blood were also found to be significant in monocytes. Interestingly, cg21352723, which was differentially methylated in PBMCs in the present study, was one of these four DMPs [5]. Another issue is that the number of RA samples in our datasets (67 in total) was

Fig. 1 Methylation data for 10 DMPs that mediate genetic risk in RA



Depicted are differences in methylation level (proportions) with 95% CIs between RA patients and controls in the datasets as presented by Liu *et al.*, Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. Nat Biotechnol 2013;31:142–7, and in two replication datasets of European and North American origin from the present study. Data on cg19321684 and cg25949002 were not available for the North American dataset. *Indicates a statistically significant difference (cg21325723 North American dataset $P = 0.0047$, combined dataset $P = 0.026$; cg01937212 North American dataset $P = 0.034$; cg14704780 North American dataset $P = 0.012$; cg06499030 European dataset $P = 0.037$). *PBX2*, *GPSM3*, *C6orf10* and *HLA-DQB2* are located at chromosome 6 in the MHC region; *GSTA2* is located at chromosome 6 outside the MHC region. DMP: differentially methylated position.

considerably smaller than the number of full blood samples of Liu *et al*. ($n = 354$), although our datasets were larger than the validation set of 12 monocyte samples. An advantage of our first replication dataset is that samples were collected at disease onset, reducing the chance that the results were the consequence of the disease itself or treatment. Further studies are required to increase our understanding of the contribution of DNA methylation on RA development and the consequences of differences in methylation at the observed positions for gene expression in different cell populations.

Rheumatology key message

- Loci within the MHC region are differentially methylated in RA.

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