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## CONCISE COMMUNICATION

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### DNA methylome signature in synoviocytes from patients with early rheumatoid arthritis compared to synoviocytes from patients with longstanding rheumatoid arthritis

Epigenetics can contribute to pathogenic mechanisms in autoimmunity, and we recently identified an imprinted DNA methylation pattern in rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS), involving multiple genes in pathways that have been implicated in cell migration, matrix regulation, and immune responses (1,2). To understand when alterations in DNA methylation occur in RA and the specificity of the methylation changes in this disease, we compared differentially methylated loci in patients with early RA, juvenile idiopathic arthritis (JIA), longstanding RA, and osteoarthritis (OA).

Genomic DNA was isolated, as previously described (1–3), from FLS of 4 patients with early RA (symptom duration 1–13 months) fulfilling the American College of Rheumatology/European League Against Rheumatism 2010 criteria for the disease (4,5). All 4 patients were female, and the mean age was 52 years. All were positive for anti–citrullinated peptide antibody. Two of the patients were being treated with methotrexate alone, 1 was being treated with methotrexate and abatacept, and 1 was not receiving antirheumatic drugs. Samples were obtained at the time of arthroscopic biopsy. FLS from 3 patients with JIA (2 female, 1 male; mean age 31 years) was isolated from synovium obtained at the time of arthroplasty. Twenty-two previously described patients (2) (11 with longstanding RA and 11 with OA) were used as controls. DNA methylation levels across 485,512 loci were measured using an Illumina Infinium HumanMethylation450 chip, and the methylation level at individual loci was reported as beta values (see Supplementary Methods, on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39123/abstract).

Small but statistically significant global hypomethylation in patients with longstanding RA compared to those with early RA was detected by summing beta values in the gene promoter region (average beta value per CpG locus 0.163 in longstanding RA and 0.168 in early RA; $P = 0.0046$). Unbiased hierarchical clustering and principal components analysis based on 15,220 previously identified loci that were differentially methylated between the 11 patients with longstanding RA and the 11 with OA (2) revealed specific clustering patterns and overlaps among FLS lines from patients with early RA, JIA, longstanding RA, and OA (Figures 1A and B, respectively). Samples from patients with early RA and patients with JIA clustered with those from patients with longstanding RA and segregated from those from patients with OA. There was a partial overlap between the patterns observed for samples from patients with early RA and those from patients with longstanding RA, which could be consistent with the notion that the methylation pattern undergoes a transition from early RA to longstanding RA. FLS from JIA patients also segregated with the longstanding RA group but formed a separate subgroup.

In methylome comparisons between longstanding RA and early RA by Welch's *t*-test, we identified 20,776 differentially methylated loci (8,059 hypomethylated and 12,717 hypermethylated in longstanding RA compared to early RA) in 5,469 differentially methylated genes (average differences of beta values $>0.1$ and q values $<0.05$) (see Supplementary Table 1, on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39123/abstract). By pathway enrichment evaluation using Ingenuity Pathway Analysis, we identified 5 potentially enriched pathways with hypomethylated genes in longstanding RA: Wnt/β-catenin signaling (enrichment ratio [ER] 0.249), integrin signaling (ER 0.233), retinoic acid receptor activation (ER= 0.233), platelet-derived growth factor signaling (ER 0.286), and superpathway of D-myoinositol 1,4,5-trisphosphate metabolism (ER 0.417) (Benjamini-Hochberg–adjusted $P$ values $< 0.1$). In addition, 340 CpG loci that most distinguished early RA from longstanding RA were identified by comparing the entire methylation data sets by the random forest method (Supplementary Table 2 and Supplementary Figure 1, http://onlinelibrary.wiley.com/doi/10.1002/art.39123/abstract). These results confirmed that early RA and longstanding RA could be distinguished from one another in this regard (Supplementary Figure 1).

Genome-wide DNA methylation patterns in inflammatory arthritis differed from those in OA and clustered together, suggesting that these diseases have common epigenetic elements of these diseases. However, the FLS formed subgroups, implying that the variations are disease specific. Of interest, the methylome of early RA exhibited a distinctive pattern compared to that of longstanding disease. These data indicate that differential methylation of RA FLS might occur early and evolve over time. Pathways that were differentially methylated between early RA and longstanding RA involved cell migration, differentiation, and adhesion, which raises the intriguing possibility that the transition to chronic RA involves epigenetic changes that alter synoviocyte hyperplasia and aggressive behavior.

One obvious limitation of this study is that the number of samples was small, in part because obtaining cell lines from early RA biopsy specimens is challenging. Despite this, these unique marks can potentially provide information on mechanisms of disease and how RA evolves over time, as well as identifying treatment targets based on the duration and type of synovitis.

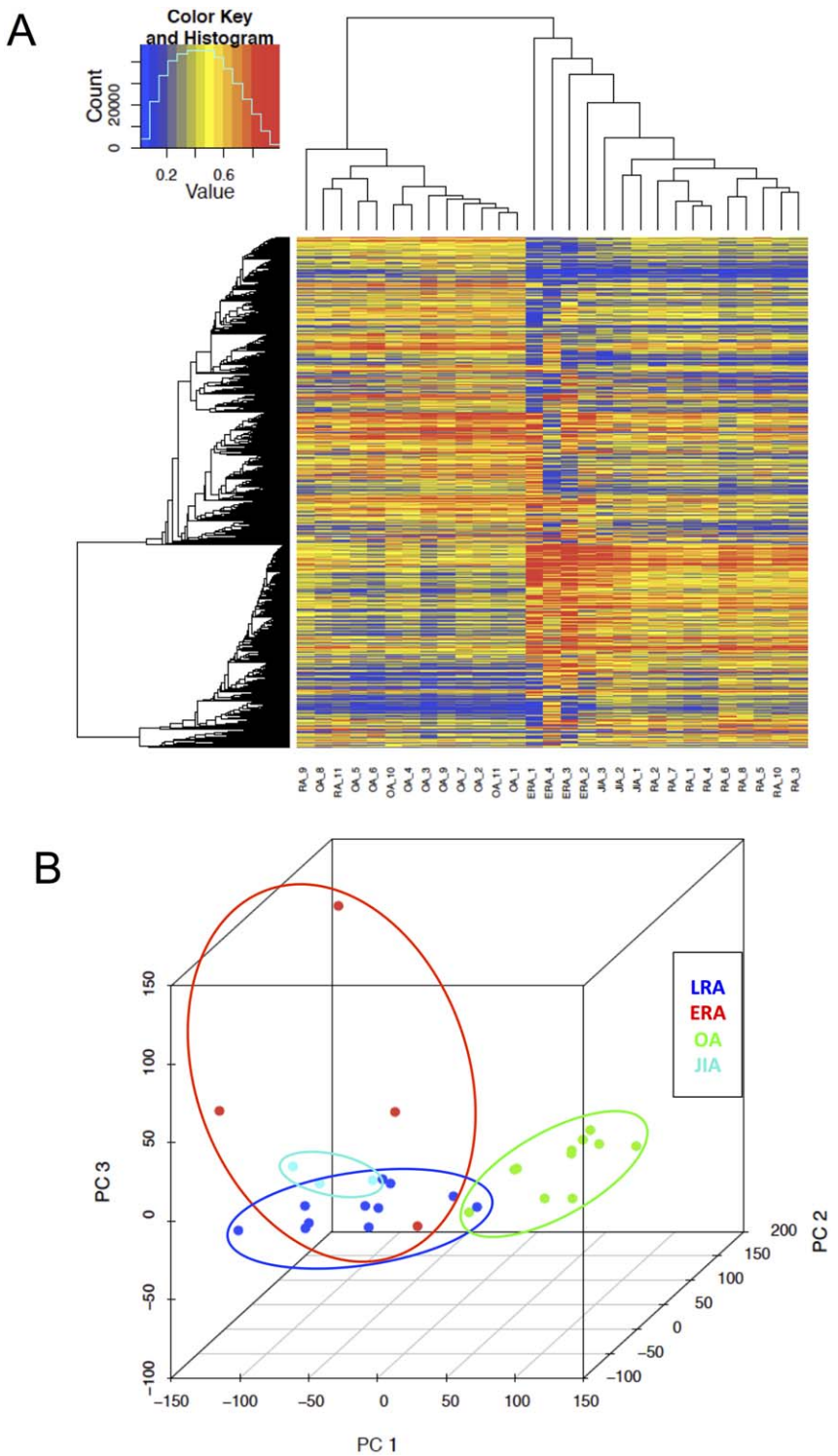


**Figure 1.** Analysis of DNA methylation patterns in early and longstanding rheumatoid arthritis (RA). **A,** Hierarchical clustering of longstanding RA (LRA), early RA (ERA), juvenile idiopathic arthritis (JIA), and osteoarthritis (OA) based on differentially methylated loci. **B,** Analysis of the first 3 principal components (PCs) of longstanding RA, early RA, JIA, and OA based on differentially methylated loci. The inflammatory arthritis samples mainly cluster together, and early RA segregates from JIA.

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All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Firestein had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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