Lymphocyte DNA Methylation as a Mediator of Genetic Risk in Rheumatoid Arthritis

Clark AD, Nair N., Skelton, AJ., Anderson AE., Thalayasingam N., Naamane N., Diboll J., Massey J., Eyre S., Barton, A., Isaacs JD., Reynard LN, Pratt AG.

Background: Genome-wide association studies have identified over 100 genomic loci at which single nucleotide polymorphisms (SNPs) confer an increased risk of developing rheumatoid arthritis (RA). These loci are enriched for lymphocyte-specific enhancer elements, consistent with a regulatory function of many disease-associated variant(s). Epigenetic modifications have also been strongly implicated in RA pathogenesis, potentially impacting cell phenotype through altered gene expression. Here, we investigate the role of DNA methylation as a mediator of genetic risk in RA.

Methods: CD4+ T- and B-lymphocytes were isolated by positive selection from fresh peripheral blood of drug-naïve patients attending an early arthritis clinic. Paired CD4+ T-lymphocyte-specific DNA and RNA were extracted from 43 RA and 60 disease control patients, respectively, and equivalent material from B-lymphocytes of 46 RA and 73 controls. Comparator groups were matched for age, sex, and acute phase response. Genotyping was performed using the Illumina Infinium Human CoreExome-24 array, and DNA methylation at >850,000 CpG sites quantified with the Illumina MethylationEPIC array. Gene expression profiling was carried out using the Illumina Human HT-12 v4 BeadChip. Having first mapped genome-wide methylation quantitative trait loci (meQTLs) in *cis* (<1Mb) and *trans*, we focussed our analysis on known RA risk loci, and integrated paired normalised gene expression measurements for transcripts within 500kb of index CpGs. Finally, to highlight RA-specific effects, we performed a meQTL analysis seeking interactions between genotype and disease diagnosis.

Results: Our analysis in CD4+ T-lymphocytes revealed a total of 45,172 independent SNP-CpG associations in *cis* with 437 acting in *trans*. Meanwhile in B-lymphocytes 46,023 and 552 effects were present in *cis* and *trans* respectively. T-lymphocyte *cis*-meQTLs colocalised with 27 independent (r^2 < 0.8) RA-associated SNPs, whilst in B lymphocytes meQTL effects were present at 29 RA SNPs, 22 of these functioning in both cell types. CpG sites subject to *cis* effects at risk loci were depleted in regions associated with cell type-specific repressed chromatin marks, with enrichment at enhancer regions in T-lymphocytes and those flanking transcription start sites in B-lymphocytes, suggesting active roles in transcriptional regulation. Linear regression identified putative regulatory effects of these CpG sites on gene expression, and causal inference testing highlighted genes for which risk SNPs most likely modulate gene expression via CpG methylation. Such effects, robust to false discovery rate, were particularly prevalent in CD4+ T lymphocytes, implicating *ANKRD55*, *ORMDL3*, and *FCRL3* amongst others as causal genes in this cell type. Similar effects were less robust in B-lymphocytes, albeit potentially implicating genes including *FCRL3*, *XKR6*, *FAM167A*, and *CCR6*. Interaction analysis revealed 1,196 instances of differential *trans* meQTL effects between RA patients and disease controls in CD4 T-lymphocytes, with 4,827 occurring in B-lymphocytes.

Conclusion: Here we demonstrate the utility of DNA methylation profiling as a tool for the prioritisation of candidate genes following GWAS studies in RA, and highlight an important mechanism through which genetic variants may contribute to altered lymphocyte phenotype. The functional roles of highlighted genes in CD4+ T cells during RA pathogenesis await clarification.