**Rheumatoid Arthritis Associated Alteration in T-cell DNA Methylation pattern: Potential for the Development of diagnostic Biomarker**

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**Background** Despite the new EULAR-2010 classification criteria and the use of Anti-citrullinated protein antibodies (ACPAs), diagnosing Rheumatoid Arthritis (RA) is still difficult particularly for sero-negative patients. Alteration in epigenetic patterns have been associated with several diseases including RA. The hypothesis underlying my PhD project is that epigenetic changes happen early in the disease and can be utilised for diagnosis. The aim of the 1st year of my project was to identify changes in DNA methylation pattern in RA patient in order to select candidate CpGs for further development of a diagnostic biomarker for RA using a methylation sensitive qPCR assay.

**Methods** The methylation patterns of 480,000 CpGs were measured using an Illumina methylation genome-wide array in CD4+T-cells and monocytes (negative control) in 6 healthy control (HC) and 10 early, drug naïve RA patients. DNA methylation data were analysed using a standard workflow (R package minfi). DNA methylation data were controlled for quality, pre-processed and methylation intensity was normalised.

**Results** The intended final qPCR assay will measure differential methylation in T-cells and normalised it to this specific cell subsets using a similar approach to an existing CD4 gene assay (industrial collaboration with Epiontis). In order to select CpG candidates for developing this qPCR assay, the 2 step approach was used. We first applied a methylation filter which selects CpGs that were highly methylated in monocytes (Beta value 0.8 to 1) and showed low methylation levels in T-cells (Beta value 0 to 0.6). Then we applied a second criteria, to find the CpGs differentially methylated between HC and RA. The first filter selected 171,787 CpGs suggestive of gene silencing in monocytes and 209,778 CpGs with a low level of methylation in T-cells. Combining the two-filters resulted in 1,791 CpGs which were specifically undermethylated in T-cells and fully methylated in monocytes. 25 of these CpGs were differently hypo-methylated between HC and RA which associated with 23 genes potentially over expressed in RA patients CD4+T-cells, and just 1 CpGs differentially hyper-methylated in RA.

**Conclusion and future plan** These 25 differentially methylated CpGs in CD4+T-cells offer the potential for the development of a methylation sensitive specific qPCR biomarker for the early diagnosis of RA. In collaboration with Epiontis, I will screen available to methylation database for CD8+T-cells, B and NK cells for candidate genes. The next step will be to validate the difference in CpG methylation for 2-3 final candidates using bisulfite sequencing in 20 DNA samples from purified CD4+T-cells obtained from HC and RA. This will also allow me to obtain a more precise picture of the selected DNA region in order to position primers for developing the methylation sensitive qPCR assay. The test will then be developed in whole blood using 250 DNA samples from patients attending the Leeds early arthritis clinic, of whom 175 developed RA.