

## **Whole Genome Scale DNA Methylation Differences in Type 1 Diabetes Disease-related Cells**

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**Purpose:** Type 1 diabetes (T1D) is due to the interaction of genetic and non-genetic factors. The latter may influence the former through epigenetic changes including DNA methylation. Thus we have initiated 1) a discovery programme to identify DNA methylation variable positions (MVPs) in disease-relevant tissues; 2) a development programme to define MVPs in disease relevant tissues when DNA amount is limited; 3) a development programme to collect thymus and obtain DNA/RNA from it.

**Methods:** In an initial study we generated genome-wide DNA methylation profiles (EWAS) using Illumina 27K arrays of purified CD14+ monocytes (an immune effector cell type relevant to T1D pathogenesis) from 15 T1DM-discordant MZ twin pairs, as well as antibody positive children pre- and post-diabetes onset and antibody positive children who are now unlikely to develop T1DM plus control subjects. In the development programme we tested three different approaches for DNA methylomics, each offering different advantages: Illumina 450K arrays, and two high-throughput sequencing (HTS)-based methods: Methylated DNA Immunoprecipitation combined with HTS (mini-MeDIP-seq); and HTS of randomly sheared, bisulfite-treated gDNA (BS-seq).

We have established a protocol for thymocyte and stromal cell isolation and good quality DNA and RNA from these paired samples from the same individual. In addition, fresh thymic tissue was mounted in preservative blocks and frozen for later use in microscopy studies and for nPOD collection.

**Summary of Results:** We identified 132 different CpG sites at which the direction of the intra-MZ pair DNA methylation difference significantly correlated with the diabetic state i.e. T1D-associated methylation variable positions (T1D-MVPs). We confirmed these T1D-MVPs display statistically significant disease-associated DNA methylation variation in an independent set T1D-discordant MZ pairs ( $P = 0.035$ ). Then, to establish the temporal origins of the T1D-MVPs, we generated two further genome-wide datasets and found that, when compared with controls, T1D-MVPs are enriched in singletons both before ( $P = 0.001$ ) and at ( $P = 0.015$ ) disease diagnosis, and also in singletons positive for diabetes-associated autoantibodies but disease-free even after 12 years follow-up ( $P = 0.0023$ ). The genes affected encompass: immune response pathways, notably HLA class II gene, HLA-DQB1, RFXAP, an HLA class II regulating element, NFKB1A, an important regulator of apoptosis, TNF, a key inflammatory cytokine, and GAD2 which encodes GAD65, a major T1D autoantigen. We developed a method to identify DNA methylation in small tissue samples and found that mini-Medip-Seq gave comparable results to the other methods and only required 200 ng DNA. To date we have 10 paired samples of thymus thymocytes and stromal cells.

**Conclusion:** These results suggest that changes in DNA methylation represented by T1D-MVPs must arise very early in the etiological process that leads to overt T1D. These changes involve genes likely associated with the immune response. In addition we have developed a method to identify MVPs in small tissue samples with limited DNA amounts. Our EWAS of T1D represents the first systematic analysis of the temporal origins of disease-associated epigenetic variation for any human complex disease. The development of mini-Medip-Seq will enable us to translate this data into different T1DM-related tissues, even when the amount of tissue is limiting.