Cohn’s disease (CD) is an incurable chronic disorder characterized by the inflammation of the gastrointestinal tract. Despite extensive research, the etiology remains unknown. While genome-wide association studies (GWAS) have reported several CD-associated loci, common variants explain only 20% of the estimated heritability. To this end, additional factors such as epigenetics and the microbiome are thought to play an important role in the pathogenesis of CD. While differentially methylated positions (DMPs) were observed in blood-derived samples, the effects sizes were modest with a maximum methylation difference of ~30% when CD patients were compared to controls [1–6]. As CD may itself primarily in the intestine, we sought to associate alterations in the DNA methylome with the different degrees of CD in ileum-derived fibroblasts.

Methods

Fibroblasts were cultured from ileal resection material obtained from 8 CD and 3 control (HC) patients. Ileal resection material acquired from patients with colon cancer whereby samples were taken at least 10 cm from the tumor. Genomic DNA was subsequently isolated, bisulfite treated and analyzed using the Illumina HumanMethylation450 BeadChip.

Data analysis was performed in the R statistical programming environment using the Bioconductor packages minfi [7] for importing the data, Methyldiff [8] for quality control, limma [9] for finding differentially methylated positions (DMPs), DMRcate [10] for finding differentially methylated regions (DMRs) and missMethyl [11] for gene ontology (GO) enrichment analyses. Linear regressions for finding DMPs and DMRs were corrected for age, gender and passage. Significance was based on a Benjamini-Hochberg-adjusted q of 0.05. Group comparisons included in this study are represented in Figure 1a.

Results

Principal component analysis (PCA) revealed no separation of the various groups, except for the stenotic samples, which separated from the non-inflamed samples on the first principal component (Figure 1b).

In total, we found 8, 65,383 and 109,494 DMPs when comparing CD versus HC, CDINF versus CDNINF and CDSTE versus CDNINF respectively (Figure 2). In addition, we found 13, 65, and 10,370 DMRs when comparing CD versus HC, CDINF versus CDNINF and CDSTE versus CDNINF respectively (Figure 3). Overall, our results revealed large effect sizes (Beta > 0.2), where most significant differences were found when comparing stenotic versus non-inflamed CD tissue.

Analysis of the genes associated to the DMPs and DMRs revealed differential methylation of HOX genes as well as genes encoding micro RNAs and long intergenic non-coding RNAs. Enrichment analysis of the DMPs associated to CDINF and CDSTE revealed significant enrichment of GO-terms associated to the development and growth of cells (GO:00044459, GO:004885 and GO:0048731).

Discussion

Our results show that large methylation differences exist between CD patients versus HC as well as between inflamed and stenotic versus non-inflamed CD tissue. As such, our results corroborate the large effects sizes observed in other methylation studies on colon-cancer-derived samples [12, 13]. Given that CD is itself predominantly in an intra-intestinal fashion, such effect sizes were not unexpected.

In line with our PCA, most DMPs and DMRs were found when comparing stenotic with non-inflamed CD tissue, while the methylation pattern associated to Crohn’s disease in mucosal-derived fibroblasts was predominantly distinct from non-inflamed tissue. Stenosis is characterized by the expansion of mesenchymal cells and the accumulation of extracellular matrix [14], which is in agreement with the differential methylation found for processes involved in cell growth.

While our study reveals a relationship between the varying degrees of CD and changes in the DNA methylome of colonic fibroblasts, the biological relevance of our reported loci remains to be tested. Our next step is thus to perform transcriptional analysis to understand the differential methylation in a biological context.

References