



# The value of blood derived DNA methylation signatures in advancing our understanding of Crohn's Disease pathogenesis

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DNA methylation is one of the main epigenetic mechanisms, known to be operative in mammals. Extensive evidence has highlighted its pivotal role in several fundamental biological processes including organogenesis, X-chromosome inactivation and genomic imprinting (1). Although our understanding of how DNA methylation impacts on gene transcription and cellular function remains incomplete, it is clear that DNA methylation plays an important role in defining cell and tissue specific cellular function (2,3). Increasing evidence suggests that altered DNA methylation can contribute to either the development and/or persistence of many human diseases. Importantly, what makes epigenetic mechanisms a particularly attractive concept when it comes to investigating disease pathogenesis of modern, multi-factorial/complex diseases is their responsiveness to environmental factors (4). The incidence of inflammatory bowel diseases (IBDs) such as Crohn's Disease (CD) and ulcerative colitis (UC) has been increasing dramatically in recent decades with disease onset shifting to younger age groups and disease phenotypes becoming more aggressive (5). Assuming an overall stable human genome combined with well documented links to several environmental factors (e.g., western lifestyle) epigenetics may provide a missing mechanistic link between environmental changes and stable alterations in cellular function either causing chronic relapsing gut inflammation

and/or contributing to its persistence (6). However, despite the plausible concept, providing supportive evidence has proven to be difficult and as a result high quality studies remain scarce (7,8).

In the June 2019 edition of *Gastroenterology*, Somineni and colleagues report on the results of a study investigating blood-derive DNA methylation signatures in children diagnosed with CD and their correlation with disease severity as well as intestinal inflammation (9). The group of investigators performed genome wide DNA methylation analyses (using Illumina EPIC arrays) of whole blood derived DNA samples obtained from 164 treatment naïve CD patients at the point of diagnosis and 74 matched non-IBD controls. Longitudinal follow-up samples were obtained from all CD patients during the course of disease between 1–3 years. In addition to DNA methylation, genotyping was performed on all samples and detailed clinical follow-up data was prospectively recorded allowing the authors to correlate epigenetic signatures with disease phenotype and outcome.

Comparative methylation analyses revealed a total of 1,189 CpGs that were found to be differentially methylated in CD patients at diagnosis compared to non-IBD controls. Moreover, methylation changes strongly correlated with systemic inflammation, i.e., serum CRP levels. Interestingly, the vast majority of methylation changes (except from

3 CpGs) disappeared in longitudinal samples, in which CRP levels were also found to be lower. The authors conclude that their findings suggest disease associated DNA methylation changes are likely to be a result of inflammation rather than a causing factor. Therefore, these changes are less likely to contribute to disease development or progression.

So, to what extent do these results provide evidence against the hypothesis that epigenetic alterations in disease relevant cell types are involved in either causing and/or maintaining chronic intestinal inflammation in IBD?

In order to answer this question, we must take a critical view on the strengths and limitations of this study. Starting with the former, this work has numerous advantages making generated results highly valuable for the field. Major strengths include, the prospective recruitment of a large paediatric CD patient cohort at diagnosis (i.e., treatment naïve), documentation of long-term clinical follow-up as well as the availability of longitudinal samples. Additionally, performing epigenetic studies in paediatric patients, including recruitment of age matched non-disease controls has major advantages as the presence of co-morbidity and the potentially confounding impact of other environmental factors are limited. Lastly, the fact that this study recruited patients from multiple centres across the US further adds to its value.

Despite the numerous advantages, there is one critical factor and major limitation that needs to be considered when interpreting the findings of this study: DNA methylation signatures were derived from mixed cell tissue samples (i.e., peripheral blood) which vary in their cellular composition according to the presence or absence of systemic inflammation.

Hence, the main question that arises is whether observed CD associated DNA methylation changes represent cell type specific epigenetic alterations or are the result of profiling different proportions of circulating immune cells, which all have unique methylation signatures (10). Whilst the former is impossible to prove given the study design, there are a number of findings that point towards a likely effect of cell composition on DNA methylation changes. Indeed, the authors demonstrate clear differences in cell composition of blood samples obtained from CD patients at diagnosis compared to controls (Figure S2). These differences largely disappear in follow-up samples and with it the vast majority of disease associated DNA methylation changes. Although the authors adjust for estimated cell subset proportions (estimates based on DNA methylation

signatures) (11) as covariates when performing differential DNA methylation analysis, this approach cannot completely exclude a confounding effect of cell composition. Hence, the finding of CD associated DNA methylation changes at diagnosis reverting to being indistinguishable from controls at follow-up must be interpreted with caution. In this respect it is important to note that once fully established (e.g., in differentiated cells), DNA methylation is a highly stable epigenetic mark and hence it would be surprising if reported large-scale methylation changes are reversible in a relatively short period of time (i.e., 1–3 years). Although the authors' conclusion that transient epigenetic changes are unlikely to play a major role in contributing to the chronic relapsing nature of IBD is correct, reported findings do not exclude their presence in individual cell subsets.

Indeed, this represents another major limitation associated with performing epigenetic profiling on mixed cell tissue samples, that is the inability to identify disease associated alterations effecting specific cell types. This makes it impossible to investigate potential functional implications. Whilst correlating DNA methylation changes with transcriptional profiles appears a reasonable approach, in the study presented, transcriptional signatures were also derived from whole blood samples and hence the confounding impact of varying cellular composition equally applies. One may expect immune related pathways to be enriched if transcriptional profiles of samples with significant differences in immune cell composition are being compared.

Nevertheless, even if disease associated methylation changes are the result of changes in cell composition, they may still be of value as biomarkers provided they correlate with clinical parameters of interest. However, unfortunately the authors did not observe a correlation between DNA methylation and specific disease phenotype and/or disease progression thereby limiting the potential value of whole blood derived DNA methylation signatures as clinical biomarkers in paediatric CD.

The concept of DNA methylation mediating genetic risk of CD is highly plausible and the study performs elegant analyses using a Mendelian randomization approach that benefits from the availability of genotype data for all patients. Identification of CD associated methylation changes at 3 CpGs, which showed significant causal associations and did not change during the course of disease provide promising evidence in support of this hypothesis despite the limited statistical power. Future studies particularly those performing single cell transcriptional

profiling are likely to shed further light on this concept.

In summary, the study by Somineni and colleagues provides an important contribution to the field particularly when interpreted in the context of its specific strengths and limitations. Importantly, whilst highlighting many challenges associated with performing epi-genome wide association studies, results do not oppose the main concept of epigenetic mechanisms playing a crucial role in disease pathogenesis of complex diseases including CD.

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## Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

*Ethical Statement:* The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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