Epigenetic modifications in ANCA Associated Vasculitis: Potential for insights into disease pathogenesis and prediction of outcome?

Thomas Oates and Alan D. Salama

UCL Centre for Nephrology, Royal Free Hospital, London, UK

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Correspondence:
Prof Alan Salama
UCL Centre for Nephrology, Royal Free Hospital
Rowland Hill Street, London NW3 2PF
a.salama@ucl.ac.uk
Phone: +44 2077940500 x36007
Fax: +44 2078302653
Methylation of DNA cytosine bases is an essential and well-studied epigenetic modification that may regulate and coordinate gene expression. Methylated gene promoter or enhancer regions are thought to block accessibility to transcriptional activators and prevent gene transcription. Numerous physiological and disease states have been associated with alterations in DNA methylation, with patterns shown to change over time in distinct cell types. The majority of this plasticity is explained by DNA variants, likely to be working through a downstream effect of sequence variation on transcription factor activity. However, more controversially, environmental exposures, including events such as famine, may affect DNA methylation patterns, which in addition, can be propagated to daughter cells during cell division and influence the transcription of associated genes through diverse and as yet poorly understood mechanisms. The potential link between environment, changes in DNA methylation and resultant variation in gene expression that may translate into particular cellular phenotypes, has made this topic attractive to many researchers in biomedicine.

Study of DNA methylation in disease may be able to define regions of DNA that are differentially methylated between cases and controls. Knowledge of these differences could aid understanding of the pathogenesis of the disease under study or allow characterization of novel biomarkers. Importantly, DNA methylation may go some way to explain the significant non-genetic contributions to autoimmune diseases, which have proven to be elusive to date. So far, use of diverse high throughput technologies has provided mechanistic information on how abnormal methylation patterns correlate with disordered gene expression. Furthermore, biomarkers based on the methylation status of small sets of genes have been validated as predictors of different outcomes in some cancers.

In this issue of the Journal of the American Society of Nephrology, Jones et al. present a study of DNA methylation in patients with Anti-neutrophil cytoplasm antibody (ANCA) associated vasculitis (AAV) using whole blood analysis. AAV is a relapsing and remitting autoimmune disease, generally
characterised by circulating ANCA, reactive to proteinase 3 (PR3) or myeloperoxidase (MPO) and thought to be triggered by certain environmental exposures, such as silica dust, infections, or drug exposure. Different disease phenotypes are recognised and more recently different genetic susceptibility loci have been identified according to ANCA serotype, with polymorphisms in HLA, PRTN3 and SERPINA1, encoding MHC molecules, PR3 and its natural inhibitor alpha-1-antitrypsin respectively, being associated with PR3-ANCA disease. In their study, Jones et al, firstly examined methylation and gene expression in AAV patients and healthy controls. Secondly, they investigated whether DNA methylation patterns can be used to predict disease relapse.

In the first part, Jones et al. selected two regions of the genes, MPO and PRTN3, which encode the autoantigens MPO and PR3 respectively, and interrogated the DNA methylation status of these two regions. They found that in both genes DNA methylation was reduced in active disease compared to healthy controls, and rebounded in disease remission. They then examined expression of MPO and PRTN3 and found a negative correlation between DNA methylation and gene expression, with higher methylation levels of the assayed gene sites being associated with less expression of associated genes.

They then narrowed their study to paired samples from a subset of patients assessed longitudinally, during active disease and remission. This investigation showed that, although statistically DNA methylation was more likely to increase at the MPO and PRTN3 loci upon remission, patients could be stratified into groups who increased or decreased methylation at these loci. Integrating expression and methylation data suggested that patients showing gene specific DNA methylation increases during remission showed a correlation with reduced expression of the associated autoantigen gene.

The second part of the study sought to establish if DNA methylation might associate with disease relapse. Patients who increased DNA methylation at the PRTN3 locus during remission were less likely to relapse regardless of their autoantigen serotype. Additionally, in univariate and multivariate
analyses, PRTN3 methylation status remained strongly associated with relapse, as patients with decreased PRTN3 promoter methylation were significantly more likely to relapse than other patients.

This study has a number of strengths. It suggests that AAV patients have distinct epigenetic phenotypes and that these differences in DNA methylation can be associated with both a cellular phenotype - that of differing autoantigen gene expression, and a key clinical consideration - disease relapse. The correlation between methylation and gene expression may in the future be able to shed light on the mechanism underlying ANCA formation and may elicit novel therapeutic targets. The possible association of DNA methylation and relapse may lead to a useful biomarker that could guide cessation or intensification of therapy.

However, there are points for consideration when building on this study in the future. As interest in methylation has grown, so has understanding of some of the challenges to interpreting human methylation data (Reviewed in 3,11), many of which are relevant to the study of Jones et al.

For example, DNA methylation is largely bimodal, with individual loci either fully methylated or not methylated at all. Therefore, observed methylation differences of approximately 10-20% may reflect varying DNA methylation signatures from varying proportions of different cell types making up the patient leukocyte samples.11 In their study, the authors could not exclude an impact of differential cell composition in the samples, as values for total white cell and neutrophil counts were missing in a significant proportion of the samples. Future studies could address this by using purified samples of a single cell subtype, or utilizing the emerging technique of single cell methylation analysis.12 However, a further challenge is then that an overall 10-20% methylation difference may be illustrative of a mosaic subset of cells with distinct methylation states.11

Another important consideration for the design of future experiments is DNA sequence variation. The clear importance of genetic influence on DNA
methylation patterns has recently been demonstrated, and the complex and context specific interaction of sequence, methylation, transcription factor activity and gene expression remains an area of intense study.\textsuperscript{13} As a result, drawing conclusions on the role of DNA methylation patterns in disease pathogenesis would be greatly enhanced by datasets assaying all of this information simultaneously.

Independent of using DNA methylation experiments to gain insight into mechanisms of disease, reproducible epigenetic signatures could be useful as biomarkers or prognostic indicators in the disease course. Again, in order to remove the confounder of sample cell compositions, assaying purified cell populations may be critical for the development of this field. Additionally, DNA methylation must be considered as a phenotype (such as proteinuria) that can change over time.\textsuperscript{11} Therefore, future experiments will need to prospectively evaluate the predictive ability of biomarkers based on epigenetic modifications over a longer follow-up period. Once the dynamic changes in methylation phenotype which occur over the course of disease are better understood, this will raise the issue of what time points to compare to provide the most robust prediction for future outcomes such as relapse. In addition, it is important to consider the impact of therapy on methylation status. Although the authors have attempted to address this by considering the impact of glucocorticoid use, the effect of cytotoxic or other depletional agents was not taken into account. Previous studies have suggested that IL-6 blockade for example can impact on methylation status in SLE patients.

The study results raise some other interesting questions regarding the fundamental biology of AAV. Why does regulation of the PR3 locus predict relapses in patients with MPO-ANCA? Does modulation of PR3 in turn regulate MPO expression? Intriguingly, why doesn't MPO methylation predict changes in MPO expression? Is this related to other epigenetic modifications such as the histone modifications of the MPO promoter which was associated with reduced MPO transcription?\textsuperscript{14} Additionally, if PR3 gene expression predicts relapse and influences MPO expression, how to explain the genome wide association study data demonstrating differential gene influence based
on ANCA serotype\textsuperscript{10} and the lower relapse rates found in MPO-ANCA patients compared to those with PR3-ANCA? Finally, what environmental factors are altering methylation status and can these be targeted and modified?

An important aspect to consider is the need for a validation cohort to ensure that the findings from the studied cohort can be generalised to other AAV populations. Clearly, the study patients were carefully selected, and although enriched for previous relapse, there appeared to be no episodes of relapse in patients in the longitudinal arm between the active and remission study time points, and unusually the ANCA serotype had no impact on frequency of disease relapse. These issues and questions can be tackled by performing future prospective studies.

Overall, the work of Jones \textit{et al.} represents a first and significant step linking epigenetic modifications and disease phenotype in AAV. There are other epigenetic mechanisms to be considered including the role of non-coding RNAs and histone modifications which could be addressed together in the future. There has been a recent explosion of epigenetic studies guided by large consortia and using sophisticated whole genome analyses\textsuperscript{15}. Much of this work has looked specifically at immune phenotypes and leukocyte subtypes, which are key players in mediating autoimmune disease in response to environmental cues. Therefore, the study by Jones should represent a springboard from which the nephrology and vasculitis communities embrace these novel approaches and further investigate the role of epigenetic modifications in immune mediated kidney diseases.

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References


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