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## Gene-specific DNA methylation in white blood cells: Association with blood cellular heterogeneity

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### ABSTRACT

**Objective:** The purpose of this study was to determine if there is a significant difference in methylation levels between whole blood and CD4+ T cells.

**Methods:** A panel of twelve (12) genes selected on the basis of their involvement in inflammation, autoimmunity and/or fibrosis was analyzed for methylation in whole blood and CD4+ T cells using EpiTect Methyl PCR Arrays technology.

**Results and conclusion:** Even though the methylation levels remain the same in both CD4+ T cells and whole blood, methylation is significantly elevated in whole blood, implying presence of methylated status of these genes in cells other than CD4+ T cells.

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### KEYWORDS

Methylation;  
Peripheral blood;  
Genomic DNA;  
CD4+ T cell;  
Autoimmunity;  
Methyl qPCR arrays.

### INTRODUCTION

Literature is very much variable with regard to the source of DNA used in methylation studies using human peripheral blood. Many studies use genomic/leukocyte DNA while an equal number use either total lymphocyte DNA or a specific lymphocyte cell type DNA. Thus given the cellular and population heterogeneity of blood and depending on whether cell purification is employed in the study care should be taken in interpreting results.

In most studies DNA is acquired from total white blood cells (WBC) isolated by simple centrifugation of whole blood and collection of the buffy coat, since it is the easiest method and provides a concentrated

source of DNA<sup>[1,2]</sup>. However when one looks closely, the centrifugation of blood over a density gradient allows the separation of the more abundant granulocytes (~85% of total WBC) from the smaller mononuclear cell fraction, which is comprised of monocytes and lymphocytes<sup>[1,2]</sup>. Many publications refer to the mononuclear cell fraction as lymphocytes even though they contain ~15% monocytes. The isolation of pure lymphocytes or specific lymphocyte subpopulations requires additional steps, such as flow cytometric separation using cell surface receptor antibody binding or magnetic bead separation, and if a richer cell harvest is required both methods require an additional enrichment step<sup>[3,4]</sup>.

Another important factor to consider is the well

known fact that there are differences in total WBC counts in healthy individuals, (ranging from 5,000–10,000/ $\mu$ l), which are to some extent controlled by certain host and lifestyle characteristics such as age, gender and alcohol drinking<sup>[5,6]</sup>. There are also significant differences in blood cell populations observed due to differences in gender, race/ethnicity, as well as during periods of stress, acute infections or inflammation, and even exercise has impact on specific cell types<sup>[6]</sup>. All these complicate investigating DNA methylation in WBC and need to be considered in designing investigations aimed at identifying associations between DNA methylation and health outcomes.

Many epigenetic studies concerned with autoimmune rheumatic disease have focused mostly on systemic erythematosus lupus (SLE) and rheumatoid arthritis (RA), and have successfully identified both global and sequence-specific methylation and expression changes of some key genes involved in immune function<sup>[7]</sup>. It is now known that the immune deregulation in SLE involves both B and T lymphocytes, cytokine production, clearance of complexes and apoptosis<sup>[8,9]</sup>, however for many years, most of the DNA methylation analyses in SLE focused on the study of CD4+ T cells. For example, there are many studies reporting on T-cell DNA hypomethylation in SLE and other connective tissue diseases, such as systemic sclerosis (SSc) and dermatomyositis<sup>[10,11]</sup>.

The central role of B cells in the patho-genesis of autoimmune diseases includes the presentation of autoantigens, activation of CD4+ and CD8+ cells and secretion of proinflammatory cytokines (12), and it is therefore relevant to investigate epigenetic deregulation in this cell type as well. As a matter of fact, several studies have shown that B cells also display alterations in the DNA methylation status of certain genes in SLE<sup>[9,11]</sup>. For example; the study of methylation patterns in CD19 (+) B lymphocytes in a group of asthmatics shows an epigenomic dysregulation of the CYP26A1 gene<sup>[13]</sup>. Similarly, CD5+ B cells have been found to be hypomethylated in lupus. Early B cell factor 1 (EBF1) has also proven to be an important regulator of signalling pathways in B cells that facilitates the demethylation of DNA, though the underlying mechanisms are yet to be revealed<sup>[14]</sup>. For example, blockade of the interleukin-6 autocrine loop in SLE B cells restores DNA methylation status, thus opening new

perspectives for therapy<sup>[15]</sup>.

We do not yet know the complete hierarchical order of epigenetic modifications and the differential mechanisms that are involved in different white blood cell types, but one important issue is that the epigenetic deregulation affects both B and T lymphocytes<sup>[15,16]</sup>. So while not all is yet known about epigenetic deregulation of T cell-specific genes, future investigations should also address the role of B cell-specific set of epigenetic changes resulting in the patho-genesis of autoimmune rheumatic disease. Despite the obvious fact that each cell type has a distinct role in the pathogenesis of each disease, it is also worth noting that there are complex interactions between the different immune cell types in autoimmune rheumatic diseases. As an example, it has been found that patients with SLE, myositis, RA and SSc share activation of a common type I interferon pathway<sup>[14,17]</sup>. So in order to develop an epigenomic signature of genes involved in the pathogenesis of these diseases both T and B lymphocyte biology have to be investigated from all angles, and this holds potential to help identify patients likely to respond to therapies that target this cohort of genes.

This study assessed whether methylation levels of genomic DNA are different from that of CD4+ T cells in healthy individuals. Using The EpiTect Methyl qPCR Arrays we analyzed the promoter hypermethylation pattern of 12 genes previously reported to exhibit epigenomic dysregulation in cancer and various autoimmune rheumatic diseases.

## MATERIALS AND METHODS

### Study population and ethics

This study was approved by the Ethics Committee for Research on Human Subjects of both the University of the Witwatersrand (Johannesburg, South Africa) and the South African National Blood Transfusion Services (SANBS). The individuals investigated in this study were volunteer blood donors recruited during a blood drive by the SANBS. These 30 individuals were randomly selected to represent both sexes and the various races found within South Africa.

### Sample collection and DNA preparation

After obtaining written informed consent from

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blood donors 5ml samples of peripheral blood were collected in EDTA tubes. Whole blood CD4<sup>+</sup> T cells were isolated from peripheral blood using MACS magnetic cell sorting technique (Miltenyi Biotec). Genomic DNA (gDNA) from both whole blood and from CD4<sup>+</sup> T cells was isolated using GenElute mammalian DNA extraction kit (Sigma-Aldrich). The isolated DNA was quantified using the Nanodrop 2000C spectrophotometer (Thermoscientific) and thereafter stored at -20°C until use.

### DNA methylation analysis

The panel of genes profiled consisted of twelve<sup>[12]</sup> genes, which were selected on the basis of their involvement in many collagen (especially autoimmune rheumatic) diseases, whose common characteristics include inflammation, autoimmunity and/or fibrosis. PCR Array analysis was performed using the EpiTect Methyl PCR Arrays technology (SABiosciences) on a 7300 Applied Biosystems real-time PCR instrument.

### Data analysis

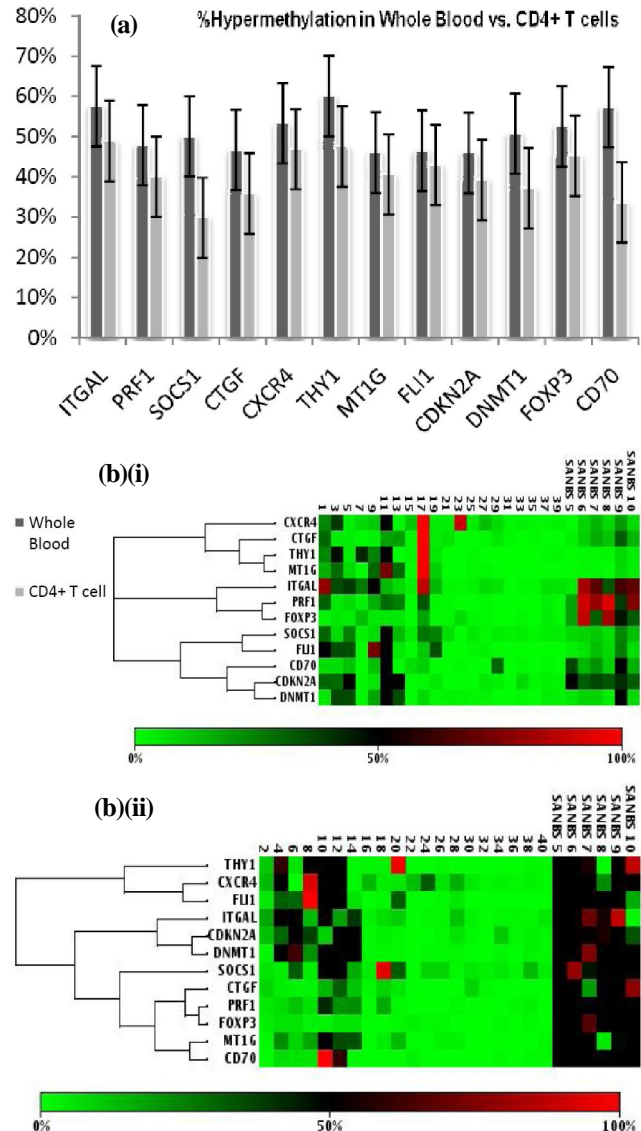
The methylation qPCR Array data was analyzed using an integrated Excel-based template provided by the MethylScreen™ technology (SABiosciences). Similarly, graphical representation (heat map) of the qPCR array data was obtained using the MethylScreen™ technology.

## RESULTS AND DISCUSSION

From the graphical data analysis the following observations can be made;

1. The majority of analyzed genes are intermediately methylated (about 50% hypermethylated) as opposed to hypermethylated in both whole blood and CD4<sup>+</sup> T cells, which somehow makes sense since these are healthy individuals and the opposite is to be expected in diseased condition.
2. In all instances, whole blood appears to significantly exhibit higher levels of methylation than CD4<sup>+</sup> cells, which may indicate that these genes are also available on other cells besides CD4<sup>+</sup> T cells.

Overall the methylation pattern, which indicates intermediate methylation of all 12 genes analyzed, remains the same in both CD4<sup>+</sup> T cells as in whole blood.



**Figure 1 :** (a) A bar chart showing hypermethylation percentage of the genes analyzed from peripheral blood of the 30 healthy individuals. (b) A heatmap showing distribution of hypermethylation among the 12 genes analyzed in (i) whole blood and (ii) CD4<sup>+</sup> T cells.

## CONCLUSION

There is growing evidence for a role for epigenetic mechanisms in the development of autoimmune diseases. Accumulating evidence indicates that epigenetic alterations contribute to exacerbated activation or deregulation of the mechanisms that maintain tolerance to self-antigens in patients with autoimmune conditions<sup>[18]</sup>. Significant evidence has also shown that there is heterogeneity in the characteristics of vasculopathies underlying different autoimmune diseases<sup>[19]</sup>. The data presented in this study further indicate that epigenetic

changes seen on human T cells are just a portion of the entire mechanisms that influence the phenotypic characteristics both in health and disease. Hence the need for further investigation into the other white blood cell subtypes.

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### REFERENCES

- [1] H.Pertoft; Fractionation of cells and subcellular particles with percoll, *J.Biochem.Biophys.Methods*, **44**, 1-30 (2000).
- [2] A.J.Ulmer, M.Ernst, E.Brandt, H.D.Flad; Isolation and subfractionation of human peripheral blood mononuclear cells (PBMC) by density gradient centrifugation on Percoll, *Immunobiology*, **166**, 238-250 (1984).
- [3] W.L.J.Haas; Separation of antigen-specific lymphocytes. I. Enrichment of antigen-binding cells, *J.Exp.Med.*, **141**, 1004-1014 (1975).
- [4] A.J.Strelkauskas, S.Dray; Enumeration and isolation of human T and B lymphocytes by rosette formation with antibody-coated erythrocytes, *Clin.Exp.Immunol.*, **22**, 62-71 (1975).
- [5] M.B.Terry, N.Vin-Raviv, H.C.Wu, R.M.Santella; DNA methylation in white blood cells: association with risk factors in epidemiologic studies, *Epigenetics*, **6**, 828-837 (2011).
- [6] F.F.Zhang, J.Carroll, K.G.Fulda, M.Kaur, K.Gonzalez, J.K.Vishwanatha, R.M.Santella, A.Morabia; Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood, *Epigenetics*, **6**, 623-629 (2011).
- [7] E.Ballestar; Epigenetic alterations in autoimmune rheumatic diseases, *Nature Reviews Rheumatology*, **7**, 263-271 (2011).
- [8] M.Bijl, P.C.Limburg, C.G.Kallenberg; Expression of costimulatory molecules on peripheral blood lymphocytes of patients with systemic lupus erythematosus, *Ann.Rheum.Dis.*, **60**, 523-526 (2001).
- [9] S.Garaud, S.Jousse-Joulin, C.Hanrotel-Saliou, A.Sarau, R.A.Mageed, P.Youinou, Y.J.Renaudineau; IL-6 modulates CD5 expression in B cells from patients with lupus by regulating DNA methylation, *J.Immunol.*, **182**, 5623-5632 (2009).
- [10] W.Lei, W.Lei, Y.Luo, K.Yan, S.Zhao, Y.Li, X.Qiu, Y.Zhou, H.Long, M.Zhao, Y.Liang, Y.Su, Q.Lu; Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis, *Scand.J. Rheumatol.*, **38**, 369-374 (2009).
- [11] B.W.Higgs, L.Richman, D.F.Fiorentino, S.A.Greenberg, B.Jallal, Y.Yao; Identification of activated cytokine pathways in the blood of systemic lupus erythematosus, myositis, rheumatoid arthritis, and scleroderma patients, *Int.J.Rheum.Dis.*, **15**, 25-35 (2012).
- [12] J.Anolik; B cell biology and dysfunction in SLE, *Bull NYU Hosp.Jt.Dis.*, **65**, 182-186 (2007).
- [13] M.Pascual, M.Isidoro-Garcia, J.Padrón, T.Turner, F.Lorente, I.Dávila, J.M.Greally; Epigenetic changes in B lymphocytes associated with house dust mite allergic asthma., *Epigenetics*, **6**, (2011).
- [14] J.Hagman, K.Lukin; B lymphocyte lineage specification, commitment and epigenetic control of transcription by early B cell factor 1, *Curr.Top. Microbiol.Immunol.*, **356**, 17-38 (2012).
- [15] Y.Renaudineau; Epigenetics and autoimmunity, with special emphasis on methylation, *Keio.J.Med.*, **60**, 10-16 (2011).
- [16] A.M.Deaton, A.R.Kerr, R.S.Illingworth, J.Guy, R.Andrews, A.Bird; Cell type-specific DNA methylation at intragenic CpG islands in the immune system, *Genome.Res.*, **21**, 1074-1086 (2011).
- [17] B.W.Higgs, B.White, W.Zhu, W.I.White, C.Morehouse, P.Brohawn, P.A.Kiener, L.Richman, D.Fiorentino, S.A.Greenberg, B.Jallal, Y.Yao; Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway., *Ann.Rheum.Dis.*, **70**, 2029-2036 (2011).
- [18] L.Mazari, M.Zouali; Subversion of B lymphocyte tolerance by hydralazine, a potential mechanism for drug-induced lupus, *Proc.Natl.Acad.Sci.U S A.*, **104**(15), 6317-22 (2007).
- [19] C.López-Pedreira, M.Ramos-Casals, M.Santos-Gonzalez, A.Rodriguez-Ariza, Cuadrado M.J.Clin; Cardiovascular risk in systemic autoimmune diseases: Epigenetic mechanisms of immune regulatory functions, *Clin.Dev.Immunol.*, **2012**, 974648 (2012).