Epigenetic Regulation of Airway Inflammation in Asthma

Lakshitha Gunawardhana

B. Biotech (Hons)

A Thesis Submitted for the Degree of Doctor of Philosophy

School of Medicine and Public Health

Faculty of Health

The University of Newcastle

June 2014
Statement of Originality

The content of this thesis is the result of work I have carried out since the commencement of my research higher degree candidature. It does not contain material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to provisions of the copyright Act 1968. I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material.

Lakshitha Gunawardhana

Date: 26th June 2014
Acknowledgement of Authorship

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Signature of Candidate:

Full Name of Candidate: Lakshitha Prabhath Gunawardhana
Date: 26th June 2014

Signature of Assistant Dean Research Training (ADRT):

Full Name of ADRT: Prof Robert Callister
Date: 27th June 2014
Acknowledgement:

Undertaking this PhD degree is perhaps the most challenging activity that I had undertaken so far and it would not have been possible to do without the support and guidance that I received from many people.

My first debt of gratitude must go to my main supervisor Prof. Peter Gibson for all the support and encouragement over these years. It’s been a privilege to being a part of his research group and having the opportunity to involve in a novel research. Peter will remain my best role model for an enthusiastic scientist and a person of choice for insightful science discussions. I appreciate all contributions of time, ideas and funding to make this PhD research productive and stimulating.

It is my great pleasure to be the first PhD student of Dr Katie Baines. I always admired her enthusiasm and persistence in scientific investigations and this has greatly helped sparking my interest in this research area. I want to especially thank her unflagging encouragement and for being a mentor to me. She has been a strong and supportive adviser providing guidance necessary for me to proceed through the doctoral program and complete my dissertation.

Dr. Jodie Simpson has been helpful in providing advice many times. She is an optimistic person having knowledge in wider subject area and especially clinical study design. Her advice was indispensable from submission of ethics and throughout clinical sample collection phase of this study.

The members of VIVA group have contributed immensely to my personal and professional time at HMRI. Thanks to all of those people including Deborah Hall, Dr Lisa Wood, A/Prof Peter Wark and Heather Powell to name a few. Members of clinical and the laboratory sample processing team have also helped immensely. Special thanks to Rebecca Oldham for teaching and helping me sample processing during the early days and subsequently, helping with clinical recruitments. Similarly, Michelle Gleeson,
Kelly Fakes, Emma Hall and Naomi Fibbens for teaching me and assisting with sputum processing also thanks to Bridgette Ridewood for assisting with sample processing. Thanks to all members of VIVA clinical team and especially Hayley Candler, Anne-Marie Gibson who made a greater contribution in the patient recruitment in the ERA study. Thanks to my early office neighbours of Kristy Parsons and Melinda Tooze for helpful talks and ideas. Apart from VIVA group, members of Genetic research group, Mothers and babies’ research group have helped with certain aspects. In particular, thanks to Tiffany Evans from Genetic research group for sharing tips and helping me with Illumina infinium workflow. Also Heather Powell and A/Prof Patrick McElduff were a great help in clarifying statistics related problems.

My fellow PhD students also deserve my sincerest thanks, their friendship and assistance has meant more to me than I could ever express. Alan Hsu, Rebecca Vanders, and Hayley See perhaps the few laboratory based PhD students I met very early on. Thanks to all of you for helping out things in the lab and sharing of ideas on many things throughout these years. Also thanks to subsequent lab-based students of Juan juan Fu, Faizul Addnen, Heng Zong. Especially thank Juan juan for the help with clinical recruitments and for being one of my later stage office neighbours who always willing to listen and share thoughtful ideas.

I gratefully acknowledge the funding received towards my PhD research from the Asthma Foundation NSW and the priority research centre for asthma, University of Newcastle.

Lastly, I would like to say a heartfelt thank my Mum, Dad and Sister for always believing in me, for their encouragement and for their help in many ways during the past few years.

Ending this formal acknowledgement, I must say that best and worst moments of my doctoral journey have been shared with many people. However, in here I just mentioned some people whose contribution is obvious because the list of the people I need to thank will not fit to a single acknowledgement section.
Publications included as a part of this thesis


2. Gunawardhana LP, Gibson PG, Simpson JL, Powell H, Baines KJ. Activity and expression of histone acetylases and deacetylases in inflammatory phenotypes of asthma. Clinical & Experimental Allergy 2014;44(1):47-57. (Chapter 4)

3. Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. Epigenetics 2014;9(9):1302-1316. (Chapter 5)
Statement of contribution of others

I, Prof. Peter Gibson, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing papers entitled:


Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. Submitted to Epigenetics

Signature:

Full Name of Co-Author: Prof. Peter Gibson
Date: 12-06-2014
Statement of contribution of others

I, Dr. Katie Baines, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing papers entitled:


Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. Submitted to Epigenetics

Signature:

Full Name of Co-Author: Dr Katherine J Baines

Date: 12/06/2014
Statement of contribution of others

I, A/prof. Jodie Simpson, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing papers entitled:


Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. Submitted to Epigenetics

Signature:

Full Name of Co-Author: A/Prof Jodie L Simpson

Date: 13th June 2014
Statement of contribution of others

I, Heather Powell, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing of papers entitled:


Signature:

Full Name of Co-Author: Gillian Heather Powell
Date: 12th June 2014
Statement of contribution of others

I, Prof. Joerg Mattes, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing papers entitled:


Signature:

Full Name of Co-Author: Prof. Joerg Mattes
Date: 12th June 2014
Statement of contribution of others

I, Dr Vanessa Murphy, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing papers entitled:


Signature:

Full Name of Co-Author: Dr Vanessa Murphy
Date: 13/6/2014
Statement of contribution of others

I, Dr. Rodney Lea, attest that Research Higher Degree candidate, Lakshitha Gunawardhana, provided substantial intellectual input and contributions to the study design, laboratory experimentation, data input, statistical analyses and manuscript preparation/writing papers entitled:

Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ. Characteristic DNA methylation profiles in peripheral blood monocytes are associated with inflammatory phenotypes of asthma. Submitted to Epigenetics

Signature:

Full Name of Co-Author: Dr Rodney Lea

Date: 13th June 2014
Statement of contribution of others

I, Dr. Miles Benton, attest that Research Higher Degree candidate, Lakshitha
Gunawardhana, provided substantial intellectual input and contributions to the study
design, laboratory experimentation, data input, statistical analyses and manuscript
preparation/writing papers entitled:

Gunawardhana LP, Gibson PG, Simpson JL, Benton MC, Lea RA, Baines KJ.
Characteristic DNA methylation profiles in peripheral blood monocytes are associated
with inflammatory phenotypes of asthma. Submitted to Epigenetics

Signature:

Full Name of Co-Author: Dr Miles Clifford Benton

Date: 13th June 2014
Publications & conference presentations related to this thesis:

Publications


Conference presentations

Biomarker Discovery Conference (BDC) in Dec 2010

Gunawardhana LP, Baines KJ, Mattes J, Murphy VE, Simpson JL, Gibson PG. Maternal asthma is associated with alterations in DNA methylation profile of peripheral blood of infants. Oral presentation at Biomarker Discovery Conference in Dec 2010.


Gunawardhana, LP, Baines, KJ, Simpson, JL, Mattes, J, Murphy, VE, Gibson, PG. Maternal asthma is associated with alterations in DNA methylation profile of peripheral blood of infants. Respirology. 2011. 16(Suppl. 1): 19. (Oral)


Table of contents:

Statement of Originality ............................................................................................................ i
Acknowledgement of Authorship ............................................................................................. ii
Acknowledgement: ................................................................................................................ iii
Publications included as a part of this thesis ........................................................................... v
Statement of contribution of others ......................................................................................... vi
Publications & conference presentations related to this thesis: ................................................ xiv
List of abbreviations: ................................................................................................................ xxv
Abstract .................................................................................................................................... 1

1. Introduction .......................................................................................................................... 3
  1.1. Asthma ............................................................................................................................ 3
  1.2. Clinical signs and pathophysiology .............................................................................. 4
  1.3. Inflammatory Phenotypes of Asthma ........................................................................... 5
    1.3.1. Eosinophilic asthma .............................................................................................. 6
      1.3.1.1. Role of Th2 cytokines in eosinophilic asthma ............................................. 7
      1.3.1.2. Role of eosinophils in allergic airway inflammation ............................... 8
      1.3.1.3. Pathophysiology, disease mechanisms and unique features .................... 10
    1.3.2. Neutrophilic asthma .............................................................................................. 11
      1.3.2.1. Role of the innate immune system in neutrophilic asthma ...................... 12
      1.3.2.2. Role of Neutrophils in airway inflammation ............................................... 13
      1.3.2.3. Pathophysiology, Disease mechanisms and unique features .................... 15
    1.3.3. Paucigranulocytic asthma .................................................................................... 17
  1.4. Role of blood monocytes and airway macrophages in asthma .................................... 19
    1.4.1. Monocytes ............................................................................................................ 19
    1.4.2. Airway Macrophages ........................................................................................... 21
    1.4.3. Environmental influences on monocyte and macrophage biology ............... 24
  1.5. Risk factors for development of asthma ........................................................................ 25
    1.5.1. Risk factors during early life: Developmental origins of asthma .................... 27
  1.6. Epigenetics and asthma .................................................................................................. 29
  1.7. DNA Methylation .......................................................................................................... 32
1.7.1. DNA methylation in asthma ................................................................. 35
1.8. Chromatin structure .............................................................................. 37
1.8.1. Histone modifications ........................................................................ 39
1.8.1.1. Histone acetylation and de-acetylation ....................................... 41
1.8.1.1.1. Histone acetylation and de-acetylation in asthma ................. 42
1.9. Overall project hypothesis .................................................................... 44
1.9.1. Specific aims and hypotheses ............................................................. 45
2. Methods ..................................................................................................... 47
2.1. Ethics and Regulatory approval ............................................................. 47
2.2. Recruitment Methods ............................................................................ 47
2.2.1. MAP, VEAP and GIA ....................................................................... 47
2.2.2. ERA ................................................................................................. 48
2.3. Clinical Study Design: .......................................................................... 48
2.3.1. MAP, VEAP and GIA ....................................................................... 48
2.3.2. ERA ................................................................................................. 49
2.4. Clinical testing and sample collection .................................................... 50
2.4.1. Spirometry: ...................................................................................... 50
2.4.2. Saline Challenge and Sputum Induction ......................................... 51
2.4.3. Blood collection ............................................................................... 51
2.4.4. Allergy skin prick testing ................................................................. 51
2.4.5. Exhaled nitric oxide ......................................................................... 52
2.4.6. Exhaled carbon monoxide ............................................................... 52
2.5. Processing of biological samples ............................................................ 53
2.5.1. Induced sputum processing .............................................................. 53
2.5.2. Induced sputum differential cell counting ....................................... 54
2.5.3. Blood processing and Ficoll density gradient ................................. 54
2.5.4. Immunomagnetic cell separation ..................................................... 55
2.5.4.1. Blood .......................................................................................... 55
2.5.4.2. Sputum ....................................................................................... 56
2.6. Molecular methods (General) ............................................................... 56
2.6.1. Nucleic acid extraction .................................................................... 56
2.6.1.1. Nucleic acid quantitation ............................................................ 57
2.6.2. Nuclear protein extraction ............................................................... 57
2.6.2.1. Nuclear protein quantification ...........................................................58
2.7. Infinium assay ..................................................................................................58
2.7.1. Bisulfite conversion: .................................................................................58
2.7.2. Infinium assay for methylation .................................................................58
2.7.3. Validation of array data .............................................................................59
2.8. ELISA ...............................................................................................................59
2.8.1. Histone acetyltransferase activity assay ....................................................60
2.8.2. Histone deacylase activity assay ...............................................................60
2.8.3. Gene expression analysis of HATs and HDACs.......................................60
2.9. Data analysis .....................................................................................................61
2.9.1. Illumina infinium methylation .................................................................61
2.9.2. Statistical methods ....................................................................................63
2.10. Exploratory data analyses .............................................................................63
3. Differential DNA Methylation Profiles of Infants Exposed to Maternal Asthma during Pregnancy .................................................................65
3.1. Abstract ............................................................................................................66
3.2. Introduction ......................................................................................................67
3.3. Methods ............................................................................................................68
3.3.1. Study design and participants.................................................................68
3.3.2. DNA extraction and bisulfite conversion ..................................................69
3.3.3. Genome-wide methylation assay ............................................................69
3.3.4. EpiTect methyl II DNA Methylation qPCR Primer Assay .......................70
3.3.5. Data analysis .............................................................................................70
3.4. Results ..............................................................................................................72
3.4.1. Clinical features ........................................................................................72
3.4.2. Differential DNA methylation in peripheral blood of infants due to maternal asthma ................................................................................................74
3.4.3. Effects of asthma medication and atopy ...................................................78
3.4.4. Clinical associations ...............................................................................79
3.5. Discussion ........................................................................................................ 81
3.5.1. Conclusion ................................................................................................ 85
4. Activity and expression of histone acetylases and deacetylases in inflammatory phenotypes of asthma .................................................................................................. 86
4.1. Abstract ............................................................................................................ 87
   Background .......................................................................................................... 87
   Objective .............................................................................................................. 87
   Methods ................................................................................................................... 87
   Results ...................................................................................................................... 87
   Conclusions & clinical relevance ............................................................................ 88
4.2. Introduction ...................................................................................................... 88
4.3. Methods .......................................................................................................... 90
   4.3.1. Participants ................................................................................................ 90
   4.3.2. Induced sputum processing ....................................................................... 90
   4.3.3. Asthma inflammatory phenotype and severity definition ......................... 91
   4.3.4. Cell isolations ............................................................................................ 91
   4.3.5. Immunomagnetic cell separation .............................................................. 91
   4.3.6. Gene expression analysis .......................................................................... 92
   4.3.7. Preparation of nuclear extracts .................................................................. 92
   4.3.8. Histone acetyltransferase (HAT) activity assay ........................................ 92
   4.3.9. Histone deacetylase (HDAC) activity assay ............................................. 93
   4.3.10. Statistical analysis ................................................................................. 93
4.4. Results .............................................................................................................. 94
   4.4.1. Clinical characteristics of asthma participants .......................................... 94
   4.4.2. HAT and HDAC activity and expression in asthma ................................. 96
   4.4.3. Clinical characteristics of inflammatory phenotypes of asthma .......... 97
   4.4.4. HAT and HDAC Activity and Expression in Monocytes in Inflammatory Phenotypes of Asthma ................................................................. 100
   4.4.5. Effects of severity, ICS use and Age on HAT and HDAC Activity ...... 102
4.5. Discussion ...................................................................................................... 103
   4.5.1. Conclusion .............................................................................................. 108
5. Characteristic DNA methylation profiles in peripheral blood monocytes associated with inflammatory phenotypes of asthma .............................................................................. 109
5.1. Abstract ........................................................................................................... 110
Background ............................................................................................................ 110
Methods ................................................................................................................. 110
Results .................................................................................................................... 110
Conclusions ............................................................................................................ 110
5.2. Introduction .................................................................................................... 111
5.3. Materials and methods.................................................................................... 112
  5.3.1. Participants .............................................................................................. 112
  5.3.2. Induced sputum processing ..................................................................... 112
  5.3.3. Asthma inflammatory phenotype classification ...................................... 113
  5.3.4. Peripheral blood monocyte isolation ....................................................... 113
  5.3.5. DNA isolation and bisulphite conversion ............................................... 113
  5.3.6. Genome-wide methylation assay ............................................................ 114
  5.3.7. Data analysis ........................................................................................... 114
    5.3.7.1. Network Analysis ............................................................................ 115
  5.4. Results ............................................................................................................ 116
    5.4.1. Clinical features and airway inflammation ............................................. 116
    5.4.2. Differential DNA methylation of blood monocytes in asthma inflammatory phenotypes ............................................................ 119
    5.4.3. Differential DNA methylation comparisons ........................................... 121
    5.4.4. Pathway network analysis of differentially methylated genes in asthma inflammatory phenotypes ............................................................ 123
  5.5. Discussion ...................................................................................................... 131
    5.5.1. Conclusion .............................................................................................. 136
6. General Discussion ............................................................................................ 137
  6.1. Primary findings of this thesis ........................................................................ 137
    6.1.1. Effects of maternal asthma on DNA methylation in infancy .............. 137
    6.1.2. Clinical associations of differentially methylated genes...................... 138
    6.1.3. Effects of maternal atopy and ICS use on methylation of PM20D1 ......... 139
    6.1.4. Increased histone acetylation in neutrophilic asthma ......................... 140
    6.1.5. Monocyte DNA methylation in asthma inflammatory phenotypes ...... 140
  6.2. Strength and Limitations ................................................................................ 143
    6.2.1. MAP/VEAP and GIA Study ................................................................. 143
    6.2.2. ERA study .............................................................................................. 144
6.3. Future Research.............................................................................................. 145
6.4. Summary ........................................................................................................ 145
6. Appendices............................................................................................................. 147
   6.1. Appendix 1: Supplementary material relevant to the chapter 3 ............... 147
7. References............................................................................................................. 152
**List of Tables:**

1. Table 1.1: Features of inflammatory phenotypes of asthma .......................................................... 19
2. Table 3.1: Maternal and Infant Characteristics ............................................................................ 73
3. Table 3.2: Maternal and infant blood cell count ........................................................................... 74
4. Table 3.3: Differentially methylated CpG loci in infants born to mothers with asthma .............. 76
5. Table 3.4: PANTHER biological process and molecular function categories enriched for positive selection. Underlined genes indicated more methylation in asthma. .......................... 78
6. Table 4.1: Demographic and clinical characteristics ....................................................................... 95
7. Table 4.2: Protein enzyme activity and gene expression of selected HATs and HDACs in blood monocytes from subjects with and without asthma ............................................ 96
8. Table 4.3: Demographic and clinical characteristics in inflammatory phenotypes of asthma ............................................................................................................................ 98
9. Table 4.4: HAT and HDAC activity in blood monocytes from subjects with asthma inflammatory phenotypes who were taking ICS ........................................................... 101
10. Table 4.5: Relative gene expression of selected HATs and HDACs of blood monocytes in inflammatory phenotypes of asthma ................................................................. 101
11. Table 4.6: Relative gene expression of selected HATs and HDACs of blood monocytes in inflammatory phenotypes of asthma ................................................................................. 101
12. Table 4.7: HAT and HDAC activity in blood monocytes from subjects with asthma inflammatory phenotypes who were taking ICS ................................................................. 103
13. Table 5.1: Demographic and clinical characteristics of the subjects with asthma and healthy controls .......................................................................................................................... 117
14. Table 5.2: Differentially methylated CpG loci common to all three inflammatory phenotypes ................................................................. 122
15. Table 5.3: Summary of top ranked genes associated with EA. Based on network diagram, genes with more than 3 interconnections with other genes have been included. .......................................................................................................................... 125
16. Table 5.4: GATHER KEGG Pathway Analysis of the gene clusters from EA vs Healthy analysis ............................................................................................................................. 126
17. Table 5.5: Genes with more than 3 interconnections within the 2 determined PGA networks ............................................................................................................................. 128
Table 5.6: GATHER KEGG Pathway Analysis of the gene clusters from PGA vs Healthy analysis. ........................................................................................................... 129

Table 5.7: GATHER KEGG Pathway Analysis of the gene clusters from NA vs Healthy analysis. ........................................................................................................................................... 130
List of Figures

Figure 1.1: Airway inflammation in asthma. ................................................................. 4
Figure 1.2: Induced sputum cytospins showing four inflammatory phenotypes. .......... 6
Figure 1.3: The cells of acquired immune system such as Th2 CD4+ lymphocytes are involved in eosinophilic airway inflammation ......................................................... 7
Figure 1.4: Eosinophil derived mediators important in airway inflammation .............. 9
Figure 1.5: The cells of the innate immune system such as epithelial cells, macrophages are involved in neutrophilic airway inflammation ............................................... 12
Figure 1.6: Neutrophil derived mediators in airway inflammation ............................. 14
Figure 1.7: A pictorial representation of the interaction of the environment in shaping organismal phenotypes ................................................................. 31
Figure 1.8: Stylistic diagram of a gene in relation to the double helix structure of DNA and to a chromosome. ................................................................. 32
Figure 1.9: Mechanism of CpG methylation ............................................................... 34
Figure 1.10: Schematic representation of the assembly of the core histones into the nucleosome ................................................................. 39
Figure 1.11: Histone modifications ............................................................................ 40
Figure 1.12: Role of HATs and HDACs in asthma .................................................... 44
Figure 3.1: Differential methylation levels expressed as % methylation for 8 CpG loci in peripheral blood DNA of infants’ born to mothers with or without asthma ............ 77
Figure 3.2: Methylation of FLJ32568 in infants born to mothers with asthma with and without ICS use during pregnancy (A), and atopic and non-atopic mothers with and without asthma (B) ................................................................. 79
Figure 3.3: Correlation of MAPK8IP3 and AURKA methylation with infant and maternal clinical parameters ............................................................. 80
Figure 3.4: Methylation of PIWIL-1 in infants born to mothers with or without asthma as determined by EpiTect Methyl II qPCR assay ..................................................... 81
Figure 4.1: Correlation of total histone acetyltransferases and histone deacetylases enzyme activities in peripheral blood monocytes ............................................. 97
Figure 4.2: Total histone acetyltransferases (HAT) (a), histone deacetylases (HDAC) (b) enzyme activities and their ratio (HAT: HDAC) (c) in peripheral blood monocytes in inflammatory phenotypes of asthma .................................................. 100
Figure 5.1: Clustering of the subjects based on differentially methylated loci ............ 120
Figure 5.2: Venn diagram showing unique and shared gene loci between and among three inflammatory phenotypes of asthma ............................................................. 121
Figure 5.3: The differentially methylated genes in EA interact in three networks......124
Figure 5.4: Sixty seven of the differentially methylated genes in PGA interact in two
networks........................................................................................................................127
Figure 5.5: In NA, only 8 the differentially methylated genes associated with each other
and formed a single network..........................................................130
List of abbreviations:

ACQ  
asthma control questionnaire

AHR  
airways hyperresponsiveness

AM  
alveolar macrophage

BMI  
body mass index

C2R  
chromotrope 2R

cAMP  
cyclic adenosine monophosphate

CD  
cluster of differentiation

CGIs  
CpG islands

COPD  
obstructive pulmonary disease

CS  
corticosteroids

DAMPs  
damage associated molecular patterns

DCs  
dendritic cells

DEP  
diesel exhaust particulate

DLCO  
diffusing capacity of the lung for carbon monoxide

DNMTs  
DNA methyltransferases

DRR  
damage recognition receptors

DTT  
dithiothreitol

EA  
eosinophilic asthma

ECP  
eosinophil cationic protein

EPO  
eosinophil peroxidase

FE\textsubscript{NO}  
exhaled nitric oxide (fractional)

FEV\textsubscript{1}  
forced expiratory volume in the first second

FVC  
forced vital capacity

GINA  
global initiative of asthma

GM-CSF  
granulocyte-macrophage colony stimulating factor

GR  
glucocorticoid receptor

GRE\textsubscript{s}  
glucocorticoid response elements

HATs  
histone acetyl-transferases

HDAC\textsubscript{s}  
histone deacetylases

ICS  
inhaled corticosteroids

IFN  
interferon

IgE  
immunoglobulin E

IL  
Interleukins

LABA  
long acting β2 agonists

LPS  
lipopolysaccharides

MBD  
methyl binding domains

MBP  
major basic protein

MGA  
mixed granulocytic asthma

MGG  
May-Grünwald-Giemsa

MIP  
macrophage inflammatory protein
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMPs</td>
<td>matrix metalloproteinases</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NA</td>
<td>neutrophilic asthma</td>
</tr>
<tr>
<td>NEA</td>
<td>non-eosinophilic asthma (includes PGA, NA and MGA)</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor kappa beta</td>
</tr>
<tr>
<td>NLR</td>
<td>NOD-like receptor</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>OCS</td>
<td>oral corticosteroid</td>
</tr>
<tr>
<td>oxLDL</td>
<td>oxidised low-density lipoprotein</td>
</tr>
<tr>
<td>PAMPs</td>
<td>pathogen associated molecular patterns</td>
</tr>
<tr>
<td>PBMCs</td>
<td>peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PD15</td>
<td>provocation dose</td>
</tr>
<tr>
<td>PGA</td>
<td>paucigranulocytic asthma</td>
</tr>
<tr>
<td>PMA</td>
<td>phorbol myristate acetate</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PPM</td>
<td>parts per million</td>
</tr>
<tr>
<td>PRRs</td>
<td>pattern recognition receptors</td>
</tr>
<tr>
<td>RLR</td>
<td>RIG-1-like receptor</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>TCC</td>
<td>total cells count</td>
</tr>
<tr>
<td>TGF</td>
<td>tissue growth factor</td>
</tr>
<tr>
<td>Th</td>
<td>T-helper type</td>
</tr>
<tr>
<td>Th1</td>
<td>T-helper type 1</td>
</tr>
<tr>
<td>Th2</td>
<td>T-helper type 2</td>
</tr>
<tr>
<td>TLR</td>
<td>toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
</tr>
</tbody>
</table>
Abstract

Asthma is an inflammatory disease that manifests in the airways. There are an estimated 300 million people worldwide currently suffer from asthma. Common asthma symptoms include dyspnea and wheezing. These are consequences of the reversible airflow obstruction associated with airway inflammation. The symptoms can be mild or can be as severe as life threatening depending on nature of underlying inflammation. Although heredity plays a role in the disease pathogenesis, the high and rising prevalence of asthma, particularly in recent decades highlights a strong influence of the environment. To this end, epigenetic phenomena including alteration of DNA methylation and chromatin structure are likely contributors to the pathogenesis of asthma as well as a plausible source of phenotype heterogeneity. Especially subtle alteration of DNA methylation patterns which occur early in life may impact on disease development. However, the exact role of epigenetic mechanisms in the pathogenesis of asthma and inflammatory phenotypes of asthma are not well understood. This thesis investigates; 1) Alterations in infant peripheral blood DNA methylation profiles associated with pre-natal exposure to maternal asthma, 2) The role of chromatin structure by analysing histone acetyl-transferases (HAT) and histone de-acetylases (HDAC) activity of peripheral blood monocytes in inflammatory phenotypes of adult asthma, 3) Alterations in the DNA methylation profile of peripheral blood monocytes associated with inflammatory phenotype of adult asthma.

The primary findings of this thesis are:

1) Maternal asthma during pregnancy is associated with alterations in peripheral blood DNA methylation in infants’.

2) Inflammatory phenotypes of asthma are associated with differential DNA methylation in peripheral blood monocytes. Gene network analyses of these differentially methylated genes revealed distinct molecular pathways, suggesting possible implications in the disease pathogenesis.
3) Neutrophilic asthma is associated with lower HDAC activity and higher HAT activity of peripheral blood monocytes compared to both eosinophilic and paucigranulocytic asthma.

Collectively, the findings of this thesis emphasised the significance of epigenetic factors playing a role in the development of asthma and inflammatory phenotypes of asthma. An association of peripheral blood methylation profiles of infants with maternal asthma suggests a potential inheritance of the disease susceptibility. The characteristic alterations of DNA methylation in blood monocytes suggest an underlying epigenetic basis for the inflammatory phenotypes while the differences in HAT/HDAC activity in monocytes further emphasise a role for the epigenome in the development of inflammatory phenotypes. The findings of this thesis warrant further investigation and may help us get one step closer to understanding the role of epigenetics in airway inflammation in asthma.