Antenatal causes of cerebral palsy and adverse pregnancy outcomes: Investigating associations between inherited thrombophilia, cytokine polymorphisms and viral infections

Catherine S. Gibson BSc (Biomed Sci) (Hons)

Thesis submitted for the degree of
Doctor of Philosophy

Department of Obstetrics and Gynaecology
The University of Adelaide
Australia

October 2005
**Chapter 5**  The association between inherited cytokine polymorphisms and cerebral palsy

<table>
<thead>
<tr>
<th>Abstract</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>61</td>
</tr>
<tr>
<td>The Fetal Inflammatory Response</td>
<td>61</td>
</tr>
<tr>
<td>Cytokine Responses</td>
<td>61</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>64</td>
</tr>
<tr>
<td>Patient Selection</td>
<td>64</td>
</tr>
<tr>
<td>DNA Extraction</td>
<td>64</td>
</tr>
<tr>
<td>MBL Genotyping</td>
<td>64</td>
</tr>
<tr>
<td>DNA Sequencing</td>
<td>65</td>
</tr>
<tr>
<td>TNF-α Genotyping</td>
<td>65</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>65</td>
</tr>
<tr>
<td>Results</td>
<td>66</td>
</tr>
<tr>
<td>Prevalence of Cytokine Polymorphisms in Control Population</td>
<td>66</td>
</tr>
<tr>
<td>Cytokines and Cerebral Palsy</td>
<td>69</td>
</tr>
<tr>
<td>All Controls</td>
<td>69</td>
</tr>
<tr>
<td>Cerebral Palsy All Gestational Ages</td>
<td>69</td>
</tr>
<tr>
<td>Cerebral Palsy Gestational Ages ≥37 weeks</td>
<td>69</td>
</tr>
<tr>
<td>Cerebral Palsy Gestational Ages 32-36 weeks</td>
<td>69</td>
</tr>
<tr>
<td>Cerebral Palsy Gestational Ages &lt;32 weeks</td>
<td>70</td>
</tr>
<tr>
<td>Term Controls</td>
<td>76</td>
</tr>
<tr>
<td>Cerebral Palsy All Gestational Ages</td>
<td>70</td>
</tr>
<tr>
<td>Cerebral Palsy Gestational Ages ≥37 weeks</td>
<td>70</td>
</tr>
<tr>
<td>Cerebral Palsy Gestational Ages 32-36 weeks</td>
<td>71</td>
</tr>
<tr>
<td>Cerebral Palsy Gestational Ages &lt;32 weeks</td>
<td>71</td>
</tr>
<tr>
<td>Discussion</td>
<td>80</td>
</tr>
<tr>
<td>Tumour Necrosis Factor α</td>
<td>80</td>
</tr>
<tr>
<td>Mannose Binding Lectin</td>
<td>82</td>
</tr>
</tbody>
</table>

**Chapter 6**  Neurotropic viruses are associated with cerebral palsy

<table>
<thead>
<tr>
<th>Abstract</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>85</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>87</td>
</tr>
<tr>
<td>Patient Selection</td>
<td>87</td>
</tr>
<tr>
<td>Virus Detection</td>
<td>87</td>
</tr>
</tbody>
</table>
Virus sensitivity, limits of detection and positive control stocks
Extraction
DNA Viruses
RNA Viruses
Amplification
Sequencing Analysis
Internal Controls
Statistical Analysis
Results
Prevalence of viruses in control population
Viruses and Cerebral Palsy
All Gestational Ages
Gestational Ages ≥37 weeks
Gestational Ages <37 weeks
Combination viruses at any gestational age
Discussion
Chapter 7 Associations between inherited thrombophilia and adverse pregnancy outcomes
Abstract
Introduction
Materials and Methods
Patient Selection
Statistical Analysis
Results
Intrauterine Growth Restriction <10^th percentile
Hypertension
Pregnancy-Induced Hypertensive Disorders
Pregnancy-Induced Hypertensive Disorders and IUGR <10^th percentile
Pre-existing Hypertension
Antepartum Haemorrhage
Antepartum Haemorrhage and IUGR <10^th percentile
Preterm Birth <37 weeks
Preterm Birth and IUGR <10^th percentile
Discussion
Chapter 8 Associations between cytokine polymorphisms and adverse pregnancy outcomes

Abstract
Introduction
The Fetal Inflammatory Response
Cytokine Responses
Materials and Methods
Patient Selection
Statistical Analysis
Results
Intrauterine Growth Restriction <10th percentile
Hypertension
Pregnancy-Induced Hypertensive Disorders
Pregnancy-Induced Hypertensive Disorders and IUGR <10th percentile
Pre-existing Hypertension
Antepartum Haemorrhage
Antepartum Haemorrhage and IUGR <10th percentile
Preterm Birth <37 weeks
Preterm Birth and IUGR <10th percentile
Discussion
Tumour Necrosis Factor-α
TNF-α and Intrauterine Growth Restriction
TNF-α and Hypertension
TNF-α and Antepartum Haemorrhage
TNF-α and Preterm Birth
Mannose Binding Lectin
MBL and Intrauterine Growth Restriction
MBL and Hypertension
MBL and Antepartum Haemorrhage
MBL and Preterm Birth
Conclusions
Chapter 9  Neurotropic viruses are associated with adverse pregnancy outcomes

Abstract  131
Introduction  132
Materials and Methods  134
  Patient Selection  134
  Statistical Analysis  134
Results  135
  Intrauterine Growth Restriction <10th percentile  135
  Hypertension  135
    Pregnancy-Induced Hypertensive Disorders  135
    Pregnancy-Induced Hypertensive Disorders and IUGR <10th percentile  135
    Pre-existing Hypertension  135
  Antepartum Haemorrhage  136
    Antepartum Haemorrhage and IUGR <10th percentile  136
  Preterm Birth <37 weeks  136
    Preterm Birth and IUGR <10th percentile  136
Discussion  140

Chapter 10  Do inherited thrombophilic polymorphisms, cytokine polymorphisms and perinatal viral exposure interact to further increase the risks of cerebral palsy?

Abstract  144
Introduction  145
Materials and Methods  146
  Patient Selection  146
    Genotyping for inherited thrombophilic polymorphisms  146
    Genotyping for inherited cytokine polymorphisms  146
    Detection of viral nucleic acids  146
    Statistical Analysis  146
Results  147
  Main Effects Model including prematurity  147
    Interactions between inherited thrombophilia, cytokine polymorphisms and viral infections  147
Discussion  149
Chapter 11  General Discussion

Introduction 151
Inherited Thrombophilic Polymorphisms 151
Inherited Cytokine Polymorphisms 152
Viral Infection 154
Interactions between main outcome measures 155
Population Attributable Risk 156
Prevention 156
Study Caveats 157
Conclusions 160
Future Implications and Directions 160

Chapter 12  References 164

Appendices
Appendix 1: Published Literature Review 186
Appendix 2: Published Inherited Thrombophilia Prevalence 198
Appendix 3: Inherited Thrombophilia Detection Methods (Thesis Ch 2) 202
Appendix 4: Supplementary Results for Inherited Thrombophilia and Cerebral Palsy (Thesis Ch 4) 204
Appendix 5: Inherited Cytokine Polymorphism Detection Methods (Thesis Ch 5) 212
Appendix 6: Viral Infection Detection Methods (Thesis Ch 6) 215
Appendix 7: Supplementary Results for Inherited Thrombophilia and Adverse Pregnancy Outcomes (Thesis Ch 7) 218
Appendix 8: Supplementary Results for Inherited Cytokine Polymorphisms and Adverse Pregnancy Outcomes (Thesis Ch 8) 226
Appendix 9: Supplementary Results for Viral Infection and Adverse Pregnancy Outcomes (Thesis Ch 9) 224
Abstract

Objective: The objective of this thesis was to investigate three potential antenatal risk factors — inherited thrombophilic polymorphisms, cytokine polymorphisms and exposure to viral infections — and their possible association with the development of cerebral palsy (CP) and other adverse pregnancy outcomes (APO), including intrauterine growth restriction, pregnancy-induced hypertensive disorders, antepartum haemorrhage and preterm birth.

Methods: Newborn screening cards from 1,326 babies (443 CP cases and 883 non-CP controls for the CP study; 717 APO cases and 609 non-APO controls for the APO study) were tested for inherited thrombophilic polymorphisms, cytokine polymorphisms and exposure to viral infections using polymerase chain reaction technology in the largest study of its kind worldwide. The four inherited thrombophilic polymorphisms tested were: Factor V Leiden (FVL G1691A), Prothrombin gene mutation (PGM G20210A) and Methyleneetetrahydrofolate reductase gene (MTHFR) C677T and MTHFR A1298C. Five cytokine polymorphisms were genotyped: Tumour necrosis factor alpha -308 (TNF-α -308), Mannose binding lectin -221, (MBL -221) and three polymorphisms in Exon 1 of the MBL gene at codons 52, 54 and 57. The newborn screening cards were also tested for viral nucleic acids from enteroviruses and herpesviruses.

Results: Inherited thrombophilic polymorphisms may play a role in the development of CP and adverse pregnancy outcomes, as suggested by previous small studies. This thesis determined that the MTHFR C677T thrombophilic polymorphism approximately doubled the risk of CP in preterm infants, and a combination of homozygous MTHFR C677T and heterozygous PGM increased the risk of quadriplegic CP five-fold at all gestational ages. The results also suggested that some fetal thrombophilia, in particular PGM, may be related to such adverse pregnancy outcomes as intrauterine growth restriction.

The role of the TNF-α -308 polymorphism and four polymorphisms within the MBL gene had not previously been described for the subsequent development of CP. Carriage of polymorphisms in the TNF-α and MBL genes were associated significantly with an increased risk of CP. The TNF-α -308 polymorphism was also found to be associated, with intrauterine growth restriction, pregnancy-induced hypertensive disorders, antepartum haemorrhage and preterm birth, and the MBL polymorphisms were associated with antepartum haemorrhage, pregnancy-induced hypertensive disorders and preterm birth.
Viral nucleic acid sequences were detected from newborn screening cards in 46.1% of cases, compared with 39.8% of controls (OR 1.30, 95% CI 1.00-1.67). This study was the first to demonstrate that evidence of direct infection with herpesviruses is associated with CP. In particular, detection of herpes group B viruses were associated with the development of CP (OR 1.68, 95% CI 1.09-2.59). These viral nucleic acid sequences were also found to be associated with adverse pregnancy outcomes, in particular preterm birth and pregnancy-induced hypertensive disorders.

Multivariable analysis demonstrated no significant interactions between the three main outcome measures listed above and the development of CP. Bivariable analyses showed increased risks of CP. The combination of herpes group B viruses and carriage of any cytokine polymorphism was associated with an increased risk of CP (OR 2.47, 95% CI 1.43-4.27). This relationship was linear and showed no significant synergistic relationship between the two outcome measures in the causation of CP.

Conclusions: This research has shown that thrombophilic polymorphisms, cytokine polymorphisms and viral infections are all independently associated with the subsequent development of CP. These three factors do not interact to further increase the risk of CP, and this may reflect different pathological pathways to the brain white matter damage and periventricular leukomalacia that ultimately leads to CP. Together, their potential attributable risk is 15% of cerebral palsy cases, but further studies of new polymorphisms and infections are likely to increase this attributable risk. This dataset has also shown that these same inherited thrombophilic and cytokine polymorphisms and viral infections are associated with adverse pregnancy outcomes such as intrauterine growth restriction, pregnancy-induced hypertensive disorders, antepartum haemorrhage and prematurity. These associations suggest interaction between genes and environmental risk factors.

Implications: Future research should investigate interactions between genes and the environment. Possible preventative strategies should be explored, such as vaccination programmes against the neurotropic viruses identified in this thesis as being associated with CP. This research also has medico-legal and political implications. The possible causal pathways for most CP outcomes currently cannot be influenced by obstetric practice. Their detection in retrospect may lead to prospective testing and research into the antenatal causes of cerebral palsy and its eventual prevention, saving hundreds of millions of dollars annually.