Angiogenesis Regulating Gene Polymorphisms in Adverse Pregnancy Outcomes

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Thesis submitted to the degree of Doctor of Philosophy

The Discipline of Obstetrics and Gynaecology
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Introduction

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General Discussion

Placental expression of VEGF family gene mRNA is reduced in pregnancy complications

Polymorphisms in angiogenesis regulating genes are associated with pregnancy complications

Paternal angiogenesis regulating gene polymorphisms are associated with pregnancy complications

Angiogenesis regulating gene polymorphisms may have a role in the pathogenesis of pregnancy complications

Gene-environment interactions modify the risk of pregnancy complications

Evidence for a genetic contribution to vascular diseases

Limitations in candidate gene association studies

Future implications

Conclusions

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Abstract

Introduction: Both placental vascular defects and a genetic contribution are documented in pregnancies complicated by preeclampsia, small-for-gestational-age infants (SGA) and spontaneous preterm birth (sPTB). Our primary aim was to investigate the association between polymorphisms in genes regulating placental vascular integrity including vascular endothelial growth factor (VEGFA), placenta growth factor (PGF), kinase insert domain receptor (KDR), fms-like tyrosine kinase 1 receptor (FLT1), angiopoietin 1 (ANGPT1) and thrombospondin 1 (TSP1) and these pregnancy complications in a Caucasian cohort. The secondary aims were to investigate the association between these polymorphisms and (1) preeclampsia in Sri Lankan women (2) first trimester placental gene expression (3) abnormal uterine and umbilical artery Doppler (4) environment and lifestyle interactions that modify the risk of pregnancy complications and to (5) compare term placental angiogenic gene mRNA expression in complicated pregnancy with uncomplicated pregnancy.

Methods: Nulliparous pregnant women, their partners and infants (3234 trios) were recruited to a prospective multicenter cohort study (SCOPE study) in Adelaide, Australia and Auckland, New Zealand. Pregnancy outcomes were classified using international guidelines. Uterine and umbilical artery Doppler was performed at 20 weeks gestation. Mean uterine or umbilical artery resistance indices (RI) above the 90th percentile or the presence of bilateral notching of the uterine artery waveform were considered abnormal. A second Sri Lankan cohort comprised 175 nulliparous preeclamptic women and 171 matched controls. The polymorphisms in the Caucasian parent-infant trios, Sri Lankan women and first trimester placental tissue from elective pregnancy terminations (n = 74) were genotyped using the Sequenom Mass ARRAY system. Term placentae were collected from women with preeclampsia (n = 18), gestational hypertension (n = 15), normotensive SGA infants (n = 13), spontaneous
preterm birth (n = 10) and uncomplicated pregnancy (n = 30). Placental mRNA expression was analysed by quantitative RT-PCR.

**Results:** In the Caucasian cohort, maternal *ANGPT1* 1414T/A and paternal and infant *KDR* -604T/C polymorphisms were associated with preeclampsia; maternal *ANGPT1* 1414T/A, paternal and infant *KDR* -604T/C, paternal and infant *TSP1* 2210A/G and infant *VEGFA*+936C/T were associated with SGA. In the Sri Lankan cohort, *PGF* 642C/A was associated with preeclampsia. The *ANGPT1* 1414T/A was associated with abnormal uterine Doppler and the *VEGFA* +936C/T was associated with abnormal uterine and umbilical artery Doppler and reduced first trimester placental *VEGFA* mRNA expression suggesting that these polymorphisms may have a role in the pathogenesis of pregnancy complications. We also found that the maternal *ANGPT1* 1414T/A and *VEGFA* -2578C/A polymorphisms interact with maternal BMI to modify the risk of sPTB and that the maternal *KDR* -604T/C interacts with smoking to influence the risk of preeclampsia and SGA. In all these polymorphisms, genotypes associated with pro-angiogenic phenotypes reduced the risk and genotypes associated with anti-angiogenic phenotypes increased the risk of pregnancy complications. We were also able to demonstrate that term placental expression of *VEGFA*, *PGF*, *KDR* and *FLT1* mRNA were reduced in pregnancy complications compared to uncomplicated pregnancy.

**Conclusion:** This project demonstrates that inherited susceptibility to altered angiogenic gene expression in the placenta contributes to the risk of pregnancy complications.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Prabha Andraweera 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.

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2. PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, CT Roberts. A functional variant in the thrombospondin-1 gene and the risk of small for gestational age infants. *Journal of Thrombosis and Haemostasis* 2011;9:2221-8 – copyright resides with the International Society on Thrombosis and Haemostasis (ISTH)

3. PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, CT Roberts. A functional variant in ANGPT1 and the risk of pregnancies with hypertensive disorders and small for gestational age infants. *Molecular Human*

5. **PH Andraweera, GA Dekker, CT Roberts.** Placental expression of VEGF family mRNA in adverse pregnancy outcomes. *Placenta*, In Press - copyright resides with Elsevier

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There are no conflicts of interest to declare for myself or my supervisors. This study was funded by the Premier’s Science and Research Fund, Government of South Australia in Australia, the Foundation for Research Science and Technology, Health Research Council and Auckland District Health Board Charitable Trust in New Zealand, the National Health and Medical Research Council Australia and the Channel 7 Children’s Research Foundation.

Prabha Andraweera

March 2012
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Publications arising from this thesis


3. **PH Andraweera**, GA Dekker, SD Thompson, LME McCowan, RA North, CT Roberts. A functional variant in *ANGPT1* and the risk of pregnancies with hypertensive disorders and small for gestational age infants. *Molecular Human Reproduction*, 2011, Published Online; doi: 10.1093/molehr/gar081 (IF 3.5)


6. **PH Andraweera, GA Dekker, CT Roberts.** The Vascular endothelial growth factor family in adverse pregnancy outcomes. *Human Reproduction Update*, In Press (IF 8.7)

7. **PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, CT Roberts.** Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth, Revision submitted to *Molecular Human Reproduction* – Manuscript ID – MHR-12-0007-R1

Conference presentations and abstract publications arising from this thesis

2011

1. **PH Andraweera**, GA Dekker, SD Thompson, RA North, LME McCowan, CT Roberts. Single nucleotide polymorphisms in angiogenesis regulating genes and the risk of preeclampsia and small for gestational age birth. *12th International Congress of Human Genetics, October 2011, Montreal, Canada.*

   “Awarded the best poster award”


2010


8. GA Dekker, CT Roberts, DL Furness, **PH Andraweera**, Paternal Factors Involved in the Causation of Preeclampsia, *17th World Congress of the Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia, Abstracted in Hypertension in Pregnancy 2010*


**2009**

15. **PH Andraweera**, SD Thompson, RC Nowak, VJ Zhang, GA Dekker, CT Roberts. Single nucleotide polymorphisms in angiogenesis regulating genes are associated with pregnancy complications. *Annual Conference of the International Federation of Placental Associations (IFPA), October 2009, Adelaide, Australia , Abstracted in Placenta 2009*

Awards received for presentations arising from this thesis

Conference Awards

1. **Best Poster Award.** Awarded at the Research Day 2011, Robinson Institute, University of Adelaide, Australia, November 2011

2. **Best Poster Award.** Awarded at the Postgraduate Research Conference, Faculty of Health Sciences, University of Adelaide, August 2011

3. **Frederick P Zuspan Award.** This was awarded for the most outstanding Basic science work submitted to the 12th world congress of the International Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia

4. **ISSHP Young Investigator Award.** This was awarded for excellence in abstracts submitted to the 12th world congress of the International Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia

Travel Awards

1. Healthy Development Adelaide (HDA) Postgraduate Travel Award 2011

2. Faculty of Health Sciences, University of Adelaide, Postgraduate Travelling Fellowship 2011

3. Research Centre for Reproductive Health, University of Adelaide, Travel Awards 2011, 2010 and 2009

4. Society for Reproductive Biology, Postgraduate Student Travel Award 2010

5. ANZPRA New Investigator Travel Award 2009. This was awarded by the Australian and New Zealand Placental Research Association
Contribution made by the candidate

Statement of Authorship: Prabha H Andraweera

I, Prabha H Andraweera declare that I am the principal author of the following manuscripts.


6. **PH Andraweera, GA Dekker, CT Roberts.** The Vascular endothelial growth factor family in adverse pregnancy outcomes. *Human Reproduction Update – In Press*

7. **PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, CT Roberts.** Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth. Revision submitted to *Molecular Human Reproduction*


I declare that I contributed to the design of the SCOPE candidate gene association study and the Sri Lankan candidate gene association study, performed the laboratory experiments for the evaluation of the first trimester and term placental gene expression, conducted the statistical analyses of the data and wrote the manuscripts. I declare that I have no conflicts of interest.

Prabha H Andraweera
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I, Claire T Roberts declare that I am a co-author of the following manuscripts.

1. PH Andraweera, GA Dekker, SD Thompson, RC Nowak, VJ Zhang, LME McCowan, RA North, **CT Roberts**. Association of vascular endothelial growth factor +936 C/T single nucleotide polymorphism with pregnancies complicated by small for gestational age babies. *Archives of Pediatrics & Adolescent Medicine*, 2011;165(12):1123-1130

2. PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, **CT Roberts**. A functional variant in the thrombospondin-1 gene and the risk of small for gestational age infants. *Journal of Thrombosis and Haemostasis* 2011;9:2221-8

3. PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, **CT Roberts**. A functional variant in *ANGPT1* and the risk of pregnancies with hypertensive disorders and small for gestational age infants. *Mol Hum Reprod*;doi: 10.1093/molehr/gar081


5. PH Andraweera, GA Dekker, JA Laurence, **CT Roberts**. Placental expression of VEGF family mRNA in adverse pregnancy outcomes. *Placenta*, In Press

6. PH Andraweera, GA Dekker, **CT Roberts**. The Vascular endothelial growth factor family in adverse pregnancy outcomes. *Human Reproduction Update* – In Press
7. PH Andraweera, GA Dekker, SD Thompson, LME McCowan, RA North, CT Roberts. Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth. Revision submitted to *Molecular Human Reproduction*


I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed to the design and supervision of the SCOPE clinical trial and the candidate gene association study and critically reviewed these manuscripts for important intellectual content. I declare that I have no conflicts of interest.

I give consent for these manuscripts to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

Claire T Roberts
Statement of Authorship: Gustaaf A Dekker

I, Gustaaf A Dekker declare that I am a co-author of the following manuscripts.


3. PH Andraweera, **GA Dekker**, SD Thompson, LME McCowan, RA North, CT Roberts. A functional variant in *ANGPT1* and the risk of pregnancies with hypertensive disorders and small for gestational age infants. *Mol Hum Reprod*; doi: 10.1093/molehr/gar081


7. PH Andraweera, **GA Dekker**, SD Thompson, LME McCowan, RA North, CT Roberts. Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth. Revision submitted to *Molecular Human Reproduction*


I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed to the design and supervision of the SCOPE clinical trial and the candidate gene association study and critically reviewed these manuscripts for important intellectual content. I declare that I have no conflicts of interest.

I give consent for these manuscripts to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

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I, Lesley ME McCowan declare that I am a co-author of the following manuscripts.


4. Andraweera PH, Dekker GA, Thompson SD, McCowan LME, North RA, Roberts CT. Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth. Revision submitted to *Molecular Human Reproduction*

I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed to the design and supervision of the SCOPE clinical trial and critically reviewed these manuscripts for important intellectual content. I declare that I have no conflicts of interest. I give consent for these manuscripts to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

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Statement of Authorship: Robyn A North

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4. Andraweera PH, Dekker GA, Thompson SD, McCowan LME, North RA, Roberts CT. Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth. Revision submitted to *Molecular Human Reproduction*

I wish to attest the contribution made by Prabha Andraweera. I wish to declare that I contributed to the design and supervision of the SCOPE clinical trial and critically reviewed these manuscripts important intellectual content. I declare that I have consultancy relationships with Pronota and Alere and declare the patent number WO/2009/108073. I give consent for these manuscripts to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

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I, Rohan W Jayasekara declare that I am a co-author of the following manuscript.


I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed to the design of the Sri Lankan candidate gene association study and critically reviewed the manuscript for important intellectual content. I declare that I have no conflicts of interest.

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5. PH Andraweera, GA Dekker, **SD Thompson**, LME McCowan, RA North, CT Roberts. Interaction between maternal BMI and angiogenesis regulating gene polymorphisms associates with the risk of spontaneous preterm birth. Revision submitted to *Molecular Human Reproduction*
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I give consent for this manuscript to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

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I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed by providing administrative and technical support. I declare that I have no conflicts of interest.

I give consent for this manuscript to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

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I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed by performing the bioinformatics assessment on the role of the PGF polymorphism. I declare that I have no conflicts of interest.

I give consent for this manuscript to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

Tina Bianco-Miotto
Statement of Authorship: Jessica A Laurence

I, Jessica A Laurence declare that I am a co-author of the following manuscript.


I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed to the RNA extraction from the term placentae. I declare that I have no conflicts of interest.

I give consent for this manuscript to be included in the thesis submitted to the degree of Doctor of Philosophy by Prabha Andraweera.

Jessica A Laurence
Thesis explanation

This thesis is arranged as a portfolio of published/accepted or submitted manuscripts.

Manuscript 1 provides a comprehensive review of literature on the role of the vascular endothelial growth factor family of angiogenic growth factors and a brief overview of the role of the angiopoietin family and the thrombospondin family in normal and complicated pregnancies. This is followed by the hypotheses and aims of the project.

The work described in the thesis mainly arises from the Adelaide, Australia and Auckland, New Zealand arms of the SCOPE (Screening for Pregnancy Endpoints) study. The SCOPE study is an international, multicenter, prospective cohort study with the aim of developing screening tests to predict the risk of pregnancy complications namely preeclampsia, small for gestational age infants and spontaneous preterm birth. The SCOPE study is registered in the Australian and New Zealand clinical trial registry and the details are given below.

**Trial Registry Name:** Screening nulliparous women to identify the combinations of clinical risk factors and/or biomarkers required to predict preeclampsia, small for gestational age babies and spontaneous preterm birth.

**URL:** [https://www.anzctr.org.au](https://www.anzctr.org.au)

**Registration number:** ACTRN12607000551493

Manuscripts 2 - 6 describe the associations of polymorphisms in candidate genes that regulate angiogenesis in the SCOPE cohort. As these papers are from the same study there is some repetition in the methods sections of the manuscripts.

Manuscript 7 describes the same polymorphisms in a case-control study population comprising preeclamptic women and matched controls from Sri Lanka.

Manuscript 8 describes the association of placental mRNA expression of the VEGF family in complicated compared to uncomplicated pregnancy. This study population is a randomly selected subset of those recruited to the SCOPE study in Adelaide.
The final section of the thesis comprises a general discussion based on the overall significance of the findings, the problems encountered and future directions of the work.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>cDNA</td>
<td>Complementary dioxyribonucleic acid</td>
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<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>DNA</td>
<td>Dioxyribonucleic acid</td>
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<tr>
<td>FLT1</td>
<td>Fms-like tyrosine kinase receptor 1 gene</td>
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<tr>
<td>FLT-1</td>
<td>Fms-like tyrosine kinase receptor 1 protein</td>
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<tr>
<td>KDR</td>
<td>Kinase-insert domain receptor gene</td>
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<tr>
<td>KDR</td>
<td>Kinase-insert domain receptor protein</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PGF</td>
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<td>Vascular endothelial growth factor protein</td>
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