

Angiogenesis Regulating Gene Polymorphisms in Adverse Pregnancy Outcomes

Prabha H Andraweera M.B.,B.S

**Thesis submitted to the degree of
Doctor of Philosophy**

**The Discipline of Obstetrics and Gynaecology
Research Centre for Reproductive Health
University of Adelaide
Australia**

March 2012

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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*

Reproduction, 2011, doi: 10.1093/molehr/gar081- copyright resides with the European Society of Human Reproduction and Embryology

4. **PH Andraweera**, GA Dekker, SD Thompson, CT Roberts. A Single nucleotide polymorphisms in the KDR gene in pregnancies complicated by gestational hypertensive disorders and small for gestational age infants. *Reproductive Sciences*, 2012, doi: 10.1177/1933719111428520 – copyright resides with the Society for Gynecologic Investigation
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

Acknowledgements

First and foremost, I would like to extend an enormous thank you to my supervisor Professor Claire Roberts. It was a great pleasure to work with someone so dedicated to Science as you. Thank you so much for all the encouragement and support you gave me in writing the manuscripts and presenting my work at national and international conferences. I greatly appreciate the many hours spent in reading numerous manuscript drafts, helping with conference presentations and having the time to discuss whenever I needed. It was a wonderful experience and I loved each day that I worked in your group.

Secondly, I would like to say a huge thank you to my co-supervisor Professor Gustaaf Dekker. Thank you so much for all the guidance, support and the responses to many queries. I really appreciate your prompt feedback on all the manuscripts, without which I would not have been able to submit eight manuscripts. Thank you very much also for taking time off your busy schedule to listen to my many conference practice presentations. It was a great opportunity and a wonderful experience to have worked with you.

I would also like to thank Professor Lesley McCowan from the University of Auckland, New Zealand and Professor Robyn North from King's College London, United Kingdom, our international collaborators on the SCOPE project, for their valuable comments on four of the manuscripts (manuscripts 2, 4, 5 and 6).

I would like to thank Professor Rohan Jayasekara, the Dean of the Faculty of Medicine, and the Director of the Human Genetics Unit at the Faculty of Medicine, University of Colombo, Sri Lanka, for inspiring my interest in Genetics and for the encouragement

given in pursuing Doctoral studies. I would also like to thank Professor Vajira Dissanayake from the Department of Anatomy and Human Genetics of the Faculty of Medicine, University of Colombo, Sri Lanka for the help given in the Sri Lankan component of the study, for generously offering the Sri Lankan samples for the project and for the feedback on manuscript 7.

In addition, I wish to thank all my colleagues, Denise Furness, Amanda Highet, Ang Zhou, Gary Heinemann, Steven Thompson, Jessica Laurence, Rachael Nowak, Tina Bianco-Miotto, Shalem Lee, Dylan McCullough and Jamie Zhang in my research group from the Department of Obstetrics and Gynaecology for all their support and friendship. I would like to extend a special thank you to Gary Heinemann and Steven Thompson for their technical assistance with the laboratory experiments.

I would like to acknowledge the Australian Leadership Award, my PhD scholarship which was funded by the Australian government. I also acknowledge the travel grants received from the Robinson Institute, the Faculty of Health Sciences, University of Adelaide and Healthy Development Adelaide that supported my conference travel.

Last but not least, I would like to thank my family for all the support, encouragement and love during my candidature, a special thank you to my parents and my brother for all the long distance calls and the encouraging words, to my husband Nalinda for the understanding and love and a very special thank you to my daughter Oshana for being such a great kid and helping me through all the lengthy periods of writing.

Publications arising from this thesis

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 (IF 4.0)
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 (IF 5.4)
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)
4. **PH Andraweera**, GA Dekker, SD Thompson, CT Roberts. A Single nucleotide polymorphisms in the KDR gene in pregnancies complicated by gestational hypertensive disorders and small for gestational age infants. *Reproductive Sciences*, 2012, Published online; doi: 10.1177/1933719111428520 (IF 2.6)
5. **PH Andraweera**, GA Dekker, CT Roberts. Placental expression of VEGF family mRNA in adverse pregnancy outcomes. *Placenta*, In Press (IF 2.9)

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

8. **PH Andraweera**, GA Dekker, VHW Dissanayake, TB Miotto, RW Jayasekara, CT Roberts. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri-Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine* – Manuscript ID - DJMF-2012-0029

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.*
2. **PH Andraweera**, GA Dekker, SD Thompson, RA North, LME McCowan, CT Roberts. Single nucleotide polymorphisms in angiogenesis regulating in preeclampsia and small for gestational age infants. *Postgraduate Research Conference, August 2011, University of Adelaide, Adelaide, Australia.*
“Awarded the best poster award”
3. **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: evidence from parent-infant trios, *ASMR June 2011, Adelaide, Australia*

2010

4. **PH Andraweera**, G.A. Dekker, S.D. Thompson, V.J. Zhang, C.T. Roberts. Association of maternal, paternal and fetal single nucleotide polymorphisms in vascular endothelial growth factor family genes with preeclampsia. *17th World Congress of the Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia, Abstracted in Hypertension in Pregnancy 2010*

5. **PH Andraweera**, G.A. Dekker, S.D. Thompson, L.M.E. McCowan, R.A. North, C.T. Roberts. Association of single nucleotide polymorphism in the vascular endothelial growth factor gene with small for gestational age birth. *17th World Congress of the Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia, Abstracted in Hypertension in Pregnancy 2010*
6. **PH Andraweera**, R.C. Nowak, S.D. Thompson, V.H.W. Dissanayake, R.W. Jayasekara, G.A. Dekker, C.T. Roberts. Association of single nucleotide polymorphisms in cytokine genes with preeclampsia in a Sinhalese population from Sri-Lanka. *17th World Congress of the Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia, Abstracted in Hypertension in Pregnancy 2010*
7. **PH Andraweera**, S.D. Thompson, V.H.W. Dissanayake, R.W. Jayasekara, G.A. Dekker, C.T. Roberts. Insulin like growth factor family genes and the risk of preeclampsia in a Sinhalese population from Sri-Lanka. *17th World Congress of the Society for the Study of Hypertension in Pregnancy, October 2010, Melbourne, Australia, Abstracted in Hypertension in Pregnancy 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*

9. **PH Andraweera**, R.C. Nowak, S.D. Thompson, D.L. Furness, V.H.W. Dissanayake, R.W. Jayasekara, G.A. Dekker, C.T. Roberts. Single nucleotide polymorphisms in genes modulating inflammation and the risk of preeclampsia in a Sinhalese population from Sri-Lanka. *11th International Congress of Reproductive Immunology, August 2010, Cairns, Australia, Abstracted in the Journal of Reproductive Immunology 2010*

10. **P.H. Andraweera**, D.L. Furness , R.C. Nowak, S.D. Thompson, V.H.W. Dissanayake, R.W. Jayasekara, G.A. Dekker, C.T. Roberts. Single nucleotide polymorphisms in genes regulating inflammation and the risk of Recurrent Pregnancy Loss in a Sinhalese population from Sri-Lanka. *11th International Congress of Reproductive Immunology, August 2010, Cairns, Australia, Abstracted in the Journal of Reproductive Immunology 2010*

11. **PH Andraweera**, G.A. Dekker, S.D. Thompson , L.M.E. McCowan, R.A. North, C.T. Roberts. Vascular endothelial growth factor gene polymorphisms in placental impairment and small for gestational age birth. *Annual Scientific Conference of the Society for Reproductive Biology, August 2010, Sydney, Australia. "ANZPRA new Investigator Award finalist"*

12. **PH Andraweera**, S.D. Thompson, V.H.W. Dissanayake, R.W. Jayasekara, G.A. Dekker, C.T. Roberts. Hypoxia-Inducible factor-1 α gene polymorphisms and the risk of preeclampsia in a Sinhalese population from Sri-Lanka, *Annual Scientific Conference of the Endocrine Society of Australia, August 2010, Sydney, Australia*

13. **PH Andraweera**, G.A. Dekker, S.D. Thompson, L.M.E. McCowan, R.A. North, C.T. Roberts. Single nucleotide polymorphisms in the vascular endothelial growth factor gene and the risk of small for gestational age birth, *ASMR June 2010, Adelaide, Australia*
14. **PH Andraweera**, G.A. Dekker, S.D. Thompson, V.J. Zhang, C.T. Roberts. Maternal, paternal and fetal single nucleotide polymorphisms in vascular endothelial growth factor family genes associate with preeclampsia. *Annual Scientific Sessions of the Society for Maternal and Fetal Medicine, February 2010, Chicago, USA, Abstracted in American Journal of Obstetrics and Gynaecology 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*
16. **PH Andraweera**, SD Thompson, RC Nowak, VJ Zhang, GA Dekker, CT Roberts, Paternal and fetal single nucleotide polymorphisms in KDR gene associate with preeclampsia and intrauterine growth restriction, *Annual Scientific Conference of the Society for Reproductive Biology, August 2009, Adelaide, Australia*

17. **PH Andraweera**, SD Thompson, RC Nowak, VJ Zhang, GA Dekker, CT Roberts. Angiogenesis regulating gene polymorphisms in pregnancy complications. *Proceedings of the Annual Scientific Sessions of the Australian Society for Medical Research*. June 2009, Adelaide, Australia

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.

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
4. **PH Andraweera**, GA Dekker, SD Thompson, CT Roberts. A Single nucleotide polymorphisms in the *KDR* gene in pregnancies complicated by gestational hypertensive disorders and small for gestational age infants. *Reproductive Sciences*, 2012, doi: 10.1177/1933719111428520
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*
8. **PH Andraweera**, GA Dekker, VHW Dissanayake, T Bianco-Miotto, RW Jayasekara, CT Roberts. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine*

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

Contributions made by co-authors

Statement of Authorship: Claire T Roberts

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
4. PH Andraweera, GA Dekker, SD Thompson, **CT Roberts**. A Single nucleotide polymorphisms in the *KDR* gene in pregnancies complicated by gestational hypertensive disorders and small for gestational age infants. *Reproductive Sciences*, 2012, doi: 10.1177/1933719111428520
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*
8. PH Andraweera, GA Dekker, VHW Dissanayake, T Bianco-Miotto, RW Jayasekara, **CT Roberts**. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine*

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.

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
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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*
8. PH Andraweera, **GA Dekker**, VHW Dissanayake, T Bianco-Miotto, RW Jayasekara, CT Roberts. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine*

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

Gustaaf A Dekker

Statement of Authorship: Lesley ME McCowan

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

1. Andraweera PH, Dekker GA, Thompson SD, North RA, **McCowan LME** and Roberts CT. A functional variant in the thrombospondin-1 gene and the risk of small for gestational age infants. *J Thromb Haemost.* 2011;9(11):2221-8.
2. Andraweera PH, Dekker GA, Thompson SD, Nowak RC, Zhang JV, **McCowan LME**, North RA and Roberts CT. Association of Vascular Endothelial Growth Factor +936 C/T Single-Nucleotide Polymorphism With Pregnancies Complicated by Small-for-Gestational-Age Babies. *Arch Pediatr Adolesc Med.* 2011;165(12):1123-30..
3. Andraweera PH, Dekker GA, Thompson SD, North RA, **McCowan LME** and Roberts CT. A functional variant in *ANGPT1* and the risk of pregnancies with hypertensive disorders and small for gestational age infants. *Mol Hum Reprod.* 2011; doi: 10.1093/molehr/gar081.
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.

Statement of Authorship: Robyn A North

I, Robyn A North declare that I am a co-author of the following manuscripts.

1. Andraweera PH, Dekker GA, Thompson SD, **North RA**, McCowan LM and Roberts CT. A functional variant in the thrombospondin-1 gene and the risk of small for gestational age infants. *J Thromb Haemost.* 2011;9(11):2221-8.
2. Andraweera PH, Dekker GA, Thompson SD, Nowak RC, Zhang JV, McCowan LM, **North RA** and Roberts CT. Association of Vascular Endothelial Growth Factor +936 C/T Single-Nucleotide Polymorphism With Pregnancies Complicated by Small-for-Gestational-Age Babies. *Arch Pediatr Adolesc Med.* 2011;165(12):1123-30.
3. Andraweera PH, Dekker GA, Thompson SD, **North RA**, McCowan LM and Roberts CT. A functional variant in *ANGPT1* and the risk of pregnancies with hypertensive disorders and small for gestational age infants. *Mol Hum Reprod.* 2011; doi: 10.1093/molehr/gar081.
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.

Statement of Authorship: Rohan W Jayasekara

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

1. PH Andraweera, GA Dekker, VHW Dissanayake, T Bianco-Miotto, **RW Jayasekara**, CT Roberts. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine*

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.

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

Rohan W Jayasekara

Statement of Authorship: Vajira HW Dissanayake

I, Vajira HW Dissanayake declare that I am a co-author of the following manuscript.

1. PH Andraweera, GA Dekker, **VHW Dissanayake**, T Bianco-Miotto, RW Jayasekara, CT Roberts. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri-Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine*

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.

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

Vajira HW Dissanayake

Statement of Authorship: Steven D Thompson

I, Steven D Thompson 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
4. PH Andraweera, GA Dekker, **SD Thompson**, CT Roberts. A Single nucleotide polymorphisms in the *KDR* gene in pregnancies complicated by gestational hypertensive disorders and small for gestational age infants. *Reproductive Sciences*, 2012, doi: 10.1177/1933719111428520
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*

I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed by providing administrative and technical support in conducting the SCOPE candidate gene association study. 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.

Steven D Thompson

Statement of Authorship: Rachael C Nowak

I, Rachael C Nowak declare that I am a co-author of the following manuscript.

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

I wish to attest the contribution made by Prabha Andraweera. I declare that I contributed to the RNA extraction from the first trimester 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.

Rachael C Nowak

Statement of Authorship: Jamie V Zhang

I, Vouleng J Zhang declare that I am a co-author of the following manuscript.

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

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.

Jamie V Zhang

Statement of Authorship: Tina Bianco-Miotto

I, Tina Bianco-Miotto declare that I am a co-author of the following manuscript.

1. PH Andraweera, **GA Dekker**, VHW Dissanayake, T Bianco-Miotto, RW Jayasekara, CT Roberts. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri Lanka. Under review by the *Journal of Maternal-Fetal and Neonatal Medicine*

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.

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

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>

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

cDNA	Complementary dioxyribonucleic acid
CI	Confidence interval
DNA	Dioxyribonucleic acid
<i>FLT1</i>	Fms-like tyrosine kinase receptor 1 gene
FLT-1	Fms-like tyrosine kinase receptor 1 protein
<i>KDR</i>	Kinase-insert domain receptor gene
KDR	Kinase-insert domain receptor protein
OR	Odds ratio
PCR	Polymerase chain reaction
<i>PGF</i>	Placenta growth factor gene
PIGF	Placenta growth factor protein
RNA	Ribonucleic acid
SNP	Single nucleotide polymorphism
<i>TSP1</i>	Thrombospondin 1 gene
TSP-1	Thrombospondin 1 protein
<i>VEGFA</i>	Vascular endothelial growth factor gene
VEGF-A	Vascular endothelial growth factor protein