

# **Renin Angiotensin System Polymorphisms and Pregnancy Complications**

Ang Zhou BHIthSc (Hons)

Thesis submitted for the degree of  
Doctor of Philosophy

The Discipline of Obstetrics and Gynaecology

Research Centre for Reproductive Health

University of Adelaide

Australia

July 2012

# Table of content

Abstract	iv
Declaration	vi
Acknowledgments	vii
Abstracts arising from this thesis	ix
List of figures	xi
List of tables	xii
Abbreviations	xiii
<b>CHAPTER 1: Renin angiotensin system and pregnancy complications</b>	<b>1</b>
Overview	2
1.1 The renin angiotensin system	4
1.2 RAS during normal pregnancy	5
1.2.1 Maternal systemic RAS	5
1.2.2 Uteroplacental RAS	7
1.3 RAS and pregnancy complications	9
1.3.1 Pregnancy complications	9
1.3.2 RAS and preeclampsia	10
1.3.2.1 Maternal systemic RAS in preeclampsia	11
1.3.2.2 Uteroplacental RAS in preeclampsia	13
1.3.3 RAS and other pregnancy complications	14
1.4 The association of RAS polymorphisms with pregnancy complications	15
1.4.1 RAS polymorphisms	15
1.4.1.1 <i>Renin T/G</i>	15
1.4.1.2 <i>AGT M235T</i>	15
1.4.1.3 <i>AGT T174M</i>	16
1.4.1.4 <i>ACE A11860G</i>	16
1.4.1.5 <i>AGT1R A1166C</i>	17
1.4.1.6 <i>AGT2R C4599A</i>	17
1.4.1.7 <i>AGT2R A1675G</i>	17
1.4.1.8 <i>AGT2R T1334C</i>	18
1.4.2 Paternal genotype in association studies	19
1.4.3 Gene and environment interactions in association studies	19
1.4.4 Fetal sex in association studies	20
1.5 Aims and structure of the current thesis	22
<b>CHAPTER 2: Materials and Methods</b>	<b>26</b>
2.1 The study population	27
2.1.1 Participants	27
2.1.2 Ethics	27
2.1.3 Recruitment	27
2.1.4 Data collection	28
2.1.5 Biological sample collection	29
2.1.6 Pregnancy outcomes	29
2.1.6.1 Uncomplicated pregnancy	29
2.1.6.2 Preeclampsia	30
2.1.6.3 Small for gestational age (SGA)	30
2.1.6.4 Spontaneous preterm birth (sPTB)	30
2.1.6.5 Gestational hypertension (GHT)	31
2.1.6.6 Gestational diabetes (GDM)	31
2.1.6.7 Placental abruption	31

2.2	DNA Extraction	31
2.2.1	DNA extraction from buffy coats	31
2.2.2	DNA extraction from Whatman FTA cards	32
2.2.3	DNA extraction from saliva samples	32
2.3	Genotyping assay	32
2.4	Plasma prorenin concentration	33
2.5	Plasma ACE concentration	33
2.6	Plasma ANG II concentration	34
2.7	Plasma ANG 1-7 concentration	34
<b>CHAPTER 3: Characteristics of the study population</b>		<b>37</b>
3.1	Introduction	38
3.2	Results	39
3.3	Discussion	42
<b>CHAPTER 4: The association of RAS polymorphisms with maternal circulating RAS profile at 15 weeks' gestation</b>		<b>51</b>
4.1	Abstract	52
4.2	Introduction	53
4.3	Materials and Methods	54
4.4	Results	57
4.5	Discussion	58
<b>CHAPTER 5: The association of RAS polymorphisms with maternal blood pressure and uterine and umbilical artery Doppler</b>		<b>63</b>
5.1	Abstract	64
5.2	Introduction	65
5.3	Materials and Methods	67
5.4	Results	70
5.5	Discussion	72
<b>CHAPTER 6: The association of maternal <i>ACE A11860G</i> with SGA is modulated by the environmental factors and fetal sex</b>		<b>81</b>
6.1	Abstract	82
6.2	Introduction	83
6.3	Materials and Methods	85
6.4	Results	88
6.5	Discussion	93
<b>CHAPTER 7: The association of <i>AGT2R</i> polymorphisms with preeclampsia and uterine artery bilateral notching is modulated by maternal BMI</b>		<b>106</b>
7.1	Abstract	107
7.2	Introduction	110
7.3	Materials and Methods	110
7.4	Results	114
7.5	Discussion	118
<b>CHAPTER 8: General discussion</b>		<b>126</b>
8.1	Paternal <i>AGT2R C4599A</i> is associated with preeclampsia	128
8.2	Environmental factors modify the association of RAS polymorphisms with SGA and preeclampsia	129
8.3	Fetal sex affects the association of RAS polymorphisms with SGA and uterine and	130

umbilical artery resistance indices at 20 weeks' gestation	
8.4 Limitations in genetic association studies	131
8.5 Implications	133
8.6 Conclusion	134
<b>REFERENCES</b>	<b>135</b>

## **Abstract**

**Introduction:** The renin angiotensin system (RAS) plays an important role in blood pressure regulation and salt-water homeostasis. Aberrant maternal circulating and uteroplacental RAS profiles have been implicated in pregnancy complications, in particular preeclampsia. Our primary aims were to determine associations of polymorphisms in the RAS genes including renin, angiotensinogen (AGT), angiotensin converting enzyme (ACE), angiotensin II type 1 receptor (AGT1R) and type 2 receptor (AGT2R) with pregnancy complications, including preeclampsia, small for gestational age (SGA) and spontaneous preterm birth (sPTB) and to identify potential gene-environment and gene-fetal sex interactions that may modify risks for these pregnancy complications. The secondary aims were to determine the association of RAS polymorphisms with maternal plasma RAS profile at 15 weeks' gestation, maternal blood pressure at 15 weeks' gestation and uterine and umbilical artery resistance at 20 weeks' gestation.

**Methods:** Healthy nulliparous women, their partners and babies (3234 trios) were recruited prospectively in Adelaide, Australia and Auckland, New Zealand. Data analyses were confined to 2121 Caucasian parent-infant trios, among which 123 had preeclampsia, 216 had SGA and 116 had sPTB. Uncomplicated pregnancies (n=1185) served as controls. DNA was genotyped by Sequenom MassARRAY. Maternal blood samples were taken at 15 weeks' gestation and measured for plasma prorenin, ACE, angiotensin II (ANG II) and angiotensin 1-7 (ANG 1-7) concentrations. Maternal blood pressure was measured at 15 weeks' gestation. Doppler sonography on the uterine and umbilical arteries was performed at 20 weeks' gestation. Mean uterine or umbilical artery resistance indices (RI) above the 90<sup>th</sup> percentile were considered abnormal.

**Results:** Maternal and neonatal *ACE A11860G* GG genotype was associated with elevated maternal plasma ACE concentration. Neonatal *renin T/G* and maternal *AGT M235T* were

associated with maternal plasma ANG 1-7 concentration. In female-bearing pregnancies, maternal *AGT1R A1166C* CC genotype was associated with an increased risk for abnormal uterine artery RI, whereas in male-bearing pregnancies, the *AGT M235T* TT genotype in mothers and neonates was associated with an increased risk for abnormal umbilical artery RI. In the Australian SCOPE cohort, maternal *ACE A11860G* GG genotype was associated with an increased risk for SGA and a reduction in customized birth weight centile compared with the AA or AG genotype. Interestingly, these associations were only observed in female-bearing pregnancies and in women with socio-economic index <34 or pre-pregnancy green leafy vegetable intake <1 serve/day. Furthermore, maternal, neonatal and paternal *AGT2R C4599A* was associated with preeclampsia in women with BMI  $\geq 25$  kg/m<sup>2</sup>. In the same subset of women, paternal *AGT2R C4599A* and paternal *AGT2R A1675G* were associated with uterine artery bilateral notching. Finally, no significant associations, gene-environment interactions or gene-fetal sex interactions were found for sPTB.

**Conclusion:** The association of RAS polymorphisms with preeclampsia, SGA and abnormal uterine and umbilical artery RI indicates the involvement of the RAS in the pathogenesis of pregnancy complications, in particular preeclampsia and SGA. Furthermore, future genetic association studies into pregnancy complications should take account of fetal sex and appropriate environmental factors, otherwise genetic associations hidden by these factors may be left unrecognized.

## **Declaration**

I certify that 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 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signature:

Date: July 2012

## **Acknowledgements**

The first person I would like to thank is my supervisor, Professor Claire Roberts. Thank you for the opportunity to pursue my Ph.D. in your laboratory and your unconditional support through the course of these studies. It has been an honour to work with someone so dedicated and passionate about medical research, you have been an inspiration. Thanks for your patience, encouragement and valuable feedback on my conference presentations, manuscripts preparation and thesis writing. It has been a fantastic journey and life-changing experience for me. I enjoyed every bit of it.

I would like to express my sincere thanks to co-supervisor Professor Gus Dekker for his invaluable insight and advice regarding the interpretation of the data. Thanks for your prompt feedback on my thesis drafts. It is such a privilege to have regular Friday meetings with you and Claire. I benefited enormously from our discussions and this process really transformed me from a stubborn person to an open-minded person.

I would also like to thank Professor Eugenie Lumbers for her valuable input into my Ph.D. project and Shane D. Sykes for his contribution on the plasma prorenin data.

I would like to extend my sincere thanks to all my colleagues, Prabha Andraweera, Shalem Lee, Gary Heinemann, Steven Thompson, Jessica Laurence, Dylan McCullough, Denise Furness, Amanda Highet, Tina Bianco-Miotto, and Jamie Zhang. In particular, I would like to thank Prabha Andraweera for generously sharing her Ph.D. experience with me, Gary Heinemann for his technical supports with the ACE ELISA, Steven Thompson for his help with cleaning up the database and Shalem Lee for her assistance on statistical analysis. I am blessed with working with you guys. Thanks for making my journey so enjoyable.

I also would like to thank my dearest friends in Australian and China, Chelsea Liu, Qiao Jun (Pang Zi), Yang Dan (Shou Zi), Nan Hao and Sun Yue Rui for all your unwavering support throughout my Ph.D. journey. You guys are true angels!

I would like to thank my twin brother, Xuan Zhou for his constant support and inspirational advice throughout my Ph.D. journey.

At last but not least, I would like to extend a huge thanks to my parents. This journey would not have been possible without your love and support from China.

## Abstracts arising from this thesis

1. S.D. Sykes, Y. Wang, **A. Zhou**, G.A. Dekker, C.T. Roberts, E.R. Lumbers, K.G. Pringle. Fetal sex alters the expression of the uteroplacental and maternal circulating renin-angiotensin systems; implications for maternal cardiovascular function and fetal outcome. *Gordon Research Seminar, 2012, USA*
2. C.T. Roberts, **A. Zhou**, S. Thompson, G.A. Dekker. Interactions between genes encoding renin-angiotensin system peptides and fetal sex in pregnancy complications. *International Federation of Placenta Associations 2011, Geilo, Norway.*
3. **A. Zhou**, S. Thompson, G. Heinemann, J. Zhang, G.A. Dekker, C.T. Roberts. Polymorphisms in RAS genes are associated with being born small for gestational age in an Australian cohort. *Renin Angiotensin System workshop, 2010, Melbourne, Australia.*
4. **A. Zhou**, S. Thompson, R. Nowak, G. Heinemann, J. Zhang, G.A. Dekker, C.T. Roberts. Paternal renin angiotensin system polymorphisms are associated with pregnancy complications. *International Federation of Placenta Associations annual meeting, 2009, Adelaide, Australia.*
5. **A. Zhou**, S. Thompson, R. Nowak, G. Heinemann, J. Zhang, G.A. Dekker, C.T. Roberts. Renin angiotensin system polymorphisms in paternal genotypes are associated with pregnancy complications. *The Australian Society for Medical Research annual meeting, 2009, Adelaide, Australia.*
6. **A. Zhou**, S. Thompson, R. Nowak, G. Heinemann, J. Zhang, G.A. Dekker, C.T. Roberts. Renin angiotensin system polymorphisms are associated with pregnancy complications. *39<sup>th</sup> Society for reproductive biology annual meeting, 2008, Melbourne, Australia.*

7. **A. Zhou**, S. Thompson, R. Nowak, G. Heinemann, J. Zhang, G.A. Dekker, C.T. Roberts. Renin angiotensin system polymorphisms and pregnancy success. *ARC/NHMRC Research Network in Genes & Environment in Development annual meeting, 2008, Cairns, Australia.*

## List of figures

<b>Figure 1.1</b> The activation cascade of the RAS.	24
<b>Figure 2.1</b> Sequenom iPLEX™ MassARRAY system.	35
<b>Figure 3.1</b> Flow chart of participant recruitment.	44
<b>Figure 5.1</b> Flow chart of participant recruitment.	77
<b>Figure 6.1</b> Flow chart of participant recruitment.	97
<b>Figure 6.2</b> The association of maternal <i>ACE A11860G</i> with maternal plasma ACE concentration at 15 weeks' gestation in uncomplicated and SGA pregnancies.	98
<b>Figure 6.3</b> Maternal plasma ACE concentration at 15 weeks' gestation in uncomplicated and SGA pregnancies.	99
<b>Figure 7.1</b> Flow chart of participant recruitment.	121

## List of tables

<b>Table 1.1</b> Location of RAS components in the uteroplacental unit.	25
<b>Table 2.1</b> Primers for genotyping the RAS polymorphisms.	36
<b>Table 3.1</b> The prevalence of pregnancy outcomes in the combined SCOPE.	45
<b>Table 3.2</b> Demographic characteristics of the combined SCOPE cohort.	46
<b>Table 3.3</b> Demographic characteristics of the Australian SCOPE cohort.	47
<b>Table 3.4</b> Demographic characteristics of the New Zealand SCOPE cohort.	48
<b>Table 3.5</b> The differences in demographic characteristics between the Australian and New Zealand SCOPE cohorts.	49
<b>Table 3.6</b> The prevalence of RAS polymorphisms in the combined SCOPE.	50
<b>Table 4.1</b> Characteristics of the study population.	61
<b>Table 4.2</b> The association of RAS polymorphisms with maternal plasma RAS peptide concentrations at 15 weeks' gestation.	62
<b>Table 5.1</b> Characteristics of the study population.	78
<b>Table 5.2</b> The association of <i>AGT1R A1166C</i> with abnormal uterine artery resistance index at 20 weeks' gestation, stratified by fetal sex.	79
<b>Table 5.3</b> The association of <i>AGT M235T</i> with abnormal umbilical artery resistance index at 20 weeks' gestation, stratified by fetal sex.	80
<b>Table 6.1</b> Demographic characteristics of the study population.	100
<b>Table 6.2</b> Maternal, paternal and neonatal <i>ACE A11860G</i> genotype distribution in uncomplicated pregnancies and SGA pregnancies, stratified by fetal sex and cohorts.	102
<b>Table 6.3</b> The association of maternal <i>ACE A11860G</i> with newborn growth parameters, stratified by fetal sex and cohorts.	103
<b>Table 6.4</b> The association of maternal <i>ACE A11860G</i> with SGA and customised birthweight centile, stratified by fetal sex and cohorts.	104
<b>Table 6.5</b> The association of maternal <i>ACE A11860G</i> with customised birthweight centile in the Australian SCOPE, stratified by maternal SEI, pre-pregnancy green leafy vegetable intake and fetal sex.	105
<b>Table 7.1</b> Demographic characteristics of the study population.	122
<b>Table 7.2</b> The association of <i>AGT1R</i> and <i>AGT2R</i> polymorphisms with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation.	123
<b>Table 7.3</b> The association of <i>AGT2R C4599A</i> with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation, stratified by maternal BMI.	124
<b>Table 7.4</b> The association of <i>AGT2R A1657G</i> with preeclampsia and uterine artery bilateral notching at 20 weeks' gestation, stratified by maternal BMI.	125

## Abbreviations

ACE	Angiotensin converting enzyme
ACE2	Angiotensin converting enzyme 2
AGRF	Australian Genome Research Facility
AGT	Angiotensinogen
AGT1R	Angiotensin II type 1 receptor
AGT1R-AA	Angiotensin II type 1 receptor agonistic autoantibody
AGT1R-B2	Angiotensin II type 1 receptor-bradykinin B2 receptor heterodimer
AGT2R	Angiotensin II type 2 receptor
ANG 1-7	Angiotensin 1-7
ANG 1-9	Angiotensin 1-9
ANG I	Angiotensin I
ANG II	Angiotensin II
BMI	Body mass index
C.I.	Confidence interval
dBp	Diastolic blood pressure
DNA	Deoxyribonucleic acid
dNK	Decidual natural killer cell
ELISA	Enzyme-linked immunosorbent assay
EMT	Epithelial mesenchymal transition
EVT	Extravillous trophoblasts
FCS	Fetal Calf Serum
GDM	Gestational diabetes
GHT	Gestational hypertension
hAGT	Human angiotensinogen
hCG	Human chorionic gonadotropin
hrenin	Human renin
HUVEC	Human umbilical vein endothelial cells
IL-6	Interleukin-6
IUGR	Intrauterine growth restriction
NEP	Neutral endopeptidase 24.11
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor-1
PCR	Polymerase chain reaction
PEP	Prolyl-endopeptidase
PIH	Pregnancy-induced hypertension
RAS	Renin angiotensin system
RI	Resistance index
RR	Relative risk
sBP	Systolic blood pressure
SCOPE	Screening for Pregnancy Endpoints
SEI	Socio-economic index
sFlt1	Soluble fms-like tyrosine kinase 1
SGA	Small for gestational age

SNP	Single nucleotide polymorphism
sPTB	Spontaneous preterm birth
VEGF	Vascular endothelial growth factor