Original Paper

Fetal But Not Maternal Angiotensin Converting Enzyme (ACE)-2 Gene Rs2074192 Polymorphism is Associated with Increased Risk of Being a Small For Gestational Age (SGA) Newborn

Jing He^a Yong-Ping Lu^{a,b,c} Jian Li^d Tao-Yuan Li^a Xin Chen^e Xu-Jing Liang^a
Wen-Jing Chen^f Sai-Nan Pi^a Berthold Hoche^{b,d,g,h} You-Peng Chen^{a,i}

^aDepartment of Infectious Diseases, the first Affiliated Hospital, Jinan University, Guangzhou, China,

^bFifth Department of Medicine (Nephrology/Endocrinology/Rheumatology), University Medical Centre

Mannheim, University of Heidelberg, ^cDepartment of Nephrology, Charité - Universitätsmedizin

Berlin, Campus Mitte, Berlin, Germany, ^dDepartment of Clinical Medicine, Medical college of Hunan

Normal University, Changsha, ^eDepartment of Obstetrics and Gynecology, the first Affiliated Hospital,

Jinan University, Guangzhou, ^fDepartment of Laboratory Medicine, the first Affiliated Hospital, Jinan

University, Guangzhou, ^gReproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China, ^hLADR

GmbH Medizinisches Versorgungszentrum Neuruppin, Neuruppin, Germany, ⁱDepartment of Infectious

Diseases, the Seventh Affiliated Hospital, Sun Yat-Sen University, Shenzhen, China

Keywords

ACE2 • single nucleotide polymorphisms (SNPs) • fetal growth • small-for-gestational-age (SGA)

Abstract

Background/Aims: The maternal and fetal Renin-Angiotensin-System is involved in the control of pregnancy outcomes such as blood pressure control and gestational age. However, very little is known about the impact of the angiotensin converting enzyme 2 (ACE2) on pregnancy outcome. We thus performed a prospective clinical observational study analyzing the association of maternal and fetal ACE2 gene rs2074192 polymorphism with fetal growth during pregnancy. **Methods:** 898 singleton pregnant women were prospectively recruited. 739 pregnant women finally participated in the study and were genotyped. 474 women also donated umbilical cord blood for gene analysis of their offspring. All data such as basic demographic information, data from birth records, biochemical and immunological parameters, as well as Doppler ultrasonographic findings during pregnancy were collected. Fetal and maternal ACE2 gene rs2074192 polymorphism was genotyped by DNA sequencing.

Results: Our analysis showed that the maternal ACE2 gene rs2074192 polymorphism was not associated with gestational hypertension, gestational diabetes mellitus, anemia, postpartum hemorrhage and fetal growth. However, neonates having rs2074192 T allele were more likely to be born as small for gestational age (SGA) babies. After multivariable logistic regression considering known confounding, we could demonstrate that the neonatal rs2074192 T allele was an independent risk factor for SGA (OR: 22.93, 95%CI: 1.26~418.77, $P < 0.05$). **Conclusion:** This is the first study showing that the babies but not their mothers with ACE2 gene rs2074192 T allele had a high risk for SGA, which contributes to metabolic syndrome and cardiovascular diseases in later life.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Introduction

Being small for a given gestational age is considered as clinical manifestation of intrauterine growth retardation. Newborn with birth weight below the 10th percentage of the same gestational age and genetic background are defined as small for given gestational age (SAG) cases [1, 2]. According to new data from the INTERGROWTH-21st birth weight standard, Child Health Epidemiology Reference Group (CHERG), nearly 20% of all participating newborns were classified as SGA in 14 cohort studies from 10 middle or low-income countries in 2012 [2]. The incidence rate of SGA in 47 cities of China was approximately 6.6% in 2005 [3]. Because of physiological immaturity, SGA babies underwent many hard challenges at later growth.

Compared to normal term newborns, small for gestational age (SAG) infants were developmental delay at height and weight [4]. A multi-center prospective research from European (The North European Small for Gestational Age Study) revealed that insulin sensitivity and secretion affect height and weight growth of SGA children [5, 6]. SGA newborns have an increased risk for neonatal morbidity and mortality, and they are also associated with CNS diseases and metabolic disorders in later life [7]. A recent study from Taiwan showed that the risk of metabolic diseases, such as hypertension, diabetes and/or hyperlipidemia was more than threefold higher in SGA infants [8].

The rennin-angiotensin-aldosterone system (RAAS) includes two arms: angiotensin converting enzyme (ACE)-Ang II-Ang II type 1 receptor axis and angiotensin converting enzyme 2 (ACE2)-angiotensin (1-7)-Ang (1-7) Mas receptor axis. These two arms were described in the placenta [9, 10]. The ACE2 gene is localized in chromosome Xp22, which contains 18 exons and 17 introns [11]. ACE2-knockout mice have been shown to have higher blood pressures during pregnancy than wild-type mice. In addition, ACE2-knockout mice give birth to growth-restricted offspring [12]. In early study [13], it was shown that the single nucleotide polymorphisms (SNPs) at A1075G in intron 1, G8790A in intron 3, C28330G in intron 11, and G36787C in intron 16 were not dedicated themselves to essential hypertension in male and female subjects. Some studies suggested that genetic variants in the ACE2 gene might have its impact on cardiovascular diseases and diabetes mellitus. ACE2 rs2106809 polymorphism was associated with hypertension in Chinese women and Indian adult population [14, 15]. Lieb et al. reported that ACE2 gene rs4646156, rs879922, rs4240157, and rs233575 polymorphisms might be associated with left ventricular mass, septal wall thickness, and left ventricular hypertrophy in hemizygous men [16]. Recent Chinese studies showed that the ACE2 G8790A polymorphism, especially the A allele, was associated with diabetes mellitus type 2 (T2DM) and cerebral stroke (CS) [17]. The rs2074192 and rs2106809 polymorphisms of the ACE2 gene might be determinants of circulating Ang-(1-7) level in female patients with hypertension [18]. Our own recent study in pregnant women showed that serum Ang (1-7) levels in both mothers and their infants were associated with preterm birth [19, 20]. We have not found any studies on the association of maternal SNPs of ACE2 gene with their fetal growth yet, so we conducted this study to know if the ACE2 rs2074192 polymorphism of pregnant women is associated with restricted fetal growth.

Materials and Methods

Study subjects

898 pregnant women were prospectively recruited to our mother-child study from May, 2009 to November, 2010 at the first Affiliated Hospital, Jinan University, Guangzhou, China. The study was approved by the Ethics Committee of the first Affiliated Hospital of Jinan University. All subjects had the follow-up during pregnancy with informed consent and the delivery in our clinic. Pregnant women with 20 to 40 years old and singleton pregnancy were included. Pregnant women with syphilis, tuberculosis or HIV, HAV, HCV, HDV, HEV, HSV, CMV and *toxoplasma gondii* infections, drug or alcohol abuse before and during pregnancy, and pregnant women with tumor, congenital, immunological or mental diseases were excluded. Finally, 739 pregnant women met the included criteria and participated in the study. 265 mothers refused to donate umbilical cord blood. After written consent, 474 cord blood were collected (For more details, see Fig. 1).

Sample collection and DNA extraction

Maternal peripheral whole blood samples were taken at the first day of ward admission before delivery. The umbilical cord blood samples were collected immediately after delivery. After anticoagulant processed, 1-2mL blood sample was removed and stored at -20°C until DNA extraction. DNA extraction was used by the SIMGEN DNA mini kit (SIMGEN, Hangzhou, China, FCC ID: 3001050).

Genetic analysis

ACE2 gene rs2074192 polymorphism was genotyped by polymerase chain reaction (PCR) with primers and sequencing. Forward primer: 5'-GAAGAACTGGCAAAGGTCCC-3', and reverse primer: 5'-GCCTAGGTGACAGAGCAAGACTCT-3'. The PCR amplicon is 461bp. PCR reaction container included 50μL volume with 36.75μL ddH₂O, 5μL 10×Buffer, 2μL genomic DNA template, 1μL forward primer (10pmol/μL), 1μL reverse primer (10pmol/μL), 4μL dNTP (2.5mM) and 0.25μL EX TAQ HS (5U). PCR was programmed as followed: the initial degeneration at 94°C for 3 minutes, and 40 amplification cycles with degeneration at 93°C for 30 seconds, annealing at 55°C for 45 seconds, elongation at 72°C for 45 seconds and then a final extension at 72°C for 5 minutes (C1000 Thermal cycler, Bio-Rad, American). The PCR products were proved by electrophoresis in 2% agarose gel with 120 Volt for 40 minutes (EPS-300, Tanon, China). DNA sequencing was done by automatic DNA sequencing analyzer (ABI 3730, Applied Biosystems, USA).

Clinical data collection

Databases were recorded from included pregnant women in our clinic. All data such as basic information, birth records, diagnosis, biochemical and immunological assays, Doppler ultrasonographic findings during pregnancy, etc were collected and put to our databases. The criteria of small for gestational age (SGA) babies was according to both international and Chinese diagnosis [21, 22].

Fig. 1. Flow chat. *: 898 pregnant women were willing to attend the research. They had delivery at the first affiliated hospital of Jinan University from May, 2009 to December, 2010.

Statistical analysis

SPSS software version 19.0 was used for data analysis. Quantitative data was analyzed with analysis of variance (ANOVA) or *t*-Test, and these results were shown as arithmetic Mean±SD. Enumeration data was analyzed with Chi-Square and Fisher's Exact Test. To identify the risk factors of SGA, multiple logistic regression models were used to correct for known confounding factors. Relevant confounding factors of SGA such as maternal age, BMI, gestational age, maternal smoking, gestational diabetes, gestational hypertension, hepatitis B virus infection, infant gender and oligohydramnios were included into the models [23-29]. Significant difference was defined as *P*<0.05.

Results

Basic information

Table 1 showed basic information of pregnant women and their newborns in the study. The mean maternal BMI before pregnancy was 20 kg/m². 384 (81.01%) mothers were primiparity. Maternal mean blood pressure at the third trimester was 120mmHg systolic and 73mmHg diastolic, and the mean systolic and diastolic blood pressures at the second trimester was 111 mmHg and 66 mmHg. 220 boys and 254 girls were born to enter into the study. 312 (65.82%) mothers had cesarean delivery in the study. More details were shown in Table 1.

According to ACE2 rs2074192 variants and/or alleles, three groups of CC, CT and TT genotypes have been classified in the mothers and their newborns (Fig. 1).

Association of ACE2 gene rs2074192 polymorphism with maternal complications

Maternal age, BMI before pregnancy, and gestational age were not associated with the ACE2 gene rs2074192 polymorphisms in neonates (Tables 2, 3, and 4). Blood pressures as a continuous parameter of the pregnant women at the second and third trimesters was not affected by the rs2074192 polymorphisms (*P*>0.05 in all cases). Neither maternal nor neonatal ACE2 gene rs2074192 polymorphism was associated with gestational hypertension (*P*>0.1). Gestational diabetes mellitus, anemia and/or postpartum hemorrhage was not related to the genotypes of both mothers and their neonates (*P*>0.05).

Table 1. Detailed descriptive data of the mother and child (n =474). Data are given as Mean ± SE or %

Variables	Mean±SE or %
Maternal age, y	28.5±3.83
Maternal weight at delivery, kg	67.46±8.36
Maternal height, cm	159.60±4.66
Maternal pre-gestational weight, kg	51.67±7.08
Maternal BMI before pregnancy, kg/m ²	20.26±2.48
the Hans/ the minority, %	98.31/1.69
Primigravida /multipara, %	81.01/18.99
Smoking before/during pregnancy, %	0.63/0.21
Hypertension before/during pregnancy, %	0.85/6.33
Diabetes mellitus before/during pregnancy, %	0.42/14.78
Gestational age at delivery, day	273.88±10.15
Cesarean/vaginal delivery, %	65.82/34.18
Maternal systolic BP at the third trimester, mmHg	120.94±13.09
Maternal diastolic BP at the third trimester, mmHg	73.60±9.67
Maternal systolic BP at the second trimester, mmHg	111.14±10.45
Maternal diastolic BP at the second trimester, mmHg	66.33±7.74
Child sex, male/female, %	53.59/46.41
Child birth weight, g	3238.11±447.14
Child birth length, cm	49.67±1.86
Child head circumference	33.16±1.31
Apgar score at 1 min	8.90±0.55
Apgar score at 5 min	9.94±0.29
Apgar score at 10 min	9.97±0.17

Association of ACE2 gene rs2074192 polymorphism with fetal growth

There were no differences in birth weight, birth length, head circumference, and APGAR scores among three groups of CC, CT and TT genotypes (*P*≥0.1; Table 5). And there were also no differences in incidence rates of preterm birth, low birth weight and fetal distress among three groups of CC, CT and TT genotypes (*P*≥0.05; Table 5).

Table 2. Maternal data in three groups of fetal ACE2 gene rs2074192 genotypes. *: Gestational diabetes mellitus

Variables	C/C (n= 201)	C/T (n=175)	T/T (n=98)	F/X ²	P
Age	28.57±3.90	28.45±3.64	28.59±4.05	0.067	0.935
Maternal BMI before pregnancy	20.30±2.53	20.22±2.40	20.26±2.54	0.048	0.953
Gestational age(day)	273.25±9.66	273.53±11.56	275.79±8.11	2.230	0.109
Maternal systolic BP at the third trimester	120.44±13.54	121.60±13.11	120.79±12.12	0.378	0.685
Maternal diastolic BP at the third trimester	73.19±9.44	74.27±9.92	73.28±9.72	0.656	0.519
Maternal systolic BP at the second trimester	111.71±10.77	110.76±10.96	110.60±8.82	0.304	0.738
Maternal diastolic BP at the second trimester	66.44±8.03	66.74±8.05	65.32±6.50	0.595	0.552
Maternal smoking before pregnancy	0(0.0%)	2(1.1%)	1(1.0%)	3.339	0.188
Maternal smoking during pregnancy	0(0.0%)	0(0.0%)	1(1.0%)	3.169	0.205
Gestational hypertension	10(5.1%)	14(8.1%)	6(6.1%)	1.386	0.500
GDM*	26(13.3%)	24(13.9%)	19(19.4%)	2.123	0.346
Anemia	59(29.4%)	58(33.3%)	33(34.0%)	0.964	0.617
Postpartum hemorrhage	2(1.0%)	3(1.7%)	2(2.0%)	0.579	0.749

Table 3. Maternal data in two groups of ACE2 gene rs2074192 alleles. *:Gestational diabetes mellitus. §:Fisher's Exact Test was used to analyze this data

Variables	C/C (n=201)	C/T and T/T (n=273)	t/X ²	P
Maternal systolic BP at the third trimester	120.44±13.55	121.31±12.75	0.717	0.474
Maternal diastolic BP at the third trimester	73.19±9.44	73.91±9.84	0.807	0.420
Maternal systolic BP at the second trimester	111.71±10.77	110.70±10.22	0.771	0.441
Maternal diastolic BP at the second trimester	66.44±8.03	66.23±7.54	0.217	0.829
Maternal smoking before pregnancy	0(0.0%)	3(1.1%)	0.822	0.364
Maternal smoking during pregnancy	0(0.0%)	1(0.4%)	NA [§]	0.390
Gestational hypertension	10(5.1%)	20(7.4%)	0.982	0.322
GDM*	26(13.3%)	43(15.9%)	0.611	0.434
Anemia	59(29.4%)	91(33.6%)	0.951	0.330
Postpartum hemorrhage	2(1.0%)	5(1.8%)	0.114	0.735

Table 4. Maternal data in two groups of ACE2 gene rs2074192 alleles. *: Gestational diabetes mellitus. §: Fisher's Exact Test was used to analyze this data

Variables	C/C and C/T (n=376)	T/T (n=98)	t/X ²	P
Maternal systolic BP at the third trimester	120.98±13.34	120.79±12.12	0.130	0.897
Maternal diastolic BP at the third trimester	73.69±9.67	73.28±9.72	0.377	0.706
Maternal systolic BP at the second trimester	111.28±10.84	110.60±8.82	0.420	0.675
Maternal diastolic BP at the second trimester	66.58±8.02	65.32±6.50	1.057	0.291
Maternal smoking before pregnancy	2(0.5%)	1(1.0%)	NA [§]	0.500
Maternal smoking during pregnancy	0(0.0%)	1(1.0%)	NA [§]	0.206
Gestational hypertension	24(6.5%)	6(6.1%)	0.019	0.891
GDM*	50(13.6%)	19(19.4%)	2.096	0.148
Anemia	117(31.2%)	33(34.0%)	0.283	0.595
Postpartum hemorrhage	5(1.4%)	2(2.0%)	0.001	0.977

Table 5. Neonatal data in three groups of ACE2 gene rs2074192 alleles. #: Small Gestational for Age

Variables	C/C (n= 201)	C/T (n=175)	T/T (n=98)	t/X ²	P
Birth weight	3232.94±403.65	3219.11±506.04	3282.65±420.61	0.657	0.519
Body length	49.80±1.52	49.44±2.25	49.85±1.66	2.290	0.102
Head circumference	33.20±1.17	33.09±1.50	33.21±1.21	0.427	0.652
APGAR 1	8.90±0.40	8.93±0.42	8.86±0.90	0.582	0.559
APGAR 5	9.94±0.27	9.94±0.36	9.96±0.20	0.242	0.785
APGAR 10	9.98±0.14	9.97±0.21	9.98±0.14	0.385	0.681
Preterm birth	10(5.0%)	13(7.4%)	3(3.1%)	2.486	0.288
Low birth weight	8(4.0%)	14(8.0%)	2(2.0%)	5.601	0.061
SGA [#]	4(2.0%)	14(8.0%)	3(3.1%)	8.306	0.016
Fetal distress	19(9.7%)	16(9.2%)	9(9.2%)	0.030	0.985

Table 6. Neonatal data in two groups of ACE2 gene rs2074192 alleles. #: Small Gestational for Age

Variables	C/C (n=201)	C/T and T/T (n=273)	t/X ²	P
Birth weight	3232.94±403.65	3241.92±477.32	0.216	0.829
Body length	49.80±1.52	49.59±2.06	1.184	0.237
Head circumference	33.20±1.17	33.13±1.40	0.538	0.591
APGAR 1	8.90±0.40	8.90±0.63	0.084	0.933
APGAR 5	9.94±0.27	9.95±0.31	0.358	0.720
APGAR 10	9.98±0.14	9.97±0.19	0.594	0.553
Preterm birth	10(5.0%)	16(5.9%)	0.175	0.676
Low birth weight	8(4.0%)	16(5.9%)	0.852	0.356
SGA#	4(2.0%)	17(6.2%)	4.908	0.027
Fetal distress	19(9.7%)	25(9.2%)	0.029	0.864

Babies having T allele had higher incidence of SGA compared to babies having CC homozygote ($P=0.027$, Table 6). 21 SGA infants (15 female and 6 male) were shown in Fig. 2. Six babies had preterm birth, fifteen babies had term birth. Birth weights of 14 SGA babies were below 2500 grams. However, the biometric data of the neonates were no different between CC and CT genotypes versus TT genotype ($P\geq 0.05$, Table 7). Maternal ACE2 gene rs2074192 polymorphisms were not associated with both maternal complications and fetal growth parameters (data not shown).

Fig. 2. 21 SGA newborns.

Birth weights of 2 female babies were lower than 1500 grams and the two babies were immediately admitted to neonatal ward. One baby with 1300 grams of birth weight had fetal distress. Her mother had preeclampsia with blood pressures of 143/103 mmHg and urine protein of 1.5 g/L, and her level of serum uric acid was 489 $\mu\text{mol/L}$. She had preterm delivery at 33th gestational weeks. The other baby had birth weight of 1150 grams born at 30th gestational weeks and suffered from neonatal asphyxia. Her mother had gestational hypertension of 134/93 mmHg at the third trimester. The two girls were discharged from hospital after two months. Their body weights increased to 2500g and had normal organ function at that time.

High risk factors associated with SGA

Following the *t*-Test, multivariable logistic regression models were calculated to identify babies having T allele was independently associated with SGA. Multivariable logistic regression models were adjusted for maternal age, BMI, gestational age, maternal smoking, gestational diabetes, gestational hypertension, hepatitis B virus infection, infant gender and oligohydramnios. The final logistic regression model demonstrated an independent association of the ACE2 gene rs2074192 T allele with SGA (OR: 22.93, 95%CI: 1.26~418.77, $P<0.05$, Table 8).

Table 7. Neonatal data in two groups of ACE2 gene rs2074192 alleles. #: Small Gestational for Age

Variables	C/C and C/T (n=376)	T/T (n=98)	t/X ²	P
Birth weight	3226.50±453.61	3282.65±420.61	1.107	0.269
Body length	49.63±1.90	49.85±1.66	1.051	0.294
Head circumference	33.15±1.33	33.21±1.21	0.434	0.664
APGAR 1	8.91±0.41	8.86±0.90	0.621	0.536
APGAR 5	9.94±0.31	9.96±0.20	0.694	0.488
APGAR 10	9.97±0.18	9.98±0.14	0.320	0.749
Preterm birth	23(6.1%)	3(3.1%)	1.400	0.237
Low birth weight	22(5.9%)	2(2.0%)	1.622	0.203
SGA#	18(4.8%)	3(3.1%)	0.215	0.643
Fetal distress	35 (9.5%)	9(9.2%)	0.008	0.928

Discussion

Our study showed for the first time that newborns carrying the ACE2 gene rs2074192 T allele are at higher risk for fetal growth restriction, *e.g.* SGA infants, whereas the maternal ACE2 gene rs2074192 polymorphism had no association with fetal growth. This association is independent of known risk factors of SGA as shown by multivariate analysis considering confounding factors.

ACE2 is a counterpart of the ACE enzyme in RAAS, capable of cleaving Ang II into biologically active Ang (1-7). The ACE2 and Ang-(1-7) tissue concentrations in kidneys of pregnant rats are 1.5~2 times higher as compared to non-pregnant rats [30]. Furthermore, Levy et al. reported that ACE2 mRNA expression in uterus of pregnant rats was significant higher versus non-pregnant rats [31]. If high salt diet was fed in pregnant rats, ACE2 activity in uterus was much higher compared to non-pregnant rats [31]. In the placenta, ACE2 gene expression was positively associated with gestational age from gestational day 12 to day 20 [32]. Few studies of the association between ACE2 and pregnancy outcomes have been reported. Valdés G et al. found that ACE2 gene expression in arterial endothelium of umbilical cord was lower in normal pregnancy compared to pregnancy with preeclampsia [10]. These observations fit very well with the results of our study.

SGA infants are not only at higher risk for perinatal morbidity and mortality, but they are also more likely to develop central adiposity, insulin and leptin resistance, metabolic syndrome such as hypertension and type 2 diabetes mellitus in later life than infants born with normal weight [33-35]. Infants can compensate for their small birth weight by later

Table 8. Logistics regression of SGA. *: Maternal smoking before pregnancy. #: CC versus CT+TT

Variables	B	P	OR	OR: 95%CI
Model A				
Maternal age	0.05	0.34	1.05	0.95~1.17
Maternal pre-pregnancy BMI	0.31	0.00	1.37	1.12~1.67
Gestational age	0.05	0.0	1.05	1.02~1.08
Maternal smoking*	15.26	0.99	4.25×10E6	0.00~NA
GDM	0.48	0.52	1.62	0.37~7.04
Gestational hypertension	1.65	0.00	5.21	1.72~15.74
HBV	-1.53	0.15	0.22	0.03~1.70
Model B				
Maternal age	0.07	0.22	1.07	0.96~1.20
Maternal pre-pregnancy BMI	0.32	0.00	1.37	1.12~1.68
Gestational age	0.05	0.00	1.05	1.02~1.08
Maternal smoking*	15.06	1.00	3.48×10E6	0.00~NA
GDM	0.33	0.66	1.39	0.32~6.13
Gestational hypertension	1.81	0.00	6.10	1.93~19.22
HBV	-1.86	0.11	0.16	0.02~1.51
Infant gender	0.74	0.05	2.09	1.00~4.35
Oligohydramnios	1.23	0.03	3.41	1.15~10.08
Model C				
Maternal age	-0.01	0.93	0.99	0.75~1.30
Maternal pre-pregnancy BMI	0.33	0.14	1.40	0.90~2.18
Gestational age	0.11	0.01	1.12	1.03~1.22
Maternal smoking*	15.03	1.00	3.38×10E6	0.00~NA
GDM	-0.69	0.47	0.50	0.08~3.25
Gestational hypertension	2.87	0.01	17.67	1.78~175.79
HBV	-18.62	1.00	0.00	0.00~NA
Infant gender	1.31	0.16	3.70	0.61~22.54
Oligohydramnios	3.95	0.00	51.87	3.66~734.86
Neonatal rs2074192 T allele#	3.13	0.04	22.93	1.26~418.77
Maternal rs2074192 T allele#	-16.62	1.00	0.00	0.00~NA

catch-up growth exposed to improved nutrition, however rapid childhood growth aggravates the risk of diseases in later life [36].

The pathological mechanisms leading to SGA are complex. Alterations of the endocrine systems during pregnancy, such as a change of rennin-angiotensin system (RAS) combined with genetics, epigenetics and environment [20, 37, 38] factors might play a role. Nineteen single nucleotide polymorphisms (SNPs) were identified by Itoyama et al. in the human ACE2 gene [39]. ACE2 gene SNPs have been dedicated to diseases, such as hypertension, T2DM and T2DM combined with cerebral stroke (CS). It was found that ACE2 gene rs2074192 and rs2106809 rare alleles are associated with reduced circulating Ang-(1-7) levels in 213 female Han Chinese patients [18]. Ang-(1-7) is a peptide which is a vasodilator and has an anti-tumor activity [40]. In a Canadian teenagers' (12-13 years old) study, ACE2 gene SNPs at rs2074192 and rs233575 were associated with increased systolic blood pressures [41]. Furthermore, male Caucasians with ACE2 gene rs2074192 C allele, rs4240157 G allele and rs4646188 T allele who suffered from type 2 diabetes were more likely to be hypertensive [42]. Diabetic patients who had ACE2 gene rs2074192 T allele and rs714205 C allele had higher chance for developing diabetic retinopathy at Shandong Province, China [43]. However, some recent studies from European, East Asian and Australian populations showed no significant associations of ACE2 genotypes with hypertension and diabetes mellitus [13, 16, 44-46]. We have not found any studies about human ACE2 gene polymorphisms related to fetal growth, *e.g.* SGA.

To the best of our knowledge, our study is the first to show that neonatal ACE2 rs2074192 T allele was associated with SGA. ACE2 gene-knockout pregnant mice had lower level of Ang-(1-7) in circulation and increased placental Ang II level, and it was associated with lower fetal body weight and length at the 18th day of gestation [12]. In our previous clinical study, lower maternal and fetal Ang-(1-7) levels in plasma were at high risk for preterm birth [20]. Compared with healthy pregnant women, ACE D allele in pregnant women had an increased risk for giving SGA babies [47]. In a study from Brazil, The Ang II and ACE levels in plasma of boys who were born as SGA were higher (20% of increased levels) than those in boys at term birth [48].

There are two limitations in our study: 1. the ACE2 activity in peripheral blood and placenta was not analyzed in both mothers and their newborns. 2. The number of mothers and babies was relatively small and this was a single-center clinical study.

Conclusion

The current study it is first to show that the babies with ACE2 gene rs2074192 T allele had a high risk for SGA, which contributes to metabolic syndrome and cardiovascular diseases in later life. The effect is independent of the maternal ACE2 gene.

Acknowledgements

The study is supported by Guangzhou City Technology Scientific Research Program (No. 201510010027), Science and Technology Planning Project of Guangdong Province (No. 2014A020212220).

Y. P. C. conceived and designed the study. J. H. and Y. P. L. wrote the main manuscript text. J. H. and Y. P. L. prepared tables. J. H., Y. P. L., J. L., T. Y. L., X. C., X. J. L., W. J. C., and S. N. P. collected the data. B.H. and Y. P. C. revised the manuscript.

Disclosure Statement

All authors declare that there is no conflict of interest.

References

- de Onis M, Habicht JP: Anthropometric reference data for international use: recommendations from a World Health Organization Expert Committee. *Am J Clin Nutr* 1996;64:650-658.
- Lee ACC, Kozuki N, Cousens S, Stevens, GA, Blencowe, H, Silveira, MF, Sania A, Rosen HE, Schmiegelow C, Adair LS, Baqui AH, Barros FC, Bhutta ZA, Caulfield LE, Christian P, Clarke SE, Fawzi W, Gonzalez R, Humphrey J, Huybregts L, et al.: Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21st standard: analysis of CHERG datasets. *BMJ* 2017;358:j3677.
- Wang QH, Yang YJ, Wei KL, Du LZ: Current situation investigation and analysis of SGA in China. *Chinese Journal of Practical Pediatrics* 2009;24:177-180.
- Hediger ML, Overpeck MD, Maurer KR, Kuczmarski RJ, Mcglynn A, Davis WW: Growth of Infants and Young Children Born Small or Large for Gestational Age: Findings From the Third National Health and Nutrition Examination Survey. *Arch Pediatr Adolesc Med* 1998;152:1225-1231.
- Jensen RB, Thankamony A, Day F, Scott RA, Langenberg C, Kirk J, Donaldson M, Ivarsson SA, Söder O, Roche E, Hoey H, Juul A, Ong KK, Dunger DB: Genetic Markers of Insulin Sensitivity and Insulin Secretion Are Associated With Spontaneous Postnatal Growth and Response to Growth Hormone Treatment in Short SGA Children: the North European SGA Study (NESGAS). *J Clin Endocrinol Metab* 2015;100:503-507.
- Jensen RB, Thankamony A, O'Connell S M, Salgin B, Kirk J, Donaldson M, Ivarsson SA, Soder O, Roche E, Hoey H, Dunger DB, Juul A; NESGAS Group: Baseline IGF-I levels determine insulin secretion and insulin sensitivity during the first year on growth hormone therapy in children born small for gestational age. Results from a North European Multicentre Study (NESGAS). *Horm Res Paediatr* 2013;80:38-46.
- Ewing AC, Ellington SR, Shapiro-Mendoza CK, Barfield WD, Kourtis AP: Full-Term Small-for-Gestational-Age Newborns in the U.S.: Characteristics, Trends, and Morbidity. *Matern Child Health J* 2017;21:786-796.
- Huang YT, Lin HY, Wang CH, Su BH, Lin CC: Association of preterm birth and small for gestational age with metabolic outcomes in children and adolescents: A population-based cohort study from Taiwan. *Pediatr Neonatol* 2018;59:147-153.
- Wang X, Ye Y, Gong H, Wu J, Yuan J, Wang S, Yin P, Ding Z, Kang L, Jiang Q, Zhang W, Li Y, Ge J, Zou Y: The effects of different angiotensin II type 1 receptor blockers on the regulation of the ACE-AngII-AT1 and ACE2-Ang(1-7)-Mas axes in pressure overload-induced cardiac remodeling in male mice. *J Mol Cell Cardiol* 2016;97:180-190.
- Valdés G, Neves LA, Anton L, Corthorn J, Chacón C, Germain A M, Germain AM, Merrill DC, Ferrario CM, Sarao R, Penninger J, Brosnihan KB: Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. *Placenta* 2006;27:200-207.
- Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ: A human homolog of Angiotensin-converting Enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000;275:33238-33243.
- Bharadwaj MS, Strawn WB, Groban L, Yamaleyeva LM, Chappell MC, Horta C, Atkins K, Firmes L, Gurley SB, Brosnihan KB: Angiotensin-converting enzyme 2 deficiency is associated with impaired gestational weight gain and fetal growth restriction. *Hypertension* 2011;58:852-858.
- Benjafield AV, Wang WYS, Morris BJ: No association of Angiotensin-Converting enzyme 2 gene (ACE2) polymorphisms with essential hypertension. *Am J Hypertens* 2004;17:624-628.
- Fan X, Wang Y, Sun K, Zhang W, Yang X, Wang S, Zhen Y, Wang J, Li W, Han Y, Liu T, Wang X, Chen J, Wu H, Hui R; Study Group for Pharmacogenomic Based Antihypertensive Drugs Selection, Effects and Side Effects, in Rural Area Chinese: Polymorphisms of ACE2 gene are associated with essential hypertension and antihypertensive effects of captopril in women. *Clin Pharmacol Ther* 2007;82:187-196.

- 15 Patnaik M, Pati P, Swain SN, Mohapatra MK, Dwibedi B, Kar SK, Ranjit M: Association of angiotensin-converting enzyme and angiotensin-converting enzyme-2 gene polymorphisms with essential hypertension in the population of Odisha, India. *Ann Hum Biol* 2014;41:145-152.
- 16 Lieb W, Graf J, Götz A, König IR, Mayer B, Fischer M, Stritzke J, Hengstenberg C, Holmer SR, Döring A, Löwel H, Schunkert H, Erdmann J: Association of angiotensin-converting enzyme 2 (ACE2) gene polymorphisms with parameters of left ventricular hypertrophy in men. Results of the MONICA Augsburg echocardiographic substudy. *J Mol Med (Berl)* 2006;84:88-96.
- 17 Wu YH, Li JY, Wang C, Zhang LM, Qiao H: The ACE2 G8790A polymorphism: involvement in type 2 diabetes mellitus combined with cerebral stroke. *J Clin Lab Anal* 2017;31:e22033.
- 18 Chen YY, Zhang P, Zhou XM, Liu D, Zhong JC, Zhang CJ, Jin LJ, Yu HM: Relationship between genetic variants of ACE2 gene and circulating levels of ACE2 and its metabolites. *J Clin Pharm Ther* 2018;43:189-195.
- 19 Chen YP, Li J, Wang ZN, Xu H, Gong J, Chen GJ, Pfab T, Xiao XM, Hoher B: Renin angiotensin aldosterone system and glycemia in pregnancy. *Clin Lab* 2012;58:527-533.
- 20 Chen YP, Lu YP, Li J, Liu ZW, Chen WJ, Liang XJ, Chen X, Wen WR, Xiao XM, Reichetzeder C, Hoher B: Fetal and maternal angiotensin (1-7) are associated with preterm birth. *J Hypertens* 2014;32:1833-1841.
- 21 Villar J, Cheikh IL, Victora CG, Ohuma EO, Bertino E, Altman DG, Lambert A, Papageorgiou AT, Carvalho M, Jaffer YA, Gravett MG, Purwar M, Frederick IO, Noble AJ, Pang R, Barros FC, Chumlea C, Bhutta ZA, Kennedy SH; International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st): International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* 2014;384:857-868.
- 22 Neonatal physical development research collaboration group from 15 cities in China: The revised report of male or female newborns birth weight at different gestation from 15 Chinese cities. *Chinese Journal of Practical Pediatrics* 1992;7:306-307.
- 23 Şahin Uysal N, Gülümser Ç, Bilgin YF: Maternal and perinatal characteristics of small for gestational age newborns: Ten-year experience of a single center. *J Turk Ger Gynecol Assoc* 2017;18:90-95.
- 24 Watanabe H, Inoue K, Doi M, Ogasawara K, Fukuoka H, Nagai Y: Risk factors for term small for gestational age infants in women with low prepregnancy body mass index. *J Obstet Gynaecol Res* 2010;36:506-512.
- 25 Eskes M, Ajm W, Scherjon SA, Bergman KA, Abu-Hanna A, Ravelli ACJ: Small for gestational age and perinatal mortality at term: An audit in a Dutch national cohort study. *Eur J Obstet Gynecol Reprod Biol* 2017;215:62-67.
- 26 Manzanares S, Marotomartín MT, Naveiro M, Sánchez-Gila M, López-Criado S, Puertas A: Risk of recurrence of small-for-gestational-age fetus after first pregnancy. *J Obstet Gynaecol* 2017;37:723-726.
- 27 Tong VT, England LJ, Rockhill KM, D'Angelo DV: Risks of preterm delivery and small for gestational age infants: effects of nondaily and low-intensity daily smoking during pregnancy. *Paediatr Perinat Epidemiol* 2017;31:144-148.
- 28 Sebastian T, Yadav B, Jeyaseelan L, Vijayaselvi R, Jose R: Small for gestational age births among South Indian women: temporal trend and risk factors from 1996 to 2010. *BMC Pregnancy Childbirth* 2015;15:7.
- 29 Safir A, Levy A, Sikuler E, Sheiner E: Maternal hepatitis B virus or hepatitis C virus carrier status as an independent risk factor for adverse perinatal outcome. *Liver Int* 2010;30:765-770.
- 30 Brosnihan KB, Neves LA, Joyner J, Averill DB, Chappell MC, Sarao R, Penninger J, Ferrario CM: Enhanced renal immunocytochemical expression of ANG-(1-7) and ACE2 during pregnancy. *Hypertension* 2003;42:749-753.
- 31 Levy A, Yagil Y, Bursztyn M, Barkalifa R, Scharf S, Yagil C: ACE2 expression and activity are enhanced during pregnancy. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R1953-1961.
- 32 Vaswani K, Chan HW, Verma P, Dekker Nitert M, Peiris HN, Wood-Bradley RJ, Armitage JA, Rice GE, Mitchell MD: The rat placental renin-angiotensin system - a gestational gene expression study. *Reprod Biol Endocrinol* 2015;13:89.
- 33 Martínez-Aguayo A, Capurro T, Peña V, Iñiguez G, Hernández MI, Avila A, Salazar T, Asenjo S, Mericq V: Comparison of leptin levels, body composition and insulin sensitivity and secretion by OGTT in healthy, early pubertal girls born at either appropriate- or small-for-gestational age. *Clin Endocrinol (Oxf)* 2007;67:526-532.
- 34 Hofman PL, Cutfield WS: Insulin sensitivity in people born preterm, with low or very low birth weight and small for gestational age. *J Endocrinol Invest* 2006;29:2-8.

- 35 Evagelidou EN, Giapros VI, Challa AS, Kiortsis DN, Tsatsoulis AA, Andronikou SK: Serum adiponectin levels, insulin resistance, and lipid profile in children born small for gestational age are affected by the severity of growth retardation at birth. *Eur J Endocrinol* 2007;156:271-277.
- 36 Barker DJ, Osmond C: Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1986;1:1077-1081.
- 37 Hochoer B: More than genes: the advanced fetal programming hypothesis. *J Reprod Immunol* 2014;104-105:8-11.
- 38 Nair AV, Hochoer B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. *Proc Natl Acad Sci U S A* 2012;109:11324-11329.
- 39 Itoyama S, Keicho N, Hijikata M, Quy T, Phi NC, Long HT, Ha LD, Ban VV, Matsushita I, Yanai H, Kirikae F, Kirikae T, Kuratsuji T, Sasazuki T: Identification of an alternative 5'-untranslated exon and new polymorphisms of angiotensin-converting enzyme 2 gene: lack of association with SARS in the Vietnamese population. *Am J Med Genet A* 2005;136:52-57.
- 40 Santos RA, Ferreira AJ, Verano-Braga T, Bader M: Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin-angiotensin system. *J Endocrinol* 2013;216:R1-R17.
- 41 Malard L, Kakinami L, O'Loughlin J, RoyGagnon MH, Labbe A, Pilote L, Hamet P, Tremblay J, Paradis G: The association between the angiotensin-converting enzyme-2 gene and blood pressure in a cohort study of adolescents. *BMC Medical Genetics* 2013;14:1-7.
- 42 Patel SK, Wai B, Ord M, MacIsaac RJ, Grant S, Velkoska E, Panagiotopoulos S, Jerums G, Srivastava PM, Burrell LM: Association of ACE2 genetic variants with blood pressure, left ventricular mass, and cardiac function in Caucasians with type 2 diabetes. *Am J Hypertens* 2012;25:216-222.
- 43 Meng N, Zhang Y, Ma J, Li H, Zhou F, Qu Y: Association of polymorphisms of angiotensin I converting enzyme 2 with retinopathy in type 2 diabetes mellitus among Chinese individuals. *Eye* 2015;29:266-271.
- 44 Huang W, Yang W, Wang Y, Zhao Q, Gu D, Chen R: Association study of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms and essential hypertension in northern Han Chinese. *J Hum Hypertens* 2006;20:968-971.
- 45 Currie D, Mcknight AJ, Patterson CC, Sadlier DM, Maxwell AP, UK Warren 3/GoKinD Study Group: Investigation of ACE, ACE2 and AGTR1 genes for association with nephropathy in Type 1 diabetes mellitus. *Diabet Med* 2010;27:1188-1194.
- 46 Fröjdö S, Sjölin L, Parkkonen M, Mäkinen VP, Kilpikari R, Pettersson-Fernholm K, Forsblom C, Fagerudd J, Tikellis C, Cooper ME, Wessman M, Groop PH; FinnDiane Study Group: Polymorphisms in the gene encoding angiotensin I converting enzyme 2 and diabetic nephropathy. *Diabetologia* 2005;48:2278-2281.
- 47 Fatini C, Romagnuolo I, Sticchi E, Rossi L, Cellai AP, Rogolino A, Abbate R: ACE gene in pregnancy complications: Insights into future vascular risk. *Hypertens Pregnancy* 2016;35:62-72
- 48 Franco MC, Casarini DE, Carneiro-Ramos MS, Sawaya AL, Barreto-Chaves ML, Sesso R: Circulating renin-angiotensin system and catecholamines in childhood: is there a role for birth weight? *Clin Sci* 2008;114:375-380.