The role of the renin-angiotensin-aldosterone system in preeclampsia: genetic polymorphisms and microRNA

Jie Yang1, Jianyu Shang1, Suli Zhang1, Hao Li1 and Huirong Liu1,2
1Department of Pathophysiology, School of Basic Medical Sciences, Capital Medical University, 10 Xitoutiao, You An Men, Beijing 100069, People's Republic of China 2Department of Physiology, Shanxi Medical University, T'aiyuan, Shanxi 030001, People's Republic of China

Abstract

The compensatory alterations in the rennin-angiotensin-aldosterone system (RAAS) contribute to the salt-water balance and sufficient placental perfusion for the subsequent well-being of the mother and fetus during normal pregnancy and is characterized by an increase in almost all the components of RAAS. Preeclampsia, however, breaks homeostasis and leads to a disturbance of this delicate equilibrium in RAAS both for circulation and the uteroplacental unit. Despite being a major cause for maternal and neonatal morbidity and mortality, the pathogenesis of preeclampsia remains elusive, where RAAS has been long considered to be involved. Epidemiological studies have indicated that preeclampsia is a multifactorial disease with a strong familial predisposition regardless of variations in ethnic, socioeconomic, and geographic features. The heritable allelic variations, especially the genetic polymorphisms in RAAS, could be the foundation for the genetics of preeclampsia and hence are related to the development of preeclampsia. Furthermore, at a posttranscriptional level, miRNA can interact with the targeted site within the 3'-UTR of the RAAS gene and thereby might participate in the regulation of RAAS and the pathology of preeclampsia. In this review, we discuss the recent achievements of genetic polymorphisms, as well as the interactions between maternal and fetal genotypes, and miRNA posttranscriptional regulation associated with RAAS in preeclampsia. The results are controversial but utterly inspiring and attractive in terms of potential prognostic significance. Although many studies suggest positive associations with genetic mutations and increased risk for preeclampsia, more meticulously designed large-scale investigations are needed to avoid the interference from different variations.

Key Words

- Pre-eclampsia
- Renin-angiotensin system
- Polymorphism
- Genetic
- microRNA

Introduction

Preeclampsia, characterized as hypertension (>140/90 mmHg) after 20 weeks’ gestation accompanied by proteinuria (>300 mg/l), represents a major cause of maternal and neonatal morbidity and mortality, leading to devastating effects to mothers and their children. According to recent epidemiological studies, 5–7% of pregnant women in the world have suffered from this disease (Kuc et al. 2011). Various as the theories for pathogenesis of preeclampsia...
are, the exact underlying molecular mechanisms remain unclear but are likely to be multifactorial: endothelial cell dysfunction, excessive vasoconstriction (Roberts & Gammill 2005), inflammation, immunological disorder (Hubel et al. 2007), etc. Of the multifactorial impact factors, the renin–angiotensin–aldosterone system (RAAS) serves as the major cause for shallow trophoblast invasion and impaired spiral artery remodeling, which are the most paramount and fundamental pathological alterations followed by a series of complications. The compensatory and delicate changes of RAAS during normal pregnancy are hard to counterbalance for preeclamptics women, resulting in body capillary constriction, renal damages, disorders of salt and water balance, etc. However, the etiology of this dysfunction requires further explorations.

**There exists both a classical RAAS and a local renin–angiotensin system during pregnancy**

The RAAS contributes to blood pressure regulation and body fluid through a series of enzymatic reactions induced by its primary components: renin, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensin I (ANG I), angiotensin II (ANG II), angiotensin α receptor type I (AT1R), and angiotensin receptor type 2 (AT2R). In response to low blood pressure and low circulating sodium chloride, renin, synthesized and secreted by juxtaglomerular cells of the afferent renal arterioles, catalyzes the cleavage of AGT made in the liver to ANG I. This is the rate-limiting step of the renin–angiotensin system (RAS) cascade. Then, this ten amino acid peptide, without any biological function as we presently know, is cleaved by ACE, which is made primarily in lung endothelium, into ANG II, a biologically active eight amino acid molecule. ACE2, a homolog of ACE, is a new component of RAS and can cleave a single residue from ANG II to form angiotensin (1–7) (ANG (1–7)), or from ANG I to angiotensin (1–9) (ANG (1–9)) (Anton & Brosnihan 2008), whose functions include a vasodilator response and antidiuresis in water-loaded animals. Therefore, ACE2, ANG (1–7), and ANG (1–9) are regarded to modulate the effects of RAS on vascular tone, neutralizing excessive contraction. There exist two major types of angiotensin receptors: AT1R and AT2R, both of which belong to seven transmembrane G-protein-coupled receptor families, though their distributions and functions are totally different. Via activation of AT1R or AT2R, ANG II is regarded as a well-known versatile effector involved in vasoconstriction, sympathetic activity, cell viability, and the release of different molecules, such as aldosterone (ALD). Through a major activation of AT1R and then the primary aldosterone-synthesizing enzyme (CYP11B2), in the zona glomerulosa of the adrenal cortex, ANG II affects the production of ALD synthesis, which plays a critical role in the regulation of salt and water retention and excretion to maintain sufficient blood fluids and electrolyte balance.

It has been confirmed that the components of RAS are not unique to the kidney but are synthesized in many tissues, among which one of the major local RAS during pregnancy is in the uteroplacental unit (placenta also named fetal origin and decidua named maternal origin) (Shah 2006). Since the first-time pro-renin, AGT, ACE, ANG II and ANG I, AT1R were identified in fetal placental tissues (Li et al. 1998), the expression of renin, AGT, ACE, and AT1R has been observed in the first trimester human deciduas (Shaw et al. 1989), and more recent studies via human third trimester decidual cells also have demonstrated the presence of AGT and renin (Li et al. 2000). Other studies of the decidual spiral arteries have indicated the expression of AGT, renin, ACE, and AT1R (Morgan et al. 1998a,b). Moreover, it has been demonstrated that immunocytochemical expression of ANG (1–7) and ACE2 was widely distributed throughout the human fetal placental unit during gestation (Valdes et al. 2006, Neves et al. 2007). Therefore, all the necessary components of RAS have been found both in maternal decidua and in the fetal placental tissues.

**Compensatory regulation of RAAS during pregnancy**

During normal pregnancy, both body fluid and the RAAS undergo major changes. There is a marked enlargement of plasma volume that starts in the first trimester, accelerates in mid-gestation, and stabilizes after around the 34th week of gestation (Pirani et al. 1973). In other words, this new steady state in pregnancy reflects a downward resetting of the osmolarity thresholds for thirst sensation and vasopressin release, which finally leads to an increase in salt and water strengthening placental perfusion to protect the pregnant woman and her fetus (Escher & Mohaupt 2007). As the most principal part in the renal control of body fluid, nearly all the components of RAAS experience an increase except ACE, the only component decreasing or leveling off at the nonpregnant concentration (Merrill et al. 2002). An early increase in renin is observed due to the local release by maternal decidua and ovaries (Hsueh et al. 1982), and the growth of circulating estrogen generated by fetus placenta promotes the AGT synthesis.
in the liver, which leads to obvious rises in serum ANG II (Brown et al. 1988). ALD level shows an upward trend mainly corresponding to the requirement for volume expansion. Meanwhile, in both plasma and urinary levels, there is also an increase both in ANG (1–7) and ACE2 (Valdes et al. 2001, Oudit et al. 2003, Turner 2003). Furthermore, in rat pregnant models, a study conducted by Levy et al. (2008) indicated that the uterus and placentas contributed to Ace2 expression and activity during preeclampsia. The placenta was the foremost total contributor, although the kidney was responsible for the highest levels of ACE2 activity. They found that during pregnancy, ACE abundance in reproductive organs during the third trimester of pregnancy was commensurate with a systemic vasodilatory state both in normotensive and in hypertensive rats. ACE activity in the uterus was affected by salt-loading. These studies demonstrate the importance of transient overexpression of ACE2 and its elevated activity in modulating systemic and local hemodynamics in the uteroplacental unit during pregnancy. However, we know little about the underlying exact mechanisms.

Although ANG II levels increase during pregnancy, normotensive pregnant women are less responsive to its vasopressor effects, which was substantiated by the historic study conducted by Assali & Westersten (1961) showing that the required ANG II concentration of a pregnant woman is twice that needed in a nonpregnant woman to achieve the same vasomotor response. This reduced ANG II sensitivity is considered to be due to the increased progesterone and prostacyclins (Gant et al. 1980), as well as a little inactivation of AT1R by reactive oxygen species (ROS; Abdalla et al. 2001).

**Changes in both circulating RAAS and local RAS during preeclampsia**

Women with preeclampsia suffer from an utterly different RAAS state (Table 1) and develop a much poorer control of sodium loading and a substantial fluctuation of sodium level in sera with avid retention of sodium and slow excretion of additional sodium loads, when compared with normotensive pregnant women who have much better tolerances of sodium intake and slight changes in either weight or plasma volume (Symonds et al. 1975, Herse et al. 2007). This implies that there is a dysfunction of salt–water balance that is dominated mainly by ALD. Moreover, compared with the increase in RAAS components in normotensive pregnancy, the circulating levels of renin, ANG I, ANG II, ANG (1–7), and ALD are much lower, yet with little fluctuation of ACE levels (Granger et al. 2001, Merrill et al. 2002); stranger still, there is a relatively higher level of ALD for the given level of renin (Gallery & Brown 1987) and worse, pregnant women are highly sensitive to the pressor effects of ANG II partly due to heterodimerization of AT1R (Quitterer et al. 2004, Abdalla et al. 2005). As for local uteroplacental RAS, there are some various and controversial results: recently, Herse et al. (2007) argued that excessive expression of the AT1R receptor in maternal deciduas is the only change observed in local RAS, without any increase in renin production in decidua of preeclamptic patients. By contrast, Shah et al. (2000) demonstrated an increase in renin level in the decidua vera of preeclamptic women. A recent clinical trial performed by Anton et al. (2008) suggested that in the chorionic villi of preeclampsia patients, an essential part of the placenta responsible for regulating nutrient and oxygen exchange between the mother and the fetus, ANG II levels and AT1R mRNA were significantly higher despite a decrease in circulating ANG II when compared with normal pregnancies. Furthermore, in murine models of antenatal maternal hypoxia responsible for the development of preeclampsia, such stress elevated the expression of AGT and ACE by post-transcriptional mechanisms in murine placenta (Goyal et al. 2011). Another symptom leading to

<table>
<thead>
<tr>
<th>RAAS components</th>
<th>Normotensive pregnancy</th>
<th>Preeclamptic pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT</td>
<td>+ +</td>
<td>–</td>
</tr>
<tr>
<td>Renin</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Renin activity</td>
<td>+ +</td>
<td>=/–</td>
</tr>
<tr>
<td>ALD</td>
<td>+ +</td>
<td>–</td>
</tr>
<tr>
<td>ANG II</td>
<td>+ +</td>
<td>=/–</td>
</tr>
<tr>
<td>ANG II activity</td>
<td>–</td>
<td>+=/–</td>
</tr>
<tr>
<td>ANG (1–7)</td>
<td>+ +</td>
<td>–</td>
</tr>
<tr>
<td>ACE</td>
<td>–</td>
<td>=/–</td>
</tr>
<tr>
<td>ACE2</td>
<td>+ +</td>
<td>–</td>
</tr>
<tr>
<td>ACE2 activity</td>
<td>+ +</td>
<td>=/–</td>
</tr>
<tr>
<td>AT1R</td>
<td>–</td>
<td>+=/–</td>
</tr>
</tbody>
</table>

+ +, significantly increased over nonpregnancy; +, slightly increased over nonpregnancy; =, same as nonpregnant; –, decreased compared with nonpregnancy.
Preeclampsia is pregnancy-induced hypertension, of which the rat model suggested that ANG (1–7) concentrations decreased both in the uterus and placenta, so did Ace2 mRNA levels (Neves et al. 2008). The possible reasons for this divergence involve the different sample sizes, chosen tissues, and defining criteria for preeclampsia, which suggests that it is necessary to conduct further investigation. In summary, preeclampsia is characterized by a series of significant alterations in RAAS worthy of deeper research (Fig. 1).

Genetic mutations in preeclampsia play a big role

Right now, we still know little about the exact reasons for the dysfunction of RAAS in preeclampsia, although several pathophysiological mechanisms have been proposed including endothelial damage, oxidative stress, and autoimmunity. Yet inspiringly, from an epidemiological point of view, various genetic and environmental factors have been considered to contribute to the pathogenesis of preeclampsia due to a strong familial predisposition observed in different geographic, socioeconomic, and racial features (Hocher et al. 2008). Researchers, for instance, reported that compared with the offspring or relatives of normotensive pregnancies, daughters of preeclamptic mothers suffered from much higher incidence of risk for the disease ranging from 20 to 40%, 11 to 37% for sisters of preeclamptic women, and 22 to 47% in twin studies (Mogren et al. 1999, Esplin et al. 2001, Hocher et al. 2008). Studies also showed paternal contributors to the disease: Esplin et al. (2001) suggested an increased risk of fathering a preeclamptic pregnancy among males whose mothers once had preeclampsia, as shown among males who previously fathered a preeclamptic pregnancy with another partner (Lie et al. 1998). Trying to separate the effect of maternal and fetal genetic factors from environmental factors, Cnattingius et al. (2004) suggested that via population-based Swedish Birth and Multi-Generation Registries, genetic factors accounted for more than 50% of the liability to preeclampsia, maternal genes may contribute more than fetal genes and genetic interaction effects between spouses. Naturally, ever-increasing studies have been focusing on the role of genes as candidates for this disorder. The genetic polymorphisms involve various functional systems and each of them requires a detailed review. Considering the importance of RAAS in preeclampsia, this review will be limited to the current views on genetic mutations in RAAS.

Genetic polymorphisms associated with decreased ALD level

Deficiency of ALD, a paramount hormone regulating body fluid, has been considered to be involved in the development of reduced placental perfusion and hypertension leading to a variety of factors that cause widespread dysfunction of the maternal vasculature (Roberts et al. 2003, Sibai et al. 2005). The hypothesis favored by current researchers is that a gestational increase in plasma volume, regarded as a compensatory adaptation to support placental perfusion and fetal substrate delivery, is jeopardized in preeclampsia due to a decrease in ALD, resulting in fluid deficiency and subsequent placental ischemia (Gallery & Brown 1987, Roberts & Cooper 2001, Takeda et al. 2002). Therefore, the reduced circulating volume circumscribes placental blood supply, finally inducing an elevated blood pressure during preeclampsia. Compared with normal pregnancies, preeclamptic women are proven to experience much lower levels of circulating ALD (Shojaati et al. 2004).
CYP11B2 activity is impaired in preeclampsia partly because of genetic polymorphisms. CYP11B2, a multi-enzyme complex sharing 93% homology to CYP11B1, comprised three enzymatic reactions conducting the last but most important in ALD synthesis, whose activities can be quantified separately through the determination of urinary steroids. Compared with the increased tetrahydroaldosterone (TH-Aldo) during normal pregnancy, a metabolite of urinary ALD, preeclampsia is characterized by a higher level of TH-Aldo’s immediate precursor – 18-OH-tetrahydrocorticosterone (18-OH-THA) corticosterone and lower levels of TH-Aldo, suggesting comprised ALD synthase (Fig. 2; Shackleton 1993, Shojaati et al. 2004). To characterize the jeopardized CYP11B2 (Shojaati et al. 2004), Shojaati et al. demonstrated that the reduced ALD synthesis was due to diminished methyl oxidase activity with which genetic polymorphisms appeared to be associated. Based on the altered urinary steroid profile, they found two of five exon mutations associated with an increased risk of preeclampsia – R173K and V386A-CYP11B2. R173K CYP11B2 polymorphism was slightly less frequent in preeclampsia, as it had been reported previously (Portrat-Doyen et al. 1998), whereas other studies claimed that it was related to low renin hypertension in Chilean residents (Fardella et al. 1996). The other was V386A-CYP11B2 polymorphism that had been reported, compared with 4% in normotensive pregnancies, to show up in 18% of patients with essential hypertension closely related to preeclampsia (Pascoe et al. 1992), although it solely appeared in preeclamptic women in Kushiar’s own study.

Recently, the −344C/T CYP11B2 polymorphism disrupting a putative steroidogenic factor-1 site (SF-1) in CYP11B2 has attracted more attention and has been reported as a loss-of-function mutation associated with a high risk of preeclampsia, compared with the gain-of-function in high ALD-to-renin ratio alleles in normal pregnancies (Bauknecht et al. 1982, Lindheimer & Katz 1992, Wacker et al. 1995). The T-allele polymorphism in the 5′-flanking region of the CYP11B2 gene has been reported to be more relevant in hypertensives than in normotensives and has also been related to increased ALD levels (Davies et al. 1999, Tamaki et al. 1999, Komiya et al. 2000, Poch et al. 2001, Nicod et al. 2003, Escher et al. 2009). Nicod et al. (2003) were the first to positively associate the −344/CT CYP11B2 polymorphism with increased ALD in selected hypertensive patients. Recently, a study performed by Escher et al. (2009) confirmed the hypothesis of the correlation of higher ALD availability in normal pregnancies with lower maternal blood pressure, and heterozygosity for SF-1 preferably in preeclampsia corresponded to hypertension and deficiency in placental infusion. In other words, the prevalence of gain-of-function homozygosity for SF-1 prevented pregnancies from developing preeclampsia. Interestingly, many studies have reported the low expression of high ALD-to-renin ratio variants in preeclampsia in the healthy, normotensive, nonpregnant subjects, which strongly corroborates with the notion that −344C/T CYP11B2 polymorphism variants, instead of SF-1-344T/T CYP11B2 genotype, predisposed women to preeclampsia, as well as facilitating any additional contributing factors to the increased risk of developing preeclampsia (Lim et al. 2002, Nicod et al. 2003, Escher et al. 2009). As reported in a research study conducted by Procopciue et al. (2010), women with CC344 CYP11B2 tended to experience an increased risk of preeclampsia (3.21 vs 1.29 of those with CT344 CYP11B2), of which the tendency appeared in mild preeclampsia and severe preeclampsia, 1.49 and 2.01 with a significant statistical difference respectively. They also found the CC344 CYP11B2 polymorphism was related to curtailed delivery gestation and decreased birth weight of babies. Interestingly, still, for a woman positive for M235T AGT or I/D

![Figure 2](http://jme.endocrinology-journals.org) Adrenocortical steroidogenic pathway for ALD biosynthesis. StAR is paramount for the rate-limiting step of movement of cholesterol into the mitochondria, where cholesterol is cleaved by CYP11A1 to pregnenolone. Then the compound is catalyzed by HSD3B2, CYP21A2, and CYP11B2 into ALD. Urinary steroid metabolites can be quantified to assess the enzyme activities of CYP11B2 consisting of three different enzymatic reactions. In preeclampsia, the decreased TH-Aldo level accompanied by elevated 18-OH-THA indicates impaired CYP11B2 activity, specifically 18-oxidase activity. DOC, 11-deoxycorticosterone; THDOC, tetrahydro-11-deoxycorticosterone; THB, tetrahydrocorticosterone; THA, tetrahydro-11-dehydrocorticosterone; 18-OH-THA, 18-OH-tetrahydrocorticosterone; TH-Aldo, tetrahydroaldosterone. The red arrows indicate an increase in concentration while the blue arrows indicate a decrease in concentration.
ALD (see below), a much higher risk of preeclampsia was observed if she had a C344T CYP11B2 polymorphism. However, some studies indicated a lack of association (Mulatero et al. 2000, Percin et al. 2006). A negative relationship of −344C/T CYP11B2 with pregnancy-induced hypertension closely related to preeclampsia was supported by a recent case–control study among 100 women in 2011 (Remírez-Salazar et al. 2011). Despite the reduced serum ALD levels in gestational hypertensive women, neither was the genotype of −344T/C of CYP11B2 related with gestational hypertension nor with ALD levels at delivery. A direct comparison of these polemical results is often limited by the missing information on primiparity, the ethnic divergence between different study groups, and the concern for any other patient abnormalities, such as diabetes. Moreover, preeclampsia is regarded as a multifactorial disease with various factors contributing to the different extents, of which genetic divergence is likely to play a large role.

Moreover, decreased ALD concentration might be attributed to altered activity of the mineralocorticoid receptor (MR), due to the binding of ALD and MR conducting the major functions of ALD in renal sodium reabsorption and potassium excretion. Despite the increased competitive combinations of other hormones against ALD in preeclampsia (Myles & Funder 1996), either gaining or loss-of-function mutations of MR genes such as the S810L mutation appeared to be associated with the alteration of ALD in preeclampsia (Geller et al. 1998, 2000, Pearce et al. 2003, Procopciue et al. 2010). However, the overall assumption favored by most scientific research is that the genetic polymorphisms of MR are rare and cannot be a factor significant enough to articulate the frequency of preeclampsia (Nicod et al. 2003, Escher et al. 2009), although this assumption is supported by observations in very small subsets of preeclamptic patients by other groups (Schmider-Ross et al. 2004, Tempfer et al. 2004).

**ACE polymorphism in preeclampsia**

The *ACE* I/D polymorphism seems to be related to hypertension. Rigat *et al.* (1990) made a striking discovery after performing deeper studies of the *ACE* gene for the detection of the well-known polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287 bp sequence of DNA in intron 16 of this gene, located on the long arm of chromosome 17(17q23) with 26 exons and 25 introns. More evidence corroborate that the DD genotype is commensurate with higher ACE levels, whereas the II genotype is associated with lower ACE levels and the ID genotype with middle levels (Rigat *et al.* 1990, 1992). The reason for the higher ACE levels in the D allele is partly due to the silenter sequence in the I allele (Choi *et al.* 2004). Furthermore, Ueda *et al.* (1995) found that DD carriers suffered from a more marked increase in venous concentration of ANG II and blood pressure compared with II carriers after injection of ANG I. In consideration of the contribution of high ACE to hypertension (Wilson *et al.* 1991, Nogueira *et al.* 2009), naturally, there is the favored hypothesis of a functional polymorphism involved in pathophysiological conditions of hypertension. Based on clinical studies, some researchers failed to find a significant association between the I/D polymorphism and hypertension (Jeunemaitre *et al.* 1992, Schmidt *et al.* 1993, Agachan *et al.* 2003), whereas several other studies reported a positive association between the D allele and high blood pressure (Iwai *et al.* 1994, Schunkert *et al.* 1994, Staessen *et al.* 1997, Giner *et al.* 2000). This is probably because it is necessary to perform sensitivity analyses in subgroups based on gender, ethnicity, mean ages, and genotyping methods. According to these data, there was a substantial relationship between the D allele and hypertension in women and in Asians (Kim *et al.* 2004). Furthermore, with the help of transgenic mice models of these three functional copies of the *ACE* gene (DD, DI, II), Krege *et al.* (1997) found that there was no marked difference in blood pressure between DD type and II type, although the higher activity of ACE in serum was easily detected in the DD type. This discrepancy suggests the combination of *ACE* gene expression and additional environmental or genetic factors would greatly affect blood pressure.

The consequence of ACE in blood pressure by regulating the generation of ANG II has stimulated ever more investigations on the role of the *ACE* I/D polymorphism in the pathogenesis of preeclampsia. The results are conflicting, presumably attributable to differences in study population, ethincs, genetic basis, and sample size. It has been assumed that lack of association between the *ACE* I/D polymorphism appeared more in Koreans (Kim *et al.* 2004), Colombians (Serrano *et al.* 2006), Greeks, and Black South African women (Bouba *et al.* 2003, Roberts *et al.* 2004, Akbar *et al.* 2009). Recently, in a meta-analysis using data from 17 studies (Shaik *et al.* 2011), Shaik *et al.* argued that the presence of the *ACE* I/D polymorphism was not related to increased risk of preeclampsia. In southeastern Iran (Salmi *et al.* 2011), northern India (Aggarwal *et al.* 2011), and Turkey (Morgan *et al.* 1998a, b, Bereketoglu *et al.* 2012), however, the presence of the D allele of the *ACE* gene was related to increased risk of preeclampsia. Uma *et al.* (2010) found...
that the DD genotype of ACE was related to early-onset preeclampsia, although no marked changes in RAS were detected. A recent study by Bereketoglu et al. (2012) also suggested a higher incidence of the DD genotype in Turkish preeclampsia patients in all the three models – codominant, recessive, and dominant. Meanwhile, based on 30 case-control studies, a meta-analysis by Chen et al. (2012) showed a strong relationship between the DD genotype of ACE and a higher risk of pregnancy hypertensive disorders, especially for Asians and Caucasians. Furthermore, Rahimi et al. (2013) confirmed that the ACE D allele and DD genotype were more associated with increased risk of mild preeclampsia (1.99- and 2.34-fold respectively) than severe preeclampsia (1.61- and 1.56-fold respectively), similar to the results of the study by Mandø et al. (2009). These results substantiated the theory that severe and mild preeclampsia patients are characterized by genetic heterogeneity (Oudejans et al. 2007). Furthermore, they demonstrated that the ID genotype of ACE is associated with lower total antioxidant capacity levels compared with the II genotype, which suggested the lipid per-oxidation and oxidative stress, known to be involved in the pathogenesis of preeclampsia, and this might be influenced by polymorphisms in the genes. Moreover, a recent study by Procopciuc et al. (2011) showed that a preeclamptic woman with at least one mutated D-ACE allele suffered from a higher frequency of maternal complications, a lower gestational age, and lower birth weight babies. Although ever-increasing human studies are conducted to prove a positive association between ACE I/D polymorphism with preeclampsia, various sample size, genetic basis, and ethnicity, a more appropriate mouse model or other basic scientific research may offer more information.

Genetic polymorphisms in AT1R, AGT, and renin

Other RAS gene polymorphisms, such as AT1R A1166C, AGT Met235Thr, AGT Thr174Met, and 83A/G REN, seem related to preeclampsia. Similarly, studies are diverging on the relationship of AT1R A1166C and preeclampsia – some claiming a higher risk of pregnancy-induced hypertension commensurate with the AT1R A1166C polymorphism (Shanmugam et al. 1993, Hu et al. 2000, Seremak-Mrozikiewicz et al. 2005, Procopciuc et al. 2011, Salimi et al. 2011), yet others are failing to report marked differences (Morgan et al. 1998a, b, Li et al. 2007) partly due to the multifactorial character of preeclampsia. Moreover, Salimi et al. recently demonstrated a lack of synergistic effects between two alleles of ACE D and AT1R A116C on the risk of preeclampsia. As for AGT polymorphisms, the exon 2 polymorphism M 235T has been extensively explored (Kobashi et al. 1999, Suzuki et al. 1999). Ward et al. (1993) observed a significant association of preeclampsia with a molecular association of AGT T235. Subsequently, they found that more expression of the 235Thr variant in 235Met/Thr heterozygotes might be related to first trimester athertotic changes before pre-eclampsia (Morgan et al. 1997). Later, a study conducted by Levesque et al. (2004) among 847 French Canadian pregnancies (180 cases of preeclampsia, 310 cases of essential hypertension, 357 cases of normotensive control subjects) reported that AGT Thr174Met was markedly associated with preeclampsia and A-Met-Thr (G1035A-Thr174Met-Met235Thr) haplotype was related to a 2.1-fold increased risk of preeclampsia. However, researchers also do not agree on the association between the AGT polymorphism and preeclampsia. The studies performed by Kaur et al. (2005), Kobashi (2006), and Zafarmand et al. (2008) revealed an obvious association between Met235Thr (AGT) and hypertension in preeclampsia, which clearly contradicts the findings of Bashford et al. (2001) and Galao et al. (2004). The studies of the the Genetics of Preeclampsia Consortium (GOPEC 2005) and Akbar et al. (2009) also reported inconsistent results. As for 83A/G Ren genetic variants, the positive relationship with preeclampsia has been assumed to be very weak.

Despite all these conflicting evidences, Procopciuc et al. (2011) proved that the risk of preeclampsia increased significantly for women homozygous for Met235Thr AGT, I/D ACE, A116C AT1R, and 83A/G REN polymorphisms. More than that, their results suggested that there was a relationship between mutated Met235Thr AGT and Thr174Met AGT genotypes and lower gestational age at delivery and/or birth weight. Furthermore, the most original and inspiring part of their work is that they first analyzed the interaction of seven maternal/newborn RAS genotypes and their connection with the risk of preeclampsia: independently of the maternal genotype, newborn Thr174Met AGT and A1166C AT1R polymorphisms were associated respectively with a 1.53- and 1.22-fold risk of preeclampsia, which also increased substantially if both the mother and newborn suffered from one of the Met235Th AGT, I/D ACE, A1166C AT1R, and 83A/G REN polymorphisms. This study confirmed the hypothesis that mutated newborn RAS genes and the interaction of RAS maternal/newborn genotypes make an important contribution to the pathogenesis of preeclampsia in mothers, as well as to intrauterine growth retardation (Procopciuc et al. 2011). Although most
miRNA related to RAAS in preeclampsia

The discrepancies between studies decrease dramatically when it comes to subpopulations defined by ethnic origin (Hirschhorn et al. 2002, Sethupathy et al. 2007). For example, the literature search by Sethupathy et al. (2007) suggested a clear association of the 1166C allele with hypertension in several such major subpopulations as pregnancy hypertension. The increased frequency of +1166A/C polymorphism has been correlated with preeclampsia. However, the discordance remains about the physiological significance of this polymorphism, probably due to its location in the 3'-UTR of human AT1R genes.

Recently, miRNA seems to provide feasible biochemical mechanisms. miRNAs are ~21-nucleotide small noncoding RNA mainly involved in such post-transcriptional gene regulation as silencing gene expression by basepairing to their targets within the 3'-UTR in the complementary sequences (Kloosterman & Plasterk 2006). Interestingly, more studies have suggested that SNPs in the 3'-UTR can affect gene regulation by interfering with miRNA binding (Doench et al. 2003, Huttugner et al. 2004, Abelson et al. 2005, Clop et al. 2006). miR-155, a typical multifunctional miRNA, is a case in point. Martin et al. for the first time examined whether miRNAs play a role in regulating components of the RAS. It has been demonstrated that miR-155 binding to the 3'-UTR of human AT1R mRNA can translationally repress the endogenous expression of human AT1R and significantly reduce ANG II-induced ERK 1/2 activation (Martin et al. 2006, Zheng et al. 2010, Zhu et al. 2011). Moreover, further laboratory experiments showed that the human AT1R +1166A/C polymorphism overlaps with the miR-155 target site in the 3'-UTR of this gene and the +1166C allele decreases the ability of miR-155 to interact with the cis-regulatory site, leading to attenuation of miR-155 silencing function and enhancement of AT1R expression (Martin et al. 2006, 2007), similar to another simultaneous study conducted by Sethupathy et al. (2007). Besides, an additional hint was provided suggesting that miR-155 could participate in blood pressure regulation from low blood pressure. Overexpression of miR-155 resulted in underepression of AT1R in fibroblasts of homozygous twins discordant for trisomy 21 with the observed low blood pressure. These data strengthened the hypothesis about the association between +1166C allele and hypertension. Furthermore, Ceolotto et al., conducting a case–control trial among young untreated hypertensives, reported that compared with subjects with AA and AC genotypes, patients homozygous for the 1166C allele experienced a significant decline in miR-155 expression but a reverse trend in AT1R protein expression and blood pressure. Taken together, miR-155 seems related to hypertension via regulating the component of RAS. The abrogation of miR-155 binding suggests at least a very compelling mechanism for the correlation of 1166C with abnormal blood pressure. It is rational to hypothesize an inverse correlation between miR-155 and AT1R in preeclampsia, based on the aberrant AT1R protein expression and intractable hypertension observed in preeclamptic women.

However, the expression of miR-155, either upregulated or downregulated, differed in diverse tissues of preeclampsia. In 2007, a cross-sectional, case–control study by Pineles et al. for the first time indicated that in placenta of a preeclampsia plus small-for-gestational age (SGA) group, the expression of miR-155 was much higher than in the control group with spontaneous preterm labor and delivery (PTL; Pineles et al. 2007). Later, Zhang et al. (2010) found a similar trend in miR-155 greatly upregulated in preeclampsia placenta. By contrast, Cheng et al. (2011) demonstrated that severely preeclamptic pregnant women had less mature miR-155, yet a much higher expression of AT1R in their human umbilical vein endothelia cells (HUVECs) compared with healthy puerperant women. Similarly, a recent study indicated that overexpression of miR-155 in HUVECs (Zhu et al. 2011) suppressed the expression of AT1R via interacting…

### Table 2 Summary of candidate genes in preeclampsia reported by different groups

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Map location</th>
<th>Gene ID</th>
<th>Polymorphism</th>
<th>Remarks elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP11B2</td>
<td>8q21-q22</td>
<td>1585</td>
<td>–344C/T</td>
<td>Aldosterone concentration</td>
</tr>
<tr>
<td>ACE</td>
<td>17q23.3</td>
<td>1636</td>
<td>I/D(intronic)</td>
<td>I/D allele with ACE activity</td>
</tr>
<tr>
<td>AT1R</td>
<td>3q24</td>
<td>185</td>
<td>A1166C</td>
<td>AT1 receptor upregulation</td>
</tr>
<tr>
<td>AGT</td>
<td>1q42.2</td>
<td>183</td>
<td>M234T,T174M</td>
<td>Abnormal spiral artery remodeling</td>
</tr>
<tr>
<td>Renin</td>
<td>1q32</td>
<td>5972</td>
<td>–</td>
<td>Placental vascular contraction</td>
</tr>
</tbody>
</table>

Molecular genetic studies of preeclampsia to date have focused on maternal susceptibility genes, maternal–fetal interactions have attracted ever-increasing attention and will be the direction of future studies. The summary of discussed candidate genes in preeclampsia is shown in Table 2.
with the AT1R mRNA 3' UTR, leading to repression of ANG II-induced signal transduction in comparison with non-translated or mutant-miR-155-transfected cells. The reasons for these discrepancies probably lie in the disparate mechanisms by which miR-155 contributes to preeclampsia. In placenta, both luciferase assays and transfection assays experimentally corroborated that increased miR-155 targeted the 3' UTR of cysteine-rich protein 61 (CYR61), a molecular regulating placental vascular sufficiency, leading to a deficiency in CYR61 level and placental ischemia (Zhang et al. 2010). Despite elevated AT1Rs and an aberrant miR-155 level, little research has reported on the relationship between them in preeclampsia. Nevertheless, further studies remain necessary in the future. By contrast, when it comes to vascular remodeling and dysfunction, apart from HUVECs, miR-155 overexpression in rat aortic adventitial fibroblasts (AFs) interacted directly with ATIR mRNA 3' UTR, resulting in decreased AT1R, and attenuated ANG II-induced AF phenotypic differentiation into myofibroblasts (Zheng et al. 2010). Zheng et al. found that adult spontaneously hypertensive rats characterized themselves by reduced miR-155 in the aorta accompanied by negative correlation with blood pressure (Xu et al. 2008). The expression of miR-155 seems negatively related to blood pressure and vascular damages (Urbich et al. 2008). Therefore, aberrant miR-155 might be correlated with the pathology of preeclampsia via different mechanisms. But overall, it remains unknown whether miR-155 expression was related to ATIR genetic polymorphisms in preeclampsia.

Perspective

Epigenetics is the study of the alteration of gene function without a change in the DNA sequence. Recent evidence from emerging research suggest a possibility that epigenetic dysregulation, especially abnormal DNA methylation, might increase preeclampsia susceptibility (Chelbi & Vaiman 2008). Moreover, compared with classic mechanisms for miRNAs regulating gene expression, specific miRNAs called epi-miRNAs target effectors of such epigenetic machinery as DNA methyltransferase, thereby influencing gene expression controlled by epigenetic factors, rather than through mRNA degradation and mRNA translational inhibition (Chelbi et al. 2007). A recent study suggested that altered global DNA methylation patterns in placenta were found to be associated with maternal hypertension in preeclampsia (Kulkarni et al. 2011). Early research among patients with preeclampsia or intrauterine growth restriction (IUGR) suggested that much more gene-specific hypomethylation like TIMP3 – a gene involved in cell growth, invasion, migration, transformation – was found in early-onset preeclamptic placentas (Yuen et al. 2010). More interestingly, alterations in promoter DNA methylation of genes of RAAS were observed in the fetal programming of hypertension in animal models (Bogdarina et al. 2007, Goyal et al. 2010). For example, the AT1b receptor gene under methylation in the adrenal gland results in higher expression of the AT1b receptor and increased adrenal angiotensin responsiveness in the maternal low-protein diet rat model of programming (Bogdarina et al. 2007).

In addition, human ACE expression level was found to be under a strong DNA methylation characterized by cell-type-specific and tissue-specific regulations (Rivière et al. 2011). Moreover, it has been substantiated that several miRNAs, such as miR-130a, miR-181a, miR-222, and miR-220, seem prevalent in the placenta and serum of severe preeclamptic women (Bogdarina et al. 2007, Pineles et al. 2007, Zhu et al. 2009). Further investigations are required to decipher the precise binding sites of aberrant miRNAs to targeted genes. Nevertheless, few direct evidences have been found to identify the possibility of miRNA targeting the sites of RAAS gene mRNA. Given that preeclampsia is often accompanied by IUGR and shares similar both maternal and fetal pathology with administration of a low-protein diet during pregnancy, epi-miRNA may provide a feasible mechanism to explain the abnormal DNA methylation and miRNA expression level in preeclampsia.

Summary

RAAS is the primary system regulating blood fluids and salt and water balance. During normal pregnancy, all components of RAAS show compensatory and protective alterations. But overall, the balance was broken in preeclampsia. Various studies in different populations have identified both maternal and fetal polymorphisms associated with preeclampsia. Furthermore, at a post-transcriptional level, such miRNA as miR-155 might participate in pathology of preeclampsia via binding to the targeted sites of genes and regulating RAAS expression. However, controversial results hinder research from a definitive conclusion on the influence of genetic polymorphisms due to variations in ethnic population, genetic basis, genetic misclassification, preeclampsia criteria, sample sizes, detective methods, and so on. In the future, meta-analyses, especially cumulative meta-analyses, may
give a more determined perspective of underlying effects. Of all the limitations, small sample sizes encumber researchers most regarding deeper statistical analyses based on the principles of ratios of the amount of parameters to the subject sizes. In summary, many promising findings were reported about the interactions with genetic polymorphisms and RAAS alterations in preeclampsia but remain to be replicated.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding
This work was supported by the grants from the Natural Sciences Foundation of China (NSFC) 81070263, KZ201110025023 from Science and Technology Plan Project of Beijing Municipal Education Commission.

References
AbdAlla S, Lother H, el Massiery A & Quitterer U 2001 Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness. Nature Medicine 7 1003–1009. (doi:10.1038/nm0901-1003)
Davies E, Holloway CD, Ingram MC, Inglis GC, Friel EC, Morrison C, Anderson NH, Fraser R & Connell JM 1999 Aldosterone extraction rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene CYP11B2. Hypertension 33 703–707. (doi:10.1161/01.HYP.33.3.703)


Iwai N, Ohmichi N, Nakamura Y & Kinoshita M 1994 DD genotype of the angiotensin-converting enzyme gene is a risk factor for left ventricular hypertrophy. Circulation 90 2622–2628. (doi:10.1161/01.CIR.90.6.2622)


Ramirez-Salazar M, Romero-Gutierrez G, Zaina S, Malacara JM, Kornhauser C & Perez-Luque E 2011 Relationship of aldosterone synthase gene (C-344T) and mineralocorticoid receptor (S810L) polymorphisms with gestational

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P & Soulby F 1990
An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. Journal of Clinical Investigation 86 1343–1346. (doi:10.1172/JCI114844)


Roberts JM & Gammill HS 2005 Preeclampsia: recent insights. Hypertension 46 1243–1249. (doi:10.1161/01.HYP.0000188408.49896.c5)


Salimi S, Mokhtari M, Yaghmaei M, Jamshidi M & Naghavi A 2011


Shah AP, Sultana A, Bammidi VK, Sampathiaro K & Jamil KA 2011


Valdes G, Neves LAA, Anton L, Corthorn J, Chacon C, Germain AM, Merrill DC, Ferrario CM, Sarao R, Penninger J et al. 2006 Distribution of angiotensin-(1–7) and ACE2 in human placenta of normal...


Received in final form 20 January 2013
Accepted 31 January 2013
Accepted Preprint published online 31 January 2013