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JB Review

Pregnancy-associated homeostasis and dysregulation: lessons from genetically modified animal models

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Physiological alterations occur in many organ systems during pregnancy. These changes are necessary for the adaptation to pregnancy-specific physiological processes in mother and fetus, and the placenta plays a critical role in the maintenance of homeostasis in pregnancy. Dysregulation of these functional feto–maternal interactions leads to severe complications. There have been many attempts to create animal models that mimic the hypertensive disorders of pregnancy, especially pre-eclampsia. In this review, we summarize the physiology of pregnancy and placental function, and discuss the placental gene expression in normal pregnancy. In addition, we assess a number of established animal models focusing on a specific pathogenic mechanism of pre-eclampsia, including genetically modified mouse models involving the renin–angiotensin system. Validation of these animal models would contribute significantly to understanding the basic principles of pregnancy-associated homeostasis and the pathogenesis of pre-eclampsia.

Keywords: Genetically modified animal models/ pregnancy-associated homeostasis/placenta/ pre-eclampsia/renin–angiotensin system.

Pregnancy is a normal physiological process that requires the synchronized adaptation of multiple organ systems. Pregnancy dramatically alters energy balance, osmoregulation, and the metabolism of carbohydrates, amino acids, lipids, nutrients, vitamins and glucocorticoids in order to maintain maternal and fetal homeostasis. Dysregulation of these homeostatic controls during pregnancy leads to serious disorders.

Each year, 536,000 women die from complications of pregnancy and childbirth (*1*). Hypertensive disorders are the most common complications in pregnancy. Pre-eclampsia is defined as the presence of hypertension and proteinuria during the second or third trimester. Mortality rates in mothers with pre-eclampsia are as high as 30%, and perinatal mortality is between 6.6% and 60% (*2–4*). Although the molecular basis for pre-eclampsia is still undetermined, several factors considered to be possible causes of its pathogenesis have recently been reported. This review summarizes the current understanding of the physiology of homeostatic regulation and the functional changes in gene expression in the placenta during normal pregnancy. In addition, recent findings pertaining to pathogenic factors in pre-eclampsia based on genetically modified animal models are discussed.

Homeostasis of pregnancy

Physiology of pregnancy and placental function

Pregnancy is defined as the period between fertilization and delivery. A variety of physiological changes take place in a woman's body during pregnancy, most of which are reversed after birth.

The amount of circulating blood in the mother during pregnancy increases significantly; in the late pregnancy it is 40–45% higher than that prior to pregnancy (*5*, *6*). With regard to cardiac function, heart rate and cardiac output increase, but maternal blood pressure is not elevated because of decreased resistance in systemic blood vessels caused by desensitization of the vascular wall to vasoconstrictors such as angiotensin II (Ang II). In the respiratory system, tidal volume and minute ventilation increase as the pregnancy progresses. In combination with elevations in circulating haemoglobin and cardiac output, the mother is able to get enough oxygen corresponding to the increased requirement by the pregnancy.

Maternal metabolism is marked by a slight decrease in fasting blood glucose, post-prandial hyperglycaemia, hyperinsulinaemia and mild insulin resistance (*7*). The insulin resistance in the maternal peripheral tissues facilitates the supply of glucose to the embryo (*8*). Additionally, as placental lactogen (hPL) increases with progression of pregnancy and its growth hormone-like action elevates the levels of free fatty acids in blood (*9*), the energy source of the mother shifts from glucose to lipids.

The placenta is a temporary organ required for the development of the fetus. During early pregnancy, the placenta synthesizes glycogen, cholesterol and fatty acids, which serve as sources of nutrients and energy for the fetus. Oxygen and nutrients are transferred from mother to fetus across the very large surface area of the placenta. The fetus is also provided with

passive immunity against certain diseases through antibodies received from the mother through the placenta. The placenta actively synthesizes hormones, and the syncytiotrophoblast, which produces both protein and steroid hormones including chorionic gonadotropin (hCG), oestrogens and progesterone, hPL, placental growth hormone, chorionic thyrotropin (hCT), chorionic adrenocorticotropin (hACTH) and relaxin, is an important endocrine organ for mother and fetus. The placental barrier creates a state of immunotolerance, ensuring that the mother's immune system does not target the fetus as a foreign body. Although the placenta's functions are well-known, their molecular basis is poorly understood.

Regulation of gene expression needed to maintain homeostasis during pregnancy

It is widely accepted that appropriate placental development, combined with environmental factors, plays a major role in the maintenance of normal pregnancies. In this review, we used full genome expression profiling to characterize the placenta at a molecular level during normal pregnancy and to conduct a search for factors involved in pregnancy-associated homeostasis. This allowed for an unbiased search for factors that are up- or down-regulated during the development of normal placentas. We compared expression levels of over 30,000 genes in the placentas of normal mouse sibling beginning at embryonic day 13 (E13) and continuing to E16 and E19, just prior to initiation of labour. Quality control-passed values were compared to those of E13 placenta over the array.

We found significantly down-regulated expression of a number of metabolism-related genes and up-regulated expression of several vascular remodelling-related genes (Fig. 1A). Relative to those from E13, samples from E16 and E19 showed decreased mRNA levels of the genes important for amino acid and lipid metabolism, such as Maob (monoamine oxidase B), Ehhadh (enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase) and Aldh1b1 (aldehyde dehydrogenase 1) genes. In addition, gene expression of the key enzymes for glucose metabolism, such as *Pfkm* (phosphofructokinase), *Pgk1* (phosphoglycerate kinase 1) and *Aldob* (aldolase 2, B isoform), was also attenuated in samples from E16 and E19. On the other hand, tissues from E16 and E19 showed elevated expression of the genes responsible for the vascular remodelling necessary for the normal development of the placenta, such as *MMP3* (matrix metallopeptidase 3), *MMP12*, *Adamts5* (a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 5), *Adamts9*, *TIMP3* (tissue inhibitor of metalloproteinase 3), *Angpt1* (angiopoietin 1), *Amot* (angiomotin), *VEGF-A* (vascular endothelial growth factor A) and *Kdr* (kinase insert domain protein receptor). Furthermore, relative to those from E13, samples from E16 and E19 showed increased mRNA levels of IL-1β (interleukin 1-β), M-CSF (macrophage colony-stimulating factor), GM-CSF (granulocyte macrophage colony-stimulating factor), CCL3 (chemokine ligand 3), CCL4 (chemokine ligand 4) and CXCL10 (chemokine ligand 10), which were recently recognized as vascular remodelling factors in addition to having cytokine and chemokine properties (*10–13*). These results suggest that in the normal placenta, amino acid, lipid and glucose metabolism was elevated for placental formation at E13, and then blood vessel remodelling was actively reinforced to ensure that the amount of circulating placental blood was sufficient to meet the embryo's oxygen requirements (Fig. 1B).

With regard to the cell-mediated responses of the immune system, the expression of IL-1β and M-CSF, both of which contribute to macrophage proliferation, were increased in the placenta at E16 compared with E13. The expression of *CD44* (CD44 antigen), *Lilrb3* [leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3] and *Fcgr2B* (Fc receptor, IgG, low affinity IIb), all genes expressed in B cells, was also elevated in the placenta at E16 and E19. The expression of T-cell-related genes, such as *CD3* (CD3 antigen, gamma polypeptide), *CCL3*

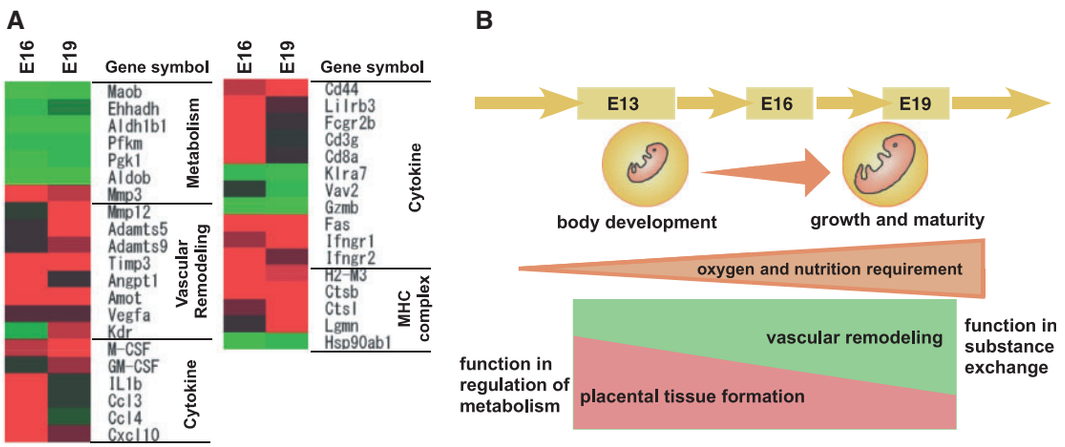


Fig. 1 Gene expression profiles in the placentas of WT and PAH mice. (A) The heatmap reflects normalized gene expression ratios for each mouse. The highest expression is indicated by bright red and the lowest expression by bright green. (B) Schematic of placental function in normal pregnancy. The gene expression profile in the normal placenta suggests that metabolism-related genes are activated for placental formation at E13, and then genes related to blood vessel remodelling are expressed more highly to address the oxygen requirements of the embryo.

[chemokine (C–C motif) ligand 3], *CCL4* [chemokine (C–C motif) ligand 4], *CXCL10* [chemokine (C–X–C motif) ligand 10], and *CD8* (CD8 antigen, α chain), were activated in the placenta at E16. The expression of two genes related to natural killer (NK) cells, *Klra7* (killer cell lectin-like receptor, subfamily A, member 7), *Vav2* (Vav2 oncogene) and *Gzm B* (granzyme B), was suppressed in the placenta at E16 and E19. On the other hand, the expression of *Fas* (tumor necrosis factor) receptor superfamily member, *Ifngr1* (interferon-γ receptor 1) and *Ifngr2* (interferon-γ receptor 2) was activated in the placenta at E16 and E19. In addition, the expression of *H2-M3* (histocompatibility 2, M region locus 3), *Ctsb* (cathepsin B), *Ctsl* (cathepsin L) and *Lgmn* (legumain), which are important for the formation of the major histocompatibility complex complex, was elevated at E16 and E19, while that of *Hsp90ab1* (heat shock protein 90 kDa α (cytosolic), class B member 1) was decreased.

Although the embryo is a foreign body from the perspective of the mother's immune system, it is able to grow until birth without being rejected. In recent years, it has become clear that the interactions between the immune systems of the embryo and the mother in the deciduous membrane of the placenta positively contribute to the maintenance of pregnancy. That is, the mechanism underlying the immunotolerance of the embryo is responsible for pregnancy-associated homeostasis. Microarray results have suggested that innate immunity is activated in the placenta at E13 and continues to E16. It has also been shown that acquired immunity, consisting mainly of antibody production in the placenta, was activated toward E19, because activation of NK cells and cytotoxic T cells and increased numbers of T cells and B cells were observed in the placental gene expression profile. These findings suggest that the switching mechanism of placental immune response from innate immunity to acquired immunity greatly contributes to the maintenance of a normal pregnancy (Fig. 1A).

Pregnancy-related diseases result from the failure of pregnancy homeostasis

Hypertension during pregnancy is one of the most serious risks to both maternal and neonatal health. In normal pregnancy, cardiac output is elevated as a result of increased circulatory blood flow and decreased peripheral vascular resistance (*14*). Because of the dilation of maternal vessels, blood pressure is slightly lowered (*15*), and this permits the maternal fluid expansion that plays an important role in the prevention of placental hypoperfusion (*16*). Dysfunction of these cardiovascular regulatory mechanisms during gestation leads to elevated blood pressure in the mother as well as development of pregnancy-related disorders such as pre-eclampsia. Pre-eclampsia is defined as the presence of maternal hypertension and proteinuria in the second or third trimester of pregnancy. In pre-eclamptic patients, plasma volume is significantly decreased, which results in reduced systemic perfusion. Reduction of the systemic fluid perfusion is a risk factor that potentially correlates with damage to both maternal and fetal organs (*17*). In addition, it has been reported that the vascular sensitivity for vasoactive substances is augmented in pre-eclamptic patients (*18*), but the precise mechanism of this hypersensitivity is still unclear.

Normal placental development is an additional factor contributing to sustained pregnancy homeostasis. During placental development in humans, cytotrophoblasts differentiate into invasive trophoblasts, which consist of multinuclear syncytiotrophoblasts and extravillous trophoblasts (*19*, *20*). It is well-known that extravillous trophoblasts invade uterine endometrium and also inner myometrium. Invasive trophoblasts interact with the uterine vasculature and undergo endothelial-like specialization to replace the smooth muscle layer of spiral arteries. This process, called 'epithelial–endothelial transformation', allows the vessels to acquire resistance to vasoactive substances, independent from maternal blood pressure regulation. In pre-eclampsia, it has been shown that trophoblast invasion is shallow and the remodelling of the spiral arteries is inadequate (*21*). As a consequence, the placental vessels have high resistance and are poorly dilative, leading to an insufficient blood supply to the embryo.

Low blood flow in the placenta is a fundamental factor in the pathogenesis of pre-eclampsia (*22–24*). It has been presumed that abnormal implantation at the onset of pregnancy is a major risk factor for decreased placental circulation and placental hypoxia (*21*, *25*). However, in pre-eclampsia, the molecular basis for placental hypoperfusion is not yet clear. It is thought that a large number of circulatory substances originate in the placenta as a result of reduced placental perfusion, and these cause the maternal features of pre-eclampsia.

A wide variety of angiogenic molecules play critical roles in placentation and the development of the placental circulatory system. During pregnancy, VEGF, fibroblast growth factor (FGF) and placenta growth factor (PlGF) are essential (*26*, *27*). Excessive soluble-fms–like tyrosine kinase-1 (sVEGFR-1/sFlt-1), an endogenous inhibitor of VEGF and PlGF, causes widespread endothelial cell (EC) dysfunction by interfering with the physiological effects of VEGF and PlGF (*28*, *29*). sFlt-1 has also been identified as an important serum marker of pregnancy-induced hypertension (PIH) (*30*). In PIH patients, overabundant sFlt-1 seems to be released from the placenta into the maternal circulation (*31*). In addition to sFlt-1, pre-eclamptic patients show increased serum levels of soluble endoglin (sEng/sCD105), especially in severe cases that constitute the HELLP (Haemolysis, Elevated Liver enzyme, Low Platelets) syndrome (*32*). CD105 is a cell surface co-receptor for transforming growth factor-β1 (TGF-β1) and TGF-β3, and is expressed in ECs and syncytiotrophoblasts. In the vasculature, CD105 is thought to regulate the expression of endothelial nitric oxide synthase (eNOS) and affect eNOS-dependent vascular tonus (*33*, *34*). It is also suggested that sEng inhibits TGF-β1 signalling in the vasculature in pre-eclamptic condition (*32*).

It has been suggested that the maternal immune response to the placenta and fetus plays a significant role in the pathogenesis of pre-eclampsia. Maternal

immune cells, mostly NK cells, interact with trophoblasts that invade into the maternal decidua. In normal pregnancy, the NK cell count decreases towards parturition. In contrast, in pre-eclamptic decidua, NK cells remain active (*35*). It is believed that in pre-eclampsia, activated NK cells are important for the predominant Th1-type inflammatory response. NK cell-derived Th1 cytokines, such as tumour necrosis factor-α and interferon-γ, may inhibit trophoblast invasion and play a significant role in the pathogenesis of pre-eclampsia through the reinforcement of systemic inflammation with endothelial damage (*36*).

Animal models of pre-eclampsia

Although pre-eclampsia is widely recognized as a serious disorder of pregnancy, its pathogenesis has not been yet clarified. On the other hand, several hereditary and genetically modified animal models have been used for studying PIH and pre-eclampsia.

Hereditary animal models of pre-eclampsia

Davisson *et al.* (*37*) reported that BPH/5 mice, an inbred mouse strain with moderately elevated blood pressure, developed a pregnancy-induced hypertensive syndrome. Pregnant BPH/5 mice showed a further rise in blood pressure in the third trimester compared with non-pregnant mice, and also exhibited late-gestational proteinuria and progressive glomerulosclerosis. It has also been reported that the abnormalities in pregnant BPH/5 mice were prevented by the chronic administration of a membrane-permeable radical scavenger, tempol (*38*).

In another study, Sharkey *et al.* (*39*) reported that the SHHF/Mcc-fa(cp) (spontaneous hypertension and heart failure) rat exhibits both spontaneous pregnancy-related hypertension and small offspring size. SHHF rat placentas also showed altered expression of several genes that have been implicated in pre-eclampsia, including serotonin receptor, sodium channel, oestrogen receptor regulator, carbonic anhydrase, superoxide dismutase, major histocompatibility complex proteins and angiotensiogen.

Genetically modified mouse models of pre-eclampsia

By using gene knockout techniques, Kanayama *et al.* (*40*) demonstrated that $p57^{Kip2}$, a potent inhibitor of several cyclin/cyclin-dependent kinase complexes, was partially responsible for symptoms similar to those of pre-eclampsia. During late pregnancy, pregnant $p57^{+/-}$ (heterozygotes for $p57^{Kip2}$) female mice that were mated with $p57^{+/-}$ males showed hypertension, proteinuria, thrombocytopenia, decreased antithrombin III activity and increased endothelin levels. The pregnant $p57^{+/-}$ mice had conceptuses both with and without $p57^{Kip2}$ expression. Placentas without $p57^{Kip2}$ expression showed trophoblastic hyperplasia, which mimics the notable characteristic proliferation of intermediate trophoblasts in pre-eclampsia, suggesting that these pre-eclamptic abnormalities in pregnant $p57^{+/-}$ mice were of placental origin.

In another work, eNOS-deficient mice showed sustained hypertension in the non-pregnant state, and the blood pressure of pregnant eNOS-deficient mice further increased in the third trimester (*41*). In addition, Chan *et al.* (*42*) demonstrated that mice deficient for Corin, a cardiac transmembrane serine protease that has been shown to process pro-atrial natriuretic peptide (pro-ANP) to active ANP, had spontaneous hypertension as compared with wild-type (WT) mice, and pregnant Corin-deficient mice demonstrated late-gestation proteinuria and elevated blood pressure during pregnancy. These studies suggest the importance of endothelial nitric oxide production in the regulation of blood pressure during pregnancy.

Catechol-*O*-methyltransferase (COMT) is a catabolic enzyme that plays a role in the degradation of catecholamines and catecholestrogens. Oestradiol-derived 17-hydroxyoestradiol, a substrate for COMT, converts 17-hydroxyoestradiol into 2-methoxyoestradiol (2-ME); this is the rate-limiting step in oestrogen catabolization. Additionally, 2-ME inhibits hypoxia-inducible factor (HIF)-1α, which serves as an angiogenic factor for trophoblasts. Kanasaki *et al.* (*43*) reported that pregnant mice deficient in COMT ($Com^{-/-}$) showed a pre-eclampsia-like phenotype resulting from an absence of 2-ME, which is elevated during the third trimester of normal human pregnancy. $Com^{-/-}$ pregnant mice exhibited hypertension, placental hypoxia, HIF-1α expression and sFLT-1 elevation in late pregnancy, and exogenous 2-ME ameliorated all abnormal features without toxicity. In addition, the levels of COMT and 2-ME are significantly lower in women with severe pre-eclampsia, suggesting the significance of 2-ME in the pathophysiology of pre-eclampsia.

Investigating both newly generated animal models of pre-eclampsia as well as those mentioned above should help to clarify the condition's pathogenesis, and also opportunities to test preventative and therapeutic strategies in the management of pre-eclampsia and related hypertensive disorders of pregnancy.

Renin–angiotensin system in the pathogenesis of pre-eclampsia

Renin–angiotensin system

The renin–angiotensin system (RAS) is a major determinant of blood pressure and sodium balance in both the pregnant and non-pregnant states (*44*, *45*). The reaction between renin as an aspartyl protease and its substrate, angiotensinogen, is the initial and rate-limiting step in the enzymatic cascade that generates the decapeptide angiotensin I. Angiotensin I is further processed to the potent octapeptide vasopressor angiotensin II (Ang II) by angiotensin-converting enzyme (ACE) (*46*). The actions of Ang II are mediated by several types of receptors expressed in a variety of target tissues. Two Ang II receptors have been identified in humans: AT1 (type 1) and AT2 (type 2). Rodents, on the other hand, express Ang II type 1a (AT1a) and Ang II type 1b (AT1b). In rodents, blood pressure and pathophysiological effects of RAS are primarily mediated by the AT1a receptor (*47*) (Fig. 2A). It has been shown that AT1 signalling accelerates atherosclerosis, cardiac hypertrophy and

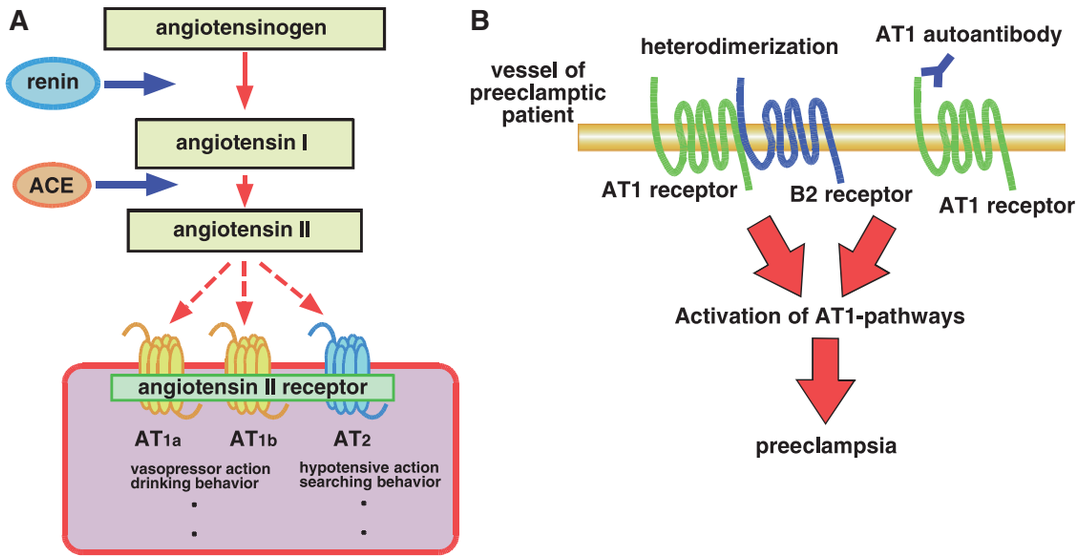


Fig. 2 Schematic representation of the RAS. (A) Angiotensinogen is a unique substrate of the enzyme renin, which generates angiotensin I. Angiotensin I is subsequently converted to angiotensin II by ACE. Angiotensin II stimulates a wide variety of physiological responses, including vasopressor action and drinking behaviour, through its specific receptors (AT1a, AT1b, AT2). (B) The putative mechanisms of angiotensin II hypersensitivity in pre-eclampsia. Increased heterodimerization of AT1 and the bradykinin B2 receptor has been implicated in increased responsiveness to angiotensin II in pre-eclampsia. In addition, agonistic AT1 autoantibody-mediated AT1 receptor activation leads to increased intracellular signal transduction in pre-eclampsia.

nephrosclerosis (*48*). In normal pregnancy, both tissue and circulating levels of angiotensinogen and renin are increased. Consequently, plasma levels of Ang II are increased in association with the elevation of angiotensinogen levels and plasma renin activity during pregnancy (*49*, *50*). It is also known that normal pregnant women are resistant to the pressor effects of Ang II (*18*, *51*, *52*), and they remain normotensive despite a 2-fold increase in Ang II. On the other hand, it is widely accepted that vascular sensitivity to Ang II is elevated in pre-eclamptic women (*18*).

Pregnancy-associated hypertensive mice

We previously reported that mice generated by cross-mating human angiotensinogen transgenic ($hAG^{+/+}$) female mice with human renin transgenic ($hRN^{+/+}$) male mice showed hypertension in late pregnancy due to an overactive RAS, and named them pregnancy-associated hypertensive (PAH) mice (Fig. 3B) (*53*). In PAH mice, maternal blood pressure began rising at 13 days of gestation (E13) and continued until delivery (E19–20). Systolic blood pressure at E19 in PAH mothers reached 160 mmHg, whereas that in normal pregnant mice remained ~100 mmHg. Blood pressure returned to normal levels by 3 days after delivery. This elevation was attributable to the generation of excessive angiotensin I, a precursor of Ang II, as a result of hRN secretion from the feto–placental side (*53*). Other phenotypes, such as proteinuria, cardiac hypertrophy, delayed delivery and often convulsions were also found in PAH mice. Both biological and physiological data demonstrated that RAS-mediated maternal hypertension beginning in the second half of gestation caused PAH mothers to develop pathological conditions that satisfied the criteria of PIH.

PAH mice also exhibited fetal defects. The fetuses from PAH pregnancies showed severe intrauterine growth retardation (IUGR) (Fig. 4A) with placental morphological changes. The mean body weight of PAH fetuses at E19 was about 62% of that seen in fetuses from normal pregnant mothers (WT) (Fig. 4B) (*54–56*). In addition, PAH fetuses were anaemic, and except for the heart ventricles, their organ sizes were smaller than those in WT mice (*56*). The cardiothoracic ratio (CTR) was significantly higher in PAH fetuses than that WT fetuses (Fig. 4C). Furthermore, in PAH neonates, electrocardiographic analysis revealed that the QRS duration was elevated and S action potential was lowered compared with that in WT neonates (Fig. 4D), suggesting cardiac dysfunction in PAH neonates. These pathologies in PAH fetuses and neonates were probably due to defects in maternal–fetal circulatory exchange induced by impaired fetoplacental vascular maturation and uteroplacental tissue remodelling in the later stages of pregnancy (*56*).

AT1 plays a critical role in the development of PAH

As mentioned above, it is now widely recognized that the vascular sensitivity to Ang II is elevated in pre-eclamptic patients, but the mechanism of elevated Ang II sensitivity in pre-eclampsia has remained an open question for several decades. Although AT1a is thought to be significantly associated with hypertension and tissue damage, it is unknown whether the maternal and foetal phenotypes observed in PAH mice are mediated through AT1a. Therefore, to

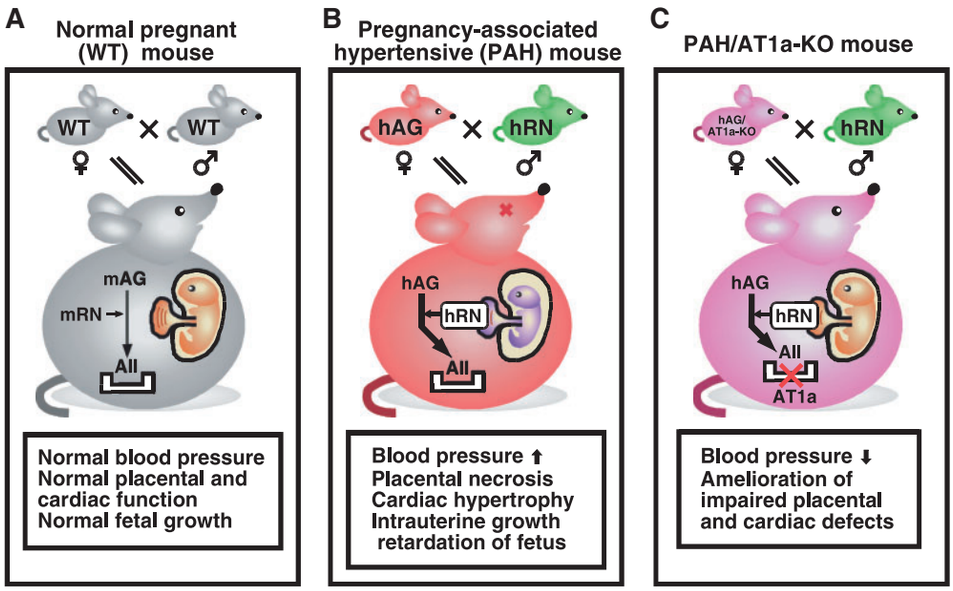
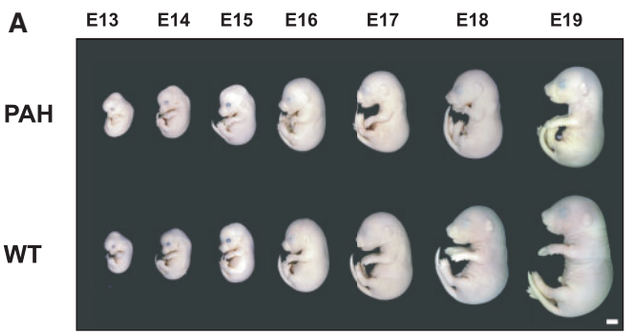
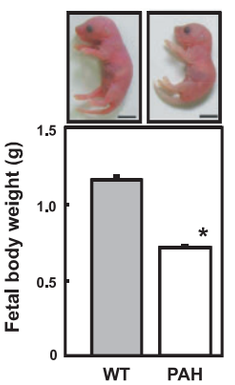
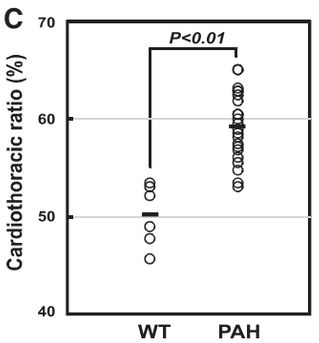


Fig. 3 Generation of PAH and PAH/AT1a-KO mice. (A) Normal pregnancy in WTmice. (B) Generation of PAH mice. Transgenic females expressing human angiotensinogen (hAG) mated with transgenic males expressing hRN. (C) Mating strategy of PAH/AT1a-KO mice. The AT1a gene was removed from hAG transgenic mice by cross-mating with AT1a-deficient mice (hAG/AT1a-KO mice), and then PAH/AT1a-KO mice were generated by mating with hAG/AT1a-KO mice and hRN male mice.







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Parameter	WT	PAH
PR interval (ms)	77 ± 2.1	74 ± 4.5
QRS duration (ms)	11 ± 0.7	14 ± 0.5 *
QT interval (ms)	117 ± 6.1	126 ± 9.3
Q action potential (μV)	-0.1 ± 0.1	-0.4 ± 0.2
R action potential (μV)	29 ± 3.8	30 ± 2.4
S action potential (μV)	-28 ± 2.7	-45 ± 3.2 **

*; p<0.01 v.s. WT, **; p<0.001 v.s. WT

Fig. 4 Pathophysiological analysis in PAH fetuses. (A) A time course comparison of growth among fetuses from WT and PAH mice. Scale bar, 1 mm. (B) Comparison of fetal body weights in each group. Fetuses were obtained from mice on gestation Day E19. Scale bars, 5 mm. Data are means ± SE; $n = 16$–20 fetuses per group. *$P < 0.001$ versus WT mice. (C) Measurement of the fetal CTR of each group. Fetuses were obtained from mice on gestation day E19. Bars indicate the mean value of each group. (D) Analysis of electrocardiograms of fetuses in each group. Fetuses were obtained from mice at E19, and underwent electrocardiographic examination under unaesthetic condition. Data are means ± SE.

clarify the significance of AT1 signalling in PAH, we evaluated the effects of AT1 deficiency in this model (*54*). hAG mothers that lacked AT1a ($hAG^{+/+}/mAT1a^{-/-}$) were generated and mated with hRN males (Fig. 3C). $hAG^{+/+}/mAT1a^{-/-}$ mothers (PAH/AT1a-KO) did not show hypertension during pregnancy and maternal pathologies and fetal IUGR were significantly improved (*54*). These results clearly demonstrated that AT1a greatly contributed to pregnancy-associated hypertension with IUGR.

Table I. Animal models of hypertensive disorders in pregnancy.

Manuscript	Year	Treatment	Molecule	Phathology			
Takimoto *et al.* (*53*)	1996	Transgene	hAG, hRN	Hypertension	Proteinuria	Tissue damage	
Saito *et al.* (*54*)	2004	Transgene	hAG, hRN	Hypertension	Proteinuria	Tissue damage	IUGR
Takimoto-Ohnishi *et al.* (*55*)	2005	Transgene	hAG, hRN	Hypertension	Proteinuria	Tissue damage	IUGR
Venkatesha *et al.* (*32*)	2006	Adenovirus	sEng, sFlt-1	Hypertension	Proteinuria	Tissue damage	IUGR
Zhou *et al.* (*64*)	2007	Infusion	Ang II	Hypertension			IUGR
Zhou *et al.* (*62*)	2008	Infusion	AT1AA	Hypertension	Proteinuria	Tissue damage	IUGR
Kumasawa *et al.* (*63*)	2011	Lentivirus	sFlt-1	Hypertension	Proteinuria	Tissue damage	IUGR

sEng, soluble endoglin; AT1AA, agonistic autoimmune antibodies against AT1; IUGR, intrauterine growth retardation.

In humans, although the aetiology and pathology of PIH remain enigmatic, it is thought that aberrantly activated AT1 signalling is one of the major causes of increased Ang II sensitivity, which in turn induces pre-eclamptic pathogenesis. For example, AT1 is up-regulated in the placentas of pre-eclamptic patients (*57*); enhanced expression of AT1 is observed in the placentas of rats with endotoxin-induced hypertension (*58*); and a genetic polymorphism, A1166C, in AT1 is associated with an increased risk of pre-eclampsia (*59*). Recently, AbdAlla *et al.* (*60*) reported that the protein levels of the bradykinin B2 receptor increased in pre-eclamptic patients and that the heterodimerization of AT1–bradykinin B2 accelerated GPCR signal transduction. On the other hand, Wallukat *et al.* (*61*) reported that agonistic autoimmune antibodies against AT1 (AT1-AA) were detected in the sera of pre-eclamptic women (Fig. 2B). Recently, the relationship between AT1-AA and the pathogenesis of pre-eclampsia was evaluated in animal models. Zhou *et al.* (*62*) showed that the key features of pre-eclampsia, including hypertension, proteinuria, glomerular endotheliosis, placental abnormalities and small fetus size, appeared in pregnant mice after injection with affinity-purified AT1-AA from women with pre-eclampsia. These disease states were prevented by co-injection with an AT1 antagonist or by a neutralizing antibody that recognizes a 7-amino-acid epitope peptide on the second extracellular loop of AT1.

The abnormal balance of vasoactive factors in pre-eclamptic pregnant patients has also been studied. An animal model of pre-eclampsia involving co-administration of sFlt-1 and sEng was reported in the rat (*32*). In another study, Kumasawa *et al.* (*63*) demonstrated the lentiviral vector-mediated placenta-specific expression of sFlt-1 in mice. The model mice showed maternal hypertension, proteinuria and fetal intrauterine growth restriction, and treatment with pravastatin induced VEGF-like angiogenic factor placental growth factor (PGF) and ameliorated the symptoms. Excess production of circulating anti-angiogenic proteins, such as sFlt-1 and sEng, is emerging as a prominent component in the onset of endothelial damage, which is thought to play a pivotal role in the pathogenesis of pre-eclampsia. Interestingly, PAH mice were shown to have significantly elevated plasma levels of sFlt-1 at E19 (*56*). This was consistent with another study's finding of significantly increased circulating levels of sFlt-1 induced by the infusion of Ang II in pregnant mice (*64*). In addition, Zhou *et al.* (*65*) showed that the inhibition of AT1 signalling by administration of AT1 antagonist or FK506 resulted in reduced sFlt-1. Taken together, it appears likely that maternal anti-angiogenic factors can be elevated by AT1 activation in which Ang II, AT1-AA and AT1-bradykinin B2 heterodimers are potentially implicated.

Pre-eclampsia is an AT1 receptor–mediated signal transduction disease

At present, the molecular basis for the pathogenesis of pre-eclampsia is not fully understood, but the pathophysiological importance of the RAS and its functional mediator, the AT1 receptor, have been assessed as mentioned above (Table I). Mechanisms underlying the enhanced AT1-mediated signalling in pre-eclamptic pathology and its deteriorative effects, including up-regulation of anti-angiogenic factors, on the development of disease were evaluated in various animal models. Very recent study has reported a novel mechanism of enhanced angiotensin production that may lead to enhanced AT1 signalling in pre-eclampsia. Zhou *et al.* (*66*) demonstrated that the oxidized form of angiotensinogen, which preferentially interacts with receptor-bound renin and will more effectively release angiotensin, was more abundantly present in the maternal circulation in pre-eclamptic patients than that in those with normal pregnancies. Given all of the above evidence, pre-eclampsia can be said to be an AT1-mediated signal transduction disease.

In conclusion, more work is required to better understand the molecular basis for the maintenance of normal pregnancy and the development of pre-eclampsia. Pathophysiological studies using genetically modified animal models would contribute tremendously to deciphering the mechanisms of homeostasis in pregnancy and the pathogenesis of this pregnancy-associated signal transduction disorder.

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