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Role of Corin in Trophoblast Invasion and Uterine Spiral Artery Remodeling in Pregnancy

Yujie Cui[1,*], **Wei Wang**[1,*], **Ningzheng Dong**[2,3,*], **Jinglei Lou**[1,*], **Dinesh Kumar Srinivasan**[1], **Weiwei Cheng**[4], **Xiaoyi Huang**[4], **Meng Liu**[2], **Chaodong Fang**[2], **Jianhao Peng**[1], **Shenghan Chen**[1], **Shannon Wu**[1], **Zhenzhen Liu**[2], **Liang Dong**[2], **Yiqing Zhou**[2], and **Qingyu Wu**[1,2]

[1]Molecular Cardiology, Nephrology and Hypertension, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA

[2]Cyrus Tang Hematology Center, Jiangsu Institute of Hematology, the First Affiliated Hospital, Soochow University, Suzhou, China

[3]Key Lab of Thrombosis and Hemostasis, Jiangsu Institute of Hematology, the First Affiliated Hospital, Soochow University, Suzhou, China

[4]The International Peace Maternity and Child Health Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Summary

In pregnancy, trophoblast invasion and uterine spiral artery remodeling are important for lowering maternal vascular resistance and increasing uteroplacental blood flow. Impaired spiral artery remodeling has long been implicated in preeclampsia, a major complication of pregnancy, but the underlying mechanisms remain unclear[1, 2]. Corin is a cardiac protease that activates atrial natriuretic peptide (ANP), a cardiac hormone important in regulating blood pressure[3]. Unexpectedly, corin expression was detected in the pregnant uterus[4]. Here we identify a novel function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling. We show that pregnant corin- or ANP-deficient mice developed high blood pressure and proteinuria, characteristics of preeclampsia. In these mice, trophoblast invasion and uterine spiral artery remodeling were markedly impaired. Consistently, we find that ANP potently stimulated human trophoblasts in invading Matrigels. In patients with preeclampsia, uterine corin mRNA and protein levels were significantly lower than that in normal pregnancies. Moreover, we have identified corin gene mutations in preeclamptic patients, which decreased corin activity in processing pro-ANP. These results indicate that corin and ANP are essential for physiological changes at the maternal-fetal interface, suggesting that defects in corin and ANP function may contribute to preeclampsia.

Pregnancy poses a serious challenge for maintaining normal blood pressure. Pregnancy-induced hypertension, a major cause of maternal and fetal deaths, occurs in ~10% of pregnancies[5, 6]. During pregnancy, the uterus undergoes profound morphological changes,

Correspondence should be addressed to Q.W. (wuq@ccf.org).
*These authors contributed equally to this work.

AUTHOR CONTRIBUTIONS

Y.C., W.W., N.D., J.L., D.K.S., M.L., C.F., J.P., S.C., S.W., Z.L. and L.D. designed and performed experiments. N.D., W.C. and X.H. collected patient samples and analyzed clinical data. Q.W. conceived the study and designed experiments. Y.Z. and Q.W. wrote the manuscript. All authors analyzed and interpreted data, and critically read the manuscript.

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COMPETING FINANCIAL INTERESTS

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including trophoblast invasion and spiral artery remodeling. In preeclampsia, impaired spiral artery remodeling is common, but the underlying mechanisms are unclear[1, 2, 7-9]. Studies indicate that vascular growth factor receptors, angiotensin and estradiol are involved in the disease[10-14].

Corin is a cardiac protease that activates ANP, a cardiac hormone regulating blood pressure and sodium homeostasis[15]. In mice, lack of corin prevents ANP generation and causes hypertension[16]. In humans, corin variants are associated with hypertension[17]. Interestingly, corin expression was detected in the pregnant mouse[4] (Fig. 1a) and human uterus (Supplementary Fig. 1). As a transmembrane protein, corin is expected to act at the expression sites, suggesting a possible function in the pregnant uterus.

To understand the role of corin in pregnancy, we created a mouse model, in which a *corin* transgene was expressed under a cardiac promoter (Fig. 1b). The transgenic (Tg) and corin knockout (ko) mice were crossed to generate ko/Tg mice expressing corin only in the heart (Fig. 1c, d). In ko/Tg mice, transgenic corin expression restored pro-ANP processing in the heart (Supplementary Fig. 2) and normalized blood pressure (Fig. 1e), indicating that cardiac corin was sufficient to maintain normal blood pressure in non-pregnant (np) mice.

In pregnant corin ko mice, blood pressure increased at ~17 days post-coitus and rose further before returning to the np level after delivery (Fig. 1f), which resembled late gestational hypertension in preeclamptic women. In corin ko/Tg mice, which were normotensive, blood pressure increased similarly during pregnancy (Fig. 1g), indicating that cardiac corin expression did not prevent pregnancy-induced hypertension. The data also show that in these mice hypertension in pregnancy was not due to preexisting high blood pressure. In addition to in the uterus, corin mRNA was detected in the umbilical cord and placenta (Supplementary Fig. 3). To distinguish the role of maternal corin from that of placental or other fetal organs, corin ko females were mated with either wt or ko males. The resulting fetuses carried one or no copy of the functional *corin* gene. Normally, enzymes encoded by one gene copy are sufficient for their function. As shown in Fig. 1h, pregnant corin ko females, mated with either wt or ko males, had similarly increased blood pressure, indicating that lack of maternal, but not fetal, corin caused hypertension in pregnancy.

Proteinuria is a hallmark of preeclampsia. WT, corin ko and ko/Tg mice had similar urinary protein levels before pregnancy and at mid gestation. The levels, however, increased in corin ko and ko/Tg mice at late gestation (Fig. 1i), consistent with reported proteinuria in mouse models of preeclampsia[18]. Ischemic glomeruli, indicated by fewer red blood cells, were found in pregnant corin ko and ko/Tg mice (Fig. 1j, i-vi) but not in np mice (Supplementary Fig. 4). PAS staining revealed increased extracellular matrixes and collapsed glomerular capillaries in pregnant corin ko and ko/Tg mice (Fig. 1j, vii-ix). Electron microscopy showed narrow glomerular capillary lumens and thick basement membranes (Fig. 1k), suggesting endotheliosis and increased extracellular matrixes. Additional pathological features such as necrotic cells and calcium deposits in the placental labyrinth also existed in these mice (Supplementary Fig. 5), indicating insufficient uteroplacental perfusion. Consistently, corin ko and ko/Tg mice had smaller litters (7.1 ± 2.3 (n=28) and 6.8 ± 2.7 (n=28), respectively, *vs.* wt 9.1 ± 1.2 (n=21) pups/litter; p values <0.001).

We examined embryos of E12.5 day, an early time point before blood pressure increase in corin ko and ko/Tg mice, and E18.5 day before delivery. WT E12.5 embryos exhibited obvious trophoblast invasion, shown by cytokeratin (cytok) staining (Fig. 2a), and large vessels mostly in the deep decidua, shown by smooth muscle actin (SMA) staining (Fig. 2b), indicating that smooth muscles in the superficial decidua were replaced by invading trophoblasts. In contrast, trophoblast invasion in corin ko and ko/Tg embryos was markedly

reduced (Fig. 2a) and smaller arteries were found in both superficial and deep decidua (Fig. 2b). In E18.5 wt embryos, more abundant trophoblasts were found in the decidua and myometrium compared with those in corin ko and ko/Tg mice (Fig. 2c, d). By H&E staining, larger and more abundant decidual spiral arteries were observed in wt than corin ko or ko/Tg mice (Fig. 2e). Fig. 2f-h showed strong cytok (trophoblasts) staining but weak von Willebrand factor (vWF) (endothelial) and SMA (smooth muscles) staining in wt decidual and myometrial arteries. These data indicate that trophoblast invasion and spiral artery remodeling were impaired in corin ko and ko/Tg mice and that this defect occurred before blood pressure increased in these mice.

Corin activates ANP in the heart[15]. It was unknown if the corin function in pregnancy also was mediated by ANP. Pro-ANP is expressed in the np and pregnant uterus (Supplementary Fig. 6). If corin acts on pro-ANP to promote trophoblast invasion and spiral artery remodeling, thereby preventing hypertension in pregnancy, ANP and corin ko mice should have similar phenotypes. ANP ko mice are hypertensive (Fig. 3a) but their blood pressure was not monitored during pregnancy[19]. We found similarly increased blood pressure in pregnant ANP ko mice (Fig. 3b). The mice also had late gestational proteinuria (Fig. 3c) and smaller litters (4.4 ± 1.7 (n=25) *vs.* wt 9.1 ± 1.2 (n=21) pups/litter, $p<0.001$). By immunostaining, impaired trophoblast invasion and smaller spiral arteries were observed in E12.5 embryos (Fig. 3d, e). In E18.5 embryos, ANP ko mice had far fewer trophoblasts (Fig. 3f, g) and smaller arteries (Fig. 3h) in the decidua and myometrium than those in wt. Consistently, weak cytok-staining but strong vWF-staining were found in arteries in ANP ko mice (Fig. 3i). Thus, ANP and corin ko mice had very similar phenotypes, indicating that the role of corin in pregnancy is most likely mediated by ANP.

In the heart, corin produces ANP, which in turn regulates blood pressure by promoting natriuresis and vasodilation[3]. Here we found that lack of corin and ANP impaired trophoblast invasion and spiral artery remodeling, which was not rescued by cardiac corin expression in corin ko/Tg mice. ANP is known to relax vascular smooth muscles. Recently, ANP and its downstream cGMP-dependent protein kinase were shown to be important in angiogenic processes by promoting endothelial regeneration[20, 21]. Thus, ANP may function locally to remodel uterine arteries. Our results also suggest that ANP may directly promote trophoblast invasion (Fig. 4a). This hypothesis was tested. We found that ANP markedly stimulated human trophoblasts to invade Matrigels (Fig. 4b) (Supplementary Fig. 7a). In these cells, ANP receptor mRNA expression was confirmed (Supplementary Fig. 7b) and ANP-stimulated intracellular cGMP production was detected (Fig. 4c) (Supplementary Fig. 7c).

Our findings underscore the importance of locally produced ANP by corin, which acts on trophoblasts and vascular cells in the uterus. Because heart-derived ANP circulates inside the vessel, our model may explain why cardiac corin failed to promote trophoblast invasion and uterine artery remodeling, as shown in corin ko/Tg mice. To verify this hypothesis, we quantified corin mRNA and protein in human uteruses by RT-PCR and ELISA. The levels were low in np women but increased in pregnant women (Fig. 4d, e). In preeclamptic women, the levels were significantly lower than in normal pregnancies. Similar results were found by immunostaining (Supplementary Fig. 8). Consistently, pro-ANP levels in uterine tissues were significantly higher in preeclamptic women than normal pregnant women (Fig. 4f), indicating that reduced uterine corin expression impaired pro-ANP processing in these patients. Corin is a membrane-bound protein[4, 15]. Recent studies showed that corin can be shed from cardiomyocytes and that soluble corin was detected in human plasma[22, 23]. We found that plasma corin levels were higher in preeclamptic patients than np or normal pregnant women (Fig. 4g). Thus, plasma corin levels did not reflect that in tissues, indicating that plasma corin was likely derived from the heart, where corin expression was

increased in response to high blood volume and pressure in pregnancy. These results further support a local corin function in the pregnant uterus.

We then sequenced the *CORIN* gene[24] in preeclamptic patients and identified a mutation altering Lys to Glu at position 317 in LDL receptor repeat 2 in one woman (Fig. 4h, j) and another mutation altering Ser to Gly at position 472 in frizzled 2 domain in two women from the same family who had preeclampsia (Fig. 4i, j). In functional studies, K317E and S472G mutations did not affect corin expression in HEK293 cells but markedly reduced corin activity in processing pro-ANP (Fig. 4k-n). The data was consistent with previous findings that LDL receptor repeats and frizzled domains are critical for corin activity[25], suggesting that the mutations may impair corin function in the patients, thereby contributing to preeclampsia. Interestingly, corin variants in frizzled 2 domain that impaired corin function have been reported in African Americans[17, 26], a high risk population for preeclampisa.

Previously, high levels of plasma pro-ANP/ANP were detected in preeclamptic patients[27, 28]. As shown with our plasma soluble corin data, plasma protein levels may not reflect those in tissues. Together, we have identified a novel local function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling to prevent hypertension in pregnancy. Our data suggest that impaired corin expression or function in the pregnant uterus may represent an important mechanism underlying preeclampsia. Studies to further understand impaired uterine corin expression in preeclamptic patients may help to develop new strategies to enhance the corin/ANP pathway to prevent or treat this life-threatening disease.

METHODS SUMMARY

Corin and ANP ko mice were described previously[16, 19]. Tg mice with cardiac corin expression were generated using a heart-specific promoter. Blood pressure was measured by radiotelemetry[16]. Tissue sections from np and pregnant mice were stained with H&E, Masson's trichrome, PAS or von Kossa or immunostained with antibodies against cytok, SMA, vWF or corin. Renal sections were also examined by electron microscopy. Transwell invasion assay was done with human primary villous trophoblasts (ScienCell) and trophoblastic JEG3, BeWo, JAR cell lines (ATCC) in Matrigel Invasion Chambers (BD Biosciences). ANP-stimulated cGMP production in trophoblasts was assayed in 96-well plates. Intracellular cGMP levels were determined using an EIA kit (Enzo Life Sciences). Corin levels in human blood and uterus tissue samples were measured by ELISA[22]. Pro-ANP levels in human uterus tissues were measured by ELISA (Alpco Diagnostics). *CORIN* gene exons[24] from preeclamptic patients were PCR amplified and directly sequenced. Corin gene mutations identified were studied by expressing mutant corin proteins in HEK293 cells and testing their activities in pro-ANP processing assays, as described previously[26].

SUPPLEMENTARY ONLINE METHODS

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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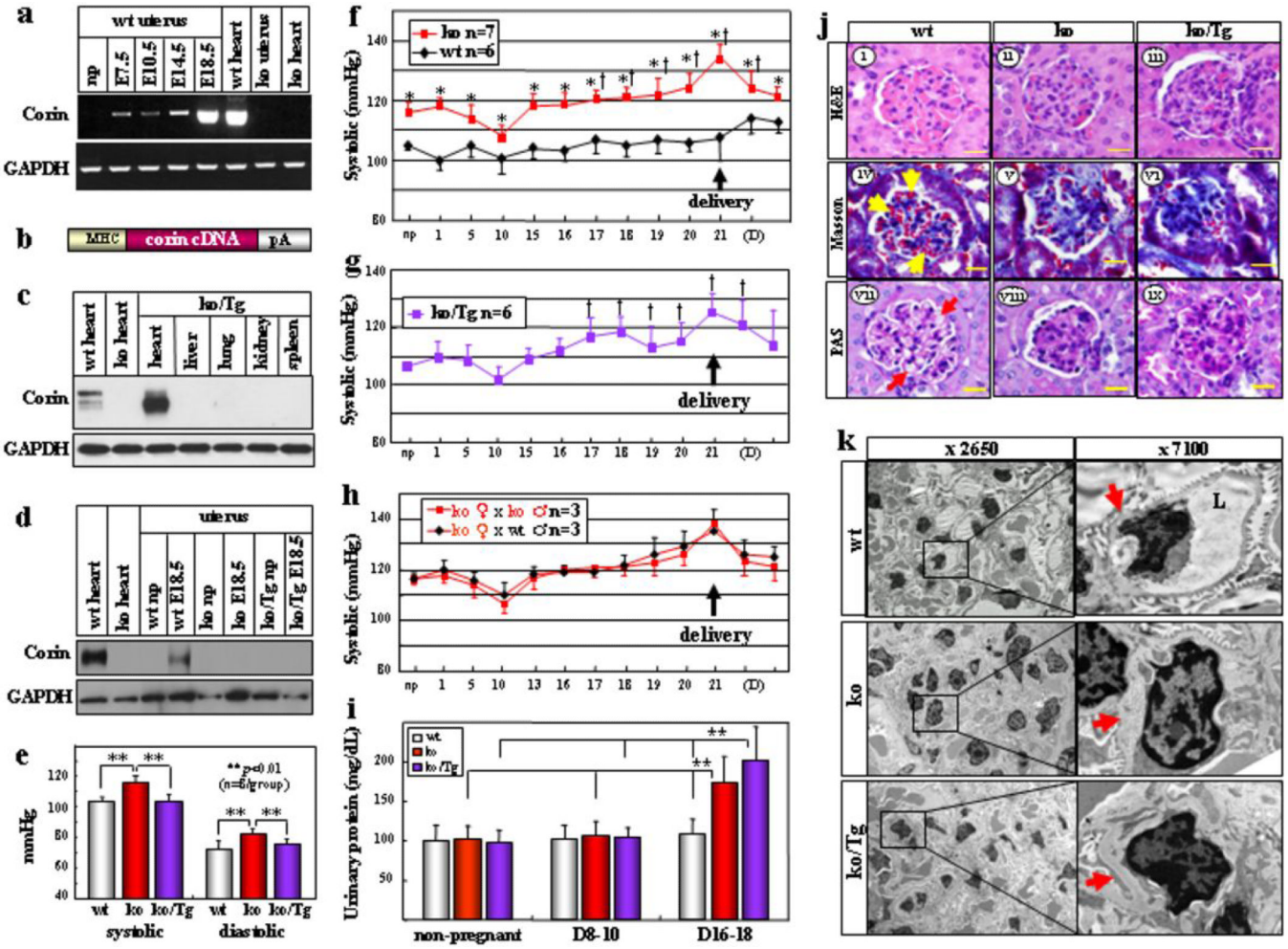

Figure 1. Hypertension, proteinuria and renal pathology in pregnant corin ko and ko/Tg mice

a, Corin mRNA expression in mouse uteruses. **b,** Corin Tg construct. pA: poly A. **c, d**, Western analysis of corin protein in wt, corin ko and ko/Tg mice. **e,** Blood pressure (BP) (mean ± s.d.) in np females. BP increased in corin ko (**f**) and ko/Tg (**g**) mice in pregnancy. Data represent mean ± s.d. **P*<0.05 *vs.* wt of the same time point. †*P*<0.05 *vs.* np level of the same genotype. **h,** Similar BP changes in corin ko females mated with ko or wt males. **i,** Late gestational proteinuria in corin ko and ko/Tg mice. Data represent mean ± s.d. ***P*<0.01, n=7-8 per group. **j,** Renal ischemia in pregnant corin ko and ko/Tg mice, shown in H&E (i-iii), Masson trichrome (iv-vi) or PAS (vii-ix) stained E18.5 sections. bar: 20 μm. Red blood cells (yellow arrows) and open capillaries (red arrows) in wt glumeruli are indicated. **k,** Narrow glomerular capillary lumen (L) and thick basement membranes (red arrows) in corin ko and ko/Tg mice at E18.5 shown by electron microscopy.

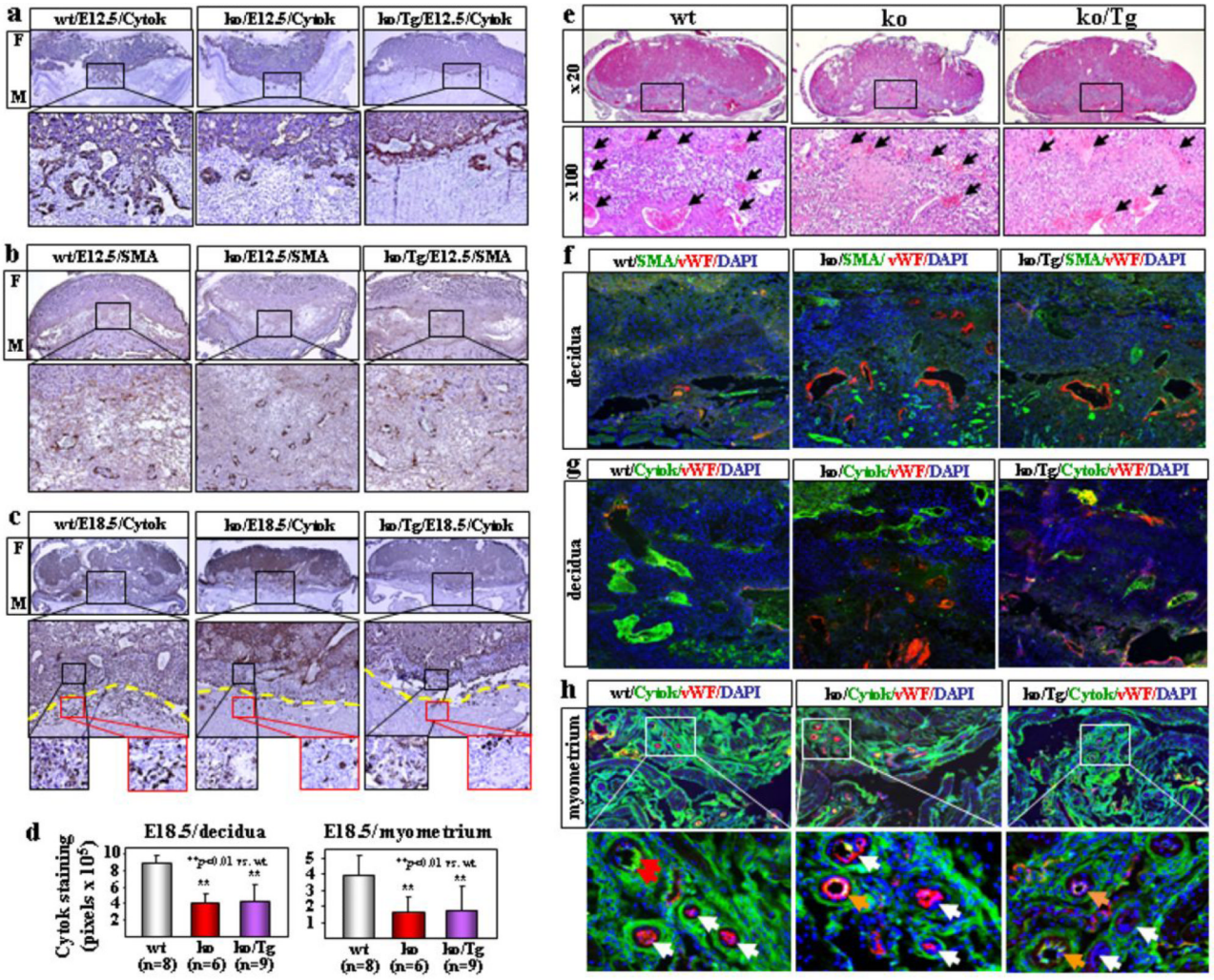

Figure 2. Impaired trophoblast invasion and spiral artery remodeling in corin ko and ko/Tg mice

E12.5 embryo sections were stained for trophoblasts (**a**) or smooth muscles (**b**). Fetal (F) and maternal (M) sides are indicated. Boxed areas in top panels are shown at a higher magnification (x200). **c,** E18.5 embryo sections were stained for trophoblasts. In lower panels (x100), yellow lines indicate the decidua and myometrium boundary. **d**, Quantitative data (mean ± s.d.) of cytok staining. **e,** Fewer and smaller decidual spiral arteries (arrows) in H&E-stained E18.5 corin ko and ko/Tg embryos. **f-h**, Co-staining of SMA, vWF, cytok and nuclei in E18.5 embryos. Red arrows indicate cytok (green) signals, white arrows vWF (red) signals, and orange arrows mixed (yellow) signals.

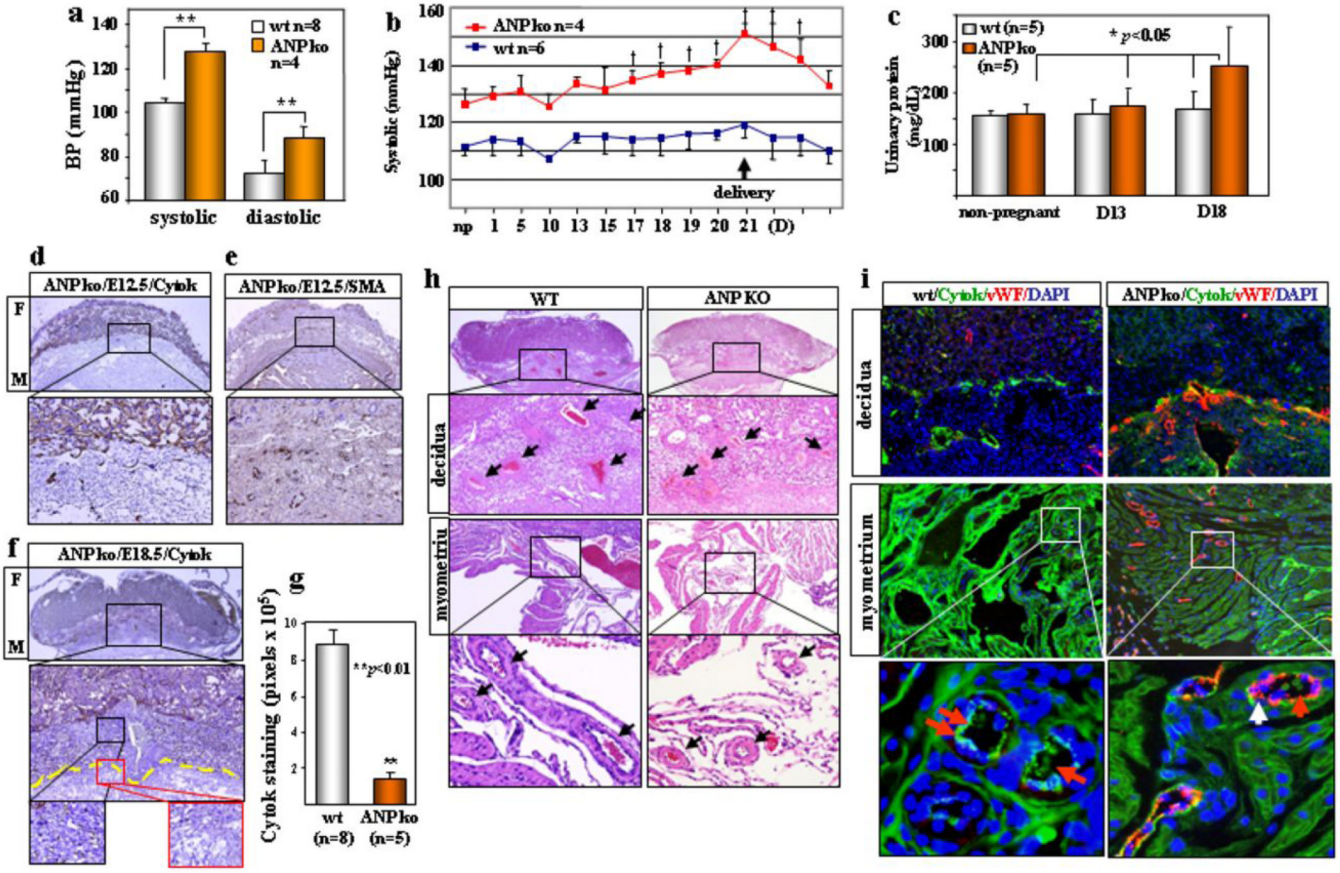

Figure 3. Hypertension, proteinuria and uteroplacental pathology in pregnant ANP ko mice

a, BP (mean ± s.d.) in np females, $^{**}P<0.01$. **b,** Elevated BP (mean ± s.d.) in pregnant ANP ko mice. †$P<0.05$ *vs.* np level. **c,** Gestational proteinuria in ANP ko mice. Data represent mean ± s.d. Impaired trophoblast invasion and smooch muscle remodeling in E12.5 embryos stained for cytok (**d**) or SMA (**e**). Boxed areas in top panels are shown at a higher magnification (x200). **f,** Impaired trophoblast invasion in E18.5 embryos stained for cytok. **g**, Quantitative data (mean ± s.d.) of cytok staining in E18.5 ANP ko embryos. **h,** Impaired decidual and myometrial artery remodeling (arrows) in H&E-stained E18.5 ANP ko embryos. **i,** Co-staining of cytok, vWF and nuclei in E18.5 ANP ko embryos. Red arrows indicate cytok (green) signals and white arrows vWF (red) signals.

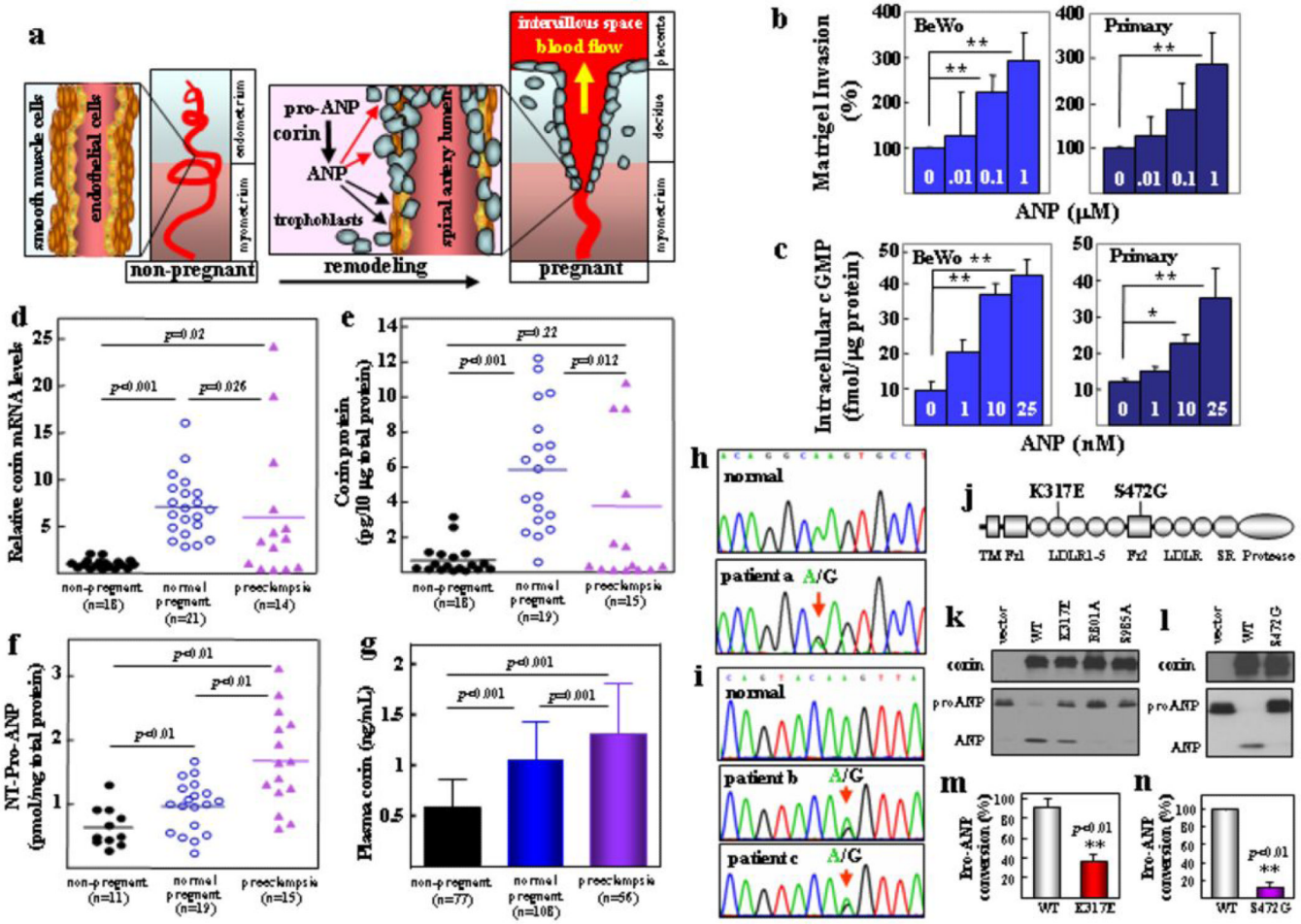

Figure 4. ANP-stimulated human trophoblast invasion, and impaired uterine corin expression and corin mutations in preeclamptic patients

a, A model illustrating that corin-produced ANP in the pregnant uterus promotes trophoblast invasion (red arrows) and vascular wall remodeling (black arrows). ANP stimulated human BeWo and primary trophoblasts in Matrigel invasion (**b**) and intracellular cGMP production (**c**). Data represent mean ± s.d. **P*<0.05; ***P*<0.01 *vs.* control. Corin mRNA (**d**) and protein (**e**) and N-terminal (NT) pro-ANP levels (**f**) in human uterus samples. Horizontal lines indicate mean values. **g**, Plasma soluble corin levels (mean ± s.d.) in preeclamptic patients and normal controls. **h-j**, *CORIN* gene mutations causing K317E (**h**) and S472G (**i**) changes in corin (**j**). TM, transmembrane; Fz, frizzled; LDLR, LDL receptor; SR, scavenger receptor. **k**,**l**, Expression of K317E and S472G mutants in HEK293 cells (top panels). Vector, wt corin and inactive corin R801A and S985A mutants were controls. K317E and S472G mutations reduced pro-ANP processing activity (bottom panels). **m,n**, Quantitative data (mean ± s.d.) from ≥3 experiments.