LETTER

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Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy

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In pregnancy, trophoblast invasion and uterine spiral artery remodelling are important for lowering maternal vascular resistance and increasing uteroplacental blood flow. Impaired spiral artery remodelling has been implicated in pre-eclampsia, a major complication of pregnancy, for a long time but the underlying mechanisms remain unclear[1,2]. Corin (also known as atrial natriuretic peptide-converting enzyme) is a cardiac protease that activates atrial natriuretic peptide (ANP), a cardiac hormone that is important in regulating blood pressure[3]. Unexpectedly, corin expression was detected in the pregnant uterus[4]. Here we identify a new function of corin and ANP in promoting trophoblast invasion and spiral artery remodelling. We show that pregnant corin- or ANP-deficient mice developed high blood pressure and proteinuria, characteristics of pre-eclampsia. In these mice, trophoblast invasion and uterine spiral artery remodelling were markedly impaired. Consistent with this, the ANP potently stimulated human trophoblasts in invading Matrigels. In patients with pre-eclampsia, uterine *Corin* messenger RNA and protein levels were significantly lower than that in normal pregnancies. Moreover, we have identified *Corin* gene mutations in pre-eclamptic 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 pre-eclampsia.

Pregnancy poses a serious challenge for maintaining normal blood pressure. Pregnancy-induced hypertension, a major cause of maternal and fetal deaths, occurs in approximately 10% of pregnancies[5,6]. During pregnancy, the uterus undergoes profound morphological changes, including trophoblast invasion and spiral artery remodelling. In pre-eclampsia, impaired spiral artery remodelling is common, but the underlying mechanisms are unclear[1,2,7–9]. Studies indicate that vascular growth factor receptors, angiotensin and oestradiol are involved in the disease[10–14].

Corin is a cardiac protease that activates ANP, which is a cardiac hormone that regulates 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 and *Corin* knockout mice were crossed to generate mice expressing *Corin* only in the heart ('knockout/transgenic mice'; Fig. 1C, D). In knockout/transgenic 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 mice.

In pregnant *Corin* knockout mice, blood pressure increased at approximately 17 days post coitus and rose further before returning to the non-pregnant blood pressure level after delivery (Fig. 1F), which resembled late gestational hypertension in pre-eclamptic women. In *Corin* knockout/transgenic 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 pre-existing high blood pressure. As well as 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* knockout females were mated with either wild-type or knockout males. The resulting fetuses carried one or no copy of the functional *Corin* gene. Normally, enzymes that are encoded by one gene copy are able to function. As shown in Fig. 1H, pregnant *Corin* knockout females that were mated with either wild-type or knockout males had similarly increased blood pressure, indicating that lack of maternal, but not fetal, *Corin* caused hypertension in pregnancy.

Proteinuria is a hallmark of pre-eclampsia. Wild-type, *Corin* knockout and knockout/transgenic mice had similar urinary protein levels before pregnancy and at mid gestation. However, the levels increased in *Corin* knockout and knockout/transgenic mice at late gestation (Fig. 1I), consistent with reported proteinuria in mouse models of pre-eclampsia[18]. Ischaemic glomeruli, indicated by fewer red blood cells, were found in pregnant *Corin* knockout and knockout/transgenic mice (Fig. 1J, a–f) but not in non-pregnant mice (Supplementary Fig. 4). Periodic acid–Schiff staining revealed increased extracellular matrixes and collapsed glomerular capillaries in pregnant *Corin* knockout and knockout/transgenic mice (Fig. 1J, g–i). Electron microscopy showed narrow glomerular capillary lumens and thick basement membranes (Fig. 1K), suggesting endotheliosis and increased extracellular matrices. 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. Consistent with this, *Corin* knockout and knockout/transgenic mice had smaller litters (7.1 ± 2.3 ($n = 28$) and 6.8 ± 2.7 ($n = 28$) pups per litter, respectively, versus wild-type mice, which had 9.1 ± 1.2 ($n = 21$) pups per litter; $P < 0.001$ in both cases).

We examined embryos at embryonic day 12.5 (E12.5), an early time point before blood pressure increase in *Corin* knockout and knockout/transgenic mice, and E18.5 (two days before delivery). Wild-type E12.5 embryos showed obvious trophoblast invasion, shown by cytokeratin staining (Fig. 2a), and large vessels mostly in the deep decidua, shown by smooth-muscle α-actin (SMA) staining (Fig. 2b), indicating that

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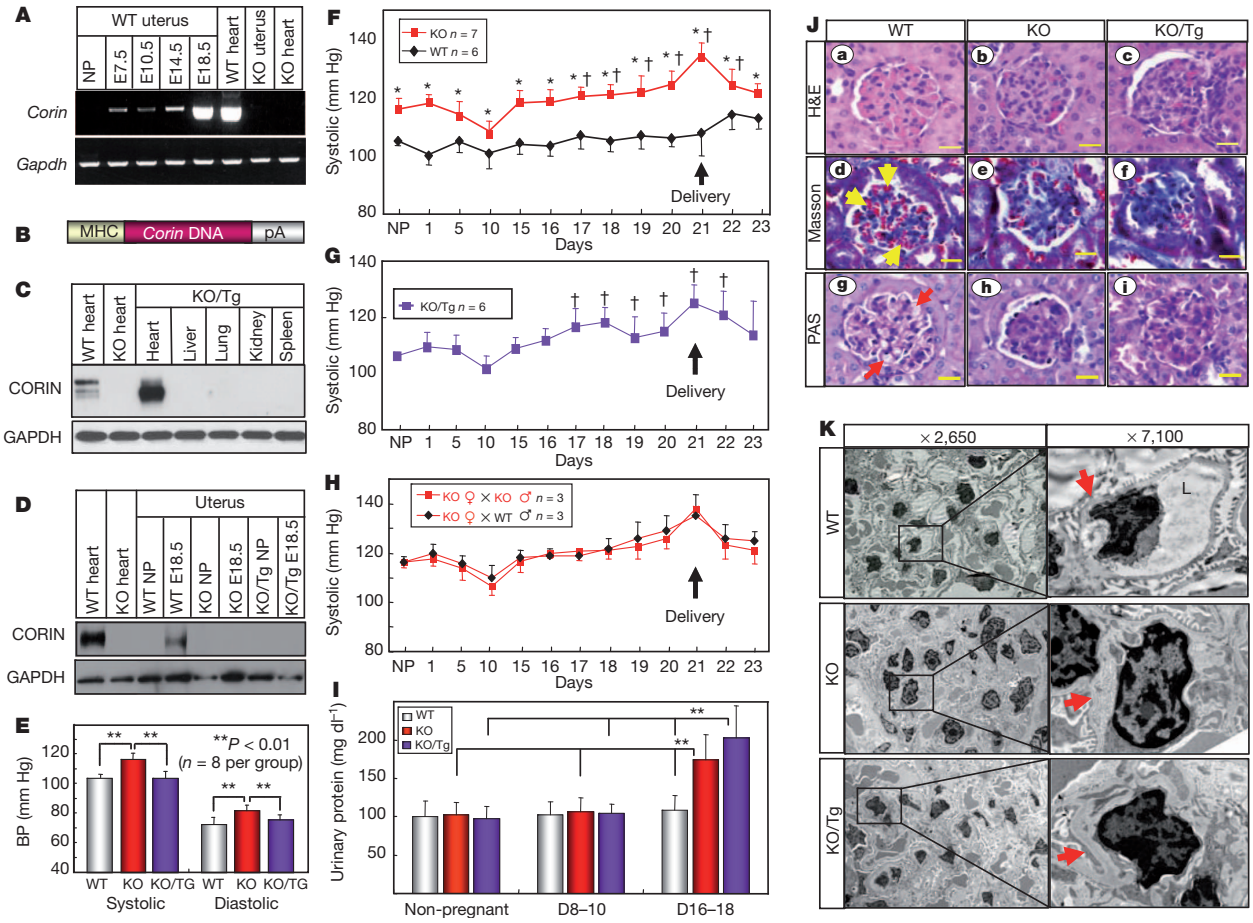

Figure 1 | Hypertension, proteinuria and renal pathology in pregnant *Corin* knockout and knockout/transgenic mice. **A**, Corin mRNA expression in mouse uteruses. **B**, Corin transgenic (Tg) construct. **C**, **D**, Western blot analysis of CORIN protein in wild-type (WT), *Corin* knockout (KO) and knockout/transgenic mice. **E**, Blood pressure (BP, mean ± s.d.) in non-pregnant females. **F**, **G**, Blood pressure increased in *Corin* knockout (**F**) and knockout/transgenic (**G**) mice in pregnancy. Data are mean ± s.d. $*P < 0.05$ versus WT of the same time point. †$P < 0.05$ versus non-pregnant level of the same genotype. **H**, Similar blood-pressure changes in *Corin* knockout females mated with knockout or WT males. **I**, Late gestational proteinuria in *Corin* knockout and knockout/transgenic mice. Data are mean ± s.d. $**P < 0.01$, $n =$ 7 or 8 per group. **J**, **a**–**i**, Renal ischaemia in pregnant *Corin* knockout and knockout/transgenic mice. E18.5 sections are stained with haematoxylin and eosin (H&E), Masson trichrome or periodic acid–Schiff (PAS). Scale bar, 20 μm. Red blood cells (yellow arrows) and open capillaries (red arrows) in WT glomeruli are shown. **K**, Narrow glomerular capillary lumen (L) and thick basement membranes (red arrows) in *Corin* knockout and knockout/transgenic mice at E18.5 shown by electron microscopy. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NP, non pregnant; pA, poly A.

smooth muscles in the superficial decidua were replaced by invading trophoblasts. In contrast, trophoblast invasion in *Corin* knockout and knockout/transgenic embryos was markedly reduced (Fig. 2a) and smaller arteries were found in both superficial and deep decidua (Fig. 2b). In E18.5 wild-type embryos, more abundant trophoblasts were found in the decidua and myometrium compared with those in *Corin* knockout and knockout/transgenic mice (Fig. 2c, d). By haematoxylin and eosin staining, larger and more abundant decidual spiral arteries were observed in wild-type than in *Corin* knockout or knockout/transgenic mice (Fig. 2e). Figure 2f–h shows strong cytokeratin (trophoblasts) staining but weak von Willebrand factor (endothelial) and SMA (smooth muscle) staining in wild-type decidual and myometrial arteries. These data indicate that trophoblast invasion and spiral artery remodelling were impaired in *Corin* knockout and knockout/transgenic mice, and that this defect occurred before blood pressure increased in these mice.

CORIN activates ANP in the heart[15] but it was unknown whether the CORIN function in pregnancy was also mediated by ANP. Pro-ANP is expressed in the non-pregnant and pregnant uterus (Supplementary Fig. 6). If CORIN acts on pro-ANP to promote trophoblast invasion and spiral artery remodelling, thereby preventing hypertension in pregnancy, *ANP* (also known as *Nppa*) and *Corin* knockout mice should have similar phenotypes. *ANP* knockout mice are hypertensive (Fig. 3a) but their blood pressure was not monitored during pregnancy[19]. We found similarly increased blood pressure in pregnant *ANP* knockout mice (Fig. 3b). The mice also had late gestational proteinuria (Fig. 3c) and smaller litters (4.4 ± 1.7 ($n = 25$) versus wild-type, 9.1 ± 1.2 ($n = 21$) pups per 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* knockout mice had far fewer trophoblasts (Fig. 3f, g) and smaller arteries (Fig. 3h) in the decidua and myometrium than those in wild-type mice. Consistent with this, weak cytokeratin-staining but strong von Willebrand factor-staining were found in arteries in *ANP* knockout mice (Fig. 3i). Thus, *ANP* and *Corin* knockout mice had very similar phenotypes, indicating that the role of CORIN in pregnancy is probably mediated by ANP.

In the heart, CORIN produces ANP, which then regulates blood pressure by promoting natriuresis and vasodilation[3]. Here we found that lack of CORIN and ANP impaired trophoblast invasion and spiral artery remodelling, which was not rescued by cardiac *Corin* expression in *Corin* knockout/transgenic mice. ANP is known to relax vascular smooth muscles. Recently, ANP and its downstream cyclic GMP-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 indicate that ANP may directly promote trophoblast invasion (Fig. 4a), and we

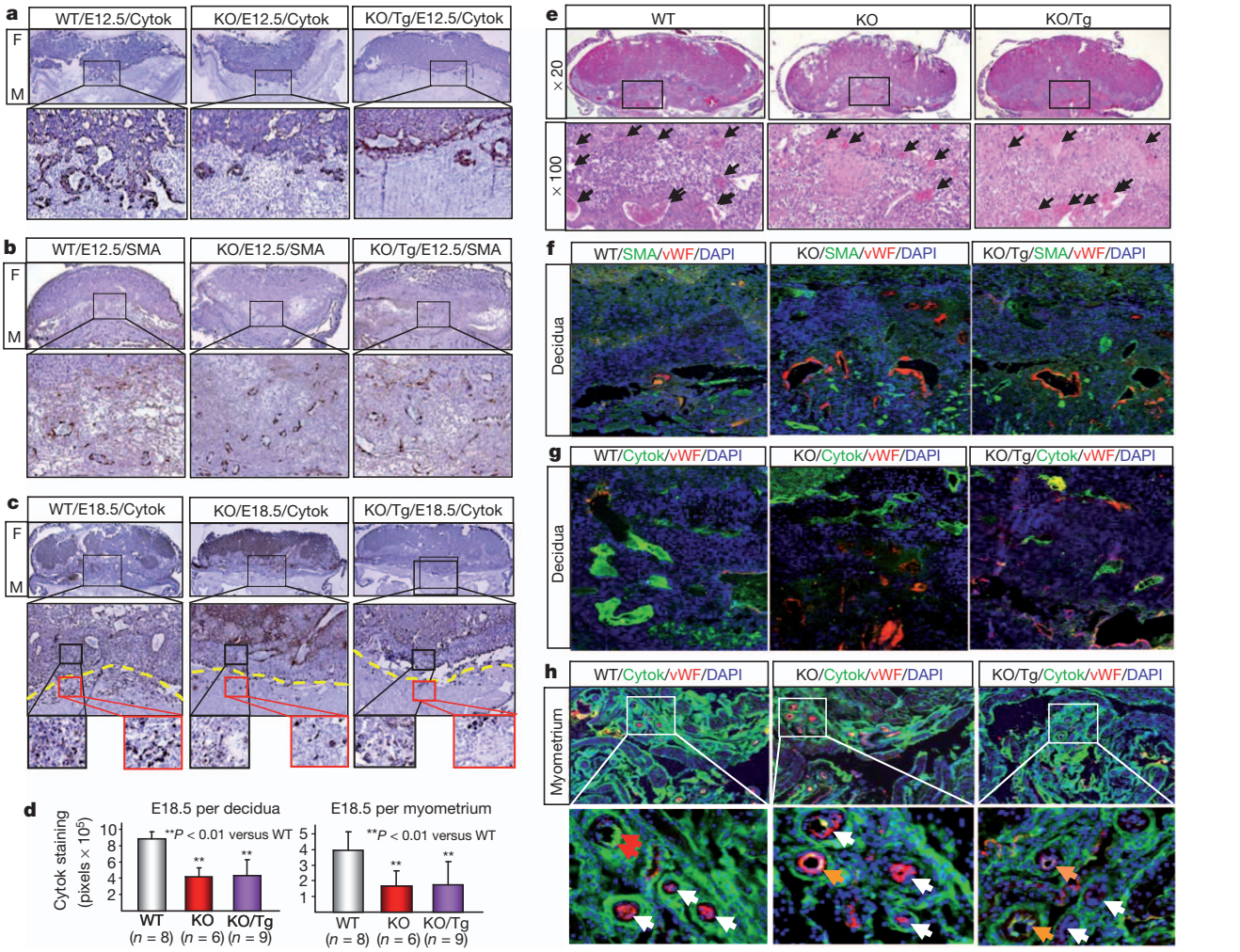

Figure 2 | Impaired trophoblast invasion and spiral artery remodelling in *Corin* knockout and knockout/transgenic mice. **a**, **b**, E12.5 embryo sections were stained for trophoblasts (**a**) or smooth muscles (**b**). Fetal (F) and maternal (M) sides are indicated. Boxed areas in top panels are shown at a higher magnification (×200). **c**, E18.5 embryo sections were stained for trophoblasts. In lower panels (×100), yellow lines show the decidua and myometrium boundary. **d**, Quantitative data (mean ± s.d.) of cytokeratin (Cytok) staining. **e**, Fewer and smaller decidual spiral arteries (arrows) in H&E-stained E18.5 *Corin* knockout and knockout/transgenic embryos. **f**–**h**, Co-staining of SMA, von Willebrand factor (vWF), cytokeratin and nuclei in E18.5 embryos. Red arrows indicate cytokeratin (green) signals, white arrows indicate von Willebrand factor (red) signals and orange arrows indicate mixed (yellow) signals.

therefore tested this idea. We found that ANP markedly stimulated human trophoblasts to invade Matrigels (Fig. 4b) (Supplementary Fig. 7a). In these cells, ANP receptor (also known as atrial natriuretic peptide receptor 1) mRNA expression was confirmed (Supplementary Fig. 7b) and ANP-stimulated intracellular cGMP production was detected (Fig. 4c) (Supplementary Fig. 7c).

Our findings emphasize the importance of local ANP production 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 remodelling, as shown in *Corin* knockout/transgenic mice. To verify this hypothesis, we quantified *Corin* mRNA and protein in human uteruses by polymerase chain reaction with reverse transcription (RT–PCR) and enzyme-linked immunosorbent assay (ELISA). The levels were low in non-pregnant women but increased in pregnant women (Fig. 4d, e). In pre-eclamptic women, the levels were significantly lower than in normal pregnancies. Similar results were found by immunostaining (Fig. 4d and Supplementary Fig. 8). Consistent with this, pro-ANP levels in uterine tissues were significantly higher in pre-eclamptic women than in 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], and recent studies showed that CORIN can be shed from cardiomyocytes and that soluble CORIN is found in human plasma[22,23]. We found that plasma CORIN levels were higher in pre-eclamptic patients than non-pregnant or normal pregnant women (Fig. 4g). Thus, CORIN levels in plasma did not reflect the levels in tissues, indicating that plasma CORIN was probably derived from the heart, where *Corin* expression increased in response to high blood volume and high blood pressure in pregnancy. These results provide further support for a local function of CORIN in the pregnant uterus.

We next sequenced the *CORIN* gene[24] in pre-eclamptic patients and identified a mutation that alters Lys to Glu at position 317 in low-density lipoprotein receptor repeat 2 in one woman (Fig. 4h, j) and another mutation altering Ser to Gly at position 472 in the frizzled 2 domain in two women from the same family who had pre-eclampsia (Fig. 4i, j). In functional studies, Lys317Glu and Ser472Gly mutations did not affect CORIN expression in HEK293 cells but markedly reduced CORIN activity in processing pro-ANP (Fig. 4k–n). The data were consistent with previous findings that Low-density lipoprotein 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 pre-eclampsia. Interestingly, CORIN variants in the frizzled 2 domain that impaired CORIN function have been reported in African American people[17,26], a high-risk population for pre-eclampsia.

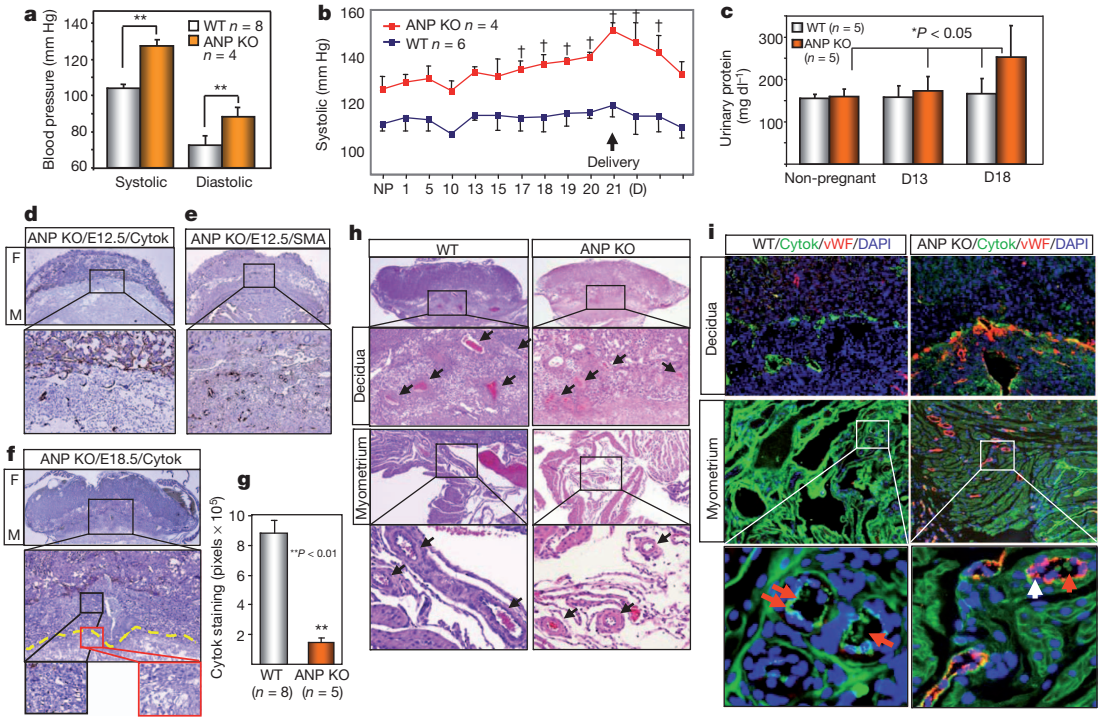

Figure 3 | Hypertension, proteinuria and uteroplacental pathology in pregnant *ANP* knockout mice. **a**, Blood pressure (mean ± s.d.) in non-pregnant females, $^{**}P < 0.01$. **b**, Elevated blood pressure (mean ± s.d.) in pregnant *ANP* knockout mice. †$P < 0.05$ versus non-pregnant level. **c**, Gestational proteinuria in *ANP* knockout mice. Data are mean ± s.d. **d**, **e**, Impaired trophoblast invasion and smooth muscle remodelling in E12.5 embryos stained for cytokeratin (**d**) or SMA (**e**). Boxed areas in top panels are shown at a higher magnification (×200). **f**, Impaired trophoblast invasion in E18.5 embryos stained for cytokeratin. **g**, Quantitative data (mean ± s.d.) of cytokeratin staining in E18.5 *ANP* knockout embryos. **h**, Impaired decidual and myometrial artery remodelling (arrows) in H&E-stained E18.5 *ANP* knockout embryos. **i**, Co-staining of cytokeratin, von Willebrand factor and nuclei in E18.5 *ANP* knockout embryos. Red arrows indicate cytokeratin (green) signals and white arrows indicate von Willebrand factor (red) signals.

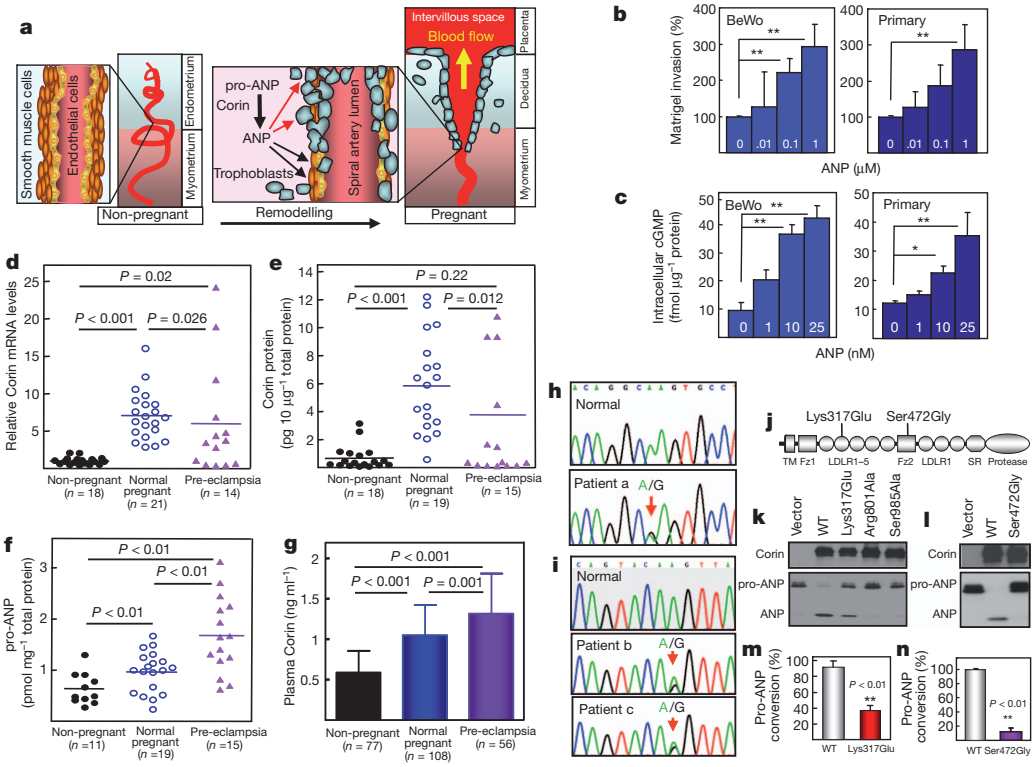

Figure 4 | ANP-stimulated human trophoblast invasion, and impaired uterine *Corin* expression and *Corin* mutations in pre-eclamptic patients. **a**, A model showing that CORIN-produced ANP in the pregnant uterus promotes trophoblast invasion (red arrows) and vascular-wall remodelling (black arrows). **b**, **c**, ANP-stimulated human BeWo trophoblasts and primary trophoblasts in Matrigel invasion (**b**) and intracellular cGMP production (**c**). Data are mean ± s.d. $^{*}P < 0.05$; $^{**}P < 0.01$ versus control. **d**–**f**, Corin mRNA (**d**) and protein (**e**), and pro-ANP levels (**f**) in human uterus samples. Horizontal lines indicate mean values. **g**, Plasma-soluble CORIN levels (mean ± s.d.) in pre-eclamptic patients and normal controls. **h**–**j**, *CORIN* gene mutations causing Lys317Glu (**h**) and Ser472Gly (**i**) changes in CORIN (**j**). **k**, **l**, Expression of Lys317Glu and Ser472Gly mutants in HEK293 cells (top panels). Vector, WT CORIN and inactive CORIN Arg801Ala and Ser985Ala mutants were controls. Lys317Glu and Ser472Gly mutations reduced pro-ANP processing activity (bottom panels). **m**, **n**, Quantitative data (mean ± s.d.) from three experiments or more. Fz, frizzled; LDLR, LDL receptor; SR, scavenger receptor; TM, transmembrane.

Previously, high levels of plasma pro-ANP or ANP were detected in pre-eclamptic patients[27,28]. As shown by our plasma soluble CORIN data, plasma protein levels may not reflect those in tissues. Taken together, our data show a novel local function of CORIN and ANP in promoting trophoblast invasion and spiral artery remodelling to prevent hypertension in pregnancy. The data suggest that impaired *Corin* expression or function in the pregnant uterus may be an important mechanism underlying pre-eclampsia. Studies to better understand impaired uterine *Corin* expression in pre-eclamptic patients may help to develop new strategies to enhance the CORIN–ANP pathway and prevent or treat this life-threatening disease.

METHODS SUMMARY

Corin and *ANP* knockout mice have been described previously[16,19]. Transgenic mice with cardiac *Corin* expression were generated using a heart-specific promoter. Blood pressure was measured by radiotelemetry[16]. Tissue sections from non-pregnant and pregnant mice were stained with haematoxylin and eosin, Masson's trichrome, periodic acid–Schiff or von Kossa, or immunostained with antibodies against cytokeratin, SMA, von Willebrand factor or CORIN. Renal sections were also examined by electron microscopy. Trans-well invasion assay was carried out 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 enzyme immunoassay kit (Enzo Life Sciences). Corin levels in human blood and uterus tissue samples were measured using ELISA[22]. Pro-ANP levels in human uterus tissues were also measured using ELISA (Alpco Diagnostics). *Corin* gene exons[24] from pre-eclamptic patients were PCR-amplified and sequenced directly. *Corin* gene mutations that were identified were studied by expressing mutant CORIN proteins in HEK293 cells and testing their activities in pro-ANP processing assays, as described previously[26].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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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 analysed clinical data. Q.W. conceived the study and designed experiments. Y.Z. and Q.W. wrote the manuscript. All authors analysed and interpreted data, and critically read the manuscript.

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METHODS

Knockout and transgenic mice. Corin knockout mice were described previously[16]. *ANP* knockout mice (B6.129P2-*Nppa* ^{tm1Unc}/J)[19] were from the Jackson Laboratory. To make transgenic mice expressing *Corin* in the heart, the full-length mouse *Corin* cDNA was inserted into a construct driven by the mouse α-myosin heavy chain (α-MHC) promoter. Pro-nuclear microinjection and breeding of transgenic mice were carried out at the Case Western Reserve University Transgenic Core. Corin knockout and transgenic mice were crossed to generate knockout/transgenic mice. Littermates were used as controls. The animal study was conducted in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee at the Cleveland Clinic.

Blood-pressure monitoring. Radiotelemetry was used for real-time blood-pressure monitoring in conscious and unrestrained mice[16]. Female mice (8–12 weeks old) were chronically instrumented in the left carotid artery with a PA-C10 device (Data Sciences International) and rested for at least 7 days to recover from the surgery. The mice were mated and checked for vaginal plugs to establish gestation timing. The day on which a plug was observed was defined as E0.5. The mating mice were homozygous except for those in the fetal testing experiment. Telemetry receivers (model RPC-1) were placed under individual cages for data acquisition using the Dataquest A.R.T. 4.0 Gold System (Data Sciences International). Data presented were from continuous recording of at least 6 h per day (10:00 to 16:00).

Urinary protein measurement. Urine samples were collected from non-pregnant mice and pregnant mice at mid (8–10 days post coitus) and late (16–18 days post coitus) gestational stages. Urinary protein levels were measured using a colorimetric assay based on a modified Bradford method (Bio-Rad).

RT–PCR, western blot analysis and ELISA. Total RNAs were isolated from cultured cells or mouse and human tissues using TRIzol reagent (Invitrogen) or an RNeasy kit (Qiagen), and were used to synthesize the first strand cDNAs. RT–PCR was carried out using oligonucleotide primers that were specific for the mouse or human CORIN, mouse ANP, or human ANP receptor genes. Quantitative RT–PCR for human CORIN mRNA expression in uterus tissues was carried out using the PRISM 7500 System (Applied Biosystems). The β-actin gene was used as an internal control. Quantitative RT–PCR for mouse *ANP* mRNA in uteruses was carried out using the iCycler system (Bio-Rad). For western blot analysis of CORIN protein, membrane fractions from tissue homogenates were isolated by ultracentrifugation, as described previously[29]. Proteins were analysed by SDS–polyacylamide agarose gel electrophoresis (SDS–PAGE) and western blot using a polyclonal antibody (Berlex Biosciences). Western blot analysis of pro-ANP in heart samples was carried out using a polyclonal antibody (Santa Cruz). Processing pro-ANP by CORIN in transfected cells was analysed by western blot analysis, as described previously[30]. Pro-ANP in human uterus tissues was measured by an amino-terminal (NT) pro-ANP ELISA kit from Alpco Diagnostics. Human CORIN in uterus tissues or plasma was measured by ELISA, as described previously[31].

Histology and immunohistochemistry. Tissues were fixed with 4% paraformaldehyde and embedded in paraffin. Sections were stained with H&E, Masson's trichrome, PAS or von Kossa. For immunohistochemical or immunofluorescent analysis, antibodies against SMA (Sigma-Aldrich), von Willebrand factor (Sigma-Aldrich) and cytokeratin (Dako) were used to label smooth muscle cells, endothelial cells and trophoblasts, respectively. For human CORIN, an antibody from Berlex Biosciences was used. Secondary antibodies were conjugated with horseradish peroxidase or Alexa Fluor 488 (green) or Alexa Fluor 594 (red) (Invitrogen). Tissue sections were mounted with or without DAPI-containing (blue) mounting medium (Dako). For *ANP* expression in mouse uterus tissues, a polyclonal antibody from Millipore was used. Control sections were treated similarly but without the primary antibodies. Photographs were taken with a light or fluorescent microscope equipped with a digital camera (Olympus). Data are from experiments using five or more mice per study group.

For immunohistochemical analysis of trophoblast invasion in mouse embryos, tissue samples from at least five mice per group, and at least two implant sites per mouse were used. Serial sections (>50 per embryo) of 5 μm in thickness were prepared. The position of the maternal artery was used as a guide to orient section positions. At least 4–6 sections from the centre of the placenta of each embryo were used for immunohistochemical analysis. Slides that were stained for cytokeratin were examined by two individuals. The sections that showed the deepest trophoblast invasion are presented. These sections were also analysed by ImagePro software to quantify cytokeratin staining. For each section that was analysed, the entire area of the decidua and myometrium was scanned by the software.

Electron microscopy. Kidneys from pregnant mice at E18.5 were fixed in 3% glutaraldehyde, treated with 1% osmium tetroxide and embedded in an Araldite–Epon mixture. Semi-thin sections (0.6 μm) were prepared and examined with a transmission electron microscope (JEOL JEM-1210) at the Lerner Image Core of the Cleveland Clinic. Data are from experiments using at least three mice per study group.

Trans-well invasion assay. Human trophoblastic JEG3, BeWo and JAR cells from the American Type Culture Collection were cultured in Minimum Essential Medium (JEG3), RPMI1640 (JAR) and F-12K (BeWo) medium, respectively, with 10% FBS at 37 °C. Primary human villous trophoblasts from ScienCell Research Labs (Carlsbad) were cultured in the Trophoblast Medium (ScienCell) with 10% FBS. Transwell invasion assays were carried out using the BioCoat Growth Factor Reduced Matrigel Invasion Chambers (pore size of 8 μm) and control inserts (no Matrigel coating) (BD Biosciences) in 24-well plates. Culture medium containing human ANP (Calbiochem) was added to the bottom wells, and cell suspension (5×10^4) was added to the top wells and incubated at 37 °C for 24 h. Non-invading cells were removed from the upper surface of the Matrigel layer by gentle scrubbing. The cells on the lower surface of the membrane were stained using Diff-Quick staining solutions. The membranes were excised and mounted onto glass slides. Invasion indices were determined by counting the number of stained cells on the membrane under a light microscope. The assay was carried out in duplicate in at least three independent experiments.

cGMP assay. ANP-stimulated intracellular cGMP production assay was performed with JEG3, JAR and BeWo cells and primary human trophoblasts using a method described previously[32]. The cells were grown in 96-well plates. Confluent cells were washed once with serum-free medium. Human ANP was added to serum-free medium and incubated with cells at 37 °C for 30 min. In these experiments, ANP was more potent in stimulating intracellular cGMP production when serum-free medium was used (data not shown). The cells were lysed with 0.1 M HCl. Intracellular cGMP levels in ANP-stimulated cells were determined using an EIA kit (Enzo Life Sciences). Each experimental condition was assayed in duplicate in at least three independent experiments.

Human blood and tissue samples. The study was approved by local ethics committees and participants gave informed consent. Women of normal pregnancy or with pre-eclampsia, and age-matched non-pregnant normal controls who underwent routine medical check-ups were recruited. All participants were ethnic Han Chinese. Hypertension was defined as diastolic pressure >90 mm Hg and/or systolic pressure >140 mm Hg on at least two occasions. Pre-eclampsia was defined as hypertension that appeared after 20 weeks of gestation with proteinuria (>300 mg urinary protein per 24 h). Patients with chronic hypertension, chronic kidney disease, diabetes and heart disease were excluded. Uterus tissues were obtained during caesarean sections in pregnant women or operations for uterine leiomyoma in non-pregnant women. Clinical characteristics of women who provided blood and those who provided uterus tissue samples are summarized in Supplementary Tables 1 and 2, respectively.

***CORIN* gene sequences in patients.** Blood samples from 56 patients with pre-eclampsia were collected into tubes containing EDTA as an anticoagulant. Genomic DNA was extracted from white blood cells using the QIAamp DNA Mini kit (Qiagen) and used in PCR to amplify exon sequences of the *CORIN* gene[24]. PCR products were used for direct DNA sequencing. Mutations that were identified were verified by independent PCR and DNA sequencing. Additional PCR and DNA sequencing were carried out with DNA samples from more than 100 normal controls to verify that mutations that were identified in patients did not exist in the normal population.

Expression and functional analysis of *Corin* mutants. Plasmids expressing human wild-type *Corin* and two inactive mutants Arg801Ala and Ser985Ala, in which the activation cleavage site and catalytic site residues were mutated, respectively, were described previously[33]. Plasmids expressing *Corin* mutants Lys317Glu or Ser472Gly were constructed by PCR-based mutagenesis. Recombinant CORIN proteins that were expressed by these plasmids contained a carboxy-terminal V5 tag to be detected by an anti-V5 antibody (Invitrogen)[30]. Plasmids were transfected into HEK293 cells using Lipofectamine 2000 (Invitrogen). Cells were lysed and proteins were analysed by western blot using an anti-V5 antibody. To analyse the function of CORIN, recombinant human pro-ANP in conditioned medium was added to HEK293 cells expressing *Corin* wild-type or mutants and incubated at 37 °C for 2 h. Pro-ANP and ANP in the medium were immunoprecipitated and analysed by western blot. Protein bands on X-ray films were scanned by densitometry. The percentage of pro-ANP to ANP conversion was calculated as described previously[30].

Statistical analysis. Results are presented as mean ± s.d. Differences between two groups were analysed with the Student's *t*-test. Data involving more than two groups were analysed by analysis of variance followed by the Tukey multiple comparison test. Comparisons for *Corin* mRNA and protein and pro-ANP levels in human uterus samples were carried out using the Mann–Whitney–Wilcoxon test. A *P* value of less than 0.05 was considered statistically significant.

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