Interleukin-11 alters placentation and causes preeclampsia features in mice

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Preeclampsia (PE) is a pregnancy-specific disorder characterized by hypertension and proteinuria after 20 wk gestation. Abnormal extravillous trophoblast (EVT) invasion and remodeling of uterine spiral arterioles is thought to contribute to PE development. Interleukin-11 (IL11) impedes human EVT invasion in vitro and is elevated in PE decidua in women. We demonstrate that IL11 administered to mice causes development of PE features. Immunohistochemistry shows IL11 compromises trophoblast invasion, spiral artery remodeling, and placentation, leading to increased systolic blood pressure (SBP), proteinuria, and intrauterine growth restriction, although nonpregnant mice were unaffected. Real-time PCR array analysis identified pregnancy-associated plasma protein A2 (PAPPA2), associated with PE in women, as an IL11 regulated target. IL11 increased PAPPA2 serum and placental tissue levels in mice. In vitro, IL11 compromised primary human EVT invasion, whereas siRNA knockdown of PAPPA2 alleviated the effect. Genes regulating uterine natural killer (uNK) recruitment and differentiation were down-regulated and uNK cells were reduced after IL11 treatment in mice. IL11 withdrawal in mice at onset of PE features reduced SBP and proteinuria to control levels and alleviated placental labyrinth defects. In women, placental IL11 immunostaining levels increased in PE pregnancies and in serum collected from women before development of early-onset PE, shown by ELISA. These results indicate that elevated IL11 levels result in physiological changes at the maternal–fetal interface, contribute to abnormal placentation, and lead to the development of PE. Targeting placental IL11 may provide a new treatment option for PE.

placenta | trophoblast | cytokines | pregnancy

Preeclampsia (PE) is a pregnancy-induced disorder characterized by hypertension and proteinuria, unique to humans, affecting ∼8% of pregnancies (1). The etiology is poorly understood (2); nevertheless, there is substantial evidence showing abnormal placentation is the key underlying cause. During pregnancy, highly invasive extravillous trophoblasts (EVT) acquire vascular-like properties to remodel uterine spiral arterioles. This creates low-resistance, large-diameter vessels that promote uteroplacental blood supply to sustain fetal growth (3, 4). It is widely accepted that inadequate trophoblast invasion and impaired uterine spiral artery remodeling is an initiating factor in the development of PE (5). This is thought to impair uteroplacental arterial flow and lead to placental oxidative stress (6). PE is associated with increased placental secretion of proinflammatory cytokines (7) and angiogenic regulators (8), thought to contribute to widespread maternal endothelial dysfunction. The clinical symptoms of PE are hypertension, proteinuria, and peripheral and/or cerebral edema. Symptoms can differentially manifest during the second (early-onset, EO), or third (late-onset, LO) trimester (9). In addition to the maternal symptoms, PE is also frequently associated with prematurity (1) and intrauterine growth restriction (IUGR), related to impaired insulin-like growth factor (IGF1) signaling (10).

It is well established that cytokines produced within the local uterine environment can alter trophoblast action (11). Interleukin-11 (IL11) is a pleiotropic cytokine that regulates cell cycle, invasion, and migration in numerous cell types (12, 13), all roles critical to placental development. IL11 is a member of the IL-6-type cytokines and signals via the IL11 receptor (R) α chain and signal transducer gp130 (14) to activate the Janus kinase (JAK)/Signal transducers and activators of transcription (STAT)3 pathway in human endometrium (15) and primary human EVT (16, 17). IL11 is required for decidualization in humans (18) and mice (19). IL11 levels are elevated in PE decidual tissue (20). More recently, IL11 has been shown to impede human EVT invasion in vitro (16, 21), although its function in placentation in vivo has not been investigated.

We investigated the effect of elevated IL11 levels on placentation and PE features in mice. We demonstrated that IL11 contributes to impaired trophoblast invasion, spiral artery remodeling, and altered placental labyrinth morphology, leading to the development of PE-like features including elevated systolic blood pressure (SBP), proteinuria, kidney glomerular pathology, and IUGR. We identified IL11 as a potential valuable biomarker that could anticipate the development of PE in women. Finally, we demonstrated that IL11 withdrawal after the onset of PE features in mice could rescue PE features. Thus, we identify IL11 as a key regulator of EVT invasion during early gestation in mice and provide evidence that dysregulation of IL11 could contribute

Significance

Preeclampsia is an insidious disease, unique to humans, affecting ∼8% of pregnancies. There are no early detection tests or pharmacological treatments. Impaired placentation is widely accepted to contribute to the pathogenesis. However, the mechanisms remain elusive, given the complications of studying first-trimester placental development in women. A major limitation for the study of new treatments is the lack of available animal models that recapitulate the full spectrum of preeclampsia features. We have developed a mouse model characterized by elevated levels of the cytokine Interleukin-11 (IL11). This study provides evidence of a novel pathway causative of preeclampsia features in vivo. It also provides a novel in vivo mouse model that is useful for preclinical studies to test potential therapeutics.

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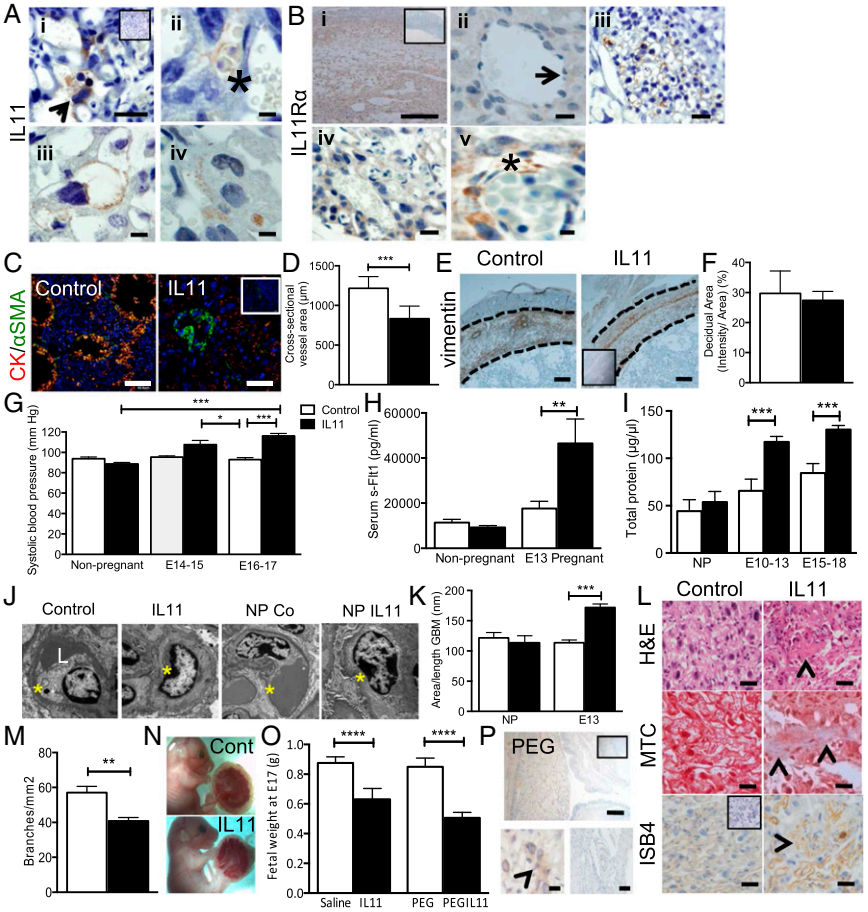

Fig. 1. IL11 and IL11Rα localize to mouse placental trophoblasts and vasculature, and IL11 administration during pregnancy in mice alters placentation and contributes to maternal and fetal features of PE in mice. (*A*) Representative photomicrographs of wild-type mid-gestation (E13) implantation site sections immunostained for IL11 (n = 3 mice/time-point). (*i*) IL11 localized to labyrinth mononuclear trophoblasts (arrow) associated with maternal blood sinuses, (*ii*) labyrinth fetal capillary endothelial cells (asterisk), (*iii*) spongiotrophoblast glycogen trophoblasts, and (*iv*) trophoblast giant cells at the maternal–fetal interface. [Scale bar: 100 μm (*i*) and 50 μm (*ii–iv*).] (*B, i*) IL11Rα was produced abundantly throughout the decidua and placenta, specifically within (*ii*) decidual EVTs (arrow), (*iii*) glycogen trophoblasts, (*iv*) labyrinth trophoblasts, and (*v*) fetal capillary endothelial cells (asterisk). [Scale bar: 200 μm (*i*), 50 μm (*ii–iv*), and 20 μm (*v*).] Nonpregnant or pregnant mice were treated with saline vehicle control or IL11 (500 $\mu g \cdot kg^{-1} \cdot d^{-1}$) from E8 through E13 ($n$ = 5) or E10 through E17, or 8-d equivalent in nonpregnant mice (n = 8). (*C*) E13 embryo sections were stained for trophoblasts (cytokeratin; red) or smooth muscle (α-SMA; green). (Scale bar: 50 μm.) (*D*) Decidual vessel area was measured in placental cross sections at E13. Data are mean ± SEM, Students t test, $^{***}P < 0.001$, n = 5. (*E*) Vimentin immunostaining highlights decidual area at E13. (Scale bar: 200 μm.) (*F*) Staining intensity was quantified as pixel intensity/area (percent). Data are mean ± SEM, n = 5. (*G*) SBP was measured by tail cuff plethysmography. Data are mean ± SEM, one-way ANOVA, $^{*}P < 0.05$, $^{***}P < 0.001$, n = 8. (*H*) Circulating s-Flt1 levels were quantified by ELISA in mouse sera. Data are mean ± SEM, one-way ANOVA, $^{**}P < 0.01$, n = 5. (*I*) Total urinary protein was quantified by Bradford colorimetric assay. Data are mean ± SEM, Students t test, $^{**}P < 0.01$, n = 5. (*J*) Glomerular pathology at E17, including narrow glomerular capillary lumen (L) and basement membrane thickening (asterisk) shown by electron microscopy at high power magnification, 8000×. (*K*) Glomerular basement membrane thickening was quantified across all treatment groups. Data are mean ± SEM, one-way ANOVA, $^{***}P < 0.001$, n = 8. (*L*) Haematoxylin and eosin (H&E; *Top*) staining shows abnormal labyrinth structure following IL11 administration at E17. Masson's trichrome staining (MTC; *Middle*) shows collagen deposition (arrow heads). Isolectin B4 (ISB4; *Bottom*) highlights maternal sinusoid branches. Labyrinth vascular branching development is impaired (arrow head) in IL11-treated mice. *Inset* is negative control. (Scale bar: 200 μm.) (*M*) Number of labyrinth vascular branches were counted, expressed as branches per placental area (square millimeters). Data are mean ± SEM, Students t test, $^{**}P < 0.01$, $^{****}P < 0.0001$, n = 8. (*N*) Representative photos of (*O*); fetal weight (E17) was reduced following IL11 or PEGIL11 treatment compared with saline or PEG control, respectively. Data are mean ± SEM, Students t test, $^{****}P < 0.0001$, n = 8. (*P*) Representative photomicrographs of E17 PEG-treated implantation site immunostained for PEG, localized to mouse placental trophoblasts (arrow in *Bottom Left*) but not fetal tissue (*Bottom Right*). [Scale bar: 500 μm (*Top*) and 100 μm (*Bottom* panels).] *Inset* is negative control.

to PE development in women. This suggests that targeting IL11 may provide a new treatment option for PE.

Results

IL11 Administration Impairs EVT Invasion and Spiral Artery Remodeling in Vivo. In vivo evidence for a functional role of IL11 in placentation is lacking. We localized IL11 and IL11Rα in the mouse placental endovascular trophoblast and endothelial cells in implantation sites throughout gestation (Fig. 1 *A* and *B*) and determined mRNA and protein expression levels (Fig. S1). This reflects localization patterns in women (Fig. S2), implying a role in placentation in vivo. To model elevated levels of IL11 as in women with PE (20), mice were administered with physiologically relevant doses of IL11 (Fig. S3) or saline vehicle control twice daily from embryonic day (E)8 through E13 to determine effects on spiral artery remodeling and placentation (22), or E10 through E17 to determine effects on PE features. At E13, IL11 enhanced phosphorylated (p) STAT3 in the placental labyrinth and maternal spiral arterioles, suggesting that STAT3 is one of the signaling pathways that mediate IL11 action at these sites (Fig. S3). Elevated IL11 did not alter the expression of IL11Rα in the mouse placenta (Fig. S3). Control implantation sites at E13 showed decidual trophoblast invasion and displacement of α-smooth muscle actin (α-SMA) positive vascular smooth muscle cells (VSMCs), indicating normal spiral artery remodeling (Fig. 1 *C* and *D* and Fig. S4). In IL11-treated mice, trophoblast invasion and spiral artery remodeling was impaired, decidual vessel area was significantly reduced, and VSMCs lining decidual spiral arterioles were retained (Fig. 1 *C* and *D* and Fig. S4), supporting in vitro findings in humans (16, 21). Because IL11 plays a crucial role in decidualization from E3 through E6 (19), we investigated the effect of elevated IL11 during mid gestation on the decidua. In mice treated with IL11 from E8 through E13, vimentin and desmin immunostaining (decidual markers) were unchanged between groups (Fig. 1 *E* and *F* and Fig. S4).

IL11 Administration Recapitulates the Features of PE in Mice. IL11 impaired trophoblast invasion in vivo; thus, we determined the effect of elevated IL11 on hallmark features of PE in mice. In pregnant mice treated with IL11 from E10 through E17, SBP increased by 20% at E15 (116.20 mm/Hg ± 2.37 versus control 92.96 mm/Hg ± 1.85, $P < 0.001$) (Fig. 1*G*). IL11 had no effect on

SBP in nonpregnant mice. The antiangiogenic factor soluble (s)-Flt1, associated with endothelial dysfunction and PE in mice and women (23), was increased in a pregnancy-specific manner in response to IL11 at E13 (46,455 pg/mL ± 10,836 versus control 18,750 pg/mL ± 2,723, $P < 0.01$) (Fig. 1*H*). IL11 treatment from E10 through E17 increased urinary protein in pregnant mice at middle and late gestation (117.4 μg/μL ± 5.86 versus control 65.64 μg/μL ± 12.38, $P < 0.001$) (Fig. 1*I*). Kidney glomeruli from IL11-treated pregnant mice were enlarged and ischemic compared with pregnant controls (Fig. S3). Glomeruli had narrow capillary lumen and thickened basement membrane that facilitates filtration (Fig. 1 *J* and *K*), suggesting endotheliosis and extracellular matrix deposition in the maternal kidneys.

IL11 Impairs Placental Labyrinth Vasculature Development and Promotes IUGR via Placental Insufficiency. Elevated IL11 dramatically altered placental labyrinth structure and morphology at mid and late gestation compared with control (Fig. 1*L* and Fig. S5), which may alter maternal–fetal exchange (22). We demonstrated impaired labyrinth endothelial cell differentiation (*Mest*) and reduced invasive trophoblasts (*Prlb*) at the maternal–fetal interface (Fig. S5). Labyrinth fetal vascular branching (Isolectin B4) was significantly reduced (Fig. 1 *L* and *M*) and collagen deposition was evident in IL11-treated placenta compared with control at E17 (Fig. 1*L*). IL11 reduced fetal weight at E17 (0.63g ± 0.02 versus control 0.87g ± 0.01, $P < 0.01$) (Fig. 1 *N* and *O*). To confirm that IL11 likely acts via the placenta and not directly on the fetus to cause IUGR, polyethylene glycol (PEG) was ligated to IL11. Very little PEGIL11 crossed the placenta (Fig. 1*P* and Fig. S6). PEGIL11 resulted in a similar reduction in fetal weight to IL11 alone, compared with respective controls (Fig. 1*O*). We identified IGF1, an important regulator of placental and fetal growth (10), as an IL11 regulated target in the mouse placenta at mid-gestation. IL11 down-regulated IGF1 mRNA by 2.05-fold at E13 ($P < 0.01$) (Fig. 2*A*) and significantly reduced serum IGF1 levels at E17 (Fig. 2*B*).

IL11 Impedes Human EVT Invasion via the Pregnancy-Associated Plasma Protein A2 Protease. We identified IL11 targets in the mouse placenta at E13 by quantitative PCR (qPCR) array. IL11 up-regulated three genes, and down-regulated four genes (>twofold) (Fig. 2*A*). Pregnancy-associated plasma protein A2 (PAPPA2) was up-regulated 2.6-fold ($P < 0.001$). PAPPA2 protein is elevated in EO PE placenta (24) and maternal serum before PE onset (25) and expressed by first-trimester EVT in women and by invasive trophoblasts in mice (26). However, the functional role of PAPPA2 in EVT invasion is not known. IL11 regulated PAPPA2 mRNA and protein in mouse EVTs and decidual cells and in IL11-treated mouse serum, in a pregnancy-specific manner (Fig. 2 *C* and *D* and Fig. S7). IL11 regulated PAPPA2 protein in human placental villous cytotrophoblast and syncytiotrophoblast (Fig. 2 *E* and *F*). In first-trimester placental explants, PAPPA2 reduced trophoblast outgrowth by 55% ± 8% compared with control ($P < 0.05$), to a similar extent as did IL11 (Fig. 2 *G* and *H*). Knockdown of endogenous PAPPA2 (Fig. S7) did not significantly alter outgrowth ($P > 0.05$) (Fig. 2*H*), suggesting that only abnormally elevated PAPPA2 levels alter trophoblast invasion. PAPPA2 knockdown rescued IL11-impaired trophoblast outgrowth (79% ± 6%) compared with IL11 (50% ± 4%, $P < 0.05$) (Fig. 2*H*).

IL11 Alters Decidual Immune Cells Required for Normal Spiral Artery Remodeling. Immune cells function at the maternal–fetal interface to mediate maternal tolerance and facilitate decidual and arterial tissue remodeling during EVT invasion (27). IL11 reduced placental gene expression of *IL10, IL15*, and *IL18* (Fig. 2*A*), which play important roles in regulating decidual immune cell functions. As indicated by DBA lectin staining (Fig. 2*I*), IL11 dramatically reduced the number of decidual uterine natural killer (uNK) cells at mid-gestation (17 ± 2 versus control 50 ± 3, $P < 0.0001$) (Fig. 2*J*) and also promoted a significant increase in the number of senescent binucleate decidual uNK cells ($P < 0.05$) (Fig. 2*K*). We found a trend in reduced decidual macrophages (Fig. S4), although this was not significant.

IL11 Withdrawal After Onset of PE Features in Mice Rescues the Placenta and Alleviates PE Hallmarks. Our data suggest that IL11 contributed to the pathogenesis of PE features in mice via placental alterations. We investigated normalizing elevated IL11 levels during pregnancy in mice, in particular, after the onset of placental dysfunction/PE features. Mice received IL11 from E10 through E14 so that administration was ceased at the onset of IL11-induced PE features. By late gestation, IL11 withdrawal restored IL11-induced elevated SBP in pregnant mice to control levels (withdrawal 100.21 mm/Hg ± 1.32 versus IL11 117.65 mm/Hg ± 1.89, $P < 0.0001$) (Fig. 3*A*). Proteinuria was alleviated (withdrawal 50.22 μg/μL ± 10.75 versus IL11 121.20 μg/μL ± 2.53, $P < 0.001$) (Fig. 3*B*), and kidney glomerular hypertrophy seen in pregnant IL11-treated mice subsided (Fig. S8). Fetal weight by E17 was significantly increased by 18% (withdrawal 0.72 g ± 0.04 versus IL11 0.61 g ± 0.04, $P < 0.05$) (Fig. 3*C*). To determine how quenching midlate gestation elevations in IL11 may ameliorate PE features after their onset, we examined implantation sites from IL11 treated and IL11 withdrawal mice at E17. IL11 withdrawal did not rescue IL11-impaired spiral artery remodeling (Fig. 3 *D* and *E*). However, differences in placental labyrinth morphology were evident in the IL11 withdrawal group compared with IL11-treated mice at E17 (Fig. 3*D*). The IL11 withdrawal labyrinth morphology resembled the control (Fig. 3*D*). Isolectin B4 staining highlighted impaired labyrinth branching and structure in IL11-treated placenta but normal branching in the IL11 withdrawal placenta (Fig. 3 *D* and *F*). We investigated the potential for IL11 to modify syncytialization, because the syncytium is the barrier between the maternal and fetal circulations in the human and mouse placenta. However, IL11 had no affect on *Syna* in the mouse placenta (Fig. S5) or syncytins *SYN1*, *SYN2*, or *E-CADHERIN* gene transcription, or human chorionic gonadotrophin (hCG) protein secretion in primary human first-trimester placental explants (Fig. S9). IL11 withdrawal alleviated IL11-induced STAT3 activation in the mouse placenta (Fig. S8). Quantitative gene array analysis was performed on E17 placental tissue samples from mice administered with IL11 or saline from E10 through E17 or E10 through E14 (IL11 withdrawal group). Placental growth factor (*PLGF*) showed a 2.2-fold increase and follistatin-like-3 (*FSTL3*) showed a twofold decrease in the IL11 withdrawal mouse placenta compared with IL11-treated mice at E17 ($P < 0.05$) (Fig. 3*G*).

IL11 in PE in Women and the Potential to Target IL11. Circulating IL11 was significantly increased in women with EO PE (35.48 pg/mL ± 6.29) compared with LO PE (9.77 pg/mL ± 0.58) or normal pregnant gestation-matched controls (4.83 pg/mL ± 1.88) before PE development ($P < 0.001$) (Fig. 4*A*) and also following diagnosis ($P < 0.01$) (Fig. 4*B*) (Table S1). In PE placenta, there was a trending increase in *IL11* and *IL11Rα* mRNA expression (Fig. 4*C*), and IL11 protein was significantly up-regulated (Fig. 4 *D* and *E*). IL11 immunostaining was increased in syncytiotrophoblast and cytotrophoblast (Fig. 4*D*).

Discussion

Our data answer an outstanding question regarding the role of IL11 in trophoblast invasion and placental development in vivo. Evidence for a functional role of IL11 in placentation in vivo was lacking. Female IL11Rα$^{-/-}$ mice are infertile (19), attributed to defective decidualization between E3 and E6, leading to mid-gestation pregnancy loss (28), and thus are not a useful model for studying placentation. This is the first study, to our knowledge, to demonstrate that IL11 is causal of PE features in a mouse model.

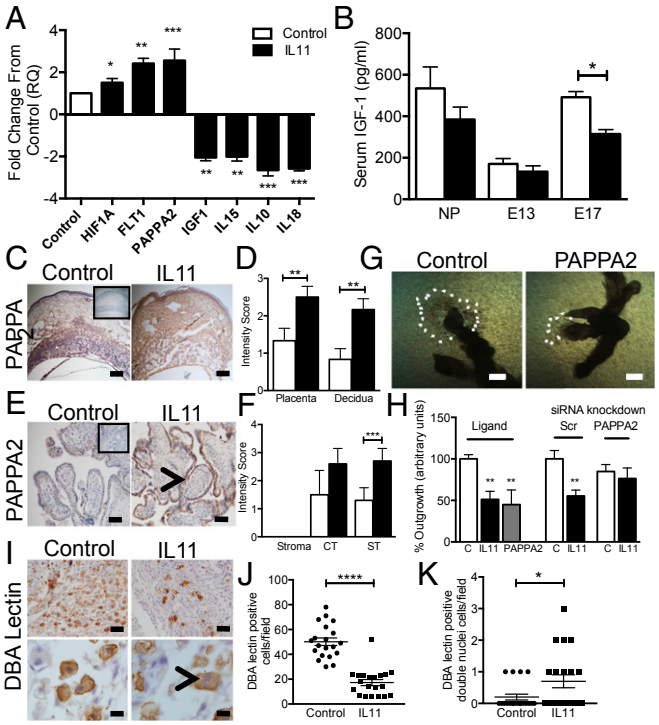

Fig. 2. IL11 impairs IGF1 and up-regulates pappasylin-A2 (PAPPA2) in mouse and human placenta to alter EVT function and alters decidual uNK cells. (*A*) Effects of single-dose IL11 treatment (500 μg/kg), after 2 h, on mouse placental gene expression at E13, normalized to control, from highest to lowest abundance quantified by real-time PCR array; $n = 3$. (*B*) Circulating IGF1 levels were quantified by ELISA in mouse sera. Data are mean ± SEM, one-way ANOVA, $^{*}P < 0.05$, $n = 5$. (*C*) PAPPA2 immunostaining shows increased placental and decidual levels following IL11 administration in E8 through E13 treated mouse embryo sections. *Inset* is negative control. (Scale bar: 500 μm.) (*D*) Data are mean ± SEM, Students *t* test, $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$. (*E*) First-trimester human placental villous explants cultured with saline or IL11 (100 ng/mL) for 48 h were immunostained for PAPPA2 or negative control IgG (*Inset*). (Scale bar: 200 μm.) (*F*) PAPPA2 levels were increased in the syncytiotrophoblast (arrows). Data are mean ± SEM, Students *t* test, $^{***}P < 0.001$, $n = 5$. (*G*) Representative images of first-trimester human placental villous explants cultured on collagen drops to model villous outgrowth and invasion. (Scale bar: 500 μm.) (*H*) Treatment with recombinant human PAPPA2 (100 ng/mL) or IL11 (100 ng/mL) similarly reduced trophoblast outgrowth compared with saline vehicle control. PAPPA2 knockdown by siRNA rescued IL11-impaired EVT outgrowth. Data are mean ± SEM, ANOVA, Tukey's post hoc, $^{**}P < 0.01$, $n = 5$. (*I*) Dolichos biflorus agglutinin (DBA) lectin staining highlights decidual uNK cells in E13 implantation sites (*Top*), and high-power images show double-nuclei uNK cells in IL11-treated implantation sites (*Bottom*). (Scale bar: *Top*, 100 μm; *Bottom*, 50 μm.) (*J*) Decidual uNK cells and (*K*) double-nuclei uNK cells were quantified expressed as number of positive cells per field (20× magnification; three fields per tissue were analyzed from three placentas per mouse). Data are mean ± SEM, Students *t* test, $^{***}P < 0.0001$, $n = 5$.

Defective decidual trophoblast invasion, spiral artery remodeling, and placental labyrinth development were associated with IL11 elevation in mice. Moreover, IL11-administered pregnant mice exhibit diagnostic criteria for PE, including elevated SBP, sFlt-1 dysregulation, and proteinuria with kidney pathology. Our data suggest that elevated SBP and proteinuria can be reversed after damage to the placenta, vasculature, and the kidney has ensued, whereas totally reversing abnormal EVT invasion is not necessary in this model. Furthermore, IL11 is elevated in the serum of women with EO PE before diagnosis and disease onset, strongly suggesting that IL11 is up-regulated early during placentation before PE. IL11 is therefore a likely causal factor of PE in women. Similar to IL11-treated mice that exhibit impaired embryo growth, EO PE in women is almost always associated with IUGR.

Nonpregnant female mice administered with IL11 did not develop PE features. These data demonstrate a pregnancy-specific effect of IL11 in eliciting PE features in mice, strongly suggesting this occurs via placental alterations. Administration of PEGIL11 confirmed at least that the IUGR phenotype seen in IL11-treated mice was attributed to placental insufficiency and not to direct IL11 signaling in fetal tissues. PEGIL11 did not cross the placenta, likely due to the large hydrodynamic volume displayed by the PEG moiety. This finding in itself is novel, suggesting that PEGylation of potential pharmacological therapeutics may reduce potential embryotoxic effects in pregnancy. In accordance with IUGR, IL11-treated mice exhibited placental fibrosis, associated with placental insufficiency and IUGR in women (29). We identified that IL11 reduced placental gene expression and circulating protein levels of IGF1, an important regulator of placental and fetal growth (10), suggesting a mechanism by which IL11 contributes to IUGR via the placenta. Interestingly, IGF1 itself can also alter trophoblast migration (30).

We highlighted a role for exogenous IL11 activating STAT3 in the human and mouse placenta. Other pathways activated by IL11 include the mitogen-activated protein kinase (31), Src-family kinases (32), and phosphatidylinositol 3-kinase signaling pathways (33). The relative importance of each signaling pathway is tissue-specific (34). Our results are consistent with previous findings in female reproductive tissues, where IL11 has been shown to signal exclusively via the JAK/STAT3 pathway in the human endometrium (15) and primary human EVT (16, 17). IL11 withdrawal alleviated IL11-induced STAT3 activation, supporting that IL11 likely regulates placentation in vivo at least in part via STAT3. To confirm this, use of STAT3 inhibitor or gene silencing studies should be performed.

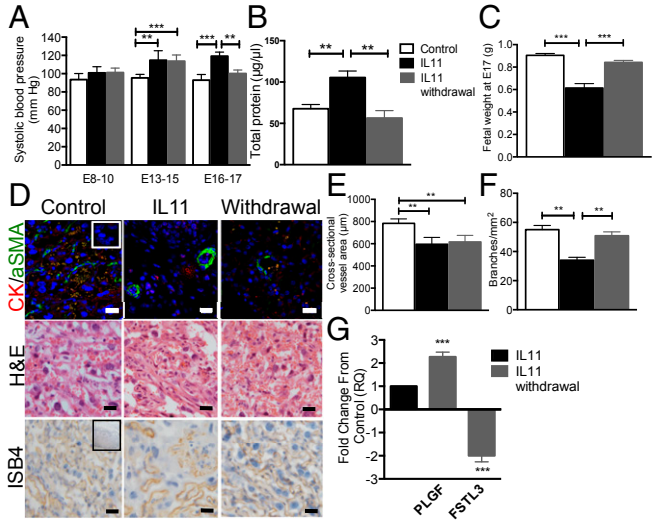

Fig. 3. IL11 withdrawal at mid gestation rescues PE features in mice. Pregnant mice were treated with IL11 (500 $\mu g \cdot kg^{-1} \cdot d^{-1}$) from E10 through E14 (IL11 withdrawal), $n = 8$. (*A*) In the IL11 withdrawal treatment group, SBP was reduced at late gestation (E16 through E17); (*B*) total urinary protein was reduced and (*C*) fetal weight (E17) was significantly increased at late gestation compared with IL11-treated mice from E10 through E17. Data are mean ± SEM, one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{***}P < 0.0001$, $n = 8$. (*D*) E17 embryo sections were stained for trophoblasts (cytokeratin; red) or smooth muscle (α-SMA; green) (*Top*), hematoxylin and eosin H&E (*Middle*), and isolectin B4 (*Bottom*). *Insets* are negative controls. (Scale bar:50 μm.) (*E*) Decidual vessel area was measured in cross sections from three placentas per mouse at E17. Data are mean ± SEM, one-way ANOVA, $^{**}P < 0.01$, $n = 8$. (*F*) IL11 withdrawal rescues IL11-impaired vascular branching in the placental labyrinth, one-way ANOVA, $^{**}P < 0.01$, $n = 8$. (*G*) IL11 withdrawal altered placental growth factor (PLGF) and follistatin-like-3 (FSTL3) placental gene expression when normalized to IL11 treated placenta at E17, quantified by real-time PCR array, $n = 3$.

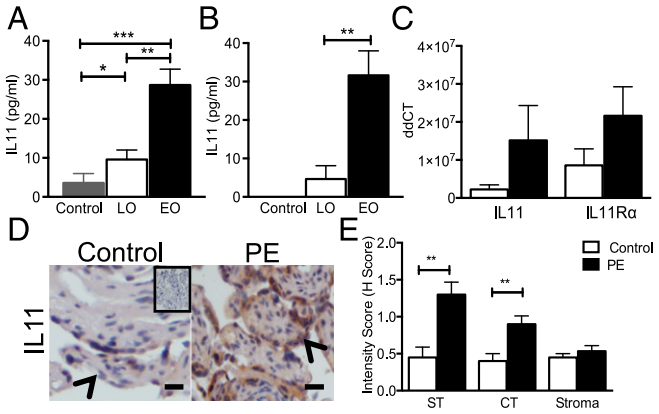

Fig. 4. IL11 levels are elevated in the sera of women before onset of PE. IL11 protein was quantified in pregnant human serum using ELISA (*A*) during the first or second trimester, before the development LO or EO PE, or (*B*) after PE onset. Data are expressed as mean ± SEM, Mann–Whitney *u* test, $*P < 0.05$, $**P < 0.01$, $**P < 0.001$, $n = 15$. (*C*) IL11 and IL11Rα mRNA levels in PE placental villous or preterm match control villous whole tissue were determined by quantitative real-time PCR, normalized to 18S, and expressed as mean delta-delta cycle threshold (ddCT) values ± SEM, Students *t* test, $n = 15$. (*D*) IL11 protein levels in PE placental villous or preterm match controls were determined by immunohistochemistry. *Inset* is negative control. (Scale bar: 50 μm.) (*E*) Staining intensity in the syncytiotrophoblast (ST) (arrow heads), cytotrophoblast (CT), and stroma were scored from 0 (no staining) to 3 (intense staining) by two independent, blinded assessors. Data expressed as mean ± SEM, Students *t* test, $**P < 0.01$, $n = 15$.

Although it is established that IL11 regulates human EVT function (16), previously, the mechanism of IL11-impaired trophoblast invasion and a potential causative role of IL11 in the development of PE were unknown. IL11 did not regulate common proteases associated with human EVT invasion and PE, including matrix metallopeptidases, tissue inhibitors of matrix metallopeptidases, plasminogen activator urokinase, plasminogen activator urokinase receptor, and serpin peptidase inhibitors (16). We have now identified that IL11 significantly up-regulates PAPPA2 in the human and mouse placenta and also circulating levels in pregnant mice. The functional role of PAPPA2 in EVT invasion has not previously been investigated. We demonstrated that PAPPA2 ligand impaired primary human EVT outgrowth, and PAPPA2 knockdown was able to rescue IL11-impaired EVT outgrowth, implying that IL11 impedes trophoblast invasion, at least partly via PAPPA2. This demonstrates a novel mechanism by which IL11 impairs trophoblast invasion and spiral artery remodeling.

Additionally, we have shown that IL11 alters decidual immune cells required for normal spiral artery remodeling. IL10, among other factors, can induce M2 antiinflammatory macrophage polarization, which promotes tissue remodeling and immune tolerance (35). In the IL11-treated mouse placenta, *IL10* was significantly down-regulated, suggesting that IL11 may promote M2 macrophage polarization. Although total macrophages were not altered in the IL11-treated mouse decidua, the polarization status was not investigated. Interestingly, IL10 is also reduced in women with PE (36). Uterine natural killer cells are the most abundant immune cell type at the maternal–fetal interface (27). IL11 significantly reduced placental *IL15*, required for uNK cell maturation and differentiation (37), and *IL18*, secreted by uNKs to mediate normal tissue remodeling (38). In accordance, decidual uNK cell numbers were reduced in IL11-treated pregnant mice at mid-gestation. Binucleate uNK cells, representing senescent cells (39), were also significantly increased. This finding contradicts a previous report on IL11Rα$^{-/-}$ mice, in which IL15 production was compromised and uNK cell localization within the decidual compartment was virtually absent (40). Together, these findings suggest that IL11 signaling is required for normal uNK cell recruitment, but elevated levels also affect recruitment and/or differentiation and normal function. Our findings highlight a complex role for IL11 in impairing spiral artery remodeling, possibly attributed to impaired immune cell recruitment and/or differentiation.

IL11 withdrawal after the development of PE features in mice alleviated elevations in blood pressure and proteinuria and also reduced fetal weight. Differences in placental labyrinth morphology were evident in the IL11 withdrawal group compared with IL11-treated mice at E17, proposing that this layer is dynamic during late gestation. IL11 did not alter the expression of syncytialization genes or hCG in the human or mouse placenta, suggesting that IL11 does not affect syncytialization. Thus, functionally, the precise mechanism of restored placental function remains to be investigated. Impaired labyrinth branching and structure in IL11-treated placenta was restored in the IL11 withdrawal placenta, implying that rescue of the labyrinth may mediate reduced blood pressure and proteinuria. Labyrinth defects alone have been shown to induce hypertension in mice (41). This finding could potentially be attributed to a significant increase in placental growth factor (PLGF) in the IL11 withdrawal compared with IL11-treated mouse placenta at E17. Inducing PLGF has been shown to ameliorate PE symptoms in mice (42), indicating a potential mechanism by which alleviating high levels of IL11 could rescue PE features in vivo in our model.

Clinically diagnosed most often in the late second or third trimester, the only currently available treatment for PE is placental delivery by labor induction or Cesarian section. Therefore, identification of biomarkers in early stages of PE could help to target women at elevated risk for closer follow-up, optimizing delivery timing, and avoiding unnecessary premature deliveries. In this study, we provide evidence that circulating IL11 was significantly increased in women with EO PE. Screening in a large-scale cohort could determine the value of IL11 as a potential biomarker to predict PE. IL11 protein was also significantly increased in human PE villous and EVT. This supports a previous study demonstrating increased decidual IL11, but contradicts reported unchanged IL11 levels in EVT in PE at term, relative to gestation matched controls (20), likely due to population and/or methodological differences.

Despite its well-characterized role in decidualization in humans and mice, IL11 withdrawal had no effect on the decidua, confirming our previous findings that IL11 is required early postimplantation in decidua formation (28). In women and mice, decidual IL11Rα protein levels are significantly reduced during the second trimester or mid-gestation, respectively, highlighting the potential feasibility for targeting IL11 to ameliorate PE in women, without affecting the decidua or pregnancy viability.

In summary, this is the first study, to our knowledge, to demonstrate that IL11 is causal of PE features in a mouse model and likely in women. These findings highlight the potential of IL11 inhibition to rescue PE symptoms in women. We have established an appropriate model to test potential therapeutics for the treatment of PE, of which there are few that have all these features.

Materials and Methods

Nonpregnant or pregnant female mice were injected with recombinant human IL11 (500 $\mu g \cdot kg^{-1} \cdot d^{-1}$, i.p.) or saline control from E8-13, or E10-17. To normalize IL11 levels, mice were i.p. injected with IL11 from E10 through E14 (IL11 withdrawal). SBP was measured by tail cuff plethysmography. Total urinary protein was quantified by Bradford colorimetric protein assay. Kidney glomerular morphology was assessed by electron microscopy. Placental tissue sections were stained with hematoxylin and eosin or Masson's trichrome or immunostained with antibodies against vimentin, cytokeratin, α-SMA, pSTAT3, IL11, or IL11Rα. Mouse Preeclampsia qPCR-Arrays (QIAGEN) were performed on placental RNA. Targets were validated in human and mouse placental tissue by qPCR (Table S2), immunohistochemistry and Western blot. Human first-trimester explants were cultured with IL11

(100 ng/mL), PAPPA2 (100 ng/mL), or PBS. PAPPA2 knockdown was performed by siRNA, and EVT outgrowth was measured using Adobe Photoshop. IL11 levels in human blood and placental tissue samples were measured using ELISA and immunohistochemistry, respectively. All animal procedures were approved by the Monash Medical Centre (B) Animal Ethics Committee, and this study followed the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Human blood collections were approved by the ethical committee of the University of Tokyo and Mushashino Red Cross Hospital, with informed written consent obtained from all women. Human placenta ethics approval was granted by the Southern Health Human Research and Ethics committee. Informed consent was obtained from all participating patients.

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