Effects of Reduced *Gcm1* Expression on Trophoblast Morphology, Fetoplacental Vascularity, and Pregnancy Outcomes in Mice

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Abstract—Preeclampsia is a life-threatening disorder characterized by maternal gestational hypertension and proteinuria that results from placental dysfunction. Placental abnormalities include abnormal syncytiotrophoblast and a 50% reduction in placental expression of the transcription factor *Gcm1*. In mice, homozygous deletion of *Gcm1* prevents syncytiotrophoblast differentiation and is embryonic lethal. We used heterozygous *Gcm1* mutants ($Gcm1^{+/-}$) to test the hypothesis that hypomorphic expression of placental *Gcm1* causes defective syncytiotrophoblast differentiation and maternal and placental phenotypes that resemble preeclampsia. We mated wild-type female mice with $Gcm1^{+/-}$ fathers to obtain wild-type mothers carrying ≈50% $Gcm1^{+/-}$ conceptuses. $Gcm1^{+/-}$ placentas had syncytiotrophoblast abnormalities including reduced gene expression of *Gcm1*-regulated *SynB*, elevated expression of *sFlt1*, a thickened interhemal membrane separating maternal and fetal circulations, and electron microscopic evidence in syncytiotrophoblast of necrosis and impaired maternal-fetal transfer. Fetoplacental vascularity was quantified by histomorphometry and microcomputed tomography imaging. In $Gcm1^{+/-}$, it was ≈30% greater than wild-type littermates, whereas placental vascular endothelial growth factor A (*Vegfa*) expression and fetal and placental weights did not differ. Wild-type mothers carrying $Gcm1^{+/-}$ conceptuses developed late gestational hypertension (118±2 versus 109.6±0.7 mm Hg in controls; $P<0.05$). We next correlated fetoplacental vascularity with placental *Gcm1* expression in human control and pathological pregnancies and found that, as in mice, fetoplacental vascularity increased when GCM1 protein expression decreased ($R^2=-0.45$; $P<0.05$). These results support a role for reduced placental *Gcm1* expression as a causative factor in defective syncytiotrophoblast differentiation and maternal and placental phenotypes in preeclampsia in humans. (***Hypertension*. 2012;59:732-739.**) ● **Online Data Supplement**

Key Words: syncytin ■ placenta ■ preeclampsia ■ angiogenesis ■ VEGFA ■ placental growth factor ■ sFlt1

Placental dysfunction is believed to be the major cause of one of the most common and serious complications of human pregnancy, preeclampsia (PE).[1] This potentially life-threatening hypertensive disorder adversely impacts ≤5% of all pregnancies and has no known cure. In PE, growing evidence implicates abnormalities in syncytiotrophoblast (SynT) in the villous exchange region of the placenta.[1–6] SynTs are thin, fetal-derived cells that lie between the maternal blood flowing through the villous exchange region of the placenta and the fetal blood flowing through fetal vessels in the highly vascularized villi. This surface is essential for fetomaternal communication and exchange and is formed through the differentiation of SynT from the underlying villous cytotrophoblast cells under the control of the transcription factor GCM1 (glial cells missing 1).[7] *Gcm1* in the conceptus is almost exclusively expressed in a subset of trophoblast in the placenta[8,9] and is a critical regulator of the SynT cell type.[10]

In the PE placenta, *Gcm1* expression is reduced by ≈50%,[4] as is expression of the immediate downstream target of *Gcm1*,[11] *Syn-1*.[5,6] That SynT is abnormal and potentially causative in PE is supported by abnormal levels of SynT-produced factors, such as pregnancy-associated plasma protein A, human chorionic gonadotropin, and inhibin in maternal blood during early pregnancy in women at high risk for the later development of PE.[12,13] When PE develops, the abnormal syncytial surface is prothrombotic, leading to infarctions within the intervillous space,[14] and is excessively

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shed as aponecrotic fragments into the maternal circulation[1,3]; both are believed to be factors contributing to clinical signs of disease.[1,3]

The availability of *Gcm1* deletion mutants provides an opportunity to explore the function of *Gcm1* in vivo. Homozygous deletion mutants show the critical importance of *Gcm1*; embryonic lethality results from absent SynT differentiation and failure to form the exchange region known as the labyrinth in mice.[15,16] Relative to placental differences between species, the placentas of humans and mice are strongly similar,[17,18] although differences exist. In both species, maternal blood perfuses an intervillous space lined by fetal-derived trophoblasts that encase the fetoplacental vasculature within the exchange region. In both species, *Gcm1* is expressed in trophoblast cells differentiating into SynT.[19,20] However, whereas humans have a single SynT layer, mice have 2 layers; SynT-II is adjacent to the fetal vasculature, and it is intimately connected to SynT-I via gap junctions and nutrient transporters[21,22] and, unlike SynT-I, only SynT-II expresses *Gcm1*.[10] In humans, the SynT surface is fully exposed to maternal blood, whereas in mice the SynT-I surface is partially covered by a discontinuous layer of sinusoidal trophoblast giant cells (sTGCs).[23] Given the difficulty in establishing causal relationships in human studies, here we exploit similarities between humans and mice by using heterozygous *Gcm1* deletion mutants to determine whether reduced *Gcm1* expression in mice causes changes in trophoblast morphology, fetoplacental vascularity, and pregnancy outcomes that resemble PE in humans.

Materials and Methods

Mouse and human experiments were approved by institutional review committees. For complete methods please see the online-only Data Supplement.

Mouse Model

CD1 females mated to males with heterozygous deletion of *Gcm1* (*Gcm1*$^{+/-}$)[15,16] carried ≈50% *Gcm1*$^{+/-}$ and ≈50% wild-type conceptuses. Control pregnancies were CD1 females mated with CD1 males. At embryonic day 13.5 (E13.5) and E17.5, placentas were microdissected to remove decidual tissue and frozen for protein assays or fixed for histology. Others were microdissected to obtain labyrinth-enriched samples for mRNA quantification by quantitative RT-PCR (primers in Table S1, available in the online-only Data Supplement).

Human Patients

Placental villous tissue and matched histological samples were obtained from a BioBank (http://biobank.lunenfeld.ca). Samples were from pregnancies with PE, intrauterine growth restriction (IUGR; and without PE), and from gestation-matched preterm controls (Table). PE was diagnosed as new-onset gestational hypertension, proteinuria, and reversal of hypertension and proteinuria by 12 weeks postpartum.[24] IUGR was diagnosed as fetal sex and gestation-adjusted birth weights less than the third centile[25] and absent end-diastolic blood flow velocity in umbilical artery Doppler waveforms.

Histology and Imaging

In mice, images from midline placental sections were quantified by histomorphometry[26] to obtain areas of placental components or evaluated by transmission electron microscopy. Counts of Ki67 positive cells in the labyrinth excluded sinusoidal trophoblast giant cells. In humans, the proportion of fetoplacental vasculature in villi was quantified in hematoxylin-eosin–stained sections. Microcomputed tomography was used to quantify fetoplacental arteries and arterioles ≥50 μm in diameter, as published.[27–29] Capillaries were examined in vascular corrosion casts as published.[17,27]

Table. Human Clinical Outcomes and Histomorphometry Data

Variables	Preterm Controls (N=5)	PE (N=5)	IUGR (N=5)
Gestational age at delivery, wk	30.9±0.8	30.5±1.2	30.7±1.2
Range, wk	28–33	28–35	28–34
Systolic BP, mm Hg	112±5	185±5†	138±7
Diastolic BP, mm Hg	61±6	109±4†	86±4*
Fetal weight, g	1772±127	1364±200	998±179*
Placental weight, g‡	271±23	189±31	163±23*
Fetal vasculature in villi, % area	11.3±1.1	19.1±1.2†	11.1±1.4

BP indicates blood pressure; PE, preeclampsia; IUGR, intrauterine growth restriction.

*$P<0.05$ vs preterm controls.

†$P<0.05$ vs preterm controls and vs IUGR.

‡Data show placental weight after fixation; mean±SE.

Maternal Phenotypes

Arterial pressure in awake mice was measured by tail-cuff plethysmography, urine protein by the Bradford method, urine creatinine by the Jaffé reaction, and sFLT1 protein in maternal plasma by ELISA.

Statistical Analysis

Wild-type littermates from CD1×*Gcm1*$^{+/-}$ matings were used as controls for *Gcm1*$^{+/-}$ conceptuses. Statistical significance ($P<0.05$) was determined using Wilcoxon sign-ranked test for quantitative RT-PCR data, 2-tailed Student *t* test (genotype effects), or 2-way ANOVA (genotype and gestation effects), followed by the Tukey post hoc test. Results show mean±SE.

Results

Abnormal Trophoblast Differentiation

Gcm1 in mice is specifically expressed in the SynT-II layer of the labyrinth where it regulates expression of its downstream target, *SynB*[10]. In the *Gcm1*$^{+/-}$ labyrinth, expression of *Gcm1* mRNA and protein was decreased by ≈50% relative to wild-type littermates (Figure S1 in the online-only Data Supplement). *Gcm1* deficiency resulted in a dysregulation of trophoblast differentiation as shown by an ≈35% decrease in *SynB* mRNA expression (Figure S1A), an ≈2-fold increase in cell proliferation within the labyrinth (Figure S2), and prominent ultrastructural abnormalities of the 2 syncytial layers (SynT-I and SynT-II) at E17.5 (Figure 1). There was evidence of necrosis[2,30] in SynT-II including pale cytoplasm, numerous vacuoles, and swollen and degenerating mitochondria in transmission electron microscopy images (Figure 1A through 1C). The *Gcm1*$^{+/-}$ SynT-I layer appeared to differentiate normally in that expression of its marker gene, *SynA*, was not significantly affected (1.0±0.2- and 1.3±0.3-fold change relative to wild-type littermates, at E13.5 and E17.5, respectively; $P>0.05$). Nevertheless, the SynT-I layer exhibited ultrastructural abnormalities, including increased electron density and the presence of large fluid-filled vacuoles (Figure 1A and 1B). SynT-I and SynT-II appeared thicker in

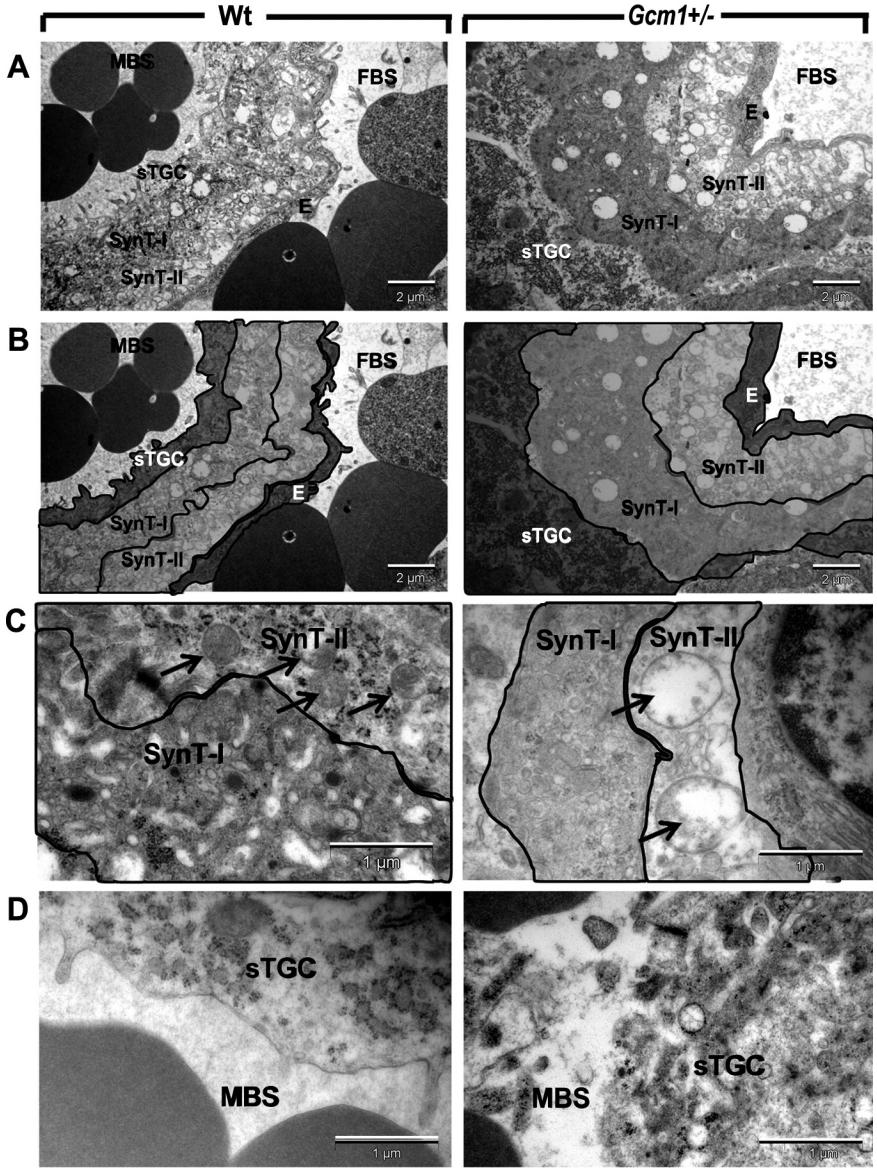

Figure 1. Transmission electron micrographs of the interhemal membrane that separates the maternal and fetal blood spaces in the labyrinth. Representative images for the wild-type (Wt; left) and *Gcm1*$^{+/-}$ (right) labyrinths at embryonic day 17.5 are shown (from N=4 Wt and N=4 *Gcm1*$^{+/-}$). **A**, Images showing more numerous vacuoles in a darker syncytiotrophoblast (SynT) I and paler SynT-II in *Gcm1*$^{+/-}$ placentas compared with Wt. **B**, Images in **A** with shading to show cell layers. **C**, Image showing abnormal mitochondria in SynT-II of *Gcm1*$^{+/-}$ placenta (right) in contrast to Wt (left). Arrows point to mitochondria. Added lines demarcate layers. **D**, Example showing cellular debris from sinusoidal trophoblast giant cells (sTGCs) in adjacent maternal blood space in the *Gcm1*$^{+/-}$ labyrinth (right) in contrast to Wt (left). FBS indicates fetal blood space; MBS, maternal blood space; E, fetal endothelial cells. Arrow, mitochondria. Scale bar=2 μm (**A** and **B**); 1 μm (**C** and **D**).

Gcm1$^{+/-}$ placentas compared with wild-type littermate controls at both E13.5 and E17.5 (Figure 1A and 1B). In addition, loss of apical membrane integrity in focal areas of the sTGCs (in direct contact with maternal blood) was observed in transmission electron microscopy images of the *Gcm1*$^{+/-}$ placenta at E17.5 (Figure 1D).

Abnormal Placental Histomorphology and Vascularity

At E13.5, the *Gcm1*$^{+/-}$ labyrinth was significantly decreased in cross-sectional area, and there were significant increases in the proportion of maternal blood spaces and in the thickness of the interhemal membrane relative to wild-type littermates (Figure 2A through 2D). There was also an increase in the area of clustered cuboidal cells within the *Gcm1*$^{+/-}$ labyrinth (Figure 2C). In contrast, at E17.5, *Gcm1*$^{+/-}$ placentas had a significantly higher proportion of fetal blood spaces in the labyrinth compared with wild-type littermates (Figure 2G). The latter is consistent with increased fetoplacental vascularity at E15.5 in *Gcm1*$^{+/-}$ placentas, as shown by significant increases in the total number and length of arterial segments with diameters >50 μm by microcomputed tomography (Figure 3) and apparent increases in the density of capillaries in vascular casts and in histological sections (Figure S3A through S3C).

Intriguingly, the mRNA and protein expression profile of VEGFA, a potent proangiogenic molecule thought to play an important role in placental angiogenesis,[31] demonstrated *decreased* expression across gestation within the *Gcm1*$^{+/-}$ placenta relative to that of wild-type littermates (Figure 4A and 4B). A similar examination of the mRNA and protein expression of another proangiogenic molecule, placental growth factor (PGF) demonstrated a modest increase in expression at E13.5 in the *Gcm1*$^{+/-}$ placenta and no difference in expression by E17.5 (late gestation) compared with littermate controls (Figure 4A and 4C).

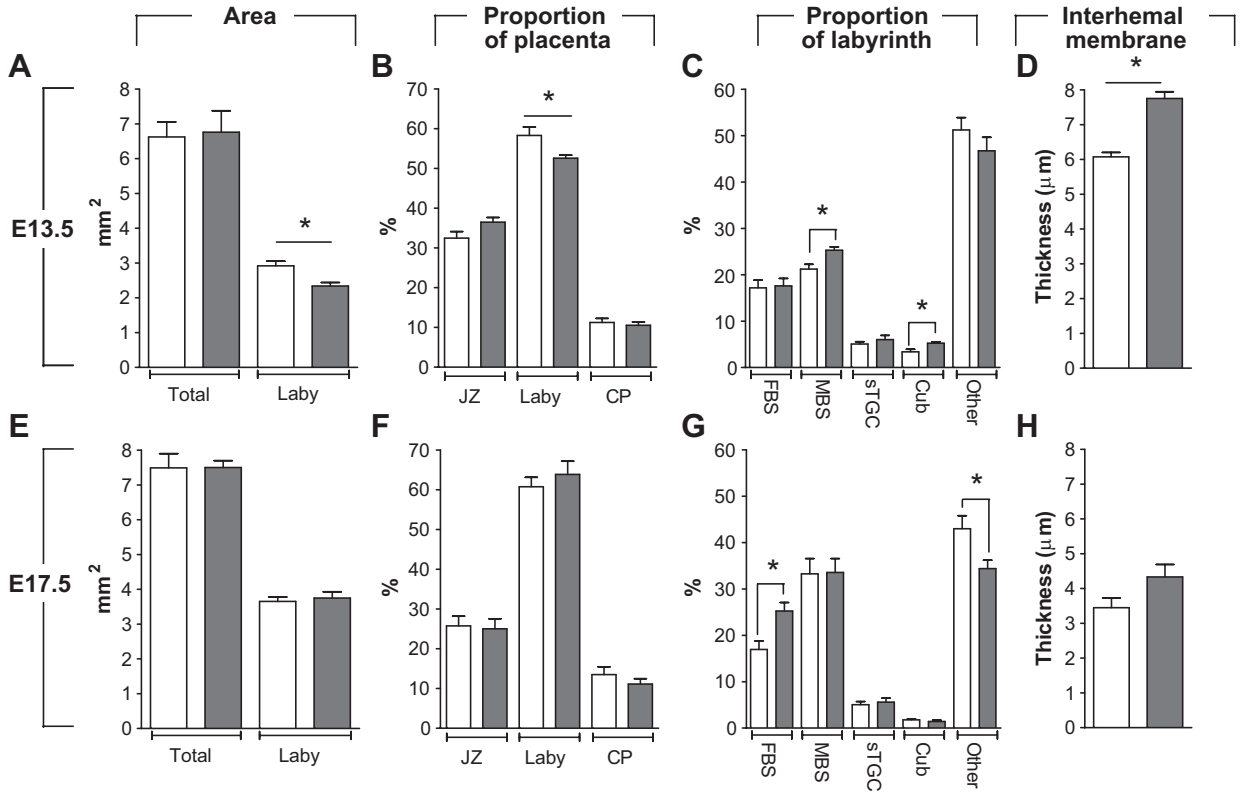

Figure 2. Histomorphometry of *Gcm1*$^{+/-}$ placentas (■) vs wild-type littermate controls (Wt; □; N=5 pregnancies). *Gcm1*$^{+/-}$ placentas had a smaller labyrinth (**A** and **B**) and increased maternal blood space (**C**) and cuboidal cell area (**C**) within the labyrinth, as well as a thicker interhemal membrane (**D**) at E13.5. By E17.5, there was no difference in labyrinth size in *Gcm1*$^{+/-}$ placentas (**E** and **F**) or interhemal membrane thickness (**H**), but fetal blood space was significantly increased (**G**) vs Wt. Laby indicates labyrinth; JZ, junctional zone; CP, chorionic plate; FBS, fetal blood space; MBS, maternal blood space; sTGC, sinusoidal trophoblast giant cells; Cub, cuboidal cells. *$P<0.05$.

Fetal and Placental Growth

Despite pronounced abnormalities in trophoblast differentiation and placental morphology, *Gcm1*$^{+/-}$ conceptuses grew normally; at E17.5 there were no significant differences between fetal weights of *Gcm1*$^{+/-}$ (1.2±0.1 g) versus wild-type littermates (1.1±0.1 g) or in their placental weights (92±15 versus 90±10 mg, respectively). There was no evidence for an effect of paternal genotype on litter size or number of resorptions.

Clinical Signs of PE in Mothers

CD1 females mated with *Gcm1*$^{+/-}$ males carried litters in which ≈50% of conceptuses were *Gcm1*$^{+/-}$. These mothers had significantly higher mean arterial pressure at E17.5 compared with CD1 females mated with CD1 males (118±2 versus 109.6±0.7 mm Hg, respectively; Figure 5A). The increase in maternal pressure in late gestation was positively correlated with the number of *Gcm1*$^{+/-}$ pups within the litter ($R^2=0.54$; $P<0.05$; Figure 5B). CD1 mothers carrying mixed litters did not have proteinuria in late gestation; they excreted 1.8±0.5 mg of protein per milligram of creatinine versus 2.1±0.7 mg of protein per milligram of creatinine in controls. As in human PE placentas,[32] the *Gcm1*$^{+/-}$ labyrinth demonstrated a 2-fold increase in *sFlt1* mRNA expression relative to the wild-type littermate controls (Figure 5C). However, in contrast to human PE,[32] there was no significant increase in maternal plasma sFLT1 protein (Figure 5D).

Correlation Between GCM1 and Fetoplacental Vascularity in Humans

Human clinical outcomes for patients with PE, with IUGR, and for gestation-matched controls are shown in the Table. The proportion of fetal vascular space within the villous tissue of patients with PE was significantly elevated relative to gestation-matched controls and IUGR placentas (Table). Across all of the groups, the proportion of fetal vascular space within villous tissue was negatively correlated with GCM1 protein expression ($R^2=-0.45$; $P<0.05$; Figure 6).

Discussion

In the current study, hypomorphic expression of placental *Gcm1* caused defective SynT differentiation and maternal and placental phenotypes resembling PE in humans. As anticipated, heterozygous *Gcm1* gene deletion reduced placental *Gcm1* expression by ≈50%, a decrement similar to that in human PE placentas.[4] SynT-II cells exhibited prominent abnormalities, including reduced expression of *SynB* and ultrastructural evidence of necrosis.[30] Both SynT-I and SynT-II appeared thickened and ultrastructurally abnormal. Nevertheless *Gcm1*$^{+/-}$ fetuses were normally grown. Similarly, fetuses are normally grown in the majority of PE pregnancies,[33] despite evidence of hypomorphic *Gcm1* expression and SynT dysfunction.[1–6] An unexpected and striking finding of the current study was increased fetoplacental

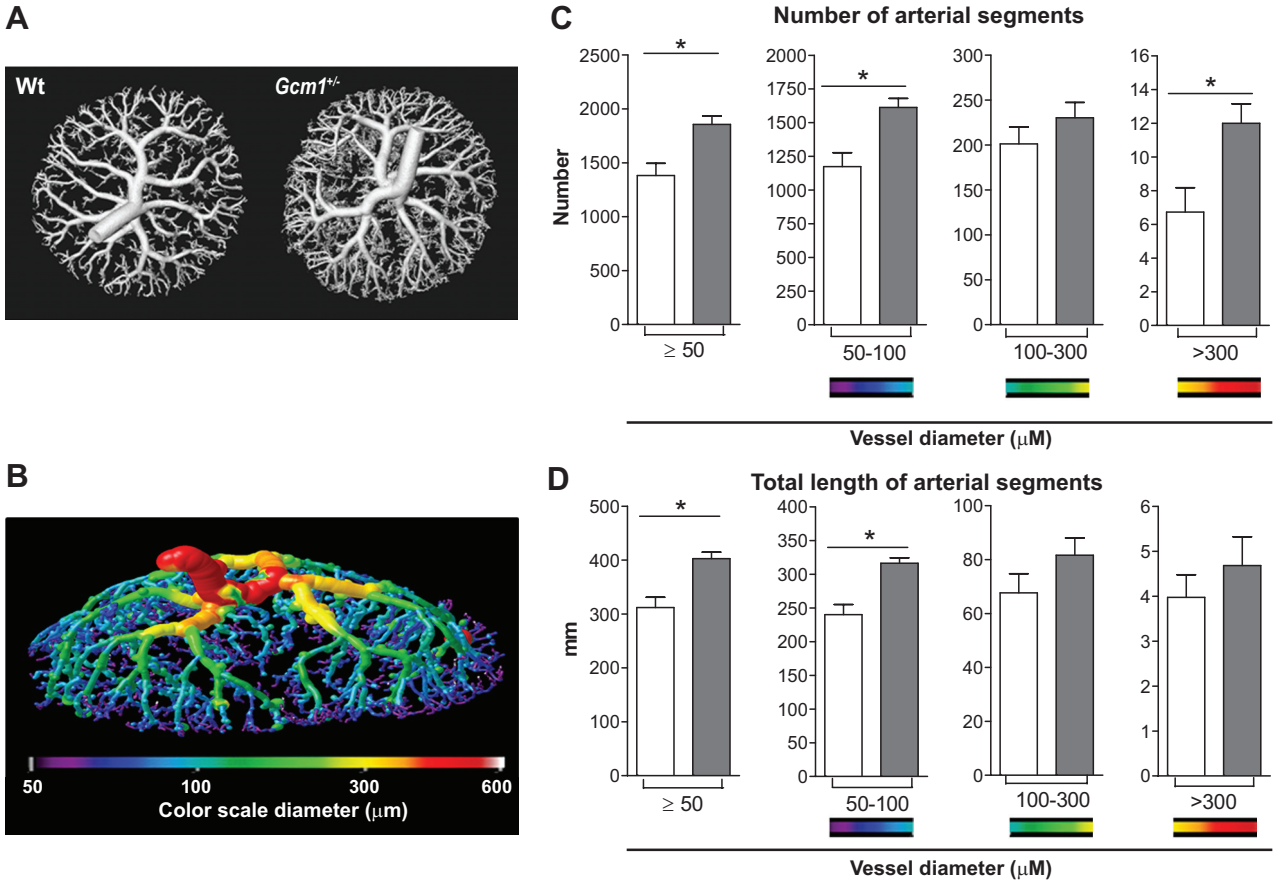

Figure 3. Arterial vascularity in the labyrinth at embryonic day (E) 15.5 by microcomputed tomography (CT). **A**, Images from a *Gcm1*$^{+/-}$ and wild-type littermate control (Wt). **B**, Color-coded arterial tree to show the anatomic distribution of vessel diameters. **C** and **D**, Quantitative analysis showing significant overall increases in (**C**) the total number of vessel segments and (**D**) the total length of vessel segments in arterial trees of *Gcm1*$^{+/-}$ placentas (■) vs Wt (□). Increased vascularity was most prominent in the smallest diameter range (ie, 50–100 μm). *$P<0.05$. N=11 per genotype (2–3 conceptuses per genotype from 5 pregnancies).

vascularity in the *Gcm1*$^{+/-}$ mouse placentas. We show a similar increase in fetoplacental vascularity correlates with decreased GCM1 protein expression in human placental villi. Finally, results show that conceptuses with hypomorphic *Gcm1* expression caused maternal hypertension in late gestation in otherwise healthy, wild-type mothers.

Hypomorphic *Gcm1* expression caused abnormal differentiation of SynT-II cells, as shown by a reduction in expression of

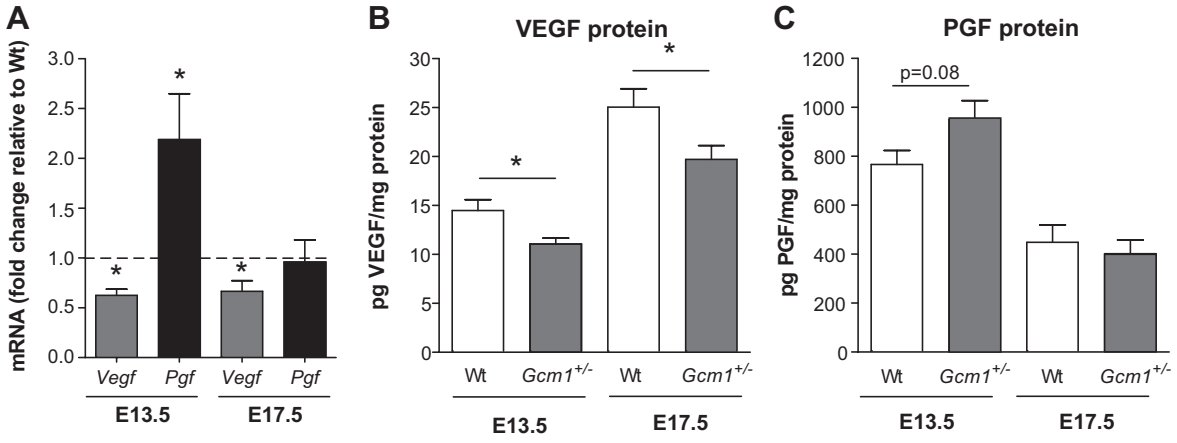

Figure 4. Expression of proangiogenic factors at embryonic day (E) 13.5 and E17.5. **A**, mRNA expression of *Vegfa* and *Pgf* in labyrinth-enriched *Gcm1*$^{+/-}$ samples normalized using 3 housekeeping genes and expressed as a fold change relative to wild-type littermate controls (Wt; hashed line at 1.0). *Vegfa* mRNA was significantly reduced at E13.5 and E17.5 in *Gcm1*$^{+/-}$, whereas *Pgf* mRNA was significantly increased at E13.5 only. **B**, Placental VEGFA protein by ELISA was significantly decreased at both ages. **C**, Placental PGF protein by ELISA was not significantly changed. Number of pregnancies for each gestational age and genotype was N=6. *$P<0.05$.

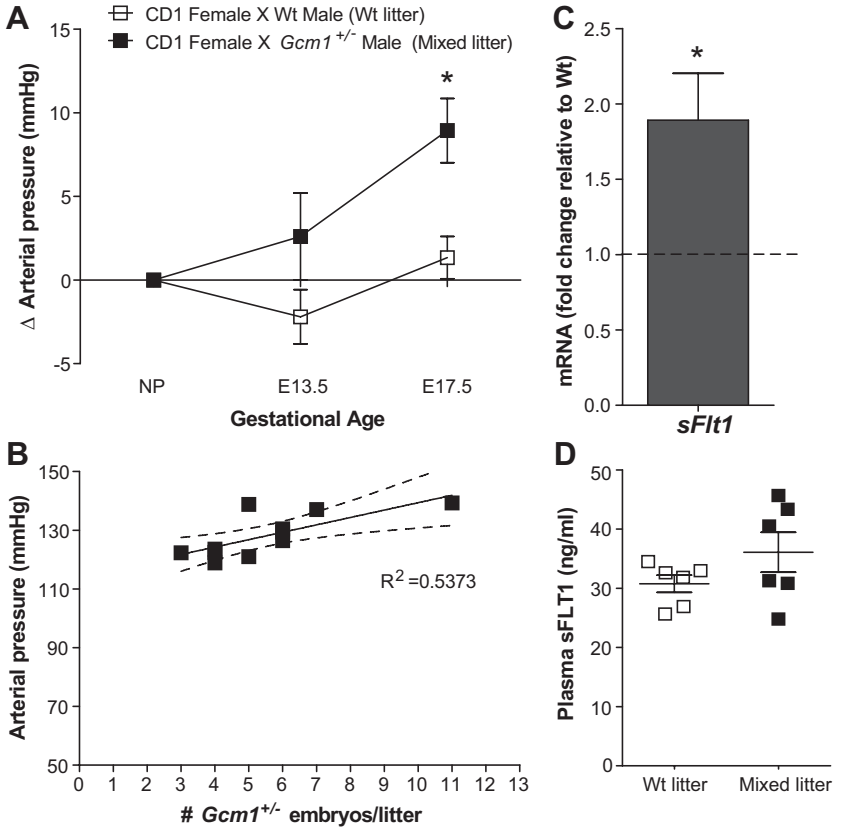

Figure 5. Effect of *Gcm1*$^{+/-}$ conceptuses in CD1 mothers on maternal arterial pressure and placental and plasma sFlt1 levels. **A**, Arterial pressure in CD1 mothers carrying mixed litters of wild-type (Wt) and *Gcm1*$^{+/-}$ conceptuses (■) was significantly higher at E17.5 than in CD1 mothers with Wt conceptuses only (□) when expressed as a change (Δ) from the nonpregnant state (NP). **B**, Maternal arterial pressure was positively correlated with the number of *Gcm1*$^{+/-}$ conceptuses at E17.5. R^2 value $P<0.05$. **C**, mRNA expression of the antiangiogenic factor *sFlt1* was higher in *Gcm1*$^{+/-}$ placentas expressed relative to Wt littermate controls at E17.5 (hashed line at 1.0). **D**, Maternal plasma sFLT1 protein was not significantly different whether CD1 mothers carried only Wt fetuses (□) or mixed litters (■) at E17.5. Number of pregnancies for each gestational age and paternal genotype was N=6 in **A**, **C**, and **D**, and N=10 in **B**. *$P<0.05$.

the *Gcm1*-regulated fusogenic protein *SynB* and by prominent ultrastructural abnormalities that directly paralleled the described focal necrosis abnormalities observed in the SynT layer in human PE.[30,34] SynT-II is tightly integrated with the adjacent SynT-I layer. SynT-I uniquely expresses *Syncytin A* (*SynA*).[10] We found that *SynA* expression was not altered in *Gcm1*$^{+/-}$ placentas suggesting normal differentiation of SynT-I, a non-*Gcm1*–expressing cell type.[10] Similarly, no change in *SynA* expression was observed in homozygous *Gcm1* knockout placentas.[10] Nevertheless, morphology of the SynT-I layer was markedly abnormal in *Gcm1*$^{+/-}$ placentas; transmission electron microscopy imaging showed that it appeared thicker, contained more electron dense material, and contained many large, fluid-filled vacuoles. SynT-I and SynT-II are intimately connected by gap junctions and ultimately function as a single syncytial layer across which maternal-fetal transfer occurs.[21,22] Thus, it is likely

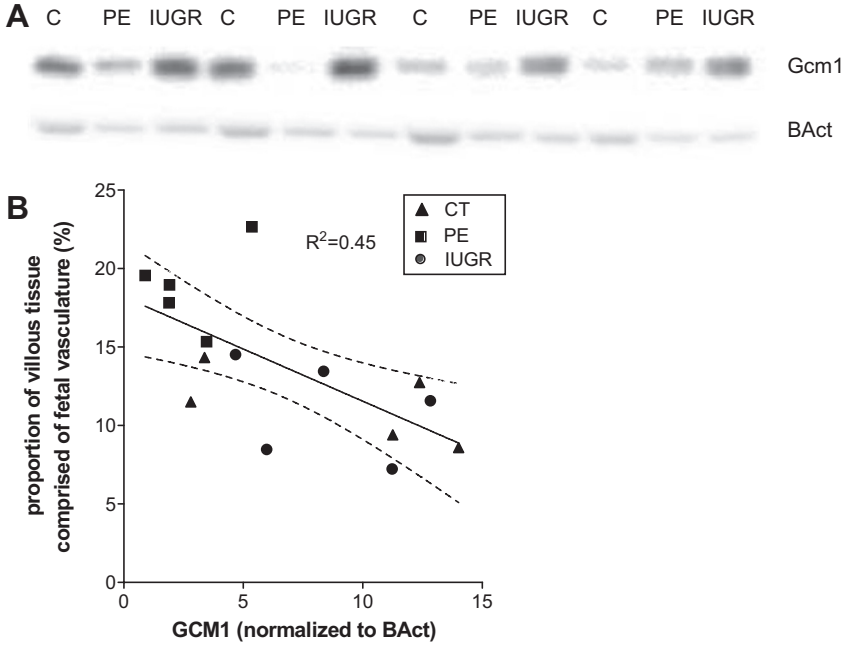

Figure 6. GCM1 protein expression and its correlation with villous fetoplacental vascularity in human placentas. **A**, Western blot showing GCM1 protein expression in placentas from preterm control (C), preeclamptic (PE), and intrauterine growth-restricted (IUGR) pregnancies at delivery. β-Actin (BAct) was used as the loading control. **B**, There was a significant negative correlation between placental GCM1 expression and the proportion of villous tissue composed of fetal vasculature in C, PE, and IUGR placentas (N=5 per group). R^2 value $P<0.05$.

that a primary limitation in transport across the abnormal SynT-II layer contributed to abnormal SynT-I morphology by impeding the egress of protein and vacuoles from SynT-I.

Abnormal SynT-II in *Gcm1*$^{+/-}$ placentas additionally influenced the discontinuous sTGCs[23] that overlie SynT-I in the maternal blood spaces of the labyrinth. Apical membrane integrity of sTGC was lost in localized areas, and cytoplasmic cellular contents were visible within the adjacent maternal blood space. This was unexpected given that the sTGCs are only loosely connected to the underlying SynT-I layer through desmosomal attachments[23] and are not in direct contact with SynT-II cells. Nevertheless, results suggest that hypomorphic *Gcm1* expression in the PE placenta in human pregnancy may play a similar causative role in promoting the shedding of placental debris into the maternal circulation. This debris is thought to be responsible, at least in part, for the widespread maternal endothelial dysfunction and hypertension in PE.[1,2] In mice, sTGCs release hormones such as placental lactogen, into the maternal circulation,[10] so abnormalities observed in this cell type in the current study may also alter maternal responses to pregnancy by an endocrine mechanism.

A remarkable finding in the current study was that fetal growth was not compromised despite marked abnormalities in SynT-I, SynT-II, and sTGC cell morphology, as well as augmented interhemal membrane thickness. It is possible that normal fetal growth was protected by increased fetoplacental vascular density in the *Gcm1*$^{+/-}$ labyrinth. Increased fetoplacental vascularity may have been caused by feedback mechanisms invoked by impaired transfer caused by the thicker, malfunctioning fetal-maternal exchange barrier. However, abnormal SynT-I in *SynA* knockout placentas also causes abnormal interhemal membrane morphology, but in that case results in fetoplacental hypovascularity, fetal growth restriction, and fetal lethality by E13.5.[35] Furthermore, *Gcm1* overexpression results in fetoplacental hypovascularity, leading to fetal growth restriction in late-gestation in mice.[36] Thus, it is more likely that dysregulated SynT-II differentiation directly influences fetoplacental vascularization through altered signaling to the adjacent fetal endothelial and mesenchymal cells. How angiogenesis is augmented in the face of decreased mRNA and protein expression of the potent proangiogenic factor VEGFA, increased mRNA expression of the antiangiogenic *sFlt1*, and only modest increases in PGF at E13.5 is not known. It is possible that alternate unidentified angiogenic mechanisms are activated within the *Gcm1*$^{+/-}$ placenta. The same paradox among villous trophoblast pathology,[2,30,36] high placental expression of the antiangiogenic sFlt1,[32] and augmented villous angiogenesis (current study) occurs in PE where the majority of women give birth to infants of normal weight.[33] Indeed, the current results in *Gcm1*$^{+/-}$ mice prompted the examination and discovery of a similar negative correlation between placental vascularity and GCM1 protein expression in placental biopsies from normal and pathological pregnancies (PE and IUGR). Thus, this mouse model provides an excellent opportunity to explore this apparent incongruity and to advance our understanding of the influence of SynT on fetoplacental vascularization.

Hypomorphic *Gcm1* expression resulted in significantly increased maternal arterial pressure in late gestation, although how hypomorphic *Gcm1* in SynT-II caused this change is not known. Mechanisms other than increased circulating sFLT1 must be driving maternal hypertension, because, despite increased placental mRNA expression of *sFlt1* in the *Gcm1*$^{+/-}$ placenta, sFLT1 protein in the maternal circulation was not significantly elevated. Maternal proteinuria was also absent, suggesting that renal function was unimpaired. It is possible that placental debris from sTGC entered the maternal circulation and caused endothelial cell inflammation, increased peripheral vascular resistance, and hypertension (as in human PE[1,2]), or that abnormal endocrine signals from the dysfunctional sTGC are responsible. The increase in arterial pressure in this mouse model was modest, and this could mean that, alone, the hypomorphic expression of *Gcm1* in the placenta of women with PE is insufficient to completely explain their hypertension. This is consistent with evidence suggesting that a combination of placental dysfunction and underlying maternal predispositions to endothelial dysfunction may be required for PE in women.[37,38] Alternatively, it is also possible that relatively mild maternal effects were observed, because not all of the conceptuses were *Gcm1*$^{+/-}$; this cannot be achieved in a wild-type mother by natural breeding, because *Gcm1* deletion is homozygous lethal. Wild-type conceptuses may diminish maternal effects by effectively diluting circulating factors released by *Gcm1*$^{+/-}$ placentas and/or by releasing protective factors into the maternal circulation. Indeed, a positive correlation was observed when maternal arterial pressure was plotted against the number of *Gcm1*$^{+/-}$ conceptuses within the pregnancy. Interestingly, evidence supports a similar mechanism in human twin pregnancies complicated by IUGR where a healthy co-twin appears to be protective; PE affects 6.8% of singleton pregnancies complicated with IUGR versus 1.5% in twin pregnancies when only 1 twin has IUGR.[39] Thus, results of the current study show that hypomorphic *Gcm1* expression in SynT can elevate maternal arterial pressure in late-gestation in mice. This means that hypomorphic placental *Gcm1* expression may not just correlate with human PE but could also play a causative role in maternal hypertension, one of the hallmark signs of PE. This conclusion is supported by the recent identification of a fetal variant in the *Gcm1* gene that is associated with gestational hypertension in human pregnancy.[40]

Perspectives

Hypomorphic placental *Gcm1* expression caused dysregulated SynT-II differentiation, abnormal morphology and dysfunction of the interhemal membrane, and augmented fetoplacental vascularity, and caused maternal hypertension in late gestation. In addition, we showed in humans that there is a similar inverse correlation between fetoplacental vascularity of placental villi and *Gcm1* expression. These results, therefore, support a role for reduced placental *Gcm1* expression as a causative factor in defective SynT differentiation and maternal and placental phenotypes in PE in humans.

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Disclosures

None.

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