Investigation of a Paternal-Mediated Preeclampsia-Like Pregnancy Phenotype Mouse Model

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ABSTRACT

Preeclampsia, a pregnancy specific syndrome characterized by new onset hypertension and proteinuria, is a leading cause of maternal and neonatal morbidity and mortality. While no animal model perfectly mimics the human syndrome, breeding $C1q^{-/-}$ (male) to C57 (female) mice results in a preeclampsia-like pregnancy including pregnancy-specific hypertension, vascular dysfunction and altering placental phenotype. As the placental genotype is primarily paternally driven, lack of paternal C1q is likely driving this preeclampsia-like phenotype. However, more work is needed to investigate whether a lack of maternal C1q also contributes to this preeclampsia-like phenotype. The aim of this study was to investigate the pregnancy phenotype of genetic control ($C1q^{-/-}$ female bred to C57 male) mice. Blood pressure was monitored during pregnancy and vascular function assessed during late pregnancy (gestation day 17.5) in genetic control females. These data were compared to similar data obtained from control (C57 male bred to C57 female) and preeclampsia-like ($C1q^{-/-}$ male bred to C57 female) pregnant mice. Genetic control blood pressure and vascular function data were similar to that of the control pregnancy group, indicating no significant effect of maternal C1q deficiency on the “preeclampsia-like” pregnancy phenotype. As understanding preeclampsia and its effect on women’s health is critical, the work presented is important to confirm the $C1q^{-/-}$ x C57 mouse model as a useful model for studying this syndrome further.

INTRODUCTION

Preeclampsia is a pregnancy specific syndrome characterized by new onset gestational hypertension and end-organ dysfunction with or without proteinuria, and typical onset after 20 weeks of gestation (3). Preeclampsia causes serious maternal and fetal morbidity and mortality and affects about 3-6% of all pregnancies (3, 4). Although the pathophysiology of preeclampsia is not well understood, there is a proposed two stage model of preeclampsia that the syndrome is initiated by impaired maternal blood flow to the placenta due to placental malperfusion (stage 1), which then leads to the maternal syndrome (stage 2) (4, 19, 20). There is a specific theory that failed remodeling of the maternal spiral arteries in the placenta is caused by impaired trophoblast invasion into and around the uterine spiral arteries during placental development (19). This idea is supported by the finding that endovascular trophoblasts are present in spiral artery remodeling during early pregnancy and are important and present throughout pregnancy, and play a role in the development of preeclampsia (25).

Complement component C1q is hypothesized to play an important role in placental development by regulating trophoblast migration and spiral artery remodeling (2). C1q deficiency is associated with impaired placental labyrinth development and

poor vessel formation and remodeling (17). A novel mouse model of preeclampsia, the C1q$^{-/-}$ (male) bred to a wildtype C57 (female) model, originally reported by Girardi et al. mimics the pregnancy-specific hypertension and proteinuria of preeclampsia (11, 21). Results from this study show the effect of male C1q deficiency on gestational health of both wildtype and C1q deficient dams. Specifically, there is evidence of impaired trophoblast invasion in the placentas of pregnant dams (21). Since the placenta is more heavily influenced by paternally expressed genes (6, 8), it is possible that the deficiency in paternal C1q in this model is driving the preeclampsia-like phenotype. This idea is supported by normal blood pressure in C1q deficient female mice crossed with wildtype control males, indicating that a maternal C1q deficiency does not affect the dam's health during pregnancy (21). However, more work is necessary to establish differences in vascular function and offspring outcome in C1q knockout females bred to wildtype control males to better understand the effect of maternal C1q deficiency.

Clinical studies have shown that there are signs of maternal endothelial dysfunction during preeclampsia, as endothelial-dependent relaxation is impaired in women with preeclampsia (15, 19). Research in Dr. Powers' lab has extended this work by assessing vascular function during pregnancy and postpartum in C57 x C57 and C1q$^{-/-}$ x C57 dams (22).

The aim of this study was to investigate the vascular phenotype of the C1q x C57 model, specifically the C57 (male) x C1q$^{-/-}$ (female) pregnancy. Verified animal models are imperative for studying human syndromes since it is difficult to employ invasive, *in vivo* procedures to study the early mechanisms of preeclampsia. Our study aimed to discover if the preeclampsia-like (PE-like) phenotype, evidenced by gestational hypertension and vascular dysfunction in the C1q$^{-/-}$ x C57 model, is observed in C1q deficient females bred to wildtype C57 males (19). Findings from the current study will further confirm the C1q$^{-/-}$ x C57 mouse model as a unique model for investigating preeclampsia.

MATERIALS AND METHODS

Animals: Female C1q$^{-/-}$ mice (Jackson Labs) eight-weeks of age were time-mated to C57BL/6J (also abbreviated as C57) male mice (genetic control, n=7). For comparison, eight-week-old C57 female mice and male C1q$^{-/-}$ mice (Jackson Labs) (PE-like, n=19), and C57 female and male mice (control, n=13), were also time-mated. For time-mating, female mice were placed in male cages in the afternoon with the presence of a copulation plug the following morning indicating gestation day 0.5. Female mice were assessed during late pregnancy (gestation day 17.5) for outcomes including blood pressure, fetal data/litter characteristics, and vascular function (detailed below). Mice were euthanized by carbon dioxide asphyxiation with cervical dislocation as a secondary method. The uterus was removed, and each pup and placenta were dissected and weighed for litter characteristics analysis. Mesenteric arteries were isolated for vascular function measurement by wire myography (detailed below). Up until experiment day, all mice were multi-housed with ad libitum access to standard chow and water. Mice lived in a temperature controlled room with a 12 hour light:dark cycle before and during pregnancy. This study was approved by the Institutional Animal Care and Use Committee of the Magee-Womens Research Institute and the University of Pittsburgh, Pittsburgh, PA, USA.

Blood Pressure: Volume-pressure recording by tail cuff method was utilized to measure blood pressure during mid (gestation day 9.5-11.5) and late pregnancy (gestation day 14.5-17.5) using the CODA-2 blood pressure monitoring system (Kent Scientific, Torrington, CT) (10, 22). Mice were acclimated for 10-20 minutes per day for a minimum of two days before data collection. Each mouse was gently placed into a warmed restrainer and platform for data collection. Collection consisted of 10 acclimation cycles and then 10 measurement cycles over the course of 10 minutes. Collection was at the same time each day by the same operator. The last five valid cycles for each mouse were recorded and averaged to obtain the average systolic blood pressure (SBP) and diastolic blood pressure (DBP). Mean Arterial Pressure (MAP) was calculated using the following equation: MAP = (SBP + 2*DBP)/3.

Fetal Data and Litter Characteristics: Fetal data and litter characteristics were collected and analyzed for each mouse. The uterus was removed, and each individual pup and placenta were dissected and weighed (grams). The length of each pup was measured (cm). Measurements were collected by the same individual for all mice.

Vascular Function: Endothelial-dependent and -independent vascular function was measured by a Mulvaney isometric wire-myograph system using dissected mesenteric arteries about 190 µm in diameter (16, 22). Dissected arteries were mounted with 10 µm wires and maintained in 7mL organ baths with HEPES-buffered saline at 37°C (142 mmol/L NaCl, 4.7 mmol/L KCl, 1.18 mmol/L KH2PO4, 1.17 mmol/L MgSO4 7H2O, 2.5 mmol/L CaCl2 2H2O, 10 mmol/L HEPES, and 5.5 mmol/L dextrose, pH 7.4). Arteries were equilibrated for 20 minutes and exposed to a condition stretch of 1.5 mN. Optimal tension responses were confirmed by preliminary tests of the passive and active tension-internal circumference relationship. Arteries were equilibrated at 95% of the calculated arterial internal circumference that would be obtained if the artery was at 100mmHg pressure utilizing the Law of LaPlace. Dose response curves were generated by aggregate addition of agonist, followed by replacement of HEPES buffer and a 20-minute re-equilibration.

To assess contraction of mesenteric arteries α-adrenergic agonist phenylephrine at a dosage of 10^{-8} to 10^{-5}mol/L was used. Endothelial-dependent vasodilation was assessed by methacholine at a dosage of 10^{-10} to 10^{-5}mol/L. Endothelial-independent vasodilation was assessed by sodium nitroprusside at a dosage of 10^{-10} to $3x10^{-7}$mol/L. In addition, phenylephrine and methacholine dosage response curves were produced following 20 min preincubations in 10^{-4} mol/L NG-nitro-L-arginine methyl ester (L-NAME) to analyze the role of nitric oxide (NO) in contraction and vasodilation. All chemicals were purchased from Sigma (St. Louis, MO). Percent contraction was the reported value for vascular response to phenylephrine. Percent contraction remaining was the reported value for vascular response to methacholine and sodium nitroprusside. Percent contraction was calculated using the following equation: [dose specific tension (mN/mm)/maximal tension (mN/mm)]*100. Percent contraction remaining was calculated using the following equation: [dose specific tension (mN/mm)/pre-constriction tension (mN/mm)]*100.

Statistical Analysis: The software Statistica (Dell Inc.) was utilized for data analysis. All data distributions were assessed for normality. Summary data are reported as mean ± standard deviation. ANOVAs were used to compare three groups: control (wildtype C57 males x C57 females), PE-like ($C1q^{-/-}$ males x C57 females), and genetic control (C57 males x $C1q^{-/-}$ females). ANOVAs were used for comparisons of litter characteristics to assess pup weight, placental weight, and pup to placental ratio with litter size as a covariate. Individual group differences were compared by a Fisher LSD post hoc test to analyze significant interaction effects. Data were considered significant if p values <0.05. Data variable and sample sizes for each group are designated in table and figure legends.

RESULTS

Maternal C1q deficiency does not significantly affect maternal blood pressure in late pregnancy.

Systolic (p=0.01, Figure 1A), diastolic (p=0.01, Figure 1B), and mean arterial blood pressure (p=0.01, Figure 1C) were elevated in genetic control and PE-like dams compared to control dams in mid pregnancy (GD 9.5-11.5). However, systolic, diastolic, and mean arterial blood pressure were similar between control and genetic control dams in late pregnancy (GD 14.5-17.5), and both groups had significantly lower blood pressure compared to the PE-like dams (Figure 2).

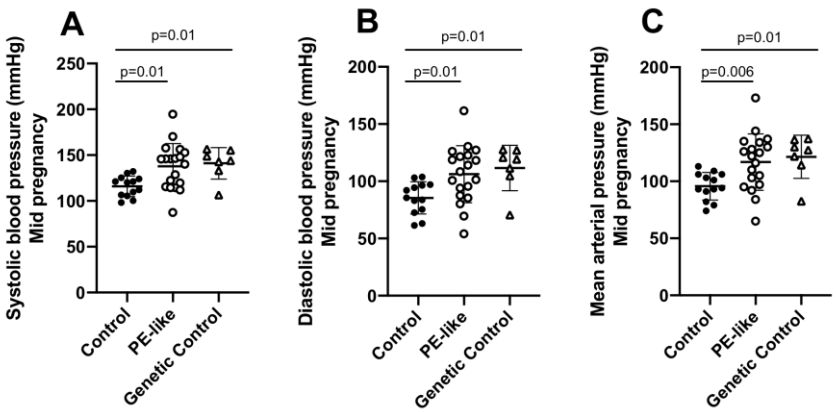

Figure 1. Blood pressure for dams during mid pregnancy (GD 9.5-11.5). No significant difference in the A) Systolic, B) diastolic, and C) mean arterial pressure for genetic control dams and preeclampsia-like dams. (significance determined when p<0.05. (n=7-19/group)).

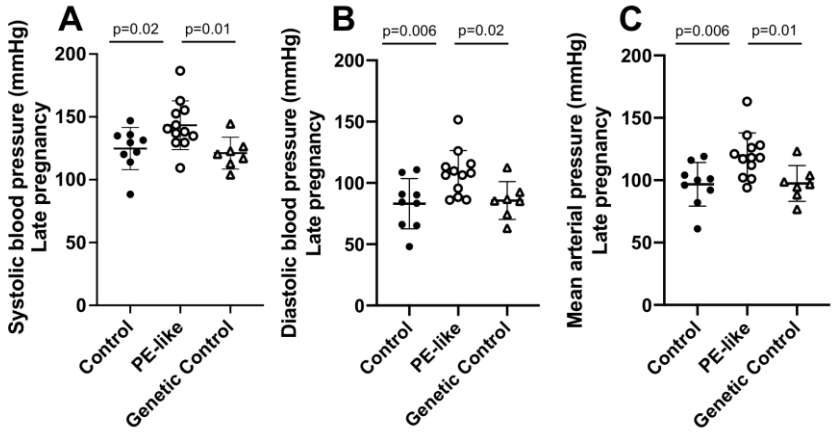

Figure 2. Blood pressure for dams during late pregnancy (GD 14.5-17.5). A) Systolic, B) diastolic, and C) mean arterial pressure are significantly lower for genetic control dams during late pregnancy compared to preeclampsia-like dams. (significance determined when p<0.05. (n=7-19/group)).

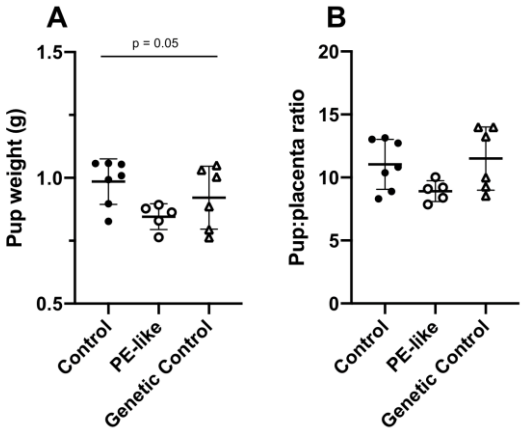

Figure 3. Litter characteristics. Genetic control females tended to have restored A) pup weight (p=0.05) on GD 17.5 compared to the preeclampsia-like pregnancy dams. B) The pup:placenta ratio is not statistically different between pregnancy groups on GD 17.5 (p=0.40) (n=7-19/group).

Vascular function is not adversely affected by maternal C1q deficiency.

The mesenteric arteries of genetic control dams demonstrated a lower sensitivity to phenylephrine compared to control and preeclampsia-like dams (Figure 4A, $p \leq 0.01$). Endothelial-dependent relaxation was not different in the genetic control dams compared to controls (Figure 4C, $p > 0.05$). In contrast, mesenteric arteries from PE-like dams showed a significant impaired endothelial-dependent relaxation response compared to arteries from control and genetic control dams (Figure 4C, $p < 0.03$). Endothelial-independent relaxation by sodium nitroprusside was not impaired in the genetic control dams compared to control dams (Figure 4E), however endothelial-independent relaxation was significantly impaired in arteries from PE-like dams compared to both control and genetic control dams (Figure 4E, $p \leq 0.03$).

Interestingly, following exposure to L-NAME, which inhibits nitric oxide synthase, arteries from genetic control

Maternal C1q deficiency does not significantly affect pup weight and growth.

As shown in Figure 3, pup weight and pup:placenta weight ratio was significantly less in offspring of PE-like dams compared to control dams. Conversely, pup weight and pup:placenta weight ratio was not different between the genetic control dams compared to control pregnant mice ($p \geq 0.40$) on GD 17.5.

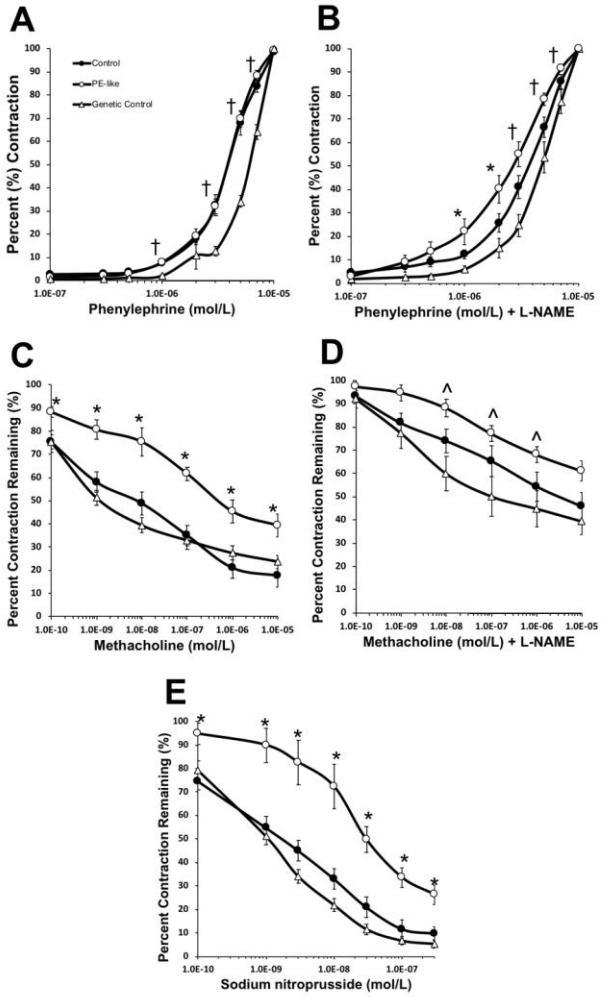

Figure 4. Vascular function during late pregnancy. Mean (±SEM) percent contraction and relaxation responses during isometric wire myography at GD 17.5. *p<0.05 of C157 from all other groups, †p<0.05 of 57C1 from all other groups, ^p<0.05 of C157 from 57C1. Control pregnancy, closed circle, n=13; preeclampsia-like pregnancy, open circle, n=9; genetic control pregnancy, open triangle, n=7.

dams still exhibited lower sensitivity to contraction by phenylephrine compared to the other pregnancy groups (Figure 4B, $p \leq 0.01$). In addition, genetic control dams also still exhibited increased relaxation response compared to arteries from PE-like dams and trended towards increased relaxation response compared to arteries from control dams (Figure 4D, $p \leq 0.01$).

DISCUSSION

This study highlights a healthy pregnancy phenotype in female $C1q^{-/-}$ mice bred to wildtype male C57 mice (genetic control). Main findings show that female C1q deficiency during pregnancy does not adversely impact maternal vascular function as compared to the PE-like pregnancy model. This is evidenced by similar systolic, diastolic and mean arterial blood pressure, similar pup weight, and ex-vivo vascular function compared to pregnant wildtype control mice, and all of these measures are significantly different in the PE-like pregnant mouse model. The improved ex-vivo vascular function of the genetic control pregnancy, compared to the PE-like pregnancy, indicates that maternal C1q deficiency does not have an adverse effect on pregnancy health, and that paternal C1q deficiency drives the previously observed preeclampsia-like phenotype (22).

C1q deficiency and Blood Pressure:

Systolic, diastolic, and mean arterial blood pressure were significantly elevated in mid-pregnancy in genetic control dams compared to control dams (GD 9.5-11.5). However, systolic, diastolic, and mean arterial blood pressure were significantly lower compared to the PE-like dams in late pregnancy (GD 14.5-17.5). This lower blood pressure in late pregnancy in the genetic control females supports the findings of previous work. Genetic control dams were found to have normal blood pressure compared to the PE-like dams with a paternal C1q deficiency (21). Previous studies, however, have not reported hypertension in mid pregnancy for the genetic control dams (21). This observation compared to the data of previous studies may be the result of differences in methodology and the timepoint when mid-pregnancy blood pressure was investigated. In addition, previous studies have found no difference in blood pressure until gestation day 13 (21). Furthermore, this model may display different subtypes of pregnancy which cause mid-pregnancy hypertension. Nevertheless, differences in blood pressure in late pregnancy may be more indicative of preeclampsia as studies have shown that symptoms are typically encountered during the third trimester, and therefore likely are not expected until after mid-pregnancy (9). Overall, normal blood pressure in late pregnancy seen in genetic control mice indicate a healthy pregnancy similar to that of the control females (21, 22). However, future studies are still needed to investigate the long-term effects of maternal C1q deficiency.

C1q deficiency and Fetal Health:

Pup weight was higher on GD 17.5 for offspring of genetic control dams compared to PE-like dams. However, there was no significant difference in pup:placenta ratio of genetic control dams compared to PE-like dams on GD 17.5. Previous studies have found that genetic control dams have fetal resorption frequencies and litter sizes similar to wildtype control dams, and lower fetal resorption compared to PE-like dams (21, 22).

Furthermore, lack of intrauterine growth restriction (IUGR) in pups from genetic control dams suggests an otherwise uncomplicated pregnancy as IUGR is typically a symptom of preeclampsia (14, 20). Therefore, findings suggest that genetic control pregnancies are as healthy as wildtype control pregnancies. This data and previous studies suggest that maternal C1q does not significantly affect placentation and fetal growth, but more work is still needed investigating the role of C1q in the placenta (21).

C1q deficiency and Maternal Vascular Health:
Arteries from genetic control dams had lower sensitivity to contraction by phenylephrine compared to the other pregnancy groups. In previous studies it was found that arteries from wildtype controls and PE-like dams showed no difference in vasoconstriction (22). This difference in phenylephrine mediated vasoconstriction in arteries from genetic control mice could be due to different subtypes of pregnancy that contributed to decreased contractile sensitivity in genetic control dams compared to the other pregnancy groups (7, 24). Further, endothelial-dependent relaxation was not impaired in arteries from genetic control dams compared to wildtype control dams and increased compared to arteries from preeclampsia-like dams. Previous studies have shown a loss of endothelium-dependent relaxation in women who experience preeclampsia, and this phenotype is also seen in arteries from the PE-like mouse model (1, 5, 15, 18). This difference in relaxation response to methacholine, as described in a previous study, could indicate that there is difficulty in smooth muscle utilization for the PE-like model (22). In addition, endothelial-independent relaxation by sodium nitroprusside was also not impaired in genetic control dams, who exhibited significantly increased relaxation response compared to PE-like dams. However, healthy pregnancies experience more vasodilation, so increased vasodilation in genetic control dams indicate a healthier pregnancy overall (7, 24).

C1q deficiency and Nitric Oxide:
Arteries from the PE-like dams show an increased response to phenylephrine and a decreased response to methacholine with nitric oxide synthase inhibition by L-NAME. This likely indicates an impairment in smooth muscle NO utilization and signaling in the PE-like dams (22). Arteries from genetic control dams had a comparative lack of phenylephrine response and an increased response to methacholine similar to the arteries from wildtype control dams which suggests a healthy and undisrupted NO pathway, indicating a healthier pregnancy compared to the PE-like dams. With normal NO pathways, it is likely that placental development is not disrupted, and the model is not adversely affected by the lack of maternal C1q (12, 13, 23). In addition, drugs that increase nitric oxide (NO) availability are highly effective for reducing blood pressure (7). This supports the vascular function findings that the genetic control and control dams with reduced blood pressure could have more NO availability and more efficient NO mechanisms compared to the PE-like dams. Importantly, nitric oxide production has been found to occur during trophoblast invasion, which indicates that uncomplicated placentation supports NO pathways and uncomplicated vasoconstriction and vasodilation (13, 23). These data suggest that genetic control and control dams have similar and undisrupted NO pathways, and therefore it is likely that maternal C1q

deficiency has minimal impact on the nitric oxide pathways and vascular health during pregnancy.

Female C1q$^{-/-}$ and Vascular Dysfunction:
Overall, this study offers evidence that paternal C1q deficiency is more important in the determination of maternal pregnancy phenotype and outcome, and maternal C1q deficiency has no significant adverse effects on maternal pregnancy phenotype and outcome. Previous studies have shown that paternal C1q deficiency causes poor placental perfusion, which leads to impaired remodeling of the spiral arteries by endovascular trophoblasts (2, 19, 20, 25). Preeclampsia has been linked to absence of these physiological changes in the uterine spiral arteries (14). Since the placenta genotype is primarily paternally driven, these changes are not expected when only the female is C1q deficient (6, 8). The classical symptoms of preeclampsia (gestational hypertension and vascular dysfunction) were not observed in the present study of genetic control dams. Overall these data support the use of the unique paternal driven C1q57 mouse model of preeclampsia.

Unfortunately, because preeclampsia is strictly a human syndrome and the exact cause of the syndrome is unknown, there is no perfect animal model of preeclampsia. However, future work utilizing this model may lead to a better understanding of the human disorder. Specifically, future work is needed to investigate how male C1q deficiency affects the development of the placenta and how these developments change over the time course of pregnancy. It is also unclear from this work if the C1q57 model displays different subtypes of disease and how this severity is related to blood pressure, pup weight, and ex-vivo vascular function. In order to understand this model better, it will also be important to investigate the biological mechanism of pregnancy in this model when the C1q deficiency is maternal and when the C1q deficiency is paternal. Lastly, further investigation of the mechanisms underlying vascular dysfunction is required in this model of preeclampsia. Specifically, future studies can look at the NO signaling mechanism of both models and investigate endogenous inhibitors of NO signaling such as asymmetric dimethylarginine (ADMA). ADMA is closely linked to the inhibition of endothelial NO synthase and is elevated in preeclampsia, and consequently may be an indicator of the integrity of the NO signaling pathway (13, 23).

This study shows that a C1q deficiency affects pregnancy health when it is paternally driven. Thus, it is most likely that the PE-like phenotype derives from impaired placentation, similar to the proposed mechanism for the human syndrome (19, 20). This confirms the C1q$^{-/-}$ x C57 model as a unique model for further preeclampsia research. As a result, current and future studies with this model of preeclampsia will be able to be translated to clinical settings with more confidence.

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