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Discovery of a Spontaneous Genetic Mouse Model of Preeclampsia

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Abstract—Preeclampsia remains a leading cause of maternal and fetal morbidity and mortality but has an unknown etiology. Women with elevated baseline blood pressure have an increased risk of this disorder. We hypothesized that BPH/5 mice, an inbred mouse strain with mildly elevated blood pressure, would develop a pregnancy-induced hypertensive syndrome. Nonpregnant female BPH/5 and C57BL/6 mice underwent thoracic aortic implantation of telemeters. After 7 days of recovery and 5 days of baseline mean arterial blood pressure (MAP) recording, strain-matched timed matings were carried out. MAP was recorded continuously during pregnancy and for 1 week after birth. In separate mice in metabolic cages, urinary protein was tracked, followed by renal histological analysis. Before pregnancy, the BPH/5 strain had elevated baseline MAP compared with the C57BL/6 strain, but both strains had similar total urinary protein levels and renal histology. MAP remained stable in both groups during the first 2 weeks of pregnancy. However, at the start of the last trimester, MAP began to rise further in the BPH/5 mice; it rose to peak levels just before delivery and returned to prepregnancy levels by 2 days after delivery. This was accompanied by late-gestational proteinuria and progressive glomerulosclerosis. No changes were observed in the C57BL/6 group except for a small decrease in MAP at mid gestation. The BPH/5 group delivered significantly smaller litters despite normal numbers of fetuses early in gestation, and longitudinal ultrasound studies documented fetal demise before the onset of hypertension and renal disease. This is the first report of an animal model that spontaneously develops a syndrome that bears close resemblance to preeclampsia, and it should have an impact on our understanding of the pathophysiology of this disorder. (Hypertension. 2002;39[part 2];337-342.)

Key Words: hypertension, gestational blood pressure pregnancy proteinuria preeclampsia

Preeclampsia, the most prevalent hypertensive disorder of pregnancy, is defined by the development of hypertension and proteinuria after the 20th week of pregnancy. It is thought to have an impact on 6% to 10% of pregnancies and is the leading cause of maternal mortality in Western countries.1 A distinguishing feature of the disorder is its complete resolution after delivery of the fetus and placenta, the only known effective means to avoid cataclysmic progression to overt eclampsia. The necessity for urgent preterm delivery, along with progressive intrauterine growth restriction, implicates preeclampsia as a leading cause of perinatal morbidity and mortality.2

The preeclampsia/eclampsia syndrome was described by ancient civilizations. Despite considerable research effort to date, we still understand very little about its etiology and pathophysiology, which are complex and multifactorial.3 Clinical research is difficult because of the logistics of testing hypotheses related to pathogenesis or treatment in an urgent high-risk setting. Development of an animal model that fully recapitulates this complex hypertensive disorder may help to broaden our understanding and may hold great potential for the design and implementation of effective prevention and treatment.4

Women with elevated baseline blood pressure before pregnancy have an increased risk of developing preeclampsia.5 A genetically “borderline hypertensive” mouse strain derived from the well-known hypertensive inbred strain BPH/2 was reported by Schlager, Lester, and colleagues6–8 some years ago. BPH/5 is an inbred subline generated from brother-sister matings of fully inbred BPH/2 mice over many generations,6 resulting in mild blood pressure elevation throughout adult life.6,7,9 The etiology of blood pressure elevation in these mice, as in humans, remains incompletely defined. We hypothesized that this borderline hypertensive mouse strain would be at an increased risk of developing pregnancy-related hypertension and other hallmark signs associated with preeclampsia.
Methods

Animals and Husbandry
Experiments were performed in female (19- to 24-g) C57BL/6 (C57) or BPH/5 mice (kind gift of G. Schlager, University of Kansas, Lawrence), an inbred subline derived from >20 generations of brother-sister matings of the inbred spontaneously hypertensive strain BPH/2.6-9 BPH/5 mice exhibit baseline blood pressures that are significantly lower than those of BPH/2 mice but that are elevated above control blood pressures.6-9 Mice were housed in standard polycarbonate cages placed in a temperature- and humidity-controlled facility, maintained on a 12-hour light/dark cycle, and fed standard mouse chow with water available ad libitum. Timed matings were carried out by strain-matched pairing of males and females overnight. Females were checked the next morning for the presence of a vaginal plug by a gentle probe of the vaginal orifice with a blunt tapered glass rod.9 The day of plug detection was designated day 0 of pregnancy, at which time the males were removed from the cage for the duration of the pregnancy. Mice were weighed 3 times weekly to confirm pregnancy. It should be noted that all cohorts of the BPH/5 and C57 groups were composed of both primiparous and multiparous females. Gestational stages are defined as follows: early, days 7 to 9; middle, days 11 to 14; and late, days 16 to 20. Care of the mice met or exceeded the standards set forth by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All procedures were approved by the University Animal Care and Use Committee at the University of Iowa.

Longitudinal Measurement of Blood Pressure Throughout Pregnancy With Telemetry
Nonpregnant female BPH/5 (n = 8) and C57 (n = 9) mice underwent thoracic aortic implantation of telemeters (Data Sciences Int) as described in detail by us previously.9 Briefly, mice were anesthetized (90 mg/kg IP ketamine and 1.8 mg/kg IP acepromazine), a ventral midline neck incision was made, the left common carotid artery was isolated and retracted (7-0 silk), and a tiny incision was made. The pressure-sensing catheter was carefully inserted into the left carotid artery by use of vessel cannulation forceps, and it was advanced 10 mm so that the tip resided just inside the thoracic aorta. The catheter was secured by ligatures, and the transmitter body was tunneled subcutaneously to a small pouch along the right ventral flank. The neck incision was closed with 6-0 silk, and the mice were kept warm until they had fully recovered from anesthesia. After 7 days of recovery in home cages (placed atop telemetric receivers), telemeters were magnetically activated, and baseline mean arterial blood pressure (MAP) was recorded continuously for 5 days (with sampling every 5 minutes for 10-second intervals). On day 13 after surgery, telemeters were switched off, and strain-matched breeding was carried out (see above). On detection of a vaginal plug, telemeters were reactivated, and MAP was recorded continuously throughout the pregnancy (20 to 21 days) and for an additional 1 week after delivery. Data were collected online and stored by using a Dataquest ART data acquisition system (Data Sciences Int) as described.9

Urinary Protein Measurements and Renal Histopathology
In separate timed mating experiments, BPH/5 and C57 mice without telemeters were placed in individual metabolic cages with free access to food and water, and 24-hour urine samples were collected over 2 days. Three separate cohorts of each strain were studied. Mice were either not pregnant (BPH5, n = 4; C57, n = 5), middle gestational (BPH5, n = 4; C57, n = 4), or late gestational (BPH5, n = 5; C57, n = 6). Body mass (in grams) on entry and removal from the cage was recorded. Urine was frozen at −20°C until analysis for total protein content with the use of a protein assay kit (Bio-Rad) according to the manufacturer’s instructions.10 Immediately after urine collection, a subset of these mice was euthanized by CO₂ asphyxiation, and the kidneys were removed and fixed in 10% neutral-buffered formalin. Tissue was embedded in paraffin, sectioned (4 μm sagittal), and stained with periodic acid–Schiff stain (PAS) for histopathologic analysis. Kidneys from a minimum of 2 different mice from each strain and gestational stage were examined.

Analysis of Pregnancy Outcomes
The numbers of live pups born and neonatal body mass (in grams) were recorded for BPH/5 (n = 12) and C57 (n = 15) mothers that had been allowed to proceed to term. Fetus numbers at different stages of pregnancy were analyzed by ultrasonography or by visualization at euthanasia. Fetal ultrasonography was performed in utero with an Acuson Sequoia c256 imager fitted with a 15-MHZ linear array transducer. Gestation (defined as the period of pregnancy) was calculated from the day of fertilization, except in cases of partial reabsorption. The day of parturition was recorded. The number of live births and stillbirths were recorded. Intrauterine fetal deaths were scored in utero and at necropsy with the use of criteria to determine fetal viability. These criteria included the presence of a heartbeat, movement, and breathing. Photographs of the skulls of dead fetuses were taken for confirmation of viability. Recordings were made of fetal cardiac activity within a given sac after gestational day 12. Ultrasonic findings were confirmed in a separate cohort that underwent necropsy during early (BPH/5, n = 8; C57, n = 7), middle (BPH/5, n = 5; C57, n = 6), or late (BPH/5, n = 8; C57, n = 9) gestation. A ventral midline incision was made, the uterine horn was exposed, and the fetuses were counted. Fetal resorptions, identified by necrotic/hemorrhagic appearance and smaller sizes compared with normal viable fetuses, were noted.

Analysis of Endothelium-Dependent Vasodilation In Vitro
Timed matings were carried out, and vascular function studies were performed in separate nonpregnant mice (BPH5, n = 9; C57, n = 8) or late-gestational mice (BPH5, n = 5; C57, n = 5). Mice were euthanized, and mesenteric resistance arteries (≈100 μm) were excised and cleaned of fat and connective tissue. Vessels were double-cannulated between 2 glass micropipettes, given intraluminal resting pressurization (20 mm Hg), and placed in a 37°C oxygenated Krebs bath. Vessels were precontracted with phenylephrine (10⁻⁴ mol/L), which was followed by incremental doses of the endothelium-dependent vasorelaxant acetylcholine (10⁻⁸–10⁻⁶ mol/L). Vessels were visualized via an inverted microscope connected to a closed-circuit video system, and changes in luminal diameter were measured by NIH Image.

Data Analysis
Data are expressed as mean±SEM (calculated for the gestational day or stage of pregnancy). Telemetric data are plotted as 24-hour averages every 2 days. Urinary protein levels are the average of two 24-hour samples for each mouse. Ultrasonographic data were analyzed by assigning the number 1 to a pregnancy with at least 1 demise and the number 0 to pregnancies with no demises and by using the Student t test. Litter sizes and pup weights were also analyzed by the Student t test. Remaining data were analyzed by repeated-measures or 1-way ANOVA followed by the Student modified t test and the Bonferroni correction for multiple comparisons between means with use of the modified-error mean-square term from the ANOVA.

Results

BPH/5 Mice Developed Late-Gestational Hypertension That Resolved After Delivery
BPH/5 mice developed late-gestational hypertension that resolved after delivery. We recently developed a new radio-
telemetric approach for continuous hands-off recording of blood pressure longitudinally in pregnant, unrestrained, un tethered mice. We have demonstrated that this is a reliable method for obtaining highly accurate blood pressure recordings during pregnancy without interfering with conception, gestation, delivery, or postnatal care of the neonates. Using this technology, we compared MAP before, during, and after pregnancy in BPH/5 and C57 mice (Figure 1). Consistent with previous reports, BPH/5 mice had significantly elevated baseline MAP compared with control mice before pregnancy (128/110 mm Hg, respectively; P<0.01). MAP remained stable in BPH/5 and C57 mice throughout the first 2 weeks of pregnancy. However, beginning in the last trimester (day 14), MAP began to rise even further in BPH/5 mice, continued to increase to peak levels just before delivery, and returned to prepregnancy levels by postpartum day 2 or 3. In sharp contrast, MAP fell at the end of the second trimester in C57 mice for a short period but returned to prepregnancy levels by several days before delivery, where it remained throughout the postpartum period. No differences were observed between primiparous and multiparous BPH/5 or C57 mice with regard to blood pressure or any of the other parameters studied (data not shown).

BPH/5 Mice Exhibited Renal Disease in the Last Trimester of Pregnancy
The BPH/5 mice exhibited renal disease in the last trimester of pregnancy. Because of the importance of proteinuria secondary to renal pathology in the diagnosis of preeclampsia, we analyzed urinary protein levels and renal histopathology. Before pregnancy, C57 and BPH/5 mice had similar total urinary protein levels, and these remained unchanged through mid gestation (Figure 2). However, compared with earlier time points, by the late stages of pregnancy (days 17 to 19), the BPH/5 mice exhibited increased protein excretion, whereas the C57 mice did not. Proteinuria was accompanied by marked renal histological changes by the start of the third trimester in BPH/5 mice (Figure 3). By gestational day 14 to 15 in BPH/5 mice, glomeruli showed early signs of focal and segmental sclerosis with collapse of glomerular capillaries (Figure 3C). These changes were more often noted in the vascular pole of the glomeruli. Occasional glomeruli showed segmental adhesion of the collapsed glomerular tuft to Bowman’s capsule. By day 19, these changes were more pronounced and ranged from focal and segmental glomerulosclerosis (Figure 3D) to global sclerosis (Figure 3E). Glomeruli showed prominent focal and segmental sclerosis, with collapse of glomerular capillaries and accumulation of PAS-positive hyaline material in the collapsed capillaries. In addition, the afferent/effenter arterioles of these glomeruli showed accumulation of PAS-positive hyaline material in the walls (Figure 3D and 3E). In contrast, kidneys from the C57 mice showed normal glomeruli with open glomerular capillary loops, normal cellularity, and delicate mesangium (Figure 3A); no changes were observed at any stage of pregnancy in these mice. Nonpregnant and early gestational BPH/5 mice...
showed similar normal renal histology (Figure 3B). It should be noted that the extraglomerular interstitium of kidneys from late-gestation BPH/5 mice did not show significant changes; in particular, there was no evidence of sclerosis of the larger vessels.

Intrauterine Fetal Demise, Small Live-Born Litters, and Low-Birth-Weight Pups Were Observed in BPH/5 Pregnancies

Intrauterine fetal demise, small live-born litters, and low-birth-weight pups were observed in BPH/5 pregnancies. Preeclampsia is associated with perinatal morbidity and mortality and an increased risk of poor fetal growth. Thus, we examined litter sizes, neonatal birth weights, and fetus numbers at different stages of pregnancy. Compared with C57 mothers, BPH/5 mothers delivered significantly smaller litters of live pups (Figure 4A), and of those BPH/5 pups born, the average body weight was significantly less than that of C57 pups (Figure 4B). To determine whether reduced litter sizes were due to failure to conceive, failure to implant, or fetal demise, we examined fetuses in utero by ultrasound or visually at necropsy. BPH/5 and C57 mice had similar numbers of fetuses early in gestation, but BPH/5 mice had progressively fewer viable fetuses at middle and late gestation (Figure 5A). Evidence of fetal resorption was commonly observed during middle and late pregnancy in BPH/5 mice, whereas it was rare in C57 mice at any time point (data not shown). These findings were confirmed in ultrasound studies of a separate cohort in which BPH/5 and C57 fetuses were followed longitudinally. In a typical image of a BPH/5 mouse at day 12 of gestation (Figure 5B), we observed fetuses of varying status. Fetus 2 was apparently healthy and of normal size for this stage. Pulse-wave Doppler interrogation of umbilical blood flow (Figure 5B, bottom) revealed a typical murine fetal heart rate (200 bpm). In contrast, signs of fetal distress were observed in fetus 3. In addition to being small for this stage, it was bradycardic (140 bpm). The fetal sac on the left (labeled 1) contained homogeneous fluid, indicating completed fetal demise and autolysis. Interestingly, ultrasound images of the same BPH/5 mouse a week later revealed a single large empty sac without septa and no apparently viable fetuses (data not shown). Indeed, there were no pups born of this pregnancy. Overall, ultrasonographic evidence of fetal demise was detected in 13 of 15 pregnant BPH/5 mice that were studied beginning at gestational day 11, but fetal demise was detected in only 2 of 14 pregnant C57 mice (P<0.0001).

A fetal echo movie can be found in an online data supplement available at http://www.hypertensionaha.org.
in BPH/5 and C57 mice. Endothelium-intact mesenteric resistance arteries from BPH/5 mice at 19 days of gestation, compared with C57 vessels at the same time point, showed diminished relaxation (Figure 6). Dose-response curves showed not only poor relaxation to acetylcholine but also an early transition to acetylcholine-induced contraction; both are suggestive of endothelium dysfunction. Although vessel segments from nonpregnant BPH/5 mice, compared with those from nonpregnant C57 mice, showed a somewhat blunted response to the highest doses of acetylcholine, pregnancy induced a striking decrease in endothelium-dependent relaxation in BPH/5 mice but had little effect on C57 mice. Vessels obtained from mice at early gestation were indistinguishable from those of nonpregnant mice (data not shown). Relaxation to the endothelium-independent nitrovasodilator sodium nitroprusside was not different between groups, nor were there differences in the contractile response to acetylcholine in vessel segments denuded of endothelium by intraluminal infusion of air bubbles (data not shown).

**Discussion**

Preeclampsia is a serious hypertensive complication of pregnancy that increases maternal and perinatal morbidity and mortality. The etiology and pathogenesis of preeclampsia remain poorly understood, leading to the search for appropriate experimental models to study this disorder. This is the first report of a nonprimate animal model that spontaneously exhibits relevant clinical features of human preeclampsia. Pregnant BPH/5 mice, an inbred strain of the genetically hypertensive model BPH/2,6–8 develop hypertension, proteinuria, glomerular disease, and endothelial dysfunction. Intrauterine fetal demise and growth retardation are also observed.

Many attempts have been made to generate animal models of preeclampsia, including uteroplacental ischemia,13,14 chronic NO synthase inhibition,15 adriamycin nephropathy,16 and transgenic expression of human renin-angiotensin system genes.10 Each has been important for the understanding of certain aspects of the disease, although no model has recapitulated the full syndrome.4 In most, but not all,14 of these models, hypertension does not resolve on delivery, and pathophysiological changes are observed in both pregnant and nonpregnant animals.4 BPH/5 mice develop a syndrome that is strikingly reminiscent of the clinical disorder only when they are pregnant. The onset of hypertension and renal disease during the last trimester (third week) of pregnancy in BPH/5 mice is corroborated with the time course of the clinical disorder. Furthermore, the return of elevated blood pressure to prepregnancy levels immediately after delivery in BPH/5 mice is also consistent with the definitive response of hypertension to delivery in humans. When this information is taken into account together with the knowledge that mice have a similar hemochorial type of placentation as humans17 (which is an important consideration inasmuch as preeclampsia is thought to involve abnormal placentation), the BPH/5 strain is a promising new model for the study of this complex disease.

The long-term recording potential of telemetry9 allowed us to implant transmitters before the mice were bred, to provide adequate recovery time for the mice after surgery, and to record blood pressure continuously throughout the pregnancies without interruptions such as restraint, handling, or anesthesia/surgery. In the prepregnancy state, BPH/5 mice had baseline blood pressure levels that were intermediate between the hypertensive parent strain BPH/2 and normotensive control mice. This corroborated earlier analyses of this model subsequent to its generation as an inbred subline of BPH/2.6–7,9 Because preeclampsia is manifested as new-onset or worsening hypertension and because patients with borderline hypertension are known to be at increased risk of developing the disease,5 we were interested in whether this inbred strain with elevated blood pressure levels would develop hypertension during pregnancy. Indeed, the increase in blood pressure at the start of the last trimester and the subsequent rise to peak levels until delivery that were observed in BPH/5 mice are the heralding signs of preeclampsia.12 Given that pregnancy is actually antihypertensive during middle to late gestation in normotensive and hypertensive women and animal models,18–20 the rise in blood pressure is even more striking. Maintenance of normal or transiently decreased blood pressure in C57 mice recapitulates the findings in normal women, although the timing of the blood pressure decline is slightly different.20

A clinical diagnosis of preeclampsia includes an increase not only in blood pressure but also in proteinuria. Without proteinuria, a diagnosis of gestational hypertension is made.12 Although the significance of this classification with regard to pathogenesis is not clearly understood, it is an important consideration in establishing a model of preeclampsia. In the prepregnancy state and through mid gestation, urinary protein levels were normal in BPH/5 mice. However, proteinuria was detected during late gestation in these mice, and it was concomitant with the rise in blood pressure and consistent with clinical observations of proteinuria during the last trimester of pregnancy. In patients, this abnormality is resolved on delivery20; however, this is a difficult parameter to measure in an early postpartum mouse because the mice are removed from the metabolic cages for the delivery and care of the pups. Early signs of renal histological changes in BPH/5 mice were first detected at the start of the last trimester (days

**Figure 6.** Resistance arteries from pregnant BPH/5 mice have impaired endothelium-dependent relaxation. In vitro mesenteric arteries (~100-μm internal diameter) from late-gestational (BPH/5, n=5; C57, n=5) or nonpregnant (BPH/5, n=9; C57, n=8) mice constricted with 10⁻⁷ mol/L phenylephrine and relaxed with incremental doses of the endothelium-dependent vasodilator acetylcholine. Data are expressed as mean±SEM. *P<0.05 vs all other groups; †P<0.05 vs BPH/5 pregnant group.
14 to 15) and became more pronounced during the last few days of pregnancy. Abnormalities ranged from focal and segmental glomerulosclerosis to global sclerosis, reminiscent of pathological changes seen in the kidneys of preeclamptic patients.

The present study indicates that BPH/5 mothers deliver significantly smaller litters of low-birth-weight pups. This appears to be the result of intrauterine fetal demise and/or compromise rather than a failure of conception or implantation, inasmuch as BPH/5 mice have a normal number of fetuses in early gestation. Interestingly, fetal demise began to occur before the onset of hypertension and renal disease. This supports a prominent theory suggesting that the primary defect in preeclampsia originates at the fetoplacental interface. Abnormal placental vasculature causes inadequate maternal-fetal circulation, leading to intrauterine fetal compromise. This is thought to result in the release of fetoplacental factors that damage the maternal vascular endothelium, which leads to the various systemic manifestations of preeclampsia. Interestingly, in vitro vessel data show that BPH/5 mice also exhibit a striking pregnancy-related impairment in endothelial function. We believe that this model, coupled with noninvasive longitudinal analysis of fetal status with the use of ultrasonography, may provide the opportunity to experimentally test this theory.

The major contribution of the present study is the characterization of an animal model that bears a close pathophysiological resemblance to the clinical disorder preeclampsia. Given that preeclampsia likely has an important genetic component, this inbred genetic strain may provide a particularly exciting opportunity for studying its molecular genetic pathophysiology. Furthermore, the model should prove useful for preclinical testing of therapeutic approaches to this disorder.

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