Impact of Corticosteroid-Binding Globulin Deficiency on Pregnancy and Neonatal Sex

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Context: Plasma corticosteroid-binding globulin (CBG) transports cortisol but high progesterone levels at the maternal-fetal interface can displace cortisol from its steroid-binding site. A secretion-deficient CBG mutant (A51V) in ~1 of 36 Chinese causes low circulating CBG levels.

Objective: Assess the implications of a CBG deficiency on pregnancy outcomes.

Participants and Design: From 1978 Chinese women screened at 12–16 weeks' gestation, 50 A51V carriers were identified and 46 were followed with 60 controls throughout pregnancy. Blood samples from another 2051 pregnant women were obtained at term to determine the secondary sex ratio (SSR) of newborns in an extended cohort (n = 101) of A51V mothers.

Outcome Measures and Results: Among women recruited at 12–16 weeks' gestation, serum CBG increased progressively during pregnancy but was lower ($P < .0001$) in heterozygous A51V carriers than controls. Two women homozygous for A51V had very low serum CBG but their pregnancies progressed normally. The A51V mothers did not differ from controls in body mass index, gestational age at delivery, duration of parturition, blood pressure, gravidity, infant birth weight and size, or placental weights, and reported no unusual clinical symptoms. Peripheral CBG and progesterone levels correlated (r = 0.459) during first and second trimesters. Progesterone levels were much higher in intervillous blood and correlated (r = 0.637) with CBG levels. A female-skewed SSR in newborns of A51V mothers (0.77) differed ($P < .05$) from the SSR (1.17) in a reference cohort.

Conclusions: CBG influences progesterone levels in peripheral blood and at the maternal-fetal interface. The female-skewed SSR suggests that male fetal survival is compromised in CBG-deficient mothers. **(*J Clin Endocrinol Metab* 100: 1819–1827, 2015)**

Plasma corticosteroid-binding globulin (CBG) binds glucocorticoids (cortisol and corticosterone) and progesterone with high affinity (1). In peripheral blood, plasma cortisol concentrations (2, 3) and the free fraction of cortisol that is "bioavailable" to cells (4) are controlled primarily by plasma CBG levels. By contrast, the influence of CBG on plasma progesterone levels and bioavailability remains to be defined. It is also thought that CBG regulates the local delivery of its steroid ligands in a targeted manner. For example, neutrophil elastase cleaves CBG and releases its bound cortisol, providing a highly efficient delivery of cortisol to sites of inflammation (5).

Circulating CBG levels increase 2–3-fold during the second and third trimesters of human pregnancy (6) in step with increased total and free plasma cortisol levels (6, 7, 8). At the same time, a dynamic 3–8-fold increase in maternal plasma progesterone occurs between the first trimester of pregnancy and term (8, 9), and it has been pro-

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Abbreviations: BMI, body mass index; BP, blood pressure; CBG, corticosteroid-binding globulin; EIA, enzyme immunoassay; HPA, hypothalamic-pituitary-adrenal; SNP, single nucleotide polymorphism; SERPIN, serine proteinase inhibitor; SSR, secondary sex ratio.

posed that high levels of progesterone in the circulation protect pregnant women from excessive cortisol exposures (8). Moreover, whereas human CBG is mainly occupied by cortisol in peripheral blood, the very high concentrations of progesterone at the maternal-fetal interface have the capacity to displace cortisol from the CBG steroid-binding site (10). In this unique anatomical context, CBG therefore acts as the major plasma progesterone-binding protein with the ability to influence the local concentrations and bioavailability of progesterone.

Increasing numbers of nonsynonymous single nucleotide polymorphisms (SNPs) in gene (*SERPINA6*) encoding human CBG have been described (11–19). Most of these mutations disrupt the production or steroid-binding activity of CBG and involve the amino acid substitutions L93H (11), D367N (12), and E102G (17), which are characterized by reduced steroid-binding affinity or capacity; G237V (13) and W371S (14) that have no detectable cortisol-binding activity, and two null mutations: W11stop (15) and L5stop (16), both of which are not produced at all due to premature termination of CBG translation. Patients with these mutations usually have low plasma cortisol levels, are often overweight and present with chronic pain and/or fatigue among other symptoms (12–16, 20).

Although CBG deficiencies are generally considered as rare, five pedigrees of Mediterranean/Middle Eastern descent with the CBG D367N mutant have been identified (12, 14, 15, 20, 21), and some CBG mutants are therefore likely enriched in specific ethnic groups or geographic regions. In support of this, the *SERPINA6* SNP (rs146744332) encoding CBG A51V occurs in Han Chinese at a much higher frequency (1:35 in men, n = 1011 and 1:37 in women, n = 1031) than expected from global frequency data in SNP databases (17). Moreover, inspection of SNP databases suggests that this mutation occurs at approximately the same frequency in both Chinese and Japanese populations. The alanine-to-valine substitution at residue 51 in the mature CBG sequence disrupts its cellular production in vitro, and this explains the lower plasma CBG levels in individuals heterozygous for CBG A51V (17). The effect CBG A51V has on plasma CBG levels is much greater than that associated with another more common CBG A224S variant, which binds steroids normally but is present at only ~15% lower levels in the blood (19). Thus, CBG A51V is the most common mutation known to disrupt CBG production or function, and its relatively high frequency in Chinese subjects provides an opportunity to assess the clinical consequences of CBG deficiencies in large patient cohorts.

The present study was designed to identify CBG A51V carriers among several thousand pregnant Chinese women, and to assess the implications of a CBG deficiency on pregnancy outcomes in these women.

Materials and Methods

Subjects and samples

Studies were performed at the Beijing Obstetrics and Gynecology Hospital, Capital Medical University from 2011 to 2013. Approval to obtain blood and tissue samples from mothers and neonates was granted by the Ethical Committee and the Review Board of the Institute of Zoology, the Chinese Academy of Sciences, and the Beijing Obstetrics and Gynecology Hospital. Samples were taken with informed consent and anonymized.

Blood samples were collected initially from a cohort of women (n = 1978) between gestational weeks 12 and 16, and among them we identified 48 heterozygous and two homozygous carriers of CBG A51V. Genomic DNA was extracted from maternal peripheral blood using a commercial kit (Newprobe Biotechnology Corp., Beijing, China), and the SNP (rs146744332) encoding CBG A51V was identified, as previously described (17). At that point, 63 age-matched women homozygous for the wild-type (CBG A51) rs146744332 allele were randomly selected as controls to study the clinical outcomes of mothers and neonates, and for blood sample collection at later time points. Maternal blood samples were taken in the morning after routine analyses at 12–16 and 24 weeks' gestation. At term, maternal blood samples were taken just prior to delivery from the CBG A51V carriers and the age-matched controls. When possible, umbilical artery blood was taken at delivery after clamping the cord, and intervillous blood was collected from the basal plate of the placenta immediately after delivery from a subset of these subjects. Serum or EDTA-plasma was freshly separated from blood samples and stored at −80°C. Genomic DNA was also extracted from the umbilical artery blood samples to identify the SNP (rs146744332) encoding CBG A51V.

Pregnancy records were checked anonymously from this cohort of women to ensure they had no preexisting illnesses or pregnancy-related complications. The following clinical information was obtained anonymously from maternal and neonatal medical records: maternal age, body mass index (BMI), blood pressure (BP), gravidity, parity, mode of birth (cesarean section or spontaneous labor), and weeks of gestation at delivery, as well as infant outcome information, including birth weight, birth size, placenta weight, Apgar score, and secondary sex ratio (SSR) at birth.

In a follow-up study of SSR, we screened an additional 2051 term pregnant women at delivery and identified 55 CBG A51V heterozygous carriers for whom the sex of their offspring was recorded.

Measurements of CBG, progesterone, and albumin concentrations

Serum CBG concentrations were determined using a cortisol-binding capacity assay with [^{3}H]cortisol (PerkinElmer Life Sciences, Piscataway, NJ) as the labeled ligand (22). Serum progesterone levels were measured using an enzyme immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI). Freeze and thaw cycles of the samples were avoided and each sample was assayed in duplicate. Albumin levels were determined using a rapid dye-binding method (23) in the maternal peripheral and intervillous blood samples as an internal plasma protein concentration marker.

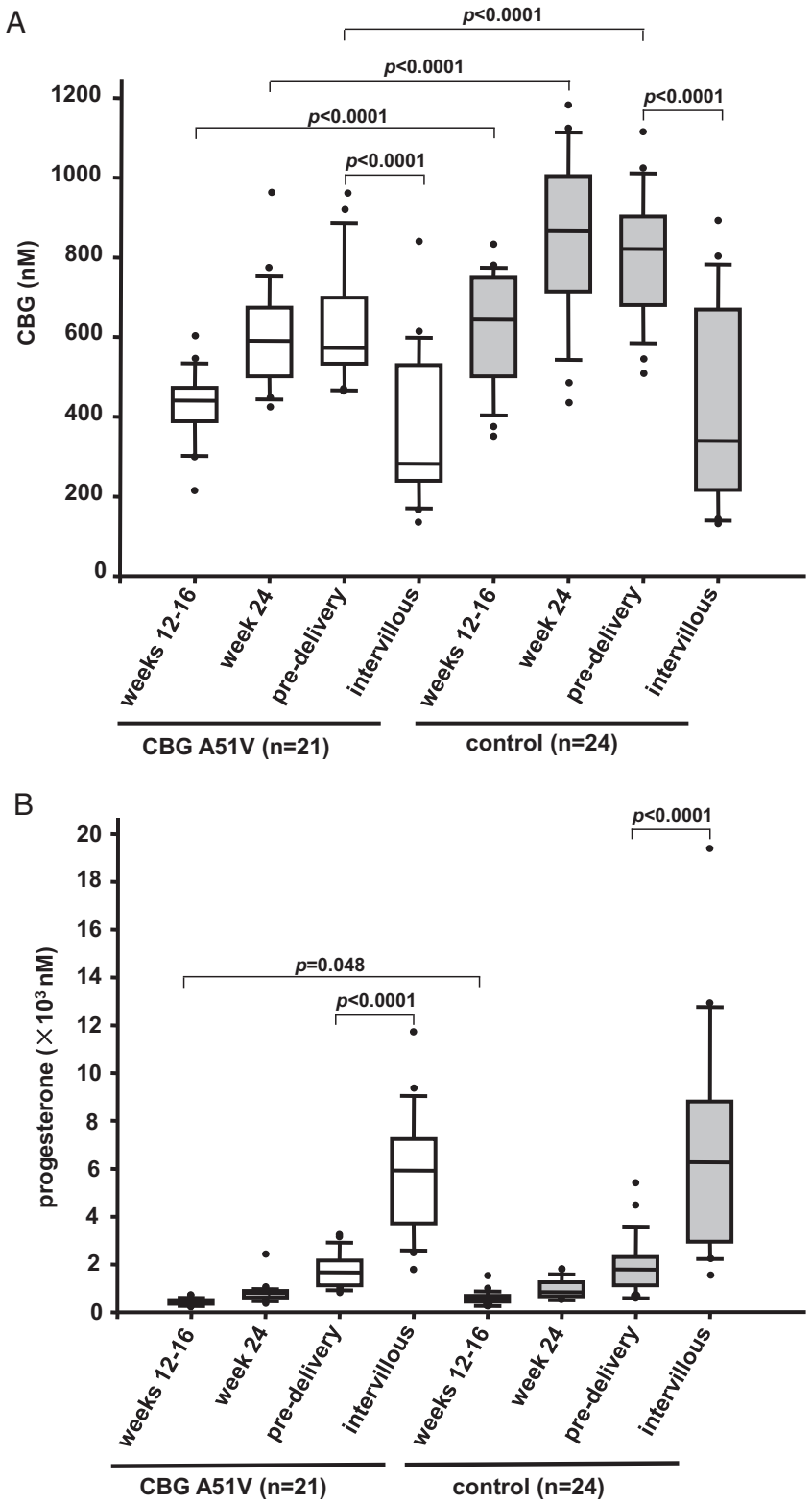

Figure 1. Box plots of serum CBG and progesterone levels of CBG A51V carriers and controls during gestation. A, CBG concentrations were measured using a cortisol-binding capacity assay in peripheral blood taken from pregnant women heterozygous for CBG A51V (n = 21) or control pregnant women (n = 24) at weeks 12–16 and week 24 of gestation, and at term (predelivery), as well as from intervillous blood taken from placentas. B, Progesterone was measured in the same samples from pregnant women heterozygous for CBG A51V or control pregnant women. The central boxes represent the values from the lower to upper quartile (25–75 percentiles). The middle lines represent medians and bars represent 95% confidence interval (CI) ranges. Outliers are displayed as separate points. Mann-Whitney *U* test was used to analyze the differences between groups.

Statistical analysis

Differences between maternal ages were established with the Mann-Whitney *U* test for independent samples. The Student *t* test was used to analyze continuous variables including BMI, BP, weeks of gestation at delivery, birth weight, birth size, and placental weight. To compare differences of primigravida percentage, primipara percentage, ratio of cesarean section to spontaneous labor, neonate CBG A51V genotype, etc., we used a χ^2 test. The linear Pearson correlation method was used for the association analyses of CBG and progesterone levels in peripheral and intervillous blood samples. Because repeated measures from the same individuals were performed on samples at weeks 12–16 and 24, the generalized estimating equations method (24) was used to assess the relationship between progesterone and CBG levels in these samples. Statistical analyses were performed using SPSS software (version 11.5; SPSS Inc., Chicago, IL), and $P < .05$ was considered significant.

A χ^2 test and a binary proportion test (two-sample test of proportions) were both used to analyze the difference in SSR between the CBG A51V group and a reference group derived from 13 439 male and 11 448 female infants delivered at the same hospital (25). In addition, a binary proportion test (one-sample test of proportions) was used for comparison with the Chinese National SSR (from 2009–2012) obtained from the National Bureau of Statistics of China (http://data.stats.gov.cn/). These analyses were conducted using STATA (version 12; StataCorp, College Station, TX), and $P < .05$ was considered significant.

Results

Maternal and infant characteristics

From the 1978 pregnant Chinese women recruited at 12–16 weeks of gestation, 48 heterozygous and two homozygous carriers of CBG A51V (frequency ~1:40) were identified. As expected, both women homozygous for the mutant *SERPINA6* rs146744332 allele expressing CBG A51V had very low plasma CBG levels (mean of 168nM) at gestational weeks 12–16, whereas the median

levels in the control and heterozygous CBG A51V groups were 646nM and 441nM, respectively (Figure 1A). We obtained blood samples later during pregnancy from only one of the women homozygous for the mutant rs146744332 allele. Her plasma CBG levels were 157nM, 208nM, and 364nM at gestational weeks 15, 24, and term, respectively. One homozygote reported chronic pain and depression while the other did not, but both had normal labor at term and delivered healthy singleton babies of both sexes.

Among the 48 heterozygous CBG A51V carriers, one had a pregnancy termination at 25 weeks because of fetal trisomy 21. There were also two premature stillbirths at 26 and 31 weeks of gestation because of umbilical cord torsion and hydrothorax/ascites, respectively. The CBG A51V carrier who prematurely delivered at 31 weeks was carrying a female CBG wild-type fetus. Compared with the stillbirth frequency recorded by the Beijing Obstetrics and Gynecology Hospital (0.09%), the relative number of stillbirths in the A51V mothers seems higher.

We followed 46 CBG A51V pregnant women and 60 of the 63 randomly selected controls clinically until term. There were no differences in maternal characteristics between A51V carriers and controls in BMI, BP, number of previous pregnancies, mode of delivery, and gestational age at delivery (Table 1), or adverse symptoms including chronic pain/fatigue (Supplemental Figure 1). The maternal *SERPINA6* genotype had no influence on infant birth weight or size, placental weight, Apgar score (Table 2), or the birthing process (Supplemental Figure 2).

Serum CBG and progesterone levels in CBG A51V carriers and controls during gestation

Maternal serum samples were obtained from 21 heterozygous A51V carriers and 24 controls at gestational weeks 12–16, 24, and at term (37–41 wk), as well as intervillous blood samples from placentas postdelivery. As expected, peripheral serum CBG levels increased progressively across gestation, and were significantly ($P < .0001$) lower in CBG A51V carriers than in the controls at all gestational stages; ie, when compared with the median serum CBG levels of controls, those in CBG A51V mothers were 32% lower at 12–16 and 24 weeks of gestation and 30% lower just prior to delivery (Figure 1A). In both CBG A51V and control mothers, peripheral CBG levels at term were not significantly different from those at 24 weeks of gestation (Figure 1A). The median intervillous CBG levels were much lower than maternal peripheral levels ($P < .0001$) in A51V carriers (573nM vs 307nM, respectively) and controls (822nM vs 340nM, respectively). Surprisingly, the CBG levels in the intervillous samples of CBG A51V carriers and controls were similar, despite being much lower in the peripheral blood samples of CBG A51V carriers at term (Figure 1A).

We also noted that the relative amounts of CBG in the intervillous vs the predelivery peripheral samples of both A51V carriers and controls varied considerably between individuals, irrespective of the *SERPINA6* genotype (Figure 2A). In 10 of 45 cases, intervillous CBG levels were within 10% of maternal peripheral levels at term, ie, within the range of assay variability (Figure 2A, left panel).

Table 1. Clinical Profiles of Pregnant Women Heterozygous for CBG A51V Versus Controls With a Wild-Type *SERPINA6* rs 146744332 Allele

Clinical Profiles	A51V (n = 46)	Control (n = 60)	*P* Value
Maternal age, y	28.8 ± 3.1	29.9 ± 2.9	.07
Pre-pregnancy BMI, kg/m^{2a}	21.0 ± 3.8	21.0 ± 2.8	.92
Pre-delivery BMI, kg/m^{2a}	27.1 ± 3.9	27.5 ± 3.1	.61
Systolic BP in labor, mm Hg	120.8 ± 12.5 (n = 40)	122.6 ± 12.2 (n = 55)	.47
Diastolic BP in labor, mm Hg	76.4 ± 8.9 (n = 40)	77.2 ± 10.5 (n = 55)	.68
Primigravida, %	26 (56.5)	34 (56.7)	.99
Multigravida, n = 2–5	20	26	
Primipara, %	43 (93.5)	59 (98.3)	.19
Multipara, n = 2	3	1	
Delivery mode	12:31	24:36	.20
Cesarean section: spontaneous labor	(n = 43)^{b}	(n = 60)	
Gestation at delivery, wk	38.9 ± 1.0 (n = 43)^{b}	38.9 ± 1.1 (n = 60)	.94
Gestation at delivery, wk	39.1 ± 0.8	39.1 ± 1.0	.93
Spontaneous labor	(n = 31)	(n = 36)	

When appropriate results are expressed as the means ± SD. $P < .05$ was accepted as significant.

[a] BMI is defined as body mass (kg) divided by the square of the height (m).

[b] One pregnancy termination at 25 weeks of gestation because of fetal trisomy 21 and two premature stillbirths at 26 and 31 weeks of gestation because of umbilical cord torsion and hydrothorax/ascites were not included.

Table 2. Characteristics of Infants Delivered by CBG A51V Carriers and Control Mothers

	A51V Carrier		Control		
Characteristic	**Mean ± SD**	**n**	**Mean ± SD**	**n**	***P* Value**
Weight at birth, g	3392.4 ± 356.4	43	3365.2 ± 365.5	60	.71
Size at birth, cm	50.1 ± 0.7	35	50.2 ± 1.0	54	.49
Placental weight, g	506.3 ± 105.0	34	601.4 ± 138.1	55	.12
Apgar score at 5 min	10	43	10	60	—
Sex ratio, M:F	17:29	46	35:25	60	.029[b]
Genotype, A51V:WT	13:11	24	2[a]:25	27	.0005[b]

Abbreviations: F, female; M, male; WT, wild type.

[a] Neonates were both female.

[b] $P < .05$ was accepted as significant.

However, CBG levels in the other 35 samples were reduced by 55 ± 20% in intervillous samples when compared with the corresponding predelivery peripheral samples, and in 17 cases by as much as 62–85% (Figure 2A, right panel). In addition, these data (Figure 2A) reveal that the CBG levels in intervillous blood samples of women (n = 17) undergoing a cesarean delivery (272 ± 159nM) were lower ($P = .001$) when compared with mothers (n = 28) who delivered spontaneously at full term (488 ± 226nM). These wide variations in CBG levels in the intervillous vs peripheral samples contrast with much smaller differences in albumin levels between the corresponding maternal peripheral and intervillous blood samples (Figure 2B), which were approximately 8% lower in the intervillous samples, although CBG and albumin levels in the intervillous samples correlated with each other (r = 0.317, $P = .002$).

Serum progesterone levels from both A51V carriers and controls exhibited the expected increases throughout gestation and levels were consistently higher in intervillous samples, irrespective of the *SERPINA6* genotype (Figure 1B). The progesterone levels in peripheral samples taken during the first trimester (12–16 wk) were lower ($P = .048$) in A51V mothers than in the controls, but not at later stages of pregnancy (Figure 1B). In addition, there was a positive (r = 0.459, $P = .0003$) correlation between progesterone and CBG levels during the first (12–16 wk) and second (24 wk) trimesters of pregnancy (Figure 3A), but not in the predelivery samples (Figure 3B). Importantly, progesterone levels in intervillous samples also correlated positively (r = 0.637; $P < .0001$) with the CBG levels (Figure 3C).

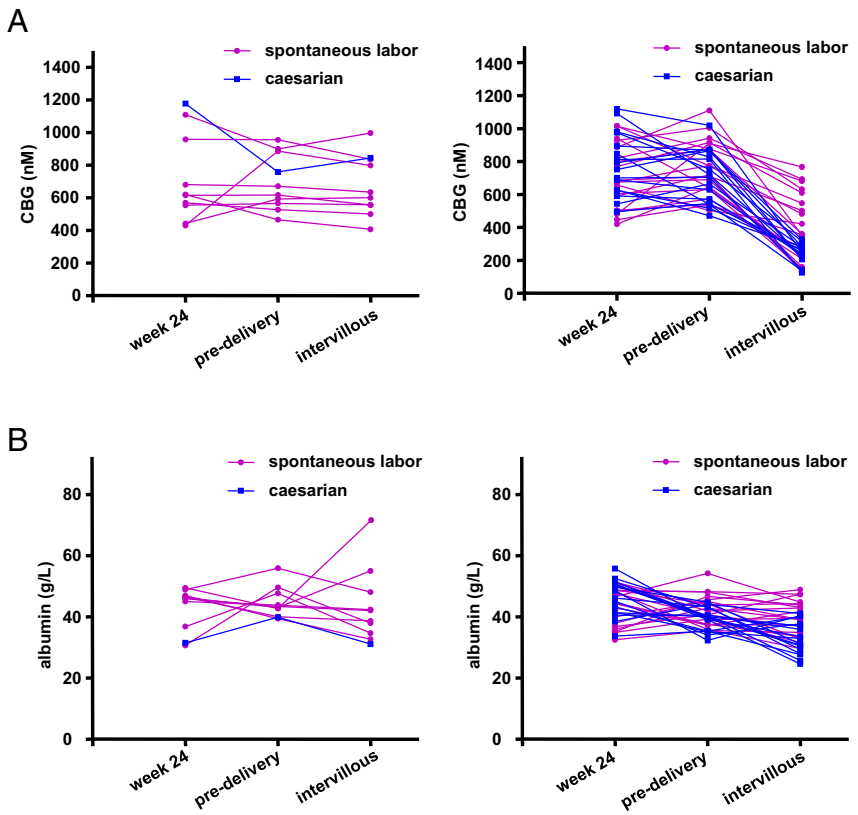

Figure 2. Individual changes in CBG (A) and albumin (B) levels in peripheral serum at gestational week 24 and term (predelivery), as well as in intervillous blood samples. The 10 cases whose intervillous CBG levels were within 10% of maternal peripheral levels at term (ie, within the range of assay variability) are shown in the left panel. The other 35 samples whose intervillous CBG levels were reduced by 55 ± 20% when compared with the corresponding predelivery peripheral samples are delineated in the right panel. Purple and blue lines represent spontaneous and cesarean delivery, respectively.

Female-skewed sex ratio in infants born to CBG A51V mothers

From the original cohort of approximately 2000 women, we collected cord blood at term from neonates of 24 heterozygous A51V carriers and 27 controls. As expected, approximately 50% of the

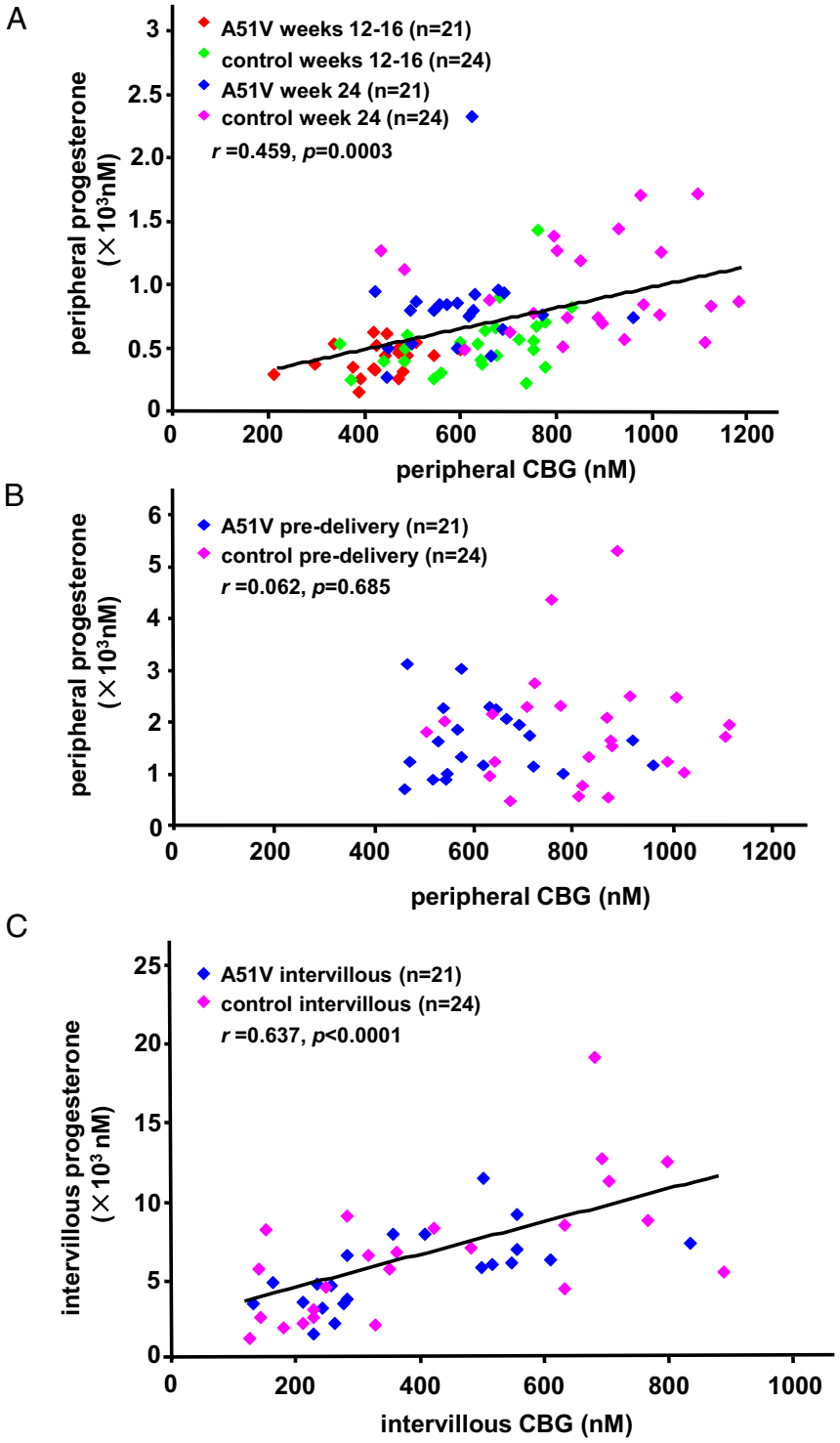

Figure 3. Relationship between progesterone and CBG levels in peripheral and intervillous blood samples. A, In peripheral serum from women at gestational weeks 12–16 and week 24. B, In peripheral serum from women at term (predelivery). C, In intervillous blood from placentas at delivery. The Pearson correlation method was used to obtain correlation coefficients and their significance, except for panel A in which the generalized estimating equations method was used to assess the significance of the correlation between progesterone and CBG levels.

A51V mothers (n = 13) had offspring with the mutant *SERPINA6* rs146744332 allele ($P = .0005$). These infants were all heterozygous for the A51V mutant but their sex ratio was remarkably female skewed (ie, three males and 10 females), whereas the sex ratio in the 11 wild-type infants delivered by the A51V mothers was at the expected norm (ie, six males and five females). Moreover, when the sex of infants born to all 46 heterozygous A51V mothers and 60 controls in this cohort was reviewed, the SSR (ie, the ratio of male-to-female live births) of infants delivered by A51V mothers was 0.59 (ie, 17 males and 29 females), and was much lower ($P = .029$) than the SSR of 1.4 in the control group (ie, 35 males and 25 females).

This surprising difference in SSR within the original cohort prompted us to collect blood from another 2051 pregnant women at term. Among these subjects, we identified 55 CBG A51V heterozygous carriers (frequency ~1:37) for whom the sex of their offspring was known. In total, we therefore identified 105 A51V carriers of 4029 pregnant women, and the SSR in 101 of these women was 0.77 (ie, 44 males and 57 females; male proportion, 0.436). The SSR in the offspring of these A51V carriers is also significantly different from both the SSR (1.17; male proportion, 0.540; $P = .036$) in a reference cohort from the same hospital (25) and the Chinese National SSR (ie, 1.18; male proportion, 0.541; $P = .034$) (Table 3).

Discussion

Patients with CBG deficiencies usually present with low morning cortisol levels, low total cortisol levels, and low ACTH-stimulated peak cortisol levels, but their free cortisol levels in serum, saliva, or urine are within normal ranges (12–16). These biochemical measures imply that the hypothalamic-pituitary-adrenal (HPA) feedback loop readjusts the homeostatic balance between adrenal cortisol output and pituitary ACTH secretion, so that tissues of these individuals are exposed to appropriate levels of cortisol. What is not known is how

Table 3. Male Proportions and SSR at Birth for Neonates Delivered by Heterozygous CBG A51V and Reference Mothers

A51V Mothers (n = 101)		Reference Mothers			
Male Proportion (95% CI)	**SSR**	**Male Proportion (95% CI)**	**SSR**	**Statistical Analyses[c]**	***P* Value**
0.436 (0.339–0.532)	0.77	0.540[a] (0.534–0.546)	1.17	χ^2 test	$\chi^2 = 4.410$ $P = .036$
				Two-sample test of proportions diff = proportion (x)-proportion (y)	Ha (diff < 0), $P = .018$ Ha (diff $\neq 0$), $P = .036$ Ha (diff > 0), $P = .98$
0.436 (0.339–0.532)	0.77	0.541[b]	1.18	One-sample test of proportions P = proportion (x)	Ha (P $< .541$), $P = .017$ Ha (P $\neq .541$), $P = .034$ Ha (P $> .541$), $P = .98$

Abbreviation: CI, confidence interval.

[a] Male proportion recorded for 24 887 neonates delivered at the same hospital (25).

[b] Male proportion from the Chinese national database (2009–2012) (http://data.stats.gov.cn).

[c] Variables x and y represent the male proportions for neonates delivered by CBG A51V and reference mothers, respectively.

CBG-deficient individuals cope with stress, acute inflammation, and infection, or situations when the HPA axis is not mature or is influenced by changes in physiological state. During pregnancy, the fetal HPA axis is both maturing and exposed to other drivers such as CRH from the placenta, whereas their mothers experience dynamic endocrine changes involving the HPA axis (26). We therefore examined how a CBG deficiency in Chinese women might influence their pregnancy outcomes.

Although peripheral CBG levels in our control group of Chinese women resemble those in Australian women at different stages of gestation (27), they were significantly lower in A51V carriers throughout pregnancy. The reduction in CBG levels in pregnant women heterozygous for the A51V mutation was not as marked as observed in nonpregnant Chinese women (17), but this might reflect an increased hepatic production of CBG during pregnancy (28), or a longer half-life of CBG due to a pregnancy-specific difference in its glycosylation (29). Importantly, the CBG levels measured during the first trimester in two women homozygous for the mutation suggest that CBG A51V is produced at approximately one fifth the level associated with a normal CBG. This provides a direct measure of the effect that the reduced secretion of CBG A51V has on circulating CBG levels in vivo, and this is consistent with a more than 40% reduction in serum CBG levels in nonpregnant women heterozygous for CBG A51V (17).

Low circulating CBG levels in pregnant women expressing the CBG A51V mutant were not associated with symptoms of secondary adrenal insufficiency, and did not affect BMI, gestational age at delivery, duration of parturition, infant birth weight or size, placental weight, etc., (Table 1). There was a higher than expected incidence of fetal complications in women with a CBG A51V mutant, but the small number of cases (n = 2) precludes any conclusions regarding this. However, a CBG deficiency would have most likely exacerbated the extreme fetal stress caused by the underlying pathologies in these cases.

Adequate maternal ovarian progesterone production is essential for embryo implantation and early placental development, and the very high amounts of progesterone produced by the placenta are necessary for maintaining the maternal-fetal unit (30). Given that the uterus and placenta are both critical sites of progesterone action in these situations, an understanding of how the amounts and bioavailability of progesterone are controlled at the maternal-fetal interface may help inform how maternal progesterone supplementation might be used to prevent early pregnancy loss (31) or preterm birth (32).

Pregnant women were recruited at the first and second trimester transition, shortly after the syncytiotrophoblast takes over as the major source of circulating progesterone. Our results provide evidence that CBG is a key determinant of progesterone concentrations in the maternal circulation, at least between the first and second trimesters when pregnancy loss frequently occurs (33). They also demonstrate that the CBG concentration determines the amount of progesterone in intervillous blood. Taken together, these new findings suggest that exogenous progesterone given to promote establishment of a viable maternal-fetal unit, or during late pregnancy to prevent preterm birth, may rely on adequate levels of CBG to reduce its metabolic clearance and to modulate cortisol and progesterone bioavailability at the maternal-fetal interface.

We also noted that intervillous blood levels of CBG from placentas of women undergoing cesarean sections were approximately 50% lower than those undergoing spontaneous delivery, irrespective of the *SERPINA6* ge-

notype. Moreover, whereas very low CBG levels were observed in intervillous samples in 16 of 17 placentas from women who had cesarean deliveries, the same was observed in only nine of 28 women who delivered spontaneously, and this may be related to differences in the mode of delivery and recovery of the placentas. However, the loss of albumin in these same samples was much lower than that observed for CBG, suggesting a preferential disappearance of CBG at the maternal-fetal interface. Our results also confirm that the concentrations of progesterone in the intervillous blood are always approximately 20 times greater than those of CBG (10), and it is therefore unlikely that CBG has a role in regulating free progesterone levels within the placenta or the adjacent maternal uterine cells. Nevertheless, the very low levels of CBG in the intervillous blood of some women at term is clearly linked with lower total progesterone levels in this location, and it will be of interest to determine the biochemical basis for these differences and their physiological relevance.

The small family sizes of pedigrees with CBG deficiencies have raised the possibility of increased pregnancy loss and/or decreased fertility (20), but we observed no obvious effects of CBG A51V on pregnancy outcome. However, women were not recruited until after most early pregnancy losses may have occurred (33). Thus, whereas we cannot rule out an effect on early pregnancy loss, the parity data in the CBG A51V carriers and control mothers suggest this is unlikely. By contrast, the female-skewed SSR of infants born to CBG A51V carriers whose pregnancy outcomes were monitored was unexpected. This prompted us to re-examine this in an additional ~2000 mothers and their infants, which provided more robust evidence that the SSR of infants born to CBG A51V carriers is female skewed.

Although the SSR among different populations generally manifests as a small male preponderance (34, 35), a female-skewed SSR is frequently observed after stress-inducing adverse life events (36). The biological basis for a female-skewed SSR remains obscure, but contributing factors include altered parental peri-conception hormone levels or preferential male fetal loss due to poor maternal health (37), and to stress in particular (36). Mortality is also known to be greater in male vs female fetuses and neonates, as evidenced by sex differences in stillbirths and neonatal deaths (37).

Our finding that CBG A51V is associated with a female-skewed SSR in Chinese women builds on observations in isolated pedigrees that women with a CBG deficiency have more female offspring (12, 14, 15), and suggests that CBG deficiencies might contribute to male fetal demise due to inappropriate exposures of cortisol or progesterone during gestation. In the 13 cases in which both mother and fetus carry the A51V mutation, the SSR was extremely female-skewed and this needs to be confirmed. In future studies of CBG deficiencies during pregnancy and its relationship with SSR, more attention will must be focused on maternal health status. In particular, it will be of interest to determine whether genetic CBG deficiencies exacerbate the female-skewed SSR of infants born to mothers experiencing unusual levels of stress at specific stages of pregnancy (38).

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References

1. **Hammond GL.** Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev*. 1990; 11:65–79.
2. **Bolton JL, Hayward C, Direk N, et al.** Genome wide association identifies common variants at the SERPINA6/SERPINA1 locus influencing plasma cortisol and corticosteroid binding globulin. *PLoS Genet*. 2014;10:e1004474.
3. **Perogamvros I, Ray DW, Trainer PJ.** Regulation of cortisol bioavailability—effects on hormone measurement and action. *Nat Rev Endocrinol*. 2012;8:717–727.
4. **Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW.** The serum transport of steroid hormones. *Recent Prog Horm Res*. 1982;38:457–510.
5. **Hammond GL, Smith CL, Paterson NA, Sibbald WJ.** A role for corticosteroid-binding globulin in delivery of cortisol to activated neutrophils. *J Clin Endocrinol Metab*. 1990;71:34–39.
6. **Jung C, Ho JT, Torpy DJ, et al.** A longitudinal study of plasma and

urinary cortisol in pregnancy and postpartum. *J Clin Endocrinol Metab*. 2011;96:1533–1540.
7. **Demey-Ponsart E, Foidart JM, Sulon J, Sodoyez JC.** Serum CBG, free and total cortisol and circadian patterns of adrenal function in normal pregnancy. *J Steroid Biochem*. 1982;16:165–169.
8. **Abou-Samra AB, Pugeat M, Dechaud H, et al.** Increased plasma concentration of N-terminal beta-lipotrophin and unbound cortisol during pregnancy. *Clin Endocrinol (Oxf)*. 1984;20:221–228.
9. **O'Leary P, Boyne P, Flett P, Beilby J, James I.** Longitudinal assessment of changes in reproductive hormones during normal pregnancy. *Clin Chem*. 1991;37:667–672.
10. **Benassayag C, Souski I, Mignot TM, et al.** Corticosteroid-binding globulin status at the fetomaternal interface during human term pregnancy. *Biol Reprod*. 2001;64:812–821.
11. **Smith CL, Power SG, Hammond GL.** A Leu—His substitution at residue 93 in human corticosteroid binding globulin results in reduced affinity for cortisol. *J Steroid Biochem Mol Biol*. 1992;42:671–676.
12. **Emptoz-Bonneton A, Cousin P, Seguchi K, Avvakumov GV, Bully C, Hammond GL, Pugeat M.** Novel human corticosteroid-binding globulin variant with low cortisol-binding affinity. *J Clin Endocrinol Metab*. 2000;85:361–367.
13. **Perogamvros I, Underhill C, Henley DE, et al.** Novel corticosteroid-binding globulin variant that lacks steroid binding activity. *J Clin Endocrinol Metab*. 2010;95:E142–E150.
14. **Hill LA, Vassiliadi DA, Simard M, et al.** Two different corticosteroid-binding globulin variants that lack cortisol-binding activity in a Greek woman. *J Clin Endocrinol Metab*. 2012;97:4260–4267.
15. **Torpy DJ.** Familial corticosteroid-binding globulin deficiency due to a novel null mutation: Association with fatigue and relative hypotension. *J Clin Endocrinol Metab*. 2001;86:3692–3700.
16. **Torpy DJ, Lundgren BA, Ho JT, Lewis JG, Scott HS, Mericq V.** CBG Santiago: A novel CBG mutation. *J Clin Endocrinol Metab*. 2012;97:E151–E155.
17. **Lin HY, Underhill C, Lei JH, et al.** High frequency of SERPINA6 polymorphisms that reduce plasma corticosteroid-binding globulin activity in Chinese subjects. *J Clin Endocrinol Metab*. 2012;97:E678–E686.
18. **Simard M, Hill LA, Lewis JG, Hammond GL.** Naturally occurring mutations of human corticosteroid-binding globulin. *J Clin Endocrinol Metab*. 2015;100:E129–E139.
19. **Torpy DJ, Bachmann AW, Gartside M, et al.** Association between chronic fatigue syndrome and the corticosteroid-binding globulin gene ALA SER224 polymorphism. *Endocr Res*. 2004;30:417–429.
20. **Cizza G, Bernardi L, Smirne N, et al.** Clinical manifestations of highly prevalent corticosteroid-binding globulin mutations in a village in southern Italy. *J Clin Endocrinol Metab*. 2011;96:E1684–E1693.
21. **Brunner E, Baima J, Vieira TC, Vieira JG, Abucham J.** Hereditary corticosteroid-binding globulin deficiency due to a missense mutation (Asp367Asn, CBG Lyon) in a Brazilian kindred. *Clin Endocrinol (Oxf)*. 2003;58:756–762.
22. **Hammond GL, Lähteenmäki PL.** A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin binding capacities. *Clin Chim Acta*. 1983;132:101–110.
23. **Dumas BT, Watson WA, Biggs HG.** Albumin standards and the measurement of serum albumin with bromcresol green. 1971. *Clin Chim Acta*. 1997;258:21–30.
24. **Zeger SL, Liang KY.** Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42:121–130.
25. **Yang X, Li Y, Li C, Zhang W.** Current overview of pregnancy complications and live-birth outcome of assisted reproductive technology in mainland China. *Fertil Steril*. 2014;101:385–391.
26. **Braun T, Challis JR, Newnham JP, Sloboda DM.** Early-life glucocorticoid exposure: The hypothalamic-pituitary-adrenal axis, placental function, and long-term disease risk. *Endocr Rev*. 2013;34:885–916.
27. **Ho JT, Lewis JG, O'Loughlin P, et al.** Reduced maternal corticosteroid-binding globulin and cortisol levels in pre-eclampsia and gamete recipient pregnancies. *Clin Endocrinol (Oxf)*. 2007;66:869–877.
28. **Rosenthal HE, Slaunwhite WR, Jr, Sandberg AA.** Transcortin: a corticosteroid-binding protein of plasma. X. Cortisol and progesterone interplay and unbound levels of these steroids in pregnancy. *J Clin Endocrinol Metab*. 1969;29:352–367.
29. **Mitchell E, Torpy DJ, Bagley CJ.** Pregnancy-associated corticosteroid-binding globulin: High resolution separation of glycan isoforms. *Horm Metab Res*. 2004;36:357–359.
30. **Simpson ER, MacDonald PC.** Endocrine physiology of the placenta. *Annu Rev Physiol*. 1981;43:163–188.
31. **Wahabi HA, Fayed AA, Esmaeil SA, Al Zeidan RA.** Progestogen for treating threatened miscarriage. *Cochrane Database Syst Rev*. 2011;12:CD005943.
32. **Norwitz ER, Caughey AB.** Progesterone supplementation and the prevention of preterm birth. *Rev Obstet Gynecol*. 2011;4:60–72.
33. **Norwitz ER, Schust DJ, Fisher SJ.** Implantation and the survival of early pregnancy. *N Engl J Med*. 2001;345:1400–1408.
34. **Grech V, Savona-Ventura C, Vassallo-Agius P.** Research pointers: Unexplained differences in sex ratios at birth in Europe and North America. *BMJ*. 2002;324:1010–1011.
35. **Garenne M.** Sex ratios at birth in African populations: A review of survey data. *Hum Biol*. 2002;74:889–900.
36. **Tourikis JD, Beratis IN.** Community psychological stressor-induced secondary sex ratio decline after a seismic sequence in the Greek island of Zakynthos. *J Biosoc Sci*. 2013;45:231–238.
37. **Clifton VL.** Review: Sex and the human placenta: Mediating differential strategies of fetal growth and survival. *Placenta*. 2010;31(Suppl):S33–S39.
38. **Doger E, Cakıroglu Y, Köpük SY, Ceylan Y, Simsek HU, Calıskan E.** Impact of earthquakes on sex ratio at birth: Eastern Marmara earthquakes. *J Turk Ger Gynecol Assoc*. 2013;14:92–97.