Published in final edited form as:

Hum Genet. 2009 January ; 124(6): 659–668. doi:10.1007/s00439-008-0589-2.

The joint associations between *F5* gene polymorphisms and maternal smoking during pregnancy on preterm delivery

Yunxian Yu^{1,3,6,*}, Hui-Ju Tsai^{1,3,#,*}, Xin Liu^{1,3}, Karen Mestan^{2,3}, Shanchun Zhang^{1,3}, Colleen Pearson⁴, Katherin Ortiz⁴, Xiping Xu⁵, Barry Zuckerman⁴, and Xiaobin Wang^{1,3,#}

¹Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center, Chicago, IL, USA

²Division of Neonatology, Children's Memorial Hospital and Children's Memorial Research Center, Chicago, IL, USA

³Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

⁴Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

⁵Center for Population Genetics, University of Illinois at Chicago, School of Public Health, Chicago, IL, USA

⁶Department of Epidemiology and Health Statistics, Zhejiang University, School of Public Health, Hangzhou, Zhejiang, China

Abstract

Factor V (*F5*) genetic variants and maternal smoking during pregnancy individually has been associated with increased risk of preterm delivery (PTD). We hypothesize that *F5* gene and maternal smoking may synergistically increase the risk of PTD. Three single nucleotide polymorphisms (SNPs) in *F5* gene (rs6019, rs2213869 and rs6022) were genotyped in 542 mothers with PTD and 1,141 mothers with term deliveries at the Boston Medical Center. The individual and interactive effects of *F5* SNPs and maternal smoking on PTD and gestational age were examined, respectively. The results suggested that maternal smoking, three *F5* SNPs and *F5* haplotype were individually associated with PTD and gestational age. More importantly, we found significant interactions between the two *F5* SNPs (rs6019 and rs6022) and maternal smoking on PTD and gestational age. Compared with non-smoking mothers carrying rs6019 GG genotype, persistently smoking mothers carrying genotypes GC or CC were associated with significantly increased risk of PTD (OR(95%CI): 2.1(1.2–3.6) for GC; 5.7(2.1–15.0) for CC; p-interaction=0.02). A significant interaction was also observed for gestational age. Similar pattern

#Correspondence and reprint requests should be addressed to: Hui-Ju Tsai, MPH, PhD, Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center; Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; telephone: 312-573-7735, fax: 312-573-7825, htsai@childrensmemorial.org; h-tsai@northwestern.edu, Xiaobin Wang, MD, MPH, ScD, Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center; Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; telephone : 312-573-7738, fax: 312-573-7825, xbwang@childrensmemorial.org.

*Drs. Yu and Tsai have made equal contributions to the manuscript.

of interactions was found between rs6022 and maternal smoking on PTD. In summary, our data indicated that *F5* gene variants and maternal smoking may synergistically increase the risk of PTD.

Introduction

Preterm delivery (PTD, < 37 weeks of gestation) is one of the leading causes of death among infants and is associated with significant postnatal morbidities and developmental disabilities (Goldenberg 2002). The overall rate of preterm birth was 12.7% in the U.S. in 2004 (Martin et al. 2006). There is compelling evidence that PTD is a complex disease influenced by multiple genetic and environmental factors and gene-environment (G×E) interactions (2006; Crider et al. 2005; Goldenberg et al. 2008; Macones et al. 2004). This report focused on the influence of factor V (*F5*) gene and maternal smoking during pregnancy on PTD.

Maternal smoking has been recognized as a common and preventable risk factor for PTD (Cnattingius 2004; Fantuzzi et al. 2007; Pollack et al. 2000; Wang et al. 2002). The biological mechanisms by which cigarette smoking affects PTD are not yet fully understood, but may be partially due to its well-established procoagulant effects (Wannamethee et al. 2005). Factor V (*F5*) serves as a critical cofactor for the prothrombinase activity of factor Xa, a central regulator of hemostasis. The mutation in *F5* has been found to be associated with resistance to activated protein C (APC) (Bertina et al. 1994; Dahlback and Hildebrand 1994; Dawood et al. 2007) and results in increasing level of procoagulant factor Va (Rosing et al. 1998). Our group has previously demonstrated that *F5* haplotype was associated with PTD in 758 mothers enrolled at Boston Medical Center (BMC) (Hao et al. 2004). Given smoking and *F5* both affect coagulation status and PTD, we hypothesize that they may have synergistic effects on the risk of PTD, which has not been examined in published literature.

This study had two purposes. First, we intended to replicate our previous findings (Hao et al. 2004) of haplotype association of *F5* genetic variants (rs6019, rs2213869 and rs6022) with PTD using newly enrolled subjects in the BMC cohort as well as in pooled samples. Second, we sought to test the new hypothesis that there was a significant interaction between maternal smoking during pregnancy and *F5* gene variants on the risk of PTD in the pooled samples.

Materials and Methods

Study population and data collection

The study sample for this report included the subjects from our previous report (Hao et al. 2004) (1st sample) and new subjects recruited subsequently (2nd sample). The pooled sample (combining 1st and 2nd sample) included 1,683 mothers (542 preterm mothers and 1,141 term controls) enrolled at the Boston Medical Center (BMC) during the period of 1998 to 2004 (Wang et al. 2002). Case mothers were defined as those delivering a singleton live birth at less than 37 weeks gestation, and controls were defined as mothers delivering at greater than or equal to 37 weeks gestation. Birth weight was not included as part of the definition

of preterm cases or full-term controls. Pregnancies resulting in multiple births and newborns with major birth defects were excluded. Detailed description was published previously (Wang et al. 2002). Briefly, we collected comprehensive epidemiologic data, clinical data and maternal venous blood samples in this study. After informed consent was obtained, a maternal questionnaire interview was administered 1 day post normal spontaneous vaginal delivery and 2–3 days post c-section to obtain relevant information including demographics, medical and reproductive history, cigarette smoking, alcohol consumption, illicit drug use, diet and stress. Maternal and infant medical records were reviewed to obtain clinical information including prenatal care, pregnancy complications, and birth outcomes. Overall the participation rate was ~90%. The Institutional Review Boards at the Boston University Medical Center, the Massachusetts Department of Public Health, the Children's Memorial Hospital in Chicago, and the Harvard School of Public Health approved the study protocol. All participants gave written informed consent.

Definition of phenotypes

Preterm delivery (PTD) was evaluated using both continuous and binary (< 37 gestational weeks vs. ≥ 37 gestational weeks) outcomes. Gestational age was assessed using an algorithm based on last menstrual period and the result of early ultrasound (<20 weeks gestation). The last menstrual period estimate was used only if confirmed by ultrasound within 7 days or if no ultrasound estimate was obtained, otherwise, the ultrasound estimate was used. This approach has been used in previous studies (Kramer et al. 1998; Wang et al. 2002).

Maternal Cigarette Smoking

The information on maternal smoking was obtained from the postpartum questionnaire for 4 time periods: 3 months before the index pregnancy and the first, second, and third trimesters of the index pregnancy. Maternal smoking data were clustered into 3 groups: 1) Those who did not smoke throughout the index pregnancy (never smokers); 2) Those who smoked during pregnancy but quit smoking during any trimester (intermittent smokers); and 3) Those who smoked continuously during the index pregnancy (persistent smokers).

Genotyping

F5 SNP genotyping and quality assurance—Three *F5* SNPs (rs6019, rs2213869 and rs6022) were genotyped in 1,683 mothers. DNA was extracted from samples of venous whole blood in accordance with standard protocol (Sambrook et al. 1989). SNP genotyping was performed either by the BeadArray™ technology of Illumina Inc (Hao et al. 2004) or by the TaqMan allelic discrimination method (Hong et al. 2007). For quality assurance, each plate included one blank control (no DNA template). A random 5% of the samples were independently repeated using both genotyping platforms. The concordance of these duplicated samples were greater than 99.5% for each SNP.

Ancestry informative markers (AIMs) genotyping—To estimate individual admixture proportions in African Americans in the BMC cohort, we genotyped a panel of 61 AIMs with averaged δ (difference of allele frequencies between African and European ancestral populations) equal to 0.59 in 735 African American mothers (296 preterm cases

and 439 controls). Detailed information regarding polymorphic site, flanking sequence and other relevant information for all the 61 AIMs can be found in previous publication (Yang et al. 2005).

Statistical analyses

We first assessed whether each *F5* SNP was under Hardy-Weinberg equilibrium (HWE) among controls in three self-reported ethnic groups (African Americans, Hispanics and Whites) using the exact tests implemented in the SNP-HWE program (Wigginton et al. 2005). We also calculated pair-wise linkage disequilibrium (LD) (r^2 measures) for three *F5* SNPs among controls in three ethnic groups, separately, using the Haploview program (Barrett et al. 2005).

For replication of the haplotype association, we first reconstructed haplotype for three *F5* SNPs (rs6019, rs2213869 and rs6022) in three ethnic groups, separately, using EM algorithm implemented in gap, an R package (Zhao 2007). Next, the *F5* haplotype associations with PTD were performed in the 1st and 2nd sample as well as the pooled sample (pooled the 1st and 2nd sample together), respectively, using chi-square test. Likewise, similar replication analysis was carried out in African American mothers only (the 1st, 2nd and the pooled sample, individually).

For testing *F5* × smoking interaction, we applied logistic regression and linear regression to examine the individual and interactive effects of *F5* genetic polymorphisms and maternal smoking on the risk of PTD in pooled samples, African American mothers and White/Hispanic mothers, respectively. The interactions of maternal smoking and *F5* SNPs were tested by adding a product term in the model. Since the sample size of intermittent smoking during pregnancy was small, the mothers with intermittent smoking were only included in the analyses for testing individual effects, not in those for testing interactive effect. To minimize false positive results due to multiple testing issue, we applied false discovery rate (FDR) to correct for multiple testing by using more stringent significance cutoff point (Benjamini and Yekutieli 2001). The threshold of statistically significant *p* value after correcting for multiple testing is 10^{-2} .

All analyses included the following covariates: maternal age (< 20, 20–24, 25–29, and 30 years), ethnicity (African Americans, Hispanics and Whites), education (less than high school, high school, and greater than high school), parity (0, 1, and 2), marital status (married, other), maternal pre-pregnant body mass index (BMI) (< 20, 20–24, 25–29, and 30), alcohol use (yes/no), passive smoke exposure (yes/no), drug use (yes/no), infant gender and small for gestational age (SGA) status (Wang et al. 2006). We performed parsimonious model and a model including well-recognized risk factors of PTD by the literature and our previous work. We found consistent results regardless of the numbers of covariates included in the models, indicating the robustness of our results. Therefore, we reported the results with the adjustment of the above covariates.

In addition, we previously identified the interaction of two metabolic genes (*CYP1A1* and *GSTT1*) with maternal smoking during pregnancy on PTD in this study population (Tsai et al. 2008). To assess if these genetic variants and their interactions with smoking could affect

our analysis, we included the genetic variants of *CYP1A1* and *GSTT1* and their interaction with maternal smoking in the regression models for testing $F5 \times$ smoking interaction.

Due to data availability, we were able to perform a subset analysis to assess potential population stratification confounding in African American mothers. We first estimated individual admixture proportions using 61 AIMs data by applying a Bayesian approach implemented in the Structure program (Falush et al. 2003; Pritchard et al. 2000). We then repeated regression analyses in 735 African American mothers with and without adjusting for ancestral proportions.

Data analyses were performed using statistical packages R 2.6.2 and SAS (Version 9.0, SAS Institute, Inc., Cary, NC).

Results

Demographic, clinical and genetic characteristics

The pooled sample consists of a total number of 1,683 mothers (542 PTD cases, and 1,141 term controls) in this study. The distributions of maternal pre-pregnant BMI, maternal age, ethnicity, educational status, parity, alcohol use, passive smoking exposure and infant sex were similar in preterm vs. term groups (Table 1). The proportions of unmarried mothers and maternal smoking and drug use during pregnancy were higher in the preterm cases than those in the term group. The prevalence of SGA was higher in the term group than in the preterm group. All three *F5* genetic polymorphisms were under HWE in each of the three ethnic groups. The distributions of genotype frequencies for these three SNPs were different between preterm and term groups (Table 1). Additionally, the pair-wise LD measures (r^2 measures) between rs6019, rs2213869 and rs6022 among three ethnic groups were as follows: rs6019 vs. rs2213869, rs6019 vs. rs6022 and rs2213869 vs. rs6022 in African American: 0.74, 0.37 and 0.50; in White: 1.0, 0.24 and 0.24; and in Hispanic: 0.92, 0.26 and 0.29.

Replication of the *F5* haplotype association

We previously reported that *F5* haplotypes (rs6019, rs2213869 and rs6022) were associated with PTD in the 1st sample (Hao et al. 2004). In this study, we analyzed the *F5* haplotype association with PTD in the 1st and 2nd samples, separately, and then repeated the analysis in the pooled sample. Of note, 60% of the study sample was African Americans, therefore, similar analysis was carried out in the African American mothers. As shown in Supplemental Table 1, we observed a similar trend of haplotype association in the 2nd sample with marginal significance ($P = 0.11$); and importantly we replicated the association in the 2nd sample among the African American mothers ($P = 0.02$). In addition, the previously reported significant haplotype association with PTD was confirmed in the pooled sample ($P < 10^{-3}$). However, the distribution of GAA haplotype was not concordant between batch 1 and batch 2 samples of both pooled samples and AA mothers.

Individual associations of maternal smoking and F5 variants with PTD

Table 2 showed the individual associations of maternal smoking during pregnancy and *F5* polymorphisms with PTD and gestational age among all mothers, after adjusting for important covariates. Compared with never smoking mothers, persistent smoking was significantly associated with the increased risks of PTD (OR (95% CI) = 1.6 (1.1–2.3)), and with lower gestational age (β (SE) and P = -0.86 (0.29) and 3.1×10^{-3}). Intermittent smoking also increased the risk of PTD (OR (95% CI) = 1.1 (0.7–1.7)) and decreased the gestational age (β (SE) and P = -0.23 (0.35) and 0.51), but not statistically significant.

All three SNPs of *F5* gene were individually associated with the increased risk of PTD. For rs6019, in comparison with the GG genotype, subjects carrying one or two C alleles had ORs for PTD of 1.3 (95% CI, 1.0–1.6) and 1.8 (95% CI, 1.2–2.6), respectively. For rs2213869, in comparison with the GG genotype, subjects carrying one or two A alleles had ORs for PTD of 1.1 (95% CI, 0.9–1.4) and 1.7 (95% CI, 1.3–2.3), respectively. For rs6022, in comparison with the CC genotype, subjects carrying one or two A alleles had ORs for PTD of 1.1 (95% CI: 0.9–1.5) and 1.6 (95% CI: 1.1–2.1), respectively. Similarly, compared to mothers with homozygous genotype (GG for rs6019, GG for rs2213869 and CC for rs6022), those with high-risk homozygous genotype (CC for rs6019, AA for rs2213869 and AA for rs6022) had lower gestational age (β (SE) and P = -1.06 (0.31) and 7×10^{-4} for rs6019; -0.68 (0.25) and 6.3×10^{-3} for rs2213869; and -0.65 (0.25) and 8.6×10^{-3} for rs6022). Similar associations were found in stratified analysis for AA mothers and White/Hispanic mothers, respectively (Supplement Table 2).

Interactions of maternal smoking and F5 variants on PTD

Because pair-wise LD of rs6019 and rs2213869 was very high, we further investigated the interactions between two *F5* SNPs on exons (rs6019 and rs6022) and maternal smoking on PTD. As shown in Table 3, we found significantly interactive effects of *F5* SNPs (rs6019 and rs6022) and maternal smoking on PTD and gestational age. Using never smoking mothers with low-risk allele as the reference group, persistent smoking mothers with one or two copies of high-risk allele of *F5* SNPs (rs6019 and rs6022) had significantly higher risk of PTD and lower gestational age, respectively (Table 3a and Figure 1). Using rs6019 as an example, compared with never smoking mothers with GG genotype, those who smoked during pregnancy and carried genotypes GC or CC were associated with substantially increased risk of PTD (OR for GC: 2.1 (95% CI: 1.2–3.6); OR for CC: 5.7 (95% CI: 2.1–15.0); p -interaction = 0.02). Such an interactive association was also observed for gestational weeks (β (se) = -0.97 (0.36); p -interaction = 0.007). Likewise, the interactive effects of rs6022 and maternal smoking were also observed for PTD and gestational weeks. Of note, the joint effects on PTD and gestational age remained statistically significant after correcting for multiple testing (Table 3a). Our data also indicated that the interactive associations appear to be consistent across ethnic groups, as illustrated in Figure 1 and in Table 4, respectively. Additionally, the results in African American mothers were very similar with or without the adjustment of ancestral proportions (Supplemental Table 3).

Adjustment of other potential PTD-related risk factors

In addition to smoking, several environmental factors have been previously reported to be associated with PTD, including pre-pregnant BMI, alcohol use and illicit drug use. Therefore, we also examined whether these factors could interact with *F5* gene or modify the association between *F5* gene and maternal smoking on PTD. We found that none of these risk factors was interactive with *F5* gene on PTD (data not shown). However, these potential risk factors were also taken as covariates in interaction models. Furthermore, since our group demonstrated a significant interaction between two metabolic genes (*CYP1A1* and *GSTT1*) and maternal smoking on the increasing risk of PTD (Tsai et al. 2008), we repeated the above analyses with the adjustment of these two metabolic genetic variants and their interaction with maternal smoking during pregnancy. The joint effects between *F5* variants and maternal smoking on PTD remained statistically significant (Tables 3). Taken together, our data provided strong evidence that the interaction between *F5* gene and maternal smoking on PTD was independent of major environmental confounders, population admixture, and the interaction between *CYP1A1*, *GSTT1* and maternal smoking.

Discussion

Consistent with previous reports, we observed that maternal smoking during pregnancy was associated with a moderately increased risk of PTD (Cnattingius 2004; Fantuzzi et al. 2007; Ness et al. 1999; Pollack et al. 2000; Shah and Bracken 2000; Wang et al. 1997). We also replicated the previous findings (Hao et al. 2004) that *F5* haplotypes were associated with the increased risk of PTD in the pooled sample and in African American mothers. More importantly, this is the first report that demonstrated a significant interaction between *F5* gene variants and maternal persistent smoking which led to a substantially increased risk of PTD.

The underlying biologic mechanism of the observed interaction between maternal smoking and *F5* genetic variants remains to be elucidated, but appears to be biologically plausible. The effect of smoking on procoagulation has been well documented. First, previous findings have suggested that tobacco smoke increases blood viscosity and hematocrit or/and plasma viscosity (Wannamethee et al. 2005), impairs t-PA (tissue plasminogen activator) releasing from the vascular endothelium (Eliasson et al. 1995) and increases the plasma concentrations of t-PA antigen, plasminogen activator inhibitor (PAI) (Wannamethee et al. 2005) and prothrombin fragment F1+2 (Nagashima et al. 2007). Second, it has been reported that smoking during pregnancy reduces the activity of an anticoagulant, activated protein C (APC) (Kafkas et al. 2007). Third, several studies have suggested that smoking during pregnancy may result in hypoxia of placenta and fetus (Longo 1976) and nicotine, a strong vasoconstrictor, may lead to reduce uterine and placental blood flow (Services. 2004; Walsh 1994). On the other hand, factor V (F5) is known as a key enzyme in the coagulation cascade. The abnormality of F5 protein can lead to an increased tendency towards thrombosis. Specifically, several studies have provided strong evidence that the factor V Leiden, a mutant in *F5* gene causing a single amino acid substitution of arginine 506 to glutamine, affects one of the APC cleaving sites, and increases procoagulant factor Va concentration (Bertina et al. 1994; Dahlback and Hildebrand 1994; Rosing et al. 1998).

Taken together, it is likely that smoking mothers with high-risk genotypes of *F5* gene may have a higher procoagulant status, especially in the third trimester given the procoagulant status increases with gestational age (Kafkas et al. 2007).

There were several strengths in the present study. First, this was one of the largest gene-environment studies on PTD. As such, it provided us sufficient power to investigate the individual effects and interactions of *F5* gene-maternal smoking. Second, the detected interactions between *F5* gene and maternal smoking appear to be independent of other known environmental confounders, and the known metabolic genes (*CYP1A1* and *GSTT1*)-smoking interactions (Tsai et al. 2008). Third, the observed associations are robust in the models regardless of numbers of covariates adjusted, or with or without adjustment for population admixture in African Americans. Fourth, the interactive effects of *F5* gene and maternal smoking on PTD appear to be consistent across ethnic groups. Therefore, we believe that our findings are likely generalizable to other U.S. populations. Finally since multiple testing may potentially lead to false-positive results, we used FDR to minimize false positive result. Interestingly, the interactions between *F5* gene and maternal smoking remain statistically significant after accounting for multiple testing.

On the other hand, a number of limitations should be considered. First, information on maternal smoking may be subject to recall bias. However, previous studies have demonstrated fair agreement between self-reported smoking amounts with serum and urinary levels of cotinine, a biochemical marker of cigarette smoke (George et al. 2006; Klebanoff et al. 2001; Peacock et al. 1998). However, those studies in which reported smoking was validated against cotinine were by and large prospective cohorts. To address this concern, we compared 277 subjects' smoking status between self-report in the postpartum questionnaire and medical record documentation. Of note, the data in medical record documentation was collected prospectively during each trimester and may be subject to less recall bias. There was over 99% agreement between self-reported smoking and medical record documentation. Although the mothers might over or under report their smoking amounts and duration, they were unaware of their genotypes. Thus, recall bias should be independent of maternal genotypes. Recall bias, if any, most likely biased the $G \times$ smoking interactions towards the null. Second, due to sample size constraints, we did not evaluate the interactive effect in White and Hispanic mothers separately. Future investigation of these two groups is warranted. Third, this report only focused on three *F5* SNPs that we reported previously. It will be of interest to examine the other candidate *F5* genetic variants, especially the factor V Leiden (the minor allele frequency was very low ($< 1\%$) in this study sample). Fourth, even though both rs6019 and rs6022 locate in exons of *F5* gene, no functional study of these two SNPs has been reported. It will be of importance to further investigate the underlying biological function of the two SNPs. Fifth, the sample size of testing the interaction of *F5* gene and maternal smoking with PTD among White / Hispanic mothers was very small (the sample size of persistent smoking + rs6019 CC and persistent smoking + rs6022 AA was 5 and 9, respectively), so the association was not robust. Sixth, we did not genotype AIMs for the entire study sample. However, our analysis on AIMs in African American mothers (the largest ethnic group in our sample) indicated that population admixture was not a major confounder in our analysis. Last, we did not genotype newborns

in this analysis. It would be useful to simultaneously investigate the joint effect of maternal and fetal genotypes on PTD in future studies.

In summary, we have demonstrated coherent evidence of the synergistic interaction between maternal smoking and *F5* genetic polymorphisms on PTD. Smoking women carrying high-risk genotypes of *F5* gene may be at a substantially higher risk of PTD. Our findings have raised the possibility that women at high risk of PTD can be identified by simultaneously examining both environmental exposures and genetic polymorphisms. The findings in this study may help identify high-risk individuals well before preterm birth occurs, so that effective and targeted preventive and therapeutic measures can be implemented.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

1. Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center; Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA: Yunxian Yu, Hui-Ju Tsai, Xin Liu, Shanchun Zhang, Xiaobin Wang; 2. Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA: Colleen Pearson, Katherin Ortiz, Barry Zuckerman; 3. Center for Population Genetics, University of Illinois at Chicago School of Public Health, Chicago, IL, USA: Xiping Xu

The study was supported in part by grants from the National Institute of Child Health and Human Development (R01 HD41702), National Institute of Environmental Health Sciences (R01ES11682, R21ES11666), and March of Dimes Birth Defects Foundation (20-FY98-0701, 20-FY02-56 and #21-FY07-605).

We thank the nursing staff of Labor and Delivery at Boston Medical Center for their continuous support and assistance to the study and Lingling Fu for data management, and Ann Ramsey for administrative support. We would like to particularly thank the outstanding expert consultants of the BMC Preterm Study team: Drs. Paul Wise, Jerome Klein, John M. Kasznica, and Milton Kotelchuck.

Reference

- Preterm birth: cause, consequences, and prevention. Washington, D.C.: Institute of medicine of the national academies (Committee on understanding premature birth and assuring healthy outcomes board on health sciences policy); 2006.
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265. [PubMed: 15297300]
- Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann. Statist.* 2001; 29:1165–1188.
- Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature*. 1994; 369:64–67. [PubMed: 8164741]
- Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine Tob Res.* 2004; 6(Suppl 2):S125–S140. [PubMed: 15203816]
- Crider KS, Whitehead N, Buus RM. Genetic variation associated with preterm birth: a HuGE review. *Genet Med.* 2005; 7:593–604. [PubMed: 16301860]
- Dahlback B, Hildebrand B. Inherited resistance to activated protein C is corrected by anticoagulant cofactor activity found to be a property of factor V. *Proc Natl Acad Sci U S A.* 1994; 91:1396–1400. [PubMed: 8108421]

- Dawood F, Mountford R, Farquharson R, Quenby S. Genetic polymorphisms on the factor V gene in women with recurrent miscarriage and acquired APCR. *Hum Reprod.* 2007; 22:2546–2553. [PubMed: 17640947]
- Eliasson M, Asplund K, Evrin PE, Lundblad D. Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA Study. *Atherosclerosis.* 1995; 113:41–53. [PubMed: 7755654]
- Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics.* 2003; 164:1567–1587. [PubMed: 12930761]
- Fantuzzi G, Aggazzotti G, Righi E, Facchinetti F, Bertucci E, Kanitz S, Barbone F, Sansebastiano G, Battaglia MA, Leoni V, Fabiani L, Triassi M, Sciacca S. Preterm delivery and exposure to active and passive smoking during pregnancy: a case-control study from Italy. *Paediatr Perinat Epidemiol.* 2007; 21:194–200. [PubMed: 17439527]
- George L, Granath F, Johansson AL, Cnattingius S. Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. *Acta Obstet Gynecol Scand.* 2006; 85:1331–1337. [PubMed: 17091413]
- Goldenberg RL. The management of preterm labor. *Obstet Gynecol.* 2002; 100:1020–1037. [PubMed: 12423870]
- Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* 2008; 371:75–84. [PubMed: 18177778]
- Hao K, Wang X, Niu T, Xu X, Li A, Chang W, Wang L, Li G, Laird N, Xu X. A candidate gene association study on preterm delivery: application of high-throughput genotyping technology and advanced statistical methods. *Hum Mol Genet.* 2004; 13:683–691. [PubMed: 14976157]
- Hong X, Hsu YH, Terwedow H, Arguelles LM, Tang G, Liu X, Zhang S, Xu X, Xu X. CYP19A1 polymorphisms are associated with bone mineral density in Chinese men. *Hum Genet.* 2007; 121:491–500. [PubMed: 17216495]
- Kafkas S, Kalkan U, Bolaman Z, Sanci M, Yuksel H, Odabasi AR. Evaluation of Smoking as a Risk Factor for Activated Protein C Resistance during Pregnancy. *Gynecol Obstet Invest.* 2007; 64:89–94. [PubMed: 17314487]
- Klebanoff MA, Levine RJ, Morris CD, Hauth JC, Sibai BM, Ben Curet L, Catalano P, Wilkins DG. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. *Paediatr Perinat Epidemiol.* 2001; 15:140–143. [PubMed: 11383579]
- Kramer MS, Platt R, Yang H, Joseph KS, Wen SW, Morin L, Usher RH. Secular trends in preterm birth: a hospital-based cohort study. *Jama.* 1998; 280:1849–1854. [PubMed: 9846780]
- Longo LD. Carbon monoxide: effects on oxygenation of the fetus in utero. *Science.* 1976; 194:523–525. [PubMed: 973133]
- Macones GA, Parry S, Elkousy M, Clothier B, Ural SH, Strauss JF 3rd. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. *Am J Obstet Gynecol.* 2004; 190:1504–1508. discussion 3A. [PubMed: 15284722]
- Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S. Births: final data for 2004. *Natl Vital Stat Rep.* 2006; 55:1–101.
- Nagashima J, Musha H, Takada H, Matsumoto N, Fujimaki R, Ishige N, Aono J, Murayama M. Influence of physical fitness and smoking on the coagulation system in hypertensive patients: effect on prothrombin fragment F1+2. *Intern Med.* 2007; 46:933–936. [PubMed: 17603229]
- Ness RB, Grisso JA, Hirschinger N, Markovic N, Shaw LM, Day NL, Kline J. Cocaine and tobacco use and the risk of spontaneous abortion. *N Engl J Med.* 1999; 340:333–339. [PubMed: 9929522]
- Peacock JL, Cook DG, Carey IM, Jarvis MJ, Bryant AE, Anderson HR, Bland JM. Maternal cotinine level during pregnancy and birthweight for gestational age. *Int J Epidemiol.* 1998; 27:647–656. [PubMed: 9758120]
- Pollack H, Lantz PM, Frohna JG. Maternal smoking and adverse birth outcomes among singletons and twins. *Am J Public Health.* 2000; 90:395–400. [PubMed: 10705857]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics.* 2000; 155:945–959. [PubMed: 10835412]

- Rosing J, Hemker HC, Tans G. Molecular biology and pathophysiology of APC resistance: current insights and clinical implications. *Semin Thromb Hemost.* 1998; 24:329–335. [PubMed: 9763350]
- Sambrook, J.; Fritsch, EF; Maniatis, T. Molecular cloning : a laboratory manual. 2nd. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory; 1989.
- Services. UDoHaH. The Health Consequences of Smoking: A Report of the Surgeon General. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Diseases Prevention and Health, Promotion, Office on Smoking and Health; 2004.
- Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. *Am J Obstet Gynecol.* 2000; 182:465–472. [PubMed: 10694353]
- Tsai HJ, Liu X, Mestan K, Yu Y, Zhang S, Fang Y, Pearson C, Ortiz K, Zuckerman B, Bauchner H, Cerda S, Stubblefield PG, Xu X, Wang X. Maternal cigarette smoking, metabolic gene polymorphisms, and preterm delivery: new insights on G×E interactions and pathogenic pathways. *Hum Genet.* 2008; 123:359–369. [PubMed: 18320229]
- Walsh RA. Effects of maternal smoking on adverse pregnancy outcomes: examination of the criteria of causation. *Hum Biol.* 1994; 66:1059–1092. [PubMed: 7835872]
- Wang L, Wang X, Laird N, Zuckerman B, Stubblefield P, Xu X. Polymorphism in maternal LRP8 gene is associated with fetal growth. *Am J Hum Genet.* 2006; 78:770–777. [PubMed: 16642433]
- Wang X, Tager IB, Van Vunakis H, Speizer FE, Hanrahan JP. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. *Int J Epidemiol.* 1997; 26:978–988. [PubMed: 9363518]
- Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, Niu T, Wise PH, Bauchner H, Xu X. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *Jama.* 2002; 287:195–202. [PubMed: 11779261]
- Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *Eur Heart J.* 2005; 26:1765–1773. [PubMed: 15817606]
- Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet.* 2005; 76:887–893. [PubMed: 15789306]
- Yang N, Li H, Criswell LA, Gregersen PK, Alarcon-Riquelme ME, Kittles R, Shigeta R, Silva G, Patel PI, Belmont JW, Seldin MF. Examination of ancestry and ethnic affiliation using highly informative diallelic DNA markers: application to diverse and admixed populations and implications for clinical epidemiology and forensic medicine. *Hum Genet.* 2005; 118:382–392. [PubMed: 16193326]
- Zhao J. gap: genetic analysis package. *Journal of Statistical Software.* 2007; 23:1–18.

Figure 1.

Using non-smoking mothers with homogenous genotype as a reference group (GG + never for rs6019 and CC + never for rs6022), odds ratios of PTD among other groups were plotted in all mothers, African American (AA) mothers and White/Hispanic mothers, separately.

Note: never and persistent indicate never smoking and persistent smoking during pregnancy, respectively.

Table 1

The epidemiologic characteristics of mothers, grouped by term and preterm birth.

Variables	Term (n=1,141)	Preterm (n=542)	P
Prepregnancy BMI, kg/m² (Mean ± SD)	25.7 ± 5.8	26.17 ± 6.6	0.17
Gestational Age, wks (Mean ± SD)	39.6 ± 1.1	33.54 ± 3.4	<10 ⁻⁴
Birth Weight, g (Mean ± SD)	3,241.0 ± 536.6	2,135.5 ± 747.7	<10 ⁻⁴
Maternal Age (%)			
<20	144(12.6)	60(11.1)	0.28
20~24	314(27.5)	136(25.1)	
25~29	286(25.1)	132(24.4)	
>=30	397(34.8)	214(39.5)	
Maternal Ethnicity (%)			
Black	662(58.0)	329(60.7)	0.14
White	144(12.6)	78(14.4)	
Hispanic	335(29.4)	135(24.9)	
Education Level (%)			
<High school	399(35.0)	165(30.4)	0.07
High school	371(32.5)	205(37.8)	
>=High school	371(32.5)	172(31.7)	
Marital Status, married (%)	409(35.8)	165(30.4)	0.03
Parity (%)			
0	437(38.3)	217(40.0)	0.13
1	353(30.9)	142(26.2)	
>=2	351(30.8)	183(33.8)	
Alcohol Consumption (%)	46(4.0)	25(4.6)	0.58
Maternal Passive Smoking During Pregnancy (%)	283(24.8)	157(29.0)	0.07
Maternal Smoking During Pregnancy (%)			
Never	909(79.7)	387(71.4)	<10 ⁻³
Intermittent	80(7.0)	44(8.1)	
Persistent	152(13.3)	111(20.5)	
Drug use (%)	127(11.1)	97(17.9)	<10 ⁻³
Infant Gender, male (%)	529(46.4)	276(50.9)	0.08
Small for gestational age (SGA) (%)	193(16.9)	50(9.2)	<10 ⁻³
F5 Genetic Variant (%)			
rs6019			
GG	669(58.6)	280(51.7)	6×10 ⁻³
GC	378(33.1)	196(36.2)	
CC	94(8.2)	66(12.2)	
rs2213869			
GG	407(35.7)	167(30.8)	10 ⁻³
GA	512(44.9)	227(41.9)	
AA	222(19.5)	148(27.3)	

Variables	Term (n=1,141)	Preterm (n=542)	P
rs6022			
CC	356(31.2)	146(26.9)	0.01
CA	504(44.2)	227(41.9)	
AA	281(24.6)	169(31.2)	

Table 2

Individual association* of maternal smoking during pregnancy and *F5* polymorphisms with PTD and gestational age among all mothers.

Variables	PTD (< 37 wks)				Gestational age		
	Case %	Control %	OR (95% CI)	<i>P</i>	Mean ± SD (N)	β (se)	<i>P</i>
Smoking							
Never	387(29.9)	909(70.1)	1.0	--	37.8±3.5(1296)	Ref	--
Intermittent	44(35.5)	80(64.5)	1.1(0.7–1.7)	0.59	37.4±3.9(124)	−0.23(0.35)	0.51
Persistent	111(42.2)	152(57.8)	1.6(1.1–2.3)	6.9×10^{−3}	36.9±3.8(263)	−0.86(0.29)	3.1×10^{−3}
Rs6019							
GG	280(29.5)	669(70.5)	1.0	--	37.9±3.3(949)	Ref	--
GC	196(34.1)	378(65.9)	1.3(1.0–1.6)	0.05	37.5±3.8(574)	−0.28(0.2)	0.17
CC	66(41.3)	94(58.7)	1.8(1.2–2.6)	2.8×10^{−3}	36.7±4.3(160)	−1.06(0.31)	7.0×10^{−4}
Rs2213869							
GG	167(29.1)	407(70.9)	1.0	--	38±3.32(574)	Ref	--
GA	227(30.7)	512(69.3)	1.1(0.9–1.4)	0.37	37.64±3.6(739)	−0.29(0.2)	0.15
AA	148(40)	222(60)	1.7(1.3–2.3)	4.0×10^{−4}	37.18±3.83(307)	−0.68(0.25)	6.3×10^{−3}
Rs6022							
CC	146(29.1)	356(70.9)	1.0	--	38.1±3.2(502)	Ref	--
CA	227(31.1)	504(68.9)	1.1(0.9–1.5)	0.35	37.7±3.5(731)	−0.28(0.21)	0.18
AA	169(37.6)	281(62.4)	1.6(1.1–2.1)	5.1×10^{−3}	37.2±4.0(450)	−0.65(0.25)	8.6×10^{−3}

* Adjusted for maternal age, drug use and passive smoking during pregnancy, education status, parity, marital status, alcohol consumption during pregnancy, prepregnancy BMI, infant gender, maternal ethnicity and small for gestational age.

Bold *p* value indicated statistically significant *p* value after correcting for multiple testing (threshold of significant *p* value is 10^{−2}).

Table 3

Interaction* between maternal smoking during pregnancy and *F5* polymorphisms on PTD and gestational age among all mothers.

	PTD (< 37 wks)				Gestational age		
	Case %	Control %	OR (95% CI)	P	Mean ± SD (N)	β (se)	P
Smoking + rs6019							
Never + GG	198(27.8)	514(72.2)	1.0	--	38.0±3.3(712)	Ref	--
GC	143(31.3)	314(68.7)	1.2(0.9–1.6)	0.18	37.8±3.5(457)	−0.06(0.22)	0.78
CC	46(36.2)	81(63.8)	1.5(1.0–2.3)	0.06	37.0±4.1(127)	−0.81(0.35)	0.02
Persistent + GG	56(34.6)	106(65.4)	1.1(0.7–1.8)	0.62	37.7±3.1(162)	−0.13(0.35)	0.71
GC	39(50.0)	39(50.0)	2.1(1.2–3.6)	0.01	36.0±4.4(78)	−1.36(0.46)	3.2×10^{−3}
CC	16(69.6)	7(30.4)	5.7(2.1–15.0)	5×10^{−4}	34.9±4.5(23)	−2.65(0.76)	5×10^{−4}
Test of interaction			1.7(1.1–2.7)	0.02		−0.97(0.36)	7×10^{−3}
Smoking + rs6022							
Never + CC	101(27.9)	261(72.1)	1.0	--	38.1±3.3(362)	Ref	--
CA	163(28.5)	408(71.5)	1.1(0.8–1.5)	0.67	37.9±3.4(571)	−.17(0.24)	0.47
AA	123(33.9)	240(66.1)	1.4(1.0–1.9)	0.07	37.6±3.7(363)	−.37(0.27)	0.18
Persistent + CC	31(31.3)	68(68.7)	1.0(0.6–1.8)	0.95	38.1±2.7(99)	0.05(0.44)	0.90
CA	47(43.1)	62(56.9)	1.5(0.9–2.5)	0.12	36.8±3.7(109)	−.73(0.42)	0.09
AA	33(60.0)	22(40.0)	3.3(1.7–6.4)	4.2×10^{−4}	35.1±4.7(55)	−.32(0.54)	1.7×10^{−5}
Test of interaction			1.5(1.0–2.2)	0.05		−.95(0.32)	2.6×10^{−3}

* Adjusted for maternal age, passive smoking during pregnancy, drug use during pregnancy, education status, parity, marital status, alcohol consumption during pregnancy, prepregnancy BMI, infant gender, small for gestational age, maternal ethnicity, *CYP1A1*, *GSTT1* and the interaction of *CYP1A1*, *GSTT1* and smoking during pregnancy.

Bold *p* value indicated statistically significant *p* value after correcting for multiple testing (threshold of significant *p* value is 10^{−2}).

Table 4

Interaction* between maternal smoking during pregnancy and *F5* polymorphisms on PTD and gestational age among African American mothers and white / Hispanic mothers, separately.

	PTD (< 37 wks)				Gestational age		
	Case %	Control %	OR (95% CI)	P	Mean ± SD (N)	β (se)	P
African American Mothers							
Smoking + rs6019							
Never + GG	82(26.9)	223(73.1)	1.0		37.9±3.6(305)	Ref	--
GC	113(31.3)	248(68.7)	1.2(0.9–1.8)	0.22	37.8±3.6(361)	−0.15(0.29)	0.61
CC	42(36.2)	74(63.8)	1.6(1.0–2.5)	0.07	36.9±4.3(116)	−0.97(0.41)	1.7×10 ^{−2}
Persistent + GG	17(34.0)	33(66.0)	1.2(0.6–2.4)	0.69	37.6±3.3(50)	0.11(0.62)	0.85
GC	33(53.2)	29(46.8)	2.5(1.3–4.9)	6×10^{−3}	35.5±4.7(62)	−1.95(0.59)	9×10^{−4}
CC	12(66.7)	6(33.3)	5.7(1.9–17.7)	2.5×10^{−3}	34.9±4.8(18)	−2.61(0.95)	6.1×10^{−3}
Test of interaction			1.8(1.0–3.3)	0.07		−1.13(0.52)	0.03
Smoking + rs6022							
Never + CC	34(26.6)	94(73.4)	1.0	--	37.8±3.8(128)	Ref	--
CA	101(28.8)	250(71.2)	1.1(0.7–1.8)	0.66	37.8±3.5(351)	−0.08(0.38)	0.84
AA	102(33.7)	201(66.3)	1.4(0.9–2.2)	0.18	37.5±3.9(303)	−0.34(0.39)	0.40
Persistent + CC	6(27.3)	16(72.7)	1.0(0.3–3.0)	0.94	38.4±2.5(22)	0.63(0.9)	0.49
CA	30(48.4)	32(51.6)	2.0(1.0–4.1)	0.06	36.3±4.1(62)	−0.94(0.64)	0.14
AA	26(56.5)	20(43.5)	2.9(1.3–6.4)	7.1×10^{−3}	35.1±5.0(46)	−2.21(0.69)	1.4×10^{−3}
Test of interaction			1.4(0.8–2.6)	0.25		−1.21(0.51)	0.02
White / Hispanic Mothers							
Smoking + rs6019							
Never + GG	116(28.5)	291(71.5)	1.0	--	38.1±3.1(407)	Ref	--
GC	30(31.3)	66(68.7)	1.1(0.7–1.9)	0.60	38.0±3.2(96)	0(0.34)	0.99
CC	4(36.4)	7(63.6)	1.7(0.5–6.3)	0.40	38.4±1.6(11)	0.24(0.91)	0.79
Persistent + GG	39(34.8)	73(65.2)	1.1(0.6–1.9)	0.83	37.7±3.0(112)	−0.31(0.4)	0.44
GC	6(37.5)	10(62.5)	1.0(0.3–3.7)	0.95	38.0±2.3(16)	0.71(0.82)	0.39
CC	4(80.0)	1(20.0)	9.9(1.0–99.4)	0.05	34.6±3.7(5)	−3.19(1.35)	0.02
Test of interaction			1.6(0.7–3.8)	0.31		−0.49(0.61)	0.42
Smoking + rs6022							
Never + CC	67(28.6)	167(71.4)	1.0	--	38.2±3.1(234)	Ref	--
CA	62(28.2)	158(71.8)	1.0(0.7–1.6)	0.85	38.0±3.2(220)	−0.25(0.28)	0.37
AA	21(35.0)	39(65.0)	1.4(0.8–2.7)	0.26	38.0±2.5(60)	−0.15(0.43)	0.73
Persistent + CC	25(32.5)	52(67.5)	1.0(0.5–1.9)	0.96	38.0±2.8(77)	−0.2(0.46)	0.66
CA	17(36.2)	30(63.8)	1.1(0.5–2.5)	0.77	37.5±3.1(47)	−0.44(0.54)	0.41
AA	7(77.8)	2(22.2)	8.6(1.6–47.6)	1.3×10^{−2}	35.3±3.1(9)	−2.49(1.06)	0.02
Test of interaction			1.6(0.8–3.2)	0.15		−0.58(0.46)	0.21

* Adjusted for maternal age, passive smoking during pregnancy, drug use during pregnancy, education status, parity, marital status, alcohol consumption during pregnancy, prepregnancy BMI, infant gender, small for gestational age, *CYP1A1*, *GSTT1* and the interaction of *CYP1A1*, *GSTT1* and smoking during pregnancy.

Bold p value indicated statistically significant p value after correcting for multiple testing (threshold of significant p value is 10^{-2}).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript