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Genetic Variation Associated with Preterm Birth in African-American Women

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Abstract

Background—Preterm birth is considered a multifactorial condition, however emerging evidence suggests that genetic variation among individuals may have an important role. Prior studies have suggested that single nucleotide polymorphisms associated with genes related to the immune system, and particularly the maternal inflammatory response, may be associated with an increased risk of preterm delivery.

Objective—To identify single nucleotide polymorphisms (SNPs) associated with spontaneous preterm birth <37 weeks within a cohort of African-American women.

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Study Design—This is a secondary analysis of a randomized trial that evaluated periodontal disease and preterm birth. Women were enrolled between 6-20 weeks' gestation at three prenatal care clinics between 2004-2007. Maternal DNA samples were collected and analyzed using a custom 1536-SNP genotyping array designed to assess genes involved in inflammation. Women were included in this study if they self-identified as African-American. We excluded women with a multiple gestation or an indicated preterm delivery. We performed allele- and genotype-based analyses to evaluate the association between spontaneous preterm birth and tag SNPs. We used logistic regression to adjust for prior preterm birth in our genotype-based analysis. In a subgroup analysis, we compared women who delivered at <34 weeks gestation to women who delivered at term. Within the microarray, we identified ancestry informative markers and compared global ancestry estimates among women who delivered preterm to those who delivered at term.

Results—Of the 833 African-American women in the study with genotype data, 77 women (9.2%) had a spontaneous preterm birth, while 756 women delivered at term. In allele-based analysis, 4 SNPs related to the genes for protein kinase C-α (*PRKCA*) were associated with increased risk of spontaneous preterm birth <37 weeks, while a single SNP related to fms-related tyrosine kinase 1 (*FLT1*) was associated with spontaneous preterm birth <34 weeks. Genotype-based analysis revealed similar associations between SNPs related to the *PRKCA* genes and spontaneous premature delivery. Additionally, SNPs related to matrix metalloproteinase-2 (*MMP2*), tissue inhibitor of matrix metalloproteinase-2 (*TIMP2*), and interleukin 16 (*IL16*) genes were associated with spontaneous preterm birth <37 weeks in genotype-based analysis. Genetic variants related to *MMP2*, matrix metalloproteinase-1 (*MMP1*), and leukemia inhibitory factor receptor antisense RNA 1 (*LIFR-AS1*) genes were associated with higher rates of preterm birth <34 weeks. Ancestry estimates were similar between the women who had a spontaneous preterm birth and those who delivered at term.

Conclusion—We identified tag SNPs related to 7 genes that are critical to inflammation, extracellular remodeling, and cell signaling that were associated with spontaneous preterm birth in African-American women. Specifically, we found a strong association with the *PRKCA* gene. Genetic variation in these regions of the genome may be important in the pathogenesis of preterm birth. Our results should be considered in the design of future genomic studies in prematurity.

Keywords

Preterm birth; genetics; inflammation

Introduction

Preterm birth is a leading cause of infant morbidity and mortality. [1-3] The pathophysiology of preterm birth is largely unknown but genetic predisposition is likely an important component of this multifactorial process. This assumption is supported by twin studies, [4,5] data demonstrating that family history influences the risk of preterm delivery,[6,7] and high recurrence rates among women with a history of preterm birth.[8] Genetic variation may also account, in part, for the differences in preterm birth rates among individuals of different ancestry groups in the U.S.[9-13]

The role of the immune system and specifically maternal inflammatory response in the pathogenesis of preterm birth has also been supported by strong evidence.[14-23] Therefore, analyses of genetic variation associated with preterm birth have focused on identifying polymorphism primarily associated with genes involved in the inflammatory pathways.[24-28] Previously reported polymorphisms associated with preterm birth were related to tumor necrosis factor-alpha (*TNFA*),[27,29,30] IL-1,[26] IL-6,[24,31] type 1 insulin-like growth factor receptor (*IGF1R*),[32] and tissue inhibitor of metalloproteinase-2 (*TIMP2*).[25]

To expand upon the existing evidence that specific genetic variants are involved in the pathogenesis of preterm birth, we sought to identify single nucleotide polymorphisms (SNPs) associated with spontaneous preterm births less than 37 weeks' gestation using multiple inheritance models within a cohort of African-American women.

Materials and Methods

This was a secondary analysis of data collected by the Periodontal Infections and Prematurity Study, a randomized controlled trial performed to evaluate the effect of periodontal disease and its treatment on preterm birth.[33] Women were enrolled from 2004 to 2007 at three prenatal clinics in the Philadelphia area between 6 and 20 weeks' gestation. Gestational age was calculated using the participant's reported last menstrual period and first ultrasound evaluation in the pregnancy. Results of the trial demonstrated that neither periodontal disease nor its treatment was associated with preterm delivery.[33,34]

A secondary aim of the trial was to evaluate the relationship between genetic variation within candidate genes and preterm birth. An additional observational cohort of women without periodontal disease was enrolled for this purpose. As periodontal disease was not associated with preterm delivery, women enrolled in both the randomized trial and observational cohort were included in this genetic study. Approval for this study was granted by the Institutional Review Board at the University of Pennsylvania.

After obtaining informed consent, maternal DNA samples using buccal swabs were collected at the time of enrollment. A customized 1536-SNP Illumina Golden Gate genotyping array was utilized in this study. In the development of the customized SNP chip, genes of interest were identified using two reference databases that provide integrated information about the genome and biomolecular pathways, Biocarta and Kyoto Encyclopedia of Genes and Genomes. Genes associated with the inflammatory pathway and those previously reported in the literature to be associated with preterm birth were of interest. We used information from the International HapMap Project to identify specific tag SNPs associated with the genes of interest to include in the customized SNP chip panel. Specific SNPs within each gene were selected based on evidence from the literature that the polymorphisms are functional, or the nature of the variation suggests potential function (e.g. promotor polymorphisms) if no specific functional data had been published. Tag, or “tagging”, SNPs are specific polymorphisms within the human genome that provide information about allelic variation within a larger segment of a chromosome. This is possible because of the co-inheritance of SNP alleles within regions found in close proximity within the human genome, a phenomenon referred to as linkage disequilibrium.

Utilizing tag SNPs is thus an indirect approach that allows researchers to study genetic variation within a genomic region of interest and associations with disease.[35]

We designed a nested case-control study to examine the relationship between genetic polymorphisms and spontaneous preterm birth prior to 37 weeks' gestation. A spontaneous preterm birth was defined as birth prior to 37 weeks that resulted from idiopathic preterm labor or preterm premature rupture of membranes (PPROM). Women with an indicated preterm delivery were excluded, as were women who delivered prior to 20 weeks' gestation. Only women who self-identified as being African American who had a singleton gestation were included in the analysis. We restricted our analysis to African-American women for two reasons – 1) very few participants in the randomized controlled trial were not African-American (15.9%); 2) this restriction would reduce systematic differences in allele frequency due to ancestry (population stratification).

Extensive demographic and clinical data were collected as part of the study and this information was entered into a research database by trained obstetric research staff. Delivery information including gestational age at the time of delivery was obtained from the participant's inpatient delivery medical record.

Statistical analysis

Baseline characteristics, including maternal age, parity, history of prior preterm delivery, body mass index (BMI) and smoking status, were compared between women who delivered prior to 37 weeks' and those who delivered at term. Categorical variables were compared using the Chi-square test. Maternal age and BMI were not normally distributed and therefore median values were compared using the Mann-Whitney U test. This analysis was performed using Stata 10.0 statistical software (Stata Corp, College Station, TX).

All genetic association analyses were conducted using PLINK 1.9 software, a publically available genetic association analysis program developed by the Center for Human Genetic Research at Massachusetts General Hospital and the Broad Institute.[36,37] Individuals with a low call rate (>5% missing SNP data) (n=28), and SNPs with no data reported for >5% of the study cohort were excluded (n=72). We assessed for deviations from Hardy-Weinberg equilibrium (HWE) within our population for each SNP using the Chi-square test in Plink 1.9. While deviations from HWE are often tested only in the control group in genetic case-control studies, we performed the test within our entire cohort since control status was established prospectively.[38] We included all SNPs in our analysis regardless of whether a SNP was in HWE within our sample. While genotypes that deviate from HWE should be interpreted with caution because they may indicate genotyping error, population heterogeneity or selection bias, deviations from HWE may also be seen when the SNP is associated with the disease of interest.[39]

We used a stepwise approach to explore genetic variation within our cohort. First, an allele association analysis was performed in women with spontaneous preterm birth (<37 weeks) and those women who delivered at term. The minor allele was designated as the risk allele and the frequency of the minor allele was compared in the groups. We then performed genotype-based analyses considering recessive, dominant and additive genetic models for

each SNP. If a SNP was significantly associated with preterm birth using more than one model, then the best model was selected by comparing the strength of the association as measured by the p-value in each model. We then performed logistic regression adjusting for prior preterm birth. If a SNP was not significantly associated with preterm birth in unadjusted analysis, but a significant association was seen after controlling for preterm birth then this was reported.

We also performed a subgroup analysis comparing women who had an early spontaneous preterm delivery, defined as preterm birth <34 weeks gestation, to women who delivered at term. Similar to the primary analysis, we performed allelic and genotype-based analyses.

All analyses used a p-value $<7.64 \times 10^{-4}$ as the criterion for statistical significance. This accepted level of error is less conservative than that suggested by the Bonferroni procedure based on the number of individual SNPs in our analysis. This value was calculated based on the number of genes (n=67) associated with the SNPs in our genotyping panel. Our reasoning for this is two-fold. In this study, we have used tag SNPs, and as such, it is not the particular SNP itself but genetic variation in the larger region of the genome linked to the SNP that is of interest. We evaluated multiple tag SNPs that may be in linkage disequilibrium with the same genomic region, therefore focusing on fewer genomic regions than actual SNPs. Second, this study, like many genetic association studies, is exploratory and we sought to avoid missing any potentially important associations.

To further assess the impact of ancestry on risk of preterm delivery, we identified 177 ancestry informative markers (AIM) that were included in the SNP microarray. These specific polymorphisms were selected using the International HapMap Project based on ≥70% difference in allele frequencies in West African and European ancestral populations. A two-way model of admixture was generated using ADMIXMAP (http://www.ucd.ie/geneepi/admixmap/tools.html).[40, 41] Differences in global ancestry estimates among women who delivered preterm and those who delivered at term were compared using the Welch two sample t-test.

Results

In all, 870 African-American women included in the trial delivered after 20 weeks' gestation and had complete (<5% missing) SNP data. We excluded 37 women who had an indicated preterm delivery. Overall, 77 women (9.2%) had a spontaneous delivery prior to 37 weeks' gestation, while 756 women delivered at term. Women who delivered preterm were similar to those women who delivered at term with regard to maternal age, parity, body mass index (BMI), and smoking status. However, women who delivered preterm were more likely to have history of prior preterm birth. Of the women with a spontaneous preterm birth, 55% had PPROM (Table 1). The median gestational age of women who had a spontaneous preterm birth was 35.3 weeks (interquartile range, 33.0-36.3) and 39.6 (interquartile range, 38.7-40.3) in women who delivered at term.

Allele-based analysis

Minor allele frequency was compared in women who had a spontaneous preterm delivery and women who delivered at term. Four SNPs were identified that were associated with a higher minor allele frequency among women who delivered preterm compared to women who delivered at term (Table 2). All four SNPs were associated with the region of the genome encoding protein kinase C-alpha (*PRKCA*).

Genotype-based analysis

In our genotype-based analysis, we considered recessive, dominant and additive genetic models for each SNP. The best fitting model for each SNP was determined by comparing the strength of the association between the SNP and spontaneous preterm birth based on the p-value in unadjusted analysis. Six SNPs were significantly associated with spontaneous preterm birth in unadjusted analysis. After performing logistic regression adjusting for prior preterm birth, an additional SNP (rs4486944) was also found to be associated with a higher risk of spontaneous preterm delivery. In contrast, another SNP (rs7171517) that was associated with preterm birth in unadjusted analysis, was no longer statistically significant after controlling preterm birth history. All four SNPs associated with spontaneous preterm birth in the allele-based analysis were significantly associated with preterm delivery in genotype-based analysis as well (Table 3).

Subgroup Analysis of Spontaneous Preterm Birth <34 weeks

The minor allele frequency of a SNP (rs12428494) related to the fms-related tyrosine kinase 1 (*FLT1*) gene was significantly associated with spontaneous preterm birth prior to 34 weeks gestation in the subgroup analysis (Table 2). Three additional SNPs were associated with early spontaneous preterm birth using a recessive genotype-based model (Table 4).

Ancestry-Informative Markers

We assessed 177 ancestry informative markers in the SNP microarray to assess the impact of ancestry on risk of preterm delivery. Based on the two sample t-test African ancestry estimates were similar between women who delivered preterm and those who delivered at term (mean 0.799+/- standard deviation 0.080 vs. 0.799+/-standard deviation 0.081, p=0.98).

Comment

Within a prospectively observed cohort of pregnant African-American women, we identified a number of SNPs associated with spontaneous preterm birth. These SNPs were related to multiple genes involved in the maternal inflammatory response. In the primary analysis, seven individual SNPs were found to be associated with spontaneous preterm birth <37 weeks gestation, however many of these SNPs were associated with the same gene *PRCKA*. Interestingly, when we evaluated spontaneous preterm births <34 weeks, none of the SNPs associated with preterm birth <37 weeks were found to be significantly associated with earlier preterm birth, with the exception of a SNP associated with *MMP2*. While this may be due to chance, it may also reflect the differences in the etiology of early and late preterm deliveries.

Our data suggests that genetic variation within the region of chromosome 17 that encodes *PRKCA* potentially has an important role in the pathogenesis of preterm birth. Multiple SNPs in this region were found to increase the risk of spontaneous preterm birth <37 weeks in both allele-based and genotype-based analyses. This association between polymorphisms related to the *PRKCA* gene and preterm birth is further supported by a prior analysis utilizing data from the Periodontal Infections and Prematurity Study, which demonstrated that multiple tag SNPs associated with *PRKCA*, but different than the SNPs identified in this study, were associated with spontaneous preterm birth in women with bacterial vaginosis.[28]

PRKCA has a central role in many different cellular processes including cell signaling through phosphorylation of a variety of proteins.[42] In cardiac myocytes,[43, 44] vascular,[45] and colonic smooth muscle cells,[46, 47] as well as myometrial cells,[48, 49] *PRKCA* effects contractility. However, the role of *PRKCA* in regulating uterine contractions during pregnancy remains debated. Fomin et al. found that both reduced expression and inhibition of *PRKCA* in myometrial cells in vitro resulted in decreased contractility.[48] However, more recent evidence suggests that *PRKCA* may have a role in uterine quiescence. These researchers found that while the content of *PRKCA* within the myometrium did not significantly change throughout pregnancy, there were changes in the activity of the protein. *PRKCA* activity was significantly reduced in myometrial samples obtained from term laboring and non-laboring women as well as women in preterm labor compared to samples from preterm non-laboring women. This suggests that it is possible that decreased *PRKCA* activity could lead to the preterm contractions and labor.[49] While this is a potential mechanism by which PRKCA could affect preterm birth, further investigation is required to determine if genotypic variation within the gene effects protein expression or activity.

The role of the other genes related to the tag SNPs that were significantly associated with preterm birth in our study have been variably described in the obstetric and non-obstetric literature. IL16 is a proinflammatory cytokine. Higher concentrations of IL16 in the amniotic fluid have been associated with increased rates of preterm birth,[50,51] with the highest levels of IL16 found among women with culture-proven microbial invasion of the amniotic cavity.[50] Matrix metalloproteinase-2 is a member of a class of proteins involved in extracellular matrix remodeling and in embryo implantation and trophoblast invasion.[53,54] A role for MMP-2 in preterm parturition has been suggested by the finding of lower concentrations of the active form of the MMP-2 enzyme in the amniotic fluid of women with PPROM.[55] Another study found that serum MMP-2 concentrations were reduced in women in preterm labor compared to women who delivered at term.[56] The functional activity of MMP-2 is regulated by tissue inhibitor of matrix metalloproteinase-2 (TIMP-2). Interestingly, we found that polymorphisms related both to the *MMP2* gene and *TIMP2* gene were associated with higher rates of spontaneous preterm delivery within our cohort. We are not the first to report that genetic variation in the region *TIMP2* gene may be associated with prematurity. Romero and colleagues found that a polymorphism in a coding exon of the *TIMP2* gene (rs2277698) was associated with an approximately 2-fold increased risk of spontaneous preterm delivery in women with both intact[57] and ruptured membranes.[25] The relationships between the polymorphisms, the reported alterations in MMP-2 activity, and the pathophysiology of prematurity remain to be elucidated.

Because there may be differences in etiologic pathways of early and late preterm birth, we performed a subgroup analysis comparing women who delivered <34 weeks gestation to those who delivered at term. Women with the polymorphism related to the *MMP2* gene that was associated with preterm birth <37 weeks were also at risk for preterm birth <34 weeks. Polymorphisms related to *MMP1*, *Flt1* and the leukemia inhibitory factor receptor antisense RNA 1 gene (*LIFR-AS1*) were also associated with early preterm birth.

MMP-1, like MMP-2, is an enzyme that degrades collagen and may have a role in the pathogenesis of PPROM.[58] In a previous study, a polymorphism in the promotor region of the *MMP1* gene of the fetus was associated with an increased risk of PPROM.[59] There is a well-described association between elevated levels of soluble Flt1 (sFlt1) and the development of preeclampsia.[60] Although an association between elevated sFlt1 levels and risk of preterm birth was suggested by one small study,[61] further research is needed to determine if spontaneous preterm birth risk is related to Flt1 expression. SNP rs6451398 is in the non-coding *LIFR* antisense RNA gene that overlaps the coding *LIFR* gene. Leukemia inhibitory factor receptor appears to have an important role in blastocyst implantation in early pregnancy,[62] but it has not been previously reported to have an association with premature delivery.

The nested case-control design within a well-characterized cohort of women with prospectively collected data is a major strength of this study. In addition, the 1536-SNP array utilized in the study was customized to specifically evaluate genes associated with inflammation. Finally, as the relationship between genotype and phenotype in preterm birth is largely unknown, we assessed multiple genetic models.

This study was performed within a cohort of African-American women, so our findings may not extend to women of different ancestral backgrounds. The proportion of West African ancestry was similar among self-reported African-American women who delivered preterm and those who delivered at term. We included all self-reported African-American women in our analysis because these women may experience similar environmental exposures, even if they vary in genomic ancestry composition. While in this study we did not examine the interaction between environment and genetic variation on the risk of premature delivery, this should be one of the focuses of future prematurity research since the etiology of the condition is multifactorial.

Based on the size of our cohort, the approach of focusing on genetic variation within candidate genes that were selected based on previous research was most appropriate in our study. However, genome-wide association studies and whole exome sequencing are more advanced genomic techniques that can be used to assess for putative markers across a wider breadth of the genome. While much larger cohorts will be required to be adequately powered to perform these types of studies, they are needed. Results of genomic association studies such as this also require validation in other cohorts. While resource constraints make it difficult to replicate studies of this magnitude, researchers should consider these and other previously published investigations when designing future genetic studies related to preterm birth. This collaborative approach would allow for the advancement of knowledge on the genetic basis of preterm birth.

While epidemiologic studies suggest that there is an inherited predisposition to preterm birth, the relationship between genetic variation and preterm birth remains unclear. This study contributes to the growing body of information regarding genetic variation associated with preterm birth. While our study, like most prior genomic studies in prematurity, is exploratory, our results identify regions of the genome not previous reported to be associated with preterm birth. The region with the strongest evidence in our study is associated with *PRKCA*. Further investigation of the role of functional genetic polymorphisms and gene expression within the *PRKCA* gene, in particular, are warranted.

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Table 1

Baseline characteristics of women who had a spontaneous preterm delivery and women who delivered at term

Characteristic	Spontaneous Preterm Delivery N=77	Term Delivery N=756	P
Maternal age (years)[a]	22 (19-27)	23 (20-27)	0.45
Nulliparity	31 (40.3)	301 (39.9)	0.95
History of prior PTB	17 (22.1)	75 (9.9)	<0.01
Body mass index (kg/m^2)[a]	25.8 (22.3-31.4)	25.9 (22.4-31.3)	0.97
Smoking	21 (27.6)	267 (35.4)	0.18
Birthweight[a]	2440 (1940-2723)	3260 (2973-3560)	<0.01
Periodontal disease			
Yes	59 (76.6)	568 (75.1)	0.77
No	18 (23.4)	188 (24.9)	
Preterm premature rupture of membranes			
Yes	42 (54.5)		
No	35 (45.5)		

[a]Data presented as median values (interquartile range), in all other cases data represents n (%).

Table 2

Allele-based analysis comparing women with spontaneous preterm birth to women who delivered at term

Gene	SNP	Minor Allele	Minor allele frequency		Odds Ratio	p	HWE[a]
			Preterm	Term			
PRETERM BIRTH <37 WEEKS							
PRKCA	rs7225452	A	0.58	0.42	1.92	1.2^{-4}	Yes
PRKCA	rs4486944	C	0.62	0.47	1.79	7.0^{-4}	Yes
PRKCA	rs6504424	A	0.61	0.47	1.82	5.8^{-4}	Yes
PRKCA	rs16960070	A	0.12	0.05	2.45	6.3^{-4}	Yes
PRETERM BIRTH <34 WEEKS							
FLT1	Rs12428494	A	0.11	0.03	3.81	3.7^{-4}	Yes

[a]Based on frequencies of the alleles in the entire cohort

Table 3

Single SNPs genotype-based analyses for spontaneous preterm birth <37 weeks

Gene	SNP	Model	OR	p	aOR[a]	p	HWE[b]
MMP2	rs11639960	Recessive	8.26	4.8^{-5}	9.10	2.6^{-5}	No
TIMP 2	rs2277698	Recessive	8.70	4.7^{-4}	8.44	6.8^{-4}	Yes
IL1 6	rs7171517	Dominant	2.31	5.8^{-4}	2.26	8.6^{-4}	Yes
PRKCA	rs16960070	Dominant	2.84	3.2^{-4}	3.02	1.7^{4}	Yes
PRKCA	rs7225452	Additive	1.92	1.7^{-4}	1.98	1.2^{-4}	Yes
PRKCA	rs6504424	Additive	1.84	6.4^{-4}	1.90	4.8^{-4}	Yes
PRKCA	rs4486944	Additive	1.79	8.5^{-4}	1.86	5.4^{-4}	Yes

[a] Adjusted for prior preterm birth

[b] Based on frequencies of the alleles in the entire cohort

Table 4

Single SNPs genotype-based analyses for spontaneous preterm birth <34 weeks

Gene	SNP	Model	OR	p	aOR[a]	p	HWE[b]
MMP1	rs7945189	Recessive	17.05	3.0^{-4}	20.25	1.41^{-4}	No
MMP2	rs11639960	Recessive	10.32	2.0^{-4}	12.37	7.62^{-5}	No
LIFR-AS1	rs6451398	Recessive	7.00	3.2^{-4}	6.93	4.03^{-4}	Yes

[a]Adjusted for prior preterm birth

[b]Based on frequencies of the alleles in the entire cohort