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Polymorphisms in *NR5A2*, gene encoding Liver Receptor Homolog-1 are associated with Preterm Birth

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Abstract

Background.—Preterm birth (PTB) is a major cause of neonatal mortality and morbidity. There is strong evidence of genetic susceptibility. Objective of the study was to identify genetic variants contributing to PTB.

Methods.—Genotyping was performed for 24 SNPs in 4 candidate genes (NR5A2, FSHR, FOXP3, SERPINH1). Genotyping was completed on 728 maternal triads (mother & maternal grandparents of a preterm infant). Data were analyzed with Family Based Association Test.

Results.—For all maternal triads rs2737667 of NR5A2 showed significant association at P= 0.02. When stratifying by gestational age three SNPs in NR5A2 had p values <0.05 in the <32 weeks gestational age group (rs12131233, p=0.007; rs2737667, p=0.04; rs2816949, p=0.02). When PPROM cases were excluded rs2737667 of NR5A2 showed the strongest association with a p value <0.0002. This association remained significant after correction for multiple testing.

Conclusions.—This study suggests a potential association between intronic SNPs in the NR5A2 gene and PTB. NR5A2 gene encodes for the Liver receptor homolog 1(LRH1) protein, which plays a critical role in regulation of cholesterol metabolism, steroidogenesis and progesterone synthesis. These findings suggest NR5A2 may be important in the pathophysiology of PTB and exploring of non-coding regulators of NR5A2 is warranted.

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Background

Preterm birth (PTB), defined as a birth before 37 weeks gestation, is a major perinatal health problem. Approximately 10% of all births worldwide are preterm[1]. There is wide variation by geography and socioeconomic status. Africa, Asia and North America have the highest rates of preterm birth. PTB rate in United States was 11.4% in 2013[2], significantly higher than other developed nations. Preterm birth is directly responsible for an estimated one million neonatal deaths annually[3]. It is also an important contributor to long term morbidities such as cerebral palsy, developmental delay, visual and hearing impairments and chronic lung disease[4–5].

The etiology of PTB is multifactorial and there is strong evidence for genetic susceptibility[6–7]. Preterm births tend to recur in mothers[8]. Familial trends and racial disparities of prematurity also suggest that genetics influence this trait[9–11]. A number of case control and family based studies have evaluated candidate genes chosen from some of the pathways associated with preterm birth[12–17].

In the current study we hypothesize that single nucleotide polymorphisms (SNPs) in four candidate genes (*NR5A2, FSHR, FOXP3, SERPINH1)* contribute to genetic predisposition to preterm birth. We evaluated the association between PTB and SNPs in the four candidate genes.

The four candidate genes we studied act on fundamental mechanisms associated with the establishment and maintenance of pregnancy. *NR5A2* gene encodes Nuclear Receptor Subfamily 5, Group A, Member 2, also known as liver receptor homolog 1(LRH1). LRH1 has been shown to play an important role in establishing and sustaining pregnancy in animal models[18]. Polymorphisms in *FSHR* encoding follicle-stimulating hormone receptor have been found to be associated with preterm birth in a Finnish as well as an African American population[19–20]. Dysregulation of *FSHR* may contribute to early uterine contractility. *SERPINH1* encodes heat shock protein 47, which serves as a chaperone stabilizing the collagen triple helix. Polymorphism in *SERPINH1* increase risk of preterm premature rupture of membranes (PPROM) in African Americans[21]. *FOXP3* encodes a member of the FOX protein family that appears to function as a master regulator in the development and function of regulatory T cells an important pathway in modulating the immune interactions between mother and fetus. While many studies have focused on the fetus as the “risk case” in studies of preterm birth in this study we used the mother as the risk case and a family based approach using DNA from the mother and her parents to improve the chance for identifying risk alleles acting through the mother.

Results

We analyzed the genotype data of 728 maternal triads from United States, Argentina and Denmark. Maternal triad refers to mother and maternal grandparents of a preterm infant. A description of the study population is provided in Table 1. A total of 24 SNPs in four candidate genes (*NR5A2, FSHR, FOXP3, SERPINH1)* were studied (Table 2).

All SNPs tested had a minor allele frequency greater than 20%. Genetic predisposition for PTB was associated ($p<0.05$) in multiple SNPs in our study as shown in Table 3. Analysis of all maternal triads found a significant association of PTB with rs2737667 in *NR5A2* ($p = 0.02$). Analysis of the US triads found a significant association of PTB with rs2737667 ($p = 0.01$) and rs603647 in *SERPINH1* ($p = 0.02$). There were no significant associations in the maternal triads from Argentina or Denmark.

When stratifying by gestational age, three SNPs in *NR5A2* (rs12131233, p=0.007; rs2737667, p=0.04; rs2816949, p=0.02) and rs6165 in *FSHR* (p=0.01) had p values <0.05 in the <32 weeks gestational age group. There were no significant findings in the 32–36 weeks group.

When cases of PPROM were excluded rs2737667 in *NR5A2* showed the strongest association with PTB ($p = 0.0002$) for US and Argentina maternal triads. This association was the only one to remain significant after correction for multiple testing (Bonferroni-corrected p value <0.0006). In the PPROM group, rs6165 in *FSHR* had a p value of 0.02.

Discussion

Preterm birth is a common complex trait. The etiology of PTB depends upon the interplay between genetics and environmental factors such as nutrition, infection, stress, trauma and drug use. In the current study we investigated the association of PTB and four candidate genes with different biological mechanisms. To the best of our knowledge this is the first candidate gene study investigating the role of *NR5A2* gene in the etiology of PTB.

A SNP in *NR5A2*, rs2737667 showed an association with PTB in all maternal triads, <32 weeks gestation age group and in the spontaneous preterm with intact membranes group. Of these, association with spontaneous preterm with intact membranes reached formal level of significance (p=0.0002). Two other SNPs in the same gene (rs12131233 and rs2816949) were associated with PTB in <32 weeks group but did not reach formal level of significance.

The *NR5A2* gene encodes an orphan nuclear receptor named Liver receptor homolog1 (LRH1). Orphan nuclear receptors regulate transcription independent of known ligands. LRH1 plays a vital role in bile acid synthesis, cholesterol metabolism and steroidogenesis[22]. It also has a role in the regulation of progesterone synthesis[22]. LRH1 is expressed in high levels in the ovary with highest expression in granulosa cells and corpus lutea. It is also expressed in endometrium, liver, intestine and pancreas. Zhang et al. concluded that LRH 1 is necessary for maintenance of the corpus luteum, for promotion of decidualization and for formation of the placenta, therefore playing multiple roles in establishing and sustaining pregnancy[18]. Mice lacking NR5A2 in granulosa cells had extremely low progesterone levels in one study[23]. Progesterone is critical for maintaining pregnancy and decline of progesterone action is implicated in the onset of parturition. Prenatal administration of progesterone has significantly decreased the PTB in women considered to be at increased risk[24, 25].

SNP rs2737667 is an intron variant in NR5A2 gene located 15 base pairs from a non-coding regulatory element. There are no reports of clinical significance for this variant in the current

literature. We can hypothesize that this intronic variant may affect the function of non-coding regulatory element and affect expression of liver receptor homolog 1. Changes in LRH1 expression may result in variations of cholesterol metabolism, steroidogenesis and progesterone synthesis contributing to PTB.

One SNP in *FSHR* gene (rs6165) showed a p value <0.05 in the gestation age <32 week group and PPROM group, but did not reach formal levels of significance once corrected for multiple comparisons. Notably our results didn't replicate the previously described associations of *FSHR* polymorphisms and PTB[19,20]. In addition SNPs in *SERPINH1* didn't show an association with PPROM cases as shown previously[21]. Previously described associations for both of these genes were in Finnish and African American cohorts. It is possible that the causative alleles have different frequencies in different populations, since our cohort was mostly Caucasian and Hispanic we might haven't see the previously described associations.

Our study has several limitations. We didn't study PTB in relation to different ethnicities and most of our study population consisted of Caucasian and Hispanic ethnicities. We had to exclude Denmark maternal triad from PPROM analysis due to the unavailability of PPROM status.

Notable strengths of the study include large sample size across three different regions of the world. We studied the true spontaneous preterm birth excluding indicated preterm birth and multiple gestations, leaving a clean cohort of preterm births. We further divided the cohort into spontaneous preterm birth with intact membranes and PPROM in our sub group analysis as the mechanisms of PTB can be different in these two groups.

In conclusion, this study suggests a potential association between intronic SNPs in the *NR5A2* gene and PTB. *NR5A2* gene encodes for the Liver receptor homolog 1(LRH1) protein, which plays a critical role in regulation of cholesterol metabolism and steroidogenesis. It also has a role in the regulation of progesterone synthesis. These findings suggest *NR5A2* may be important in the pathophysiology of PTB and exploring of non-coding regulators of *NR5A2* is warranted.

Methods

Study Population

The study population used for genotyping consisted of 728 maternal triads (662 pedigrees, 2049 individuals) from United States (4 sites: University of Iowa Hospitals and Clinics in Iowa City, IA; Magee-Women's Hospital in Pittsburgh, PA; University of Rochester Medical Center in Rochester, NY, and Wake Forest University in Wake Forest, NC), Argentina (two sites: Instituto de Maternidad y Ginecología Nuestra Señora de las Mercedes in Tucumán and Hospital Provincial de Rosario in Rosario), and Denmark (Island of Funen and the Danish National Birth Cohort)(Table 1). Inclusion criteria were spontaneous preterm birth before 37 weeks of gestation. Gestational age was based on best obstetrical estimate (last menstrual period or ultrasound examination). Multiple gestation and major fetal chromosomal or structural anomalies were excluded.

All individuals provided signed informed consent for study enrollment in accordance with the protocols approved by research ethics committees in the U.S. (the University of Iowa Institutional Review Board (IRB), University of Pittsburgh IRB, University of Rochester Research Subjects Review Board, Wake Forest University Health Sciences IRB), Argentina (the Research Ethics Committee of Centro de Educación Médica e Investigaciones Clínicas), and Denmark (the Scientific-Ethical Committee of the Southern Danish Region and the Biomedical Research Ethics Committee of the Capital City Region of Denmark). DNA was extracted from venous blood, buccal swabs or saliva from the mothers and maternal grandparents. Demographic information was collected through medical chart abstraction.

SNP Genotyping

A total of 24 SNPs in four candidate genes (*NR5A2, FSHR, FOXP3, SERPINH1)* were studied (Table 2). SNPs for *FSHR* and *SERPINH1* were chosen based on previously reported associations with PTB. SNPs for *FOXP3* and *NR5A2* were selected using Haploview to get 80% coverage of the gene with a minor allele frequency ≥ 20% for each SNP. SNPs were genotyped using Taqman probes(Applied Biosystems) and the Fluidigm genotyping platform. Genotyping was completed on 728 maternal triads from Denmark (180 triads), Argentina (372 triads), and the United States (176 triads). Genotypes were entered into a laboratory database (Progeny, South Bend, IN) to generate datasets for analysis.

Statistical Analysis

Genotyping data were analyzed using transmission disequilibrium test (TDT) using the Family Based Association Test (FBAT) to identify nonrandom allele transmission from parents to offspring and p values were obtained from the maternal effect analysis. We were able to test for maternally-mediated genetic effects by using the maternal triad as the analysis unit. We analyzed the data for all maternal triads together as well as for each of the populations individually (US, Argentina and Denmark). We then performed subgroup analysis for gestational age categories (<32 and 32–36 weeks) and PPROM status (preterm labor with PPROM and without PPROM). The earliest gestational age was used to determine the gestation age for any mothers who experienced multiple preterm births. Mothers who experienced multiple PTBs and had discrepant PPROM status were excluded from analysis when conducting PPROM analyses. PPROM analysis was only completed for the US and Argentina maternal triads as PPROM data were not available for Denmark triads.

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Table 1.

Demographic characteristics of study population*

	SNP Genotyping		
	US	**Argentina**	**Denmark**
Number of Maternal Triads	176	372	180
Number of Pedigrees	168	371	123
Number of Individuals	512	1114	423
Gestational age (weeks)[1]	31.8 (+/− 3.8)	32.5 (+/−3.1)	34 (+/−2)
Infant Birth Weight (grams)[1]	1957 (+/−778) (Unknown 5)	1762 (+/−569) (Unknown 2)	2434 (+/−651)
Infant Gender (%male)	46	53	51
Maternal age at delivery (years)	30.1 (+/−4.8) (Unknown 16)	23.6 (+/−5.6) (Unknown 28)	27.5 (+/−3.9)

*for mothers who had more than one preterm delivery, data on first preterm delivery was used.

[1]Data are presented as mean ± standard deviation, with range in parenthesis.

Table 2.

List of SNPs

Gene	SNP	Chromosome	Position	Allele	MAF
NR5A2	rs2821330	1	200082464	A/G	0.416
NR5A2	rs12133107	1	200089274	G/A	0.25
NR5A2	rs2737670	1	200071286	G/A	0.482
NR5A2	rs2363573	1	200108427	T/C	0.293
NR5A2	rs12131233	1	200135175	A/T	0.264
NR5A2	rs6658424	1	200049302	T/A	0.319
NR5A2	rs2690036	1	200141801	G/A	0.314
NR5A2	rs2246209	1	200145533	A/G	0.357
NR5A2	rs2246923	1	200139350	C/T	0.254
NR5A2	rs2821367	1	200016146	T/C	0.32
NR5A2	rs2737667	1	200062858	T/G	0.25
NR5A2	rs10919806	1	200037727	C/T	0.192
NR5A2	rs3790844	1	200007432	T/C	0.212
NR5A2	rs2821312	1	200050204	G/A	0.467
NR5A2	rs2816949	1	199997778	A/G	0.228
FSHR	rs11686474	2	49287983	T/C	0.442
FSHR	rs11680730	2	49288060	G/T	0.432
FSHR	rs12473870	2	49292341	G/A	0.442
FSHR	rs12473815	2	49292362	C/T	0.438
FSHR	rs6165	2	49191041	A/G	0.403
SERPINH1	rs667531	11	75272716	G/C	0.155
SERPINH1	rs681390	11	75274630	T/C	0.296
SERPINH1	rs603647	11	75276721	T/C	0.46
FOXP3	rs2280883	X	49109128	T/C	0.412

Table 3.

Significant SNPs

Gene	SNP	P Value
All Triads		
NR5A2	rs2737667	0.02
US Triads		
NR5A2	rs2737667	0.01
SERPINH1	rs603647	0.02
Gestation Age <32 Weeks		
NR5A2	rs12131233	0.007
NR5A2	rs2737667	0.04
NR5A2	rs2816949	0.02
FSHR	rs6165	0.01
Spontaneous Preterm Birth with Intact Membranes		
NR5A2	rs2737667	0.0002 *
Spontaneous Preterm Birth with PPROM		
FSHR	rs6165	0.02

* remained significant after correction for multiple testing (Bonferroni-corrected p value <0.0006)