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Influence of *MBL2* and *NOS3* Polymorphisms on Spontaneous Preterm Birth in North East Brazil

Genetics and Preterm Birth

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Abstract

Background: The mannose-binding lectin (*MBL2*) and nitric oxide synthase 3 (*NOS3*) genes are associated with the immune response against inflammatory processes, have been reported as possibly related with premature birth. Until now, most of the researches regarding the genetic influence of prematurity have revealed limited results because only investigating the child or the mothers' genotypes, thus not exploring the possible effects of interactions between these genotypes or the interactions with environmental factors related to the duration of pregnancy. **Objective:**We performed a replica study investigating the influence of single nucleotide polymorphisms (SNPs) in *MBL2* and *NOS3* genes on premature birth, also considering socioeconomic, demographic and gestational factors. **Materials and methods**: We conducted a case-control study with 189 mother-infant dyads, with 104 spontaneous preterm births and 85 term births from Recife, Brazil. We used peripheral blood samples and umbilical cord samples to extract DNA. Functional SNPs at exon 1 and promoter region of *MBL*2 and *NOS3* rs1799983 SNP were genotyped using direct sequencing and fluorescent allelic specific TaqMan® assays respectively. Data were analyzed using the *Statistical Package for the Social Sciences* (SPSS®) program with bivariate association and logistic multivariate regression tests. **Results:** We observed a prevalence of *MBL2* wild-type genotype in the mother-infant dyad of the preterm group and polymorphic genotype in the mother-infant dyad

of term birth. The haplotype LYA predominated in our sample, being more frequent in the preterm group, while the haplotype LYB, correlated with lower levels of MBL protein, was more frequent in the term birth group. About *NOS3* rs1799983 SNP, the G/G genotype was more frequent throughout the sample. The heterozygous genotype predominated among women from the preterm group, showed a borderline difference between the groups. When *MBL2* genotypes of the mother and son were analyzed together, codon 54 of *MBL2* remained associated with prematurity. When the variables with *p* value lower than 0.20 in the bivariate analysis were analyzed by logistic regression, the low weight of the pregnant woman in relation to the gestational age, the occurrence of PPROM, urinary tract infection during birth and maternal history of other premature births were risk factors to prematurity. On the other hand, the presence of B allele at codon 54 of maternal *MBL2* was a protective factor for the occurrence of spontaneous premature birth. In contrast, a borderline association was established between the maternal genetic variation within *NOS3* gene and the outcome studied. **Conclusions:** Our study, limited by the small number of patients enrolled, indicates that *MBL2* and *NOS3* functional SNPs are associated with the occurrence of spontaneous prematurity and the regulation of the maternal inflammatory response. Despite these results are in agreement with previously reports, our findings do not replicate the ones reported in a large GWAS performed on quite high number of subjects. Thus, we can conclude that *MBL2* and *NOS3* functional SNPs are plausible candidate risk factors just in few preterm birth cases, and consequently they cannot be included in the general diagnostic practice.

Introduction

The spontaneous preterm birth can occur from spontaneous onset of labor with preservation of the integrity of amniotic membranes or with preterm premature rupture of membranes (PPROM) [1,2]. About 50% prematurity cases are still considered idiopathic [3,4,5]. It is established that premature birth results from the interaction of factors that cause a change in the state of quiescence from the beginning of uterine contractions and culminates with the delivery before 37 weeks of pregnancy, which may be related to an autoimmune reaction with a poorly defined pathophysiology [3,6].

Single nucleotide polymorphisms (SNP) at the mannose-binding lectin encoding gene (*MBL2*) are widely known to alter MBL protein serum levels, thus impacting the modulation of the immune response [7-9]. There are indications that this modification can predispose woman and fetus to spontaneous preterm birth [8,9]. On the other hand, the SNPs within nitric oxide synthase 3 (NOS3) can alter the synthesis of eNOS enzyme and nitric oxide [10], affecting the mediation mechanisms of the inflammatory response and regulation of uterine contractility and possibly contributing to the onset of premature labor [11,12].

Until now, most of the research regarding the genetic influence on prematurity has revealed limited results. Limitations in these studies include only investigating the child or the mothers genotype, and not exploring the possible effects of interactions between these genotypes or the interactions with environmental factors related to the duration of human pregnancy.

The aim of our research was to replicate previous literature findings obtained on populations of different background with respect to the one analyzed in our study, reporting a possible role of *MBL2* and *NOS3* SNPs in the modulation of the susceptibility to pre-term birth. To this end we investigated the influence of *MBL2* and

NOS3 functionalSNPs on spontaneous preterm birth in a population from North East Brazil by assessing both groups of mothers and fetuses and considering their possible interactions also controlling for the risk factors observed in our population, specifically the socioeconomic, demographic and pregnancy factors.

Materials and methods

We conducted a case-control study enrolling mothers with spontaneous preterm birth and mothers with term birth as well as their neonates. This study was developed in maternity hospitals of Clinical Hospital, Federal University of Pernambuco and Agamenon Magalhães Hospital both localized in the city of Recife, Brazil. These hospitals are public and considered as reference in high-risk pregnancy, serving a population of women with similar socioeconomic, demographic and gestational characteristics.

Both patients and controls were from Recife metropolitan region (Pernambuco, Brazil); Ethnicity self-classification according to skin-color was confirmed to be a poor indicator of population substructure in Brazilians, so we did not consider this classification in our study. In fact we already described the genetic ancestry of Pernambuco population using 12 ancestry markers observing an admixture European (59.7%), African (23%) and Amerindian (17.3%) genomes [13]. This genome configuration is present in basically all individuals from Pernambuco and represents in our study the common ancestry background shared by patients and controls.

The sample consisted of 189 postpartum mother-infant dyads, 104 of spontaneous preterm birth (55%) and 85 of term delivery (45%). The *MBL2* gene was analyzed in 71 mothers and 42 premature neonates, and compared with 62 mothers and 53 term newborns. The *NOS3* gene was analyzed in 97 mothers of preterm infants and 77 mothers of term neonates.

The exclusion factors for both groups were: multiple pregnancy, malformed fetus and neonates with clinical features of congenital infection. The data regarding the socioeconomic, demographic factors and pregnancy characteristics were obtained from both medical records and interviews conducted with the pregnant women.

The diagnosis of spontaneous preterm birth was given by the medical staff of the hospitals based on the presence of preterm labor, with or without premature preterm membrane rupture, which resulted in delivery before 37 weeks or 259 days gestation. The premature labor was characterized by the presence of regular and persistent uterine contractions in a minimal frequency of twice every 10 minutes, cervical dilatation equally or superior to one centimeter, cervical disappearance equally or greater than 80% and progression of cervical changes. The gestational age of the newborn was verified by the date of last menstrual period or by ultrasound examination, and confirmed by the Capurro somatic method [14].

Blood collection and DNA extraction

Blood samples from women who have recently given birth and newborns were supplied by the blood bank of hospitals, originated from peripheral blood and umbilical cord blood, respectively. The DNA extraction was performed using the *Salting-out* protocol, suited for small volumes of blood.

MBL2 and NOS3 genotyping

In our study, we sequenced exon 1 and promoter region of *MBL2* gene in order to genotype the three functional variants (codons 52, 54, 57 at exon 1) as well as the two

promoter (at -550 and -221 positions) polymorphisms involved in the regulation of MBL protein production [15].

MBL2 genotyping was performed using the following primers: for promoter polymorphisms, forward 5' CCA GGG CCA ACG TAG TAA GA 3' and reverse 5' GAG GGG TTC ATC TGT GCC TA 3', for exon 1 polymorphisms, forward 5' GGG CAT GCT CGG TAA ATA TG 3' and reverse 5' TGC CCA GAG AAT GAG AGC TGA 3'. Polymerase chain reactions (PCR), carried out in Gene-Amp 9700 Thermal cycler (Applied Biosystems - Life Technologies, Foster City, California, USA) using PCR buffer 1x, 1 unit of Taq Gold, 0.2 mM dNTPs and 2 mM MgCl2. PCR was performed as follows: denaturation, 30" at 95°C; annealing, 30" at 55°C; extension, 30" at 72°C for 40 cycles. PCR products were visualized using electrophoresis run (2% agarose gel) to check the successful DNA amplification and the absence of non-specific reaction products. DNA sequences were run on an automated ABI Prism 3130 Genetic Analyser, PE, using the 3130 Data Collection Software (Applied Biosystem). The sequence manual analysis was executed in software *CodonCode Aligner 5.0* (CodonCode Corporation), and the consensus sequence of MBL gene to accomplish the comparison was obtained in NCBI database. The haplotype recognition was performed using the *Haploview* software. We computed haplotypes and combined genotypes associated with high, low or deficient MBL production [15].

Genotyping of SNP rs1799983 within *NOS3* was performed using commercially available TaqMan® probes (Applied Biosystem, USA) respecting the protocol determined by the manufacturer. The samples were amplified by qualitative real time polymerase chain reaction (qPCR) using the ABI7500 platform (Applied Biosystem) which utilizes the SDS v.2.3 software to accomplish the allelic discrimination (Applied Biosystem).

Statistical Analysis

We used *Statistical Package for the Social Sciences* program(SPSS®, version 20) for statistical analysis. The chi-square and Fisher test were used to compare the polymorphisms frequency between the groups, considering statistically significant values of *p* equal or lower than 0.05. Afterward, the explanatory variables with *p* values lower than 0.20 in the bivariate analysis were selected to perform the multivariate regression logistic.

Results

Regarding the gestational age of preterm infants, there was a predominance of cases of borderline or late preterm births (N = 54, 54%) in this study. We observed 17 cases of moderate prematurity (17%), 18 cases (18%) of severe prematurity and 11 (11%) of extreme. The mean gestational age was 32.6 weeks. Most premature infants had low birth weight (N = 58, 56.9%). In addition, there were 16 cases (15.7%) of newborns with very low birth weight and 11 cases (10.8%) of extremely low birth weight, according to the criteria of weight classification at birth from the World Health Organization. Otherwise, among the group of term birth, eight neonates presented low birth weight at birth, while others were born with adequate weight (N=77, 90.6%). Concerning the demographic aspects both groups presented similar results, except for some differences in gestational aspects (Table 1).[Table 1 near here]

Allele, genotype and haplotype frequencies are represented in Tables2 and 3.[Table 2 and 3 near here].

Table 4 shows the sample distribution of mothers and infants with regard to the production level of the MBL protein inferred from the combination of haplotypes.[Table 4 near here].

The haplotype LYA predominated in our sample, being more frequent in the preterm group, while the haplotype LYB, correlated with lower levels of MBL protein, was more frequent in the term birth group.

The variables with *p* value lower than 0.20 in the bivariate model were then analyzed by logistic regression: the low weight of the pregnant woman in relation to the gestational age, the occurrence of PPROM and urinary tract infection during birth, as well as the maternal history of other premature births remained associated with prematurity. In addition, the presence of SNP in codon 54 within maternal genotype was associated with the outcome, presenting itself as a protective factor for the occurrence of spontaneous premature birth in the sample investigated, since the genotype and the polymorphic allele were more frequent in the women of the control group (Table 5). [Table 5 near here].

Discussion

MBL2 gene

Our findings suggest that women with genotypes related with a lower production of MBL protein had births at term, thus pointing to a protective effect of B allele at codon 54 within *MBL2*gene on spontaneous preterm birth. This effect remained statistically significant after controlling for socioeconomic, demographic and gestational variables using the multivariate model of logistic regression.

Our results agree with Van de Geijn [8] e de Crider et al [16], who suggested that the polymorphisms that increase the magnitude and duration of the inflammatory response are associated with prematurity, while those that lead to a lower inflammatory response may reduce the risk of this outcome. Nevertheless, our date differ from those observed by Annells et al [9], who found B allele in codon 54 associated with a higher incidence of premature birth with gestational age lower of 29 weeks. However, in this study a distinction between types of prematurity (spontaneous and indicated) was not performed. In our analyses we only considered the spontaneous preterm birth and the data stratification according to gestational age was not performed.

Similar to allele distribution between the mothers, L and Y alleles at *MBL2* promoter region of neonates were also prevalent among term and preterm groups, without statistical difference between them. When considering *MBL2* coding region, the polymorphic alleles were less frequent. These data are similar to those observed by Bodamer e et al [17], that found an association between codon 52 and the genotype O/O of neonates with preterm birth. This research only found the O/O genotype among premature births, suggesting a lower concentration of serum levels of functional MBL. However, no difference was found on the distribution of A and O alleles among neonates groups. Previous research indicated that the fetal genotype did not influence the serum levels or the activity of maternal MBL protein during pregnancy, but can influence the serum levels of MBL protein in neonates, and participate in the defense against pathogens as well as regulate the immune response [17,18].

The detection of elements belonging to the complement system in the fetus occurs in the first weeks of pregnancy and their synthesis begins at 9 weeks, however protein levels remain low until the first days of postnatal life. At the end of term gestational,

these components can reach 10% of maternal levels, which may be lower among preterm newborns [17,19,20]. Thus, we believe that the regulation of the inflammatory response by the fetal MBL protein exerts a lower contribution over the duration of human pregnancy than the regulation made by maternal MBL protein.

Otherwise, it is possible that an interaction exists between the genotypes found in the mother and neonate, and this could influence the risk of premature birth [21,22]. Therefore, when combined the *MBL2*genes of mother-infant dyad, the codon 54 remained associated with prematurity, with a higher frequency of wild genotype (A/A) in the preterm group and the variant genotype (A/B and B/B) among the dyad term birth.

In relation to haplotype frequency, the haplotype LYA predominated in our studied population, in concordance with Boldt et al [23], who observed a higher prevalence of LYA haplotype in a Brazilian population with African descent or without ethnic defined characterization, such as prevalent in this study. This haplotype is associated with high/ intermediate levels of MBL protein [7,24,25], and was more frequently observed among the pregnant women and neonates from the preterm birth group. Further, the haplotype LYB, correlated with lower levels of MBL protein was more frequent in the term birth group.

The proposal of an excessive inflammatory profile during gestation and the important role played by this through onset of preterm labor are reported in the literature as pregnancy itself considered an inflammatory event [8,9,17,26,27]. Therefore, the elevated levels of MBL protein found in blood may confer a biological disadvantage and aggravate the inflammatory response to an infection, increasing the magnitude of inflammation and favoring the production and liberation of inflammatory cytokines that can induce labor and cervix dilation [6,8,28].

NOS3 gene

NOS3 rs1799983 SNP results in a substitution of glutamic acid by aspartic acid at amino acid position 298 (Glu298Asp). The Glu298Asp polymorphism was reported as associated with altered NOS3 enzyme activity, reduced nitric oxide production, and reduction blunted endothelial-dependent vasodilation [28,29]. However, the rs1799983 SNP has been classified as "*Conflicting interpretations of pathogenicity, risk factor*" in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/14015/#clinical-assertions), so its functional impact is still debated.

In this study, *NOS3*rs1799983 SNP G/G genotype was the most frequent in the sample, with higher frequency of G/T genotype in women from the preterm group and boarderline statistical difference between the groups. The alleles frequencies (G and T) did not differ in the sample.

Our results agree with Luo et al [29], Parveen et al [31] and Almawi et al [32], that revealed a significant association between the rs1799983 genotype distribution in recurrent miscarriage patients. In these studies, women with recurrent spontaneous abortion had a significantly higher frequency of G/T genotype and lower frequency of the G allele compared to control women. Among children with cerebral palsy, preterm birth was associated with NOS3 -922, but not with polymorphisms in rs1799983 [4].

The reduced NO production may predispose pregnant women to pregnancy-related vascular disorders, including pre-eclampsia, placental abruption, recurrent spontaneous abortion [29,30] and spontaneous preterm birth [33], and also can lead to impaired placental perfusion and a compromised oxygen and nutrient supply to the fetus which might affect the ability of the embryo to resist maternal rejection in pregnancy [31].

NO is necessary for the establishment and maintenance of pregnancy, regulating the quiescent state of uterine contractions through means of tissue connectivity regulation

and myometrium looseness, and also functions in inflammation injury [4,12,26,29,31]. Experimental studies in rats have shown that endothelial nitric oxide isoform (NOS3) is present in various types of uterine cells, and that the production of nitric oxide in these cells varies according to the stage of gestation. Thus, during most of gestation, the production of NO remains high to suppress uterine contractility and maintain pregnancy, and reduces in moments prior to labor, allowing increased contractions [34-38]. On the other hand, the levels remain low in the cells of the cervix throughout the gestation, maintaining the basal state of contraction of the cervix, and elevate especially during the delivery, to allow the relaxation of the tissue and the passage of the concept [34-38].

The onset of labor may be considered an autoimmune reaction regulated by the signaling of different neurotransmitters. In the uterine cervix, this reaction is characterized by the infiltration of leukocytes, neutrophils and macrophages followed by the production of proinflammatory cytokines and collagenase, which causes dissociation of collagen from the connective tissue. The production of cytokines increases the release of nitric oxide, especially by the activation of the enzyme iNOS. The released NO, in turn, potentiates the cytokine cascade and, together with the action of prostaglandins (PGE2 and PGE2α), initiates a potent vasodilatation and promotes degradation of the extracellular matrix, allowing relaxation and cervical dilatation [39].In addition, the mechanical pressure exerted by the blood on the vessel wall elevates the release of calcium and increases the production of nitric oxide by the enzyme eNOS, potentiating vasodilation and relaxation of the cervix.

Thus, NO is considered a relaxation inflammatory mediator of uterine cervix during the labor, since it regulates the cytokine cascade and promotes the cervix vasodilatation [39].

Genetics and environmental factors association with spontaneous preterm birth

Multivariate logistic regression analysis showed that the variant genotype (A/B and B/B) at codon 54 of the maternal *MBL2* gene, the low weight of pregnant women according to the gestational age, the incidence of premature rupture of membranes, urinary tract infection at birth and history of other premature births remained associated with spontaneous preterm birth. Only the presence of the maternal genetic variant was considered a protective factor, while the other variables presented a risk for the occurrence of spontaneous preterm birth in the sample investigated. These findings confirm the hypothesis that the prematurity etiology is multifactorial, involving genetics and environmental factors.

A urinary tract infection (UTI) during pregnancy affects almost half of the sample, with nearly all having been treated with antibiotic therapy. It is known that a UTI promotes a higher concentration of phospholipase-A in blood and subsequently the conversion of prostaglandin E^2 (PGE^2) into prostaglandin E^{2d} (PGE^{2d}) and the stimulus of uterine fibers for the start of contractions. Even in lower levels, this uterine contractile activity may reduce the placenta chances and favor the onset of preterm labor [5,40]. Thus, from a clinical standpoint, the presence of a UTI during the labor may further influence the labor anticipation in the preterm group of women, as they were found to produce higher levels of MBL protein, which exacerbates the inflammatory response to infection.

Lastly, the recurrence of spontaneous premature birth demonstrates a relationship with the outcomes investigated in multivariate analysis. This data supports the interaction among genetics factors and environmental risk factors to preterm birth and indicates the

history of previous preterm birth as one of the main factors for the identification of women with higher risk of preterm birth [41,42].

A recent GWAS study of Zhang et al. [43] performed on a very high number of patients identified genetic variants at *EBF1, EEFSEC, AGTR2, WNT4, ADCY5*, and *RAP2C* genes as associated with gestational duration and variants at *EBF1, EEFSEC*, and *AGTR2* genes as related to preterm birth. The fact that *MBL2* and *NOS3*variants, considered in our study and others previously reported in the literature, were not present in the very robust findings of Zhang et al. could be due to the low number of subjects enrolled in our study as well as to the different ethnic background of the two populations analyzed (European in the Zhang manuscript, North Eastern Brazilian in our work).

In conclusion, acknowledging the limitations due to the low number of subjects enrolled in our study and the lack of a full genome (GWAS) analysis, we observed in our study population the association of *MBL2* and *NOS3* genetic variants with susceptibility to pre-term birth. However, we are aware that our findings do not replicate the ones reported in a large GWAS performed on quite high number of subjects. Thus, we can conclude that *MBL2* and *NOS3* functional SNPs are plausible candidate risk factors just in few PTB cases, and consequently they cannot be included in the general diagnostic practice.

Preterm birth is a complex trait with multifactorial etiology, depending upon environmental factors and genetics. The latter could play a marginal role due to the low penetrance, variable expression, and mother-fetus interactions of several genes (some of them to be discovered yet). So the introduction of genetic markers in the diagnostic practice of PTB is still premature and not reliable at present time; thus we emphasize the importance of adequate evaluation of the pregnant woman at the beginning of the prenatal follow-up aimed at identifying family history of preterm birth or previous preterm birth, as well as the identification of preventable factors of spontaneous preterm birth, such as the occurrence of urinary tract infection and the low birth weight of the pregnant woman according to the gestational age and offer measures to support the mother-child dyad.

Declaration of interest statement: The authors report no conflicts of interest.

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Table 1: Socioeconomic, demographic and gestacional profile of the mothers according to the type of birth.

Maternal variables	Preterm		Term		*p*
	N	%	N	%	
Age (years)					
<20	39	37.5	29	34.1	0.182††
20 a 34	62	59.6	48	56.5	
≥35	03	2.9	08	9.4	
College level (years)					
<5	11	10.6	08	9.4	0.919†
5 a 8	36	34.6	28	32.9	
≥9	57	54.8	49	57.6	
Reading ability					
Yes	93	89.4	72	84.7	0.333†
With difficulty or not read	11	10.6	13	15.3	
Occupation					
Yes	36	34.6	31	36.5	0.791†
No	68	65.4	54	63.5	
***Per capita* family income in minimum wage**					
≤ 0,25	40	38.5	29	34.1	0.187†
0,26-0,50	43	41.3	29	34.1	
> 0,50	21	20.2	27	31.8	
Skin colour					
White	10	9.6	15	17.6	
Brown	81	77.9	54	63.5	0.089†
Black	13	12.5	16	18.8	
Marital status					
United	84	80.8	71	83.5	
Separated	20	19.2	14	16.5	0.623†
Smoke					
Yes	06	5.8	08	9.4	0.341†
No	98	94.2	77	90.6	
Alcohol					
Yes	17	16.3	16	18.8	0.655†
No	87	83.7	69	81.2	
Prenatal care onset					
1º trimester	78	78.8	67	78.8	0.995†
From the 2º trimester	21	21.2	18	21.2	
Number of prenatal consults					
< 6	62	62.6	22	25.9	<0.001†
≥ 6	37	37.4	63	74.1	
Prenatal assistance quality					
Great or good	76	76.8	68	80	0.596†
Regular or bad	23	23.2	17	20	
Nutritional state of pregnant					
Low weight	35	38.5	15	18.3	0.002†
Proper	32	35.2	27	32.9	
Overweight/Obesity	24	26.4	40	48.8	
Parity					
Primiparous	63	60.6	45	52.9	0.291†
Multiparous	41	39.4	40	47.1	
Gestational interval in months					
Primiparous	63	60.6	45	52.9	0.101†
≤18	12	11.5	05	5.9	

>18	29	27.9	35	41.2	
UTI during gestation					
Yes	44	42.3	38	44.7	0.741†
No	60	57.7	47	55.3	
UTI during birth					
Yes	21	20.2	07	8.2	0.021†
No	83	79.8	78	91.8	
Bacterial vaginitis					
Yes	13	12.5	10	11.8	0.878†
No	91	87.5	75	88.2	
Chorioamnionitis					
Yes	07	6.7	0	0	0.017††
No	97	93.3	85	100	
Premature rupture of membrane					
Yes	41	39.8	12	14.1	<0.001†
No	62	60.2	73	85.9	
Premature mothers					
Yes	10	10	09	10.6	0.896†
No	90	90	76	89.4	
Family history of prematurity					
Yes	52	50	29	34.1	0.028†
No	52	50	56	65.9	
Previous premature birth					
Yes	19	46.3	04	10	<0.001†
No	22	53.7	36	90.0	
Abortion					
Yes	20	19.2	18	21.2	0.740†
No	84	80.8	67	78.8	
Gestational diabetes					
Yes	06	5.8	01	1.2	0.131††
No	98	94.2	84	98.8	
Cervical incompetence					
Yes	03	2.9	1,0	1.2	0.629††
No	101	97.1	84	98.8	
Gestational hypertension					
Yes	10	9.6	46	54.1	<0.001†
No	94	90.4	39	45.9	
Preeclampsia					
Sim	07	6.7	38	44.7	<0.001†
Não	97	93.3	47	55.3	
Neonate sex					
Male	47	46.5	49	57.6	0.131†
Female	54	53.5	36	42.4	

†Chi-square test

†† Exact Fisher test

Table2: Frequency of SNPs within maternal and neonate *MBL2* gene and maternal *NOS3* gene accordance with type of birth.

Genetics variables	Puerperal women					Neonates				
	Preterm		Term		*p*	Preterm		Term		*p*
	N	%	N	%		N	%	N	%	
***MBL2*gene**										
rs11003125 (H/L)										
H/H	08	11.3	06	9.7	0.886††	0	0.0	05	9.4	0.093††
H/L	27	38.0	26	41.9		17	40.5	23	43.4	
L/L	36	50.7	30	48.4		25	59.5	25	47.2	
Allelic frequency H/L										
H	43	30.0	38	31.0	0.949†	17	20.2	33	31.1	0.090†
L	99	70.0	86	69.0		67	79.8	73	68.9	
rs7096206 (X/Y)										
X/X	02	2.8	03	4.8	0.633††	0	0.0	06	12.2	0.002††
X/Y	19	26.8	13	21.0		14	32.6	05	10.2	
Y/Y	50	70.4	46	74.2		29	67.4	38	77.6	
Allelic frequency X/Y										
X	23	16.0	19	15.0	0.845†	14	16.3	17	17.3	0.847†
Y	119	84.0	105	85.0		72	83.7	81	82.7	
Codon 52										
A/A	65	91.5	55	93.2	0.396††	37	88.1	36	97.3	0.057††
A/D	06	8.5	03	5.1		05	11.9	0	0,0	
D/D	0	0.0	01	1.7		0	0.0	01	2.7	
Allelic frequency A/D										
A	136	96.0	113	96.0	1.0††	79	94.0	72	97.3	0.449††
D	06	4.0	05	4.0		05	6.0	02	2.7	
Codon 54										
A/A	67	94.4	47	81.0	0.012††	35	89.7	24	75.0	0.096††
A/B	04	5.6	05	8.6		01	2.6	0	0.0	
B/B	0	0.0	06	10.3		03	7.7	08	25.0	
Allelic frequency A/B										
A	138	97.0	99	85.0	0.001††	71	91.0	48	75.0	0.010†
B	04	3.0	17	15.0		07	9.0	16	25.0	
Codon 57										
A/A	64	91.1	45	90.0	0.001††	38	90.5	36	92.3	0.504††
A/C	07	9.9	0	0.0		02	4.8	0	0.0	
C/C	0	0.0	05	10.0		02	4.8	03	7.7	
Allelic frequency A/C										
A	135	95.0	90	90.0	0.129†	78	92.9	72	92.3	0.894†
C	07	5.0	10	10.0		06	7.1	06	7.7	
Genotype A/O										
A/A	56	78.9	28	60.9	<0.001††	26	68.4	17	65.4	0.004††
A/O	15	21.1	06	13.0		08	21.1	09	34.6	
O/O	0	0.0	12	26.1		04	10.5	0	0.0	
Allelic frequency A/O										
A	127	89.4	62	67.4	<0.001†	60	79.0	43	82.7	0.600†
O	15	10.6	30	32.6		16	21.0	09	17.3	
***NOS*3 gene**										
rs1799983 (G/T)										
G/G	55	56.7	51	66.2	0.078††	-	-	-	-	
G/T	38	39.2	19	24.7		-	-	-	-	
T/T	04	4.1	07	9.1		-	-	-	-	
Allelic frequency G/T										

G	148	76.3	121	78.6	0.614†	-	-	-	-
T	46	23.7	33	21.4		-	-	-	-

†Chi-square test

†† Exact Fisher test

Table3: Haplotype frequency of *MBL2*gene between maternal and neonates in accordance with type of birth.

***MBL2* Haplotype**	**Maternal genotype**					**Neonate genotype**				
	Preterm N=126	**%**	**Term N=86**	**%**	***p***	**Preterm N=60**	**%**	**Term N=40**	**%**	***p***
HYA	30	23.81	20	23.26	0.926†	06	10.00	11	27.50	0.022†
LYA	74	58.73	30	34.88	0.001†	38	63.33	13	32.50	0.003†
LXA	12	9.52	10	11.63	0.622†	08	13.33	06	15.00	0.814†
HYD	02	1.59	03	3.49	0.397††	0	0.00	0	0.00	-
LYB	02	1.59	14	16.28	<0.001†	04	6.67	10	25.00	0.010†
LYC	06	4.76	09	10.47	0.112†	04	6.67	0	0.00	0.148††

†Chi-square test

†† Exact Fisher test

Table4: Production of MBL protein from haplotype combination between puerperal women and neonates.

Maternal and neonate MBL protein production*	**Preterm**		**Term**		***p***	**OR**	**IC (95%)**
	N	**%**	**N**	**%**			
Maternal production							
Insufficiency	04	7.0	11	27.5	0.021†	1.00	-
Low	16	28.1	10	25.0		4.40	1.09-17.68
High	37	64.9	19	47.5		5.35	1.50-19.09
Neonate production							
Insufficiency	02	8.3	07	41.2	0.005††	-	-
Low	08	33.8	0	0.0		-	-
High	14	58.3	10	58.8		-	-

†Chi-square test
††Exact Fisher test

***** Inferred from haplotype combination, considered higher in HYA and LYA haplotype combination; lower in HYA or LYA with LXA, HYD, LYB or LYC haplotype combinations; and insufficiency when combinations between LXA, HYD, LYB and LYC occurred (GIBSON et al., 2011).

Table5: Logistic regression of spontaneous premature birth risk factors

Variables	OR not adjusted	*p*	OR adjusted	IC (95%)	*p*
Level 1					
Skin colour					
Black	1.22	0.093	1.16	0.38-3.53	0.092
Medium	2.25		2.24	0.92-5.46	
White	1.00		1.00		
Level 2					
Pregnant nutritional state					
Low weight	3.89	0.003	3.47	1.55-7.78	0.010
Proper	1.97		1.78	0.85-3.74	
Overweight/Obesity	1.00		1.00		
Level 3					
Maternal *MBL2* gene (codon 54)					
A/B or B/B (polymorphic)	0.25	0.026	0.20	0.045-0.91	0.037
A/A (wild-type)	1.00		1.00		
UTI during birth					
Yes	2.82	0.026	10.23	1.80-58.20	0.009
No	1.00		1.00		
Level 4					
Previous premature birth					
Primiparous	2.29		2.21	0.77-6.33	0.018
Yes	7.77	0.001	14.12	2.22-89.80	
No	1.00		1.00		
Family history of prematurity					
Yes	1.93	0.029	1.79	0.69-4.61	0.229
No	1.00		1.00		
Premature rupture of membranes					

Yes	4.02	<0.001	2.97	1.08-8.13	0.034
No	1.00		1.00		

Level 1: adjusted by maternal age and *per capita*monthly familiar income
Level 2: adjusted by gestational interval
Level 3: adjusted by maternal SNP within *NOS3*gene (rs1799983)