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The Effect of Inflammation on Preterm Birth

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1. Introduction

1.1 General considerations

The World Health Organization defines preterm birth as the delivery of an infant between 20 and 37 weeks of gestation (Martin et al., 2005, Beck et al., 2010). Preterm birth (PTB) is a worldwide health problem and remains the leading cause of perinatal morbidity and mortality. PTB rates have been reported as 11% in the United States, and approximately 5 to 7% in Europe (Goldenberg, 2002). In industrialized countries, preterm delivery is responsible for 70% of mortality and 75% of morbidity in the neonatal period, contributing to significant long-term neurodevelopmental problems, pulmonary dysfunction, and visual impairment (Challis et al., 2001; Wen et al., 2004; Iacovidou et al., 2010). In South America, more than 10% of newborns are preterm, and the incidence is between 8% and 10% in Uruguay (Rey et al., 2008; Sosa et al., 2011). Unfortunately, the rate of PTB continues to rise according to recent incidence reports, and is currently 12–13% in the USA (Ananth et al., 2006).

1.2 Etiology

Over the last decade, it has become increasingly apparent that the cause of PTB and preterm premature rupture of membranes (PPROM) is multifactorial and involves both genetic and environmental factors (Romero et al., 1994; Romero et al., 2000; Crider et al., 2005; Romero et al., 2006; Goldenberg et al., 2008). Similar observations have been made in other complex diseases such as coronary heart disease (Talmud & Humphries, 2004), hypertension (Hamet et al., 1998; Zhu, 2005) and psychiatric conditions (Lau & Eley, 2004). Consequently, researchers focused on the search for a genetic background that predispose to complex diseases when unfavorable environmental conditions are present. Preterm labor is considered the final common pathway of different complications of pregnancy such as infection, placental abruption, smoking, poor nutrition, alcoholism, multiple gestation, endocrine and coagulation disorders and fetal or maternal genetic susceptibility (Goldenberg, 2002; Wen et al., 2004; Goldenberg et al., 2008; Iacovidou et al., 2010). Regarding genetic susceptibility the single best predictor of PTB among multiparous pregnant women is past history of preterm delivery: women with one prior PTB have a recurrence risk of PTB of 15% and those with two or more prior PTBs have a recurrence risk of 32% (Pennell et al., 2007). The risk of PTB tends to remain with the mother through multiple pregnancies, even with increased levels of prenatal surveillance and preventive interventions (Carr-Hill & Hall, 1985). Twin studies have suggested that heritability for PTB ranges from 17% to 36% (Clausson et al., 2000). Women

who themselves were born preterm have an increased risk of PTB. The risk of PTB increases as the gestational age of the mother's birth decreases, with mothers born at less than 30 weeks having a 2.4-fold (95% CI 1.4 - 4.2) increase in risk of PTB (Porter et al., 1997). Furthermore, there is a well-described ethnic difference in the rate of PTB (even when controlling for mother contributing etiologic factors). In 2005, the incidence of preterm delivery among African Americans and European Americans in the U.S. was 18.4% and 11.7%, respectively (Anum et al., 2009). This ethnic disparity in PTB between African Americans and European Americans has been present for decades. The inequity is likely the consequence of multiple factors, including socioeconomic status, access to care, environment, and differences in genetic background (Anum et al., 2009).

1.3 Inflammatory pathways and preterm birth

Preterm labor is considered a syndrome because there are many causes that lead to different pathological processes. Most of them involve an inflammatory cytokine-mediated response. The fetus loses some of its immunological defense in their presence and becomes vulnerable to destruction by the immune system (Vrachnis et al., 2010). Infection has been regarded as one of the most heralded causes of preterm birth due to the drastic link between underlying infectious agents and their ability to promote inflammatory responses (Goldenberg et al., 2000; Thaxton et al., 2010). Infections like chorioamnionitis may initiate preterm labor. Bacterial vaginosis is associated with increased PTB risk even without infection of the fetal membranes (Slattery & Morrison, 2002). Increased inflammation occurs in normal parturition, and inflammatory cytokines are higher in women who deliver preterm (Keelan et al., 2003). Infection, stress, and obesity promote inflammation, suggesting that these environmental exposures may promote an inflammation-mediated mechanism resulting in early parturition (Crider et al., 2005). Inflammation and immune response are universal mechanisms of host defense against infection. They function on the basis of special receptors called pattern-recognition receptors which recognize conserved microbial structures called pathogen-associated molecular patterns. Due to pattern-recognition receptors, the human organism is able to discriminate between self- and non-self-antigens (Li et al., 2010).

1.4 The role of toll-like receptors

A strong explanation for initiation of distinct immune pathways could be the activation of toll-like receptors (TLRs). TLRs are a diverse set of innate immune sentinel receptors of pathogens highly conserved throughout evolution (Bauer et al., 2009). TLRs are a group of pattern-recognition receptors that play a crucial role in 'danger' recognition and the induction of immune response. The human TLR family comprises at least 13 distinctive proteins (TLR1 – TLR13) that are able to recognize typical microbial agents and subsequently facilitate an early immune response via downstream different signaling pathways (Medzhitov et al., 1997; O'Neill, 2008). The human TLR member best characterized to date is toll-like receptor 4 (TLR4), as this signaling molecule is essential for the recognition of bacterial lipopolysaccharides (LPS) among other microbial agents (Krishnan et al., 2007). LPS represent a key element of gram-negative bacteria and contain fatty acids in their lipid-A domain anchoring LPS into the bacterial cell wall (Raetz & Whitfield, 2002). LPS are thought to play a key role in eliciting an inflammatory response including the activation of the immune cells and the release of enzymes involved in the remodeling of the extracellular matrix leading to preterm delivery. TLRs are highly expressed at the maternal-fetal interface on trophoblasts and uterine immune cells (Patni et

al., 2007). It is likely that differential uterine immune responses occur due to the diversity of pathogens that ensues activation of any one of the TLRs, ultimately leading to deleterious inflammation and preterm birth (Li et al., 2010; Thaxton et al., 2010). In particular, TLR4 is widely expressed in different cell types of the placenta compared to others members of the TLR family (Ma et al., 2007). Human TLR4 gene is polymorphic. Combination of distinct alleles of polymorphic genes could account for individual and ethnic differences in the rate and extent of production of individual proteins as well as variations in the acquisition and/or severity of a particular disorder. Sequencing of the human TLR4 revealed that most of the variation in non-synonymous polymorphisms is located in the third exon (Smirnova et al., 2001). The non-synonymous polymorphism localized on position 896 (rs4986790) is an A/G transition causing an aspartic acid/glycine substitution at amino acid location Asp299Gly. Arbour *et al.* were the first to report that individuals with Asp299Gly polymorphism had a blunted response toward inhaled LPS (Arbour et al., 2000; Ferwerda et al., 2008). Individuals carrying the variant alleles are at increased risk of Gram-negative infections (Agnese et al., 2002) and premature birth (Lorenz et al., 2002a) but not for preterm premature rupture of membranes (PPROM) in African Americans (Ferrand et al., 2002). Animal models seem to confirm the critical role of TLR4 in induced preterm delivery by inflammation (Li et al., 2010).

1.5 Our goal

More evidences support the complexity of preterm birth (Plunkett & Muglia, 2008). Modeling procedures used by twin studies suggest that additive genetic factors and environmental risk factors that are not shared among siblings both influence preterm birth (Clausson et al., 2000). Additionally, interactions between genes (Kalish et al., 2006; Menon et al., 2006) have been associated with preterm birth risk. Several studies suggest that gene-environment interactions, such as interactions between inflammatory gene risk alleles and bacterial infections (Macones et al., 2004b; Engel et al., 2005) also influence the disorder. Together, these studies imply that the etiology of preterm birth likely involves genetic as well as environmental factors in complex interactions. However the physiological and molecular bases of these interactions are not well understood. Our goal is to advance on the knowledge of how genetic and environmental factors combine to affect the risk of PTB and, more specifically, how the variations in the human genome (polymorphisms) can modify the effects of demographic conditions and exposures to environmental health hazards. We focused on TLR4 pathway. TLR4 genetic variations of our population have been looked closely trying to associate single nucleotide polymorphisms (SNP) to the commonly demographic, social, and ethnic accepted factors that contribute or cause PTB. Since PTB likely depends on a number of interacting risk factors, our genetic studies try to identify markers or pathways involved in the disorder. The goal is to find risk factors in the context of particular populations that might predict preterm birth more accurately than those currently identified.

2. Materials and methods

2.1 Subjects

A case-control study was conducted. Subjects were offspring of women receiving obstetrical care at the Pereira Rossell Hospital, Montevideo, Uruguay. Cases (n = 276) were neonates from pregnancies complicated by spontaneous PTB. Preterm birth was considered the

delivery of an infant between 20 and 37 weeks of gestation (Martin et al., 2005). Two subgroups were distinguished; neonates born between 33 and 36 weeks were catalogued as moderate PTB (n = 169) and neonates of gestational age (GA) less than 33 weeks were included in a group of severe PTB (n = 117). Control subjects (n = 295) were neonates delivered at term (≥ 37 weeks of gestation) but not after 42 weeks of GA. The study protocol was approved by the School of Medicine Ethics Committee of the University of the Republic, Uruguay.

Sociodemographic characteristics, obstetric history, risk factors for PTB and data from the new born were collected from the perinatal informatics system. Perinatal informatics system registers all pregnancies in Uruguay since 1990 (Díaz A. et al., 1990). Confidentiality was assured by assigning numbers to the samples and deleting names and other information of patients in data collection forms. Informed consent was obtained from the mothers in all cases.

2.2 DNA extraction and Asp299Gly TLR4 genotyping

DNA was extracted either from umbilical cords or from newborn cheek swabs by conventional methods (Rey et al., 2008). To detect Asp299Gly polymorphism, we used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) strategy previously described by Lorenz et al. (2001) (Figure 1a) and a High Resolution-Melting Analysis (HRM) developed in our laboratory (Figure 1b).

2.3 Real time PCR and high-resolution -melting analysis

HRM analysis was performed on the Rotor-Gene 6000™ real-time instrument (Corbett Life Science) with Eva Green, a saturating dye technology (Type-it HRM PCR Kit, Qiagen). PCRs were performed in 10µl reaction volumes, containing 1X Qiagen HRM-typing buffer. The primers used were forward 5´ATTTGACCATTGAAGAATTCCG3´ and reverse 5'TGTTGCCATCCGAAATTATAAG3'. PCR reaction was carried out with an initial hold at 95ºC for 5 min, followed by 35 cycles of 95ºC for 15 s and 60ºC for 1 min, and then HRM ramps were generated by acquiring florescence data at a temperature ramp from 72 to 80ºC. Individuals with homozygous and heterozygous genotypes were included as controls in all experiments. HRM curves were normalized and genotype was assigned according to HRM curve shape by the Rotor-Gene software and visual inspection (Figure 1b).

2.4 Immunohistochemistry

A total of 6 chorioamniotic membranes were collected immediately after either preterm or term labor and processed for immunochemistry by standard methods. Briefly, all samples were formalin-fixed, paraffin-embedded, and 10 µm sections of the tissues were obtained. Blocking was performed by application of 5% goat blocking serum, followed by incubation with 2 µg/ml polyclonal goat anti-human TLR4 antibody (sc-8694, Santa Cruz Biotechnology, Santa Cruz, CA) with demonstrated specificity in placenta (Holmlund et al., 2002). Sections were incubated overnight at 4°C. A streptavidin-conjugated secondary antibody-mediated peroxidase development system (Elite Universal Vectastain ABC kit Vector Laboratories, Burlingame, CA) was used and antibody complexes visualized using DAB (BioFX Labs, Owing Mills, Maryland, USA). Tissues were counterstained with Mayer's hematoxylin and imaged by light microscopy (Nikon Eclipse E200). Images were imported into Adobe Photoshop Elements (Adobe, San Jose, California).

2.5 Statistics

Comparisons of allele frequencies were performed using the χ^2 test or Fisher's exact test. Means between groups were compared using Student's *t*-Test. The association between TLR4 genotypes and PROM related to GA was examined by logistic regression analysis. The presence of differential effects of TLR4 genotypes on the risk of PROM was explored by the inclusion of interaction or conditional terms. The analysis was performed with the Epi Info 2000 software (Center for Disease Control and Prevention, Atlanta, GA, USA). Probability values of 0.05 or less were considered significant.

3. Results

A total of 571 women were included in the study. Main characteristics of our study population are shown in the Table 1. No differences were detected in both groups regarding mother's age mean (25.8 ±0.9 mother's age who delivered at term vs. 25.0 ±0.9 at preterm), parity (26% mothers delivering at term were nuliparous vs. 29% of mothers who delivered preterm their first child) and poor obstetric history (8% of mothers delivering at term vs. 16% of mothers who delivered preterm). Poor obstetric history includes previous preterm birth, low birth weight, or recurrent miscarriage. No differences were found when the three causes of poor obstetric history were discriminated. In contrast with other studies, no statistical differences were found between mothers that delivered at term and preterm regarding their history of previous PTB (7% of mothers delivering at term had at least one previous PTB vs. 17% of mothers who delivered preterm). Teenage mothers tend to have more preterm children than older ones. Also, pregnant women that do not attend medical visits have an elevated frequency of preterm delivery. However, education level does not affect the incidence of PTB. Smoking strongly associates to PTB. Hypertension, pregnancy bleeding, premature rupture of membranes (PROM) and intra uterine growth restriction (IUGR) are conditions statistically significant associated to preterm birth. Anemia and previous mother pathologies are not (Table 1). Maternal infections during pregnancy do not associate to PTB. Infections included here were urinary tract infections, low genital infections and syphilis but not HIV that was excluded of the study. We did not find statistically significance neither when infections were considered together nor when they were analyzed individually (Table 1).

Genotyping was assigned by RFLP (Figure 1b) in most of the sample. HRM analysis was applied in 100 newborns with the procedure designed in our laboratory. In a blinded study, fifty random samples from our previous data assigned by RFLP were used and re genotyped by real time followed by HRM (Figure 1b). Consistency was one hundred percent, indicating an excellent accuracy. Hardy-Weinberg (H-W) disequilibrium exact test indicates that TLR4 Asp299Gly variation is in Hardy-Weinberg equilibrium ($p > 0.05$), even when case and control groups are discerned, or the data are stratified by medical conditions (data not shown). Only the wild type (wt) and the Asp299Gly allele were present in our sample. No individual carrying the homozygous variant was found. Association tests indicate none significant value with respect to preterm birth and the SNP, with OR's yielding non-significant values ($p > 0.05$) (Table 2). Logistic regression analysis (Table 3) included only most powerful variables that determine PTB in our model, based on data from Table 1. Under this consideration, TLR4 SNP is not significantly associated to PTB

(data not shown). However, a significant association is found when TLR4 Asp299Gly is conditionally by PROM between severe preterm newborns and babies born at term (table 4). Immunolocalization of TLR4 performed in fetal membranes demonstrated the presence of the protein in the epithelium of both, preterm and term amnios (Figure 2).

	Term (%) N= 271	Preterm (%) N=261	OR (CI 95%)	p-value
Maternal Age (<19)	10.6	19.3	2.01 (1.23 - 3.27)	0.003
Education Level *	47.0	48.1	0.96 (0.68 - 1.34)	0.432
Prenatal visits (< 5)	5.2	9.4	1.88 (0.90 - 3.92)	0.063
Smoking	46.1	63.9	2.07 (1.46 - 2.93)	0.0000039
Hypertension	2.9	10.5	3.89 (1.74 - 8.67)	0.0000021
Pregnacy bleeding	1.8	5.1	2.87 (1.02 - 8.09)	0.031
Infections	30.1	34.7	1.23 (0.86 - 1.77)	0.149
IUGR	3.0	6.8	2.41 (1.03 - 5.63)	0.029
PPROM	15.9	36.0	2.98 (1.98 - 4.51)	0.0000004
Anemia	2.6	3.0	1.73 (0.42 - 3.28)	0.482

* at least one year of high school
PROM, premature rupture of membranes
IUGR, intra uterine growth restriction

Table 1. Sociodemographic and medical characteristics of mothers and their newborns. Odds ratios and p-values between term and preterm births.

TLR4*	Term	Preterm
Asp299Gly/wt	10.6 (7.2 - 14.8)	11.2 (7.7 - 15.5)
wt/wt	89.4 (85.2 - 92.8)	88.8 (84.5 - 92.3)

Table 2. Asp299Gly TLR4 Genotype frequencies. * Fisher Test, p value=0.463

Trait	OR (CI 95%)	P-value
Hypertension	4.978 (2.068 - 11.985)	0.0003
Smoking	2.182 (1.512 - 3.149)	0.000002
PROM	3.185 (2.078 - 4.881)	0.0000003
TLR4	0.834 (0.445 - 1.561)	0.5694

Table 3. Logistic Regression model built using main socio-demographic and medical mother's characteristics. P-value: preterm vs. term.

Gestational Age (GA)	**Without PROM n = 415**	**With PROM n = 159**
> 37 weeks	0.0438	0.067
33-36 weeks	0.038	0.040
< 33 weeks	0.043	0.109*

Table 4. Minor Allele Frequency (MAF) of Asp299Gly according to GA. *Fisher test p value: 0.007 severe preterm vs. term.

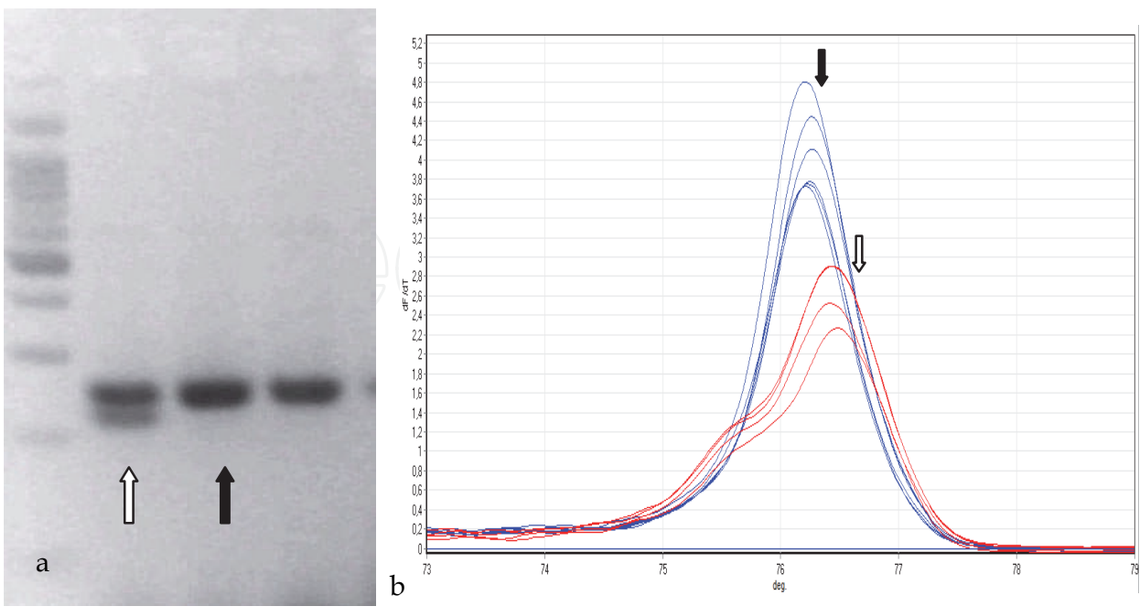

Fig. 1. a: Genotype patterns after PCR-RFLP. b: representative HRM melting curve. Black arrows: homozygous AA, White arrows: heterozygous GA.

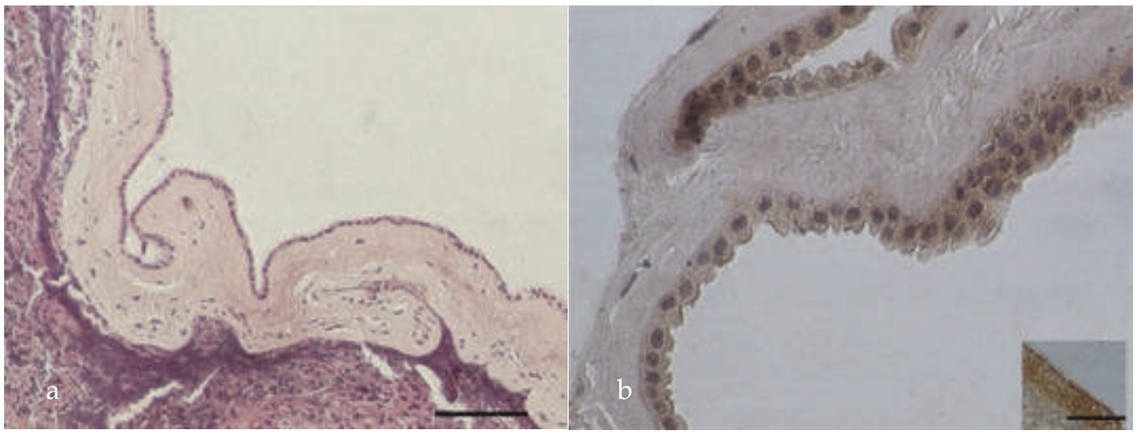

Fig. 2. Section of fetal membranes. a: 36 weeks gestation: HE (bar 100 μm).
b: Immunolocalization of TLR4 human protein at 36 weeks gestation.
Inset: term membranes (bar20μm).

4. Discussion

For the last eight years our group has been analyzing clinical, obstetric and environmental characteristics in association to genetic background of mothers and their offspring who have delivered at a public hospital in Montevideo, Uruguay. A case control study was conducted with the intention of advancing a research agenda to understand and diminish the population health burden embodied by PTB.

In view of the fact that PTB is considered a complex condition, it was not surprising the finding of strong association of the syndrome to several binomial mother-fetus socio-economical and clinical factors. Linkage of PTB to hypertension, PROM, bleedings, IUGR, and smoking suggested the possibility of vasoconstriction and/or an altered liberation of cytokines as a common basis of the process that leads to preterm labor. In addition, recent studies demonstrated associations between elevated levels of circulating proinflammatory cytokines, particularly interleukin (IL) 6, IL-1beta, and tumor necrosis factor alpha (TNF-alpha) and preterm birth (Lyon et al., 2010). These inflammatory cytokines might link the pathology of uterine contraction, uterine cervical ripening, and preterm premature rupture of membranes (Noguchi et al., 2010). Stimulation of TLRs with their ligands has been shown to induce proinflammatory cytokine release in uterine epithelial cells, fetal membranes and placenta (Schaefer et al., 2004; Noguchi et al., 2010). Moreover, the presence of TLR4 detected by immunohistochemistry in the amnios of women at labor suggested the possibility that stimulation of TLRs with their ligands induce proinflammatory cytokine release, conducting to labor (Schaefer et al., 2004; Noguchi et al., 2010). We were able to localize TLR4 in term and preterm membranes. It is probable that TLR4 pathway is playing a role in normal delivery but at the same time, some environmental conditions should also be present in order to produce PTB. Namely, when some environmental factors are present, interaction between unfavorable external conditions (e.g., infections) and TLR4 could be the ultimate cause of PTB.

Since TLRs play a key role in those pathways and the Asp299Gly variation has been associated to a blunted response toward pathogens (Arbour et al., 2000; Ferwerda et al., 2008), an association of the SNP and PTB was expected. The biological relevance of this

TLR4 SNPs has been widely investigated; individuals carrying the variant alleles are at increased risk of Gram-negative infections (Arbour et al., 2000; Agnese et al., 2002; Lorenz et al., 2002b). An association between the presence of TLR4 Asp299Gly polymorphisms and the occurrence of serious infections in HIV-1 infected patients was also reported (Papadopoulos et al., 2010). The same variant protects individuals from atherosclerosis (Kiechl et al., 2002; Ameziane et al., 2003). As we mentioned earlier, regarding preterm birth, results vary according to different studies. Lorenz et al. (2002a) in a Finish population found that fetuses carrying the Asp299Gly variation had an increased risk for preterm birth. On the other hand, there is no association between the rare variant and the risk for PPROM in African Americans (Ferrand et al., 2002). However, in our study PTB was not linked directly to the presence of TLR4 variation. We did find an association between severe PTB (less than 34 weeks of gestation), PROM and the presence of the Asp299Gly variation (see also previous work; Rey et al., 2008), suggesting possible differences between the pathogenesis of severe and moderate preterm birth. Is it possible that genetic component plays a key role in severe preterm but not in moderate preterm? Is PTB in accordance with other complex diseases where younger affected individuals show a greater genetic component? While prematurity is a continuum, with the whole population of births before 37 weeks' gestation experiencing some increased risk compared with term birth, it is the extreme of the distribution, very preterm birth, that accounts for a third of all infant deaths, 67% of neonatal mortality (Callaghan et al., 2006), and a significant prevalence of acquired developmental disabilities and severe cognitive disability in USA (Kramer & Hogue, 2009). Several issues should be considered in order to explain the differences found between studies that associates genetics and PTB.

4.1 Ethnic disparities

As far as our knowledge concerns, there is not enough evidence in South American populations that could conclusively indicate that there is Hispanic disparity in PTB. Although the utility of considering genetics at all in understanding ethnic disparities in health is being vigorously debated (Ioannidis et al., 2004; Kaufman & Cooper, 2008), the goal of gene-environment studies in specific populations is to advance in the knowledge of how genetic and environmental factors combine to affect the risk of the disease (Kelada et al., 2003) for a specific group of people. The studies of the impact of different polymorphism on inflammatory diseases in South American population have only recently started and sufficient data have yet to be collected and reported (Santos et al., 2006; Garza-Gonzalez et al., 2007; Jorge et al., 2010; Pontillo et al., 2010). Regarding PTB Romero et al. (2010) conducted a large, candidate gene association study of women and their offspring who experienced birth after preterm labor with intact membranes, in a Hispanic population from Chile. They identified sequence variants in genes involved in inflammation and extracellular matrix biology that were associated with preterm birth. TLR4 SNPs were included in their study but no differences were reported between cases and controls. Moura et al. (2009) in a study that included more than 400 Brazilian women did not find any association between spontaneous PTB and any of six gene variations of cytokines included in their studies. They did find a significant association between PTB and a combination of variants by a multi-locus analysis. TLR SNPs were not included (Moura et al., 2009).

Uruguayan population has been described fundamentally as of European origin. However, more recently, genetic admixture analysis demonstrated a Native American and African

contribution to the Uruguayan population of 10.4% and 5.6%, respectively (Hidalgo et al., 2005; Sans et al., 2006). The Uruguayan Asp299Gly observed frequency is lower than that of Europeans or Africans and equal to the admixed Chilean population (Rey et al., 2008), which suggests the effect of admixture on this gene. We published the first report concerning association between TLR4 and PTB or PPROM on a South American country and the data presented here confirm this link. The body of literature that mentions genetics as an explanation for the racial disparity in preterm birth is large, but notable for rarely measuring genes; most studies suggest that residual racial variation in preterm birth risk is genetic after statistical control for measured socioeconomic and behavioral risk factors (Kramer & Hogue, 2009). Our data combine severe PTB, PROM and the association of the SNPs which is not considered in those previous studies. Specifically, severe PTB birth could be linked stronger to genetic factors than moderate PTB. Interestingly, while severe preterm birth is a poor outcome for any pregnancy, African-American women experience nearly 3 times the risk of severe PTB birth when compared with non-Hispanic white women (Kramer & Hogue, 2009), meaning that probably genetic component is stronger when birth is produced before 33 weeks of gestational age.

4.2 Interactions with environmental factors

Again, it is possible that the aforementioned SNP could influence the PTB only when some environmental conditions are present. Indeed, some of these genetic variations require the presence of certain environmental stimuli to have any clinical significance. To date, however, only relatively few studies on the association of gene-environment interactions with preterm birth have been published (e.g. Wang et al., 2002; Genc et al., 2004; Macones et al., 2004; Nukui et al., 2004; Genc & Schantz-Dunn, 2007; Gracie et al., 2010; Menon et al., 2010; Genc & Onderdonk, 2011). Macones et al. (2004) described a gene–environment interaction between the tumor necrosing factor (TNF) alpha polymorphism and bacterial vaginosis. Women found to have both the TNF alpha polymorphism and bacterial vaginosis were at synergistically increased risk of PTB when compared with women with only the TNF alpha polymorphism or bacterial vaginosis alone (Macones et al., 2004). In our study neither environmental nor clinical conditions were found to have association to TLR4 Asp299Gly variation when preterm labor is considered as a whole. Interestingly, infection was not always present at detectable levels. Probably this is not surprising. The characteristics of intrauterine inflammation are histologic chorioamnionitis and elevated amniotic fluid concentrations of cytokines or metalloproteases. The major cause of PTB prior to 32 weeks of gestation is intrauterine infection and/or inflammation (Romero et al., 2007; Goldenberg et al., 2008; Keelan, 2011) but only a minor proportion of such pregnancies exhibit clinical signs of chorioamnionitis (Romero et al., 2007; Keelan, 2011). Intrauterine infection most commonly arises from the ascending route (associated with abnormal vaginal flora), with colonization first of the decidual membrane, followed by chorion, amnion, amniotic fluid and fetus. Chorioamnionitis can be present at all stages of the colonization process (Grigsby et al., 2010). Intrauterine infection arising from a haematogenous route is an alternative cause of PTB, although considered less common (Keelan, 2011). Various findings suggest that in some pregnancies, the infective organism may be present in placental tissues from mid-gestation. In fact, it has been suggested that intrauterine infections may originate pre-conceptually, possibly arising from organisms residing in the endometrium prior to implantation which subsequently activate the maternal innate

immune system as a result of a change in growth or immune surveillance (Oh et al., 2010). It is possible that an altered cytokine expression can cause *per se* preterm labor, namely an overproduction of cytokines can be harmful to the fetus because of the activation of the cytokine flow (Vrachnis et al., 2010). Since inflammation-driven preterm labor can happen in the absence of overt amniotic infection, it is assumed that this cascade of events can also occur as a consequence of exposure to blood organisms or endotoxins, or possibly via exposure to non-microbial triggers of the innate immune system (Romero et al., 2007; Keelan, 2011).

4.3 Genetic background, interaction between SNPs

Inflammatory signaling is highly complex and subject to modulation by numerous factors responding to multiple external and internal signals (Keelan, 2011). TLR signaling employs the NF-κB signal transduction pathway, a key regulator of inflammatory gene expression (Medvedev et al., 2000; Lu et al., 2008; Vallabhapurapu & Karin, 2009). Key steps in the TLR4 signal pathway include recruitment of adapter proteins (MyD88, IRAK1/4 and TRAF6), activation of intermediate kinases (RIP1, TAB2/3, TAK1 and IKKα/β) and phosphorylation/degradation of the chaperone protein IκB (Martinon & Tschopp, 2007). In theory, variations in any of the genes that codify for the proteins involved may affect inflammatory pathway and labor. That means that few candidate gene studies could be an endless trade with the danger of leading to an imperfect understanding of the biological pathways that end in PTB. It was also shown that the associations found are difficult to replicate (Todd, 2006; Manolio et al., 2009). Genome-wide association studies (GWAS), in which several hundred thousand to more than a million SNPs are assayed in thousands of individuals, represent a powerful new tool for investigating the genetic architecture of complex diseases (Hardy & Singleton, 2009; Manolio, 2010). The GWAS represent an important advance compared to 'candidate gene' studies, in which sample sizes are generally smaller and the variants assayed are limited to a selected few. However, most common variants individually or in combination confer relatively small increments in risk (1.1 to 1.5-fold) and explain only a small proportion of heritability (Hindorff et al., 2009). According to Dolan et al. (2010) genetic associations with preterm birth reveals a paucity of research in the area, compared with more mature genetic association fields like schizophrenia and Alzheimer's disease (Allen et al., 2008; Sun et al., 2008; Dolan et al., 2010). So far, no robustly replicated genetic variants contributing to this complex disease have been identified (Dolan et al., 2010).

GWAS research focusing on known pathways of PTB like inflammatory pathways, involved in host defense mechanisms, innate immunity activation and infection, could narrow the list of SNPs to study and produce more reliable data. Web-based reference databases that link the genome to biologic systems (e.g. Kyoto Encyclopedia of Genes and Genomes, www.genome.jp/kegg/pathway.html) are excellent tools to manage this information. The data collected so far in our group could be the first step to conduct a GWAS that associates wide amount genetic variations to a well studied population of preterm and term newborns. Uruguay has 3.5 million people (July 2010) and shares population characteristics with both developed countries and Latin American underdeveloped regions. Example of the foremost is the low birth rate (13.91 births/1,000 population; https://www.cia.gov/library/publications/the-world-factbook/). In Uruguay, nearly 10% of total newborns are preterm. The demographic characteristic of Uruguay, namely the

comparatively low population and the lack of natural hazards, will make it easy to increase the number of cases and analyze not only the newborns but also their family in order to link genetic studies to social, environmental or pathological conditions where the newborns are raised. Finally genetic profiles will help to develop treatments according different susceptibilities. Because preterm labor is a final common pathway for multiple etiologies, it is reasonable to think that it will not be a single therapy that will work optimally for all binomial mother-fetus. As patient-specific pharmacogenomic profiles are developed, it will be possible to create patient-specific treatment regimens. It may become possible to identify those women and their fetuses who are predisposed to preterm labor and to institute patient-specific preventive measures (Esplin & Varner, 2005).

5. Conclusions

Understanding causes of PTB is especially important in underdeveloped countries. Complications of PTB are associated with increase of medical expenses, representing billions of dollars of direct costs and unrealized potential each year, sometimes unaffordable for poor countries. On another hand individual and ethnic differences exist both in the prevalence of infection related preterm birth and in the extent of immune responses to infection. Diversity of immune responses may influence the levels of susceptibility in women to preterm labor. Given individual genetic variations and differential environmental exposures, stratification of study subjects by genotype may allow the detection of risk of preterm birth among individuals exposed to different environmental effects. Furthermore, enhanced understanding of pathologic mechanisms may allow the development of drugs or interventions that can be used to prevent or treat preterm birth. On the basis of our current understanding of the role of intrauterine inflammation in the etiology of preterm birth, therapeutics should be directed to the well known pathways that conduct to inflammation.
Finally, preterm birth research will likely benefit greatly from GWAS findings and PTB Gene will incorporate data from such studies. Large scale multi-center collaborations using common standards and high throughput platforms that accounts for ethnic and environmental local conditions are needed to improve newborn health. They will direct progress in deciphering the genetic factors underlying preterm birth and consecutively help to bring basic research discoveries to the bedside.

6. Acknowledgments

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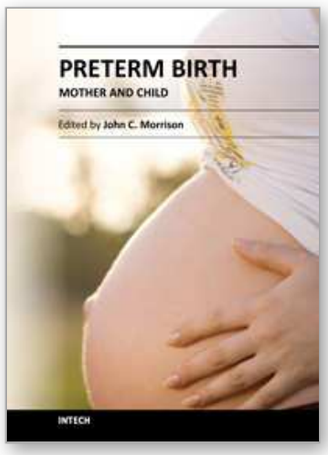

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While there are many studies and books regarding preterm birth, both the obstetric and in the neonatal/pediatric literature, what is missing is the integration of data from obstetrics through neonatal course and into pediatrics as the neonate transverses childhood. A continued dialogue between specialties is essential in the battle against preterm birth in an attempt to relieve the effects or after-effects of preterm birth. For all of our medical advances to date, preterm birth is still all too common, and its ramifications are significant for hospitals, families and society in general.

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