

Modern Approach of Assessment of the Risk of Preterm Birth

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Abstract Premature birth (PR) continues to be at the epicenter of attention of obstetricians and gynecologists around the world. Case-control association study. A total of 200 pregnant women were included in the study, including 150 preterm births and 50 control women (labor ≥ 37 weeks). For the study, methods of genotyping using TaqMan probes and traditional sequencing were carried out. Descriptive statistics were performed using Fisher's exact test and Wilcoxon's rank sum test.

Keywords Premature birth, Risk factors, Factor polymorphism, Interleukin1-beta, Hemodynamic parameters

1. Introduction

Premature birth, defined as delivery before 37 weeks, complicates many pregnancies and has been closely associated for decades with an increased risk of neonatal morbidity and mortality [1,2,5]. Long-term complications of preterm birth include cerebral palsy, respiratory illness, blindness, and deafness. In many countries, the incidence has increased in recent years [3,4,6]. There are four major pathogenic pathways of preterm labor, each of which leads to uterine contraction and cervical changes: 1) activation of the hypothalamic-pituitary-adrenal axis, 2) decidual hemorrhage, 3) abnormal uterine distension, and 4) inflammation. The latter pathway suggests that an infection involving the maternal / fetal organism, such as chorioamnionitis, may lead to preterm labor caused by a local endotoxin and / or inflammatory cytokine response [7,8,9]. The primary inflammatory response, consisting of the proinflammatory cytokines TNF- α and IL-1 β , upon initiation will promote the production of IL-6 by the chorion and decidua, resulting in the production of proteases and / or prostanoid-secreting cells that may rupture the membranes or provoke contraction uterus and, thus, cause premature labor [10,11,12,13,17]. All genetic data were tested for Hardy - Weinberg equilibrium and analyzed using logistic regression, proportions of 2×2 or χ^2 . Haplotypes were evaluated for each gene and permutation used to test the association. Results. Women carrying the rare allele (T) TNFA -857 C> T and women homozygous for the rare alleles IL1B -31 T> C and IL1B -511 C> T (C and T) have an increased risk of preterm birth with OR 3.1 (95% CI: 1.0–10.3) and OR 6.4 (95% CI: 1.3–60.5), respectively. Two putative TNFA haplotypes

were associated with preterm labor with OR 3.1 ($p = 0.037$) and OR 2.7 ($p = 0.045$). Conclusion. Polymorphism in the cytokine genes TNFA and IL1B may increase the risk of preterm birth, possibly due to dysregulated immune system regulation during pregnancy. [14,15]

Several observations support a genetic influence on the risk of preterm birth. Prior preterm birth is the leading risk factor for preterm birth, and preterm mothers themselves also have an increased risk of preterm birth. In addition, there is a relationship between ethnicity / race and PTB that persists even when socioeconomic factors are taken into account. [16,18,19]

TNF- α , IL-1 β are known to play a key role in the regulation of events related to inflammation and immunity, and SNPs in their genes have been found to be associated with a wide range of diseases, including ulcerative colitis, Crohn's disease, diabetes mellitus and disseminated sclerosis [6]. The relationship between polymorphism of these genes and preterm birth has also been investigated, but the results are conflicting and require further study. [14,18]

We hypothesized that the polymorphism in the regulatory region of the genes encoding the cytokines TNF- α , IL-1 β may be associated with preterm labor.

Purpose. Examine the relationship between 19 selected single nucleotide polymorphisms of three cytokine genes, tumor necrosis factor alpha (TNFA), interleukin 1-beta (IL1B), and preterm labor (<37 weeks gestation).

2. Research Objective

Assessment of the risk of preterm birth by assessing the polymorphism of tumor necrosis factor alpha and interleukin 1-beta.

3. Materials and Methods

We recruited 150 cases (at birth <37 weeks gestation) and 50 controls. Inclusion criteria: singleton pregnancy, no neonatal malformations, and a healthy baby after delivery. All cases were spontaneous premature birth. Gestational age was determined using ultrasound dating based on the length of the crown of the fetus. Genomic DNA was isolated from three stamped dried blood spots with a diameter of 3.2 mm using the QIAamp DNA Blood Mini (QIAGEN Nordic, Denmark) and the manufacturer's instructions.

Before genotyping, four pre-PCR reactions were performed to enhance the regions of interest. Primer sequences and PCR conditions are available upon request. Two genotyping methods were used according to manufacturer's protocols: Taqman® SNP Genotyping Assay and Big Dye Termination Mixture v. 1.1 (both Applied Biosystems, USA). Previous genotyping, unincorporated nucleotides and primers were removed using ExoSAP - IT (GE Healthcare Life Sciences, USA). Taqman® SNP genotyping test numbers, primers, and sequencing protocols are available upon request. If in doubt about the TaqMan® genotype, the assay was repeated and the sample was sequenced to ensure accurate genotype determination.

Categorical results were compared using two-sided Fisher's exact tests, normally distributed variables using t-tests, and a nonparametric test, the Wilcoxon rank sum test, was performed for variables with abnormally distributed ones. The deviation from the Hardy - Weinberg equilibrium was assessed separately for each polymorphism in cases and controls. A control sample with polymorphism deviating from the Hardy-Weinberg equilibrium (p-value <0.05) could indicate genotyping or sampling problems, so the polymorphism was excluded from further analysis. A measure of linkage disequilibrium, R^2 , was calculated for each pair of polymorphisms in each gene (data not shown). As part of associative testing, we used three genetic models: additive, dominant, and recessive. The additive model examined whether a dose-response allele was present, for example, if a woman carrying two polymorphic alleles was twice as likely to have a preterm birth as a woman carrying only one polymorphic allele. The dominant model suggests that if A is a common allele and B is a polymorphic allele carrying at least one polymorphic allele, women with genotype AB or BB have an increased / decreased risk of preterm birth. The recessive model suggests that both alleles must be polymorphic to have an increased / decreased effect on preterm labor. To study the relationship between single nucleotide polymorphism and preterm birth, the additive model was evaluated by plotting plots showing the preterm birth logit by genotype. If the additive model did not fit, the dominant or recessive model was chosen, whichever best matched the logit graph. Association tests were performed using logistic regression for additive models and simple proportions in 2×2 tables for other models. If none of the models are applicable, polymorphism will be tested using χ^2 for a 3×2 table.

The association of haplotypes for each gene was checked by first limiting the calculated contingency tables to haplotypes with the calculated frequency, both in cases and in the control, not less than 2%, and calculating the χ^2 X obs. The distribution of zero X obs was approximated by a permutation approach: at each step, the case-control status was rearranged, the haplotypes were reevaluated separately for cases and controls under the new conditions, and the χ^2 statistics X curl was calculated for the same haplotypes as in the observed and limited contingency table. After doing this a large number of times (10,000-50,000), the p-Value of the permutation test was $p(X \text{ perm} > X \text{ obs})$.

Multiple testing problems were addressed by calculating p-values for general association hypotheses using permutation tests using individual p-values as test statistics.

The significance level was set at 0.05. The measure of association was the odds ratio (OR), reported with a 95% confidence interval (95% CI) when possible. Since the permutation test is a test, it does not give us confidence intervals, but only rejects the null hypothesis or not. Stata 9.1 (Stata Corp., USA) was used for all statistical analysis. The haplotype was assessed using the Stata hapipf package, the Hardy-Weinberg equilibrium test was performed using the genhwi, and linkage disequilibrium was analyzed using pwld.

4. Results and Discussion

Table 1. Demographic and clinical characteristics of the subjects (n = 200)

Variable	Birth control ≥ 37 weeks (n = 50)	Births <37 weeks (n = 150)	p Value
Mother's age (years) *	31,4 (27,3–35,0)	30,0 (26,4–33,5)	0,100
Mother's weight (kg) #	64,3 (9,8)	66,4 (12,9)	0,332
BMI (kg / m2) *	22,6 (20,4–24,8)	22,6 (20,4–25,5)	0,661
Weeks of pregnancy *	40,5 (37,4–42,6)	33,5 (28,7–36,9)	0,0001
Bleeding (%)	12 (22)	19 (31)	0,302
Smokers (%)	11 (20)	11 (18)	0,816
Alcohol (%)	1 (1,8)	0 (0)	0,470
Infertility treatment (%)	4 (7)	5 (9)	0,517
Previous pregnancies (%)	33 (60)	26 (47)	0,206
Successful pregnancies (%)	23 (42)	18 (33)	0,175
Previous abortions (%)	15 (27)	18 (33)	0,832

* Data are presented as median (range).

Data are shown as mean (SD).wg: weeks of pregnancy, bleeding: experience of bleeding during pregnancy, smokers: daily smoking, alcohol: daily alcohol consumption. Fertility treatments: For example, artificial insemination by a husband, ovulation medications, in vitro fertilization, or artificial insemination by a donor. Previous pregnancies: Previously registered pregnancies. Successful pregnancies: Previous pregnancies with a healthy fetus at 20 weeks.

No demographic or clinical differences were observed other than gestational age when comparing cases and controls (Table 1). Only 13 of the 19 genotyped single nucleotide polymorphisms were found to be present in this population, and they are all in the Hardy-Weinberg equilibrium. The calculated allele and genotype frequencies and equilibrium p-values of Hardy - Weinberg for 19 polymorphisms, as well as equilibrium p-values of Hardy - Weinberg are shown in Table 2. IL1B -31T> C and -511C> T were in ideal linkage disequilibrium ($R^2 = 1$) and for this reason we only refer to IL1B -31T> C in the following text and analysis.

Table 2. The relationship between single nucleotide polymorphisms and preterm labor

SNP	Model *	OR	95% CI	Exact p value ‡ Gene p †
TNF - 1032T> C	Add.	1.6	(0,76–3,34)	0,215
TNF - 863C> A	Add.	1,62	(0,74–3,57)	
TNF - 857C> T	House.	3,09	(1.04–10.33)	
TNF - 307G> A	House.	1,21	(0,53–2,8)	0,699
TNF - 237G> A	House.	1,52	(0,28–10,24)	0,721
IL1B + 3953C> T	3 × 2	NA	NA	0,446
IL1B - 31T> C	Rec.	6,36	(1,3–60,5)	0,010
IL6 - 599G>	Add.	0,87	(0,51–1,47)	0,593
IL6 - 572G> C	House.	0,73	(0,19–2,76)	0,770
IL6 - 174g> C	3 × 2	NA	NA	0,882

* Model: additive model, i.e. dose-effect of allele B (add.), Dominant model, i.e. AA versus AB + BB (home), Recessive model i.e. AA + AB versus BB (rec.).

‡ Exact p: Fisher's exact p value calculated for each individual SNP.

† Gene p: p-value for hypotheses of SNP association in a gene with PTB, i.e. p-value adjusted for multiple comparisons at the gene level.

Two genotypes were significantly associated with preterm birth. Carriers of the rare allele TNFA -857C> T (genotypes C / T and T / T) had an OR of 3.1 (95% CI: 1.04-10.33), and carriers homozygous for the rare allele IL1B -31T> C (genotype C / C) had an OR of 6.4 (95% CI: 1.30-60.50) for preterm birth. The results of model selection and association can be seen in Table 2. When adjusting for multiple comparisons in each gene, only IL1B remained statistically significant with a p-value of 0.029. The p-value for the general hypothesis of the relationship between the studied polymorphisms and preterm birth was 0.059.

5. Conclusions

The present study shows that maternal polymorphisms IL1B -31T> C and IL1B -511C> T, which are in ideal linkage disequilibrium in our population, and TNFA -857C> T, are associated with preterm birth. It also suggests that having one of two TNFA haplotypes: -1032T / -863C / -857T / -307G / -237G or -1032C / -863A / -857C / -307G / -237G (rare allele underlined) increases the risk of preterm birth... Previous studies have shown that preterm labor is associated with TNFA -307G> A and IL6 -174G> C, while other studies currently included have not confirmed this

association. In this study, we found that IL1B + 3953C> T was not associated with preterm labor, which is consistent with previous studies.

Several cytokine gene polymorphisms have been investigated as potential markers of preterm labor. The most commonly studied maternal single nucleotide polymorphisms include IL1B -511C> T, IL1B + 3953C> T, IL6 -174G> C, and TNFA -307G> A. Some studies suggest a link between polymorphism and preterm labor, while others do not. The discrepancies are likely due to different study designs.

IL1B -31T> C and -511C> T have been studied previously, but no association with the outcome of preterm labor has been previously reported. Interestingly, if an IL1B haplotype containing both the rare alleles -511T and -31C is transfected into monocytes of the human THP-1 cell line, it exhibits an increased rate of transcription when stimulated with bacterial lipopolysaccharide compared to the common haplotype. IL1B -31T> C is located in the IL1B TATA box sequence, and the rare -31C allele has been shown to have a reduced affinity for several transcription factors (TATA Binding Protein, NF-IL6, and Spi-1 / PU.1). Despite the decreased activity of the promoter, therefore, one would expect in carriers of the rare allele -31C, in vivo studies demonstrate increased expression of IL1B. Perfect linkage disequilibrium between -31T> C and -511C> T was observed in this study and was reported. No allele-specific differences in protein binding in the -511C> T region have yet been reported, and the functional implications, if any, of this polymorphism are still unknown.

Of the 13 TNFA gene polymorphisms studied, only TNFA -857C> T was independently associated with preterm labor. To the best of our knowledge, this is the first time that -857C> T has been studied for preterm labor, although it has been associated with a number of medical conditions. Many observations indicate that the rare -857T allele can enhance gene transcription, but the biological effect of the TNFA -857C> T polymorphism is not fully understood. It has been shown that peripheral blood mononuclear cells carrying the rare -857T allele have increased TNF production when stimulated with concanavalin A, a plant protein that binds to most mammalian cells and elicits various immunological responses. The same study also showed that transiently transfected Raji cells, a human B cell line carrying the rare -857T allele, have an increased transcription rate compared to cells transfected with the common -857C allele. A recent study showed that the rare allele -857T increases transcription in response to infection by stimulating murine macrophages RAW264.7 with a bacterial endotoxin, lipopolysaccharide, whereas stimulation of human HeLa cells derived from a patient with cervical cancer was not consistent. In contrast to these studies, whole blood samples from homozygous carriers of the common -857C allele have been shown to have elevated TNF levels after stimulation with lipopolysaccharide compared to blood samples from carriers of the rare -857T allele.

In this study, two TNFA haplotypes were associated with

preterm birth. The risk of the first haplotype -1032T/-863C / -857T / -307G / -237G is due to the rare TNFA allele -857T. Interestingly, the second haplotype, -1032C / -863A / -857C / -307G / -237G, containing the rare TNFA alleles -1032C and -863A, was also associated. The rare TNFA allele -863A has been reported to be associated with preterm labor in one study, and this polymorphism is also thought to have a modulating function on gene expression. While the current study could have more room for comparison, the findings support previous findings.

The functional effects of the TNFA -863C> A and TNFA -857C> T polymorphisms are likely mediated by the Nuclear Factor kappa B (NFkB) transcription factor, which exists in two forms: an active heterodimer p65-p50 and an inhibitory p50-p50. homodimer. While the common allele -863C binds both the active heterodimer p65-p50 and the inhibitory homodimer p50-p50, the rare allele -863A is able to bind only the active heterodimer p65-p50. The rare allele -857T is thought to inhibit the NFkB heterodimer p65-p50 by binding to octamer-binding transcription factor 1.

Interestingly, the polymorphisms associated with preterm labor in this study were involved in the regulation of gene transcription, but from the available data it cannot be concluded that they play any functional role in the pathogenesis of preterm labor. Gene regulation is often cell-specific, and distant polymorphisms can interact with the studied polymorphism and modulate its effect. However, it is reasonable to assume that these polymorphisms can cause dysregulation of cytokine production in response to infections. Premature birth is considered a multifactorial condition in which inflammation appears to play a key role, and it is very interesting to speculate that pregnant patients carrying these genetic variations may have increased cytokine transcription in response to infections. which in turn can provoke premature termination of pregnancy. Unfortunately, the infectious status of the pregnant patients in this study was unknown. One of the overlooked aspects in this study is the contribution of fetal genotype to preterm labor. In a recent study, maternal carriage of null allelic variants of T1 glutathione S-transferase was shown to be associated with preterm labor. The study also found that if the mother smoked, with only one carrier, mother or fetus, the null allele of T1 glutathione S-transferase was required to increase the risk, suggesting that genotypes of the mother and fetus interact with the environment and induce premature birth. One of the overlooked aspects in this study is the contribution of fetal genotype to preterm labor. In a recent study, maternal carriage of null allelic variants of T1 glutathione S-transferase was shown to be associated with preterm labor. The study also found that if the mother smoked, with only one carrier, mother or fetus, the null allele of T1 glutathione S-transferase was required to increase the risk, suggesting that genotypes of the mother and fetus interact with the environment and induce premature birth. One of the overlooked aspects in this study is the contribution of fetal genotype to preterm labor. In a recent study, it was shown that maternal carriage of null allelic

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In conclusion, our results show that single nucleotide polymorphisms in the TNFA and IL1B promoter region, which can modulate gene transcription, increase the risk of preterm birth in Caucasian Danish patients.

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