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Original research article

Lack of association between the −1082 (A/G) IL-10 polymorphism (rs1800896) and spontaneous preterm birth in the Indonesian Acehnese population

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ABSTRACT

Introduction: Preterm birth is associated with multiple mechanisms, and a contractile state during preterm birth is typically accompanied by a shift in signaling from anti-inflammatory to pro-inflammatory pathways. It has been hypothesized that a mutation within the promoter of the interleukin-10 gene (*IL-10*) that causes hypo- or hyper-production of IL-10 might be associated with spontaneous preterm birth.

Aim: To determine the association between the −1082 (G/A) single nucleotide polymorphism (SNP) of *IL-10* and spontaneous preterm birth in Aceh, Indonesia.

Material and methods: A case-control study was conducted between June 2012 and July 2014 at Dr. Zainoel Abidin Hospital, Banda Aceh. A total of 40 preterm and 40 term births were included in the final analysis. Genotyping of the −1082 (G/A) *IL-10* SNP was conducted using real-time polymerase chain reaction (RT-PCR) and was confirmed by sequencing. An enzyme-linked immunosorbent assay (ELISA) was performed to measure the level of IL-10 in sera. The associations of the genotype distribution and allele frequency with IL-10 levels and preterm birth were assessed using the χ^2 test.

Results and discussion: There was no association between the genotype distribution or allele frequency and the level of IL-10 in serum. There was also no association between the genotype distribution or allele frequency and spontaneous preterm birth.

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Conclusions: There is no strong association between the −1082 (A/G) *IL-10* SNP and spontaneous preterm birth in the Acehnese ethnic group.

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1. Introduction

Preterm birth (birth before 37 weeks of pregnancy) is a major cause of neonatal death; it accounts for 35% of the 3.1 million neonatal deaths that occur each year worldwide and is also a significant cause of the long-term loss of human potential among survivors.[1] Preterm birth is associated with a substantial economic burden and causes emotional distress to parents.[2] In 2012, there were 15 million babies born preterm globally,[3] with a prevalence ranging from 5% in high-income countries to 25% in low-income countries.[4,5] However, the cause of spontaneous preterm labor remains unidentified in up to half of all cases.[6] Because many of the risk factors for preterm birth result in increased systemic inflammation, the increasing stimulation of infection or inflammation pathways might explain some of the increases in preterm births.[5]

Infection is a frequent and important mechanism leading to preterm birth,[7–10] during which the release of inflammatory chemokines and cytokines stimulates the production of prostaglandins, other inflammatory mediators, and matrix-degrading enzymes.[5,11,12] Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays a pivotal role in the inflammatory process.[13] There are three biallelic single nucleotide polymorphisms (SNPs) in the promoter of the IL-10 gene (*IL-10*), located −1082 (G/A), −819 (C/T) and −592 (C/A) from the transcriptional start site, and these polymorphisms are associated with changes in IL-10 production levels.[13,14] Previous work revealed that the concentration of IL-10 is increased in preterm newborns.[15] Furthermore, a combination of IL-10 and antibiotic therapy was shown to increase the interval to delivery in a rat model of infection-mediated preterm birth,[16] and IL-10 levels can predict preterm birth.[17] Therefore, it is has been hypothesized that a mutation within the *IL-10* promoter that causes hypo- or hyper-production of IL-10 might be associated with spontaneous preterm birth.

2. Aim

This study was conducted to determine the association of the −1082 (G/A) SNP of *IL-10* on spontaneous preterm birth in Aceh, Indonesia.

3. Material and methods

3.1. Ethical approval

The study protocol was approved by the Institutional Review Board of the School of Medicine, Syiah Kuala University, Banda Aceh, Indonesia (No. 111/KE/FK/2012). The aims, risks, and benefits of the study were explained to each participant, and they were asked to sign a consent form prior to enrollment in the study. All activities were carried out in accordance with the Code of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

3.2. Study setting and patients

During a two-year period (June 2012 to July 2014), a case–control study was conducted at Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia to assess the role of SNPs of pro- and anti-inflammatory genes in spontaneous preterm birth in the Acehnese ethnicity in Aceh province. Aceh is the westernmost province of Indonesia and was the most severely affected by the earthquake and tsunami of 26 December 2014. Details of the study setting have been described previously.[18] The study groups were women with preterm birth (birth between 20 and 37 weeks of gestation) and age-matched controls (birth after 37 weeks of gestation). Any patients meeting at least one of the following criteria were excluded from the study: age over 50 years with preeclampsia, signs and symptoms of infection, autoimmune disease, obesity, pregnancy with intrauterine growth restriction, twin pregnancy, placenta previa, fetal anomalies, gestational diabetes, or poly- or oligohydramnios. During the first visit, the patients' general condition, clinical signs and symptoms and obstetric and gynecologic status were assessed. Phlebotomy was conducted under hospital standard operating procedures to collect a 5 mL venous blood sample. The blood samples were processed as described previously[19] and were stored at −80°C until they were used.

3.3. Immunologic assay of IL-10

The level of IL-10 in the sera was determined using human IL-10 immunoassay, Quantikine kit (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique and a monoclonal antibody specific for IL-10 has been pre-coated onto a microplate. For each patient, 200 μL of sera were test and the absorbance was measured at 450 nm using microplate reader. The minimum detectable dose of IL-10 was less than 3.9 pg/mL.

3.4. DNA extraction and genotyping

DNA extraction was conducted from 200 μL of whole blood based on the spin column method using High Pure PCR Template Preparation Kit according to the manufacturer's protocol (Roche Diagnostics, Mannheim, Germany). Genotyping was conducted via real-time polymerase chain reaction (RT-PCR) with specific fluorescence-labeled hybridization probes using a LightCycler 2.0 (Roche Diagnostics,

Mannheim, Germany), employing primers and probes from a previous study.[20] Forward primer: 5′-CAACTGGCTCCCCT-TACCTT-3′, reverse primer: 5′-AAGCTTCTG TGGCTGGAGTC-3′, sensor probe: 5′-AAGCTTCTTTGGGAGGGGGAAGTAGG-GAT-3′FAM and anchor probe: 5′LCRed640-TAAGAGGAAAG-TAAGGGACCTCCTATCCAGCCTC-3′Phos. Amplification was performed over 45 cycles (denaturation at 95°C for 15 min, annealing at 56°C for 20 s, and extension at 72°C for 1 min), and the LightCycler FastStart DNA Master^{PLUS} HybProbe PCR reagents (Roche Diagnostics, Mannheim, Germany) were used. The total reaction volume was 20 μL, with 0.5 μM *forward* and *reverse* primers, 0.4 μM *anchor* and *sensor* probes, and 3 μL of template. Genotypes of −1082 (G/A) SNP of *IL-10* were determined via melting-curve analysis as described previously.[20]

3.5. Statistical analysis

The data were analyzed using previously described approaches.[18,21,22] The associations between demographic data and preterm births within the case and control groups were analyzed using ANOVA or Student's *t*-test, as appropriate. The associations between the genotype distribution or allele frequency and IL-10 levels and the genotype distribution or allele frequency and spontaneous preterm birth were assessed using the χ^2 test. For all analyses, two-sided testing was employed, and a $P \leq 0.05$ was designated as statistically significant. The data were analyzed using the Statistical Package for the Social Sciences (SPSS for Windows v. 15, Chicago, IL).

4. Results

4.1. Sample characteristics

We enrolled 80 participants in this study, including 40 preterm births (30.6 ± 4.9 years) and 40 term births (29.9 ± 4.2 years). There was no difference in age, educational attainment or occupation between the case and control groups (Table 1).

Table 1 – Sample characteristics between the case (*n* = 40) and control (*n* = 40) groups.

Characteristics	Case	Control	*P* value
Age, years[a]	30.6 ± 4.9	29.9 ± 4.2	0.448
Age group, years[b]			0.941
20–25	6 (15.0)	7 (17.5)	
26–30	14 (35.0)	16 (40.0)	
31–35	15 (37.5)	14 (35.0)	
≥36	5 (12.5)	3 (7.5)	
Education[b]			0.688
Primary school	6 (15.0)	5 (12.5)	
Junior high school	12 (30.0)	8 (20.0)	
Senior high school	13 (32.5)	17 (42.5)	
University graduate	9 (22.5)	10 (25.0)	
Occupation[b]			0.388
Housewife	27 (67.5)	26 (65.0)	
Private sector	9 (22.5)	10 (25.0)	
Civil servant	4 (10.0)	4 (10.0)	

[a] Calculated using Student's *t*-test. Numbers are given as mean ± SD.
[b] Calculated using ANOVA. Numbers are given as *n* (%).

4.2. Comparison of IL-10 levels between case and control groups

The concentration of IL-10 was higher in the case group than in the control group (9.3 ± 8.4 mg/dL vs. 5.6 ± 3.4 mg/dL, $P = 0.012$). However, it should be noted that there was relatively high variance among individuals in both the case and control groups.

4.3. Association of the −1082 (G/A) IL-10 SNP and IL-10 levels

Among all participants (cases and controls), there was no association between allele A or G of the −1082 (G/A) *IL-10* SNP and the level of IL-10 in serum (7.7 ± 6.8 pg/dL vs. 4.3 ± 0.8 pg/dL, $P = 0.070$). However, individuals carrying allele A appeared to exhibit higher levels of IL-10. In addition, there was no association of the genotype distribution, under either a dominant or recessive model, with IL-10 level (Table 2).

4.4. Association of the −1082 (G/A) IL-10 SNP with preterm birth

There was no association of the genotype distribution or allele frequency of the −1082 (G/A) *IL-10* SNP with preterm birth (Table 3). In addition, the dominant and recessive models showed no association with preterm birth.

5. Discussion

The present study was conducted to assess the association of the −1082 (A/G) *IL-10* SNP with spontaneous preterm birth in the Acehnese population, and we found that there was no strong association between this SNP and spontaneous preterm birth in this population. Previous studies have revealed that the level of IL-10 was inconsistently associated with spontaneous preterm birth. Some have reported increased levels of

Table 2 – Association of the −1082 (A/G) *IL-10* SNP and IL-10 levels (*n* = 80).

Genotype and allele	Frequency *n* (%)	IL-10 level, pg/dL mean ± SD	*P* value
Genotype			0.293
AA	70 (87.5)	7.9 ± 6.9	
AG	7 (8.8)	4.6 ± 1.0	
GG	3 (3.7)	3.9 ± 0.2	
Dominant model			0.117
GG+AG	10 (12.5)	4.4 ± 0.9	
AA	70 (87.5)	7.9 ± 6.9	
Recessive model			0.351
GG	3 (3.7)	3.9 ± 0.2	
AG+AA	77 (96.3)	7.6 ± 6.7	
Allele			0.070
A	147 (91.9)	7.7 ± 6.8	
G	13 (8.1)	4.3 ± 0.8	

Table 3 – Association of the −1082 (A/G) *IL-10* SNP and spontaneous preterm birth.

Genotype and allele	Group		OR (CI 95%)	P value
	Preterm *n* (%)	Normal *n* (%)		
Genotype				0.433
AA	36 (90.0)	34 (85.0)	1	
AG	2 (5.0)	5 (12.5)	0.38 (0.07–2.08)	0.226
GG	2 (5.0)	1 (2.5)	1.89 (0.16–21.79)	0.531
Dominant model				
GG+AG	4 (10.0)	6 (15.0)	1	0.369
AA	36 (90.0)	34 (85.0)	0.64 (0.22–2.41)	
Recessive model				
GG	2 (5.0)	1 (2.5)	1	0.500
AG+AA	38 (95.0)	39 (97.5)	2.05 (0.23–23.65)	
Allele				
G	6 (7.5)	7 (8.7)	1	0.500
A	74 (92.5)	73 (91.3)	0.83 (0.32–2.64)	

CI: confidence interval; OR: odds ratio.

IL-10 in preterm births, while others obtained a contrary result.[23] There is currently no strong evidence regarding the association between the −1082 (A/G) *IL-10* SNP and spontaneous preterm birth. The only evidence regarding such an association has been provided by studies conducted in Brazil[19] and Malaysia.[24] The study conducted in Brazil found that there was no independent association between this SNP and preterm birth.[19] However, in Malaysia, this SNP was shown to be associated with susceptibility to preterm birth.[24] The authors found that the A allele frequency was significantly higher in preterm births compared with term births, and the AA and GA genotypes exhibited significantly higher levels of IL-10 compared with the GG genotype.[24] These data indicate that the role of this SNP may be influenced by ethnicity.

There are some limitations of this study. First, the numbers of cases and controls in this study were relatively small. In addition, this study did not obtain prospective data examining the effect of this SNP on preterm birth. Finally, we did not assess other SNPs located within the *IL-10* promoter, including −819 (C/T) and −592 (C/A). These additional SNPs are linked, and the combination of these SNPs together produces three haplotypes (GCC, ATA and ACC), which are each correlated with different levels of IL-10 production.[14,25] This observation could provide a possible explanation for the lack of correlation between the −1082 (A/G) *IL-10* SNP and spontaneous preterm birth in this study.

6. Conclusions

This study reveals that there is no association between the −1082 (A/G) *IL-10* polymorphism and the serum level of IL-10, and this polymorphism alone appears to present no association with risk factors for spontaneous preterm birth in the Acehnese ethnic group.

Conflict of interest

None declared.

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