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Single nucleotide polymorphisms of *Toll-like receptor-4* and of *autophagy-related gene 16 like-1* gene for predisposition of premature delivery

A prospective study

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Abstract

To investigate the impact of carriage of single nucleotide polymorphisms (SNPs) of the Toll-like receptor-4 (TLR4) and of autophagy-related gene 16-like-1 (ATG16L1) in preterm delivery (PTD).

A prospective cohort of 145 pregnant women was studied. Women were prospectively followed-up until delivery. Genotyping for rs4986790 (Asp299Gly transition) and rs4986791 (Thr399Ile transition) of *TLR4* and for rs2241880 of *ATG16L1* was done by PCR-restriction fragment length polymorphism. The primary study endpoint was the impact of carriage of minor alleles of *TLR4* on early PTD before gestational week 32. Associations with human chorionic gonadotrophin (hCG) were also analyzed. Peripheral blood mononuclear cells were isolated from 15 healthy women and stimulated for cytokine production.

No difference in clinical characteristics was observed between women delivering full term and preterm. The frequency of early PTD was 25% among women carrying minor alleles of *TLR4* and 6.8% among women carrying major alleles (*P*: .032). Odds ratios for PTD were 3.85 among women carrying the GG genotype of rs2241880 and major alleles of *TLR4* and 0.26 among carriers of GG genotype and minor alleles of *TLR4* (*P*: .030). The co-presence of GG genotype of rs2241880 and hCG above 70 U/L was an independent variable for PTD. Stimulated production of interleukin-6 was greater among women with GG genotypes of rs2241880.

Minor alleles of SNPs of *TLR4* predispose to early PTD. The GG genotype of rs2241880 of *ATG16L1* is associated with PTD when hCG is supra-elevated.

Abbreviations: Asp = asparagine, ATG16L1 = autophagy-related gene 16-like-1, CI = confidence interval, DAMP = danger-associated molecular pattern, Gly = glycine, hCG = human chorionic gonadotrophin, HWE = Hardy-Weinberg equilibrium, IL = interleukin, Ile = isoleucine, LPS = lipopolysaccharide, OR = odds ratio, PAPP-A = pregnancy-associated plasma protein A, PBMCs = peripheral blood mononuclear cells, PCR = polymerase chain reaction, PTD = pre-term delivery, ROC = receiver operator characteristics, SNP = single nucleotide polymorphism, Thr = threonine, TLR = toll-like receptor, TNFα = tumor necrosis factor-alpha.

Keywords: autophagy, preterm delivery, single nucleotide polymorphisms, toll-like receptor-4

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1. Introduction

Pre-term delivery (PTD) is a global health problem amounting to 1.5 million cases every year worldwide.[1] The majority of cases of PTD are due to the early induction of inflammation that cannot be counterbalanced. Available evidence suggests that the activation of the inflammatory cascade is starting through the stimulation of the Toll-like receptor (TLR)-4 that is a transmembrane receptor expressed on the cell membrane of cells participating in the innate immune defense, particularly circulating monocytes and tissue macrophages.[2] The *TLR4* gene encoding for the TLR4 receptor protein is highly expressed in the placenta of women delivering pre-term, irrespectively whether these women conceive through assisted reproductive technology or not.[3] The most broadly studied agonist for TLR4 is bacterial lipopolysaccharide (LPS) of the cell wall of gram-negative bacteria. Although this points towards bacterial infection as a cause of PTD through TLR stimulation, it should be taken into consideration that danger-associated molecular patterns (DAMPs) that are released during cell destruction are also potent TLR agonists.[4]

One natural counterbalance of the inflammatory cascade is the process of autophagy. Autophagy is a natural process which plays critical role in inflammation through influencing development, homeostasis and survival of inflammatory cells; it is taking place intracellularly trying to engulf and eliminate destroyed organelles or remnants of the inflammasome.[5] When inflammation occurs, the production of oxygen-free radicals increases whatever stimulates the autophagy cascade. The autophagy cascade is further activated during PTD[6] but its efficiency to eliminate the over-activated inflammatory process is currently unknown.

There are broadly characterized single nucleotide polymorphisms (SNPs) for proteins implicated in the above processes. Two common non-synonymous SNPs are described in the *TLR4* gene; rs4986790 leading to A/G transition and causing the substitution of aspartic acid by glycine at amino acid position 299 (Asp299Gly); and rs4986791 leading to C/T transition and causing the substitution of threonine by isoleucine at amino acid position 399 (Thr399Ile).[7] A large-scale meta-analysis of 28 studies has failed to demonstrate an association between carriage of minor alleles of rs4986790 and of rs4986791 and the development of severe infections.[8] However there is recent evidence that carriage of these minor alleles is associated with risk for the development of non-severe infections like urinary tract infections in women[9] and opportunistic infections in patients infected by the human immunodeficiency virus and low CD4-count.[10] This susceptibility for infections may be attributed to the attenuation of pro-inflammatory host responses.[11]

The rate-limiting step in the autophagy cascade is catalyzed by the autophagy-related gene 16-like 1 (ATG16L1) enzyme that is encoded by the *ATG16L1* gene. A non-synonymous single-nucleotide polymorphism (SNP) in the *ATG16L1* (Thr300Ala c.898A>G, rs2241880) gene results in functional impairment which is associated with increased risk for the acquisition of Crohn's disease.[12] More precisely, carriage of A allele is associated with impaired intracellular trafficking of vacuoles and reduced interaction of ATG16L1 with bacteria.[13] This SNP has also been studied in 69 women with induced labor; the time from start of labor induction to delivery was shorter among women with the GG genotype.[14]

Despite the existing publications of these SNPs for many acute and chronic inflammatory disorders, scarce evidence exists for their association with PTD. The present study is a prospective cohort aiming to investigate the association of SNPs of *TLR4* and of *ATLG16L1* with PTD.

2. Patients and methods

2.1. Study design

A prospective study was conducted in pregnant women under follow-up at the Clinics of Obstetrics and Gynecology "Zoodochos Pigi" of Limassol Cyprus during the period January 2015 to December 2017. The study was approved by the Ethics Committee of Cyprus (EEBK/EΠ/2015/05) and by the Ethics Committee of the ATTIKON University hospital (approval 2/08-02-2013) where the genetic analysis took place.

Inclusion criteria were:

1. written informed consent provided by the pregnant woman;
2. adult women aged equal to or above 18 years; and
3. women of Caucasian origin.

Exclusion criteria were:

1. age below 18 years;
2. denial to consent;
3. infection by human immunodeficiency virus (HIV), and by hepatitis viruses B and C;
4. corticosteroid intake at dose greater than 1 mg/kg equivalent prednisone daily for the last month;
5. history of any autoimmune disorder;
6. multiple pregnancy;
7. pregnancy after assisted reproduction; and
8. any uterine abnormalities evidenced through ultrasound before conception.

A volume of 5 mL of whole blood was sampled on the day of pregnancy diagnosis via vein puncture of one forearm vein under sterile conditions and was directly placed into one ethyldiamine acetic acid-coated tube (Beckton Dickinson, Cockeysville, Md) and stored at −20°C until the isolation of DNA. Clinical characteristics collected for each woman were: parity, history of PTD, pre-eclampsia and eclampsia, obesity, smoking habit, history of chronic disorders, any congenital infection or bacterial infection and gestational week at delivery. PTD was considered as any fetal delivery before gestational week 37; early PTD was considered as any fetal delivery before gestational week 32. In all women, human chorionic gonadotrophin (hCG) and pregnancy-associated plasma protein A (PAPP-A) were measured in plasma between weeks 9 and 12 of pregnancy.

2.2. Laboratory methods

DNA was extracted from whole blood according to the manufacturer's instructions from the Blood Core Kit C (Qiagen, Hilden, Germany). DNA concentration and purity was measured using Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, USA). PCR was performed using 50 ng of genomic DNA at a final volume of 25 μL with 50 mM of $MgCl_2$ (New England BioLabs, Massachusetts, MA, USA), 20 mM of dNTPs (New England BioLabs) and 1 mM of *Taq* polymerase (New England BioLabs). For the Asp299Gly polymorphism the sense primer 5′-ATA CTT AGA CTA CTA CCT CCA TG-3′ and the antisense primer 5′-AGC CTT TTG AGA GAT TTG AGT-3′ were used. PCR consisted of one initial denaturation phase of

95°C for 5 min followed by 40 cycles; each cycle consisted of one annealing step of 95°C for 1 min, one polymerization step of 52°C for 1 min and one elongation step of 72°C for 1 min. Then another cycle of 72°C for 7 min was done before termination. Ten μL of the PCR product were digested after incubation for 60 min at 37°C with 0.5 U of the restriction enzyme *NcoI* (New England BioLabs). For the Thr399Ile polymorphism the sense primer 5′-GCT GTT CTC AAA GTG ATT TTG GGA-3′ and the antisense primer 5′-CAC TCA TTT GTT TCA AAT TGG AAT G-3′ were used. PCR consisted of one initial denaturation phase of 95°C for 4 min followed by 35 cycles; each cycle consisted of one annealing step of 95°C for 30 s, one polymerization step of 56°C for 30 s and one elongation step of 72°C for 30 s. Then another cycle of 72°C for 7 min was done before termination. Ten μL of the PCR product was digested after incubation for 60 min at 37°C with 0.5 U of the restriction enzyme *HinfI* (New England BioLabs).

For the rs2241880 SNP the forward primer 5′-CTC TGT CAC CAT ATC AAG CGT GG-3′ and the reverse primer 5′-TCT AGA AGG ACA GGC TAT CAA CAG ATG-3′ were used. PCR consisted of an initial denaturation step at 95°C for 4 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing of 58°C for 60 s and elongation of 68°C for 1 min and 5 min at 68°C. The PCR product was digested after incubation for 60 min at 37°C with 10 U of the *SfaNI* restriction enzyme (New England BioLabs).

All digestion products were evaluated after 2% agarose gel electrophoresis and ethidium bromide staining.

hCG and PAPP-A were measured by the BRAHMS KRYPTOR GOLD prenatal screening assays. Protein levels were expressed as U/l.

2.3. Study endpoints

The primary study endpoint was the impact of carriage of at least one minor allele of either Asp299Gly or Thr399Ile *TLR4* in the advent of early PTD.

Secondary study endpoints were:

1. the impact of carriage of at least one minor allele of either Asp299Gly or Thr399Ile *TLR4* in all cases of PTD;
2. the interaction of genotypes of rs2241880 of *ATG16L1* and of *TLR4* in PTD;
3. the impact of the studied SNPs on the time until delivery; and
4. the interaction between the levels of hCG and/or PAPP-A with the studied genotypes on PTD.

2.4. Power of the study

The study was powered for the primary endpoint taking into consideration that the frequency of the minor SNP alleles of Asp299Gly and of Thr399Ile of *TLR4* in the Caucasian population ranges between 8% and 10% whilst the incidence of early PTD is 9%. Based on this to demonstrate a difference with 80% power at the 10% level of significance between carriers of major and minor SNP alleles in the frequency of early PTD, 150 pregnant women should be enrolled.

2.5. Mechanistic cohort

In an attempt to provide mechanistic insight of the findings, 20 mL of whole blood was sampled from 15 fertile women of similar age with the women of the study cohort after venipuncture of one forearm vein under aseptic conditions. These women were healthy and processing of their blood was done after written informed consent and approval by the Ethics Committee of the ATTIKON University hospital (approval 2/08-02-2013). Five mL of sampled blood was processed as analyzed above for genotyping of Asp299Gly and Thr399Ile of *TLR4* and of rs2241880 of *ATG16L1*. The remaining blood was gradient centrifuged over Ficoll and peripheral blood mononuclear cells (PBMCs) were isolated and washed three serial times with ice-cold phosphate buffered saline pH 7.2. The number of PBMCs was measured in a Neubaeur plate and, following trypan blue exclusion of dead cells, they were cultured at a density of 5×10^6/mL in the presence of RPMI1640 (Biochrom, Berlin, Germany) enriched with 2 mM glutamate (Biochrom), 100 U/mL of penicillin and 0.1 mg/mL of gentamycin without/with 10 ng/mL of LPS of *Escherichia coli* O55:B5. After 24 h of incubation at 37°C in 5% CO_2, plates were centrifuged and concentrations of tumor necrosis factor-alpha (TNFα) and interleukin (IL)-6 were measured in supernatants by an enzyme immunoassay (eBioscience). The lowest limit of detection was 4 pg/mL for TNFα and 6 pg/mL for IL-6.

2.6. Statistical analysis

Comparison of demographics of women with PTD and term deliveries was performed using the Student's test for quantitative variables and by the Fisher exact test for qualitative variables. The distribution of genotypes according to the Hardy Weinberg equilibrium (HWE) was assessed by the Chi-square test. Comparisons between carriers and non-carriers of minor alleles were done by the Fisher exact test; odds ratios (ORs) and 95% confidence intervals (CIs) were determined according to Mantel and Haenszel's statistics. Comparisons of two ORs were done by the Breslow–Day's and by the Tarone tests. In order to investigate the interaction of levels of hCG and PAPP-A with the studied genotypes in PTD, receiver operator characteristics (ROC) curves were designed. The best cut-off level associated with PTD was defined by the Youden index. Forward step-wise logistic regression analysis was done with PTD as a dependent variable and studied genotypes and their interaction with the defined protein cut-off as dependent variables.

The time to PTD was compared by the log-rank test. Cytokines of PBMCs supernatants were compared by the Mann–Whitney *U* test.

Any value of *P* below .05 was considered as significant.

3. Results

The flow chart of the clinical study is shown in Figure 1. From a total of 172 screened pregnant women 145 were analyzed; 24 women had PTD, 10 of which delivered before gestational week 32. Clinical characteristics did not differ between women who delivered at full term and women who delivered pre-term (Table 1). As shown in Table 1, genotype distribution of all studied SNPs were at HWE with the exception of the genotype of rs2241880 of *ATG16L1* that was deviated from equilibrium within the group of women who delivered at pre-term. The frequency of the CT/TT genotypes of rs4986791 (Thr399Ile) of *TLR4* was greater among the PTD group.

Regarding analysis for the primary study endpoint, the advent of early PTD was compared between 20 women who were

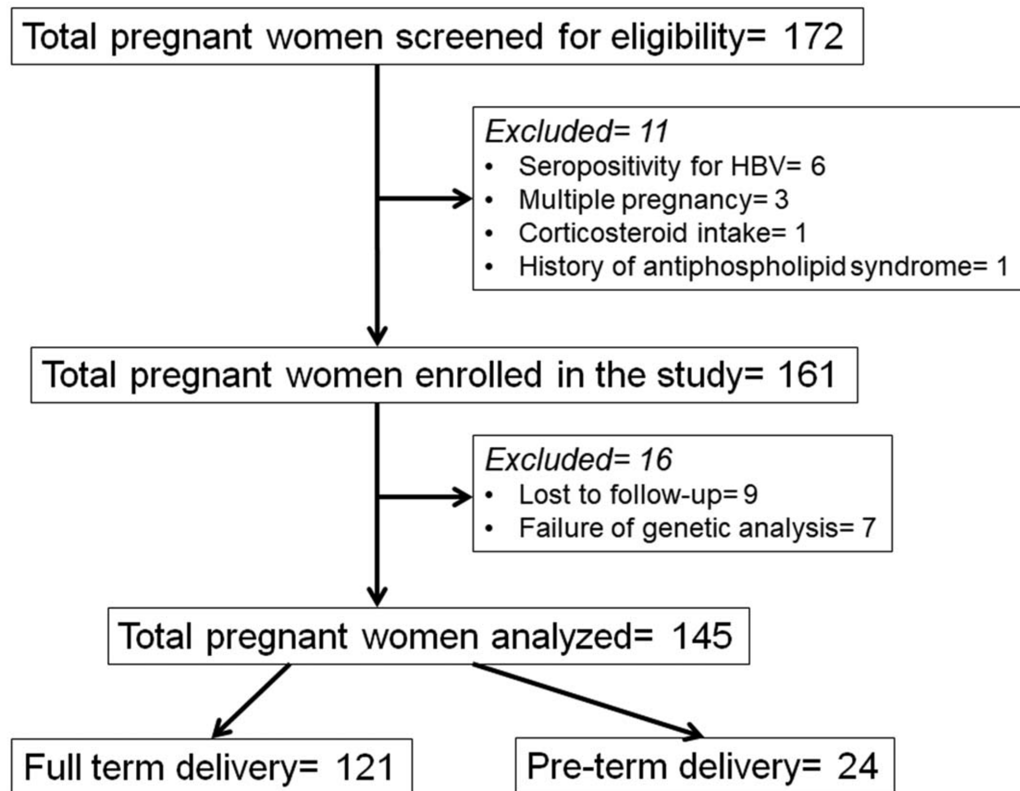

Figure 1. Clinical study flow chart. HBV=hepatitis B virus.

Table 1

Demographic characteristics of women delivering full term and preterm.

	Full-term delivery (n=121)	Preterm delivery (n=24)	*P*
Age (years, mean±SD)	29.5±4.9	30.2±6.3	.592
Smoking (n, %)	1 (0.8)	1 (4.2)	.305
Obesity (n, %)	3 (2.5)	0 (0)	1.00
Any previous abortion (n, %)	12 (9.9)	3 (12.5)	.715
Vascular hypertension of the pregnancy (n, %)	2 (1.7)	0 (0)	1.00
Gestational diabetes (n, %)	2 (1.7)	2 (8.6)	.128
Any congenital infection	0 (0)	0 (0)	1.00
Early preterm delivery (n, %)	0 (0)	10 (41.7)	<.0001
rs4986790 of *TLR4* (n, %)			
AA genotype	113 (93.4)	22 (91.7)	.671
AG genotype	8 (6.6)	2 (8.3)	
X^2 for HWE (*P*-value)	0.14 (0.706)	0.04 (0.831)	
A alleles	234 (96.7)	46 (95.8)	.689
G alleles	8 (3.3)	2 (4.2)	
rs4986791 of *TLR4* (n, %)			
CC genotype	110 (90.9)	19 (79.2)	
CT genotype	11 (9.1)	4 (16.7)	.039
TT genotype	0 (0)	1 (4.2)	
X^2 for HWE (*P*-value)	0.27 (0.600)	1.36 (0.243)	
C alleles	231 (95.5)	42 (87.5)	.043
T alleles	11 (4.5)	6 (12.5)	
rs2241880 of *ATG16L1* (n, %)			
AA genotype	32 (26.4)	6 (25.0)	
AG genotype	52 (43.0)	6 (25.0)	.144
GG genotype	37 (30.6)	12 (50.0)	
X^2 for HWE (*P*-value)	2.05 (0.152)	5.22 (0.022)	
A alleles	116 (47.9)	18 (37.5)	.207
G alleles	126 (52.1)	30 (62.5)	

HWE=Hardy-Weinberg equilibrium.

carriers of at least one minor allele of either Asp299Gly or Thr399Ile *TLR4* and 125 women who were carriers of only major alleles of both these SNPs. Early PTD occurred in four women (25%) and six women (6.8%) respectively (*P*: .032). The OR for early PTD among carriers of at least one minor allele of either Asp299Gly or Thr399Ile *TLR4* was 4.96 (95%CIs: 1.26–19.48; *P*: .022). Overall PTD happened in six (30%) and 18 (14.4%) of these women respectively (*P*: .103).

Skewing of the genotype distribution of rs2241880 of *ATG16L1* from the HWE among women with PTD focused interest on the impact of the GG genotype of this SNP for the advent of PTD. Overall, 49 women were carrying this genotype and 96 women were carriers of AA/AG genotypes. PTD happened in 12 (24.5%) and in 12 (12.5%) women respectively (*P*: .097). However, the largest impact of the GG genotype of rs2241880 of *ATG16L1* was shown among women with major alleles of *TLR4* (Table 2). The OR for PTD was significantly greater among women with the GG genotype of rs2241880 and major alleles of *TLR4* than among women with the GG genotype of rs2241880 and minor alleles of *TLR4* (*P*: .030 by the Breslow–Day's test and *P*: .030 by the Tarone's test).

The above results indicated an individualized effect of GG genotypes of rs2241880. This prompted us to study the effect of genotypes of *TLR4* according to the carriage of GG genotypes of rs2241880 and vice versa. Indeed, carriage of minor alleles of *TLR4* led to earlier delivery only among women with AA/AG genotypes of rs2241880 (Fig. 2A and B); in parallel carriage of GG genotypes of rs2241880 led to earlier delivery only among women with major alleles of *TLR4* (Fig. 2C and D).

Table 2

Interaction of the GG genotype of rs2241880 of *ATG16L1* and of minor alleles of *TLR4* for preterm delivery (PTD).

	AA/GA of rs2241880 (n, %)	GG of rs2241880 (n, %)	*P*	Odds ratio	95% confidence intervals
Carriage of major alleles of both Asp299Gly and Thr399Ile					
Full-term	76 (91.6)	31 (73.8)	.013	3.85	1.37–10.85
PTD	7 (8.4)	11 (26.2)			
Carriage of at least one minor allele of either Asp299Gly and/or Thr399Ile					
Full-term	8 (61.5)	6 (85.7)	.354	0.26	0.02–2.92
PTD	5 (38.5)	1 (14.3)			

Youden index analysis disclosed levels of hCG >70 U/L as the best trade-off for PTD. Similar cut-off for PAPP-A could not be found. As shown in Table 3, the OR for PTD was significantly greater among women with the GG genotype of rs2241880 and hCG >70 U/L compared to women with hCG < 70 U/L (*P*: .014 by the Breslow–Day's test and *P*: .014 by the Tarone's test).

Forward step-wise logistic regression analysis was done with PTD as a dependent variable and five independent variables: hCG >70 U/L, carriage of minor alleles of *TLR4*, carriage of GG genotypes of rs2241880; interaction of hCG with minor alleles of *TLR4*; and interaction of hCG with the GG genotype of rs2241880. Following two steps of analysis (Table 4), it was found that carriage of minor alleles of *TLR4* and the interaction of hCG with the GG genotype of rs2241880 were the only independent variables associated with PTD.

The mean ± SD age of the 15 women of the mechanistic cohort was 30.7 ± 2.9 years. All women were carriers of major alleles of *TLR4*; five had the GG genotype of rs2241880. Stimulated production of IL-6 was greater among those with the GG genotype of rs2241880 (Fig. 3).

4. Discussion

The present study supports the concept of individualized predisposition for PTD. Minor alleles of Asp299Gly and of Thr399Ile of *TLR4* and the GG genotype of rs2241880 of

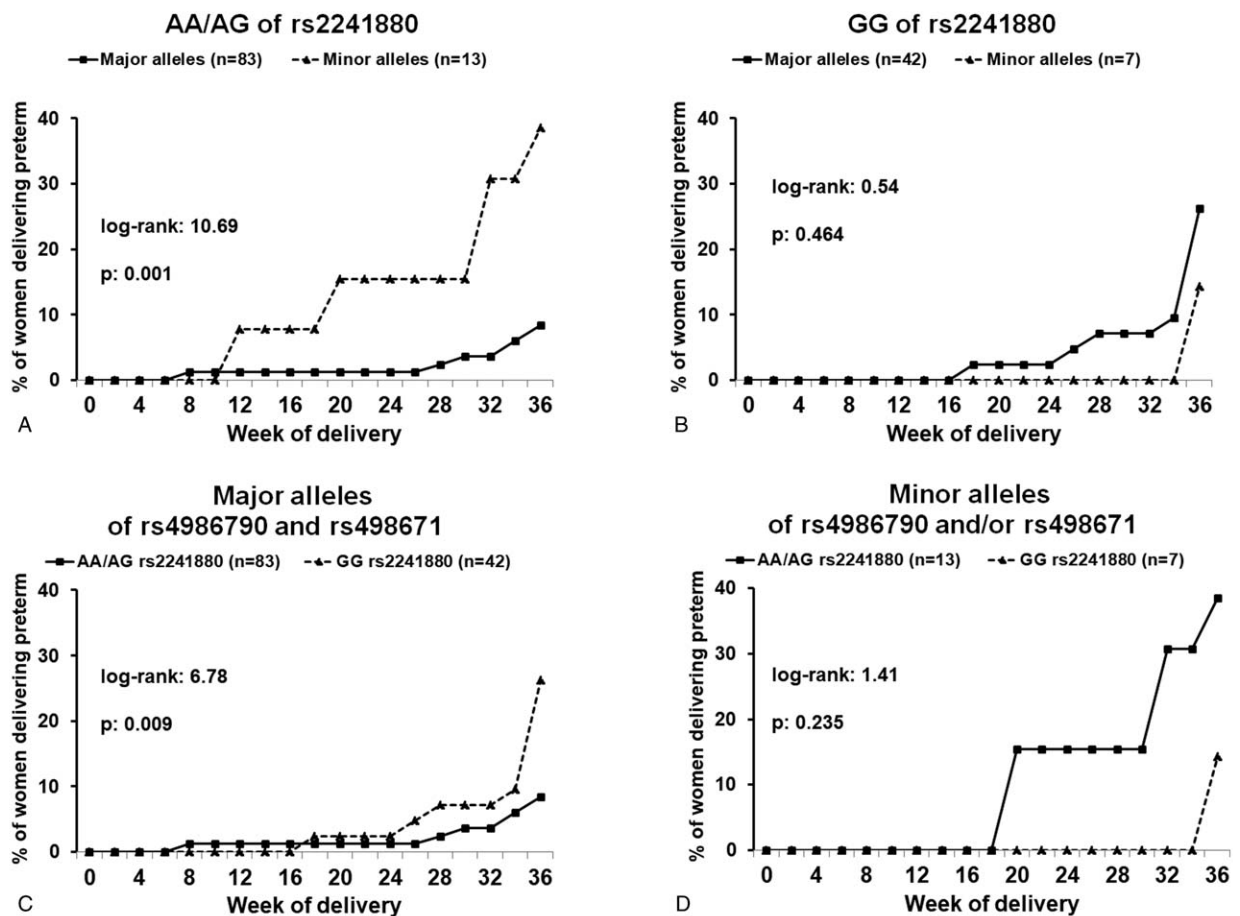

Figure 2. Time to preterm delivery. Pregnant women are split into four subgroups by genotyping (A) AA/AG of rs2241880 of *ATG16L1*; (B) GG of rs2241880 of *ATG16L1*; (C) major alleles of both Asp299Gly and Thr399Ile of *TLR4*; (D) and minor alleles Asp299Gly and/or Thr399Ile of*TLR4*. The respective log-rank tests and of the *P*-values of comparisons are shown.

Table 3

Interaction of the GG genotype of rs2241880 of *ATG16L1* and of human chorionic gonadotrophin (hCG) for preterm delivery (PTD).

	AA/GA of rs2241880 (n, %)	GG of rs2241880 (n, %)	P	Odds ratio	95% confidence intervals
hCG below 70 U/l					
Full-term	66 (85.7)	32 (84.2)	1.00	1.12	0.38–3.31
PTD	11 (14.3)	6 (15.8)			
hCG above 70 U/l					
Full-term	18 (94.7)	5 (45.5)	.004	21.60	2.09–223.65
PTD	1 (5.3)	6 (54.5)			

ATG16L1 are independent variables associated with increased risk for PTD. Minor alleles of *TLR4* predispose for early PTD, that is, delivery before gestational week 32. GG genotypes of rs2241880 of *ATG16L1* predispose to PTD under two conditions; either among women carrying only major alleles of *TLR4* or among women with hCG above 70 U/L at gestational week 9.

Autophagy is recognized as a major process that is modulated in PTD. The expression on *LC3* that encodes for the LC3 protein which elicits the formation of the autophagosome, was studied in the placenta of women who delivered preterm. The expression was greater among women whose placenta had histology of inflammation.[15] However the impact of the genotypes of genes participating in the autophagy cascade in PTD has never been studied so far. Regarding the impact of the SNP alleles of *TLR4*, only two studies have been published. Both studied only the carriage of the Asp299Gly SNP by the fetuses and they were retrospective in design. The first study came from Finland and studied 440 neonates born prematurely between 1996 and 1999 and compared them with 351 neonates born at full term in 1998. Results showed a positive association between the Asp299Gly SNP carriage and PTD.[16] The second study came from Uruguay and examined 108 neonates who were delivered before gestational week 33, 118 neonates who were delivered between gestational weeks 33 and 36 and 280 neonates who were delivered at full term. Results corroborated our findings since carriage of the Asp299Gly allele was associated with early PTD before gestational week 33.[17]

Our study did not use neonatal blood since it focused intentionally on the role of the genetic predisposition of the mother's innate immune defense. The need to study the genetic make-up of the mother is underscored by series of experimental studies of our group in which PTD is induced after the intraperitoneal administration of low-dose of LPS. Through this systemic administration of LPS, it is the innate immune response of the mother that predominates and that culminates in PTD.[18–20]

We believe that our study has three strong points:

1. the focus on the genetic composition of pregnant mothers;
2. the prospective design; and
3. the lack of difference in baseline characteristics between enrolled women who delivered at full term and those who delivered at preterm.

Table 4

Forward step-wise logistic regression analysis of variables associated with preterm delivery (PTD).

Variable	Odds ratio	95% confidence intervals	*P*-Value
Minor alleles of *TLR4*	6.47	1.47–28.51	.014
hCG >70 U/l * GG genotype of rs2241880	9.26	1.76–48.66	.009

Five independent variables were included in the equation: human chorionic gonadotrophin hCG greater than 70 U/l, carriage of minor alleles of *TLR4*, carriage of GG genotypes of rs2241880; interaction of hCG with minor alleles of *TLR4*; and interaction of hCG with the GG genotype of rs2241880. Results after two steps of analysis are shown.

The TLR4 cascade and the autophagy cascade act in opposite ways regarding the triggering of the inflammatory processes that may develop during pregnancy. TLR elicits pro-inflammatory phenomena and the autophagy clears the by-products induced by inflammatory triggers like oxygen free radicals and heat shock proteins.[5,21] Inhibition of TLR4 in animal models of PTD is associated with significant attenuation of PTD.[22,23] It may be argued that carriage of SNPs of *TLR4* leads to over-activation of the pro-inflammatory phenomena ending in modulation of prostaglandin production and PTD.[24] On the contrary, since autophagy is considered an anti-inflammatory response, it may

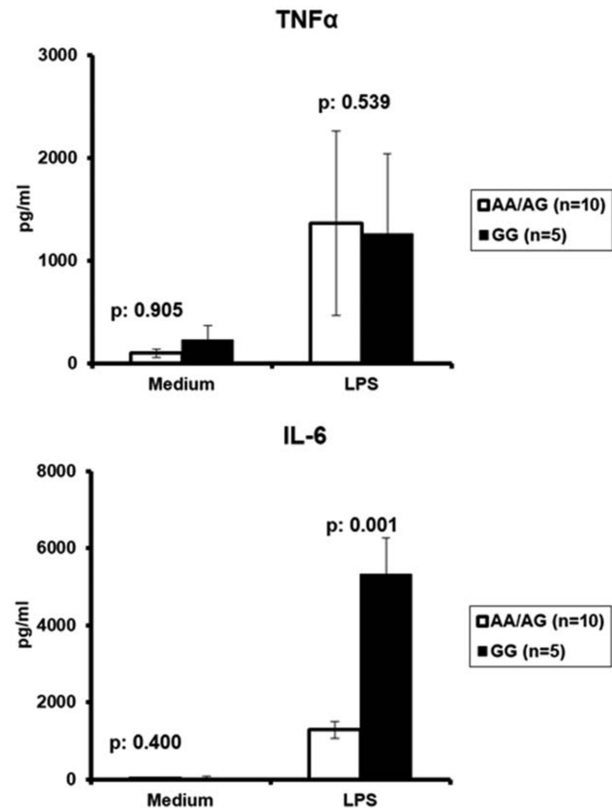

Figure 3. Cytokine stimulation and genotyping of rs2241880. The production of tumor necrosis factor-alpha (TNFα) and of interleukin (IL)-6 from peripheral blood mononuclear cells of 15 women genotyped for rs2241880 of *ATG16L1* is shown. P values refer to comparisons between women with the AA/AG genotypes and the GG genotypes. LPS = lipopolysaccharide.

be postulated that carriage of GG genotypes of rs2241880 of *ATG16L1* primes pro-inflammation. This hypothesis is compatible with the increased in vitro stimulation of IL-6 from PBMCs of women with the GG genotype. The highly stimulated IL-6 production is induced by bacterial LPS that has been studied as the trigger of PTD in female mice.[18–20] In a previous study of our group in patients with sepsis and ventilator-associated pneumonia it was shown that monocytes from patients with the GG genotypes suffering from septic shock and organ dysfunction produced greater concentrations of TNFα.[25]

The presented findings suggest that maternal carriage of minor alleles of Asp299Gly and Thr399Ile SNPs of *TLR4* predispose to early PTD before gestational week 32. Furthermore, the GG genotype of rs2241880 of *ATG16L1* among women with hCG above 70 U/L during the first 12 weeks of pregnancy are an independent risk factor for PTD. These results may pave the way of a personalized approach for the prevention of PTD.

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