BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2020, VOL. 77, NO. 2, 64–68
https://doi.org/10.1080/09674845.2019.1687151

Links between SNPs in *TLR-2* and *TLR-4* and idiopathic recurrent pregnancy loss

W Bahia[a], I Soltani[b], A Haddad[c], A Radhouani[d], Abdelkarim Mahdhi[e], S Ferchichi[a] and WY Almawi[f,g]

[a]Research Unit of Clinical and Molecular Biology, Faculty of pharmacy, University of Monastir, Monastir, Tunisia; [b]Molecular and Cellular Hematology Laboratory, Institut Pasteur de Tunis, University of Tunis El Manar, Tunis, Tunisia; [c]Department Obstetrics and Gynecology, Fattouma Bourguiba University Hospital, Monastir, Tunisia; [d]Faculty of Medicine, University of Tunis El Manar, Tunis, Tunisia; [e]Laboratory of Analysis, Treatment and Valorization of Pollutants of the Environment and Products, Faculty of Pharmacy, University of Monastir, Monastir, Tunisia; [f]Faculty of Sciences, El Manar University, Tunis, Tunisia; [g]School of Medicine, Nazarbayev University, Astana, Kazakhstan

ABSTRACT

Background: Recurrent pregnancy loss is a serious complication of pregnancy and failure of the innate immune system, one part of which are toll-like receptors (TLRs). We hypothesised links between variants of *TLR-2* and *TLR-4* with recurrent pregnancy loss.

Subjects and methods: We recruited 335 women with recurrent pregnancy loss, defined as ≥3 consecutive spontaneous miscarriage of unknown aetiology, and 331 age-matched control women. *TLR-2* rs1898830 and rs4696483 and *TLR-4* rs2770150, rs1554973 and rs7856729 genotyping were performed by allelic exclusion method (real-time PCR).

Result: Of the five tested *TLR-2* and *TLR-4* tag-SNPs, minor allele frequency of *TLR-2* rs1898830 was significantly more frequent in recurrent pregnancy loss patients than in controls. Significantly higher frequencies of homozygous (2/2) *TLR-2* rs1898830 (14.1% vs. 8.9%) genotype carriers were seen between recurrent pregnancy loss cases and control women. Haploview analysis identified 1-locus *TLR-2* haplotype (GC) that was positively associated with recurrent pregnancy loss. No *TLR-4* haplotypes associated with altered recurrent pregnancy loss risk were identified.

Conclusion: These findings confirm positive associations of *TLR-2* rs1898830 with recurrent pregnancy loss, further supporting a role for TLR signalling in defining pregnancy outcome.

ARTICLE HISTORY
Received 31 August 2019
Accepted 29 September 2019

KEYWORDS
Mutation; allele; real-time PCR; TLR-2; TLR-4; recurrent pregnancy loss

Introduction

Recurrent pregnancy loss, a clinically identified pregnancy that fails to progress, defined as two or more consecutive abortions usually before 20th week of gestation, occurs in 1–5% of all pregnancies [1]. Although anatomical, hormonal, genetic and immunological factors have been involved in the aetiology of Recurrent pregnancy loss, in more than half of the cases the aetiology is unknown [2]. The maternal-foetal interface at the placenta is a unique immunological site which provides tolerance to the allogeneic foetus. Innate immune responses against invader microorganisms at this interface may have a considerable influence on the success of a pregnancy [3]. Considerable effort has been devoted to the study of intrauterine infections and their effects on certain pregnancy complications, with the view that that innate immune disorders may lead to pathologies such as intrauterine growth restriction (IUGR), preeclampsia (PE) and Recurrent pregnancy loss [4,5].

Toll-like receptors (TLRs) play an important role in recognition of antigen determinants of viruses, bacteria, protozoa and fungi and so have a role in the activation of innate and adaptive immune responses and in determining Th1/Th2 balance [4–6]. Ten TLRs have been identified in humans: TLR4 and TLR2 are two of those most functionally investigated [7]. TLR4 and TLR2 are transmembrane type 1 glycoproteins consisting an intracellular, transmembrane and extracellular signalling domain [8]. TLR4 recognises the lipopolysaccharides (LPS) of Gram-negative bacteria and diverse exogenous or endogenous ligands. Activation of TLR4 initiates a signalling pathway through nuclear factor kappa B, leading to the release of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumour necrosis factor-α (TNF- α) [9]. TLR2 detects bacterial lipoproteins, the peptidoglycan (PDG) of Gram-positive bacteria and the lipoteichoic acid [10].

Previous studies indicate that activated TLR4 and TLR2, at the feto-maternal interface, may lead to the Recurrent pregnancy loss by perturbing the Th1/Th2 immune response balance. Chaouat et al [11] suggest that TLR4 activation generates the secretion of a variety of Th1 cytokines involved in pregnancy failure and infertility, including IL-1, IL-6, IL-8 and TNF-α. Furthermore, several authors have documented that LPS, the principal ligand for TLR4, is implicated a main cause of Recurrent pregnancy loss in a range of mammalian species [12–17]. Numerous polymorphic variants of the *TLR4* and *TLR2* have been defined, many

CONTACT W Bahia bahiawael@gmail.com Faculty of Pharmacy of Monastir, University of Monastir, Monastir 5000, Tunisia

suggesting that *TLR4* and *TLR2* single-nucleotide polymorphisms (SNPs) are linked with cardiovascular diseases, preeclampsia, cervical cancer, congenital toxoplasmosis and polycystic ovary syndrome [7,18–21].

Against this background, we hypothesised a link between any one of five polymorphisms in *TLR4* (rs2770150, rs1554973, rs7856729) and *TLR2* (rs1898830, rs4696483) and the presence of Recurrent pregnancy loss.

Subjects and methods

To test our hypothesis, we recruited 335 women with Recurrent pregnancy loss who were attending the obstretics and gynaecology service of the Frahat Hached Hospital (Sousse, Tunisia), and Fattouma Bourguiba Hospital (Monastir, Tunisia) during their 1st trimester of gestation. All Recurrent pregnancy loss patients had their first pregnancy between 19 and 34 years of age and had three or more spontaneous pregnancy losses prior to participate in this study. Recurrent pregnancy loss was assessed as per American College of Obstetricians and Gynecologists guidelines [1].

All Recurrent pregnancy loss women were screened for factors relating to known aetiologies of Recurrent pregnancy loss, including karyotyping of both partners, testosterone, prolactin dosage, anti-phospholipid antibodies, activated protein C resistance, screening for factor V Leiden and factor II G20210A mutations and pelvic ultrasound scan for uterine evaluation. Participants who tested positive for any of these procedures were excluded. Controls were 331 unrelated multiparous women with ≥2 successful natural pregnancies and free of spontaneous Recurrent pregnancy loss or negative immediate family history of miscarriage were recruited with a routine check-up following an uncomplicated pregnancy. Other exclusion criteria were Rh blood group incompatibility, older age at first pregnancy loss (>40 years), biochemical pregnancy and/or preclinical abortion, as well as history of pre-eclampsia which was characterised by elevation in systolic and diastolic blood pressure >145/95 mm Hg, or increase >30/15 mmHg on at least two blood pressure checks, thyroid dysfunction, diabetes mellitus, anatomical disorders, liver function abnormalities, systemic autoimmune disease, and infections (*Chlamydia trachomatis*, toxoplasmosis, cytomegalovirus, HIV, rubella, Group B streptococci, hepatitis B and C, and Bacterial vaginosis), past induced abortions/termination of pregnancy (TOP) due to hypertension, intrauterine infection, uterine rupture, obstetric bleeding, and malignancy. Successful pregnancy defined as live full-term birth (no preterm births). The Research and Ethics Committee of the University of Monastir and Farhat Hached University Hospital approved the study protocol, and informed written consent was obtained from each participant.

A 2–5 ml venous blood sample was taken from each participant into EDTA containing tubes for total genomic DNA extraction and a plain tube for routine biochemistry. *TLR4* (rs2770150, rs1554973 and rs7856729) and *TLR2* (rs1898830 and rs4696483) polymorphisms were genotyped using the allelic (VIC- and FAM-labelled) discrimination method. TaqMan assays were ordered from Applied Biosystems (Foster City, CA, USA). The reaction was performed in a 6-µL volume on StepOne real-time PCR system, as recommended by the manufacturer (Applied Biosystems). Replicate-blinded quality control samples were used to assess genotyping procedure reproducibility; concordance exceeded 99%. CRP, testosterone and glucose were measured by the hospital routine service by standard techniques.

Statistical analysis was performed on SPSS version 23 (IBM; Armonk, NY). Data were expressed as mean with SD for continuous variables, or as percentages of total for categorical variables, Pearson χ^2 or Fisher's exact test was used to assess inter-group significance, and Student's t-test was included to determine differences means. Allele frequencies were calculated by the gene-counting method, and each variant was tested for Hardy–Weinberg equilibrium (HWE) by Haploview (www.broad.mit.edu/mpg/haploview). Genotypic association of underlying SNPs with Recurrent pregnancy loss susceptibility was tested using SNPSstats (http://bioinfo.iconcologia.net/SNPSstats) where genotype codes are '1' for major allele and '2' for minor allele. Linkage disequilibrium (LD) analysis was done using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview). Bonferroni multiple-comparison correction method was used to determining the corrected *P* value, as per: $P_c = 1-(1-P)^n$, where n = number of comparisons. Statistical significance was set at $P < 0.05$.

Results

The demographic and clinical characteristics of study participants are presented in Table 1. There was no difference in the mean age, body-mass index, fasting glucose, previous oral contraceptive use, CRP and Testosterone between Recurrent pregnancy loss cases and control women. In contrast, significant differences between both groups were noted in menarche and smoking.

Links between *TLR4* rs2770150, rs1554973 and rs7856729 and *TLR2* rs1898830, rs4696483 and Recurrent pregnancy loss in case–control subjects are shown in Tables 2 and 3. For each SNP, the genotype distribution in the controls was not significantly different from the Hardy–Weinberg equilibrium values except for the *TLR2* rs4696483, that was not in HWE among study subjects ($P < 0.001$). The frequency of *TLR2* rs1898830 G allele was higher in patients than in controls (Table 2).

Table 1. Clinical characteristics of cases and controls.

	Cases(n = 335)	Controls(n = 331)	P^b
Age at inclusion in study [c]	33.4 ± 5.8	34.3 ± 6.7	0.097
Body-mass index (kg/m^2) [c]	25.6 ± 4.1	26.4 ± 5.8	0.058
Smokers [n (%)] [d]	62 (17)	132 (26.5)	0.001
Previous oral contraceptive use[n (%)] [d]	14 (4.2)	30 (6.0)	0.262
Menarche (years)[c]	12.1 ± 1.1	12.8 ± 1.0	<0.001
Live births[e]	0 (0–2)	3 (2–9)	<0.001
Miscarriages[e]	3 (3–12)	0 (0–0)	<0.001
CRP (mg/L)[e]	4.00(0.10–97.92)	4.07(0.01–10.52)	0.141
Testosterone (ng/mL)[c]	0.48 ± 0.12	0.47 ± 0.14	0.86
Glucose (mmol/L)[c]	5.3 ± 2.5	5.2 ± 1.8	0.85

[a]Student's *t*-test (continuous variables), Pearson's χ^2 test (categorical variables).[b]Mean ± SD[c]Percent of total within each group/subgroup[d]Median (IQR)

Table 2. *TLR4* & *TLR2* SNPs analysed.

Gene	SNP	Percent genotyped	Allele	Cases[a]	Controls[a]	*P*	*Pc*	OR (95% CI)
TLR4	rs2770150	68.5	A > G	91 (0.25) [b]	136 (0.25)	0.92	0.99	0.98 (0.72–1.33)
	rs1554973	74.9	C > T	141 (0.27)	154 (0.32)	0.12	0.31	0.81 (0.61–1.06)
	rs7856729	82.3	G > T	72 (0.14)	95 (0.16)	0.26	0.59	0.82 (0.59–1.15)
TLR2	rs1898830	72.5	A > G	188 (0.35)	111 (0.26)	**0.003**	**0.005**	**1.51 (1.14–2.00)**
	rs4696483	88.1	T > C	83 (0.15)	107 (0.18)	0.14	0.26	0.79 (0.57–1.08)

[a]Study subjects comprised 335 Recurrent pregnancy loss patients and 331 control subjects.[b]Number of alleles (frequency).

Table 3. *TLR4* & *TLR2* genotype frequencies.

		1/1 [a]			1/2 [a]			2/2 [a]		
SNP	Allele	Cases	Controls	*P* [b]	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
TLR4 rs2770150	A > G	105 (57.1)[c]	149 (54.8)	0.56	67 (36.4)	110 (40.4)	0.86 (0.58–1.28)	12 (6.5)	13 (4.8)	1.31 (0.57–2.98)
TLR4 rs1554973	C > T	140(54.5)	112 (46.3)	0.17	93 (36.2)	106 (43.8)	0.70 (0.48–1.02)	24 (9.3)	24 (9.9)	0.80 (0.43–1.48)
TLR4 rs7856729	G > T	193 (74.8)	203 (70.0)	0.43	58 (22.5)	79 (27.2)	0.77 (0.52–1.14)	7 (2.7)	8 (2.8)	0.92 (0.33–2.59)
TLR2 rs1898830	A > G	120 (44.4)	121 (56.8)	0.018	112 (41.5)	73 (34.3)	1.55 (1.05–2.28)	38 (14.1)	19 (8.9)	2.02 (1.10–3.70)
TLR2 rs4696483	T > C	214 (75.1)	215 (71.2)	0.36	59 (20.7)	67 (22.2)	0.88 (0.59–1.32)	12 (4.2)	20 (6.6)	0.60 (0.29–1.26)

[a]Genotypes were coded as per '1' = major allele, '2' = minor allele.[b]Two-way ANOVA[c]Number of subjects (frequency).

In contrast, the distribution of *TLR2* rs4696483 C allele, *TLR4* rs2770150 G allele, *TLR4* rs1554973 T allele and *TLR4* rs7856729 T allele were comparable between cases and control. Table 3 summarises the distribution of *TLR4* and *TLR2* genotypes between cases and control women. Significant differences in the distribution of *TLR2* rs1898830, but not *TLR2* rs4696483, *TLR4* rs2770150, *TLR4* rs1554973 or *TLR4* rs7856729 genotypes were noted. Only *TLR2* rs1898830 showed a significant association with Recurrent pregnancy loss, with an odds ratio of 2 for homozygous for the minor alleles.

We identified four types of haplotypes in *TLR4* (2770150, rs1554973 and rs7856729) and four types of haplotypes in *TLR2* (rs1898830 and rs4696483) with frequencies above 2%. Compared with the haplotype AC, the haplotype GC in *TLR2* was significantly associated with Recurrent pregnancy loss, while none of the identified *TLR-4* haplotypes was found to be linked with Recurrent pregnancy loss (Table 4).

Table 4. Haplotype analysis for the *TLR4* and *TLR2* genes.

Gene	Haplotype	Frequency	OR (95% CI)	P value
TLR4	ATG	0.457	1.00	
	GTG	0.244	0.89 (0.63–1.25)	0.5
	ACT	0.152	0.77 (0.54–1.11)	0.17
	ACG	0.141	0.76 (0.52–1.13)	0.18
TLR2	AC	0.542	1.00	
	GC	0.296	1.43 (1.07–1.90)	0.015*
	AT	0.151	0.96 (0.69–1.33)	0.8
	GT	0.01	0.75 (0.14–4.19)	0.75

Discussion

During pregnancy, the maternal immune system ensures both the protection of the mother's body against invader pathogens and the tolerance of the fetoplacental unit, which carries paternal alloantigens. Foetal survival relies on this crucial balance between maintenance of an immune reaction and tolerance of non-self [22]. Several studies have documented the links between TLR4 and TLR2 and the immune balance at the maternal-foetal interface, although most have focused on the genetic variation in the *TLR4* and *TLR2* and their impact on immune responses against harmful pathogens and pregnancy success [23,24]. We investigated the association between *TLR4* and *TLR2* variants and Recurrent pregnancy loss women. Five SNPs located in *TLR4* (rs2770150, rs1554973 and

rs7856729) and *TLR2* (rs1898830 and rs4696483) were genotyped to investigate their possible effect on the susceptibility to Recurrent pregnancy loss. Our findings are in agreement with the notion that genetic markers in *TLR2* influences Recurrent pregnancy loss development but not in *TLR4* gene.

We report a significant difference in the genotype distribution of *TLR2* SNP rs1898830 between Recurrent pregnancy loss and healthy women. The occurrence of rs1898830 GG genotype in combination with *Treg* markers decreases with maternal atopy [25], whilst the rs1898830 AG genotype is found less frequently in Japanese children with congenital cytomegalovirus (CMV) infection [26]. Furthermore, the *TLR2* SNP rs1898830 is associated with modified risk of Chinese neonatal severe hepatitis [27], and the rs1898830 SNP is associated with bacterial vaginosis [28]. These findings suggest that this *TLR2* SNP may affect the level of innate immunity against pathogen infections, in spite of the lack of any changes in the target amino acid sequence, and participate in the disturbance of the delicate balance of cytokines increasing the pro-inflammatory cytokine production at the maternal-foetal site which may lead to the development of Recurrent pregnancy loss. Studies have documented that synonymous SNPs have an impact on the splicing process, post-transcriptional regulation and protein folding [26,29]. The SNP in *TLR2* rs4696483 was not linked to Recurrent pregnancy loss in our population: Ryckman et al [30] described the absence of relationship between *TLR2*-rs4696483 and the cervical levels of pro-and anti-inflammatory cytokines and its association with bacterial vaginosis.

In the current examination of the effects of three SNPs of the *TLR4* gene on Recurrent pregnancy loss, no significant differences were found in the distribution of distinct genotypes. Several studies reported the role of *TLR4* SNPs in other conditions. Kolz et al [31] documented that minor the allele of rs2770150 not directly linked with the risk of type 2 diabetes, while Semlali et al [32,33] reported that rs2770150 is linked with breast cancer and colon cancer in postmenopausal women. The rs1554973 SNP is linked with cervical pro-inflammatory concentrations in women with bacterial vaginitis, which may predispose them to an increased risk of unfavourable birth outcomes such as preterm birth [30]. *TLR4* rs1554973 does not play a role in the susceptibility to multiple sclerosis, and interactions between *TLR4* rs7856729 and IL-1R2 are correlated with cervical pro-inflammatory cytokine concentrations [34,35].

Our study has a number of shortcomings. We did not perform subgroup analysis according to the type of Recurrent pregnancy loss (primary or secondary), or for euploidy/aneuploidy since most of the miscarriages were not karyotyped. In addition, we selected few SNPs in *TLR2* and *TLR4*, thus raising the speculation of possible contribution of other SNPs in modulating the Recurrent pregnancy loss, so necessitating the need for analysis of additional gene variants within *TLR2* and *TLR4* in future studies. Nevertheless, our sample size provides more than adequate power to test our hypothesis of a link between the *TLR* SNPs and Recurrent pregnancy loss. We are tempted to speculate that that rs1898830 SNP in *TLR2* has a direct causative effect in Recurrent pregnancy loss and so is a risk factor. However, our cross-sectional study can only describe a link: a well-powered prospective follow-up study in women prior to attempting pregnancy is required to determine a true genetic effect on the risk of Recurrent pregnancy loss.

Our work represents an advance in biomedical science because it provides evidence that the rs1898830 SNP in *TLR2*, but not the rs4696483 SNP, may be a diagnostic genetic marker for Recurrent pregnancy loss and that rs2770150, rs1554973 and rs78556729 in *TLR4* are not associated with Recurrent pregnancy loss.

Summary table

What is known about this subject:
- TLRs play an important role in maintaining innate host immunity.
- *TLR2* and *TLR4* are implicated in several aspects of pregnancy.
- Genetic variants in *TLR2* and *TLR4* may be involved in the imbalance of the innate immune system which is associated with Recurrent pregnancy loss

What this paper adds:
- The minor variant of *TLR-2* rs1898830 and the *TLR-2* haplotype (GC) are linked to Recurrent pregnancy loss.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ORCID

W Bahia http://orcid.org/0000-0002-5883-7415
A Haddad http://orcid.org/0000-0001-6767-4761

References

[1] American College of O, Gynecologists. ACOG practice bulletin. Management of recurrent pregnancy loss. Number 24, February 2001. (Replaces Technical Bulletin Number 212, September 1995). American college of obstetricians and gynecologists. Int J Gynaecol Obstet. 2002;78:179–190.
[2] Hyde KJ, Schust DJ. Genetic considerations in recurrent pregnancy loss. Cold Spring Harb Perspect Med. 2015;5:a023119.
[3] Bao SH, Shuai W, Tong J, et al. Increased expression of toll-like receptor 3 in decidual natural killer cells of patients with unexplained recurrent spontaneous

miscarriage. Eur J Obstet Gynecol Reprod Biol. 2012;165:326–330.
[4] Hirschfeld AF, Jiang R, Robinson WP, et al. Toll-like receptor 4 polymorphisms and idiopathic chromosomally normal miscarriage. Hum Reprod. 2007;22:440–443.
[5] Koga K, Mor G. Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy disorders. Am J Reprod Immunol. 2010;63:587–600.
[6] Chen JQ, Szodoray P, Zeher M. Toll-like receptor pathways in autoimmune diseases. Clin Rev Allergy Immunol. 2016;50:1–17.
[7] Wujcicka W, Wilczynski J, Nowakowska D. SNPs in toll-like receptor (TLR) genes as new genetic alterations associated with congenital toxoplasmosis? Eur J Clin Microbiol Infect Dis. 2013;32:503–511.
[8] Xie H, Sheng L, Zhou H, et al. The role of TLR4 in pathophysiology of antiphospholipid syndrome-associated thrombosis and pregnancy morbidity. Br J Haematol. 2014;164:165–176.
[9] Weng PH, Huang YL, Page JH, et al. Polymorphisms of an innate immune gene, toll-like receptor 4, and aggressive prostate cancer risk: a systematic review and meta-analysis. PLoS One. 2014;9:e110569.
[10] Pineda A, Verdin-Teran SL, Camacho A, et al. Expression of toll-like receptor TLR-2, TLR-3, TLR-4 and TLR-9 is increased in placentas from patients with preeclampsia. Arch Med Res. 2011;42:382–391.
[11] Chaouat G, Ledee-Bataille N, Dubanchet S, et al. Reproductive immunology 2003: reassessing the Th1/Th2 paradigm? Immunol Lett. 2004;92:207–214.
[12] Kamiyama S, Teruya Y, Nohara M, et al. Impact of detection of bacterial endotoxin in menstrual effluent on the pregnancy rate in in vitro fertilization and embryo transfer. Fertil Steril. 2004;82:788–792.
[13] Clark DA, Yu G, Arck PC, et al. MD-1 is a critical part of the mechanism causing Th1-cytokine-triggered murine fetal loss syndrome. Am J Reprod Immunol. 2003;49:297–307.
[14] Clark DA, Manuel J, Lee L, et al. Ecology of danger-dependent cytokine-boosted spontaneous abortion in the CBA x DBA/2 mouse model. I. Synergistic effect of LPS and (TNF-alpha + IFN-gamma) on pregnancy loss. Am J Reprod Immunol. 2004;52:370–378.
[15] Deb K, Chaturvedi MM, Jaiswal YK. A 'minimum dose' of lipopolysaccharide required for implantation failure: assessment of its effect on the maternal reproductive organs and interleukin-1alpha expression in the mouse. Reproduction. 2004;128:87–97.
[16] Kolben TM, Rogatsch E, Hester A, et al. Involvement of ILR4alpha and TLR4 in miscarriages. J Reprod Immunol. 2019;131:36–43.
[17] Razdaibiedina A, Khobzey M, Tkachenko V, et al. Effects of single-nucleotide polymorphisms in cytokine, toll-like receptor, and progesterone receptor genes on risk of miscarriage. Obstet Gynecol Int. 2018;2018:9272749.
[18] Frantz S, Ertl G, Bauersachs J. Mechanisms of disease: toll-like receptors in cardiovascular disease. Nat Clin Pract Cardiovasc Med. 2007;4:444–454.
[19] Molvarec A, Jermendy A, Kovacs M, et al. Toll-like receptor 4 gene polymorphisms and preeclampsia: lack of association in a Caucasian population. Hypertens Res. 2008;31:859–864.
[20] Pandey S, Mittal RD, Srivastava M, et al. Impact of toll-like receptors [TLR] 2 (−196 to −174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. Gynecol Oncol. 2009;114:501–505.
[21] Ojeda-Ojeda M, Martinez-Garcia MA, Alpanes M, et al. Association of TLR2 S450S and ICAM1 K469E polymorphisms with polycystic ovary syndrome (PCOS) and obesity. J Reprod Immunol. 2016;113:9–15.
[22] Li P, Wu HL, Dong BH. Relationship between TLR4 and CCL2 expression and recurrent spontaneous abortion. Genet Mol Res. 2016;15:gmr6882.
[23] Jiao Y, Zhao J, Hu B, et al. Toll-like receptor 4 gene is associated with recurrent spontaneous miscarriage in Uygur and Han women in Xinjiang. Exp Ther Med. 2016;12:3268–3274.
[24] Wujcicka W, Paradowska E, Studzinska M, et al. Toll-like receptors genes polymorphisms and the occurrence of HCMV infection among pregnant women. Virol J. 2017;14:64.
[25] Liu J, Radler D, Illi S, et al. TLR2 polymorphisms influence neonatal regulatory T cells depending on maternal atopy. Allergy. 2011;66:1020–1029.
[26] Taniguchi R, Koyano S, Suzutani T, et al. Polymorphisms in TLR-2 are associated with congenital cytomegalovirus (CMV) infection but not with congenital CMV disease. Int J Infect Dis. 2013;17:e1092–1097.
[27] Qiu X, Dong Y, Cao Y, et al. Correlation between TLR2, TLR3, TLR4, and TLR9 polymorphisms and susceptibility to and prognosis of severe hepatitis among the newborns. J Clin Lab Anal. 2018;32:e22292.
[28] Royse KE, Kempf MC, McGwin G Jr., et al. Toll-like receptor gene variants associated with bacterial vaginosis among HIV-1 infected adolescents. J Reprod Immunol. 2012;96:84–89.
[29] Sauna ZE, Kimchi-Sarfaty C. Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet. 2011;12:683–691.
[30] Ryckman KK, Williams SM, Krohn MA, et al. Genetic association of toll-like receptor 4 with cervical cytokine concentrations during pregnancy. Genes Immun. 2009;10:636–640.
[31] Kolz M, Baumert J, Muller M, et al. Association between variations in the TLR4 gene and incident type 2 diabetes is modified by the ratio of total cholesterol to HDL-cholesterol. BMC Med Genet. 2008;9:9.
[32] Semlali A, Reddy Parine N, Arafah M, et al. Expression and polymorphism of toll-like receptor 4 and effect on NF-kappaB mediated inflammation in colon cancer patients. PLoS One. 2016;11:e0146333.
[33] Semlali A, Jalouli M, Parine NR, et al. Toll-like receptor 4 as a predictor of clinical outcomes of estrogen receptor-negative breast cancer in Saudi women. Onco Targets Ther. 2017;10:1207–1216.
[34] Urcelay E, Blanco-Kelly F, de Las Heras V, et al. TLR4 haplotypes in multiple sclerosis: a case-control study in the Spanish population. J Neuroimmunol. 2007;192:215–218.
[35] Ryckman KK, Williams SM, Krohn MA, et al. Interaction between interleukin-1 receptor 2 and toll-like receptor 4, and cervical cytokines. J Reprod Immunol. 2011;90:220–226.