Polymorphisms in interleukin genes and their association with the risk of recurrent pregnancy loss

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Interleukins play important roles in pregnancy. Altered expression and splicing of various interleukins have been linked to the pathophysiology of recurrent pregnancy loss. Polymorphisms in interleukin genes can affect the expression and/or splicing of their respective genes and thus influence the risk of recurrent pregnancy loss. In this work, we examined the association between the *IL1B* rs16944, *IL1B* rs1143634, *IL6* rs1800795, *IL6* rs1800796, *IL10* rs1800896 and *IL18* rs187238 polymorphisms and recurrent pregnancy loss risk in a Chinese population. Study subjects comprised 598 idiopathic recurrent pregnancy loss patients and 603 controls. The genotyping was accomplished by PCR-RFLP. Regression analysis was performed to evaluate the disease association. After adjustment by Bonferroni correction, only the *IL1B* rs16944 and *IL6* rs1800796 polymorphisms were significantly associated with risk of recurrent pregnancy loss. The heterozygous TC genotype of *IL1B* rs16944 had an adjusted odds ratio (aOR) of 1.4209 (1.1302–1.8929) ($P = 0.0019$), while the homozygous CC genotype had an aOR of 1.7398 (1.2133–2.3203) ($P = 0.0008$). A significant association was also observed for the C allele [aOR = 1.3747 (1.1296–1.8972)] ($P = 0.0003$). For *IL6* rs1800796, the heterozygous CG genotype, the homozygous GG genotype and the G allele had aORs of 0.7342 (0.4412–0.8423) ($P = 0.0016$), 0.5424 (0.1768–0.7865) ($P = 0.0014$) and 0.7009 (0.4511–0.8034) ($P = 0.0007$), respectively. In summary, the *IL1B* rs16944 and *IL6* rs1800796 variants were associated with an increased and a decreased recurrent pregnancy loss risk, respectively.

Key words: recurrent pregnancy loss, association, genetic polymorphisms, odds ratio

INTRODUCTION

Recurrent pregnancy loss refers to repeated and consecutive (≥ 3 times) spontaneous abortion events that occur before the 20th gestational week of pregnancy. This condition causes substantial emotional stress to the patient and her family. It is estimated that as many as 1–2% of healthy women experience recurrent pregnancy loss (Diejomaoh, 2015). Several factors are known to contribute to this condition, such as advanced maternal age, abnormalities of the uterus and endocrine diseases (Garrido-Gimenez and Alijotas-Reig, 2015). However, these factors can only explain the etiology of the condition in a small subset of patients. Up to 60% of recur-

rent pregnancy losses have an unknown or unexplained cause, and represent a major challenge in reproductive medicine (Diejomaoh, 2015).

Genetic polymorphisms may be at least partially responsible for the incidence of these unexplained recurrent pregnancy losses (Daher et al., 2012). Cytokines, particularly interleukins, are important mediators in many reproductive events (Prins et al., 2012; Cheng and Sharma, 2015; Southcombe et al., 2015). IL-1B, for example, is crucial for the regulation of immune responsiveness of Th1/Th2 cells, which is necessary for a successful pregnancy (Agrawal et al., 2012; Sykes et al., 2012). IL-6 is involved in the modulation of embryonic implantation and placental development (Prins et al., 2012). IL-10 actively suppresses the maternal immune system to avoid rejection of the fetal allograft (Thaxton and Sharma, 2010; Cheng and Sharma, 2015). On the other hand, IL-18 may stimulate the production of

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interferon-gamma, which is needed for the remodeling of the blood vessels during pregnancy (Weissenbacher et al., 2014). Given the importance of these interleukins in pregnancy, a number of studies have investigated the association between polymorphisms in interleukin genes and the risk of recurrent pregnancy loss. Although the results obtained are often contradictory, the studies do provide some indications that functional polymorphisms, which are known to influence the expression of their respective genes, can influence the risk of recurrent pregnancy loss (Daher et al., 2012).

Among the most commonly studied interleukin polymorphisms with regard to their relationship with recurrent pregnancy loss risk are the *IL1B* rs16944, *IL1B* rs1143634, *IL6* rs1800795, *IL6* rs1800796, *IL10* rs1800896 and *IL18* rs187238 polymorphisms (meta-analyzed in Agrawal et al., 2012; Chen et al., 2015; Lee et al., 2015; Woods et al., 2015; Su et al., 2016). Except for *IL1B* rs1143634, all other polymorphisms occur in the promoter regions of their genes and are thought to affect the rate of gene transcription. On the other hand, *IL1B* rs1143634 is predicted to disrupt the splicing process (Woods et al., 2015), which can then influence the normal function of *IL-1B* and, as a result, the risk of recurrent pregnancy loss. The association of these polymorphisms has been investigated in several populations, but few studies have been conducted in the Chinese population (Agrawal et al., 2012; Chen et al., 2015; Lee et al., 2015; Woods et al., 2015; Su et al., 2016).

For the *IL1B* rs16944 polymorphism, two studies have demonstrated an association between the variant allele and the risk of recurrent pregnancy loss in a Korean population (Kim et al., 2014) and a Chinese population (Ma et al., 2017). However, another study conducted in China showed a lack of association, which indicates that the effect of the polymorphism differs even among distinct geographical areas of the same population (Ma et al., 2012). In addition, a study from the UK also failed to find an association between the polymorphism and risk of recurrent pregnancy loss (Linjawi et al., 2005). Besides, the association of the *IL1B* rs1143634 polymorphism and recurrent pregnancy loss risk has been investigated in two populations in China (Ma et al., 2012, 2017). No significant association was found in either report. Nevertheless, no study has yet been conducted on these two *IL1B* polymorphisms in the northwestern part of China.

Similarly, for *IL6* rs1800795 and rs1800796 polymorphisms, contradictory findings have been observed in several reports. For the former, Demirturk et al. (2014) and Parveen et al. (2013) demonstrated an association of the polymorphism with risk of recurrent pregnancy loss in Turkish and northern Indian populations, respectively. However, no significant association was found in Saudi Arabian (Alkhuriji et al., 2013), Iranian (Bahadori et al., 2014) or Romanian populations (Bohiletea and

Radoi, 2014). In eastern China, Ma et al. (2017) also found no significant association of the polymorphism with recurrent pregnancy loss. The effect of the polymorphism in northwestern China remains unknown. On the other hand, for rs1800796, a few studies have reported a significant association with recurrent pregnancy loss (Karhukorpi et al., 2001; Qaddourah et al., 2014; Ma et al., 2017), although there is also one study that found no significant association (Alkhuriji et al., 2013).

IL10 rs1800896 is another commonly studied polymorphism in recurrent pregnancy loss. Previous work has shown a positive link between this polymorphism and the condition in several populations (Parveen et al., 2013; Bahadori et al., 2014), but contradictory findings were again noted in other populations (Karhukorpi et al., 2001; Kamali-Sarvestani et al., 2005; Alkhuriji et al., 2013; Qaddourah et al., 2014; Ma et al., 2017). Likewise, for the *IL18* rs187238 polymorphism, two studies demonstrated an association between the variant allele and risk of recurrent pregnancy loss (Wang et al., 2014; Yue et al., 2015), but others failed to find a significant association (Ostojić et al., 2007; Al-Khateeb et al., 2011; Messaoudi et al., 2012). These inconsistent results show that the role of the polymorphisms in recurrent pregnancy loss differs from population to population. Because the effect of these polymorphisms on Han Chinese from northwestern China remains unknown, the current study aimed to investigate the association of *IL1B* rs16944, *IL1B* rs1143634, *IL6* rs1800795, *IL6* rs1800796, *IL10* rs1800896 and *IL18* rs187238 polymorphisms with the risk of recurrent pregnancy loss among Han Chinese in Shaanxi, China.

MATERIALS AND METHODS

Sample size The sample size required for this study was calculated using the Quanto program by considering the probability of exposure in both cases and controls, the difference between which was set at 7.73% based on our preliminary (unpublished) investigation. To achieve a study power of 0.9, a minimum of 603 cases and 603 controls was required. We enrolled these numbers into the study, but five cases withdrew before the completion of the research. Because of this, the final number of subjects recruited was 598 cases and 603 controls. This yielded a study power of 0.896, which is still sufficiently large for the analysis. Written informed consent was obtained from the subjects before they participated in the study. The study was conducted with approval from the Northwest Women's and Children's Hospital (approval no: 00345981231) and Xi'an Fourth Hospital (approval no: 0000001298173-AKK), Xi'an, Shaanxi, China.

Inclusion and exclusion criteria To be included in the study, cases must have experienced three or more occurrences of unexplained pregnancy loss before the 20th ges-

tational week. Cases with known causes of pregnancy loss were excluded from the study. For controls, the inclusion criterion was having had a minimum of three successful live births. Controls with a history of spontaneous abortions or preterm birth were excluded. All subjects were Han Chinese and were recruited from Xi'an, Shaanxi province between December 2014 and November 2017.

Genetic analysis All polymorphisms were genotyped with PCR-RFLP. PCR was performed with Taq PCR MasterMix (TIANGen, China) and the primers used are listed in Table 1. Following PCR, the amplicons were digested with restriction enzymes (Table 2) at 37 °C overnight. All restriction enzymes were purchased from New England Biolabs (USA). Next, the digested fragments were electrophoresed on a 2% agarose gel. The electrophoresis bands were stained with ethidium bromide and visualized on a UV transilluminator to identify the genotype of the polymorphisms based on their sizes (Table 2).

Statistical analysis Statistical analysis was performed with IBM SPSS. A chi-squared test was used to compare categorical variables between the subjects, and a *t*-test was used to compare numerical variables. A goodness-of-fit test was used to examine deviation of the polymorphisms from Hardy–Weinberg equilibrium. A *P* value of < 0.05 was considered significant for the above

analyses. Logistic regression was used to calculate the odds ratio (OR) for determining the association between the polymorphisms and the risk of recurrent pregnancy loss. The major (wild-type) alleles and genotypes were chosen as the references in this analysis. The OR was adjusted for age, smoking habits and alcohol consumption habits of the subjects. Since we examined six polymorphisms in this study, multiple comparisons error was corrected with the Bonferroni correction. For this reason, a *P* value of < 0.008 was considered significant for the

Table 1. PCR primers used for amplification of DNA

Polymorphism	PCR primers
<i>IL1B</i> rs16944	Forward, 5'TGGCATTGATCTGGTTCATC3' Reverse, 5'GTTTAGGAATCTTCCCACTT3'
<i>IL1B</i> rs1143634	Forward, 5'GTTGTCATCAGACTTTGACC3' Reverse, 5'TTCAGTTCATATGGACCAGA3'
<i>IL6</i> rs1800795	Forward, 5'GGAGTCACACACTCCACCT3' Reverse, 5'GTGGGGCTGATTGGAACCC3'
<i>IL6</i> rs1800796	Forward, 5'GAGAGGCCTTGAAGTAACTG3' Reverse, 5'AACCAAAGATGTTCTGAAGTGA3'
<i>IL10</i> rs1800896	Forward, 5'GTAAGCTTCTGTGGCTGGAGT3' Reverse, 5'TTCCAGATATCTGAAGAAGTCCTG3'
<i>IL18</i> rs187238	Forward, 5'TTGTAACATTGTAGGAATTACC3' Reverse, 5'ATGTAATATCACTATTTTATGAGA3'

Table 2. Restriction enzymes used for genotyping of polymorphisms and the band sizes obtained on agarose gels

Polymorphism	Restriction enzyme	Band(s) obtained on agarose
<i>IL1B</i> rs16944	AvaI	TT, 1 band (305 bp) TC, 3 bands (305 bp, 190 bp and 115 bp) CC, 2 bands (190 bp and 115 bp)
<i>IL1B</i> rs1143634	TaqI	CC, 2 bands (136 bp and 114 bp) CT, 3 bands (250 bp, 136 bp and 114 bp) TT, 1 band (250 bp)
<i>IL6</i> rs1800795	SfaNI	GG, 1 band (532 bp) GC, 3 bands (532 bp, 474 bp and 58 bp) CC, 2 bands (474 bp and 58 bp)
<i>IL6</i> rs1800796	BsrBI	CC, 1 band (180 bp) CG, 3 bands (180 bp, 120 bp and 60 bp) GG, 2 bands (120 bp and 60 bp)
<i>IL10</i> rs1800896	MnII	AA, 1 band (130 bp) AG, 3 bands (130 bp, 100 bp and 30 bp) GG, 2 bands (100 bp and 30 bp)
<i>IL18</i> rs187238	EcoRI	GG, 2 bands (107 bp and 24 bp) GC, 3 bands (131 bp, 107 bp and 24 bp) CC, 1 band (131 bp)

association analyses.

RESULTS

The attributes of the cases are shown in Table 3. The ages of both cases and controls ranged from 27–39 years, and mean ages were similar in both groups: 33.34 ± 3.65 years in cases and 33.17 ± 3.76 years in controls. Among the cases, 83 (13.9%) were smokers. The prevalence was similar to the controls, of whom 72 (11.9%) were smokers. With regard to alcohol consumption habits, 491 (82.1%) cases and 509 (84.4%) controls never drank or were occasional drinkers, in comparison with 93 (15.6%) cases and 86 (14.3%) who were regular drinkers and 14 (2.3%) cases and 8 (1.3%) controls who were heavy drinkers. Despite this, none of the subjects drank any alcohol during pregnancy. No significant difference was observed between cases and controls in age, smoking habits or alcohol consumption habits ($P > 0.05$, Table 3).

The distributions of *IL1B* rs16944, *IL1B* rs1143634, *IL6* rs1800795, *IL6* rs1800796, *IL10* rs1800896 and *IL18* rs187238 polymorphisms are shown in Table 4. All polymorphisms conformed to Hardy–Weinberg equilibrium (Table 4), suggesting that the distribution was random and representative of the general population.

Odds ratios (ORs) were calculated to measure the association between the polymorphisms and risk of recurrent pregnancy loss. The results are shown in Table 5. Among the six polymorphisms, only *IL1B* rs16944

and *IL6* rs1800796 polymorphisms demonstrated a significant association with risk of recurrent pregnancy loss among our subjects when the P value threshold was set at < 0.008 following Bonferroni correction. The het-

Table 4. Distribution of polymorphisms and deviation from HWE

Polymorphism	Cases	Controls	Hardy–Weinberg P value (control)
<i>IL1B</i> rs16944			0.1568
TT	155 (25.9%)	218 (36.2%)	
TC	294 (49.2%)	275 (45.6%)	
CC	149 (24.9%)	110 (18.2%)	
T	604 (50.5%)	711 (59.0%)	
C	592 (49.5%)	495 (41.0%)	
<i>IL1B</i> rs1143634			0.9296
CC	482 (80.6%)	474 (78.6%)	
CT	111 (18.6%)	121 (20.1%)	
TT	5 (0.8%)	8 (1.3%)	
C	1075 (89.9%)	1069 (88.6%)	
T	121 (10.1%)	137 (11.4%)	
<i>IL6</i> rs1800795			0.4159
GG	372 (62.2%)	369 (61.2%)	
GC	197 (32.9%)	201 (33.3%)	
CC	29 (4.8%)	33 (5.5%)	
G	941 (78.7%)	939 (77.9%)	
C	255 (21.3%)	267 (22.1%)	
<i>IL6</i> rs1800796			0.2401
CC	435 (72.7%)	369 (61.2%)	
CG	147 (24.6%)	199 (33.0%)	
GG	16 (2.7%)	35 (5.8%)	
C	1017 (85.0%)	937 (78.1%)	
G	179 (15.0%)	269 (21.9%)	
<i>IL10</i> rs1800896			0.2478
AA	524 (87.6%)	512 (84.9%)	
AG	70 (11.7%)	85 (14.1%)	
GG	4 (0.7%)	6 (1.0%)	
A	1118 (93.5%)	1109 (92.0%)	
G	78 (6.5%)	97 (8.0%)	
<i>IL18</i> rs187238			0.1430
GG	458 (76.6%)	441 (73.1%)	
GC	128 (21.4%)	144 (23.9%)	
CC	12 (2.0%)	18 (3.0%)	
G	1044 (87.3%)	1026 (85.1%)	
C	152 (12.7%)	180 (14.9%)	

Table 3. Attributes of cases and controls

Attribute	Cases	Controls	P
N	598	603	–
Age			0.4172
Mean	33.34 ± 3.65	33.17 ± 3.76	
Range	27–39	27–39	
Smoking habit			0.3162
Yes	83 (13.9%)	72 (11.9%)	
No	515 (86.1%)	531 (88.1%)	
Alcohol consumption			0.3307
Never/occasional	491 (82.1%)	509 (84.4%)	
Regular	93 (15.6%)	86 (14.3%)	
Heavy	14 (2.3%)	8 (1.3%)	
Alcohol consumption during pregnancy			1.0000
Yes	0 (0.0%)	0 (0.0%)	
No	598 (100.0%)	603 (100.0%)	

Table 5. Odds ratios for the association with recurrent pregnancy loss risk

Polymorphism	OR (95% CI)	<i>P</i>	Adjusted OR (95% CI)	Adjusted <i>P</i>
<i>IL1B</i> rs16944				
TT	Ref	–	Ref	–
TC	1.5036 (1.1553–1.9570)	0.0024	1.4209 (1.1302–1.8929)	0.0019
CC	1.9051 (1.3819–2.6265)	0.0001	1.7398 (1.2133–2.3203)	0.0008
T	Ref	–	Ref	–
C	1.4078 (1.1981–1.6542)	< 0.0001	1.3747 (1.1296–1.8972)	0.0003
<i>IL1B</i> rs1143634				
CC	Ref	–	Ref	–
CT	0.9021 (0.6770–1.2022)	0.4820	0.9493 (0.5393–1.7634)	0.6753
TT	0.6146 (0.1996–1.8923)	0.3962	0.7865 (0.3302–2.3241)	0.5031
C	Ref	–	Ref	–
T	0.8783 (0.6780–1.1377)	0.3256	0.8351 (0.5354–1.3240)	0.4623
<i>IL6</i> rs1800795				
GG	Ref	–	Ref	–
GC	0.9722 (0.7620–1.2404)	0.8205	0.7524 (0.5931–1.2012)	0.7545
CC	0.8717 (0.5186–1.4651)	0.6043	0.7440 (0.4290–1.1354)	0.5354
G	Ref	–	Ref	–
C	0.9530 (0.7850–1.1570)	0.6269	0.7435 (0.4775–1.1467)	0.6566
<i>IL6</i> rs1800796				
CC	Ref	–	Ref	–
CG	0.6266 (0.4859–0.8081)	0.0003	0.7342 (0.4412–0.8423)	0.0016
GG	0.3878 (0.2112–0.7119)	0.0022	0.5424 (0.1768–0.7865)	0.0014
C	Ref	–	Ref	–
G	0.6131 (0.4975–0.7555)	< 0.0001	0.7009 (0.4511–0.8034)	0.0007
<i>IL10</i> rs1800896				
AA	Ref	–	Ref	–
AG	0.8047 (0.5733–1.1294)	0.2089	0.7431 (0.3243–1.4289)	0.3546
GG	0.6514 (0.1827–2.3219)	0.5086	0.6680 (0.3012–1.4901)	0.5475
A	Ref	–	Ref	–
G	0.7977 (0.5854–1.0869)	0.1521	0.6952 (0.5340–1.3948)	0.4359
<i>IL18</i> rs187238				
GG	Ref	–	Ref	–
GC	0.8559 (0.6523–1.1230)	0.2615	0.6289 (0.5434–1.1396)	0.6444
CC	0.6419 (0.3056–1.3482)	0.2417	0.5333 (0.3524–1.2043)	0.4309
G	Ref	–	Ref	–
C	0.8299 (0.6577–1.0471)	0.1159	0.6027 (0.4359–1.3435)	0.3082

erozygous TC genotype of *IL1B* rs16944 polymorphism was found to contribute to a higher risk of recurrent pregnancy loss, with OR 1.5036 (1.1553–1.9570) ($P = 0.0024$). Similarly, for the homozygous CC genotype, an increased risk was observed, with OR 1.9051 (1.3819–2.6265) ($P = 0.0001$). With the above observation, it

is not surprising that the C allele also increased recurrent pregnancy loss risk, with OR 1.4078 (1.1981–1.6542) ($P < 0.0001$). After adjusting for clinical attributes of the subjects (age, smoking habits and alcohol consumption habits), all the genotypes and the allele were still significantly associated with increased risk for recurrent

pregnancy loss. The heterozygous TC genotype reported an OR of 1.4209 (1.1302–1.8929) ($P = 0.0019$). In comparison, the homozygous CC genotype increased the risk with OR 1.7398 (1.2133–2.3203) ($P = 0.0008$). For the C allele, an OR of 1.3747 (1.1296–1.8972) ($P = 0.0003$) was seen.

On the contrary, the *IL6* rs1800796 variant was significantly associated with a decreased risk of recurrent pregnancy loss. The heterozygous CG genotype had an OR of 0.6266 (0.4859–0.8081) ($P = 0.0003$) and an adjusted OR of 0.7342 (0.4412–0.8423) ($P = 0.0016$), while the homozygous GG genotype had an OR of 0.3878 (0.2112–0.7119) ($P = 0.0022$) and an adjusted OR of 0.5424 (0.1768–0.7865) ($P = 0.0014$). The G allele was also found to contribute to a decreased recurrent pregnancy loss risk, with OR 0.6131 (0.4975–0.7555) ($P < 0.0001$) and adjusted OR 0.7009 (0.4511–0.8034) ($P = 0.0007$).

DISCUSSION

In this work, we investigated the association of six interleukin gene polymorphisms with the risk of recurrent pregnancy loss. We observed a significant relationship for only two polymorphisms. First, the *IL1B* rs16944 variant was found to be significantly associated with an increased risk of recurrent pregnancy loss. This polymorphism is caused by a T-to-C substitution at the –511 position of the gene promoter. For this reason, the rs16944 polymorphism has been shown to affect the expression of the *IL1B* gene. However, contradictory findings have been observed with regard to whether the polymorphism increases or decreases *IL1B* expression (Hwang et al., 2002; Iacoviello et al., 2005), although it has been shown that the *cis*-acting effect of the *IL1B* –31T/–511C haplotype is associated with increased expression compared with the –31C/–511T haplotype (Kimura et al., 2004). Nevertheless, we postulate that if the expression of the gene is not maintained at an optimum level, it poses a risk for pregnancy loss. Thus, the expression of the gene must be kept at a well-regulated level.

Our finding that the rs16944 variant was associated with an increased recurrent pregnancy loss risk was in agreement with some previous work (Kim et al., 2014; Ma et al., 2017), although other studies have reported no association between the polymorphism and recurrent pregnancy loss risk (Linjawi et al., 2005; Ma et al., 2012). This inconsistency may be due to the different study sizes employed. Our results agreed with those of two studies with larger sample sizes (Kim et al., 2014; Ma et al., 2017), but disagreed with two studies with smaller sample sizes (Linjawi et al., 2005; Ma et al., 2012). The study by Kim et al. (2014) comprised 385 cases and 232 controls and noted an OR of 1.826 (1.130–2.953) for the variant genotype. Besides, the study by Ma et al. (2017)

comprised 775 cases and 805 controls and showed that the variant genotype had an OR of 1.377 (1.039–1.824). In comparison, Linjawi et al. (2005) analyzed only 206 cases and 224 controls, while Ma et al. (2012) studied the polymorphism in 162 cases and 156 controls. No significant association was observed for the two latter studies. Studies involving a lower sample size may generate false-negative results, as their power may not be sufficient to detect the presence of a true association.

As well as the rs16944 polymorphism, we also studied the *IL1B* rs1143634 polymorphism. We found no association between this polymorphism and the risk of recurrent pregnancy loss. rs1143634 is caused by a C-to-T substitution that may affect the splicing of the transcript (Woods et al., 2015). Our results suggest that the splicing event plays no significant role in recurrent pregnancy loss. To date, two previous studies have investigated the association between the rs1143634 polymorphism and recurrent pregnancy loss risk (Ma et al., 2012, 2017). Both studies were conducted in Chinese populations. Taking together the results from this and previous work, we conclude that the rs1143634 polymorphism is not an important factor in recurrent pregnancy loss.

We also investigated two polymorphisms in the *IL6* gene, whose product plays a role in embryonic implantation and placental development (Prins et al., 2012). We examined the rs1800795 and rs1800796 polymorphisms in our study. Both polymorphisms are located in the promoter region of the gene, which could influence the gene's expression. The rs1800795 polymorphism was not found to be associated with recurrent pregnancy loss, but the rs1800796 polymorphism was found to protect against recurrent pregnancy loss. Similar to the *IL1B* polymorphism, our observations on the rs1800795 and rs1800796 polymorphisms agreed with some previous findings and disagreed with others. For rs1800795, two previous studies observed a significant association with an increased recurrent pregnancy loss risk, with OR = 3.57 (Demirturk et al., 2014) and 1.78 (Parveen et al., 2013), while four previous studies did not (Alkhuriji et al., 2013; Bahadori et al., 2014; Bohiltea and Radoi, 2014; Ma et al., 2017). For rs1800796, the majority of previous studies reported findings similar to ours (Karhukorpi et al., 2001; Qaddourah et al., 2014; Ma et al., 2017), although one study failed to find an association between the polymorphism and risk of recurrent pregnancy loss (Alkhuriji et al., 2013). The inconsistency among study findings could be attributed to two reasons. First, as discussed above, lower sample sizes may not have sufficient power to detect an association (or lack thereof). Most of the studies above used only a small sample size: 38 cases for Karhukorpi et al. (2001), 65 for Alkhuriji et al. (2013), 69 for Bohiltea and Radoi (2014), 85 for Bahadori et al. (2014), 113 for Demirturk et al. (2014) and 296 for Qaddourah et al. (2014). Second, the population from

which the subjects were recruited may also affect the results obtained, as differences in the genetic background of different ethnic groups could result in different findings due to high variability in allele frequency among different ethnicities (Dias and Ribeiro, 2011). This may explain why our findings generally concurred with Ma et al. (2017), who also investigated a few overlapping polymorphisms in a Chinese population.

Two other interleukin polymorphisms were investigated in this work, namely *IL10* rs1800896 and *IL18* rs187238. Both polymorphisms are located in the promoter region of the gene. We found no association between either polymorphism and the risk of recurrent pregnancy loss. The association between *IL10* rs1800896 and recurrent pregnancy loss risk has been investigated since 2001, when a lack of association was observed (Karhukorpi et al., 2001). Several replicative studies that followed also found no association between the polymorphism and risk of recurrent pregnancy loss (Kamali-Sarvestani et al., 2005; Alkhuriji et al., 2013; Qaddourah et al., 2014; Ma et al., 2017), although two others did show an association (Parveen et al., 2013; Bahadori et al., 2014). Similarly, for the *IL18* rs187238 polymorphism, our results concurred with three previous reports (Ostojic et al., 2007; Al-Khateeb et al., 2011; Messaoudi et al., 2012), but not with two others that showed a significant association (Wang et al., 2014; Yue et al., 2015). As discussed above, the inconsistency in study results may be due to study size or to genetic and/or geographical features of the population from which the subjects were recruited.

In conclusion, this study demonstrated that *IL1B* rs16944 and *IL6* rs1800796 variants were associated with an increased and a decreased recurrent pregnancy loss risk, respectively. A limitation of the current work is that no replication/validation set of samples was included. Future studies in the Chinese population will be required to replicate our work.

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