Original Paper

Interactive Effects of Snps Located Within CD28/B7 Pathway and Environment on Susceptibility to Recurrent Spontaneous Abortion

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Key Words

Recurrent Spontaneous Abortion • CD28/B7 pathway • Environment Single Nucleotide Polymorphism

Abstract

Aims: This study was aimed to explore the interaction between environment and CD28/B7 pathway to provide the potential epidemiology for prevention and treatment of recurrent spontaneous abortion (RSA). **Methods:** The retrospective study included 630 RSA cases and 1320 healthy women during their middle and late prenatal care. Their living environment was investigated, and the influence of environmental factors on pregnancy abortion was analyzed. The genomic DNAs were extracted from the study subjects, and the polymorphisms of CD28 and B7 were analyzed. Finally, the interaction of gene and environment on RSA was analyzed with the logistic regression analyses. **Results:** The multi-variate regression analysis indicated that vitamin supplement, intake of fresh fruits or vegetables, night shift, staying up late, history miscarriage, as well as history induced abortion were, independently, risk factors for RSA (all $P < 0.05$). Moreover, rs3116496 (T>C), rs3181098 (G>A) and rs3181100 (G>C) of CD28, rs1915087 (C>T) of B7-2, as well as rs6804441 (A>G) and rs41271391 (G>T) of B7-1 were correlated with modified RSA risk (all $P < 0.05$). The haplotypes TGT and TAG could also regulate the risk of RSA (both $P < 0.05$). The synthetic influences of the aforementioned SNPs and environmental factors could also significantly affect the susceptibility to RSA (all $P < 0.05$). **Conclusion:** The interaction of environment and SNPs of CD28/B7 pathway on RSA risk was distinct from CD28/B7 pathway or environment alone.

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Introduction

Spontaneous abortion was manifested as the termination of pregnancy before the 28th week of pregnancy, while recurrent spontaneous abortion (RSA) was nominated when at least 2 times of terminated pregnancies were present before the 28th week of pregnancy [1]. Of note, up to 5% of the infertile couples were induced by RAS, and RAS was found to account for 15~20% of all abortions in China [2]. Besides, the pregnancy abortion that did not accomplish any births before was called primary spontaneous abortion, whereas the secondary abortion was indicated as the abortion that occurred after at least a successful pregnancy. In line with the epidemiological investigations, the odds of pregnancy loss increased with the rising number of abortions, for instance, the probability of abortion could achieve 24% after 2 miscarriages, and it even stood high at 40% after 4 miscarriages [3].

Among the parameters that contributed to RSA, the abnormal functioning of the immune system was suspected to account for certain self-related and unexplained infertilities [4]. Specifically, during the breeding process, embryo, as an antigen, would stimulate the parent body to generate antibodies, when the parent body would form immunologic tolerance to be in a status of immunologic balance. However, once this equilibrium was broken, the spontaneous abortion would take place. It has been previously summarized that self-related immune factors mainly consisted of antiphospholipid antibody (APA), anti-sperm antibody (ASA) and so on, while isoimmunization was associated with humoral NK cells, T cells and so on. Activation of T cells necessitated not only the interaction of antigen-specific T cell receptors (TCRs) and MHC composite on the antigen presented cells (APCs), but also the co-stimulatory signal. The signals were mediated by the interplay of the co-stimulatory receptor with according ligands on the T cells, which were similar in their structures and belonged to the immunoglobulin superfamily.

CD28 was discovered by Hansen in 1980, and it was constitutively expressed on the surface of CD4⁺ T cells, CD8⁺ T cells, CD3⁺ peripheral blood T cells, plasmocytes and NK cells [5]. B7 molecules were mainly expressed on the surface of APCs, and they would provide the co-stimulatory signal when T cells were activated, thereby assisting in T cells' multiplication, secretion of cytokines, and production of cytotoxic effects [6]. For instance, when compared with wide-type mice, the proportion of regulatory T cells (Tregs) within the spleen of B7-1-deficient and CD28-deficient rats, respectively, dropped to 10% and 25% [7]. Above all, the binding of CD28 and B7 could facilitate activation and clonally expansion of T cells, and highly expressed CTLA-4 within activated CD8⁺ T cells would maintain a balanced immune response through inhibiting over-proliferation of T cells [8]. Considering the vital role of CD28/B7 pathway in modifying immune responses and the crucial effects of immune dysfunctions on development of RSA, it was hypothesized that single nucleotide polymorphisms (SNPs) located within CD28 and B7 might contribute to differed consequences of RSA.

In addition, surroundings also seemed to contribute much to RSA. For example, the male animals that were exposed to phthalic acid esters (PAEs) in the early development of embryos were prone to develop such deformations as hypospadias and cryptorchism within their urinary systems [9]. Furthermore, an epidemiological survey based on female workers in the plastic industry found that their incidence of abnormal menstruation, menstrual cycle and menstrual blood volume were significantly higher than other normal industries, which were all hazard factors for menstruation [10].

To sum up, RSA appeared as the combined results of gene and environment, therefore, the current study was aimed to investigate the effects of CD28/B7 and CD28/B7-environment on susceptibility to RSA.

Materials and Methods

Subjects

This retrospective study included 630 RSA cases of Han ethnicities recruited from Affiliated Hospital of Weifang Medical University and Weifang People's Hospital from October 2013 to October 2016. They

were diagnosed as spontaneous abortion after detection of serum human chorionic gonadotropin (HCG) via B-ultrasound measurement and/or electro-chemi-luminescence (ECL) within 13 gestational weeks. The criteria for ultrasonic diagnosis and detection of serum HCG were in accordance with the Ultrasound Diagnosis in Obstetrics and Gynecology published by People's Medical Publishing House. Besides, the Han pregnant women who received perinatal health care in the hospital were incorporated into the control group. Their gestation lasted longer than 13 weeks, and we failed to find any abnormality of fetal growth and development among the pregnant women. All the subjects have signed informed consents. This study was approved by Affiliated Hospital of Weifang Medical University and Weifang People's Hospital and the ethics committee of Affiliated Hospital of Weifang Medical University and Weifang People's Hospital.

Investigation for pregnant women

General conditions included name, birth, body mass index (BMI), career, family income and educational level. The fertility indicators included onset of menstruation, number of conception, frequency of abortion, menstrual condition, eugenics inspection and so on. The indicators relevant to living habit included usage of tableware, intake of milk, fruit, vegetable, puffed food, fried food and barbecue food, drinking, smoking, usage of television, telephone, microwave oven, electromagnetic oven and electric blanket, as well as the frequency of staying up late. The parameters of living condition included existences of decoration, passive smoking, factory pollution, noise, high-tension cable, signal beacon and traffic station. Additional conditions were also investigated, including medication before and during pregnancy, diseases, sexual life, emotion, working stress, physical training and so on.

Definition of certain variables

The subjects were confirmed with smoking history when they smoked one cigarette per day for 1 year. Night shift symbolized the work that sustained from 22 pm to 6 am. The ones that did not rest until after 1 am were believed as staying up late and frequent staying up late indicates it happens at least 3 times a week. In addition, physical exercise should be continued for > 30 min each time, and the post-exercise heart rate should increase by > 30%. Passive smoking represented that the subjects unconsciously inhaled the smoke for ≥ 15 min each time.

Clinical examination for the pregnant women

The subjects all received blood examination, and they did not have abnormalities in chromosomes or thyroid function. Ultra-sound examination was performed to confirm that they did not have malformations in the genital tracts.

Genotyping

The vacuum tubes with ethylene diamine tetraacetic acid (EDTA) anti-coagulant were applied to extract 5 ml peripheral blood from each subject, and then the blood samples were preserved in the -80 °C refrigerator. Genomic DNA was extracted with assistance of the DNA extraction kit (ComWin Biotech Co., Ltd., Beijing, China). In line with the genetic sequence provided by Gene Bank database, the primers were designed based on Premier 5 software (Table 1), and were synthesized by Dalian Takara Biological Engineering Co., Ltd..

Table 1. Primers of the investigated single nucleotide polymorphisms (SNPs)

Gene	SNP	Primers		Restriction enzyme
		Sense	Anti-sense	
CD28	rs3116496	5'-ATAGCTTGCTAGTAACAGT-3'	5'-AAAATAAACACCATAGGC-3'	Acil
	rs3769684	5'-AATAGGTGTAAACGGCAAGA-3'	5'-ACAGCCGACTTCAGGAAA-3'	CviAI
	rs3181098	5'-AAATGCTCCAGAGGGCTAC-3'	5'-TTGGGAAACATCTGCATAAA-3'	Acil
	rs3181100	5'-TGCTGTAAAGTAGATTGGC-3'	5'-GAAACTGAGGTTTGGAGA-3'	Ddel
	rs4673259	5'-ATGGGAAGACTTTCAAGA-3'	5'-TCTGTGGTCATTTTCATCT-3'	Hpy188III
B7-2	rs10932017	5'-GAAGCAGCGGAGAATCCA-3'	5'-CTTCTTACCCAGGCCATT-3'	Ddel
	rs1129055	5'-TTTCTCTTATTCTCCAGA-3'	5'-ATGTATCAGTTTGTGCGC-3'	CviKI-I
	rs17281995	5'-TTACTTAGCCTACAGATGTCC-3'	5'-TTTCCTCTGGTTGCCTTG-3'	MluCI
	rs1915087	5'-AACTGCCTTTTATCTGC-3'	5'-ATACATAAGTCCCACCCA-3'	ApoI
	rs9282641	5'-CCGTCAGTCCTGGCATT-3'	5'-CACAGAAGCAAGGTGGTA-3'	CviQI
B7-1	rs6804441	5'-TGTAATTAACATAACAGGCTA-3'	5'-AGTTTCTCCATTTTCCTC-3'	MseI
	rs41271391	5'-GCGACATTTCCCTTCTA-3'	5'-CGCTTCTTCAACACCCT-3'	MspJI
	rs16829984	5'-TGCTGCCGTCTTCTTCTA-3'	5'-CAGCCACAGCCTCTACAA-3'	BfaI

Genotyping was analyzed based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR amplification system (20 µl) included forward primer (2 µl), reverse primer (2 µl), DNA template (< 1 µg), 2 × EsTaq Master Mix (25 µl) and RNase-free water (up to 50 µl). The PCR reaction conditions were arranged as: 1) pre-degeneration at 94°C for 15 min; 2) 45 cycles of degeneration at 95 °C for 30 s, annealing at 56°C for 30s, and extension at 72 °C for 30 s; and 3) eventual extension at 72 °C for 5 min. Then 10 µl PCR products were added with 5 U endonuclease, 2 µl 10× enzyme buffer and 20 µl ddH₂O, forming the 20 µl reaction system. Digestion was conducted within the water bath at the temperature of 37 °C for 2~16 h, and then the electrophoretic results were observed using gel imager (model: Gel Doc 2000; manufacturer: Bio-RAD corporation). The sequencing of PCR results was accomplished by Shanghai sangon biological engineering corporation.

Statistical analyses

All the statistical analyses were performed with assistance of the SPSS 17.0 software. The associations of genotypes with early abortion were analyzed with univariate logistic regression analysis. Moreover, the parameters of vitamin supplement, intake of fresh fruit or vegetables, alcohol consumption, frequency of smoking, frequency of night shift, frequency of physical exercise, as well as history of miscarriage were adjusted by way of multivariate logistic regression analysis. The correlation degrees were expressed by odds ratios (ORs) and their 95% confidence intervals (CIs).

Results

Effect of certain living habits on the prevalence of RSA

As was shown in Table 2, vitamin supplement, intake of fresh fruits or vegetables, as well as regular physical exercise were all, respectively, correlated with reduced incidence of RSA (OR = 0.75, 95% CI: 0.62-0.90, $P < 0.05$; OR = 0.60, 95% CI: 0.48-0.75, $P < 0.05$; OR = 0.58, 95% CI: 0.48-0.71, $P < 0.05$). In contrast, frequent night shift, frequent staying up late, history of miscarriage and previously induced abortion all appeared to elevate the possibility of suffering from RSA (OR = 1.23, 95% CI: 1.01-1.48, $P < 0.05$; OR = 1.67, 95% CI: 1.36-2.05, $P < 0.05$; OR = 2.94, 95% CI: 2.42-3.58, $P < 0.05$; OR = 2.09, 95% CI: 1.72-2.53, $P < 0.05$). Nonetheless, the results of multivariate analysis (Table 3) indicated that vitamin supplement,

Table 2. Effect of certain living habits on the prevalence of recurrent spontaneous abortion

Living Habits	Case		Control		OR	95%CI
	Number	Percent	Number	Percent		
Vitamin supplement						
Yes	339	53.81%	805	60.98%		
No	291	46.19%	515	39.02%	0.75	0.62-0.90
Fresh fruit or vegetables daily or most days						
Yes	453	71.90%	1069	80.98%		
No	177	28.10%	251	19.02%	0.60	0.48-0.75
Alcohol consumption						
Yes	94	14.92%	158	11.97%		
No	536	85.08%	1162	88.03%	1.29	0.98-1.70
Smoking before and in the first 12 wk of pregnancy (No. of cigarettes)						
No smoking	609	96.67%	1284	97.27%		
1-10/d	16	2.54%	28	2.12%	1.21	0.65-2.24
11-20/d	4	0.63%	7	0.53%	1.22	0.35-4.13
>20/d	1	0.16%	1	0.08%	2.11	0.13-33.79
Frequency of night shift						
≤1 times/wk	302	47.94%	700	53.03%		
≥2 times/wk	328	52.06%	620	46.97%	1.23	1.01-1.48
Frequent staying up late						
Yes	233	36.98%	343	25.98%		
No	397	63.02%	977	74.02%	1.67	1.36-2.05
Regular physical exercise						
Yes	323	51.27%	849	64.32%		
No	307	48.73%	471	35.68%	0.58	0.48-0.71
History of miscarriage						
Yes	344	54.60%	383	29.02%		
No	286	45.40%	937	70.98%	2.94	2.42-3.58
Previous induced abortion						
Yes	309	49.05%	417	31.59%		
No	321	50.95%	903	68.41%	2.09	1.72-2.53

Table 3. Results of multivariate analysis between living habits and the prevalence of recurrent spontaneous abortion

Living Habits	OR	95% CI	P value
Vitamin supplement	0.13	0.02-0.98	0.05
Fresh fruit or vegetables	0.39	0.20-0.78	0.01
Alcohol consumption	0.7	0.47-1.05	0.09
Smoking	0.44	0.19-1.04	0.06
Frequency of night shift	1.78	1.04-3.04	0.04
Frequent staying up late	1.63	1.05-2.52	0.03
Regular physical exercise	0.25	0.03-1.92	0.18
History of miscarriage	166.67	23.26-1000	<0.01
History of induced abortion	36.06	4.66-279.02	0.01
Constant	7.14		<0.01

Table 4. Association of CD28/B7 pathway-related single nucleotide polymorphisms with susceptibility to recurrent spontaneous abortion

Gene	SNP	Allele Change	Genotype	Case Group	Control Group	OR	95% CI	P value
CD28	rs3116496	T>C	T	969	2244	1.00		
			C	291	396	1.70	1.44-2.02	<0.01
			Dominant model			1.78	1.46-2.18	<0.01
			Recessive model			2.53	1.54-4.16	<0.01
			T	1058	2164	1.00		
			C	202	476	0.87	0.72-1.04	0.12
	rs3769684	T>C	Dominant model			0.85	0.69-1.05	0.13
			Recessive model			0.82	0.47-1.46	0.50
			G	731	1636	1.00		
			A	529	1004	1.18	1.03-1.35	0.02
			Dominant model			1.23	1.01-1.50	0.04
			Recessive model			1.26	0.98-1.63	0.07
	rs3181098	G>A	G	314	819	1.00		
			C	946	1821	1.35	1.16-1.58	<0.01
			Dominant model			1.61	1.11-2.34	0.01
			Recessive model			1.42	1.18-1.72	<0.01
			T	1034	2217	1.00		
			C	226	423	1.15	0.96-1.37	0.13
	rs4673259	T>C	Dominant model			1.16	0.95-1.43	0.15
			Recessive model			1.24	0.71-2.17	0.45
			C	654	1426	1.00		
			T	606	1214	1.09	0.95-1.25	0.22
			Dominant model			1.11	0.90-1.38	0.32
			Recessive model			1.13	0.90-1.41	0.31
	rs10932017	C>T	G	1071	2297	1.00		
			A	189	343	1.18	0.98-1.43	0.09
			Dominant model			1.20	0.97-1.48	0.10
			Recessive model			1.34	0.68-2.64	0.39
			G	367	819	1.00		
			C	893	1821	1.09	0.94-1.27	0.23
	rs17281995	G>C	Dominant model			1.14	0.81-1.59	0.46
			Recessive model			1.12	0.92-1.35	0.26
			C	404	738	1.00		
			T	856	1902	0.82	0.71-0.95	0.01
			Dominant model			0.74	0.53-1.02	0.06
			Recessive model			0.80	0.66-0.96	0.02
	rs9282641	G>A	G	781	1557	1.00		
			A	479	1083	0.88	0.77-1.01	0.07
			Dominant model			0.85	0.70-1.04	0.12
			Recessive model			0.84	0.64-1.09	0.18
			A	328	450	1.00		
			G	932	2190	0.58	0.50-0.69	<0.01
	rs41271391	G>T	Dominant model			0.42	0.27-0.65	<0.01
			Recessive model			0.55	0.45-0.67	<0.01
			G	541	950	1.00		
			T	719	1690	0.75	0.65-0.86	<0.01
			Dominant model			0.66	0.51-0.85	<0.01
			Recessive model			0.69	0.57-0.85	<0.01
	rs16829984	G>C	G	1133	2348	1.00		
			C	127	292	0.90	0.72-1.12	0.35
			Dominant model			0.89	0.70-1.14	0.36
			Recessive model			0.86	0.36-2.09	0.74

intake of fresh fruits or vegetables, night shift, staying up late, history miscarriage, as well as history induced abortion could independently regulate the incidence of RSA.

Association of CD28/B7 pathway-related SNPs and haplotypes with susceptibility to RSA

Interestingly, rs3116496 (T>C), rs3181098 (G>A) and rs3181100 (G>C) of CD28 were, respectively, found to be correlated with incremental susceptibility to RSA under the allelic model (OR = 1.70, 95% CI: 1.44-2.02, $P < 0.05$; OR = 1.18, 95% CI: 1.03-1.35, $P < 0.05$; OR =

1.35, 95% CI: 1.16-1.58, $P < 0.05$), while rs1915087 (C>T), rs6804441 (A>G) and rs41271391 (G>T) of B7 were suspected as the factors associated with reduced risk of RSA (OR = 0.82, 95% CI: 0.71-0.95, $P < 0.05$; OR = 0.58, 95% CI: 0.50-0.69, $P < 0.05$; OR = 0.75, 95% CI: 0.65-0.86, $P < 0.05$)(Table 4). Furthermore, for rs3116496, the remarkably enhance RSA risk could also be found among the individuals with genotypes CC/TC, when compared with ones carrying homozygote TT (OR = 1.78, 95% CI: 1.46-2.18, $P < 0.05$). Additionally, subjects carrying genotypes AA/GA of rs3181098 or genotypes CC/GC of rs3181100 were more prone to suffer from RSA than ones carrying homozygote GG (OR = 1.23, 95% CI: 1.01-1.50, $P < 0.05$; OR = 1.61, 95% CI: 1.11-2.34, $P < 0.05$). Slightly different from the result of allelic model, it was shown in the dominant model that merely genotypes GG/GA of rs6804441 and genotypes TT/GT of rs41271391 would significantly lessen the RSA risk in comparison to homozygotes GG and CC (OR = 0.42, 95% CI: 0.27-0.65, $P < 0.05$; OR = 0.66, 95% CI: 0.51-0.85, $P < 0.05$) (Fig. 1).

However, there showed no significant distinctions between RSA cases and healthy controls ($P > 0.05$), regarding the genotyping and allelic distributions of rs3769684 (T>C), rs4673259 (T>C), rs10932017 (C>T), rs1129055 (G>A), rs17281995 (G>C), rs9282641 (G>A) and rs16829984 (G>C). Moreover, the haplotype TGT of B7 were suggested as the protective parameter for RSA (OR = 0.65, 95% CI: 0.53-0.80, $P < 0.05$), whereas the haplotype TAG seemed as a risky element for facilitating development of RSA (OR = 1.79, 95% CI: 1.21-2.66, $P < 0.05$)(Table 5).

Fig. 1. The electrophoretic results for genotyping of eligible SNPs.

Table 5. Association of CD28/B7 pathway-related haplotypes with susceptibility to recurrent spontaneous abortion

Gene	Haplotypes	Case	Control	OR	95% CI	P value
CD28	TGC	211	480	0.88	0.72-1.08	0.22
B7	CGT	85	196	0.89	0.68-1.18	0.43
	CGG	64	110	1.24	0.90-1.72	0.19
	CAT	30	40	1.60	0.99-2.59	0.05
	TGT	181	505	0.65	0.53-0.80	<0.01
	TGG	136	284	1.00	0.80-1.26	0.97
	TAT	63	103	1.31	0.94-1.82	0.10
	TAG	48	58	1.79	1.21-2.66	<0.01

Table 6. Interactive effects of CD28/B7 pathway-related SNPs and vitamin supplement on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Vitamin supplement	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	8	6	2.70	0.92-7.91	0.06
	CC	No	27	24	2.28	1.27-4.07	<0.01
	TC	Yes	118	171	1.40	1.03-1.89	0.03
	TC	No	103	165	1.26	0.93-1.72	0.14
	TT	Yes	213	628	0.69	0.54-0.88	<0.01
	TT	No	161	326	1.00		
rs3181098	AA	Yes	21	50	0.84	0.48-1.48	0.56
	AA	No	90	141	1.28	0.90-1.84	0.17
	GA	Yes	204	445	0.92	0.69-1.24	0.59
	GA	No	103	177	1.17	0.83-1.65	0.37
	GG	Yes	114	310	0.74	0.53-1.02	0.07
	GG	No	98	197	1.00		
rs3181100	CC	Yes	169	264	1.28	0.62-2.63	0.50
	CC	No	186	364	1.02	0.50-2.09	0.95
	GC	Yes	143	438	0.65	0.32-1.34	0.24
	GC	No	93	127	1.46	0.70-3.08	0.31
	GG	Yes	27	103	0.52	0.23-1.18	0.12
	GG	No	12	24	1.00		
rs1915087	TT	Yes	118	441	0.39	0.22-0.69	<0.01
	TT	No	173	244	1.03	0.58-1.84	0.92
	CT	Yes	178	293	0.88	0.50-1.57	0.67
	CT	No	96	239	0.58	0.32-1.06	0.07
	CC	Yes	43	71	0.88	0.45-1.71	0.71
	CC	No	22	32	1.00		
rs6804441	GG	Yes	152	513	0.27	0.12-0.63	<0.01
	GG	No	193	396	0.45	0.19-1.03	0.05
	AG	Yes	156	264	0.54	0.23-1.26	0.15
	AG	No	86	108	0.73	0.31-1.73	0.47
	AA	Yes	31	28	1.01	0.39-2.66	0.98
	AA	No	12	11	1.00		
rs41271391	TT	Yes	99	197	0.38	0.23-0.61	<0.01
	TT	No	106	344	0.23	0.14-0.37	<0.01
	GT	Yes	176	476	0.28	0.18-0.43	<0.01
	GT	No	133	132	0.76	0.47-1.22	0.25
	GG	Yes	64	132	0.36	0.22-0.61	<0.01
	GG	No	52	39	1.00		

The interactive effects of CD28/B7 pathway-related SNPs and living habits on susceptibility to RSA

The results of Table 6 explained that vitamin supplement alone could effectively bring down the incidence of RSA among the carriers of rs3116496 TT (OR = 0.69, 95% CI: 0.54-0.88, $P < 0.05$), and homozygote CC was

implied as the hazard factor for RSA among the ones without vitamin supplement (OR = 2.28, 95% CI: 1.27-4.07, $P < 0.05$). Besides, the risky role of rs3116496 TC seemed to outweigh the protective function of vitamin supplement in contributing to RSA (OR = 1.40, 95% CI: 1.03-1.89, $P < 0.05$). Moreover, the combined influence of rs1915087 TT, rs6804441 GG or rs41271391 TT and vitamin supplement were associated with largely reduced RSA risk (OR = 0.39, 95% CI: 0.22-0.69, $P < 0.05$; OR = 0.27, 95% CI: 0.12-0.63, $P < 0.05$; OR = 0.38, 95% CI: 0.23-0.61, $P < 0.05$).

As for the subjects who frequently ingested fresh vegetables and fruits (Table 7), carriers of rs3116496 TT were less probable to develop RSA than ones without intake of fresh vegetables or fruits (OR = 0.38, 95% CI: 0.29-0.50, $P < 0.05$). Besides, the heterozygote TC of rs3116496 was also associated with decreased susceptibility to RSA, yet its homozygote CC was associated with elevated RSA risk (OR = 4.42, 95% CI: 1.60-12.22, $P < 0.05$). Similarly, rs3181098 AA and rs3181100 CC were tightly linked with the raised prevalence of RSA, considering the crowds who took vegetables and fruits marginally (OR = 2.90, 95% CI: 1.63-5.15, $P < 0.05$; OR = 2.31, 95% CI: 1.06-5.03, $P < 0.05$).

In addition, carriers of rs3116496 CC were more readily to suffer from RSA than carriers of TT, whether they experienced night shift or not (OR = 2.21, 95% CI: 1.06-4.62, $P < 0.05$).

Table 7. Interactive effects of CD28/B7 pathway-related SNPs and fresh fruit or vegetables on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Fresh fruit or vegetables	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	17	25	0.83	0.43-1.61	0.59
	CC	No	18	5	4.42	1.60-12.22	<0.01
	TC	Yes	190	247	0.94	0.70-1.27	0.70
	TC	No	31	89	0.43	0.27-0.68	<0.01
	TT	Yes	246	797	0.38	0.29-0.50	<0.01
rs3181098	TT	No	128	157	1.00		
	AA	Yes	68	155	1.06	0.67-1.69	0.79
	AA	No	43	36	2.90	1.63-5.15	<0.01
	GA	Yes	213	504	1.02	0.69-1.53	0.90
	GA	No	94	118	1.93	1.22-3.05	<0.01
rs3181100	GG	Yes	172	410	1.02	0.68-1.53	0.93
	GG	No	40	97	1.00		
	CC	Yes	245	509	1.20	0.57-2.55	0.63
	CC	No	110	119	2.31	1.06-5.03	0.03
	GC	Yes	179	458	0.98	0.46-2.08	0.95
rs1915087	GC	No	57	107	1.33	0.60-2.97	0.48
	GG	Yes	29	102	0.71	0.31-1.65	0.43
	GG	No	10	25	1.00		
	TT	Yes	193	535	0.54	0.27-1.09	0.08
	TT	No	98	150	0.98	0.48-2.02	0.96
rs6804441	CT	Yes	209	452	0.69	0.35-1.39	0.30
	CT	No	65	80	1.22	0.57-2.58	0.61
	CC	Yes	51	82	0.93	0.44-2.00	0.86
	CC	No	14	21	1.00		
	GG	Yes	271	737	0.59	0.19-1.81	0.35
rs41271391	GG	No	74	172	0.69	0.22-2.17	0.52
	AG	Yes	144	301	0.77	0.25-2.38	0.64
	AG	No	98	71	2.21	0.69-7.03	0.17
	AA	Yes	38	31	1.96	0.58-6.60	0.27
	AA	No	5	8	1.00		
rs41271391	TT	Yes	137	439	0.57	0.31-1.06	0.07
	TT	No	68	102	1.22	0.62-2.37	0.57
	GT	Yes	217	490	0.81	0.44-1.49	0.49
	GT	No	90	118	1.39	0.72-2.67	0.32
	GG	Yes	99	140	1.29	0.68-2.46	0.44
	GG	No	17	31	1.00		

Table 8. Interactive effects of CD28/B7 pathway-related SNPs and night shift on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Night shift	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	14	16	2.21	1.06-4.62	0.03
	CC	No	21	14	3.78	1.88-7.61	<0.01
	TC	Yes	92	178	1.30	0.96-1.77	0.09
	TC	No	129	158	2.06	1.54-2.75	<0.01
	TT	Yes	196	505	0.98	0.77-1.24	0.86
rs3181098	TT	No	178	449	1.00		
	AA	Yes	54	101	1.15	0.77-1.71	0.50
	AA	No	57	90	1.36	0.91-2.03	0.13
	GA	Yes	147	330	0.96	0.71-1.29	0.76
	GA	No	160	292	1.17	0.87-1.58	0.29
rs3181100	GG	Yes	101	269	0.81	0.58-1.11	0.19
	GG	No	111	238	1.00		
	CC	Yes	172	333	1.29	0.78-2.15	0.32
	CC	No	183	295	1.55	0.93-2.58	0.09
	GC	Yes	115	300	0.96	0.57-1.61	0.87
rs1915087	GC	No	121	265	1.14	0.68-1.92	0.62
	GG	Yes	15	67	0.56	0.27-1.17	0.12
	GG	No	24	60	1.00		
	TT	Yes	138	363	0.61	0.37-1.00	0.05
	TT	No	153	322	0.76	0.46-1.25	0.28
rs6804441	CT	Yes	129	282	0.73	0.44-1.21	0.22
	CT	No	145	250	0.93	0.56-1.53	0.77
	CC	Yes	35	55	1.02	0.55-1.90	0.95
	CC	No	30	48	1.00		
	GG	Yes	165	482	0.28	0.15-0.54	<0.01
rs41271391	GG	No	180	427	0.34	0.18-0.66	<0.01
	AG	Yes	116	197	0.48	0.25-0.94	0.03
	AG	No	126	175	0.59	0.3-1.14	0.12
	AA	Yes	21	21	0.82	0.34-1.95	0.65
	AA	No	22	18	1.00		
rs41271391	TT	Yes	88	287	0.61	0.39-0.96	0.03
	TT	No	117	254	0.92	0.59-1.43	0.71
	GT	Yes	138	322	0.86	0.56-1.32	0.48
	GT	No	171	286	1.20	0.78-1.83	0.41
	GG	Yes	76	91	1.67	1.03-2.72	0.04
	GG	No	40	80	1.00		

0.05; OR = 3.78, 95% CI: 1.88-7.61, $P < 0.05$) (Table 8). On the contrary, the rs6804441 GG was associated with depressed RSA risk than AA (OR = 0.28, 95% CI: 0.15-0.54, $P < 0.05$; OR = 0.34, 95% CI: 0.18-0.66, $P < 0.05$). Moreover, although night shift contributed to increased incidence of RSA (OR = 1.67, 95% CI: 1.03-2.72, $P < 0.05$), the rs41271391 TT could reverse the single effect of night shift (OR = 0.61, 95% CI: 0.39-0.96, $P < 0.05$).

Among the population who frequently stayed up late, carriers of rs3116496 CC (OR = 7.31, 95% CI: 3.32-16.10, $P <$

0.05) possessed a larger possibility of developing RSA than carriers of TC (OR = 2.54, 95% CI: 1.80-3.59, $P < 0.05$) and TT (OR = 1.66, 95% CI: 1.29-2.15, $P < 0.05$) (Table 9). The TC carriers

Table 9. Interactive effects of CD28/B7 pathway-related SNPs and staying up late on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Staying up late	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	22	9	7.31	3.32-16.10	<0.01
	CC	No	13	21	1.85	0.91-3.76	0.08
	TC	Yes	73	86	2.54	1.80-3.59	<0.01
	TC	No	148	250	1.77	1.38-2.28	<0.01
	TT	Yes	138	248	1.66	1.29-2.15	<0.01
	TT	No	236	706	1.00		
rs3181098	AA	Yes	61	43	3.73	2.40-5.77	<0.01
	AA	No	50	148	0.89	0.61-1.29	0.53
	GA	Yes	94	145	1.70	1.23-2.36	<0.01
	GA	No	213	477	1.17	0.91-1.52	0.22
	GG	Yes	78	155	1.32	0.94-1.85	0.10
	GG	No	134	352	1.00		
rs3181100	CC	Yes	131	157	2.95	1.80-4.84	<0.01
	CC	No	224	471	1.68	1.06-2.68	0.03
	GC	Yes	89	151	2.09	1.25-3.47	<0.01
	GC	No	147	414	1.26	0.78-2.02	0.34
	GG	Yes	13	35	1.31	0.61-2.84	0.49
	GG	No	26	92	1.00		
rs1915087	TT	Yes	62	190	0.94	0.55-1.61	0.82
	TT	No	229	526	1.25	0.78-2.03	0.36
	CT	Yes	131	153	2.47	1.48-4.11	<0.01
	CT	No	143	379	1.09	0.66-1.78	0.74
	CC	Yes	40	31	3.72	1.93-7.14	<0.01
	CC	No	25	72	1.00		
rs6804441	GG	Yes	105	244	0.63	0.30-1.32	0.22
	GG	No	240	665	0.53	0.26-1.08	0.08
	AG	Yes	98	79	1.81	0.84-3.90	0.12
	AG	No	144	293	0.72	0.35-1.50	0.37
	AA	Yes	30	20	2.19	0.89-5.42	0.09
	AA	No	13	19	1.00		
rs41271391	TT	Yes	50	106	0.87	0.53-1.45	0.60
	TT	No	155	435	0.66	0.43-1.01	0.05
	GT	Yes	108	142	1.41	0.89-2.22	0.14
	GT	No	201	466	0.80	0.53-1.21	0.29
	GG	Yes	75	95	1.46	0.90-2.38	0.12
	GG	No	41	76	1.00		

who stayed up early were also liable to suffer from RSA (OR = 1.77, 95% CI: 1.38-2.28, $P < 0.05$). Analogously, genotypes AA and AG of rs3181098 also conferred high incidence of RSA among the population who stayed up late (OR = 3.73, 95% CI: 2.40-5.77, $P < 0.05$; OR = 1.70, 95% CI: 1.23-2.36, $P < 0.05$). The genotypes CC and GC of rs3181100 were also associated with increased risk of RSA among the subjects who stayed up late (OR = 2.95, 95% CI: 1.80-4.84, $P < 0.05$; OR = 2.09, 95% CI: 1.25-3.47, $P < 0.05$). With the stay-up-late population as the research target, it could be found that genotypes CC and TC of rs1915087 were, respectively, correlated with incremental susceptibility to RSA (OR = 3.72, 95% CI: 1.93-7.14, $P < 0.05$; OR = 2.47, 95% CI: 1.48-4.11, $P < 0.05$).

Furthermore, physical exercise was found to positively affect the pregnancy of subjects (OR = 0.58, 95% CI: 0.46-0.74, $P < 0.05$), whereas genotypes CC and TC of rs3116496, respectively, led the positive direction to the negative one (OR = 2.89, 95% CI: 1.32-6.30, $P < 0.05$; OR = 1.68, 95% CI: 1.23-2.31, $P < 0.05$)(Table 10). Carriers of rs3181098 GG were less prone to suffer from RSA when they regularly did physical exercises (OR = 0.68, 95% CI: 0.49-0.95, $P < 0.05$). Moreover, the rs6804441 GG carriers were associated with less incidences of RSA whether they frequently experienced physical exercise or not (OR = 0.20, 95% CI: 0.10-0.40, $P < 0.05$; OR = 0.35, 95% CI: 0.17-0.70, $P < 0.05$). The rs41271391 TT carriers also followed the same trend (OR = 0.33, 95% CI: 0.22-0.50, $P < 0.05$; OR = 0.56, 95% CI: 0.37-0.87, $P < 0.05$).

Interestingly, only if the subjects carried the rs3116496 C allele or had a history of miscarriage, they were usually correlated with higher risk of RSA (all $P < 0.05$)(Table 11). Slightly different from rs3116496, people with the miscarriage history had a high possibility to suffer from RSA regardless of their rs3181098 genotypes (all $P < 0.05$). Besides, miscarriage also conferred ones carrying rs3181100 CC and rs1915087 CT to contract RSA more easily (OR = 6.53, 95% CI: 4.10-10.40, $P < 0.05$; OR = 2.56, 95% CI: 1.55-4.21, $P < 0.05$). The women without experiencing miscarriages were less liable to contract RSA when their genotypes were rs6804441 GG and rs41271391 TT (all $P < 0.05$). The influences brought about by the

Table 10. Interactive effects of CD28/B7 pathway-related SNPs and physical exercise on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Physical exercise	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	18	19	1.77	0.91-3.46	0.09
	CC	No	17	11	2.89	1.32-6.30	0.01
	TC	Yes	113	216	0.98	0.73-1.31	0.88
	TC	No	108	120	1.68	1.23-2.31	<0.01
	TT	Yes	192	614	0.58	0.46-0.74	<0.01
rs3181098	TT	No	182	340	1.00		
	AA	Yes	50	113	0.84	0.56-1.28	0.42
	AA	No	61	78	1.49	0.98-2.26	0.06
	GA	Yes	160	410	0.74	0.55-1.01	0.06
	GA	No	147	212	1.32	0.95-1.83	0.09
rs3181100	GG	Yes	117	326	0.68	0.49-0.95	0.02
	GG	No	95	181	1.00		
	CC	Yes	189	404	1.11	0.63-1.95	0.72
	CC	No	166	224	1.76	0.99-3.11	0.05
	GC	Yes	114	363	0.74	0.42-1.32	0.31
rs1915087	GC	No	122	202	1.43	0.80-2.56	0.23
	GG	Yes	20	82	0.58	0.28-1.19	0.14
	GG	No	19	45	1.00		
	TT	Yes	150	456	0.64	0.40-1.01	0.06
	TT	No	141	229	1.19	0.74-1.92	0.47
rs6804441	CT	Yes	145	352	0.80	0.50-1.27	0.34
	CT	No	128	180	1.38	0.85-2.23	0.19
	CC	Yes	33	41	1.56	0.83-2.92	0.16
	CC	No	32	62	1.00		
	GG	Yes	177	585	0.20	0.10-0.40	<0.01
rs41271391	GG	No	168	324	0.35	0.17-0.70	<0.01
	AG	Yes	124	239	0.35	0.17-0.70	<0.01
	AG	No	118	133	0.59	0.29-1.22	0.15
	AA	Yes	22	25	0.59	0.24-1.42	0.24
	AA	No	21	14	1.00		
rs41271391	TT	Yes	105	348	0.33	0.22-0.50	<0.01
	TT	No	100	193	0.56	0.37-0.87	0.01
	GT	Yes	158	391	0.44	0.29-0.66	<0.01
	GT	No	151	217	0.76	0.50-1.15	0.19
	GG	Yes	59	110	0.58	0.36-0.95	0.03
rs41271391	GG	No	56	61	1.00		

combined role of rs3116496/rs3181098 and induced abortions were quite similar to those by rs3116496/rs3181098 and history of miscarriage (Table 12). It could also be observed that history of induced abortion facilitated carriers of rs3181100 CC/GC and rs41271391 GG/TG more readily to suffer from RSA (all $P < 0.05$).

Discussion

According to the current study, diverse environmental factors might influence the susceptibility to RSA, such as night shift, history of repeated abortions, history of smoking and intake of vitamin supplement (Table 2-3). In fact, disorders of circadian rhythm and sleep deprivation have been demonstrated to cause a series of diseases, including somniphathy, gastrointestinal neurosis, dyssecretosis of insulin, glucocorticoid, thyroid hormones and sex hormones, hypertension and cardiopathy [11, 12]. The long-term night shift was also documented to largely elevate the possibility of suffering from diverse cancers (e.g. breast cancer, ovarian cancer, gastric cancer and intestinal cancer) and RSA [13, 14]. In addition, the repeated abortions often easily induced such gynecological diseases as endometrial base damage, endometritis and intrauterine adhesion, and the generated excessive mental pressure also increased subjects' risk of subsequent pregnancies [15-18]. Furthermore, the toxic substances inside cigarettes could seriously impact the quality of eggs, as well as hinder the division, implantation and growth of embryonic cells, finally leading to loss or dysplasia of embryos. Therefore, either passive smoking or active smoking was the risk parameter of abnormal pregnancies [19]. Moreover, the females all increasingly demanded essential micro-elements, yet 70% of the pregnant women had severe nausea and vomiting of pregnancy, which all accelerated the loss of the micro-elements. And it has been documented that low levels of zinc, copper, iron and selenium could cease the embryonic development and lead to abortion [20]. Furthermore, VD maintained the regular functioning of humoral immunity not merely through keeping the stability of CD4/CD8 ratio, but also by holding the TH1/TH2 immunity in a normal range [21, 22]. Based on the mechanisms, Harvey et al.

Table 11. Interactive effects of CD28/B7 pathway-related SNPs and history of miscarriage on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Miscarriage	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	25	16	6.02	3.15-11.50	<0.01
	CC	No	10	14	2.75	1.20-6.29	0.01
	TC	Yes	133	137	3.74	2.80-4.98	<0.01
	TC	No	88	199	1.70	1.26-2.29	<0.01
	TT	Yes	186	230	3.11	2.42-4.00	<0.01
rs3181098	TT	No	188	724	1.00		
	AA	Yes	67	39	4.72	3.02-7.38	<0.01
	AA	No	42	152	0.76	0.51-1.13	0.18
	GA	Yes	183	161	3.12	2.31-4.21	<0.01
	GA	No	126	461	0.75	0.56-1.00	0.05
rs3181100	GG	Yes	94	183	1.41	1.02-1.95	0.04
	GG	No	118	324	1.00		
	CC	Yes	223	115	6.53	4.10-10.40	<0.01
	CC	No	132	513	0.87	0.55-1.36	0.53
	GC	Yes	112	242	1.56	0.98-2.48	0.06
rs1915087	GC	No	124	323	1.29	0.82-2.04	0.27
	GG	Yes	9	26	1.17	0.49-2.76	0.73
	GG	No	30	101	1.00		
	TT	Yes	128	190	1.44	0.87-2.38	0.15
	TT	No	143	491	0.62	0.38-1.01	0.06
rs6804441	CT	Yes	179	150	2.56	1.55-4.21	<0.01
	CT	No	115	386	0.64	0.39-1.05	0.07
	CC	Yes	37	43	1.84	0.98-3.46	0.06
	CC	No	28	60	1.00		
	GG	Yes	187	270	0.92	0.49-1.75	0.81
rs41271391	GG	No	158	639	0.33	0.17-0.62	<0.01
	AG	Yes	132	98	1.80	0.92-3.49	0.08
	AG	No	110	274	0.54	0.28-1.03	0.06
	AA	Yes	25	15	2.22	0.92-5.38	0.08
	AA	No	18	24	1.00		
rs41271391	TT	Yes	111	142	1.39	0.91-2.13	0.12
	TT	No	94	399	0.42	0.28-0.63	<0.01
	GT	Yes	168	161	1.86	1.24-2.79	<0.01
	GT	No	141	447	0.56	0.38-0.83	<0.01
	GG	Yes	65	80	1.45	0.90-2.33	0.12
rs41271391	GG	No	51	91	1.00		

Table 12. Interactive effects of CD28/B7 pathway-related SNPs and history of induced abortion on susceptibility to recurrent spontaneous abortion

SNP	Genotype	Induced abortion	Case	Control	OR	95% CI	P value
rs3116496	CC	Yes	16	23	2.33	1.21-4.49	0.01
	CC	No	19	7	9.08	3.77-21.91	<0.01
	TC	Yes	124	126	3.29	2.46-4.41	<0.01
	TC	No	97	210	1.55	1.16-2.06	<0.01
	TT	Yes	169	268	2.11	1.65-2.70	<0.01
rs3181098	TT	No	205	686	1.00		
	AA	Yes	61	60	2.80	1.86-4.22	<0.01
	AA	No	50	131	1.05	0.72-1.54	0.80
	GA	Yes	162	197	2.26	1.69-3.03	<0.01
	GA	No	145	425	0.94	0.71-1.24	0.66
rs3181100	GG	Yes	86	160	1.48	1.06-2.06	0.02
	GG	No	126	347	1.00		
	CC	Yes	160	203	2.50	1.58-3.95	<0.01
	CC	No	195	425	1.45	0.93-2.27	0.10
	GC	Yes	140	182	2.44	1.53-3.88	<0.01
rs1915087	GC	No	96	383	0.79	0.50-1.27	0.33
	GG	Yes	9	32	0.89	0.38-2.08	0.79
	GG	No	30	95	1.00		
	TT	Yes	142	209	1.38	0.87-2.19	0.17
	TT	No	149	476	0.64	0.41-1.00	0.05
rs6804441	CT	Yes	136	174	1.59	0.99-2.53	0.05
	CT	No	138	358	0.78	0.50-1.23	0.29
	CC	Yes	31	34	1.85	0.98-3.50	0.06
	CC	No	34	69	1.00		
	GG	Yes	145	287	0.74	0.37-1.47	0.39
rs41271391	GG	No	215	622	0.51	0.26-1.00	0.05
	AG	Yes	136	113	1.77	0.87-3.56	0.11
	AG	No	91	259	0.52	0.26-1.04	0.06
	AA	Yes	28	17	2.42	0.99-5.89	0.05
	AA	No	15	22	1.00		
rs41271391	TT	Yes	87	140	1.28	0.84-1.96	0.26
	TT	No	118	401	0.61	0.41-0.89	0.01
	GT	Yes	158	213	1.53	1.03-2.25	0.03
	GT	No	151	395	0.79	0.54-1.15	0.22
	GG	Yes	64	64	2.06	1.27-3.32	<0.01
rs41271391	GG	No	52	107	1.00		

proposed that VD deficiency during pregnancy could cause permanently changed forms and functions of offsprings' immune organs [23, 24]. Finally, the in-vitro addition of fruits and

vegetables were demonstrated to positively stimulate IL-2, IFN- γ via modulating the secretion of Th1/Th2 cytokines [25, 26]. The underlying mechanism could be roughly summarized as that fruits and vegetables were rich in vitamin A, vitamin C, vitamin E and micro-elements, the lacking of which might contribute to the damaged immune system of organisms [27-30]. For instance, enough supply of vitamin A could promptly raise the number of peripheral blood lymphocytes, and vitamin E exerted an inhibitory role in the production of prostaglandin E2 (PGE2), the substance that suppressed the organisms' immune functions in a non-specific manner [31-33]. All in all, due to the linkage between immunity dysfunction and elevated prevalence of RSA, proper intake of fruit and vegetable were beneficial living habits against abortion.

In addition, it was previously documented that B7-1 (i.e. CD80) and B7-2 (e.g. CD86) were mainly expressed in the activated dendritic cells and macrophages, and they were the natural ligands of CD28. The combination of CD28 and B7 would boost the expressions of interleukin-2 (IL-2), IL-4 and IL-5, and this auxo-action would be enhanced when the second messenger existed [34]. Besides, the co-stimulatory signals of CD28 were involved in multiple intracellular pathways that modified the immunity responses, including PI3K pathway, GRB-2/SOS-related p21Ras pathway and ITK/EMT pathway [35-37]. Since one contributor of RSA was regarded as the immunity dysfunction, it was taken for granted that the maladjusted CD28/B7 pathway was the vital reason for RSA development. Consistent with the hypothesis, hindering B7-1 and B7-2 expressions could hold up the maternal rejection to allogenic fetus within matings that were prone to suffer from miscarriages [38]. Moreover, methods have been explored to avoid the abortion relevant to dysfunctional immunity via altering the costimulatory signal mediated by CD28 [39]. Accordingly, the immunity-related SNPs situated within CD28 and B7 were also estimated as the risk factor for RSA. To be specific, rs1129055 of B7-2 was remarkably associated with the altered risk of pneumonia-induced sepsis [40]. B7-4 rs6804441 was also investigated whether it was correlated with systemic lupus erythematosus among an Asian population [41]. A prospective study based on the Polish population also revealed the relationship between CD28 rs3116496/rs3181098 and cancer risk [42]. Above all, the SNPs that showed statistical significances could be deemed as the biomarkers for RSA development (Table 3-4). Furthermore, the interactions of SNPs and environment also derived the results that differed from SNPs and environment alone (Table 5-11)[43, 44].

Nonetheless, this study was limited by several factors. Firstly, recall bias due to memory distortion or incomplete memory would give rise to the systematic error existing between memory and the truth [45]. Secondly, the distinct outpatient rate caused by the different subjects' knowledge of the diseases would magnify the selection bias when patients were selected from the hospital. Thirdly, the sample size included was not enough for generalizing the study results into other populations. Moreover, only finite factors were investigated in this study, other environmental factors, such as coffee, were not investigated. What's more, some women might conceal their reproductive history because of the one-child policy in China, so the breeding conditions might not be a bit distinct from the real situation. Finally, we only studied the superficial association of environmental and genetic factors with risk of RSA, but we did not explore the potential molecular mechanisms underlying RSA. Hence, more in-depth studies with a large scale were demanded for the following researches.

Conclusion

The environment factors, including night shift, history of repeated abortions, history of smoking and intake of vitamin supplement, could interact with SNPs located within CD28/B7 pathway (e.g. rs3116496, rs6804441 and rs1129055) to modify the risk of RSA among the Chinese population.

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Disclosure Statement

The authors declare that they have no competing interests.

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