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Effect of miRNA-27a and Leptin Polymorphisms on Risk of Recurrent Spontaneous Abortion

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Background: The aim of this study was to investigate the possible associations of *miRNA-27a* and *Leptin* polymorphisms with the risk of recurrent spontaneous abortion (RSA).

Material/Methods: Between May 2013 and April 2015 at Shenzhen Longhua New District Central Hospital, we randomly recruited 138 RSA patients as the case group and another 142 normal pregnancy women as the control group. We used denaturing high-performance liquid chromatography (DHPLC) to determine the genotypes and allele frequencies of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A.

Results: The GG genotype and G allele frequencies of *miRNA-27a* rs895819 A/G were higher in the case group than in the control group, and the AA genotype and A allele frequencies of *Leptin* rs7799039 G/A were also higher in the case group than in the control group (all $P<0.05$). *MiRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A polymorphisms increased the risk of RSA (Exp (B)=2.732, 95% CI=1.625~4.596, $P=0.000$; Exp (B)=4.081, 95% CI=1.817~9.164, $P=0.001$). GG-AA or AG-AA carriers had a higher risk of RSA. The *miRNA-27a* expression of AA carriers of *miRNA-27a* rs895819 was lower than that of AG+GG carriers both in the case and control groups (all $P=0.024$). The plasma leptin concentration of GG carriers was lower than that of GA+AA carriers in the case group ($P=0.026$).

Conclusions: The polymorphisms of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A may contribute to an increased risk of RSA.

MeSH Keywords: Chromatography • DNA Copy Number Variations • Polymorphism, Genetic

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Background

Recurrent spontaneous abortion (RSA) is defined as the miscarriage of two or more consecutive pregnancies with unknown origin within 20 weeks of pregnancy, affecting about 1–5% of reproductive age women [1,2], which is difficult to cure in clinical practice. Generally, RSA has been caused by recognized factors including genetics, endocrine, hormonal problems, infection, placental anomalies, smoking and alcohol consumption, exposure to environmental triggers, psychological trauma, and constant stressful emotion [3,4]. However, the cause for nearly half of the patients with RSA cannot be explained [5]. Many scientists have made trials on the treatment of RSA from many fields, such as genetics, endocrinology, and immunology [6–8]. With the rapid development of molecular biology technology, the focus has been shifted to the relationship between gene polymorphism and RSA, such as the relationships between microRNAs (miRNAs) and RSA [9,10]. Some previous studies suggest that miRNAs, by affecting endometrium, pre-eclampsia, and infertility, might be essential for the normal function of the reproductive system and provide noninvasively obtained diagnostic information [11,12].

MiRNAs are a class of small endogenous noncoding RNAs that negatively regulate target gene transcription through hybridization to incomplete complementary sequences in the 3' untranslated region of their target mRNAs [13]. Previous evidence has shown the impact of miRNAs on human reproduction [14,15]. As an important member in the miRNA family, miRNA-27a is actively expressed in many cancers, such as breast cancer, colorectal cancer, and gastric cancer, and is considered as an effective indicator for early diagnosis and prognosis of tumors [16–18]. Accumulating evidence has demonstrated that single-nucleotide polymorphisms (SNPs) in miRNA precursors may influence the expression levels of miRNA. Recently, an important A to G transition SNP in *pre-mir-27a* (rs895819) was identified in common cancers [19,20]. Leptin is the gene product of the obese gene and may regulate body weight, satiety, and fertility [21]. The adipokine leptin is a lipostatic signal governing food intake and stimulating energy expenditure. It is also a pivotal metabolic regulator, which correlates with the pro-inflammatory Th1 immune response to energy balance and nutritional status [22]. In recent years, leptin regulation of immune response and inflammatory response has received much research attention due to its significant changes during infection and inflammation [21,23]. The human leptin gene is composed of three exons and two introns located on chromosome 7q31.3, which spans ~18 kb of genomic DNA [24]. *Leptin rs7799039 G/A* was shown to be a common SNP investigated in the association between leptin gene polymorphisms and plasma leptin level [25]. However, due to the limited amount of examined miRNA, and the scarcity of explorations into the effect of leptin on non-mammals, reports on the risks of RSA and its connection with gene polymorphism of *miRNA-27a* and leptin has received less attention. Therefore, our present study intended to explore the effect of gene polymorphisms of *miRNA-27a* and leptin on RSA, to evaluate the regulatory function of miRNA in the process of pregnancy, and to discuss the effect and significance of the *miRNA-27a* and leptin on RSA.

Material and Methods

Subjects

From May 2013 to April 2015, a total of 138 RSA patients with a mean age of 28.83±4.57 years and menstrual cycle of 31.52±3.03 days, admitted to the obstetric clinic of Shenzhen Longhua New District Central Hospital, were recruited into our study as the case group. All RSA cases were confirmed according to the diagnostic criteria, which were: (1) women with two or more consecutive spontaneous abortions; (2) karyotypes of couples and chorionic villus sampling (CVS) after abortion showing normal; (3) no abnormal anatomy of the reproductive tract; (4) endocrine function such as sex hormone secretion and thyroid function presenting normal; (5) negative autoantibodies, including anticardiolipin antibody, antinuclear antibody, antipaternal complement-dependent antibody (APCA) and the Toxoplasma (TOX), Other (OTH), Rubella virus (RUV), Cytomegalovirus (CMV), and Herpes simplex virus-II (HSV-II) (TORCH); (6) no thrombotic disease or thrombotic tendency; and (7) no inflammatory response in the reproductive tract or systemic inflammatory response. During the same period, we also recruited 142 normal pregnancy women as the control group, with a mean age of 29.12±4.33 years and menstrual cycle of 32.04±3.17 days. The inclusion criteria for the control group were: (1) no history of spontaneous abortion; (2) karyotypes of couples presenting normal; and (3) at least one normal live birth and no pregnancy-related complications. Baseline characteristics of the subjects between the case and control groups show no significant differences (both $P>0.05$).

Also, we excluded patients with history of smoking, drinking, drug use, and family disease; patients with chronic pain, reproductive tract abnormality, or endocrine disorder; patients with severe cardio-cerebrovascular disease, liver and kidney dysfunction; patients with comorbidity of depression and other mental disorders; and patients with reoperation due to complications within the last three months. This study was approved by the Ethics Committee of Shenzhen Longhua New District Central Hospital. All subjects signed informed consents.

Detections of *miRNA-27a* and leptin polymorphisms

The peripheral blood (5 ml) from each subject was placed in a tube containing ethylenediamine tetraacetic acid (EDTA) and

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Table 1. The PCR primer sequences of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A.

Gene	Primer sequence	Annealing temperature	Annealing time	Cycle
miRNA-27a rs895819 A/G	F: 5'- ATATGAGAAAAGAGCTTCCCTGTG -3'	61°C	45 s	35
	R: 5'- CAAGGCCAGAGGAGGTGAG -3'			
Leptin rs7799039 G/A	F: 5'- TTTCCTGTAATTTTCCCATGAG -3'	61°C	45 s	35
	R: 5'- AAAGCAAAGACAGGCATAAAA -3'			

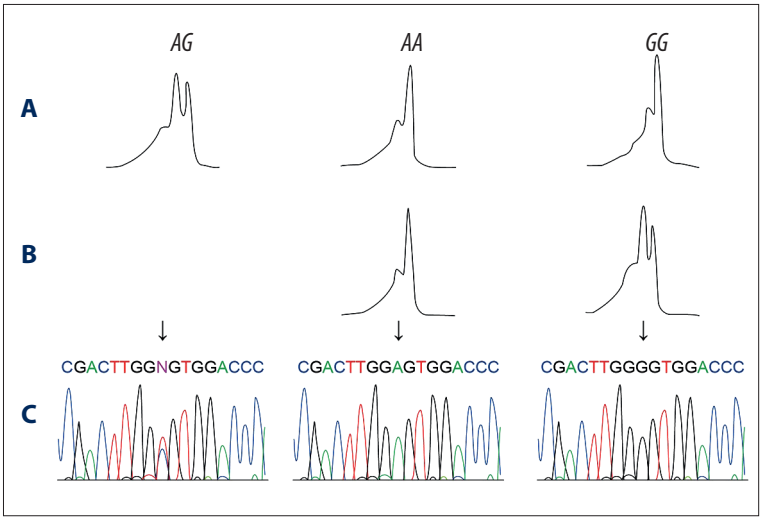

Figure 1. (**A–C**) Denaturing high-performance liquid chromatography (DHPLC) for *miRNA-27a* rs895819 A/G and sequencing of polymerase chain reaction (PCR) products.

stored at –80°C. With addition of erythrocytic segment, DNA was extracted with the use of the Blood Genome DNA Extraction Kit (TaKaRa Biotech Co., Ltd., Dalian, China). Polymerase chain reaction (PCR) amplification was conducted with primers of rs895819 A/G in *miRNA-27a* and rs7799039 G/A in leptin, and the primers were designed by Prime 5.0 software and then synthesized by Shanghai Sangon Biotech Co., Ltd. (Table 1). The PCR reaction condition was: 94°C for 2 min; 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min; 72°C extension for 5 min. The products were stored at 4°C. All genotypes of the PCR products were examined using DHPLC (Transgenomic Inc., Omaha, NE, USA). With column temperature of 59.3°C and mobile phase flow rate of 0.9 ml/min, *miRNA-27a* rs895819 A/G was genotyped through two steps (Figure 1): (1) heterozygote AG exhibited bimodal DHPLC (Figure 1A); (2) PCR samples that exhibited unimodal DHPLC mixed with equivalent AA samples verified by sequencing, and then the mixture was subject to DHPLC analysis, with AA exhibiting unimodal and GG exhibiting bimodal (Figure 1B). A and G mutations were also confirmed by sequencing (Figure 1C). *Leptin* rs7799039 G/A was genotyped through two steps (Figure 2): (1) heterozygote GA displayed bimodal DHPLC (Figure 2A); (2) PCR samples that displayed unimodal DHPLC mixed with equivalent GG samples as verified by sequencing, and then the mixture underwent DHPLC analysis, with GG displaying unimodal and AA displaying bimodal (Figure 2B). G and A mutations were also confirmed by sequencing (Figure 2C).

Real-time quantitative PCR (RT-qPCR)

Serum separated from the whole blood by centrifugation at 3000 rpm for 10 min at 4°C was transferred into a centrifuge tube. Total RNA (tRNA) was extracted from the serum using the mirVana PARIS Kit (Ambion Inc., Austen, TX, USA), and then reversely transcribed to obtained cDNA using a reverse transcription system (Thermo Fisher Scientific Fermentas, MA, USA). With the cDNA as template, the primers of *miRNA-27a* (Forward: 5' TGCGGTTCACAGTGGCTAAG 3'; Reverse: 5' CTCAACTGGTGTCGTGGA 3') were used for PCR amplification. Using the Taq-Man MicroRNA Kit (Ambion Inc., Austen, TX, USA), every cDNA sample obtained from RT-PCR was used to detect the expression level of *miRNA-27a* in a fluorescence-based RT-qPCR instrument (Model 7900; Applied Biosystems Inc., Foster City, CA, USA).

Enzyme-linked Immunosorbent Assay (ELISA)

Separated from whole blood by centrifugation at 3000 rpm for 10 min at 4°C, plasma was transferred into a centrifuge tube. The following procedures were all performed according to the kit (R&D Systems Inc, Minneapolis, MN, USA) manual. Samples were brought to 37°C, the ELISA plate was coated with diluted plasma for 30 min, then to each hole we added 50 µL enzyme liquid for 30 min. Subsequently, with the addition of 50 µL chromogenic

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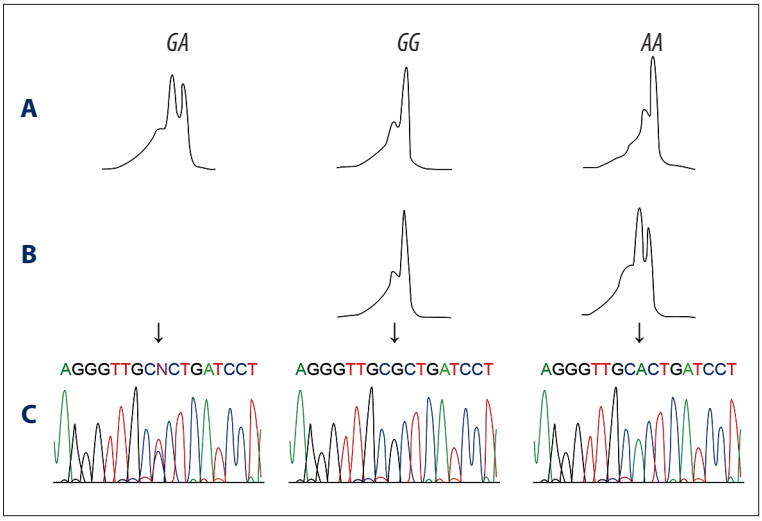

Figure 2. (**A–C**) Denaturing high-performance liquid chromatography (DHPLC) for *Leptin* rs7799039 G/A and sequencing of polymerase chain reaction (PCR) products.

reagent A and 50 μL reagent B, each hole was stored in the dark for 15 min. Consequently, each hole in the ELISA plate turned blue. It was appropriate to extend the coloration time if the color was too light. With 50 μL stopping solution added into each hole, the color changed to yellow immediately. The optical density (OD) value was measured at a wavelength of 450 nm at 15 min after the termination. The standard curve was drawn with OD value as the abscissa and concentration as the ordinate, and concentrations of the standard product were 25, 50, 100, 200, 400 ng/L successively. The optimal dilution ratio of other samples was predicted according to the concentration of plasma.

Statistical analysis

Continuous data are expressed as mean ± standard deviation (SD), with *t* test for comparison. Categorical data are exhibited as percentage or rate, with χ^2 test for comparison. $P<0.05$ was considered as statistical significance. The χ^2 test was also employed to identify whether samples between two groups met the Hardy-Weinberg equilibrium used to examine the representativeness of a study population. $P\geq0.05$ indicated samples met the genetic equilibrium and had group representation. Odds ratio (OR) with 95% confidence interval (CI) was calculated to estimate association between disease and polymorphism in single-factor and multi-factor logistic regression analyses. Data analysis was conducted using SPSS 19.0 software (SPSS, Inc. IBM, Chicago, IL, USA).

Results

Distributions of genotype and allele frequencies of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A

Distributions of genotype and allele frequencies of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A are presented in Table 2. The test for Hardy-Weinberg equilibrium revealed an agreement of *P* value to the law of genetic equilibrium, which demonstrated that the study population was representative.

With respect to *miRNA-27a* rs895819 A/G, the frequencies of GG genotype and G allele were both significantly higher in the case group than in the control group (GG genotype: 15.9% *vs.* 9.2%, OR (95%CI)=2.357 (1.095~5.075), *P*=0.026; G allele: 37.7% *vs.* 27.1%, OR (95% CI)=1.625 (1.137~2.324), *P*=0.008). For *Leptin* rs7799039 G/A, the frequencies of AA genotype and A allele were also both higher in the case group than in the control group (AA genotype: 47.8% *vs.* 32.4%, OR (95% CI)=3.262 (1.461~7.279), *P*=0.003; A allele: 69.9% *vs.* 57.4%, OR (95% CI)=1.726 (1.218~2.446), *P*=0.002).

miRNA-27a rs895819 A/G and *Leptin* rs7799039 G/A polymorphisms and risk of RSA

With AA and non-AA genotypes of *miRNA-27a* rs895819 A/G and GG and non-GG genotypes of *Leptin* rs7799039 G/A as independent variables, and RSA patients as dependent variables, the bivariate Logistic regression analyses were performed. The results signified that non-AA genotypes of *miRNA-27a* rs895819 A/G exhibited 2.732 times higher risk of RSA compared with AA genotype (95% CI=1.625~4.596, *P*=0.000). Non-GG genotypes of *Leptin* rs7799039 G/A showed 4.081 times higher risk of RSA compared with GG genotype (95% CI=1.817~9.164, *P*=0.001) (Table 3).

Gene combination of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A and the risk of RSA

We found that, compared to individuals with AA-GG combined genotypes, GG-AA carriers had 4.714 times (95% CI=1.077~20.63, *P*=0.034) and AG-AA carriers had 3.480 times (95% CI=1.114~10.87, *P*=0.028) higher risk of RSA. However,

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Table 2. Comparison of the distribution frequency of genotype polymorphism and allele gene on *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A.

Genotype	Case group (n=138)		Control group (n=142)		χ^2	*P*	OR (95%CI)
miRNA-27a rs895819							
AA	56	(40.7%)	78	(54.9%)			Ref.
AG	60	(43.4%)	51	(35.9%)	3.662	0.056	1.639 (0.987~2.721)
GG	22	(15.9%)	13	(9.2%)	4.956	0.026	2.357 (1.095~5.075)
AG + GG	82	(59.3%)	64	(45.1%)	5.775	0.016	1.785 (1.111~2.867)
A	172	(62.3%)	207	(72.9%)			Ref.
G	104	(37.7%)	77	(27.1%)	7.147	0.008	1.625 (1.137~2.324)
Leptin rs7799039 G/A							
GG	11	(8.0%)	25	(17.6%)			Ref.
GA	61	(44.2%)	71	(50.0%)	2.831	0.092	1.953 (0.888~4.292)
AA	66	(47.8)	46	(32.4%)	8.787	0.003	3.262 (1.461~7.279)
GA + AA	127	(92.0%)	117	(82.4%)	5.798	0.016	2.467 (1.162~5.236)
G	83	(30.1%)	121	(42.6%)			Ref.
A	193	(69.9%)	163	(57.4%)	9.494	0.002	1.726 (1.218~2.446)

OR – odds ratio; Ref – reference; CI – confidence interval.

Table 3. Logistic regression analysis of gene locus of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A.

Variable	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI
miRNA-27a rs895819 A/G	1.005	0.265	14.359	1	0.000	2.732	1.625~4.596
Leptin rs7799039 G/A	1.406	0.413	11.608	1	0.001	4.081	1.817~9.164

B – beta coeffient; S.E. – standard error; Wald – wald test statistic; df – difference; Sig. – significance; 95%CI – 95% confidence interval.

other related gene combinations interacting with the risk of RSA exhibited no significant difference (Table 4).

Polymorphism of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A and clinical features of RSA

We analyzed the clinical data collected from the 138 patients with RSA, and found that, compared with patients with AA genotype of *miRNA-27a* rs895819 A/G, patients with AG + GG genotype suffered more intense pregnant reactions, shorter duration of miscarriage in pregnancy, more frequent abortion, and longer interval to next pregnancy (all $P<0.05$), indicating that there were significant differences in clinical features between patients with G allele and those with non-G allele. Also, compared to patients with GG genotype of *Leptin* rs7799039 G/A, patients with GA + AA genotype suffered more intense pregnant reactions, shorter duration of miscarriage in pregnancy, more frequent abortion, and lengthened interval to next pregnancy (all $P<0.05$), revealing that there were also significant differences in clinical features between patients with A allele and those with non-A allele (Table 5).

MiRNA-27a and leptin level in plasma and RSA

MiRNA-27a was expressed in the patients with AA genotype and AG + GG genotype of rs895819 A/G in both case group and control group, with a significant difference in its total expression volume between the control group (0.50±0.09) and the case group (1.30±0.13) ($P<0.01$) (Figure 3A). In the control group, subjects with AA genotype had miRNA-27a expression of 0.49±0.08, lower than the 0.52±0.09 ($P=0.037$) in those with AG + GG genotype. In the case group, miRNA-27a expression of patients with AA genotype was 1.27±0.12, lower than the 1.32±0.13 ($P=0.024$) in patients with AG + GG genotype.

Plasma leptin level of patients with GG genotype and GA + AA genotype rs7799039 G/A was much different ($P<0.01$) between the case group (6.74±0.56) ng/ml and control group

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Table 4. Relativity of risk of RSA and combined genotype on the locus of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A.

Combined genotype		Case group (n=138)		Control group (n=142)		OR	95%CI	*P*
miRNA-27a rs895819 A/G	*Leptin* rs7799039 G/A							
AA	GG	7	(17.0)	11	(35.7)		Ref.	
AG	GA	27	(17.0)	26	(35.7)	1.632	0.549~4.855	0.376
GG	AA	12	(21.8)	4	(24.8)	4.714	1.077~20.63	0.034
AA	GA	26	(10.9)	39	(14.7)	1.048	0.359~3.054	0.932
AG	AA	31	(34.7)	14	(41.9)	3.480	1.114~10.87	0.028
GG	GG	1	(17.7)	3	(24.8)	0.524	0.045~6.095	0.601
AA	AA	22	(23.1)	28	(17.1)	1.235	0.411~3.710	0.707
AG	GG	3	(17.0)	11	(35.7)	0.429	0.087~2.102	0.291
GG	GA	9	(21.8)	6	(24.8)	2.357	0.580~9.579	0.227

OR – odds ratio; 95%CI – 95% confidence interval; Ref – reference.

Table 5. Association between clinical features of RSA and genotype of *miRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A n (%).

Genotype	Case group		Severe pregnancy reaction		Abortion time in pregnancy (≥3 months)		Number of abortion (≥3 times)		Duration to next pregnancy (≥6 months)	
miRNA-27a rs895819										
AA	56	(40.7%)	5	(8.9%)	2	(3.6%)	9	(16.1%)	1	(1.8%)
AG + GG	82	(59.3%)	23	(28.0%)	14	(17.1%)	32	(39.0%)	13	(4.8%)
χ^2			5.171		4.815		4.743		6.063	
P			0.023		0.028		0.029		0.014	
*Leptin*rs7799039										
GG	11	(8.0%)	6	(54.5%)	4	(36.4%)	8	(72.7%)	5	(45.5%)
GA + AA	127	(92.0%)	22	(17.3%)	12	(9.4%)	33	(26.0%)	9	(7.1%)
χ^2			4.586		4.729		4.438		10.39	
P			0.032		0.030		0.035		0.001	

(4.99±0.39) ng/ml (Figure 3B). In the control group, leptin level in the subjects with GG genotype was (4.87±0.36) ng/ml, slightly lower than the 5.01±0.39 ng/ml in subjects with GA + AA genotype (*P*=0.101). In the case group, leptin level in the patients with GG genotype was (6.38±0.45) ng/ml, lower than the 6.77±0.56 ng/ml (*P*=0.026) in patients with GA + AA genotype (Table 6).

Discussion

The etiology of RSA is complicated, involving genetics, endocrine, hormonal problems, placental anomalies, psychological trauma, and constant stressful emotion [3,4]. In recent years, some new ideas were put forward from the perspective of immunology and genetics, considering that the causes of RSA are related with gene polymorphism [24]. Due to the scarcity of literature on the relationship between miRNA and leptin, and the risk of RSA, we expected to make a comprehensive evaluation on the relationship between RSA and gene polymorphism of miRNA and leptin.

Our results revealed the case group had a significantly higher frequency of G allele gene and GG genotype distributed on *miRNA-27a* rs895819 A/G in comparison with the control

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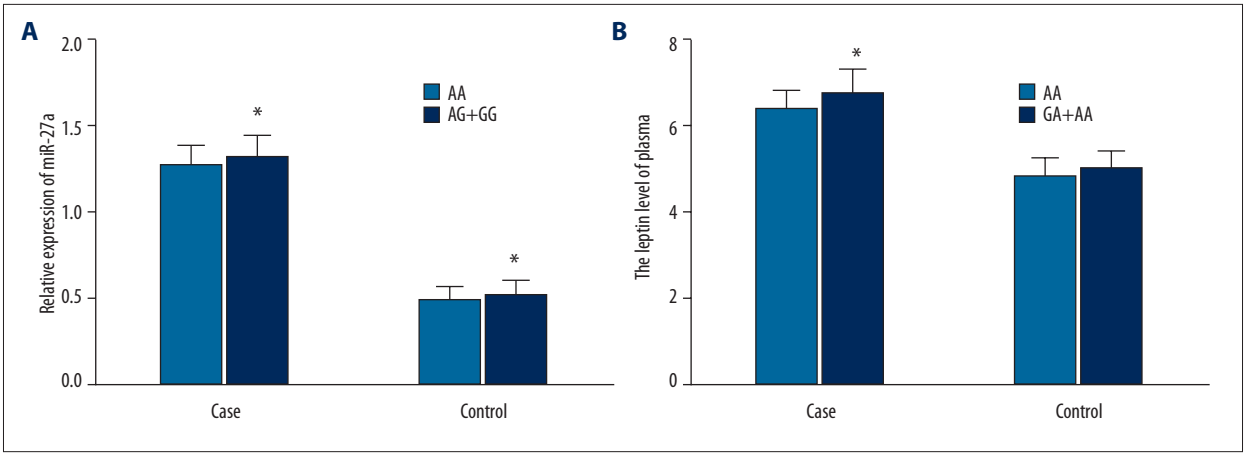

Figure 3. The expression of plasma miRNA-27a influenced by *miRNA-27a* rs895819 A/G and the level of plasma leptin influenced by *Leptin* rs7799039 G/A. (**A**) Refers to the expression of plasma miRNA-27a; * refers to *P*<0.05 when AA genotype was compared with AG + GG genotype; (**B**) Refers to the level of plasma leptin; * refers to *P*<0.05 when GG genotype was compared with AG + AA genotype.

Table 6. Expression of *miRNA-27a* and level of *Leptin* in plasma (mean ±SD).

Genotype	Case group	Control group
miRNA-27a		
AA	1.27±0.12	0.49±0.08
AG + GG	1.32±0.13	0.52±0.09
P	0.024	0.037
Leptin (ng/ml)		
GG	6.38±0.45	4.87±0.36
GA + AA	6.77±0.56	5.01±0.39
P	0.026	0.101

SD – standard deviation.

group. There was also a significant up-regulation in the frequency of A allele gene and AA genotype of *Leptin* rs7799039 G/A. Consequently, we concluded that mutation of G gene of *miRNA-27a* rs895819 A/G and mutation of gene A of *Leptin* rs7799039 G/A might increase the risk of RSA. As an important component of the miRNA class, miRNA-27a is closely related to the development of diseases, notably tumors, cardiovascular diseases, and metabolic diseases [25,26]. Recent studies have shown that the irregular expression of miRNAs might lead to recurrent miscarriage [9,10]. As a kind of protein mainly secreted by fat cells and involved in energy intake and balance of protein, leptin can influence the female reproductive endocrine system, regulate the hypothalamus-pituitary-gonadal axis, affect reproductive function and female ovulation, and participate in pubertal behavior, which plays a complex role in physiological regulation in connection with vegetative and reproductive metabolism [27,28]. Mounzih *et al.* found that leptin may directly or indirectly regulate the formation of blood vessels by affecting vascular permeability and formation of endothelial cells and trophoblastic cells in the process of forming placenta [29].

Further research deepened insights into the impact of *miRNA* and leptin gene polymorphisms on cancers, and Yang et al. has found that the protective effect of single-nucleotide polypeptide rs895819 distributed on the end ring of miRNA-G allele gene might decrease the possibility of gene mutation of *miRNA-27a* with carcinogenesis, thus reducing the risk for familial breast cancer and presents possible hormone-related effects [30]. The polymorphism of *Leptin* rs7799039 G/A had a significant association with antipsychotic drug-induced weight gain [31]. Hoffstedt et al. has reported that a common polymorphism in *Leptin* rs7799039 G/A has an effect on the expression of leptin, possibly at the transcriptional level, and therefore also adipose secretion levels of the hormone [32]. It is also reported by Mammès et al. that the A allele of *Leptin* rs7799039 G/A polymorphism was demonstrated to be associated with higher leptin levels before diet and lower BMI loss in women [35]. A

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previous study found that the AA genotype of rs7799039 G/A polymorphism and higher circulating leptin level may be associated with preeclampsia/pregnancy-induced hypertension, further leading to the occurrence of RSA [36]. Additionally, the secretion of leptin is positively correlated with body mass, and Winnie et al. reported that maternal obesity is an independent factor related to a higher risk of RSA, suggesting that leptin polymorphism may directly or indirectly contribute to the development of RSA [37]. Interestingly, reports on the risks of RSA and its connection with gene polymorphism of *miRNA-27a* and leptin remain scarce. Our logistic regression analysis showed that compared with individuals with combined AA-GG genotype distributed on *miRNA-27a* rs895819 A/G locus and *Leptin* rs7799039 G/A locus, patients with combined genotype of GG-AA and AG-AA had a higher risk of RSA, which further demonstrates the close ties between RSA and gene polymorphism of *miRNA-27a* and leptin.

Conclusions

Our study revealed polymorphisms of *MiRNA-27a* rs895819 A/G and *Leptin* rs7799039 G/A may contribute to an increased risk of RSA. Given the complicated etiology of RSA, other external triggers might have interfered with our results. Therefore, further exploration into the genetic mechanisms is needed to prevent RSA.

Competing interests

We declare that we have no conflicts of interest.

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