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Associations of serum vitamin D and Fok I polymorphism of receptor gene with unexplained recurrent spontaneous abortion

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Abstract

***Background**: To investigate the associations of serum vitamin D and Fok I polymorphism of its receptor (VDR) with unexplained recurrent spontaneous abortion (URSA). **Methods**: Ninety URSA patients and another 104 healthy pregnant women were selected as URSA and control groups, respectively. 25-Hydroxyvitamin D [25-(OH)D] level was detected by chemiluminescence. VDR gene Fok I polymorphism was analyzed by PCR, and the distribution of genotype frequency was calculated by Hardy-Weinberg equilibrium test. Association between Fok I polymorphism and susceptibility to URSA was investigated by logistic regression analysis. **Results**: Gestational age, uterine height, waist circumference, 25-(OH)D level and proportions of Fok I FF and Ff genotypes were significantly lower in the URSA group ($P<0.05$). Compared with ff genotype, risk of URSA reduced for Ff and FF genotypes. Compared with allele f, risk of URSA was lower for allele F. 25-(OH)D level of ff genotype was significantly lower in the URSA group, which was lower than that of FF genotype ($P<0.05$). Compared with women with 25-(OH)D level >30 ng/mL and F allele (FF+Ff), the risk of URSA increased 2.45-, 2.43- and 5.34-fold for those with 25-(OH)D level >30 ng/mL and ff genotype, with 25-(OH)D level ≤30 ng/mL, and with ff genotype and 25-(OH)D level ≤30 ng/mL, respectively. **Conclusions**: The 25-(OH)D level of the URSA group was significantly lower than that of normal pregnant women. Probably, VDR gene Fok I polymorphism is associated with URSA occurrence, and allele F decreases the risk. The risk of URSA dramatically increases in women with ff genotype and 25-(OH)D deficiency.*

***Keywords**: vitamin D; receptor gene; polymorphism; recurrent spontaneous abortion*

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Introduction

Recurrent spontaneous abortion (RSA) refers to successive repeated spontaneous abortions. However, agreement has not been achieved on the definition of the number of spontaneous abortions of RSA. According to standards of the American Society for Reproductive Medicine, RSA refers to 2 or more pregnancy failures (1). The Royal College of Obstetricians and Gynaecologists of the UK defines RSA as fetal loss within 24 weeks of pregnancy for ≥3 times with the same sexual partner (2). In China, the number was originally defined as 3 or more times,

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but it is found that the risk of RSA after 2 successive abortions is equal to that after 3 successive abortions (3). The probability of clinical spontaneous abortion is 15-25%. The World Health Organization estimates that the global incidence rate of RSA is 1-2% (4). Abortions that occur within 12 weeks of pregnancy account for over 80% of RSA. Since the causes of RSA cannot be determined for 50% of RSA women, this type of RSA is called unexplained RSA (URSA), which is also a difficult disease in the department of obstetrics and gynecology (5).

25-hydroxyvitamin D [25-(OH)D] is the major form of vitamin D circulating in the human body, which is a reliable index for evaluating vitamin D (6). There is an evident correlation between 25-(OH)D level in RSA patients and RSA. Deficiency of 25-(OH)D can induce oxidative stress injury in women by interfering with female hormone levels or receptors, influencing reproductive organs, germ cells and embryo implantation, and thereby affecting female reproductive health and causing adverse outcomes such as infertility and abortion (7). Vitamin D receptor (VDR), a type of ligand-dependent regulatory protein, is a member of the steroid/thyroid hormone receptor superfamily (8). 25-(OH)D can bind to VDR to regulate the downstream molecules and affect the expression levels of many proteins closely related to calcium and phosphorus regulation, bone tissue metabolism, cell differentiation and cell growth and apoptosis, thus realizing the regulation of cells at the physiological level (9). VDR gene polymorphism is closely associated with vitamin D deficiency-related diseases (10). Currently, the research on VDR polymorphism has focused on the four restriction sites of *Bsm I*, *Fok I*, *Taq I,* and *Apa I*. The genotype GG at rs9340799 has been associated with severe preeclampsia (11). Zhou et al. found that the genetic polymorphism at rs9340799 may be responsible for spontaneous abortion in patients (12). Additionally, the estrogen receptor 1 rs9340799 polymorphism is associated with an increased risk of RSA in the non-Asian group, but not in the Asian group, which may be attributed to ethnic differences (13).

The mutation of *Fok I* site is related to fetal growth restriction (14). However, there has been no report on the correlation between VDR gene polymorphism and URSA. Therefore, in this study, the *Fok I* restriction site of VDR gene was analyzed in re-pregnant URSA women and healthy pregnant women, aiming to evaluate the distribution and influence of VDR gene in URSA patients, and provide basis for clinical diagnosis and treatment of URSA.

Materials and Methods

General information

In this study, the cases were from pregnant RSA women hospitalized in our hospital from January 2017 to December 2019. Patients with URSA were selected as case group (URSA group). A total of 387 RSA cases were collected, and 90 cases were included in the URSA group, which accounted for about 23.26% of all RSA cases. The age of patients in the URSA group was 20-40 years old, with a mean of (27.85±3.55) years old, and their number of spontaneous abortion was ≥2, without childbearing. Another 104 healthy pregnant women undergoing antenatal examination during the same period were selected as the control group. Their age was 21-42 years old, with a mean of (27.63±3.54) years old. They had never had spontaneous abortion and had given birth to ≥1 healthy fetuses.

Inclusion and exclusion criteria

The inclusion and exclusion criteria in the URSA group were determined according to *Obstetrics and Gynecology* (15) and *Expert consensus on the diagnosis and treatment of recurrent spontaneous abortion* (16) as follows. Inclusion criteria: 1) Pregnant women who had two or more

successive spontaneous abortions. 2) Those who did not change spouse in the abortion period and whose spouse had normal sperm. 3) Those with elevated human chorionic gonadotrophin (HCG) in blood examination, and whose pregnancy sac and fetal heartbeat could be seen by ultrasonography. Exclusion criteria: 1) Pregnant women with histories of ectopic pregnancy, biochemical pregnancy, planned abortion, or accidental abortion. 2) Those with chromosome karyotype abnormalities in husband or wife, or confirmed chromosomal abnormalities of embryonic chorionic villi. 3) Those with infectious diseases such as hepatitis B, hepatitis C or syphilis. 4) Those with positive autoantibodies, such as KHK, ACA, β2 globulin or antithyroid antibody. 5) Those with positive test results for pathogens in vaginal secretions. 6) Those with reproductive tract malformations. 7) Those with prethrombosis, hypofibrinemia or thrombocytopenia. 8) Those with preeclampsia or other pregnancy complications.

The inclusion and exclusion criteria in the control group were as follows. Inclusion criteria: 1) Patients who gave birth to ≥1 healthy fetuses. 2) Those with elevated HCG in blood examination, and whose pregnancy sac and fetal heartbeat could be seen by ultrasonography. Exclusion criteria: 1) Patients with pregnancy history of ectopic pregnancy, biochemical pregnancy, planned abortion, accidental abortion or spontaneous abortion. 2) Those with systemic or basic diseases such as hypertension, diabetes, liver or kidney, endocrine or immune abnormalities. 3) Those with preeclampsia or other pregnancy complications. All patients signed the informed consent, and this study was approved by the Ethics Committee of our hospital (approval number: WDTX-M-2017047).

Collection of baseline clinical data

The baseline clinical data such as age, ethnic group, status of drinking, smoking and working, education level, individual income, sun exposure, and supplementation of vitamin D and calcium were collected from patients. The appropriate cubital vein was selected, and two tubes of blood were collected by using the blood collection tubes produced by BD (USA) when the patient was in a calm state. About 2-3 mL of whole blood was collected in one of the blood collection tubes containing anticoagulant EDTA-K2, which was for the extraction of genomic DNA from blood and was stored at -80°C. Another 4-5 mL of whole blood was collected in the other blood collection tube containing coagulant. After the blood coagulated and the serum was precipitated, it was centrifuged at 3,500 rpm for 5 min (2,514× g). Then 1 mL of the serum was absorbed, placed in a cryopreservation tube, and stored at -80°C for detection of serological indexes. At the time of sampling, the mean gestational age was (6.7±2.9) and (8.9±2.3) weeks in the URSA group and the control group, respectively. After being left still at room temperature for 30 min without anticoagulation, the sample was centrifuged at 2,000 r/min for 20 min (centrifugal radius: 280 mm) to separate the serum which was then stored in a -80°C refrigerator. The 25(OH)D serum level was measured according to the kit instructions (Shanghai SRK Biotechnology Co., Ltd., China).

Extraction of genomic DNA in peripheral blood

The total DNA was extracted from the blood using the blood genomic DNA extraction kit produced by Tiangen Biotech (Beijing) Co., Ltd. (China). All operations were carried out according to the instructions of the kit. The concentration and purity of extracted DNA were detected by NanoDrop One spectrophotometer. DNA concentration >20 ng/μL, A_{260}/A_{280} ≈1.8, and $A_{260/230}$ >2.0 indicated successful DNA extraction, and the subsequent experiment could be carried out.

Analysis of VDR gene Fok I site typing

VDR gene *Fok I* polymorphism was analyzed by polymerase chain reaction (PCR). The primers were designed using the Primer 5.0 software, and input into the database of the National Library of Medicine (USA) for primer homology comparison and correctness verification. The *Fok I* primers were as follows: Forward: 5'-AAGCCGTCGACCAGC-3, Reverse: 5'-AGACTAACCGAGTGAGCC-3'. The reaction system (20 μL) was added with 50 ng of genomic DNA, 10 pmol of primers, 1 U Taq of DNA polymerase, 2 μL of 10× Buffer, 2.5 mmol/L $MgCl_2$, and 200 μmol/L dNTPs. The reaction conditions were as follows: pre-denaturation at 94°C for 5 min, then 30 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 30 s, and extension at 72°C for 45 s, followed by extension at 72°C for 10 min. Finally, the 5U restriction endonuclease BseDI was added to the PCR product, digested at 55°C for 3 h, and then separated in 8% non-denaturing polyacrylamide gel for 3 h. Subsequently, the bands in each zone were observed by silver nitrate staining. Later, the genotype was interpreted using the blind method by two persons who did not know the sample source. If the interpretation results were inconsistent, the sample would be re-detected to ensure 100% successful rate of genotyping.

Statistical analysis

Double verification was adopted for data input, and SPSS 16.0 software was utilized for data analysis. The theoretical values of genotypes were calculated by Hardy-Weinberg genetic equilibrium coincidence test. The numerical data were expressed by percentage, and chi-square test was adopted for comparison between groups. The measurement data were expressed by mean ± standard deviation, and independent-samples *t*-test was performed for inter-group comparison, while paired *t*-test was conducted for intra-group comparison. Logistic regression analysis was employed to investigate the association between VDR gene *Fok I* polymorphism and susceptibility to URSA. $P<0.05$ suggested that the difference was statistically significant.

Results

Baseline clinical data

There were no statistically significant differences in age, ethnic group, status of drinking, smoking and working, education level, individual income, sun exposure and supplementation of vitamin D and calcium between the two groups ($P>0.05$). Gestational age, uterine height, waist circumference and 25-(OH)D level were significantly lower in the URSA group than those in the control group ($P<0.05$) (**Table 1**).

PCR results of VDR gene Fok I site

The alleles F and f of *Fok I* represented the absence and presence of corresponding restriction site. Under the UV lamp of gel imaging system, the PCR products of FF genotype showed only a 265 bp band, Ff genotype showed 265 bp, 196 bp and 69 bp, while ff genotype showed 2 bands of 196 bp and 69 bp (**Figure 1**).

Distribution of genotype frequencies

The frequencies of VDR gene *Fok I* polymorphism in all participants are summarized in **Table 2**. The 3 genotypes of *Fok I* site all conformed to the Hardy-Weinberg law of genetic equilibrium ($P>0.05$), indicating that they could reflect the general situation of URSA patients and healthy controls.

Genotype frequencies at Fok I site

The genotype frequencies at *Fok I* site are listed in **Table 3**. There was a statistically significant difference in the distribution of *Fok I* polymorphism between the two groups ($P<0.05$). The proportions of *Fok I* FF and Ff genotypes in the URSA group were significantly lower than those

Table 1. Baseline clinical data of the pregnant women included in the study

		URSA group	Control group	t/χ^2	P
Gestational age (week)		6.7±2.9	8.9±2.3	5.888	0.000
Age (Y)		27.85±3.55	27.63±3.54	0.431	0.667
Pre-pregnancy BMI (kg/m^2)		22.35±3.59	20.28±2.28	10.293	0.000
Uterine height (cm)		29.78±1.18	31.32±2.03	6.329	0.000
Abdominal circumference (cm)		89.17±2.97	94.14±2.95	11.666	0.000
Ethnic group	Han	89 (98.89%)	102 (98.08%)	0.209	0.648
	Others	1 (1.11%)	2 (1.92%)		
Drinking	No	10 (11.11%)	8 (7.69%)	1.248	0.742
	Yes	19 (21.11%)	21 (20.19%)		
Smoking	No	24 (26.67%)	34 (32.69%)	0.550	0.458
	Yes	37 (41.11%)	41 (39.42%)		
Working	Yes	60 (66.67%)	64 (61.54%)	0.021	0.884
	No	30 (33.33%)	40 (38.46%)		
Education level	Junior high school and below	88 (97.78%)	102 (98.08%)	5.257	0.154
	High school	2 (2.22%)	2 (1.92%)		
	Junior college	21 (23.33%)	40 (38.46%)		
	University and above	38 (42.22%)	32 (30.77%)		
Individual income (RMB/month)	≤2000	31 (34.44%)	32 (30.77%)	0.209	0.648
	2001-5000	89 (98.89%)	102 (98.08%)		
	>5000	1 (1.11%)	2 (1.92%)		
Sufficient sun exposure	No	34 (37.78%)	36 (34.62%)	0.209	0.647
	Yes	56 (62.22%)	68 (65.38%)		
Calcium supplement	No	63 (70.00%)	71 (68.27%)	0.068	0.795
	Yes	27 (30.00%)	33 (31.73%)		
Vitamin D supplement	No	83 (92.22%)	97 (93.27%)	0.079	0.779
	Yes	7 (7.78%)	7 (7.78%)		
25(OH)D (ng/mL)		27.77±5.8	29.74±6.58	2.196	0.029

in the control group. The frequency of f allele was significantly higher in the URSA group than that in the control group (32.22% *vs.* 16.83%) (P<0.05).

Logistics regression analysis results of association between VDR gene Fok I polymorphism and susceptibility to URSA

The results of *Fok I* gene analysis manifested that the risk of URSA in pregnant women carrying genotypes Ff [OR=0.35, 95% confidence interval (CI): 0.14-0.54] and FF (OR=0.45, 95% CI: 0.23-0.67) was lower than that of genotype ff. After adjustment of age, gestational age, pre-pregnancy BMI, uterine height, waist circumference, ethnic group, status of drinking, smoking and working, education level, individual income, sun exposure and supplementation of vitamin D and calcium, it was found that in contrast with that of ff genotype, the risk of URSA was still reduced in pregnant women carrying Ff (adjusted OR=0.31, 95% CI: 0.12-0.44) and FF

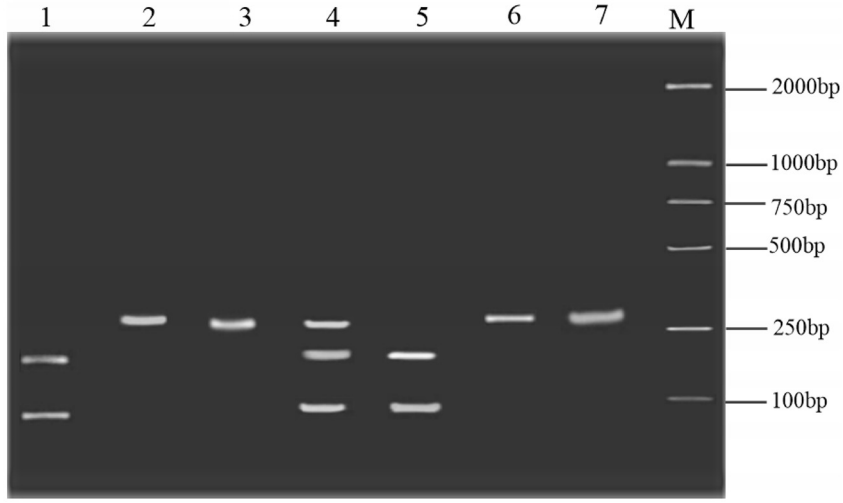

Fig. 1. PCR-RFLP electrophoretogram of VDR gene *Fok I* site. M: Marker, 1 and 5: ff genotype, 2, 3, 6 and 7: FF genotype, 4: Ff genotype.

Table 2. Hardy-Weinberg genetic equilibrium test results of genes

Genotype		URSA group (n=90)		χ^2	P	Control group (n=104)		χ^2	P
		Actual frequency	Theoretical frequency			Actual frequency	Theoretical frequency		
Fok I	ff	21	22	0.092	0.955	7	5	0.521	0.771
	Ff	16	17			21	24		
	FF	53	51			76	77		

Table 3. Genotype frequencies at Fok I site

Genotype		URSA group (n=90)	Control group (n=104)	χ^2	P
Fok I	ff	21 (23.33%)	7 (6.73%)		
	Ff	16 (17.78%)	21 (20.19%)	14.823	0.004
	FF	53 (58.89%)	76 (73.08%)		
	f	58 (32.22%)	35 (16.83%)	12.550	0.000
	F	122 (67.78%)	173 (83.17%)		

(adjusted OR=0.53, 95% CI: 0.22-0.88) genotypes. Compared with that of allele f, the risk of URSA was lower in pregnant women carrying allele F (adjusted OR=0.38, 95% CI: 0.22-0.68) (**Table 4**).

Association between Fok I genotype and vitamin D level

After stratification according to *Fok I* genotypes, the differences in 25-(OH)D level between the same genotypes in the two groups were compared (**Table 5**). The level of 25-(OH)D in pregnant women with *Fok I* ff genotype in the URSA group was significantly lower than that in the control group (P<0.05). The level of 25-(OH)D in pregnant women with Ff and FF genotypes in the URSA group was lower than that in the control group, but there was no statistically significant difference between the two groups (P>0.05). Pairwise comparison of 25-(OH)D level between different genotypes in the URSA group revealed that the 25-(OH)D level

Table 4. Logistics regression analysis results of association between VDR gene Fok I polymorphism and susceptibility to URSA

Genotype		OR (95%CI)	P	OR (95%CI)	P*
Fok I	ff	1		1.000	
	Ff	0.35 (0.14~0.54)	0.035	0.31 (0.12~0.44)	0.022
	FF	0.45 (0.23~0.67)	0.005	0.53 (0.22~0.88)	0.035
	f	1		1.000	
	F	0.38 (0.22~0.69)	0.022	0.43 (0.23~0.79)	0.027

* indicates statistical values after adjustment of age, gestational age, pre-pregnancy BMI, uterine height, waist circumference, ethnic group, status of drinking, smoking and working, education level, individual income, sun exposure and supplementation of vitamin D and calcium by using the logistic regression model. P value (the likelihood ratio test used to compare the logistic model with main effects and the model with interaction effect) = 0.011.

Table 5. Association between *Fok I* genotype and vitamin D level

Genotype		URSA group		Control group		t	P
		n	25(OH)D (ng/mL)	n	25(OH)D (ng/mL)		
Fok I	ff	21	23.83±3.12*	7	26.93±3.23	2.269	0.031
	Ff	16	25.56±3.12	21	27.82±3.72	1.960	0.058
	FF	53	27.12±4.33	76	28.42±4.58	1.622	0.107
P (interaction effect)			0.023		0.041		

P value for the interaction effect between 25-(OH)D level and Fok I gene polymorphism = 0.034.

of ff genotype was significantly lower than that of FF genotype, showing a statistically significant difference (P<0.05). However, there was no statistically significant difference in the level of 25-(OH)D between ff and Ff genotypes, and between Ff and FF genotypes, suggesting that *Fok I* homozygous genotype ff may be related to low 25-(OH)D level.

Association between VDR gene Fok I polymorphism and susceptibility to URSA

After stratification according to the differences in 25-(OH)D and *Fok I* genotypes in pregnant women, the interaction between 25-(OH)D level and *Fok I* gene polymorphism and the association between *Fok I* gene polymorphism and susceptibility to URSA were analyzed. Compared with that in pregnant women with 25-(OH)D level >30 ng/mL and F allele (FF+Ff), the risk of URSA in those with 25-(OH)D level >30 ng/mL and homozygous genotype ff, those with 25-(OH)D level ≤30 ng/mL, and those with homozygous genotype ff and 25-(OH)D level ≤30 ng/mL was increased by 2.45 (95% CI: 1.33-8.89), 2.43 (95% CI: 1.32-5.23) and 5.34 times (95% CI: 2.57-6.87), respectively (**Table 6**).

Table 6. Association between VDR gene *Fok I* polymorphism and susceptibility to URSA

Serum 25(OH)D (ng/mL)	Fok I genotype	URSA group	Control group	OR (95%CI)	OR*
>30ng/mL	FF+Ff	20	44	1	1
>30ng/mL	ff	4	2	2.23(1.45-8.24)	2.45(1.33-8.89)
≤30ng/mL	FF+Ff	49	53	2.09(1.45-5.48)	2.43(1.32-5.23)
≤30ng/mL	ff	17	5	5.02(2.45-12.19)	5.34(2.57-6.87)

* indicates statistical values after adjustment of age, pre-pregnancy BMI, uterine height, waist circumference, ethnic group, status of drinking, smoking and working, education level, individual income, sun exposure and supplementation of vitamin D and calcium by using the logistic regression model.

Discussion

According to a study in recent years, vitamin D deficiency during pregnancy is closely correlated with adverse pregnancy outcomes, such as fetal growth retardation, low birth weight and abortion (17). However, the mechanism of vitamin D deficiency affecting body growth and development is not completely clear. Currently, it has been found that mutations in several vitamin D metabolism-related substances, such as cytochrome P4502R1 gene, VDR gene and family-specific immunoglobulin gene, can lead to abnormal vitamin D level (18). VDR mRNA is expressed in placenta, decidua and ovary throughout pregnancy and may be involved in the synthesis of sex hormones (19). The purpose of this study was to analyze the base sequence of VDR gene *Fok I* site and detect the level of vitamin D in URSA, so as to understand the relationship between VDR gene polymorphism and susceptibility to URSA and the influence of interaction between vitamin D and VDR gene polymorphism on URSA, and study the pathogenesis of URSA from the perspective of molecular genetics.

Vitamin D, an essential substance to maintain human life, exerts immunomodulatory effects in the form of 25-(OH)D. During normal pregnancy, the maternal adaptive immune system is suppressed, while the innate immune system is relatively stimulated. These changes in the maternal-fetal interface are conducive to the occurrence of immune tolerance and the prevention of fetal rejection. 25-(OH)D plays an important role in maintaining an appropriate level of inflammatory response (20). Vitamin D deficiency during pregnancy is globally common, and the association between low vitamin D level and RSA has been reported by recent studies. Among pregnant women at different latitudes, 18-84% of women had an insufficient 25-(OH)D level, which varies with country, race, and dietary intake (21). After a review of sunny Mediterranean regions, it was concluded that maternal 25-(OH) D <20 ng/mL is very common, with a prevalence rate ranging from 23-90% (22). In Nordic countries, the proportion of vitamin D deficiency among pregnant women at 12 and 20 weeks of pregnancy is up to 96% (23). During pregnancy, the risk of vitamin D deficiency is elevated due to increased demand from pregnant women and fetuses, which is often correlated with adverse pregnancy. Early detection of 25-(OH) D during pregnancy is very important to avoid the deterioration of pregnancy-related complications. Li et al. reported that the concentration of 25-(OH)D in decidual tissue and serum of RSA women was lower than that of normal pregnancy (24). Another study showed that 25-(OH)D level ≥40 ng/mL can significantly reduce the risk of preterm delivery (25). In this study, the concentration of 25-(OH)D in the URSA group was lower than that in the control group, which is consistent with the results of previous studies. This suggests that 25-(OH)D deficiency may be related to the occurrence of URSA, and vitamin D supplementation during pregnancy is helpful, especially for RSA patients.

VDR gene polymorphism has been extensively studied and reported in different races and different populations. VDR gene polymorphism affects the mRNA stability, translation efficiency, and protein-protein interaction at the transcriptional level (26). VDR gene is associated with cell cycle regulation and affects the circulation, proliferation, differentiation, and apoptosis of placental cells (27). Low expression of VDR facilitates premature differentiation and apoptosis of cells, and the expression of VDR is decreased in the placenta of RSA patients (28). In addition, VDR gene polymorphism affects the transcriptional activity of VDR, changes the function of VDR protein, and directly or indirectly influences the function of vitamin D (29). Hence, the VDR signal transduction pathway is closely

associated with placental function, and placental factor is key to ensure normal pregnancy. VDR gene polymorphism is associated with genetic susceptibility to adverse pregnancy reactions or outcomes, such as gestational diabetes, preterm delivery, preeclampsia, and low-birth weight neonates (30). In this study, by analyzing the distribution characteristics of VDR gene *Fok I* site in pregnant URSA women and normal pregnant women, it was found that the number of individuals with *Fok I* Ff and FF genotypes was significantly smaller in the URSA group than that in the control group. In contrast with that of ff genotype, the risk of URSA was still reduced in pregnant women carrying Ff and FF. Compared with that of allele f, the risk of URSA was lower in pregnant women carrying allele F. Moreover, *Fok I* gene polymorphism was reported to be involved in immune regulation during early embryo implantation. Aslani et al. found that the *Fok I* genotype was associated with postpartum metabolic syndrome. Allele f was associated with prenatal obesity and familial inheritance of diabetes mellitus, and a higher proportion of patients with postpartum metabolic syndrome had the ff genotype than normal controls. They concluded that allele f and ff genotype may be risk factors for blood glucose or other metabolic abnormalities, and allele F was a protective factor (31). Therefore, we postulated that carrying *Fok I* allele F may be a protective factor against URSA.

The association between VDR gene polymorphism and diseases is influenced by vitamin D level, and there is gene-nutrient interaction. Fetal growth restriction is the result of joint action of genetic and environmental factors. A study demonstrated that the influence of VDR gene *Fok I* polymorphism on fetal growth and development is associated with maternal vitamin D level (32). When the maternal 25-(OH)D level is <50 nmol/L (25 ng/mL), the birth weight of fetus carrying *Fok I* gene FF and Ff genotypes is significantly lower than that of ff genotype, and the abdominal skinfold is thinner. Such association is not found when the maternal vitamin D level is sufficient. In this study, stratification was made according to differences in maternal 25-(OH)D level and *Fok I* genotypes. Compared with that in pregnant women with 25-(OH)D level >30 ng/mL and F allele (FF+Ff), the risk of URSA in those with 25-(OH)D level ≤30 ng/mL and allele F (Ff + FF) and those with homozygous genotype ff and 25-(OH)D level ≤30 ng/mL was increased by 2.43 and 5.34 times, respectively, suggesting that there may be an interaction between VDR gene *Fok I* polymorphism and 25-(OH)D, which affects the occurrence of URSA.

In summary, the level of 25-(OH)D in pregnant URSA women is significantly lower than that in normal pregnant women. VDR gene *Fok I* polymorphism may be associated with the occurrence of URSA, and allele F may reduce the risk of URSA. Moreover, the risk of URSA dramatically increases in pregnant women with *Fok I* gene homozygous genotype ff and 25-(OH)D deficiency. Based on the findings, particular attention should be paid to pregnant women with *Fok I* homozygous genotype ff and 25(OH)D deficiency. Nevertheless, this study is limited. This is a single-center study with a small sample size, so the results may be biased. Further multicenter studies with larger sample sizes are ongoing in our group.

Abbreviations

25-(OH)D: 25-hydroxyvitamin D; CI: confidence interval; PCR: polymerase chain reaction; RSA: recurrent spontaneous abortion; URSA: unexplained recurrent spontaneous abortion; VDR: vitamin D receptor.

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Author's contribution

HL designed this study and prepared this manuscript; YS, MZ & XY collected and analyzed clinical data. All authors approved the submission and publication of this manuscript.

Conflict of interest

There was no conflict in this work.

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