Polymorphisms of F2, PROC, PROZ, and F13A1 Genes are Associated With Recurrent Spontaneous Abortion in Chinese Han Women

Zuying Xu, MD[1,2,3], Ying Zhang, MD[1,2,3], Wei Liu, MD[4], Yunyun Liu, MD[1,2,3], Yezhou Su, MD[1,2,3], Qiong Xing, PhD[1,2,3], Xiaojin He, PhD[1,2,3], Zhaolian Wei, PhD[1,2,3], Yunxia Cao, PhD[1,2,3], and Huifen Xiang, PhD[1,2,3]

Abstract

Mutations of hemostasis/coagulation-related genes have been speculated to cause recurrent spontaneous abortion (RSA). This study investigated the genetic association between the polymorphisms of factor V (F5), factor II (F2), antithrombin (SERPINC1), protein C (PROC), protein S (PROS1), protein Z (PROZ), factor XIII (F13A1), and carboxypeptidase B2 (CPB2) genes and RSA. The 426 patients with RSA and 444 controls were recruited in this study, and single-nucleotide polymorphisms (SNPs) were analyzed by using SNPscan technology. Genotype and allele frequencies of rs3136520 in F2, rs3024731 in PROZ, and rs1050782 in F13A1 showed statistically significant differences between the 2 groups. TT genotype of rs3136520 ($P = .031$, odds ratio [OR] = 0.986, 95% confidence interval [CI] = 0.976-0.997) and AA genotype of rs2069906 in PROC ($P = .021$, OR = 0.114, 95% CI = 0.014-0.902) in their recessive models and AG + GG variants of rs1050782 ($P = .007$, OR = 0.681, 95% CI = 0.516-0.899) in the dominant model might be associated with the reduced risk of RSA. AT + TT variants of rs3024731 ($P = .010$, OR = 1.479, 95% CI = 1.098-1.994) may increase disease susceptibility in dominant model. Haplotype analysis of rs3024731 and rs3024735 in PROZ displayed that the AA and TG haplotype were inclined to decrease and increase the risk of RSA, respectively. These results suggested that rs3136520, rs2069906, rs3024731, and rs1050782 may have a significant association with the genetic susceptibility of RSA in Chinese Han women.

Keywords

recurrent spontaneous abortion, single-nucleotide polymorphisms, genetic susceptibility, thrombophilia

Introduction

Recurrent spontaneous abortion (RSA) is defined as 2 or more consecutive miscarriages with the same partner in reproductive-age women,[1] affecting approximately 1% to 5% of females.[2,3] Recurrent spontaneous abortion is a complex, multifactorial condition caused by several factors, such as uterine anatomic anomalies, immune and endocrine disorders, chromosome aberrations, and infections.[4-6] However, the pathogenesis remains unexplained in approximately 40% to 50% of cases with RSA.[7,8]

In recent decades, thrombophilia has been suggested that it could be a risk factor in women with RSA.[5,9-11] Thrombophilia could be classified into 2 categories, hereditary and acquired, presenting a blood hypercoagulable state and could result in RSA and other obstetrical complications such as placental abruption, severe preeclampsia, and intrauterine growth restriction.[10,12-14] In the RSA research field, antiphospholipid syndrome belongs to the acquired thrombophilia and is the most

established reason for thrombophilia causing recurrent miscarriage.[15] However, the role of inherited thrombophilia resulting in RSA was ambiguous. Maternal genetic variants of relevant genes that were involved in the process of blood coagulation and fibrinolysis may give rise to RSA. Previous studies

[1] Department of Obstetrics and Gynecology, Reproductive Medicine Center, the First Affiliated Hospital of Anhui Medical University, Hefei, China
[2] Anhui Province Key Laboratory of Reproductive Health and Genetics, Anhui Medical University, Hefei, China
[3] Anhui Provincial Engineering Technology Research Center for Biopreservation and Artificial Organs, Hefei, China
[4] Department of Obstetrics and Gynecology, the Second Affiliated Hospital of Anhui Medical University, Hefei, China

Corresponding Authors:
Huifen Xiang and Yunxia Cao, Department of Obstetrics and Gynecology, Reproductive Medicine Center, the First Affiliated Hospital of Anhui Medical University, 81 Meishan Road, Hefei 230022, China.
Emails: huifen521@sina.com; caoyunxia6@126.com

suggested that factor V (F5) Leiden mutation (G1691A, rs6025) and prothrombin (factor II [F2]) G20210A mutation (rs1799963) could be risk factors for RSA in Caucasians.[16-19] However, for other relevant genes such as antithrombin (SERPINC1), protein C (PROC), protein S (PROS1), protein Z (PROZ), factor XIII (F13A1), and carboxypeptidase B2 (CPB2), the exact role of genetic alterations in each of these genes in the onset of RSA in Chinese Han women remains unclear.

Therefore, the goal of this study is to investigate the association between single-nucleotide polymorphisms (SNPs) of F5, F2, SERPINC1, PROC, PROS1, PROZ, F13A1, and CPB2 genes and RSA in Chinese Han women.

Materials and Methods

Participants

This case–control study enrolled 426 women with a history of 2 or more spontaneous abortion prior to 12 weeks who were evaluated in the reproductive medicine center, the First Affiliated Hospital of Anhui Medical University, and 444 healthy women who had at least 1 live birth and no abortion history were recruited as a control group. All patients and controls belong to Chinese Han population, and they all had natural pregnancies. The participants with obesity (BMI > 30) were excluded in both groups. Patients with RSA having known causes including chromosomal abnormalities, anatomical anomalies, autoimmunity diseases, diabetes mellitus, thyroid dysfunction, and infections were also excluded in this research. All participants signed the informed consent, and the study protocol was approved by the local ethics committees.

Selection of SNPs

The selection criteria of SNPs included tag SNPs, which were chosen from the Chinese Han Beijing population of the International HapMap Consortium with the minor allele frequency greater than 0.05 and $r^2 > 0.8$ and SNPs that have been reported in the previous literature. Therefore, the total of 21 SNPs selected in this study including rs6025 in F5 (Leiden mutation), rs1799963 (20210 G>A), and rs3136520 in F2; 4 SNPs (rs2227589, rs2227612, rs5877, and rs941988) in SERPINC1; 3 tag SNPs (rs1799810, rs2069906, and rs5936) in PROC; 6 tag SNPs (rs13062355, rs4857037, rs6123, rs6803590, rs7609644, and rs8178607) in PROS1; rs3024731 and rs3024735 in PROZ; rs1050782 in F13A1; and rs1926447 and rs3742264 in CPB2.

Genotyping Technique

Peripheral blood samples were collected from all patients with RSA and controls. Genomic DNA was isolated by using DNA purification kits according to the manufacturer's instructions. SNPscan technology (Genesky Biotechnologies Inc, Shanghai, China) which is based on double ligation and multiplex fluorescence polymerase chain reaction was used for SNP genotyping. Thirty-five random samples from cases

and controls were used for repeated genotyping of all SNPs to achieve quality control, and repeated results were consistent with the original ones.

Statistical and Bioinformatics Analyses

SHEsis software was used to test Hardy-Weinberg equilibrium in controls, to calculate linkage disequilibrium (LD), and to inferred haplotypes of SNPs, and frequency <0.03 was ignored for the analysis.[20,21] If the selected SNPs were not conformed to Hardy-Weinberg equilibrium in controls, the locus should be excluded in further analysis. The statistical analyses were performed using SPSS 13.0 software. The comparison of genotype and allele frequencies between patients with RSA and controls were calculated by χ^2 test or Fisher's exact test. Multifactor dimensionality reduction software (MDR, version 3.0.2) was used to predict and analyze the gene–gene interactions. Values of $P < .05$ were regarded statistically significant.

Results

All patients with RSA who participated in this study were with 2 or more early RSA (≤12 weeks). The mean age of 426 patients was 29.26 ± 4.294 years (range, 20-44), and the mean number of miscarriages was 2.40 ± 0.710 (range, 2-5). Among them, 301 (70.7%) women had 2 abortions, 89 (20.9%) women had 3 abortions, 26 (6.1%) women had 4 abortions, and 10 (2.3%) women had 5 abortions. The mean age of controls was 34.50 ± 4.895 years (range, 21-46), statistically older than the RSA patients group.

Among the selected 21 SNPs, genotype distributions of rs2227589 and rs941988 of SERPINC1 in the control group were not consistent with Hardy-Weinberg equilibrium, so these loci were excluded for further analysis. For 19 SNPs, the comparison of genotype and allele frequencies between the 2 groups is reported in Table 1. Genotype distributions and allele frequencies of rs3136520 in F2, rs3024731 in PROZ, and rs1050782 in F13A1 were all showed the statistical significant differences between cases with RSA and controls. The frequencies of rs3136520 T allele ($P = .029$, odds ratio [OR] = 0.657, 95% confidence interval [CI] = 0.449-0.961) and rs1050782 G allele ($P = .006$, OR = 0.761, 95% CI = 0.628-0.923) were decreased, whereas rs3024731 T allele ($P = .028$, OR = 1.236, 95% CI = 1.023-1.493) was increased in patients with RSA. Regarding rs2069906 in PROC, the distribution of genotype reached statistical significance with a value of $P = .046$. However, the allele frequencies between the 2 groups failed to show significant differences. In addition, no other association was found between the rest 15 SNPs and the risk of RSA in genotypic or allelic analyses.

The genetic model analysis of rs3136520, rs2069906, rs3024731, and rs1050782 revealed statistical significance (Table 2). TT genotype of rs3136520 ($P = .031$, OR = 0.986, 95% CI = 0.976-0.997) and AA genotype of rs2069906 ($P = .021$, OR = 0.114, 95% CI = 0.014-0.902) in their recessive models and AG + GG variants of rs1050782

Table 1. Comparison of Genotype and Allele Frequencies of 19 SNPs Between the 2 Groups.[a]

SNP	Genotype	Controls, n (%)	Cases, n (%)	P	Allele	Controls, n (%)	Cases, n (%)	P	OR	95% CI
F5	CC	443 (99.8)	426 (1)	1	C	887 (99.9)	852 (1)	1	1	—
Rs6025	CT	1 (0.2)	0 (0)		T	1 (0.1)	0 (0)		0.999	0.997-1.001
F2	GG	443 (99.8)	426 (1)	1	G	887 (99.9)	852 (1)	1	1	—
rs1799963	AG	1 (0.2)	0 (0)		A	1 (0.1)	0 (0)		0.999	0.997-1.001
F2	CC	374 (85)	378 (88.9)	**.02**	C	808 (91.8)	803 (94.5)	**.029**	1	—
Rs3136520	CT	60 (13.6)	47 (11.1)		T	72 (8.2)	47 (5.5)		**0.657**	**0.449-0.961**
	TT	6 (1.4)	0 (0)							
SERPINC1	CC	199 (44.8)	200 (46.9)	.664	C	602 (67.8)	583 (68.4)	.777	1	—
Rs5877	CT	204 (45.9)	183 (43)		T	286 (32.2)	269 (31.6)		0.971	0.794-1.188
	TT	41 (9.2)	43 (10.1)							
SERPINC1	TT	211 (47.5)	215 (50.5)	.373	T	621 (69.9)	602 (70.7)	.741	1	—
Rs2227612	GT	199 (44.8)	172 (40.4)		G	267 (30.1)	250 (29.3)		0.966	0.786-1.186
	GG	34 (7.7)	39 (9.2)							
PROC	GG	340 (76.6)	333 (78.2)	**.046**	G	775 (87.3)	758 (89)	.276	1	—
Rs2069906	AG	95 (21.4)	92 (21.6)		A	113 (12.7)	94 (11)		0.851	0.636-1.138
	AA	9 (2)	1 (0.2)							
PROC	AA	308 (69.4)	297 (69.7)	.56	A	740 (83.3)	716 (84)	.691	1	—
Rs1799810	AT	124 (27.9)	122 (28.6)		T	148 (16.7)	136 (16)		0.95	0.736-1.225
	TT	12 (2.7)	7 (1.6)							
PROC	TT	126 (28.4)	131 (30.8)	.689	T	477 (53.7)	475 (55.8)	.394	1	—
Rs5936	GT	225 (50.7)	213 (50)		G	411 (46.3)	377 (44.2)		0.921	0.763-1.113
	GG	93 (20.9)	82 (19.2)							
PROS1	AA	175 (39.4)	167 (39.2)	.747	A	549 (61.8)	533 (62.6)	.752	1	—
Rs13062355	AG	199 (44.8)	199 (46.7)		G	339 (38.2)	319 (37.4)		0.969	0.798-1.177
	GG	70 (15.8)	60 (14.1)							
PROS1	GG	346 (77.9)	340 (79.8)	.715	G	786 (88.5)	761 (89.3)	.593	1	—
Rs8178607	AG	94 (21.2)	81 (19)		A	102 (11.5)	91 (10.7)		0.921	0.683-1.243
	AA	4 (0.9)	5 (1.2)							
PROS1	AA	392 (88.3)	375 (88)	.905	A	836 (94.1)	801 (94)	.909	1	—
Rs7609644	AG	52 (11.7)	51 (12)		G	52 (5.9)	51 (6)		1.024	0.687-1.524
PROS1	AA	266 (59.9)	246 (57.7)	.795	A	685 (77.1)	648 (76.1)	.594	1	—
Rs6803590	AG	153 (34.5)	156 (36.6)		G	203 (22.9)	204 (23.9)		1.062	0.851-1.326
	GG	25 (5.6)	24 (5.6)							
PROS1	AA	347 (78.2)	315 (73.9)	.337	A	785 (88.4)	735 (86.3)	.181	1	—
Rs4857037	AG	91 (20.5)	105 (24.6)		G	103 (11.6)	117 (13.7)		1.213	0.914-1.610
	GG	6 (1.4)	6 (1.4)							
PROS1	CC	143 (32.2)	136 (31.9)	.981	C	502 (56.5)	482 (56.6)	.986	1	—
Rs6123	CT	216 (48.6)	210 (49.3)		T	386 (43.5)	370 (43.4)		0.998	0.826-1.207
	TT	85 (19.1)	80 (18.8)							
PROZ	AA	142 (32.1)	103 (24.2)	**.036**	A	499 (56.3)	435 (51.1)	**.028**	1	—
Rs3024731	AT	215 (48.5)	229 (53.8)		T	387 (43.7)	417 (48.9)		**1.236**	**1.023-1.493**
	TT	86 (19.4)	94 (22.1)							
PROZ	AA	155 (34.9)	119 (27.9)	.086	A	513 (57.8)	455 (53.4)	.067	1	—
Rs3024735	AG	203 (45.7)	217 (50.9)		G	375 (42.2)	397 (46.6)		1.194	0.988-1.442
	GG	86 (19.4)	90 (21.1)							
F13A1	AA	142 (32)	174 (40.8)	**.018**	A	506 (57)	541 (63.5)	**.006**	1	—
Rs1050782	AG	222 (50)	193 (45.3)		G	382 (43)	311 (36.5)		**0.761**	**0.628-0.923**
	GG	80 (18)	59 (13.8)							
CPB2	CC	261 (58.8)	224 (52.6)	.179	C	681 (76.7)	625 (73.4)	.108	1	—
Rs3742264	CT	159 (35.8)	177 (41.5)		T	207 (23.3)	227 (26.6)		1.195	0.961-1.485
	TT	24 (5.4)	25 (5.9)							
CPB2	GG	290 (65.3)	304 (71.4)	.136	G	723 (81.4)	719 (84.4)	0.1	1	—
Rs1926447	AG	143 (32.2)	111 (26.1)		A	165 (18.6)	133 (15.6)		0.811	0.631-1.041
	AA	11 (2.5)	11 (2.6)							

Abbreviations: CPB2, carboxypeptidase B2; CI, confidence interval; F5, factor V; OR, odds ratio; PROC, protein C; PROS, protein S; PROZ, protein Z; SERPINC1, antithrombin; SNP, single-nucleotide polymorphism.
[a]P values and OR (95% CI) in bold are statistically significant.

Table 2. Genetic Model Analysis of the 4 SNPs.[a]

SNP	Genotype	Control, n (%)	Case, n (%)	P	OR (95% CI)
Rs3136520	CC	374 (85)	378 (88.9)		1
	CT	60 (13.6)	47 (11.1)	.220	0.775 (0.516-1.165)
	TT	6 (1.4)	0 (0)	**.031**	**0.984 (0.972-0.997)**
	Dominant			.086	0.705 (0.472-1.052)
	Recessive			**.031**	**0.986 (0.976-0.997)**
Rs2069906	GG	340 (76.6)	333 (78.2)		1
	AG	95 (21.4)	92 (21.6)	.946	0.989 (0.715-1.367)
	AA	9 (2)	1 (0.2)	**.021**	**0.113 (0.014-0.900)**
	Dominant			.575	0.913 (0.664-1.255)
	Recessive			**.021**	**0.114 (0.014-0.902)**
Rs3024731	AA	142 (32.1)	103 (24.2)		1
	AT	215 (48.5)	229 (53.8)	**.016**	**1.468 (1.072-2.011)**
	TT	86 (19.4)	94 (22.1)	**.038**	**1.507 (1.023-2.219)**
	Dominant			**.010**	**1.479 (1.098-1.994)**
	Recessive			.335	1.175 (0.846-1.632)
Rs1050782	AA	142 (32)	174 (40.8)		1
	AG	222 (50)	193 (45.3)	**.022**	**0.709 (0.529-0.952)**
	GG	80 (18)	59 (13.8)	**.013**	**0.602 (0.402-0.900)**
	Dominant			**.007**	**0.681 (0.516-0.899)**
	Recessive			.093	0.731 (0.507 -1.055)

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a] P values and OR (95% CI) in bold are statistically significant.

Table 3. Haplotype Analysis With Rs3024731 and Rs3024735 in PROZ Gene.[a]

Haplotype	Case (frequency)	Control (frequency)	P	OR	95% CI
AA	426.71 (0.501)	484.56 (0.547)	.037	0.817	0.676-0.988
TA	28.29 (0.033)	28.44 (0.032)	.917	1.028	0.606-1.746
TG	388.71 (0.456)	358.56 (0.405)	.039	1.222	1.010-1.480

Abbreviations: CI, confidence interval; OR, odds ratio.
[a] Frequencies <0.03 were ignored in analysis.

($P = .007$, OR = 0.681, 95% CI = 0.516-0.899) in the dominant model were associated with the reduced risk of RSA. However, in the dominant model, compared to the AA genotype, AT + TT variants of rs3024731 were inclined to confer the susceptibility of RSA ($P = .010$, OR = 1.479, 95% CI = 1.098-1.994).

Rs3024731 and rs3024735 in the PROZ gene displayed a high degree of LD (D' = 0.945, $r^2 = 0.825$). The results of haplotype analysis with these 2 SNPs were listed in Table 3. The AA haplotype was associated with decreased risk of RSA ($P = .037$, OR = 0.817, 95% CI = 0.676-0.988), whereas TG haplotype tended to increase the risk of disease ($P = .039$, OR = 1.222, 95% CI = 1.010-1.480).

Genetic interaction analysis among rs3136520 (F2), rs3024731 (PROZ), and rs1050782 (F13A1) were performed. The 3 best models were predicted from MDR software, and the prediction errors and cross-validation consistency for each of the models are displayed in Table 4. However, testing P value of these models did not show statistical significance and failed

to find an association with the 3 SNPs between cases with RSA and controls.

Discussion

Considerable researches have been done to identify possible relationship between the mutations of genes that may be involved in the inherited thrombophilia and RSA. But as far as we know, the positive results of researches were not much in Chinese Han women who experienced RSA. In this case–control study, the possible association were explored between the 19 SNPs from several genes that play vital roles in coagulation/fibrinolytic system and the risk of RSA.

F5 Leiden mutation and F2 G20210A mutation were the most studied thrombophilic gene mutations in RSA. However, this study indicated that they were not related to RSA in Chinese Han people because of the similar low mutant allele frequencies in patients and controls. Other studies did not find connections with RSA after these 2 mutation analyses in north western Iranian[22] and Israeli individuals.[23] Rs3136520 was one of the tag SNPs selected from the HapMap database and located on the intron region of the F2 gene. This study showed that TT genotype and T allele of rs3136520 were significantly lower in women with RSA than in controls and may act as the protective factor to decrease susceptibility to RSA. There have been limited investigations about this tag SNP of F2 gene in RSA research, and further explorations could focus on the effect of combining with other tag SNPs in Chinese Han women experiencing RSA.

Deficiencies of antithrombin, protein C, and protein S were regarded as risk factors for adverse pregnancy outcomes

Table 4. The Best Gene–Gene Interactions Models Predict for the Association With RSA.

Best Model	Training Balanced Accuracy, %	Testing Balanced Accuracy %	CVC	Testing *P*	OR (95% CI)
Rs1050782	54.45	53.43	8/10	.5188	1.323 (0.565-3.094)
Rs3024731, rs1050782	55.95	51.86	10/10	.7263	1.164 (0.498-2.719)
Rs3136520, rs3024731, rs1050782	57.58	52.08	10/10	.6905	1.192 (0.502-2.832)

Abbreviations: CI, confidence interval; CVC, cross-validation consistency; OR, odds ratio; RSA, recurrent spontaneous abortion.

including fetal loss.[24,25] Mutations of these anticoagulation genes may influence their activities, and some variants have been investigated in RSA. GA + AA genotypes of rs2227589 in SERPINC1 gene might be a risk factor for RSA were reported in a Brazilian study,[26] whereas an Indian study[27] and a Chinese study[28] failed to find the relationship between this polymorphisms and RSA. In the current study about the 3 genes, rs2227589 and rs941988 of SERPINC1 were excluded because of the unbalanced Hardy-Weinberg equilibrium in controls. Therefore, 11 SNPs of these 3 genes were analyzed and found that AA genotype in a recessive model of rs2069906 located on the intron region of PROC gene may have a connection to the reduced risk of RSA. Just like rs3136520 in F2 gene, not much is known about the potential function of rs2069906 in PROC and its role in the etiology of RSA.

Lack of concentration of protein Z was also considered to have an association with various thrombotic diseases including venous/arterial thrombosis[29] and pregnancy complications.[30] Genetic variants may be an important reason for the deficiency of protein Z, and previous studies have investigated the association with several polymorphisms and RSA. Rs3024735 was reported to have a connection with RSA,[31,32] whereas 1 study also demonstrated that low protein Z levels could induce the unexplained pregnancy loss but not because of the rs3024735 variants.[33] A Bahrain study[34] found the allele frequencies of rs3024731 and rs3024735 with significant difference between patients with RSA and controls. The authors further reported that GA and AA genotypes of rs3024735, GA and AA genotype of rs3024719, and AA genotype of rs3024731 were linked with the decreased protein Z levels and demonstrated that GAGAAG and AGGTAG haplotypes may increase the disease susceptibility and AGGTGG haplotypes may reduce the risk of RSA with the 6 SNPs (rs3024718, rs3024719, rs3024778, rs3024731, rs3024735, and rs3024772) of PROZ gene.[34] In this study, rs3024731 and rs3024735 were investigated in RSA with Chinese Han women. In patients with RSA, the distributions of AT + TT genotypes and T allele of rs3024731 in PROZ were higher than in the control group and may increase the risk of RSA. But it failed to find an association between the rs3024735 and the onset of RSA. However, haplotype analysis with rs3024731 and rs3024735 indicated that AA and TG haplotype could decrease and confer the genetic susceptibility to RSA, respectively. Through comparison, these results were not concordant with earlier studies. These conflicting results may be explained by the different ethnic and genetic backgrounds, the size and selection of participants, and the change in environment. Further studies are needed

to investigate the association between the polymorphisms of PROZ gene and RSA.

Coagulation factor XIII (FXIII) is known as the last zymogen and formerly named fibrin-stabilizing factor that could stabilize the fibrin clot after series of actions.[35] Factor XIII consists of 2 A subunits and 2 B subunits. The A subunits were encoded by the F13A1 gene and possess catalytic ability; the B subunits may only act as carriers.[36,37] Several studies have investigated the association between Val34Leu mutation in exon 2 of F13A1 gene and RSA.[22,38,39] However, the present study selected another polymorphism, rs1050782, which was chosen from one of the tag SNPs of F13A1 gene for analysis. Rs1050782, located in the 3′ untranslated region of F13A1, may as a functional site influence the expression of F13A1, although the specific effect of this variants remains unknown. In the current study, the frequencies of AG + GG genotypes and G allele in cases were lower than in controls, which all showed statistical significance, indicating that AG + GG genotypes and G allele of rs1050782 may be associated with decreased risk of RSA.

Carboxypeptidase B2 gene, also called thrombin-activatable fibrinolysis inhibitor gene, could inhibit fibrinolysis, and several polymorphisms including rs3742264 (505G/A) and rs1926447 (1040C/T) might influence the occurrence of venous thrombosis.[40,41] In an aspect of RSA, 1 study indicated that rs3742264 A allele was associated with RSA as a protective factor.[42] However, this study failed to reveal the connection between this SNP and RSA. Regarding rs1926447, some authors reported that this polymorphism was not related to RSA,[42,43] which is similar to the present results.

Because RSA is a complex multifactorial disease, the individual genetic variants were difficult to detect completely and may only account for a minor role in the occurrence of RSA. In the current study, 19 SNPs were analyzed and found 3 SNPs (rs3136520 of F2, rs3024731 of PROZ, and rs1050782 of F13A1) may be associated with RSA, with significant difference both in allele and in genotype distributions between the 2 groups. Therefore, the study chose these 3 SNPs and attempted to identify the gene–gene interactions using the MDR method. However, the negative results of the genetic interactions indicated that F2, PROZ, and F13A1 may play important roles participating in different pathways involved in the pathogenesis of RSA, but the specific functions and interactions with other genes remain unclear.

There are some limitations that should be considered. The patients recruited in this study, all suffered in early period of RSA, and late miscarriages were not being researched.

Coagulation and fibrinolysis status was regulated by lots of crucial genes; other candidate genes or polymorphisms were not examined in this study. Current results were identified based on the moderate sample size. Further studies should be conducted with more candidate polymorphisms of genes involved in thrombogenesis and enlarged sample size to validate our results or find new associations with RSA in Chinese Han women.

Conclusions

To the best of our knowledge, this study was the first to identify that the polymorphisms rs3136520 of F2, rs2069906 in PROC, rs3024731 of PROZ, and rs1050782 of F13A1 may be associated with the genetic susceptibility of RSA in Chinese Han women.

References

1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. *Fertil Steril*. 2008;90(suppl 5):S60.
2. Rai R, Regan L. Recurrent miscarriage. *Lancet*. 2006;368(9535):601-611.
3. Blumenfeld Z, Brenner B. Thrombophilia-associated pregnancy wastage. *Fertil Steril*. 1999;72(5):765-774.
4. Stirrat GM.Recurrent miscarriage. II: clinical associations, causes, and management. *Lancet*. 1990;336(8717):728-733.
5. Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. *Rev Obstet Gynecol*. 2009;2(2):76-83.
6. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertil Steril*. 2010;93(4):1234-1243.
7. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent miscarriage: aetiology, management and prognosis. *Hum Reprod Update*. 2002;8(5):463-481.
8. Allison JL, Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. *Curr Opin Endocrinol Diabetes Obes*. 2009;16(6):446-450.
9. Kupferminc MJ. Thrombophilia and pregnancy. *Current pharmaceutical design*. 2005;11(6):735-748.
10. Patnaik MM, Haddad T, Morton CT. Pregnancy and thrombophilia. *Expert Rev Cardiovasc Ther*. 2007;5(4):753-765.
11. Quenby S, Farquharson RG, Dawood F, Hughes AM, Topping J. Recurrent miscarriage and long-term thrombosis risk: a case-control study. *Hum Reprod*. 2005;20(6):1729-1732.
12. Kupferminc MJ, Eldor A, Steinman N, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med*. 1999;340(1):9-13.
13. Kinzler WL, Prasad V, Ananth CV. The effect of maternal thrombophilia on placental abruption: histologic correlates. *J Matern Fetal Neonatal Med*. 2009;22(3):243-248.
14. Brenner B. Clinical management of thrombophilia-related placental vascular complications. *Blood*. 2004;103(11):4003-4009.
15. McNamee K, Dawood F, Farquharson R. Recurrent miscarriage and thrombophilia: an update. *Curr Opin Obstet Gynecol*. 2012;24(4):229-234.
16. Foka ZJ, Lambropoulos AF, Saravelos H, et al. Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod*. 2000;15(2):458-462.
17. Grandone E, Margaglione M, Colaizzo D, et al. Factor V Leiden is associated with repeated and recurrent unexplained fetal losses. *Thromb Haemost*. 1997;77(5):822-824.
18. Reznikoffetiévan MF, Cayol V, Carbonne B, Robert A, Coulet F, Milliez J. Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage. *BJOG*. 2001;108(12):1251-1254.
19. Ridker PM, Miletich JP, Buring JE, et al. Factor V Leiden mutation as a risk factor for recurrent pregnancy loss. *Ann Intern Med*. 1998;128(12 pt 1):1000-1003.
20. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15(2):97-98.
21. Li Z, Zhang Z, He Z, et al. A partition-ligation-combination-subdivision em algorithm for haplotype inference with multiallelic markers: update of the shesis (http://analysis.bio-x.cn). *Cell Res*. 2009;19(4):519-523.
22. Poursadegh Zonouzi A, Chaparzadeh N, Ghorbian S, et al. The association between thrombophilic gene mutations and recurrent pregnancy loss. *J Assist Reprod Genet*. 2013;30(10):1353-1359.
23. Carp H, Salomon O, Seidman D, Dardik R, Rosenberg N, Inbal A. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. *Hum Reprod*. 2002;17(6):1633-1637.
24. Greer IA. Thrombosis in pregnancy: maternal and fetal issues. *Lancet*. 1999;353(9160):1258-1265.
25. Bennett SA, Bagot CN, Arya R. Pregnancy loss and thrombophilia: the elusive link. *Br J Haematol*. 2012;157(5):529-542.
26. Guerra-Shinohara EM, Bertinato JF, Tosin Bueno C, et al. Polymorphisms in antithrombin and in tissue factor pathway inhibitor genes are associated with recurrent pregnancy loss. *Thromb Haemost*. 2012;108(4):693-700.
27. Sharma A, Bhakuni T, Ranjan R, et al. Polymorphisms in factor V and antithrombin III gene in recurrent pregnancy loss: a case-

control study in Indian population. *J Thromb Thrombolysis*. 2015; 39(4):481-488.

28. Cao Y, Zhang Z, Xu J, et al. The association of idiopathic recurrent pregnancy loss with polymorphisms in hemostasis-related genes. *Gene*. 2013;530(2):248-252.

29. Koren-Michowitz M, Rahimi-Levene N, Volcheck Y, Garach-Jehoshua O, Kornberg A. Protein Z and its role in venous and arterial thrombosis. *Isr Med Assoc J*. 2006;8(1):53-55.

30. Paidas MJ, Ku DH, Lee MJ, et al. Protein Z, protein S levels are lower in patients with thrombophilia and subsequent pregnancy complications. *J Thromb Haemost*. 2005;3(3):497-501.

31. Dossenbach-Glaninger A, van Trotsenburg M, Helmer H, Oberkanins C, Hopmeier P. Association of the protein Z intron F G79A gene polymorphism with recurrent pregnancy loss. *Fertil Steril*. 2008;90(4):1155-1160.

32. El-Hamid SA, El-Khayat W. Relationship of the protein Z intron F G79A and il6 c634g gene polymorphisms with the risk of recurrent pregnancy loss in Egyptian women. *J Investig Med*. 2011;59(4):655-660.

33. Topalidou M, Effraimidou S, Farmakiotis D, et al. Low protein Z levels, but not the intron F G79A polymorphism, are associated with unexplained pregnancy loss. *Thromb Res*. 2009;124(1): 24-27.

34. Al-Shaikh FS, Sater MS, Finan RR, et al. Protein Z variants associated with protein Z plasma levels and with risk of idiopathic recurrent miscarriage. *Reprod Sci*. 2013;20(9):1062-1068.

35. Rijken DC, Uitte de Willige S. Inhibition of fibrinolysis by coagulation factor XIII. *Biomed Res Int*. 2017;2017:1209676.

36. Schwartz ML, Pizzo SV, Sullivan JB, Hill RL, McKee PA. A comparative study of crosslinked and noncrosslinked fibrin from the major classes of vertebrates. *Thromb Diath Haemorrh*. 1973; 29(2):313-338.

37. Lorand L. Activation of blood coagulation factor XIII. *Ann N Y Acad Sci*. 1986;485:144-158.

38. Goodman CS, Coulam CB, Jeyendran RS, Acosta VA, Roussev R. Which thrombophilic gene mutations are risk factors for recurrent pregnancy loss? *Am J Reprod Immunol*. 2006;56(4):230-236.

39. Yenicesu GI, Cetin M, Ozdemir O, et al. A prospective case-control study analyzes 12 thrombophilic gene mutations in Turkish couples with recurrent pregnancy loss. *Am J Reprod Immunol*. 2010;63(2):126-136.

40. Wang W, Ma H, Lu L, et al. Association between thrombin-activatable fibrinolysis inhibitor gene polymorphisms and venous thrombosis risk: a meta-analysis. *Blood Coagul Fibrinolysis*. 2016;27(4):419-430.

41. Qian K, Xu J, Wan H, et al. Impact of genetic polymorphisms in thrombin activatable fibrinolysis inhibitor (TAFI) on venous thrombosis disease: a meta-analysis. *Gene*. 2015;569(2):173-181.

42. Masini S, Ticconi C, Gravina P, et al. Thrombin-activatable fibrinolysis inhibitor polymorphisms and recurrent pregnancy loss. *Fertil Steril*. 2009;92(2):694-702.

43. ElDanasori N, Abulata N, Shaheen IA, ElGendy AM, El-Khayat W. Thrombin-activatable fibrinolysis inhibitor gene polymorphism (TAFI1040c/t) in women with recurrent spontaneous abortion [published online January 1, 2017]. *Clin Appl Thromb Hemost*.