Association Between *miR-605A>G*, *miR-608G>C*, *miR-631I>D*, *miR-938C>T*, and *miR-1302-3C>T* Polymorphisms and Risk of Recurrent Implantation Failure

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Abstract

Recurrent implantation failure (RIF) is diagnosed when pregnancy failure occurs after 2 consecutive in vitro fertilization–embryo transfers (IVF-ET) to the endometrium using at least 4 high-quality embryos. MicroRNAs (miRNAs) are a class of small noncoding RNA and reported to play an important role in cell proliferation as well as implantation process. Recently, it has been reported that miRNA can regulate RIF occurrence. So, we were to examine the association between the specific miRNA polymorphisms and RIF in Korean women. Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay to determine the frequency of the following polymorphisms: *miR-605A>G*, *miR-608G>C*, *miR-631I>D*, *miR-938C>T*, and *miR-1302-3C>T*. Our results demonstrate a decreased incidence of RIF in patients with the *miR-1302-3C>T* polymorphism (adjusted odds ratio [AOR], 0.234; 95% confidence interval [CI], 0.089-0.618; $P = .003$). Based on our allele combination analysis, the C-T (*miR-938/miR-1302-3*: OR = 0.259; 95% CI, 0.100-0.674; $P = .003$) allele was also associated with decreased RIF risk. From our interaction analysis with *miR-1302-3*, the *miR-1302-3CC* genotype (AOR = 43.332; 95% CI, 5.576-336.745) showed an association with RIF prevalence in participants with an activated partial thromboplastin time (aPTT) ≤ 22.6 . We found that the *miR-1302-3C>T* polymorphism is significantly associated with RIF development in Korean women. Specifically, our study suggests that the T allele of *miR-1302-3* may decrease the risk of RIF in Korean women.

Keywords

microRNA, recurrent implantation failure, polymorphism, Korean women

Introduction

Many patients who experience infertility undergo in vitro fertilization–embryo transfer (IVF-ET) to facilitate pregnancy. However, despite the fact that assisted reproductive technology (ART) has experienced rapid developments over the past several years, pregnancy rates using these techniques remain relatively low.^{1,2}

Recurrent implantation failure (RIF) is one of the most common causes of pregnancy failure after receiving ART.³ The RIF is diagnosed when clinical pregnancy failure occurs after 2 consecutive IVF-ETs to the endometrium using at least 4 high-quality embryos.^{4,5} Consequently, many patients with RIF have financial burden, physical and psychological stress, deterioration in the quality of life, and reduced marital and sexual satisfaction.³ RIF can be attributed to various factors, including endometrial receptivity, embryo quality, embryo–endometrium interaction, immunological factors, uterine anomaly, thrombophilic conditions, hormonal or metabolic disorders, and

genetics. However, in the majority of cases, the cause of RIF remains unknown.^{1,6,7}

MicroRNAs (miRNAs) are a class of small (approximately 18~22 nucleotide) noncoding RNAs that are critical for the

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regulation of gene expression. These RNA species mediate post-transcriptional gene expression by binding to the 3'-untranslated regions (UTRs), of target mRNAs in a sequence dependent manner, leading to transcript degradation and translational repression.⁸⁻¹¹ A single miRNA can bind to, and regulate, multiple targets, and consequently, miRNAs are required for the regulation of large number of genes that are required for normal cellular functions.¹²

Over one-third of human messenger RNA (mRNAs) are estimated to be regulated by miRNAs.¹³ These regulatory RNAs are involved in diverse biological processes, and their aberrant expression is associated with various pathological processes and diseases, such as cancer and diabetes.¹⁴ Additionally, miRNAs have been reported to play important roles in cellular proliferation, differentiation, and apoptosis as well as the implantation process.¹ Notably, a number of studies have shown that miRNAs can specifically affect infertility and the human reproductive system,¹⁵ and it has recently been reported that miRNAs can regulate RIF occurrence.¹⁶

In this study, we selected 5 miRNA polymorphisms that were previously identified to be indirectly related to pregnancy outcome, *miR-605A>G* (rs2043556), *miR-608G>C* (rs4919510), *miR-631I>D* (rs5745925), *miR-938C>T* (rs12416605), and *miR-1302-3C>T* (rs7589328),¹⁷⁻²⁸ and measured their frequencies in Korean women with RIF and control participants. These miRNAs have all been associated with pregnancy complications and/or fetal loss. Xiao et al showed that *miR-605* modulates the p53-mouse double minute 2 homolog (MDM2, a negative regulator of TP53) pathway.¹⁷ Additionally, Fraga et al¹⁸ reported that the MDM2 gene is associated with a risk of recurrent pregnancy loss, and TP53 is known to regulate maternal reproduction and play a key role in regulating blastocyst implantation.¹⁹ Another miRNA analyzed in this study, *miR-608*, was reported to regulate interleukin 6 (IL-6) expression,²⁰ and increased levels of IL-6 have been associated with difficulty achieving a successful pregnancy.²¹ The miRNA *miR-631* which is associated with drug metabolism was shown to regulate the expression of sulfotransferase family 1A member 1 (SULT1A1) in a genotype-specific manner.²² Over one-half of all pregnant women use prescription medications to maintain both maternal and fetal health.²³ Therefore, in some cases, drug metabolism is associated with the maintenance of pregnancy. For example, folate supplementation is recommended for pregnant women because deficient folate intake increases the risk of spontaneous abortion.²⁴ Another *miR* species investigated in this study, *miR-938*, has been reportedly associated with the transforming growth factor β (TGF- β) signaling pathway,²⁵ which plays an important role in cell survival and control of apoptosis at specific stages of pregnancy.²⁶ In addition, *miR1302-3* is known as a placenta-specific miRNA.²⁷ Because the placenta facilitates the exchange of oxygen and nutrients between the mother and the fetus, it is necessary for fetal survival.²⁸ While these 5 miRNA polymorphisms have been associated with other diseases,²⁹⁻³¹ their specific relationship with implantation and pregnancy has not yet been determined.

Materials and Methods

Study Population

Blood samples were collected from 119 patients with RIF (mean $\pm$ standard deviation [SD] age, 33.90 ± 5.64 years; mean body mass index [BMI], 21.63 ± 3.40) who were diagnosed with failure to conceive after 2 completed fresh IVF-ET cycles with more than 10 cleaved embryos. Samples were collected in the Department of Obstetrics and Gynecology, Fertility Center of CHA Bundang Medical Center in Seongnam, South Korea, between March 2010 and December 2012. For all patients, serum human chorionic gonadotropin (HCG) concentrations were <5 mIU/mL at 14 days after embryo transfer, and all transferred embryos were examined by the embryologist before transfer and considered to be of good quality.

We evaluated both the male and the female partners of couples experiencing RIF, and participants who were diagnosed with RIF due to anatomic, chromosomal, hormonal, infectious, autoimmune, or thrombotic causes were excluded from the study group. Several imaging modalities, including sonography, hysterosalpingogram, hysteroscopy, computerized tomography, and magnetic resonance imaging, were utilized to evaluate anatomical abnormalities. Karyotyping was conducted using standard protocols. Participants determined to have experienced hormonal causes of RIF, including hyperprolactinemia, luteal insufficiency, and thyroid disease, were identified by measuring prolactin, thyroid-stimulating hormone (TSH), free T4, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone levels in peripheral blood and excluded from the study. Lupus anticoagulant and anticardiolipin antibodies were further measured to rule out autoimmune diseases, such as lupus and antiphospholipid syndrome, respectively. Thrombotic disorders, defined as thrombophilia, were evaluated by protein C and protein S deficiencies and by the presence of anti- $\alpha 2$ glycoprotein antibodies. Male partners were assessed by performing semen analysis, karyotyping, and hormonal assays, including those measuring estradiol (E2), testosterone, FSH, and LH. Among the initial 167 patients selected for the study, 48 patients who had intrauterine adhesion, hypothyroidism, trisomy, or chromosomal translocation (patients or spouses) or antiphospholipid syndrome were excluded from the patient group.

The control group consisted of 212 participants (age range, 27-45 years; mean $\pm$ SD age, 34.24 ± 3.35 years; BMI, 21.01 ± 2.75) with normal karyotypes (46XX), regular menstrual cycles, a history of at least 1 naturally conceived pregnancy, and no history of pregnancy loss. The Institutional Review Board of CHA Bundang Medical Center reviewed and approved the study on February 23, 2010 (reference no. PBC09-120). Informed consent was provided from all participants, and data were collected identically for all groups.

Genotyping

Genomic DNA samples were extracted from anticoagulated peripheral blood samples from all patients and control

participants using the G-DEX Genomic DNA Extraction Kit For Blood (iNtRON Biotechnology Inc, Seongnam, Korea). The following 5 miRNA single-nucleotide polymorphisms (SNPs) were selected using the human genome SNP database (dbSNP, <http://www.ncbi.nlm.nih.gov/snp>): *miR-605A>G* (rs2043556), *miR-608G>C* (rs4919510), *miR-631I>D* (rs5745925), *miR-938C>T* (rs12416605), and *miR-1302-3C>T* (rs7589328). Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. The PCR primers and temperature used to detect each polymorphism are shown in Supplementary Table S1.

The PCRs to detect the *miR-605A>G* (rs2043556) and the *miR-608G>C* (rs4919510) polymorphisms were performed using an initial denaturation at 95°C for 15 minutes, 40 cycles of denaturation at 95°C for 20 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. We performed restriction enzyme digestion at 37°C for 16 hours with *HinfI* for *miR-605* and *PvuII* for *miR-608* (New England BioLabs, Beverly, Massachusetts). The PCRs to detect the *miR-631I>D* (rs5745925), *miR-938C>T* (rs12416605), and *miR-1302-3C>T* (rs7589328) polymorphisms were performed using an initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds at 61°C for *miR-631*, 64°C for *miR-938*, or 58°C for *miR-1302-3*, and extension at 72°C for 30 seconds; and a final extension at 72°C for 5 minutes. We performed restriction enzyme digestion at 37°C for 16 hours with *NlaIV* for *miR-631*, *HhaI* for *miR-938*, and *NlaIII* for *miR-1302-3* (New England BioLabs, Beverly, Massachusetts).

We randomly chose and repeated approximately 20% of the PCR assays for each polymorphism. To validate the PCR-RFLP analysis, DNA sequencing was performed about 20% of the samples by random selection using an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, California). The concordance of the quality control samples was 100%.

Assessment of Blood Coagulation Status

Platelet count (PLT), white blood cell (WBC), and hemoglobin (Hgb) were measured using the Sysmex XE 2100 Automated Hematology System (Sysmex Corporation, Kobe, Japan). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were measured with the ACL TOP automated photo-optical coagulometer (Mitsubishi Chemical Medience, Tokyo, Japan).

Hormone Assays

To measure FSH, LH, E2, TSH, and prolactin levels, blood samples were collected by venipuncture on the third to fifth days of the menstrual cycle. Serum was prepared as previously described, and hormone levels were determined using either a radioimmunoassay (E2, TSH, and prolactin; Beckman Coulter) or an enzyme immunoassay (FSH and LH; Siemens, Munich, Germany), according to the manufacturers' instructions.

Statistical Analysis

The differences in the frequency of miRNA gene polymorphisms in the control group and the RIF patient group were analyzed using the Fisher exact test and logistic regression. Odds ratios (ORs), adjusted odds ratios (AORs), and 95% confidence intervals (CIs) were calculated and used to investigate the association between miRNA polymorphisms and RIF risk. The data are presented as means $\pm$ SD for continuous variables or as percentages for categorical variables. Statistical analysis was performed using MedCalc, version 12.1.4 (MedCalc Software bvba, Mariakerke, Belgium) or GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA). In addition, the previous study reported the correlation between blood coagulation factors and embryo implantation success. Therefore, we tried to analyze the correlation between blood coagulation factors and miRNA polymorphisms; the analyses were performed based on the clinical indicators of blood coagulation factors which are PLT, PT, and aPTT. We performed stratified analysis by grouping the values of the relevant factors into the lower 15% and upper 15%, and miRNA polymorphisms in order to we confirmed the association between miRNA polymorphisms and blood coagulation factors in a hypercoagulated environment. The HAPSTAT program (v.3.0, <http://www.bios.unc.edu/~lin/hapstat/>) with a strong synergistic effect was used to estimate the frequency of polymorphic haplotype. *P* values <.05 were considered statistically significant. The false discovery rate (FDR) was also used to adjust for multiple comparisons; associations with an FDR-corrected *P* value <.05 were considered statistically significant.³² The statistical power of positive association was calculated using G*POWER 3.0 (Institut für Psychologie, Christian-Albrechts-Universität Kiel).

Results

The demographic characteristics and clinical profiles of patients with RIF and controls are presented in Table 1. Table 2 shows the genotype frequencies of miRNA polymorphisms in control participants and patients with RIF. All genes analyzed showed polymorphism frequencies similar to the Hardy-Weinberg equilibrium (HWE) expected value in each group.

Our data indicate that the *miR-1302-3* polymorphism is significantly associated with decreased RIF risk (CT: AOR = 0.234; 95% CI, 0.089-0.618, *P* = .003; CT+TT: AOR = 0.227; 95% CI, 0.086-0.598, *P* = .003). We further found a statistically significant negative association between this allele and RIF in patients who experienced ≥ 3 (CT: AOR = 0.261; 95% CI, 0.099-0.690; *P* = .007, CT+TT: AOR = 0.253; 95% CI, 0.096-0.668; *P* = .006) and ≥ 4 (CT: AOR = 0.301; 95% CI, 0.103-0.882; *P* = 0.029, CT+TT: AOR=0.292; 95% CI, 0.100-0.853; *P* = .024) implantation failures (Supplementary Table S2). Thus, in both groups, the *miR-1302-3* polymorphism was associated with decreased incidence of RIF.

To identify allele combinations associated with RIF prevalence, we performed allele combination analyses with 5, 4, 3,

Table 1. Clinical Characteristics of Patients With RIF and Control Participants.

Characteristic	Controls, n = 212	RIF, n = 119	P ^a
Age, years, mean $\pm$ SD)	33.90 $\pm$ 5.64	34.24 $\pm$ 3.35	.555
BMI, kg/m ² , mean $\pm$ SD	21.63 $\pm$ 3.40	21.01 $\pm$ 2.75	.150
Repeated implantation failure, n, mean $\pm$ SD	NA	4.77 $\pm$ 2.27	
Live birth, n, mean $\pm$ SD	1.73 $\pm$ 0.72	NA	
Mean gestational age, weeks, mean $\pm$ SD	39.33 $\pm$ 1.70	NA	
PT, seconds	11.42 $\pm$ 2.90 (58)	11.10 $\pm$ 1.48 (111)	.349
aPTT, seconds	33.54 $\pm$ 4.16 (58)	29.18 $\pm$ 3.42 (112)	<.0001
WBC, 10 ³ / μ L	NA	7.22 $\pm$ 2.85 (114)	
Hgb, g/dL	NA	12.57 $\pm$ 1.40 (114)	
PLT, 10 ³ / μ L	239.27 $\pm$ 65.67 (166)	233.69 $\pm$ 58.28 (114)	.466
E2 (Estradiol), mIU/mL	26.61 $\pm$ 14.73 (103)	35.61 $\pm$ 23.93 (111)	.0003^b
TSH, μ IU/mL	NA	2.26 $\pm$ 1.44 (95)	
FSH, mIU/mL	8.32 $\pm$ 2.83 (103)	8.75 $\pm$ 4.50 (95)	.971 ^b
LH, mIU/mL	3.33 $\pm$ 1.80 (103)	4.83 $\pm$ 2.29 (92)	<.0001^b
Prolactin, ng/mL	NA	12.64 $\pm$ 6.18 (95)	

Abbreviations: SD, standard deviation; BMI, body mass index; aPTT, activated partial thromboplastin time; PT, prothrombin time; WBC, white blood cell; Hgb, hemoglobin; PLT, platelet count; BUN, blood urea nitrogen; NA, not applicable; RIF, recurrent implantation failure.

^aFisher exact test.

^bMann-Whitney U test.

and 2 SNP combinations (Supplementary Table S3-S6). Among all of the interactions examined, the *miR-605A/938T/1302-3C* (OR = 31.670; 95% CI, 1.802-556.500; P = .0003) allele combination was associated with increased RIF occurrence. Conversely, the *miR-938C/1302-3T* (OR = 0.259; 95% CI, 0.100-0.674; P = .003) allele combination was associated with decreased RIF occurrence (Table 3).

From our combined gene-gene genotype analysis shown in Supplementary Table S7, the *miR-608GC/miR-938CC* (OR = 1.811; 95% CI, 1.024-3.202; P = .041) combined genotype was found to be associated with increased risk of RIF. Additionally, the *miR-631II/miR-1302-3CT* (OR = 0.244; 95% CI, 0.092-0.650; P = .005) combined genotype and the *miR-938CC/miR-1302-3CT* (OR = 0.270; 95% CI, 0.101-0.718; P = .009) combined genotype were associated with decreased RIF prevalence.

We then performed an interaction analysis to evaluate possible synergistic effects between the combined polymorphisms and the environment. We found that, particularly for *miR-608GC+CC* and *miR-1302-3CC*, there was a significant increase in RIF prevalence in association with the levels blood coagulation factors (Table 4; Supplementary FigureS1). The statistical powers of positive genetic associations measured in this study are shown in Supplementary Table S8.

Discussion

In this study, we investigated the association between 5 miRNA polymorphisms, *miR-605A>G* (rs2043556), *miR-608G>C* (rs4919510), *miR-631I>D* (rs5745925), *miR-938C>T* (rs12416605), and *miR-1302-3C>T* (rs7589328), and RIF prevalence. Notably, although these studies suggest a possible role for all 5 miRNA species in RIF, our data have shown that not all miRNA polymorphisms are significant in this context.

Specifically, we found a statistically significant association only between the *miR-1302-3C>T* polymorphism and RIF in our cohort of Korean women.

Endometrial receptivity is essential for achieving successful implantation. Wang et al have reported that the volume of specific miRNAs expressed during early pregnancy might be involved in pregnancy formation and decidualization, which is required to prepare the endometrium for pregnancy and to form the placenta.³³ This report found that these miRNAs are required for embryo implantation to occur after decidualization. In addition, another report has suggested that miRNAs are associated with blastocyst implantation failure³⁴ and play a major role in embryo implantation.¹⁴ Collectively, these studies indicate that miRNAs play critical roles during pregnancy, and our data showing that *miR-1302-3* polymorphisms can contribute to risk of RIF are in agreement with this.

Based on our interaction analysis, we further found that the *miR-608GC+CC* and *miR-1302-3CC* genotypes were significantly associated with altered levels of blood coagulation factors, including PT, aPTT, and PLT. In the early stages of implantation, prevention of excess fibrin deposition in placental vessels and intravillous spaces is critical, and secure fibrin polymerization and stabilization of the placental basal plate both require an exact balance of blood coagulation and fibrinolysis.³⁵ Women with thrombophilia also have an increased risk of fetal loss,³⁶ suggesting that alteration in blood coagulation factors, such as aPTT, PT, and PLT, may interfere with implantation and pregnancy. Interestingly, the *miR-1302-3* polymorphism showed an association with RIF, whereas the *miR-608* polymorphism did not. This suggests that the association between *miR-608* and blood coagulation does not directly affect RIF; however, further study on this will be needed for confirmation.

The 5 miRNAs that we investigated in this study have been reported to be involved in various cancers, including lung and

Table 2. Genotype Frequencies of miRNA Gene Polymorphisms Between Controls and Patients With RIF.

Genotype	Controls, n = 212	RIF, n = 119	COR (95% CI)	FDR- <i>P</i> ^a	AOR (95% CI) ^b	FDR- <i>P</i>
<i>miR-605</i> rs2043556						
AA	103 (48.6)	57 (47.9)	1.000 (reference)		1.000 (reference)	
AG	85 (40.1)	53 (44.5)	1.127 (0.703-1.806)	.620	1.120 (0.699-1.796)	.637
GG	24 (11.3)	9 (7.6)	0.678 (0.295-1.557)	.598	0.685 (0.298-1.576)	.623
Dominant (AA vs AG+GG)			1.028 (0.656-1.611)	.905	1.024 (0.653-1.606)	.917
Recessive (AA+AG vs GG)			0.641 (0.288-1.428)	.693	0.646 (0.290-1.440)	.713
HWE <i>P</i>	0.316	0.486				
<i>miR-608</i> rs4919510						
GG	68 (32.1)	29 (24.4)	1.000 (reference)		1.000 (reference)	
GC	100 (47.2)	64 (53.8)	1.501 (0.878-2.565)	.230	1.502 (0.879-2.569)	.228
CC	44 (20.8)	26 (21.8)	1.386 (0.723-2.657)	.598	1.397 (0.727-2.684)	.623
Dominant (GG vs GC+CC)			1.466 (0.882-2.436)	.235	1.476 (0.887-2.457)	.223
Recessive (GG+GC vs CC)			1.067 (0.618-1.845)	.995	1.075 (0.622-1.859)	.995
HWE <i>P</i>	0.518	0.405				
<i>miR-631</i> rs5745925						
II	189 (89.2)	112 (94.1)	1.000 (reference)		1.000 (reference)	
ID	23 (10.8)	7 (5.9)	0.514 (0.214-1.235)	.230	0.507 (0.210-1.222)	.228
DD	NA	NA	NA	NA	NA	NA
Dominant (II vs ID+DD)			0.514 (0.214-1.235)	.235	0.507 (0.210-1.222)	.223
Recessive (II+ID vs DD)			NA	NA	NA	NA
HWE <i>P</i>	0.404	0.741				
<i>miR-938</i> rs12416605						
CC	201 (94.8)	109 (91.6)	1.000 (reference)		1.000 (reference)	
CT	11 (5.2)	9 (7.6)	1.509 (0.607-3.753)	.470	1.529 (0.614-3.809)	.453
TT	NA	1 (0.8)	NA	.995	NA	.995
Dominant (CC vs CT+TT)			1.676 (0.690-4.072)	.318	1.697 (0.698-4.127)	.304
Recessive (CC+CT vs TT)			NA	.995	NA	.995
HWE <i>P</i>	0.698	0.121				
<i>miR-1302-3</i> rs7589328						
CC	178 (84.0)	114 (95.8)	1.000 (reference)		1.000 (reference)	
CT	33 (15.6)	5 (4.2)	0.237 (0.090-0.624)	.020	0.234 (0.089-0.618)	.015
TT	1 (0.5)	NA	NA	.995	NA	.995
Dominant (CC vs CT+TT)			0.230 (0.087-0.604)	.015	0.227 (0.086-0.598)	.015
Recessive (CC+CT vs TT)			NA	.995	NA	.995
HWE <i>P</i>	0.687	0.815				

Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; RIF, recurrent implantation failure.

^aFDR-adjusted *P* value^bFor AOR was adjusted by age of participants**Table 3.** Allele Combination Analysis for miRNA Gene Polymorphisms in RIF and Control Participants.

Allele Combination	Controls, 2n = 424	RIF, 2n = 238	OR (95% CI) ^a	FDR- <i>P</i> ^b
<i>miR-605/miR-938/miR-1302-3</i>				
A-C-C	266 (62.7)	151 (63.5)	1.000 (reference)	
A-C-T	20 (4.6)	5 (2.1)	0.440 (0.162-1.198)	.248
A-T-C	3 (0.7)	11 (4.6)	6.459 (1.774-23.520)	.018
A-T-T	3 (0.6)	0 (0)	0.251 (0.013-4.901)	.649
G-C-C	115 (27)	71 (29.8)	1.088 (0.761-1.554)	.649
G-C-T	13 (3.1)	0 (0)	0.065 (0.004-1.104)	.018
G-T-C	5 (1.3)	0 (0)	0.160 (0.009-2.914)	.248
<i>miR-938/miR-1302-3</i>				
C-C	380 (89.7)	222 (93.3)	1.000 (reference)	
C-T	33 (7.7)	5 (2.1)	0.259 (0.100-0.674)	.009
T-C	9 (2.1)	11 (4.6)	2.092 (0.854-5.128)	.159
T-T	2 (0.5)	0 (0)	0.342 (0.016-7.162)	.534

Abbreviations: OR, odds ratio; CI, confidence interval; RIF, recurrent implantation failure.

^aORs and 95% CIs for each allele combination were calculated with reference to frequencies of all others using Fisher exact test. *P*-value by Fisher exact test.^bFDR (false discovery rate)-adjusted *P*-value.

Table 4. Potential Interactions Between miRNA Polymorphisms and Environmental Factors, such as Advanced Age, PT, aPTT, and PLT in RIF Occurrence.

Characteristics	miR-608 GG	miR-608 GC+CC	miR-1302-3 CT+TT	miR-1302-3 CC
Age				
<34	1.000 (reference)	1.501 (0.702-3.211)	1.000 (reference)	0.118 (0.015-0.911)
≥34	1.691 (0.702-4.075)	2.403 (1.143-5.055)	1.610 (1.002-2.587)	0.471 (0.150-1.476)
PT ^a				
<11.6 seconds	1.000 (reference)	3.810 (1.693-8.572)	1.000 (reference)	2.445 (0.391-15.276)
≥11.6 seconds	8.046 (1.571-41.198)	6.260 (2.046-19.157)	1.000 (0.040-25.212)	10.140 (1.291-79.618)
aPTT ^b				
>22.6 seconds	1.000 (reference)	2.023 (0.969-4.222)	1.000 (reference)	2.569 (0.608-10.853)
≤22.6 seconds	NA	22.236 (4.783-103.369)	NA	43.332 (5.576-336.745)
PLT ^b				
>201 10 ³ /μL	1.000 (reference)	1.421 (0.760-2.659)	1.000 (reference)	5.642 (1.636-19.462)
≤201 10 ³ /μL	0.940 (0.329-2.680)	2.450 (1.117-5.376)	1.321 (0.094-18.502)	8.397 (2.239-31.490)

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; PLT, platelet count; NA, not applicable; RIF, recurrent implantation failure.

^aPT 11.6 seconds was upper 25% cutoff each level in patients and controls.

^baPTT 22.6 seconds and PLT 201 10³/μL were lower 25% cutoff each level in patients and controls.

colorectal cancer. However, to our knowledge, this is the first study to measure the association between these polymorphisms and RIF. Therefore, these data may help to enhance our understanding of the roles that miRNAs play in pregnancy and implantation. We note, however, that this study has a number of limitations. First, it remains unclear how these miRNA polymorphisms might affect RIF. In order to clarify the association of the disease more clearly, confounder requires correction of various factors such as other patient characteristics and IVF outcomes. However, our statistical results were logistic regression using age as an adjusted factor between patient and control, and additional research is required to address this question. Second, our study population was limited to Korean women, and therefore, these results should be confirmed using a more diverse patient cohort. Finally, because RIF is relatively uncommon, our patient sample size was small; more samples will be required to substantiate our observations.

In conclusion, we measured the genetic associations between RIF prevalence and the *miR-605A>G* (rs2043556), *miR-608G>C* (rs4919510), *miR-631I>D* (rs5745925), *miR-938C>T* (rs12416605), and *miR-1302-3C>T* (rs7589328) polymorphisms in Korean women. In particular, the *miR-1302-3C>T* polymorphism was found to be significantly associated with RIF risk, and specifically, the *miR-1302-3CT+TT* genotype was associated with decreased RIF prevalence. We therefore suggest that *miR-1302-3C>T* polymorphism may represent a potential biomarker for diagnosis of RIF risk.

Authors' Note

HAL performed the experiments, collected the results, involved in manuscript writing, and discussed and interpreted the data and results. EHA collected the blood samples from patients with RIF and control participants and discussed and interpreted the data and results. HGJ performed the experiments and collected the results. JOK discussed the data. JHK and WSL collected the blood samples and discussed and

interpreted the data and results. YBL collected the blood samples. NKK designed and directed the whole project.

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Authors' Contribution

Hyun Ah Lee, MS, and Eun Hee Ahn, MD, PhD, has contributed equally to this work.

Declaration of Conflicting Interests

The author(s) declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplementary material for this article is available online.

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