Association study of frameshift and splice variant polymorphisms with risk of idiopathic recurrent pregnancy loss

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Abstract. Recurrent pregnancy loss (RPL) is defined as ≥ 2 consecutive pregnancy losses, and can be caused by various factors, including genetics, chromosomal abnormalities, thrombophilia, immune disorders, nutritional factors, environmental factors, psychological stress or maternal infections; however, as many as 50% of RPL cases are idiopathic. In the present study, the role of genetic polymorphisms in RPL was investigated. Four gene polymorphisms were selected by whole exome sequencing, including membrane spanning 4-domains A14 (MS4A14)D>I (rs3217518), solute carrier family 2 member 7 (SLC2A7)D>I (rs60746313), pregnancy specific β -1-glycoprotein 9 (*PSG9*)C>T (rs3746297) and ATP binding cassette subfamily B member 5 (ABCB5) C>G (rs17143187), and the aim was to investigate their association with RPL in Korean women. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism assay. Allele combination analysis revealed that the four-allele combination I-D-T-G, (MS4A14/SLC2A7/PSG9/ABCB5) was associated with a decreased risk for RPL. Interaction analysis demonstrated that the following genotypes: MS4A14 DI+II, SLC2A DI+II and ABCB 5 CG+GG, were associated with a prothrombin time ≥ 12 sec and with RPL risk. It may be concluded that the four gene polymorphisms do not affect RPL individually, but are associated with RPL when in combination with other

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genes or blood coagulation factors. Notably, the *MS4A14* I allele, with a prothrombin time ≥ 12 sec, may be a potential biomarker for diagnosis, prevention and prognosis of RPL.

Introduction

Recurrent pregnancy loss (RPL) was initially defined as \geq 3 consecutive pregnancy losses before 20 weeks of gestation (1,2), but was later redefined by the American Society for Reproductive Medicine as ≥ 2 consecutive pregnancy losses (3). An estimated 1-5% of women of reproductive age experience a pregnancy loss, and 10-20% of pregnancies end in a miscarriage, which frequently occurs in the second or third month of pregnancy (2,4,5). RPL may be caused by genetic disorders or various other factors, including fetal chromosomal abnormalities, uterine anomalies, thrombophilia, endocrine, immune and anatomical disorders, nutritional or environmental factors, psychological stress, or maternal infections (5). However, in as many as 50% of women who suffer from recurrent miscarriages, the causes are considered idiopathic. RPL is an important reproductive health issue and remains an active field of research (4). In the present study, the potential genetic cause of RPL was investigated. Four candidate genes harboring mutations that have been associated with other diseases were selected at random as they have not been investigated in association with RPL, and their association with RPL in Korean women was assessed.

Frameshift mutations result from insertions or deletions that alter the reading frame, and affect the subsequent coding sequence as well as the stop codon. Therefore, frameshift mutations may result in final polypeptide products with an abnormal length or in nonsense-mediated mRNA decay (6). This type of mutation has been implicated in numerous diseases; for example, Crohn's disease is associated with the nucleotide-binding oligomerization domain 2 3020insC frameshift mutation (7). Alternatively, abnormalities in alternative splicing have also been implicated in various diseases, including cancer (8-10).

Next-generation sequencing (NGS) is a more advanced method of DNA sequencing compared with Sanger sequencing, which permits the sequencing of the entire human genome in

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1 day (11,12). It offers efficient analysis and rapid processing time, thereby reducing the labor and expenses associated with other sequencing options (13,14). NGS is a powerful tool used to study genetic diseases (13-16), and was therefore the technique of choice for this study.

In the present study, frameshift mutations in membrane spanning 4-domains A14 (*MS4A14*) and solute carrier family 2 member 7 (*SLC2A7*), and splice variants in pregnancy specific β -1-glycoprotein 9 (*PSG9*) and ATP binding cassette subfamily B member 5 (*ABCB5*) were investigated. These were selected through whole-exome sequencing (WES). To the best of our knowledge, the following four polymorphisms: *MS4A14*D>I (rs3217518), *SLC2A7*D>I (rs60746313), *PSG9*C>T (rs3746297) and *ABCB5*C>G (rs17143187), have not been previously studied in RPL. Therefore, the aim of the study was to examine the association between these four gene polymorphisms and RPL in Korean women.

Materials and methods

Study population. The study population included 383 female patients with RPL and 276 control women. Patients with RPL were 22-45 years old, with an average age of 33.11 ± 4.44 years and a body mass index (BMI) of 21.48 ± 3.85 kg/m². Women in the control group were 20-66 years old, with an average age of 33.09 ± 5.68 years and a BMI of 21.31 ± 3.30 kg/m².

Blood samples were collected from patients with RPL and control women between March 1999 and February 2012 at the Department of Obstetrics and Gynecology and the Fertility Center of CHA Bundang Medical Center (Seongnam, Korea). The study was approved by the Institutional Review Board of CHA Bundang Medical Center (IRB number: BD2010-123D) and written informed consent was provided by all patients. Women in the control group had regular menstrual cycles, a history of ≥ 1 naturally-conceived pregnancy, no history of pregnancy losses and a normal karyotype (46,XX). Women were diagnosed with RPL if they had a history of ≥ 2 consecutive spontaneous pregnancy losses. Before week 20 of gestation, ultrasound and/or physical examinations were performed and human gonadotropic hormone levels were assessed. The average gestational age at the time of miscarriage was 7.35±1.93 weeks, and overall patients had 3.28±1.84 miscarriages.

Patients with a history of smoking or alcohol abuse, or whose miscarriages were attributed to infection or anatomical, hormonal, chromosomal, autoimmune or thrombotic causes were excluded from the study. Anatomical causes of miscarriage were determined using hysterosalpingography, hysteroscopy, computerized tomographic scanning or magnetic resonance imaging, to identify intrauterine adhesion, septate uterus or uterine fibroids. Hormonal causes of miscarriage included hyperprolactinemia, luteal insufficiency and thyroid disease; these were determined by measuring relevant hormone levels in blood samples. The chromosomal causes for miscarriage were assessed by standard chromosome analysis using the G-banding method (17). Miscarriages caused by infection with Ureaplasma urealyticum or Mycoplasma hominis were identified by bacterial culture. The following autoimmune diseases: Lupus and antiphospholipid syndrome, were selected for their strong association with miscarriages; these were identified by measuring lupus anticoagulant and anticardiolipin antibodies. Thrombophilia was defined as a thrombotic disorder associated with miscarriages, and was identified by protein C and protein S deficiency, and presence of anti- β -2 glycoprotein.

Genotyping. Genomic DNA was extracted from peripheral blood of each study participant using the G-DEX DNA extraction kit (Intron Biotechnology, Inc., Seongnam, Korea). Macrogen, Inc. (Seoul, Korea) was commissioned to perform NGS of 20 patients with RPL. NGS was conducted using a HiSeq Instrument (Macrogen, Inc., Seoul, Korea) and paired-end sequences produced by a HiSeq Instrument were first mapped to the human genome using the mapping program 'BWA' (version 0.7.12) (https://sourceforge. net/projects/bio-bwa/files/). Based on the BAM file previously generated, variant genotyping for each sample was performed with Haplotype Caller of GATK (Broad Institute, Cambridge, MA, USA). Then, an in-house program and SnpEff (snpeff.sourceforge.net/) was applied to filter additional databases, including ESP6500 (https://esp.gs.washington. edu/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and dbNSFP2.9 (https://sites.google.com/site/jpopgen/dbNSFP). WES statistical analyses were also conducted by Macrogen, Inc. The frameshift and splice variants genes were identified from the human genome single nucleotide polymorphism (SNP) database (http://www.ncbi.nlm.nih.gov/snp), and selected from the WES statistical list. The four polymorphisms selected were as follows: MS4A14D>I (rs3217518), SLC2A7D>I (rs60746313), PSG9C>T (rs3746297) and ABCB5C>G (rs17143187). The four SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis; the primers, PCR conditions and restriction enzymes used, are detailed in Table I (18).

The *MS4A14*D>I polymorphism was amplified using forward (5'-TTGGATGGAGGGAAAGGTGTG-3') and reverse (5'-TTTTGCCGTGAAGGGAGCT-3') primers, and was amplified by the SolgTM 2X h-Taq PCR Pre-mix (SolGent co., Ltd., Daejeon, Korea) under the following conditions: 95°C for 15 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec; and a final extension at 72°C for 5 min. The PCR products were digested with the restriction enzyme *Tsp*RI (New England BioLabs, Inc., Ipswich, MA, USA) at 65°C for 16 h.

The *SLC2A7*D>I polymorphism was amplified using forward (5'-AAGATGGCGGCTACCTTC-3') and reverse (5'-CTACAACCTCTCTGTGGTCA-3') primers, and was amplified by PCR under the following conditions: 95°C for 15 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 53°C for 30 sec, and extension at 72°C for 30 sec; and a final extension at 72°C for 5 min. The genotypes of the amplified products were identified by electrophoretic separation on a 5% agarose gel using EcoDyeTM Nucleic Acid Staining Solution (BioFact Co., Ltd., Daejeon, Korea).

The *PSG9*C>T polymorphism was amplified using forward (5'-GTAATGGTAGAGGTCCGTCA-3') and reverse (5'-CGT GTGTGTATCTTCAAGGC-3') primers, and was amplified by PCR under the following conditions: 95°C for 15 min;

Polymorphism	Reference SNP	Chromosome	Position	Function	Primer sequence	Annealing temperature (°C)	Restriction enzyme	Band size (bp)
MS4A14 D>1	rs3217518	=	60397880	Frameshift	Forward: 5'-TTGGATGGAGGGAAAGGTGTG-3' Reverse: 5'-TTTTGCCGTGAAGGGAGCT-3'	55	TspRI	DD: 229, 140 DI: 370, 229, 140 II: 370
SLC2A7 D>I	rs60746313	1	9024982	Frameshift	Forward: 5'-AAGATGGCGGCTACCTTC-3' Reverse: 5'-CTACAACCTCTCTGTGGTCA-3	53	Hpy166II	DD: 109 DI: 120, 109
PSG9 C>T	rs3746297	19	43268150	Splice variant	Forward: 5'-GTAATGGTAGAGGTCCGTCA-3' Reverse: 5'-CGTGTGTGTGTATCTTCAAGGC-3'	57	BfaI	II: 120 CC: 171, 114 CT: 285, 171, 114
4 <i>BCB5</i> C>G	rs17143187	L	20626612	Splice variant	Forward: 5'-GAGAAAGGAAGCAGTTGG-3' Reverse: 5'-TAGTTCCCTCTTTCCCAC-3'	52	DdeI	11: 285 CC: 115, 32 CG: 147, 115, 32 GG: 147

The *ABCB5*C>G polymorphism was amplified using forward (5'-GAGAAAGGAAGCAGTTGG-3') and reverse (5'-TAGTTCCCTCTTTCCCAC-3') primers, and was amplified by PCR under the following conditions: 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 sec, annealing at 52°C for 30 sec, extension at 72°C for 30 sec; and a final extension at 72°C for 5 min. The PCR products were digested with the restriction enzyme *Dde*I (Enzynomics, Daejeon, Korea) at 37°C for 16 h.

To validate the PCR-RFLP analysis, DNA sequencing was performed on randomly selected samples (~20% of total samples), using a BigDye[™] Terminator v3.1 Cycle Sequencing kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), ABI 3730xL DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR-RFLP genotyping was 100% concordant with DNA sequencing.

Laboratory tests. Plasma homocysteine, folate, total cholesterol, uric acid, blood urea nitrogen (BUN) and creatinine were measured in blood samples collected from patients with RPL after 12 h of fasting. Homocysteine was measured by IMx fluorescent polarizing immunoassay using the Abbott IMx analyzer (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA). Folic acid was determined with a radioassay kit (ACS:180; Bayer AG, Leverkusen, Germany). Total cholesterol, uric acid, BUN and creatinine were measured using commercially available enzymatic colorimetric tests of the MODULAR PRE ANALYTICS PLUS system (Roche Diagnostics GmbH, Mannheim. Germany). Platelet (PLT) and white blood cell (WBC) counts 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 Gas Chemical Company, Inc., Tokyo, Japan). Blood was collected from the control group by venipuncture on the second or third day of the menstrual cycle for the measurement of FSH, LH, E2, TSH, and prolactin levels. Serum was prepared as previously described (19) and hormone levels were determined using either radioimmunoassays [E2 (cat. no. A21854), TSH (cat. no. IM3712) and PRL (cat. no. IM2121); Beckman Coulter, Inc., Brea, CA, USA], or enzyme immunoassays using IMMULITE® 1000 Systems (FSH and LH; Siemens AG, Munich, Germany) according to the manufacturer's protocols.

Flow cytometric analysis of cluster of differentiation (CD)56⁺ natural killer cells. For cell surface marker staining, peripheral blood samples (50 μ l) were mixed with monoclonal anti-CD56⁺ antibodies labeled with fluorescein isothiocyanate (cat. no. 340417, BD Biosciences, Franklin Lakes, NJ, USA) to a ratio of 5:2, for 15 min at room temperature. A total of 450 μ l 1X BD FACSTM lysing solution (cat. no. 349202, BD Biosciences) was added followed by gentle vortexing and two washes with 2 ml fluorescence-activated cell sorting buffer

Tabl	le II.	Clinica	l characteristics	of patier	its with RPI	L vs. controls.
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Parameter	Control n=276 (n=in each parameter)	RPL n=383	Р
Age (years)	33.09±5.68	33.11±4.44	0.966ª
BMI (kg/m^2)	21.31±3.30	21.48±3.85	0.304 ^b
Previous pregnancy losses (no.)	NA	3.28±1.84	
Live births (no.)	1.73±0.72	NA	
Gestational weeks	39.30±1.68	7.35±1.93	<0.0001 ^a
FSH (mIU/ml)	8.13±2.86 (110)	7.54±10.57 (195)	<0.0001 ^b
E2 (pg/ml)	25.87±14.76 (110)	35.36±29.00 (166)	0.002 ^b
LH (mIU/ml)	3.28±1.71 (110)	6.33±12.15 (196)	<0.0001 ^b
TSH (µIU/ml)	-	2.19±1.56 (211)	
Prolactin (ng/ml)	-	15.61±12.92 (206)	
CD56 ⁺ NK cells (%)	-	18.36±8.02 (131)	
Hematocrit (%)	35.35±4.24 (214)	37.31±3.38 (203)	<0.0001 ^b
PLT (10^3 platelets/ μ l)	235.37±64.08 (221)	256.31±58.83 (203)	0.001 ^a
PT (sec)	11.58±3.14 (64)	11.59±0.86 (206)	<0.0001 ^b
aPTT (sec)	30.79±4.64 (105)	32.27±4.33 (208)	0.006 ^a
Homocysteine (μ mol/l)	-	6.96±2.10 (278)	
Folate (ng/ml)	13.71±8.37 (19)	14.19±12.01 (220)	0.865ª
BUN (mg/dl)	8.07±2.02 (41)	9.96±2.74 (196)	<0.0001 ^b
Creatinine (mg/dl)	0.69±0.08 (41)	0.72±0.12 (195)	0.056 ^b
Total cholesterol (mg/dl)	239.00±85.19 (15)	187.59±49.67 (178)	0.004 ^b
Uric acid (mg/dl)	4.19±1.44 (10)	3.80±0.84 (175)	0.361 ^b

Bold text indicate significant P-values. aPTT, activated partial thromboplastin time; BMI, body mass index; BUN, blood urea nitrogen; E2, estradiol; FSH, follicle stimulating hormone; LH, luteinizing hormone; NA, not applicable; PLT, platelet count; PT, prothrombin time; TSH, thyroid-stimulating hormone. All data are presented as the means ± standard deviation. ^aFisher's exact test; ^bMann-Whitney test.

(PBS supplemented with 1% bovine serum albumin and 0.01% sodium azide; Lonza Group, Ltd., Basel, Switzerland). Cells were fixed in 200 ml 1% paraformaldehyde (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at 30 min in 4°C and washed with BD Perm/Wash[™] buffer (cat. no. 554723, BD Biosciences) prior to acquisition on a BD FACSCalibur (BD Biosciences) (20,21). The data were analyzed using Cell Quest software (BD Biosciences).

Statistical analysis. The gene frequencies of MS4A14, SLC2A7, PSG9 and ABCB5 in patients and controls were assessed by logistic regression, Fisher's exact test and Mann-Whitney test. All genotype frequencies followed the Hardy-Weinberg equilibrium (HWE). The association between the MS4A14, SLC2A7, PSG9 and ABCB5 gene polymorphisms and RPL risk factors were examined by odds ratio (OR), adjusted odds ratio (AOR) and 95% confidence intervals (CIs). Data are presented as the means ± standard deviation. P≤0.05 was considered to indicate a statistically significant difference. The correlations of each genotype or allele with the proportion of NK cells and plasma Hcy, folate, total cholesterol, and uric acid levels were assessed by the Kruskal-Wallis and Mann-Whitney tests. The false discovery rate (FDR) was used for multiple comparisons correction. An FDR adjusted P<0.05 (q-value) was deemed statistically significant (22). MedCalc version 12.1.4 (MedCalc Software bvba, Ostend, Belgium) or GraphPad Prism version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used for statistical analysis. The HAPSTAT version 3.0 (www.bios. unc.edu/~lin/hapstat/) was used to estimate the frequency of the polymorphic haplotype.

Results

Study population. In the present study, patients with RPL and control women were compared. The clinical characteristics of the two study groups are summarized in Table II. There was a significant difference between patients with RPL and control women for several of the parameters tested. The PLT values were significantly higher in the RPL patient group than in the control group. Furthermore, LH values were significantly higher in the RPL patient group than in the control group.

Combined effects between clinical factors and gene polymorphism. Interaction analysis was performed to evaluate the association between adjusted factors, such as age and the different gene polymorphisms; the results are presented in Table III. The MS4A14 DI+II genotype was associated with PT≥12 sec (AOR=3.575; 95% CI=1.115-11.464) and aPTT≤22.1 sec (AOR=0.453; 95% CI=0.211-0.973). The SLC2A DI+II genotype was associated with PT≥12 sec (AOR=6.517; 95% CI=1.783-23.825). The PSG9 CT+TT genotype was associated with PLT≥279x10³/µ1 (AOR=2.069; 95% CI=1.079-3.967) and aPTT≤22.1 sec (AOR=0.390; 95% CI=0.172-0.881). The ABCB5 CG+GG genotype was associated with PT≥12 sec

Table III. Interaction analysis of recurrent pregnancy loss and coagulation factors.

				AOR (9	95% CI)			
Parameter	MS4A14 DD	MS4A14 DI+II	SLC2A7 DD	SLC2A7 DI+II	PSG9 CC	PSG9 CT+TT	ABCB5 CC	ABCB5 CG+GG
Age <33	1.000 (reference)	0.771 (0.483-1.229)	1.000 (reference)	0.922 (0.565-1.504)	1.000 (reference)	0.866 (0.520-1.442)	1.000 (reference)	0.795 (0.490-1.290)
≥33	0.852 (0.503-1.443)	0.802 (0.501-1.285)	1.018 (0.565-1.834)	0.884 (0.542-1.443)	0.912 (0.500-1.662)	0.860 (0.514-1.438)	0.853 (0.474-1.533)	0.821 (0.507-1.329)
PLT^{a}								
$<279 \times 10^{3}/\mu$ 1	1.000 (reference)	0.991 (0.625-1.572)	1.000 (reference)	0.918 (0.564-1.495)	1.000 (reference)	1.047 (0.626-1.750)	1.000 (reference)	0.846 (0.520-1.379)
≥279x10 ³ /µ1	2.251 (1.083-4.676)	1.632 (0.873-3.050)	2.727 (1.126-6.605)	1.482 (0.800-2.747)	1.693 (0.737–3.890)	2.069 (1.079-3.967)	1.640 (0.664-4.049)	1.671 (0.909-3.072)
PT^a								
<12 sec	1.000 (reference)	0.918 (0.476-1.770)	1.000 (reference)	1.230 (0.622-2.435)	1.000 (reference)	0.612 (0.288-1.302)	1.000 (reference)	1.664 (0.842-3.288)
≥12 sec	2.110 (0.647-6.880)	3.575 (1.115-11.46)	1.716 (0.551-5.346)	6.517 (1.783-23.83)	1.015 (0.248-4.161)	2.908 (0.886-9.548)	4.351 (1.097-17.26)	4.224 (1.404-12.706)
$aPTT^b$								
>22.1 sec	1.000 (reference)	0.957 (0.528–1.735)	1.000 (reference)	1.161 (0.630- 2.141)	1.000 (reference)	0.990 (0.506-1.936)	1.000 (reference)	1.119 (0.609-2.057)
≤22.1 sec	0.390 (0.171-0.890)	$0.453\ (0.211-0.973)$	0.519 (0.250-1.075)	0.519 (0.250-1.075)	0.508 (0.188-1.371)	$0.390\ (0.172 - 0.881)$	0.289 (0.088-0.952)	0.505 (0.248-1.028)
Bold text indica member 5; AOR time; <i>SLC2A7</i> , s	te significant P-values. "T , adjusted odds ratio; aP1 olute carrier family 2 me1	The upper quartile for PL T, activated partial thror mber 7.	T and PT was $279x10^3 p$ mboplastin time; $MS4AI$.	latelets/µl and 12 sec, re 4, membrane spanning 4	spectively. ^b The lower q domains A14; PLT, plate	uartile for aPTT was 22.1 elet count; <i>PSG9</i> , pregnar	l sec. <i>ABCB5</i> , ATP bind ncy specific β-1-glycopr	ing cassette subfamily B otein 9; PT, prothrombin

Table	IV.	Genotype	e frec	juencies	in	patients	with	RPL	and	control	s.
		- /									

Genotype	Control (%) n=276	RPL (%) n=383	AOR (95% CI) ^a	\mathbf{P}^{b}	q ^c
MS4A14 (rs3217518)					
DD	92 (33.3)	141 (36.8)	1.000 (reference)		
DI	139 (50.4)	188 (49.1)	0.882 (0.627-1.242)	0.473	0.785
II	45 (16.3)	54 (14.1)	0.770 (0.478-1.239)	0.281	0.562
Dominant (DD vs. DI+II)			0.855 (0.617-1.183)	0.344	0.582
Recessive (DD+DI vs. II)			0.827 (0.537-1.273)	0.388	0.746
HWE P	0.535		0.492		
SLC2A7 (rs60746313)					
DD	74 (26.8)	112 (29.2)	1.000 (reference)		
DI	148 (53.6)	202 (52.7)	0.912 (0.635-1.311)	0.619	0.785
II	54 (19.6)	69 (18.0)	0.854 (0.538-1.356)	0.504	0.672
Dominant (DD vs. DI+II)			0.895 (0.633-1.264)	0.528	0.582
Recessive (DD+DI vs. II)			0.906 (0.610-1.346)	0.625	0.746
HWE P	0.194	0.181			
PSG9 (rs3746297)					
CC	72 (26.1)	108 (28.2)	1.000 (reference)		
СТ	149 (54.0)	195 (50.9)	0.891 (0.616-1.289)	0.540	0.785
TT	55 (19.9)	80 (20.9)	0.990 (0.627-1.562)	0.964	0.672
Dominant (CC vs. CT+TT)			0.906 (0.639-1.286)	0.582	0.582
Recessive (CC+CT vs. TT)			1.066 (0.724-1.570)	0.746	0.746
HWE P	0.164	0.642			
ABCB5 (rs17143187)					
CC	74 (26.8)	113 (29.5)	1.000 (reference)		
CG	133 (48.2)	194 (50.7)	0.950 (0.658-1.371)	0.785	0.785
GG	69 (25.0)	76 (19.8)	0.727 (0.469-1.129)	0.156	0.562
Dominant (CC vs. CG+GG)			0.872 (0.618-1.232)	0.438	0.582
Recessive (CC+CG vs. GG)			0.745 (0.514-1.079)	0.120	0.480
HWE P	0.551	0.658			

^aAOR was adjusted by age of participants. *ABCB5*, ATP binding cassette subfamily B member 5; AOR, adjusted odds ratio; CI, confidence interval; FDR, false discovery rate; HWE, Hardy-Weinberg equilibrium; *MS4A14*, membrane spanning 4-domains A14; *PSG9*, pregnancy specific β-1-glycoprotein 9; RPL, recurrent pregnancy loss; *SLC2A7*, solute carrier family 2 member 7. ^bFisher's exact test; ^cFDR-adjusted P-value.

(AOR=4.224; 95% CI=1.404-12.706). The AOR between the two different gene polymorphisms and PT are summarized in Fig. 1A and B. Overall, these genotypes appeared to be associated with coagulation indicators.

Genotype frequencies of the VEGF polymorphisms. The genotype frequencies of the four selected gene polymorphisms in patients with RPL and normal women are shown in Table IV. According to the results, there was no difference in the frequency of these genotypes between the two study populations, with respect to these polymorphisms. The genotypes between the two groups did not differ with respect to the number of pregnancy losses either, regardless as to whether normal women did not experience pregnancy loss (data not shown). In addition, to determine if various allele combinations were associated with the prevalence of RPL, allele combination analyses were performed with two, three and four allele combinations. The four-allele combination data are presented in Table V (data not shown for two and three allele combinations). Amongst all possible allele combinations of the four genes, *MS4A14I/SLC2A7D/PSG9T/ABCB5*G was associated with decreased RPL risk (OR=0.448; 95% CI=0.223-0.901; P=0.033).

Genotype combination analysis. Finally, genotype combination analysis was performed. The following combinations were significantly associated with decreased RPL occurrence: *MS4A14II/PSG9*CT (OR=0.446; 95% CI=0.200-0.995; P=0.049), *MS4A141I/ABCB5*CG (OR=0.397; 95% CI=0.185-0.851; P=0.018), *SLC2A7DI/ABCB5*GG (OR=0.485; 95% CI=0.240-0.981; P=0.044) and *SLC2A7II/ABCB5*CC (OR=0.376; 95% CI=0.152-0.932; P=0.035). A summary of the results is shown in Table VI.

Discussion

In the present study, the association between four gene polymorphisms, namely *MS4A14*D>I (rs3217518), *SLC2A7*D>I (rs60746313), *PSG9*C>T (rs3746297) and *ABCB5*C>G (rs17143187), and RPL were examined. These frameshift

Allele combination	Controls (2n ^a =552; frequency, %)	RPL (2n=766)	OR (95% CI)	\mathbf{P}^{b}	q ^c
MS4A14/SLC2A7/PSG9/ABCB5					
D-D-C-C	51 (9.2)	82 (10.7)	1.000 (reference)		
D-D-C-G	39 (7.1)	58 (7.6)	0.925 (0.541-1.581)	0.786	0.983
D-D-T-C	45 (8.1)	72 (9.4)	0.995 (0.597-1.659)	1.000	1.000
D-D-T-G	41 (7.4)	57 (7.5)	0.865 (0.508-1.472)	0.684	0.983
D-I-C-C	49 (8.8)	52 (6.8)	0.660 (0.391-1.115)	0.142	0.710
D-I-C-G	31 (5.6)	50 (6.5)	1.003 (0.568-1.771)	1.000	1.000
D-I-T-C	28 (5.0)	58 (7.6)	1.288 (0.728-2.280)	0.392	0.983
D-I-T-G	40 (7.2)	40 (5.2)	0.622 (0.355-1.090)	0.116	0.710
I-D-C-C	21 (3.9)	34 (4.5)	1.007 (0.527-1.923)	1.000	1.000
I-D-C-G	36 (6.6)	51 (6.7)	0.881 (0.508-1.530)	0.674	0.983
I-D-T-C	37 (6.8)	54 (7)	0.908 (0.526-1.566)	0.781	0.983
I-D-T-G	25 (4.6)	18 (2.3)	0.448 (0.223-0.901)	0.033	0.495
I-I-C-C	31 (5.6)	44 (5.7)	0.883 (0.496-1.573)	0.768	0.983
I-I-C-G	35 (6.3)	40 (5.2)	0.711 (0.401-1.260)	0.246	0.923
I-I-T-C	19 (3.5)	24 (3.1)	0.786 (0.392-1.576)	0.591	0.983
I-I-T-G	24 (4.3)	32 (4.1)	0.829 (0.440-1.564)	0.626	0.983

Table V. Allele combination analysis in patients with RPL and controls.

^a2n, Total number of alleles. ORs and 95% CIs of each allele combination were calculated with reference the frequency of D-D-C-C and of all others using Fisher's exact test. *ABCB5*, ATP binding cassette subfamily B member 5; OR, odds ratio; CI, confidence interval; FDR, false discovery rate; *MS4A14*, membrane spanning 4-domains A14; *PSG9*, pregnancy specific β -1-glycoprotein 9; RPL, recurrent pregnancy loss; *SLC2A7*, solute carrier family 2 member 7. ^bFisher's exact test; ^cFDR-adjusted P-value.



Figure 1. AOR between frameshift and splice variant polymorphisms and PT with respect to RPL risk. The upper quartile for PT was 12 sec in patients with RPL and control women. (A) *MS4A14* DI+II genotype was associated with an increased risk of RPL in patients with PT \geq 12 sec (AOR=3.575; 95% CI=1.115-11.464). (B) *SLC2A7* DI+II genotype was associated with an increased risk of RPL in patients with PT \geq 12 sec (AOR=6.517; 95% CI=1.783-23.825). AOR, adjusted odds ratio; CI, confidence interval; *MS4A14*, membrane spanning 4-domains A14; PT, prothrombin time; RPL, recurrent pregnancy loss; *SLC2A7*, solute carrier family 2 member 7.

mutations and splice variants have been associated with other diseases, such as colorectal cancer (23,24) and are also implicated in RPL.

The protein encoded by the *MS4A14* gene serves an important role in embryo development and fertilization in rats (25). *SLC2A7*, also known as the glucose transporter 7 gene, catalyzes the cellular uptake of sugars. During pregnancy, the transplacental nutrient transport of amino acids, lipids and carbohydrates is important for proper fetal development, and glucose from the maternal circulation is a principal source of energy for the fetus (26). The protein encoded by the *PSG9* gene is a member of the pregnancy-specific glycoprotein (PSG) family. In several studies, it was demonstrated that reduced serum concentration of PSG was associated with reduced fetal growth (27,28). The *ABCB5* gene is a biomarker for physiological and pathological stem cells, and is a mediator of cell fusion, vasculogenesis and drug efflux (29). A successful pregnancy requires the development of a complex maternal and fetal vascular network that can support the increasing oxygen and metabolic demands of the growing fetus (30). Furthermore, placental development occurs through the vasculogenesis and angiogenesis stage (31). Therefore, it may be hypothesized that a mutation in *ABCB5* would influence RPL.

Although the individual gene variations examined in this study were not associated with RPL, gene combinations were demonstrated to be associated with RPL. Allele combination analysis (*MS4A14I/SLC2A7D/PSG9T/ABCB5G*) and

Table VI.	Gene com	bination	analysis	in	patients	with	RPL	and	controls.

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Genotype	Control (%) n=276	RPL (%) n=383	AOR (95% CI)	\mathbf{P}^{a}	q-value ^b
DDDD 28 (10.1) 49 (12.8) 1.090 (reference) DDDI 45 (16.3) 66 (17.2) 0.828 (0.454-1.510) 0.555 0.613 DDDI 33 (12.0) 46 (12.0) 0.732 (0.451-1.510) 0.478 0.613 DUDI 80 (29.0) 111 (29.0) 0.781 (0.452-1.351) 0.377 0.613 DUDI 13 (4.7) 17 (4.4) 0.713 (0.255-1.252) 0.453 0.613 DIT 23 (8.3) 25 (6.5) 0.607 (0.291-1.269) 0.483 0.613 DMA 9 (3.3) 12 (3.1) 0.773 (0.285-2.094) 0.463 0.613 DDCC 21 (7.6) 39 (0.2) 1.000 (reference) DDCT 49 (17.8) 0.782 (0.498-1.496) 0.457 0.713 DDCC 21 (7.6) 39 (0.2) 1.000 (reference) 0.408 0.408 0.408 0.408 0.408 0.408 0.408 0.408 0.713 0.752 (0.409-1.496) 0.449 0.303 0.713 0.713 0.751 (0.401.404) 0.380 (0.713 0.713 0.752 (0.406) 0.406 (0.	MS4A14/SLC2A7					
DDDI 45 (16.3) 66 (17.2) 0.828 (0.454.15(0) 0.555 0.613 DD/II 19 (6.9) 26 (6.8) 0.794 (0.369-1.706) 0.555 0.613 DUDD 33 (12.0) 44 (12.0) 0.781 (0.452.1531) 0.377 0.613 DUD1 80 (29.0) 111 (29.0) 0.781 (0.452.1531) 0.377 0.613 DUD 13 (4.7) 17 (4.4) 0.733 (0.255.1270) 0.435 0.613 IDD 13 (4.7) 17 (4.4) 0.731 (0.255.1270) 0.435 0.613 DDCT 49 (17.8) 70 (18.3) 0.782 (0.499.1496) 0.457 0.713 DDCT 49 (17.8) 70 (18.3) 0.782 (0.499.1496) 0.453 0.713 DDCC 42 (15.2) 48 (12.5) 0.612 (0.312.1201) 0.153 0.408 DICT 75 (27.2) 0.2 (26.6) 0.790 (0.416.404) 0.380 0.713 DICC 42 (15.2) 67 (17.5) 0.950 (0.31.1780) 0.848 0.974 UTT 11 (4.0) 10 (2.6) 0.476 (0.	DD/DD	28 (10.1)	49 (12.8)	1.000 (reference)		
DD/II 19 (6.9) 26 (6.8) 0.794 (0.369-1.708) 0.555 0.613 DI/DD 33 (12.0) 46 (12.0) 0.792 (0.415.1510) 0.478 0.613 DI/II 26 (9.4) 31 (8.1) 0.677 (0.335-1370) 0.279 0.613 DI/II 26 (9.4) 31 (8.1) 0.677 (0.335-1370) 0.279 0.613 I/DD 13 (4.7) 17 (4.4) 0.713 (0.295-1.725) 0.433 0.613 I/II 23 (8.3) 25 (6.5) 0.607 (0.291-1.269) 0.613 0.613 MAHAIPSG9 0.773 (0.285-2.094) 0.613 0.613 DDCC 21 (7.6) 39 (10.2) 1.000 (reference) 0.133 0.408 DI/CT 49 (17.8) 70 (18.3) 0.752 (0.409-1.404) 0.308 0.713 DI/CT 75 (27.2) 102 (26.6) 0.759 (0.410-1.404) 0.308 0.713 DI/CT 75 (27.2) 102 (26.6) 0.759 (0.410-1.404) 0.308 0.739 II/T 11 (4.0) 10 (2.6) 0.476 (0.17	DD/DI	45 (16.3)	66 (17.2)	0.828 (0.454-1.510)	0.538	0.613
DVDD 33 (12.0) 40 (12.0) 0.72 (0.415.15.10) 0.478 0.613 DVD1 80 (29.0) 111 (29.0) 0.781 (0.452.1351) 0.377 0.613 DVD1 13 (4.7) 17 (4.4) 0.713 (0.295.1.25) 0.433 0.613 IMD 23 (8.3) 12 (3.1) 0.773 (0.285.2.094) 0.613 0.613 IMI 9 (3.3) 12 (3.1) 0.773 (0.285.2.094) 0.613 0.613 DDCC 21 (7.6) 39 (10.2) 1.000 (reference) 0.453 0.713 DDCT 49 (17.8) 70 (18.3) 0.782 (0.499.1.496) 0.457 0.713 DDCC 42 (15.2) 48 (12.5) 0.612 (0.312.1.201) 0.153 0.403 DVCC 42 (15.2) 48 (12.5) 0.646 (0.204.0.996) 0.99 0.799 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481.2.23) 0.933 0.933 DVCC 9 (3.3) 21 (5.5) 1.206 (0.466 3.122) 0.699 0.799 DVTT 11 (4.0) 10 (2.6) 0.476 (0.172	DD/II	19 (6.9)	26 (6.8)	0.794 (0.369-1.708)	0.555	0.613
DIDI 80 (29.0) 111 (29.0) 0.781 (0.452-1.351) 0.377 0.613 DI/II 26 (9.4) 31 (8.1) 0.677 (0.335-1.370) 0.279 0.613 II/D 13 (4.7) 17 (4.4) 0.713 (0.295-1.725) 0.433 0.613 II/D 23 (8.3) 25 (6.5) 0.607 (0.291-1.269) 0.185 0.613 MS4AL4/PSG9 0.732 (0.285-2.094) 0.613 0.782 (0.409-1.496) 0.457 0.713 DD/CT 49 (17.8) 70 (18.3) 0.782 (0.409-1.496) 0.457 0.713 DD/TT 22 (8.0) 32 (8.4) 0.786 (0.368-1.681) 0.353 0.713 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481-2.23) 0.699 0.499 0.392 ILCC 9 (3.3) 21 (5.5) 1.206 (0.466-3.122) 0.699 0.392 0.393 0.333 0.333 0.332 0.333 0.332 0.342 0.490 0.392 0.446 0.200-0.995 0.499 0.332 0.444 0.175 0.955 (0.513.1.731)	DI/DD	33 (12.0)	46 (12.0)	0.792 (0.415-1.510)	0.478	0.613
DEIT 26 (9.4) 31 (8.1) 0.677 (0.335 (1.370) 0.279 0.613 IVDD 13 (4.7) 17 (4.4) 0.713 (0.295 1.725) 0.453 0.613 II/I 9 (3.3) 12 (3.1) 0.773 (0.285 2.094) 0.613 0.613 MS4A44/PSG9 0.773 (0.285 2.094) 0.613 0.613 DDCC 21 (7.6) 39 (10.2) 1.000 (reference) DDCT 49 (17.8) 70 (18.3) 0.782 (0.409 1.496) 0.457 0.713 DDCC 42 (15.2) 48 (12.5) 0.612 (0.312 1.201) 0.153 0.498 DICT 75 (27.2) 102 (26.6) 0.759 (0.410 1.404) 0.380 0.713 DICT 25 (9.1) 22 (8.0) 34 (4.6) (2040-099) 0.499 0.999 0.993 II/CT 25 (9.1) 12 (5.5) 1.206 (0.466 -3.122) 0.933 0.133 0.493 II/CT 25 (9.1) 12 (5.5) 1.000 (reference) DD/CC 26 (9.4) 44 (11.5) 1.000 (reference) DD/CC 26 (1.4)	DI/DI	80 (29.0)	111 (29.0)	0.781 (0.452-1.351)	0.377	0.613
IVDD 13 (4.7) 17 (4.4) 0.713 (0.295-1.725) 0.453 0.613 IVD1 23 (8.3) 12 (3.1) 0.773 (0.285-2.094) 0.613 0.613 MSAIAIPSCG 0.733 (0.285-2.094) 0.613 0.613 DDVCC 19 (17.8) 70 (18.3) 0.732 (0.495-1.496) 0.457 0.713 DDVTT 22 (8.0) 32 (8.4) 0.786 (0.366-1.681) 0.353 0.713 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481-223) 0.933 0.933 DVCC 9 (3.3) 21 (5.5) 1.206 (0.466-3.122) 0.699 0.799 IL/TT 11 (4.0) 10 (2.6) 0.476 (0.172-1.318) 0.353 0.333 DVCC 26 (9.4) 14 (11.5) 1.000 (reference) DDUCG 42 (15.2) 67 (17.5) 0.955 (0.513 1.780) 0.886 0.974 DVCC 26 (9.4) 44 (11.5) 1.000 (reference) DDUCG 42 (15.2) 67 (17.5) 0.955 (0.513 1.780) 0.886 0.974 DVCC 42 (15.2)	DI/II	26 (9.4)	31 (8.1)	0.677 (0.335- 1.370)	0.279	0.613
IVDI 23 (8.3) 25 (6.5) 0.607 (0.291-1.269) 0.185 0.613 IVII 9 (3.3) 12 (3.1) 0.773 (0.285-2.094) 0.613 DD/CC 21 (7.6) 39 (10.2) 1.000 (reference) DD/CT 49 (17.8) 70 (18.3) 0.782 (0.409-1.496) 0.457 0.713 DVCC 42 (15.2) 48 (12.5) 0.612 (0.312-1.201) 0.033 0.713 DVCC 42 (15.2) 48 (12.5) 0.612 (0.312-1.201) 0.153 0.408 DVCT 75 (27.2) 102 (26.6) 0.759 (0.410-1.404) 0.380 0.713 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481 -22.23) 0.933 0.933 IVCC 9 (3.3) 2.1 (5.5) 1.206 (0.466 3.122) 0.609 0.799 IVTT 11 (4.0) 10 (2.6) 0.476 (0.172-1.318) 0.153 0.333 0.640 DVCG 26 (9.4) 44 (11.5) 1.000 (reference) DD/CG 42 (8.7) 30 (7.8) 0.732 (0.334-1.341) 0.404 0.400 0.400	II/DD	13 (4.7)	17 (4.4)	0.713 (0.295-1.725)	0.453	0.613
IVI 9 (3.3) 12 (3.1) 0.773 (0.285-2.094) 0.613 0.613 MS4A14/PSG9	II/DI	23 (8.3)	25 (6.5)	0.607 (0.291-1.269)	0.185	0.613
MS4AI4/PSG9 DD/CC 21 (7.6) 39 (10.2) 1.000 (reference) DD/TT 49 (17.8) 70 (18.3) 0.788 (0.499 - 1.496) 0.457 0.713 DD/CC 42 (15.2) 48 (12.5) 0.612 (0.312 - 1.201) 0.153 0.408 DVCT 75 (27.2) 102 (2.6.6) 0.759 (0.410 - 1.404) 0.380 0.713 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481 - 2.23) 0.039 0.733 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481 - 2.23) 0.049 0.392 IVTT 11 (4.0) 10 (2.6) 0.476 (0.172 - 1.318) 0.153 0.392 MS4A14/ABCB5 DD/CG 26 (9.4) 44 (11.5) 1.000 (reference) DD/CG 26 (9.4) 44 (1.5) 0.010 (0.561 - 3.101) 0.732 0.334 1.040 0.640 DVCG 26 (9.4) 44 (1.5) 1.000 (reference) DD/CG 26 (2.2) 17 (4.4) 1.073 (0.378 - 1.314) 0.400 0.640 DVCC 42 (15.2) 52 (13.6) 0.716 (0.358 - 4.791)	II/II	9 (3.3)	12 (3.1)	0.773 (0.285-2.094)	0.613	0.613
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	MS4A14/PSG9					
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	DD/CC	21 (7.6)	39 (10.2)	1 000 (reference)		
DD/TT 22 (8.0) 32 (8.4) 0.786 (0.368-1.681) 0.535 0.713 DVCC 42 (15.2) 48 (12.5) 0.612 (0.312-1.201) 0.153 0.408 DVCT 75 (27.2) 102 (26.6) 0.759 (0.410-1.404) 0.380 0.713 DVTT 22 (8.0) 38 (9.9) 1.034 (0.481-2.223) 0.933 0.933 IVCC 9 (3.3) 21 (5.5) 1.206 (0.466-3.122) 0.699 0.999 IVTT 11 (4.0) 10 (2.6) 0.446 (0.200-0.995) 0.049 0.392 MS4A14/ABCB5 DUCC 26 (9.4) 44 (11.5) 1.000 (reference) DD/CG 26 (9.4) 44 (11.5) 1.000 (reference) 0.301 0.640 DVCC 26 (9.4) 44 (11.5) 0.503 (0.251-1007) 0.052 0.208 DVCG 26 (9.4) 44 (11.5) 0.307 (0.354-1.514) 0.400 0.446 DVCC 42 (15.2) 52 (13.6) 0.716 (0.378-1.351) 0.301 0.640 DVCG 42 (15.2) 52 (13.6)<	DD/CT	49 (17.8)	70 (18.3)	0.782 (0.409-1.496)	0.457	0.713
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	DD/TT	22 (8 0)	32 (8 4)	0 786 (0 368-1 681)	0.535	0.713
$\begin{array}{c ccccc} 12 (2.2) & 10 (226.6) & 0.739 & 0.410 \cdot 1.404) & 0.380 & 0.713 \\ DI/TT & 22 (8.0) & 38 (9.9) & 1.034 & 0.481 \cdot 2.23 & 0.933 & 0.933 \\ I/CC & 9 (3.3) & 21 (5.5) & 1.206 & 0.466 \cdot 3.122 & 0.699 & 0.799 \\ I/TT & 11 (4.0) & 10 (2.6) & 0.446 & 0.200 \cdot 0.995 & 0.049 & 0.392 \\ I/TT & 11 (4.0) & 10 (2.6) & 0.476 & (0.172 \cdot 1.318 & 0.153 & 0.392 \\ D/CC & 26 (9.4) & 44 & (11.5) & 1.000 & (reference) \\ D/CG & 42 & (15.2) & 67 & (17.5) & 0.955 & (0.513 \cdot 1.780) & 0.886 & 0.974 \\ D/GG & 24 & (8.7) & 30 & (7.8) & 0.732 & 0.354 \cdot 1.514 & 0.400 & 0.640 \\ D/CG & 42 & (15.2) & 52 & (13.6) & 0.716 & 0.378 \cdot 1.351 & 0.301 & 0.640 \\ D/CG & 42 & (15.2) & 52 & (13.6) & 0.715 & 0.378 \cdot 1.351 & 0.301 & 0.640 \\ D/CG & 63 & (22.8) & 107 & (27.9) & 1.010 & (0.566 \cdot 1.801 & 0.974 & 0.974 \\ D/GG & 34 & (12.3) & 29 & (7.6) & 0.503 & (0.251 \cdot 1.007) & 0.052 & 0.208 \\ I/CC & 6 & (2.2) & 17 & (4.4) & 1.679 & 0.588 \cdot 4.799 & 0.333 & 0.640 \\ I/CG & 28 & (10.1) & 20 & (5.2) & 0.397 & (0.185 \cdot 0.851 & 0.018 & 0.144 \\ I/GG & 11 & (4.0) & 17 & (4.4) & 0.676 & (0.331 \cdot 1.380 & 0.282 & 0.437 \\ D/D/CT & 43 & (15.6) & 55 & (14.4) & 0.676 & (0.331 \cdot 1.380 & 0.282 & 0.437 \\ D/D/TT & 14 & (5.1) & 22 & (5.7) & 0.836 & (0.340 \cdot 2.056 & 0.696 & 0.696 \\ D/ICC & 36 & (13.0) & 48 & (12.5) & 0.657 & (0.317 \cdot 1.361) & 0.258 & 0.437 \\ D/ICT & 81 & (29.3) & 108 & (28.2) & 0.652 & 0.339 \cdot 1.252 & 0.199 & 0.437 \\ D/LT & 10 & (3.6) & 12 & (3.1) & 0.631 & (0.300 \cdot 1.455) & 0.304 & 0.437 \\ D/CC & 19 & (6.9) & 25 & (6.5) & 1.060 & (0.26 - 0.220 & 0.492 & 0.562 \\ I/CC & 19 & (6.9) & 25 & (6.5) & 1.000 & (0.450 \cdot 2.639) & 0.186 & 0.372 \\ D/D/CG & 22 & (5.1) & 32 & (8.4) & 0.661 & (0.300 \cdot 1.455) & 0.304 & 0.437 \\ D/LT & 10 & (3.6) & 12 & (3.1) & 0.631 & (0.319 \cdot 1.249) & 0.186 & 0.372 \\ D/D/CC & 20 & (7.2) & 37 & (9.7) & 1.000 & (reference) \\ D/D/CG & 12 & (4.3) & 25 & (6.5) & 1.090 & (0.450 \cdot 2.639) & 0.849 & 0.875 \\ D/LCG & 9 & (25.0) & 99 & (25.8) & 0.761 & (0.406 \cdot 1.4277) & 0.875 & 0.875 \\ D/LCG & 13 & (6.5) & 13 & (3.4) & 0.376 & (0.124 - 0.372) & 0.385 & $	DI/CC	42(0.0)	48 (12 5)	0.612 (0.312-1.201)	0.153	0.408
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	DI/CT	75 (27.2)	102 (26 6)	0.012(0.0121.201) 0.759(0.410-1.404)	0.380	0.713
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	DI/TT	$\frac{73}{22}(8.0)$	38 (9.9)	1 034 (0 481-2 223)	0.933	0.933
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		9(33)	21 (5 5)	1 206 (0 466 3 122)	0.555	0.799
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	II/CT	25 (9 1)	21(5.5) 23(6.0)	0 446 (0 200-0 995)	0.099	0.392
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	II/TT	23(9.1) 11(4.0)	10 (2.6)	$0.476 (0.172 \ 1.318)$	0.153	0.392
MM34174BCB3 DD/CC 26 (9.4) 44 (11.5) 1.000 (reference) DD/GG 42 (15.2) 67 (17.5) 0.955 (0.513-1.780) 0.886 0.974 DD/GG 24 (8.7) 30 (7.8) 0.732 (0.354-1.514) 0.400 0.640 DVCC 42 (15.2) 52 (13.6) 0.715 (0.378-1.351) 0.301 0.640 DVGG 63 (22.8) 107 (27.9) 1.010 (0.566-1.801) 0.974 0.974 DVGG 63 (22.8) 107 (27.9) 1.010 (0.566-1.801) 0.052 0.208 IVCC 6 (2.2) 17 (4.4) 1.679 (0.588-4.799) 0.333 0.640 IVCG 28 (10.1) 20 (5.2) 0.397 (0.185-0.851) 0.018 0.144 IVGG 11 (4.0) 17 (4.4) 0.912 (0.370-2.245) 0.841 0.974 SIZCA7/PSG9 DD/CT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DL/CT 81 (12.0) 0.770 (0.366-1.622) 0.492 0.562 DL/CT 81 (12.0) 0.770 (0.366-1.		11 (4.0)	10 (2.0)	0.470 (0.172-1.510)	0.155	0.372
DD/CC 26 (9.4) 44 (11.5) 1.000 (reference) DD/CG 42 (15.2) 67 (17.5) 0.955 (0.513.1.780) 0.886 0.974 DD/GG 24 (8.7) 30 (7.8) 0.732 (0.354.1.514) 0.400 0.640 D/CC 42 (15.2) 52 (13.6) 0.715 (0.378.1.351) 0.301 0.640 D/CG 63 (22.8) 107 (27.9) 1.010 (0.566.1.801) 0.974 0.974 D/GG 34 (12.3) 29 (7.6) 0.503 (0.251.1.007) 0.052 0.208 I/CC 6 (2.2) 17 (4.4) 1.679 (0.588.4.799) 0.333 0.640 I/CG 28 (10.1) 20 (5.2) 0.397 (0.185-0.851) 0.018 0.144 I/CG 28 (10.1) 20 (5.2) 0.397 (0.370-2.245) 0.841 0.974 SLC2A7/PSG9 DD/CT 43 (15.6) 55 (14.4) 0.676 (0.31-1.380) 0.282 0.437 DD/CT 43 (15.6) 55 (14.4) 0.676 (0.31-1.361) 0.258 0.437 DD/CT 43 (15.6) 55 (14.4) 0.676 (0.31-1.361) 0.258 0.437 DL/CC 17 (6.2)	MS4A14/ABCBS	26 (0, 4)	44 (11 5)	1,000 (
DD/CG 42 (15.2) 67 (17.5) 0.555 (0.51.5.1.80) 0.886 0.974 DD/GG 24 (87) 30 (7.8) 0.722 (0.354.1.514) 0.400 0.640 DI/CG 42 (15.2) 52 (13.6) 0.715 (0.378.1.351) 0.301 0.640 DI/CG 63 (22.8) 107 (27.9) 1.010 (0.566.1.801) 0.974 0.974 DI/GG 34 (12.3) 29 (7.6) 0.503 (0.251.1.007) 0.052 0.208 I/CC 6 (2.2) 17 (4.4) 1.679 (0.588.4.799) 0.333 0.640 I/CG 28 (10.1) 20 (5.2) 0.397 (0.185-0.851) 0.018 0.144 I/GG 11 (4.0) 17 (4.4) 0.912 (0.370-2.245) 0.841 0.974 SLC2A7/PSG9 DD/CT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DL/TT 14 (5.1) 22 (5.7) 0.836 (0.340-2.056) 0.696 0.696 DI/CC 36 (13.0) 48 (12.5) 0.657 (0.317-1.361) 0.258 0.437 DL/TT 31 (11.2)	DD/CC	20 (9.4)	44 (11.5)	1.000 (reference)	0.007	0.074
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	DD/CG	42 (15.2)	67 (17.5)	0.955 (0.513-1.780)	0.886	0.974
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	DD/GG	24 (8.7)	30 (7.8)	0.732 (0.354-1.514)	0.400	0.640
DI/CG 63 (22.8) 107 (27.9) 1.010 (0.566-1.801) 0.974 0.974 DI/GG 34 (12.3) 29 (7.6) 0.503 (0.251-1.007) 0.052 0.208 I/CC 6 (2.2) 17 (4.4) 1.679 (0.588-4.799) 0.333 0.640 I//CG 28 (10.1) 20 (5.2) 0.397 (0.185-0.851) 0.018 0.144 I//GG 11 (4.0) 17 (4.4) 0.912 (0.370-2.245) 0.841 0.974 SLC2A7/PSG9 D//CC 17 (6.2) 35 (9.1) 1.000 (reference) DD/CT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DL/CC 36 (13.0) 48 (12.5) 0.657 (0.317-1.361) 0.258 0.437 DL/CT 81 (29.3) 108 (28.2) 0.652 (0.339-1.252) 0.199 0.437 DL/CC 19 (6.9) 25 (6.5) 0.654 (0.283-1.511) 0.320 0.437 I//CT 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 I//CT 12 (5.2) <td>DI/CC</td> <td>42 (15.2)</td> <td>52 (13.6)</td> <td>0.715 (0.378-1.351)</td> <td>0.301</td> <td>0.640</td>	DI/CC	42 (15.2)	52 (13.6)	0.715 (0.378-1.351)	0.301	0.640
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	DI/CG	63 (22.8) 24 (12.2)	107 (27.9)	1.010 (0.566-1.801)	0.974	0.974
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	DI/GG	34 (12.3)	29 (7.6)	0.503 (0.251-1.007)	0.052	0.208
I/CG 28 (10.1) 20 (5.2) 0.97 (0.185-0.851) 0.018 0.144 II/GG 11 (4.0) 17 (4.4) 0.912 (0.370-2.245) 0.841 0.974 SLC2A7/PSG9 DD/CC 17 (6.2) 35 (9.1) 1.000 (reference) 0.282 0.437 DD/CT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DD/TT 14 (5.1) 22 (5.7) 0.836 (0.340-2.056) 0.696 0.696 DI/CC 36 (13.0) 48 (12.5) 0.657 (0.317-1.361) 0.258 0.437 DI/TT 81 (29.3) 108 (28.2) 0.652 (0.339-1.252) 0.199 0.437 DI/CC 19 (6.9) 25 (6.5) 0.654 (0.283-1.511) 0.320 0.437 II/TT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 II/TT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 II/TT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 DI/CG 20 (7.2)	II/CC	6 (2.2)	17 (4.4)	1.679 (0.588-4.799)	0.333	0.640
II/GG I1/(4.0) I1/(4.4) 0.912 (0.3/0-2.245) 0.841 0.974 SLC2A7/PSG9 DD/CC 17 (6.2) 35 (9.1) 1.000 (reference) DD/TT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DD/TT 14 (5.1) 22 (5.7) 0.836 (0.340-2.056) 0.696 0.696 DVCC 36 (13.0) 48 (12.5) 0.657 (0.317-1.361) 0.258 0.437 DVCT 81 (29.3) 108 (28.2) 0.652 (0.339-1.252) 0.199 0.437 DVTT 31 (11.2) 46 (12.0) 0.770 (0.366-1.622) 0.492 0.562 II/CC 19 (6.9) 25 (6.5) 0.654 (0.283-1.511) 0.320 0.437 II/TT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 SLC2A7/ABCB5 DD/CC 20 (7.2) 37 (9.7) 1.000 (reference) 0.186 0.372 DD/GG 12 (4.3) 25 (6.5) 1.090 (0.450-2.639) 0.849 0.875 DI/CG 69 (25.0) 99 (25.8) 0.761 (0.406-1.427) 0.395 0.632 <td< td=""><td>II/CG</td><td>28 (10.1)</td><td>20 (5.2)</td><td>0.397 (0.185-0.851)</td><td>0.018</td><td>0.144</td></td<>	II/CG	28 (10.1)	20 (5.2)	0.397 (0.185-0.851)	0.018	0.144
SLC2A7/PSG9 DD/CC 17 (6.2) 35 (9.1) 1.000 (reference) DD/CT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DD/TT 14 (5.1) 22 (5.7) 0.836 (0.340-2.056) 0.696 0.696 DV/CC 36 (13.0) 48 (12.5) 0.657 (0.317-1.361) 0.258 0.437 DI/CT 81 (29.3) 108 (28.2) 0.652 (0.339-1.252) 0.199 0.437 DI/TT 31 (11.2) 46 (12.0) 0.770 (0.366-1.622) 0.492 0.562 II/CC 19 (6.9) 25 (6.5) 0.654 (0.283-1.511) 0.320 0.437 IVCT 25 (9.1) 32 (8.4) 0.661 (0.300-1.455) 0.304 0.437 IVTT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 SLC2A7/ABCB5 DD/CC 20 (7.2) 37 (9.7) 1.000 (reference) DD/CG 42 (15.2) 50 (13.1) 0.631 (0.319-1.249) 0.186 0.372 DD/CG 42 (15.2) 50 (13.1) 0.631 (0.406-1.427) 0.395 0.632 DD/CG 69 (25.0) 99 (25.8) </td <td>II/GG</td> <td>11 (4.0)</td> <td>17 (4.4)</td> <td>0.912 (0.370-2.245)</td> <td>0.841</td> <td>0.974</td>	II/GG	11 (4.0)	17 (4.4)	0.912 (0.370-2.245)	0.841	0.974
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	SLC2A7/PSG9					
DD/CT 43 (15.6) 55 (14.4) 0.676 (0.331-1.380) 0.282 0.437 DD/TT 14 (5.1) 22 (5.7) 0.836 (0.340-2.056) 0.696 0.696 DI/CC 36 (13.0) 48 (12.5) 0.657 (0.317-1.361) 0.258 0.437 DI/CT 81 (29.3) 108 (28.2) 0.652 (0.339-1.252) 0.199 0.437 DI/TT 31 (11.2) 46 (12.0) 0.770 (0.366-1.622) 0.492 0.562 IVCC 19 (6.9) 25 (6.5) 0.654 (0.283-1.511) 0.320 0.437 IVTT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 IVTT 10 (3.6) 12 (3.1) 0.601 (0.216-1.668) 0.328 0.437 SLC2A7/ABCB5 DD/CC 20 (7.2) 37 (9.7) 1.000 (reference) 0.186 0.372 DD/GG 12 (4.3) 25 (6.5) 1.090 (0.450-2.639) 0.849 0.875 DI/CC 36 (13.0) 63 (16.4) 0.946 (0.477-1.877) 0.875 0.875 DI/CG 69 (25.0) 99 (25.8) 0.761 (0.406-1.427) 0.395 0.632 <td< td=""><td>DD/CC</td><td>17 (6.2)</td><td>35 (9.1)</td><td>1.000 (reference)</td><td></td><td></td></td<>	DD/CC	17 (6.2)	35 (9.1)	1.000 (reference)		
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	DD/CT	43 (15.6)	55 (14.4)	0.676 (0.331-1.380)	0.282	0.437
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	DD/TT	14 (5.1)	22 (5.7)	0.836 (0.340-2.056)	0.696	0.696
DI/CT $81 (29.3)$ $108 (28.2)$ $0.652 (0.339-1.252)$ 0.199 0.437 DI/TT $31 (11.2)$ $46 (12.0)$ $0.770 (0.366-1.622)$ 0.492 0.562 II/CC $19 (6.9)$ $25 (6.5)$ $0.654 (0.283-1.511)$ 0.320 0.437 II/CT $25 (9.1)$ $32 (8.4)$ $0.661 (0.300-1.455)$ 0.304 0.437 II/TT $10 (3.6)$ $12 (3.1)$ $0.601 (0.216-1.668)$ 0.328 0.437 SLC2A7/ABCB5 DD/CC $20 (7.2)$ $37 (9.7)$ $1.000 (reference)$ DD/CG DD/CG $42 (15.2)$ $50 (13.1)$ $0.631 (0.319-1.249)$ 0.186 0.372 DD/GG $12 (4.3)$ $25 (6.5)$ $1.090 (0.450-2.639)$ 0.849 0.875 DI/CC $36 (13.0)$ $63 (16.4)$ $0.946 (0.477-1.877)$ 0.875 0.875 DI/CG $69 (25.0)$ $99 (25.8)$ $0.761 (0.406-1.427)$ 0.395 0.632 DI/GG $43 (15.6)$ $40 (10.4)$ $0.485 (0.240-0.981)$ 0.044 0.176 II/CC $18 (6.5)$ $13 (3.4)$ $0.376 (0.152-0.932)$ 0.035 0.176 II/CG $22 (8.0)$ $45 (11.7)$ $1.118 (0.529-2.364)$ 0.769 0.875 II/GG $14 (5.1)$ $11 (2.9)$ $0.428 (0.164-1.117)$ 0.083 0.221 PSG9/ABCB5CC/CC $19 (6.9)$ $29 (7.6)$ $1.000 (reference)$ 0.693 0.892	DI/CC	36 (13.0)	48 (12.5)	0.657 (0.317-1.361)	0.258	0.437
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	DI/CT	81 (29.3)	108 (28.2)	0.652 (0.339-1.252)	0.199	0.437
II/CC19 (6.9)25 (6.5) $0.654 (0.283-1.511)$ 0.320 0.437 II/CT25 (9.1)32 (8.4) $0.661 (0.300-1.455)$ 0.304 0.437 II/TT10 (3.6)12 (3.1) $0.601 (0.216-1.668)$ 0.328 0.437 SLC2A7/ABCB5DD/CC20 (7.2)37 (9.7) $1.000 (reference)$ DD/CG42 (15.2)50 (13.1) $0.631 (0.319-1.249)$ 0.186 0.372 DD/GG12 (4.3)25 (6.5) $1.090 (0.450-2.639)$ 0.849 0.875 DI/CC36 (13.0)63 (16.4) $0.946 (0.477-1.877)$ $0.875 (0.875)$ DI/CG69 (25.0)99 (25.8) $0.761 (0.406-1.427)$ $0.395 (0.632)$ DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 U/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7) $1.118 (0.529-2.364)$ $0.769 (0.875)$ 0.875II/GG14 (5.1)11 (2.9) $0.428 (0.164-1.117)$ $0.083 (0.221)$ PSG9/ABCB5CC/CC19 (6.9)29 (7.6) $1.000 (reference)$ CC/CG37 (13.4)50 (13.1) $0.865 (0.421-1.777)$ $0.693 (0.892)$	DI/TT	31 (11.2)	46 (12.0)	0.770 (0.366-1.622)	0.492	0.562
II/CT $25 (9.1)$ $32 (8.4)$ $0.661 (0.300-1.455)$ 0.304 0.437 II/TT $10 (3.6)$ $12 (3.1)$ $0.601 (0.216-1.668)$ 0.328 0.437 SLC2A7/ABCB5DD/CC $20 (7.2)$ $37 (9.7)$ $1.000 (reference)$ DD/CG $42 (15.2)$ $50 (13.1)$ $0.631 (0.319-1.249)$ 0.186 0.372 DD/GG $12 (4.3)$ $25 (6.5)$ $1.090 (0.450-2.639)$ 0.849 0.875 DI/CC $36 (13.0)$ $63 (16.4)$ $0.946 (0.477-1.877)$ 0.875 0.875 DI/CG $69 (25.0)$ $99 (25.8)$ $0.761 (0.406-1.427)$ 0.395 0.632 DI/GG $43 (15.6)$ $40 (10.4)$ $0.485 (0.240-0.981)$ 0.044 0.176 II/CC $18 (6.5)$ $13 (3.4)$ $0.376 (0.152-0.932)$ 0.035 0.176 II/CG $22 (8.0)$ $45 (11.7)$ $1.118 (0.529-2.364)$ 0.769 0.875 II/GG $14 (5.1)$ $11 (2.9)$ $0.428 (0.164-1.117)$ 0.083 0.221 PSG9/ABCB5CC/CC $19 (6.9)$ $29 (7.6)$ $1.000 (reference)$ CC/CG $37 (13.4)$ $50 (13.1)$ $0.865 (0.421-1.777)$ 0.693 0.892	II/CC	19 (6.9)	25 (6.5)	0.654 (0.283-1.511)	0.320	0.437
II/TT10 (3.6)12 (3.1)0.601 (0.216-1.668)0.3280.437SLC2A7/ABCB5DD/CC20 (7.2)37 (9.7)1.000 (reference)DD/CG42 (15.2)50 (13.1)0.631 (0.319-1.249)0.1860.372DD/GG12 (4.3)25 (6.5)1.090 (0.450-2.639)0.8490.875DI/CC36 (13.0)63 (16.4)0.946 (0.477-1.877)0.8750.875DI/CG69 (25.0)99 (25.8)0.761 (0.406-1.427)0.3950.632DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)0.6930.892	II/CT	25 (9.1)	32 (8.4)	0.661 (0.300-1.455)	0.304	0.437
SLC2A7/ABCB5 DD/CC 20 (7.2) 37 (9.7) 1.000 (reference) DD/CG 42 (15.2) 50 (13.1) 0.631 (0.319-1.249) 0.186 0.372 DD/GG 12 (4.3) 25 (6.5) 1.090 (0.450-2.639) 0.849 0.875 DI/CC 36 (13.0) 63 (16.4) 0.946 (0.477-1.877) 0.875 0.875 DI/CG 69 (25.0) 99 (25.8) 0.761 (0.406-1.427) 0.395 0.632 DI/GG 43 (15.6) 40 (10.4) 0.485 (0.240-0.981) 0.044 0.176 II/CC 18 (6.5) 13 (3.4) 0.376 (0.152-0.932) 0.035 0.176 II/CG 14 (5.1) 11 (2.9) 0.428 (0.164-1.117) 0.083 0.221 PSG9/ABCB5 CC/CC 19 (6.9) 29 (7.6) 1.000 (reference) CC/CG 37 (13.4) 50 (13.1) 0.865 (0.421-1.777) 0.693 0.892	II/TT	10 (3.6)	12 (3.1)	0.601 (0.216-1.668)	0.328	0.437
DD/CC20 (7.2)37 (9.7)1.000 (reference)DD/CG42 (15.2)50 (13.1)0.631 (0.319-1.249)0.1860.372DD/GG12 (4.3)25 (6.5)1.090 (0.450-2.639)0.8490.875DI/CC36 (13.0)63 (16.4)0.946 (0.477-1.877)0.8750.875DI/CG69 (25.0)99 (25.8)0.761 (0.406-1.427)0.3950.632DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)0.6930.892	SLC2A7/ABCB5					
DD/CG42 (15.2)50 (13.1)0.631 (0.319-1.249)0.1860.372DD/GG12 (4.3)25 (6.5)1.090 (0.450-2.639)0.8490.875DI/CC36 (13.0)63 (16.4)0.946 (0.477-1.877)0.8750.875DI/CG69 (25.0)99 (25.8)0.761 (0.406-1.427)0.3950.632DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)0.6930.892	DD/CC	20 (7.2)	37 (9.7)	1.000 (reference)		
DD/GG12 (4.3)25 (6.5)1.090 (0.450-2.639)0.8490.875DI/CC36 (13.0)63 (16.4)0.946 (0.477-1.877)0.8750.875DI/CG69 (25.0)99 (25.8)0.761 (0.406-1.427)0.3950.632DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)0.6930.892	DD/CG	42 (15.2)	50 (13.1)	0.631 (0.319-1.249)	0.186	0.372
DI/CC36 (13.0)63 (16.4)0.946 (0.477-1.877)0.8750.875DI/CG69 (25.0)99 (25.8)0.761 (0.406-1.427)0.3950.632DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)CC/CG37 (13.4)50 (13.1)0.865 (0.421-1.777)0.6930.892	DD/GG	12 (4.3)	25 (6.5)	1.090 (0.450-2.639)	0.849	0.875
DI/CG69 (25.0)99 (25.8)0.761 (0.406-1.427)0.3950.632DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)CC/CG37 (13.4)50 (13.1)0.865 (0.421-1.777)0.6930.892	DI/CC	36 (13.0)	63 (16.4)	0.946 (0.477-1.877)	0.875	0.875
DI/GG43 (15.6)40 (10.4) 0.485 (0.240-0.981)0.044 0.176II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)0.6930.892	DI/CG	69 (25.0)	99 (25.8)	0.761 (0.406-1.427)	0.395	0.632
II/CC18 (6.5)13 (3.4) 0.376 (0.152-0.932)0.035 0.176II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)CC/CG37 (13.4)50 (13.1)0.865 (0.421-1.777)0.6930.892	DI/GG	43 (15.6)	40 (10.4)	0.485 (0.240-0.981)	0.044	0.176
II/CG22 (8.0)45 (11.7)1.118 (0.529-2.364)0.7690.875II/GG14 (5.1)11 (2.9)0.428 (0.164-1.117)0.0830.221PSG9/ABCB5CC/CC19 (6.9)29 (7.6)1.000 (reference)CC/CG37 (13.4)50 (13.1)0.865 (0.421-1.777)0.6930.892	II/CC	18 (6.5)	13 (3.4)	0.376 (0.152-0.932)	0.035	0.176
II/GG 14 (5.1) 11 (2.9) 0.428 (0.164-1.117) 0.083 0.221 PSG9/ABCB5 CC/CC 19 (6.9) 29 (7.6) 1.000 (reference) 0.693 0.892 CC/CG 37 (13.4) 50 (13.1) 0.865 (0.421-1.777) 0.693 0.892	II/CG	22 (8.0)	45 (11.7)	1.118 (0.529-2.364)	0.769	0.875
PSG9/ABCB5 CC/CC 19 (6.9) 29 (7.6) 1.000 (reference) CC/CG 37 (13.4) 50 (13.1) 0.865 (0.421-1.777) 0.693 0.892	II/GG	14 (5.1)	11 (2.9)	0.428 (0.164-1.117)	0.083	0.221
CC/CC19 (6.9)29 (7.6)1.000 (reference)CC/CG37 (13.4)50 (13.1)0.865 (0.421-1.777)0.6930.892	PSG9/ABCB5					
CC/CG 37 (13.4) 50 (13.1) 0.865 (0.421-1.777) 0.693 0.892	CC/CC	19 (6.9)	29 (7.6)	1.000 (reference)		
	CC/CG	37 (13.4)	50 (13.1)	0.865 (0.421-1.777)	0.693	0.892

Table VI. Continued.

Genotype	Control (%) n=276	RPL (%) n=383	AOR (95% CI)	P ^a	q-value ^b
CC/GG	16 (5.8)	29 (7.6)	1.206 (0.518-2.805)	0.665	0.892
CT/CC	41 (14.9)	59 (15.4)	0.915 (0.450-1.860)	0.806	0.892
CT/CG	72 (26.1)	104 (27.2)	0.956 (0.496-1.840)	0.892	0.892
CT/GG	36 (13.0)	32 (8.4)	0.569 (0.268-1.209)	0.143	0.892
TT/CC	14 (5.1)	25 (6.5)	1.259 (0.515-3.077)	0.613	0.892
TT/CG	24 (8.7)	40 (10.4)	1.091 (0.501-2.376)	0.827	0.892
TT/GG	17 (6.2)	15 (3.9)	0.606 (0.243-1.513)	0.284	0.892

Bold text indicate significant P-values. *ABCB5*, ATP binding cassette subfamily B member 5; AOR, adjusted odds ratio; CI, confidence interval; FDR, false discovery rate; *MS4A14*, membrane spanning 4-domains A14; *PSG9*, pregnancy specific β -1-glycoprotein 9; RPL, recurrent pregnancy loss; *SLC2A7*, solute carrier family 2 member 7. ^aFisher's exact test; ^bq-value: FDR-adjusted P-value.

genotype combination analysis (*MS4A14*II/*PSG9*CT and *MS4A14*II/*ABCB5*CG) revealed that the I allele affected RPL when the *MS4A14*D>I polymorphism was present with other genes.

The present study also demonstrated that all the selected gene polymorphisms were associated with RPL when the blood coagulation factors PLT, PT and aPTT were included in the interaction analysis. During pregnancy, fibrinolysis and coagulation must be precisely balanced so that excess fibrin deposition in placental vessels and intravillous spaces does not occur, and to ensure fibrin polymerization and stabilization of the placental basal plate. Defects in this process can have a negative impact on trophoblast transplantation and placenta development, ultimately leading to miscarriage (32). Therefore, if blood coagulation factors deviate from their normal levels this may result in an unsuccessful pregnancy.

The present study has several limitations. Firstly, it is unclear whether these polymorphisms can affect gene transcription and/or translation. Secondly, the study only included Korean women who visited the CHA Bundang Medical Center, and it would be useful to validate the findings in a different cohort. Lastly, the control group size was smaller than the patient group. Nevertheless, to the best of our knowledge, this study is the first of its kind to investigate the association between the gene polymorphisms *MS4A14D>I* (rs3217518), *SLC2A7D>I* (rs60746313), *PSG9C>T* (rs3746297) and *ABCB5C>G* (rs17143187), and RPL. These genes have been implicated in cancer and some other diseases, but have not been previously studied in the context of RPL. Therefore, these findings may help to improve our understanding of frameshift mutations and splice variants in pregnancy.

In conclusion, the association between RPL and four gene polymorphisms, MS4A14D>I (rs3217518), SLC2A7D>I (rs60746313), PSG9C>T (rs3746297) and ABCB5C>G (rs17143187), was investigated in Korean women. These four polymorphisms were not associated with RPL individually, but were associated with RPL when combined with other genes or when factoring in blood coagulation factors. Notably, the MS4A14 I allele, alongside a PT>12 sec, may be a potential biomarker for the diagnosis, prevention and prognosis of RPL. Further studies are required to clarify the associations between the four gene polymorphisms and RPL in an ethnically diverse cohort.

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Availability of data and materials

Not applicable.

Authors' contributions

WSL and NKK conceived and designed the experiments. HAL, CSR, JYL and JOK performed the experiments. EHA, JHK, CSR, JYL and SHC performed the analysis and interpretation of data for this study. WSL and NKK. prepared reagents/materials/analytical tools for experiments and analysis. HAL and EHA wrote the paper. JHK, SHC, WSL and NKK revised the manuscript for important intellectual information.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of CHA Bundang Medical Center (IRB number: BD2010-123D) and written informed consent was provided by all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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