Association of polymorphisms of cardiovascular system genes with idiopathic recurrent pregnancy loss of Kazakh populations

Asociación de polimorfismos de los genes del sistema cardiovascular con la pérdida de embarazo idiopática recurrente de poblaciones Kazajas

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Abstract

The interrelation of polymorphic variants of coagulation and cardiovascular system genes was studied: MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A), ITGB3 (Leu33Pro), PLANH1 (5G/4G); GPIa (C807T), AGTR1 (A1166C), ACE (I/D), eNOS (Glu298Asp) with development of idiopathic form of recurrent pregnancy loss (iRPL) in ethnically homogeneous population of the Kazakhs. The results of independent replicative TaqMan genotyping of 302 patients with iRPL and 300 women with normal reproduction did not reveal an association of studied polymorphisms with the development of iRPL in the Kazakh population.

Keywords: polymorphism of genes, genotypes, the idiopathic form of recurrent pregnancy loss.

Resumen

Se estudió la interrelación de variantes polimórficas de los genes del sistema cardiovascular y de coagulación: MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A), ITGB3 (Leu33Pro) , PLANH1 (5G / 4G); GPIa (C807T), AGTR1 (A1166C), ACE (I / D), eNOS (Glu298Asp) con desarrollo de forma idiopática de pérdida de embarazo recurrente (iRPL) en una población étnicamente homogénea de los kazajos. Los resultados de la genotipificación replicativa de TaqMan de 302 pacientes con iRPL y 300 mujeres con reproducción normal no revelaron una asociación de polimorfismos estudiados con el desarrollo de iRPL en la población kazaja.

Palabras clave: Polimorfismo de genes, genotipos, forma idiopática de pérdida recurrente de embarazo.

Introduction

Early recurrent pregnancy loss (further RPL), defined as three or more losses up to 12 weeks of pregnancy, is a heterogeneous disorder, affecting up to 3% of couples who are in the reproductive period[1,2].

The etiological causes of RPL are very diverse: genetic factors (chromosomal abnormalities in parents), which amount to 2-5%, presence of chromosomal disorders in embryo, anatomical factors (uterine abnormalities) -10-15%, endocrine diseases (untreated hypothyroidism, uncontrolled diabetes mellitus and etc.) -17-20%, autoimmune - 20% and infectious causes - 0.5-5%. 40-50% of RPL have not established etiology and belong to idiopathic RPL (further iRPL)[1,3-5].

Published systematic scientific reviews[6,7] of studies of SNP associations polymorphisms with iRPL, such as genes of hereditary thrombophilia, pro-inflammatory cytokine genes, angiogenesis genes, and placental function genes,

have confirmed their significant genetic contribution to the development of iRPL in not all populations.

Genome-wide association studies (GWAS) RPL were conducted in heterogeneous ethnic populations, in small sample sizes, and did not find significantly significant predisposition genes for RPL that were confirmed in independent replicative studies of other populations[7-9]. GWAS studies to identify genetic variants of iRPL development risk have not been conducted, due to the heterogeneity of the disease and lack of clear definitions of iRPL, the complexity of recruiting and small size of samples; the insufficient number of replicative studies in ethnically homogeneous populations[10,11].

According to literature data, thrombophilia makes a significant contribution to susceptibility to RPL by increasing platelet aggregation, the level of activity of coagulation factors and an excess of fibrinolytic inhibitors[11]. At iRPL, these associations may not be observed, which requires further study of the genetic contribution of new predisposing factors, including the SNP of other genes of cardiovascular system[12].

The most studied genetic associations with RPL and iRPL are the Leiden mutation FV (A506G, rs6025), the mutation in prothrombin gene FII (G20210A, rs1799963), the unfavorable genotypes of folate metabolism genes: MTHFR (C677T, rs1801133, and A1298C, rs1801131)[6,7,10,11]. Despite the contradictory results, the Practical Committee of the American Society of Reproductive Medicine[2] recommends that G20210A FII, A506G FV, C677T, and A1298C MTHFR gene be included in the mandatory screening study of couples with RPL.

Thus, associations studies of gene polymorphisms of coagulation and cardiovascular systems with the risk of iRPL development were few and showed inconsistent results in subsequent replicative studies of other ethnic populations[13-15]. This is due to the high heterogeneity of the disease and lack of clear definitions of iRPL, the complexity of recruiting and small size of samples; ethnic differences and different research methodologies.

Aim of the study: to conduct GWAS independent replicative genotyping of statistically significant polymorphisms of coagulation and cardiovascular system genes associated with iRPL in an ethnically homogeneous population of Kazakhs. The gene polymorphisms were studied: F5 (A506G), F2 (G20210A), FGB (G455A), ITGB3 (Leu-33Pro), GPIa (C807T), MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), ACE (I/D), AGTR (A1166C), eNOS3 (Glu298Asp).

Materials and methods

The study was conducted by prospective "case-control" method in the outpatient department of Scientific Center of Obstetrics, Gynecology, and Perinatology (SCOGP), the medical center "Center of Molecular Medicine." All study participants gave informed consent to the use of their blood samples and anamnestic data, the permission of the Ethical Committee of SCOGP to hold these studies is available.

RPL is classically defined as three or more spontaneous miscarriages up to 20 weeks' gestation[1,2]. The American Society of Reproductive Medicine (ASRM) believes that two consecutive pregnancy losses are sufficient for the diagnosis of RPL, since the recurrence rate and risk factors are similar to those observed after three losses[2].

The main group with iRPL consisted of 302 women of Kazakh nationality; age 18-45 years; who have two or more miscarriages before 12 weeks of gestation.

The control group is represented by 300 female Kazakh women with normal reproductive function without indication of the presence of spontaneous miscarriages with at least one child.

Recruitment criteria included: belonging to the Kazakh nationality by maternal and paternal grandparents; age 18-45 years; the presence of 2 or earlier spontaneous miscarriages, the presence of pregnancies was confirmed by ultrasound data and/or pregnancy hormones.

Criteria for exclusion from the project, according to -disorders of luteal phase in the results of endometrium biopsy, uterine anatomical abnormalities diagnosed by hysterosalpingography, hysteroscopy or sonohysteroscopy, carriers of balanced chromosomal abnormalities by karyotyping of both spouses, presence of antiphospholipid syndrome, confirmed by the analysis of anti-beta2-glycoproteinI (IgGorIgM) antibodies, anti-cardiolipin (IgGorIgM) antibodies, lupus anticoagulant; multiple pregnancies, confirmed by ultrasound, presence of sexually transmitted infections, confirmed by two different analyses of various biological materials (IgG or IgM; PCR, smear, PCR real-time), dysfunction of thyroid gland according to analyses of TSH and thyroid antibodies.

DNA isolation was performed by separating M-PVA magnetic particles on a Prepitto automatic analyzer (PerkinElmer) to isolate ChemagicPrepito nucleic acids (Wallac, Finland) using the PrepitoDNACytoPure reagent kit.

Molecular genetic studies were performed by TaqMan using a single site-specific amplification and real-time genotyping (Real-Time PCR) on a StepOnePlus instrument (AppliedBiosystems, USA) using test systems (TestGene, Russia).

Table 1 shows polymorphisms of the studied genes with an identifier (SNP Identifier), location of polymorphism on the chromosome - the physical distance in paired bases (base-pair position - bp), name of polymorphism.

Table 1. Description of studied polymorphisms of coagulation and cardiovascular system genes.

№	GENE	CHR	SNP	Type of polymorphism	POSITION
1	MTHFR	1	rs1801131	A1298C	11854476
2	MTHFR	1	rs1801133	C677T	11856378
3	MTRR	5	rs1801394	A66G	237048500
4	MTR	1	rs1805087	A2756G	7870973
5	F5	1	rs6025	A506G	169519049
6	F2	11	rs1799963	G20210A	46761055
7	FGB	4	rs4220	G455A	155491759
8	ITGB3	17	rs5918	Leu33Pro	47283364
9	PLANH1	7	rs7242	5G/4G	100781445
10	GPIa	5	rs1126643	C807T	52347369
11	ACE	17	rs4340	I/D	61565892
12	AGTR1	3	rs5186	A1166C	148742201
13	eNOS3	7	rs1799983	Glu298Asp	46761055

Statistical data processing was performed using the PLINK program. Comparative analysis of allelic and genotypic frequencies was carried out in the main group with iRLP and the group with normal reproduction using the Pearson $\chi 2$ test and/or t - Fisher test. The odds ratios (OR) and 95% confidence interval (95% CI) were calculated using unconditional logistic regression analysis.

Statistical analysis included the calculation of associations based on various models - genotypic, additive, allelic, dominant and recessive.

Obtained Results: Due to the lack of GWAS studies of iRPL, we used the results of the GWAS meta-analysis of the search for candidate RPL genes. In a systematic review, 428 case-finding studies (1990–2015) were analyzed, which differed significantly in the definition of RPL, the clinical evaluation of patients and the choice of control group[6,7,9,12,16,17].

Association with iRPL, defined as two or more spontaneous abortions without apparent etiology, was found for 13 gene polymorphisms of coagulation and cardiovascular system, immune response, angiogenesis, chromosomal segregation, and placental function.

This article presents the results of studying associations of coagulation and cardiovascular system genes with iRPL.

Analysis of allelic and genotypic frequencies in the main group with iRPL and the control group with normal reproduction is presented in Table 2. As presented in Table 2, significant differences in allelic and genotypic frequencies of polymorphisms of blood coagulation and cardiovascular system genes are MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A), ITGB3 (Leu33Pro), AGTR1 (A1166C), ACE (I/D), GPIa (C807T), PLANH1 (5G/4G), eNOS (Glu298Asp) in the compared group of patients with iRPL and control, not detected (p>0.05).

The absence of statistically significant differences in the compared groups by coagulation and cardiovascular system genes: MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A), ITGB3 (Leu33Pro), AGTR1 (A1166C), ACE (I/D), GPIa (C807T), PLANH1 (5G/4G), eNOS (Glu298Asp) due to the fact that presence of thrombophilia is an antiphospholipid syndrome, confirmed by analysis anti-beta2-glycoproteinI (IgGorIgM) antibodies, anti-cardiolipin (IgGorIgM) antibodies, lupus anticoagulant; were criteria for exclusion from recruitment of women with iRPL. The obtained results do not confirm the possible contribution of coagulation system genes and indicate the presence of another etiopathogenetic factor in the development of iRPL.

Association calculations of studied polymorphisms using various models.

Statistical analysis using PLINK includes the calculation of associations based on various models. The allelic model is based on strength evaluation of allelic frequencies association is the simplest test, but it does not take into account the general genotype of two chromosomes; therefore, we used more accurate models of genotypic tests. The genotypic test for SNP association polymorphisms with the risk of iRPL is based on the use of frequencies of three possible genotypes in the main and control groups. The unit of calculation is not allele, but three possible genotypes with df =2. The additive (trend) model suggests that the presence of two copies of the minor allele in homozygous unfavorable AA genotype is two times more associated with iRPL than single allele in heterozygous genotype. The basis of the additive mathematical model is that the more copies of the minor allele are in the study group, the greater the adverse effect on the risk of iRPL development is heterozygotes having phenotypes lying between two homozygotes. This test has 1 df and is known as the "Cochran-Armitage Trend Test".

The dominant model suggests that disease manifests itself only if there is at least one copy of the adverse allele. All subjects are classified into two groups, depending on whether or not there is a minor allele, the dominant test has 1 df. The recessive model suggests that the effect on phenotype is manifested only if the subject has two copies of the minor allele, the number of freedom degrees =1. The most significant if we are not sure of a genetic model of the association between genotypes and phenotype is the additive model, which is less based on the principle of inheritance but is statistically less effective due to the additional degree of freedom.

Table 3 presents the results of a comparative associations' analysis of studied polymorphisms with iRPL based on the use of several models at a threshold of significance at p=0.05 for multiple testing.

Table 2. Allele and genotype frequencies in the main group with iRPL and control group.

SNP	Main group	Control group	Total	χ2	Odds ratio OR
Allele/genotype	absolute number (frequency)	absolute number (frequency)	absolute number (frequency)	(p-value)*	(95% CI)
Genes of coagulation and cardiovascular system					
MTR (rs1805087) A2756G					
A	483 (0,8)	499 (0,83)	982 (0,81)	1,44 p>0,05	1,23 (0,92-1,65)
G	121 (0,20)	101 (0,17)	222 (0,19)		
AA	191 (0,63)	206 (0,68)	397 (0,66)		
GA	101 (0,33)	84 (0,28)	185 (0,31)		
GG	10 (0,04)	10 (0,04)	20 (0,04)		
MTHFR (rs1801131) A1298C					
A	440 (0,73)	432 (0,72)	872 (0,73)	0,02 p>0,05	0,98 (0,76-1,27)
C	164 (0,27)	164 (0,28)	328 (0,27)		
AA	159 (0,53)	155 (0,51)	314 (0,52)		
AC	122 (0,4)	125 (0,42)	247 (0,41)		
CC	21 (0,07)	20 (0,07)	41 (0,07)		
MTHFR (rs1801133) C677T					
C	428 (0,71)	438 (0,73)	866 (0,72)	0,79 p>0,05	1,11 (0,87-1,43)
T	176 (0,29)	162 (0,27)	338 (0,28)		
CC	152 (0,51)	157 (0,52)	309 (0,51)		
CT	122 (0,4)	123 (0,41)	245 (0,41)		
TT	28 (0,09)	20 (0,07)	48 (0,08)		
MTRR (rs1801394) A66G					
A	306 (0,51)	320 (0,54)	626 (0,52)	0,97 p>0,05	1,11 (0,88-1,4)
G	298 (0,49)	280 (0,47)	578 (0,48)		
AA	74 (0,25)	96 (0,32)	170 (0,28)		
GA	158 (0,52)	129 (0,43)	287 (0,48)		
GG	70 (0,23)	75 (0,25)	145 (0,24)		
F5 (rs6025) A506G					
A	592 (0,98)	588 (0,98)	1180 (0,98)	0,39 p>0,05	0,99 (0,44-2,23)
G	12 (0,02)	12 (0,02)	24 (0,02)		
AA	292 (0,96)	289 (0,96)	581 (0,96)		
GA	9 (0,03)	8 (0,03)	17 (0,03)		
GG	1 (0,01)	3(0,01)	4 (0,01)		
F2 (rs1799963) G20210A					
G	600 (0,992)	596 (0,993)	1196 (0,993)	0,11 p>0,05	0,99 (0,25-3,99)
A	4 (0,008)	4 (0,007)	8 (0,007)		
GG	297 (0,98)	296 (0,99)	593 (0,99)		
AG	5 (0,02)	4 (0,01)	9 (0,01)		
AA	0	0	0		
FGB (rs4220) G455A					
G	502 (0,83)	504 (0,84)	1006 (0,83)	0,42 p>0,05	1,07 (0,79-1,45)
A	102 (0,17)	96 (0,16)	198 (0,17)		
GG	207 (0,69)	211 (0,70)	418 (0,69)		
AG	86 (0,28)	83 (0,28)	169 (0,28)		
AA	9 (0,03)	6 (0,02)	15 (0,03)		
GPLa (rs1126643) C807T					
C	398 (0,66)	420 (0,7)	818 (0,68)	2,14 p>0,05	1,22 (0,96-1,55)
T	208 (0,34)	180 (0,3)	388 (0,32)		
CC	132 (0,44)	146 (0,49)	278 (0,46)		
CT	134 (0,44)	127 (0,42)	261 (0,44)		
TT	36 (0,12)	27 (0,09)	63 (0,10)		
PLANH1(rs7242) 5G/4G					
5G	302 (0,5)	312 (0,52)	614 (0,51)	0,33 p>0,05	1,08 (0,86-1,36)
4G	302 (0,5)	288 (0,48)	590 (0,49)		
5G5G	77 (0,25)	81 (0,27)	158 (0,26)		
4G5G	146 (0,48)	146 (0,49)	292 (0,49)		
4G4G	79 (0,26)	73 (0,24)	152 (0,25)		
ITGB3 (rs5918) Leu33Pro					
L	550 (0,91)	558 (0,93)	1108 (0,92)	1,33 p>0,05	1,30 (0,86-1,99)
P	54 (0,09)	42 (0,07)	96 (0,08)		
PP	1 (0,003)	1 (0,003)	2 (0,003)		
LP	50 (0,166)	39 (0,130)	89 (0,148)		
LL	251 (0,831)	260 (0,867)	511 (0,849)		
GG	26 (0,09)	20 (0,07)	46 (0,08)		
AGTR1 (rs5186) A1166C					
A	526 (0,87)	522 (0,87)	1048 (0,87)	0,016 p>0,05	0,99 (0,71-1,39)
C	78 (0,13)	78 (0,13)	156 (0,13)		
AA	244 (0,82)	226 (0,75)	450 (0,75)		
AC	75 (0,25)	69 (0,23)	144 (0,24)		
CC	3 (0,01)	5 (0,02)	8 (0,01)		
eNOS (rs1799983) Glu298Asp					
G	488 (0,81)	470 (0,78)	958 (0,80)	1,12 p>0,05	0,86 (0,65-1,14)
A	116 (0,19)	130 (0,22)	246 (0,20)		
GG	200 (0,66)	189 (0,63)	389 (0,65)		
AG	88 (0,29)	92 (0,31)	180 (0,30)		
AA	14 (0,05)	19 (0,06)	33 (0,05)		
ACE (rs4340 I/D					
I	338 (0,56)	360 (0,6)	698 (0,58)	1,86 p>0,05	1,180 (0,94-1,48)
D	266 (0,44)	240 (0,4)	506 (0,42)		
II	90 (0,30)	102 (0,34)	192 (0,32)		
ID	160 (0,53)	157 (0,52)	317 (0,53)		
DD	52 (0,17)	41 (0,14)	93 (0,15)		

Table 3. Comparative analysis of studied associations polymorphisms of coagulation and cardiovascular system.

Name of gene	Chromosome	SNP	Position	TEST	AFF	UNAFF	χ2	DF	P
MTR A2756G	1	rs1805087	7870973	GENO	10/101/191	10/84/206	2,122	2,0	0,3461
MTR A2756G	1	rs1805087	7870973	TREND	121/483	104/496	1,46	1,0	0,2269
MTR A2756G	1	rs1805087	7870973	ALLELIC	121/483	104/496	1,444	1,0	0,2295
MTR A2756G	1	rs1805087	7870973	DOM	111/191	94/206	1,97	1,0	0,1605
MTR A2756G	1	rs1805087	7870973	REC	10/292	10/290	2,283E-4	1,0	0,9879
MTHFR A1298C	1	rs1801131	11854476	GENO	21/122/159	20/125/155	0,1051	2,0	0,9488
MTHFR A1298C	1	rs1801131	11854476	TREND	164/440	165/435	0,01895	1,0	0,8905
MTHFR A1298C	1	rs1801131	11854476	ALLELIC	164/440	165/435	0,01832	1,0	0,8923
MTHFR A1298C	1	rs1801131	11854476	DOM	143/159	145/155	0,0582	1,0	0,8094
MTHFR A1298C	1	rs1801131	11854476	REC	21/281	20/280	0,01953	1,0	0,8889
MTHFR C677T	1	rs1801133	11856378	GENO	28/122/152	20/123/157	1,412	2,0	0,4937
MTHFR C677T	1	rs1801133	11856378	TREND	178/426	163/437	0,7886	1,0	0,3745
MTHFR C677T	1	rs1801133	11856378	ALLELIC	178/426	163/437	0,7868	1,0	0,3751
MTHFR C677T	1	rs1801133	11856378	DOM	150/152	143/157	0,2415	1,0	0,6231
MTHFR C677T	1	rs1801133	11856378	REC	28/274	20/280	1,392	1,0	0,2381
MTRR A66G	1	rs1801394	237048500	GENO	70/158/74	75/129/96	5,943	2,0	0,05122
MTRR A66G	1	rs1801394	237048500	TREND	298/306	279/321	0,9295	1,0	0,335
MTRR A66G	1	rs1801394	237048500	ALLELIC	298/306	279/321	0,9712	1,0	0,3244
MTRR A66G	1	rs1801394	237048500	DOM	228/74	204/96	4,174	1,0	0,04105
MTRR A66G	1	rs1801394	237048500	REC	70/232	75/225	0,273	1,0	0,6013
F5 A506G	1	rs6025	169519049	GENO	1/9/292	3/8/289	0,388	2,0	0,534
F5 A506G	1	rs6025	169519049	TREND	11/593	14/586	0,2974	1,0	0,5855
F5 A506G	1	rs6025	169519049	ALLELIC	11/593	14/586	0,3883	1,0	0,5332
F5 A506G	1	rs6025	169519049	DOM	10/292	11/289	0,056	1,0	0,813
F5 A506G	1	rs6025	169519049	REC	1/301	3/297	0,258	1,0	0,612
F2 G20210A	11	rs1799963	46761055	GENO	0/5/297	0/4/296	0,105	2,0	0,746
F2 G20210A	11	rs1799963	46761055	TREND	5/599	4/596	0,1062	1,0	0,7446
F2 G20210A	11	rs1799963	46761055	ALLELIC	5/599	4/596	0,1054	1,0	0,7455
F2 G20210A	11	rs1799963	46761055	DOM	5/297	4/296	0,106	1,0	0,745
F2 G20210A	11	rs1799963	46761055	REC	0/302	0/300	0,000	1,0	1,000
FGB G455A	4	rs4220	155491759	GENO	9/86/207	6/83/211	0,6849	2,0	0,71
FGB G455A	4	rs4220	155491759	TREND	104/500	95/505	0,426	1,0	0,5139
FGB G455A	4	rs4220	155491759	ALLELIC	104/500	95/505	0,4186	1,0	0,5176
FGB G455A	4	rs4220	155491759	DOM	95/207	89/211	0,2273	1,0	0,6335
FGB G455A	4	rs4220	155491759	REC	9/293	6/294	0,5951	1,0	0,4405
GPLa C807T	5	rs1126643	52347369	GENO	36/134/132	27/127/146	2,172	2,0	0,3376
GPLa C807T	5	rs1126643	52347369	TREND	206/398	181/419	2,128	1,0	0,1446
GPLa C807T	5	rs1126643	52347369	ALLELIC	206/398	181/419	2,142	1,0	0,1434
GPLa C807T	5	rs1126643	52347369	DOM	170/132	154/146	1,489	1,0	0,2224
GPLa C807T	5	rs1126643	52347369	REC	36/266	27/273	1,37	1,0	0,2418
PLANH1 5G/4G	7	rs7242	100781445	GENO	79/146/77	73/146/81	0,3315	2,0	0,8473
PLANH1 5G/4G	7	rs7242	100781445	TREND	304/300	292/308	0,3239	1,0	0,5693
PLANH1 5G/4G	7	rs7242	100781445	ALLELIC	304/300	292/308	0,3336	1,0	0,5636
PLANH1 5G/4G	7	rs7242	100781445	DOM	225/77	219/81	0,1757	1,0	0,6751
PLANH1 5G/4G	7	rs7242	100781445	REC	79/223	73/227	0,2658	1,0	0,6062
ITGB3 Leu33Pro	17	rs5918	47283364	GENO	1/50/251	1/39/260	1,094	2,0	0,296
ITGB3 Leu33Pro	17	rs5918	47283364	TREND	52/552	41/559	1,383	1,0	0,2396
ITGB3 Leu33Pro	17	rs5918	47283364	ALLELIC	52/552	41/559	1,332	1,0	0,2485
ITGB3 Leu33Pro	17	rs5918	47283364	DOM	51/251	40/260	1,482	1,0	0,224
ITGB3 Leu33Pro	17	rs5918	47283364	REC	1/301	1/299	0,000	1,0	0,997
ACE I/D	17	rs4340	61565892	GENO	52/160/90	41/157/102	2,073	2,0	0,3547
ACE I/D	17	rs4340	61565892	TREND	264/340	239/361	2,025	1,0	0,1547
ACE I/D	17	rs4340	61565892	ALLELIC	264/340	239/361	1,858	1,0	0,1728
ACE I/D	17	rs4340	61565892	DOM	212/90	198/102	1,221	1,0	0,2691
ACE I/D	17	rs4340	61565892	REC	52/250	41/259	1,454	1,0	0,228
AGTR1 A1166C	3	rs5186	148742201	GENO	3/75/224	5/69/226	0,016	2,0	0,901
AGTR1 A1166C	3	rs5186	148742201	TREND	81/523	79/521	0,01616	1,0	0,8989
AGTR1 A1166C	3	rs5186	148742201	ALLELIC	81/523	79/521	0,01554	1,0	0,9008
AGTR1 A1166C	3	rs5186	148742201	DOM	78/224	74/226	0,108	1,0	0,743
AGTR1 A1166C	3	rs5186	148742201	REC	3/299	5/295	0,520	1,0	0,471
eNOS Glu298Asp	7	rs1799983	46761055	GENO	14/88/200	19/92/189	1.151	2	0.5625
eNOS Glu298Asp	7	rs1799983	46761055	TREND	116/488	130/470	1.038	1	0.3082
eNOS Glu298Asp	7	rs1799983	46761055	ALLELIC	116/488	130/470	1.122	1	0.2896
eNOS Glu298Asp	7	rs1799983	46761055	DOM	102/200	111/189	0.6847	1	0.408
eNOS Glu298Asp	7	rs1799983	46761055	REC	14/288	19/281	0.8371	1	0.3602

AFFECTED - the main group of patients with iRPL; UNAFFECTED (control group) for each test; TEST - type of test; GENO = baseline genotype; TREND = additive test; DOM = dominant test; REC = recessive test

As can be seen from Table 3, statistically significant associations of iRPL, which imply a specific relationship between genotype and phenotype, include statistically highly significant differences in genotypic, additive (trend), allelic, general recessive and dominant models, were not detected for the studied polymorphisms ($p>0.05$).

Low reliable associations of A66G polymorphism (rs1801394) of MTRR folate metabolism gene with iRPL were detected using a genotypic model ($\chi 2=4.174$; $p=0.041$). The absence of similar results for other models suggests a random trend associated with a high POPULATION frequency of the adverse minor allele in this polymorphism, which was 0.43.

Despite numerous scientific studies of possible causes of RPL, such as fetal chromosomal abnormalities, infectious agents, adverse environmental factors, bad habits, anatomical defects, thrombophilic disorders, etc., etiology of RPL (up to 50% of cases) remains uncertain[1,5-10]. These cases of RPL have no explainable etiology and effective therapy, require an in-depth study of their etiopathogenesis and are considered idiopathic RPL (hereinafter iRPL).

The lack of large-scale GWAS research on iRPL is due to several objective reasons: lack of clear definitions of iRPL, difficulty in recruiting and small sample size; lack of replicative studies in ethnically homogeneous populations[6,7,9,11,12].

Published systematic scientific reviews [8–17] of more than 80 studies of associations of genetic polymorphisms with iRPL, such as genes of hereditary thrombophilia, pro-inflammatory cytokine genes, angiogenesis genes, and placental function genes, did not confirm the unambiguous connection of studied polymorphisms and iRPL. The question of choice validity of these genetic polymorphisms, based on the modern understanding of the physiology of implantation processes, which is a long and complex process of balanced interaction between the mother and the fetus, mediated through the placenta[10-17] is discussed.

Disorders of this process at all stages can lead to abortion, which led to our choice of specific polymorphisms of the maternal genome responsible for disorders of decidualization and endometrial angiogenesis[18-20].

Due to the high frequency of iRPL, its significant contribution to reproduction and fertility rates, genetic causation and lack of reliable data on genetic markers that would predict the development of iRPL, a replicative study was conducted in an ethnically homogeneous population of Kazakhs with clear criteria for recruiting and choosing etiopathogenetic polymorphisms of iRPL.

The aim of the study was to evaluate the genetic contribution of 13 potentially significant polymorphisms of coagulation and cardiovascular system genes: MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A) ITGB3 (Leu33Pro), PLANH1 (5G/4G); GPIa (C807T), AGTR1 (A1166C), ACE (I/D), eNOS (Glu298Asp) in development of iRPL in ethnically homogeneous population of Kazakhs.

Conclusion: The results of independent replicative TaqMan genotyping of 302 patients with iRPL and 300 women with normal reproduction did not reveal an association of studied polymorphisms of coagulation and cardiovascular system genes with the development of iRPL in the Kazakh population. The obtained results are consistent with the data of replicative genotyping in other ethnic populations[13,21], with published meta-analyzes[8,22-25].

The obtained contradictory results in different ethnic populations may reflect the methodological errors associated with an insufficient sample size, ethnic heterogeneity and the methodology of research conducted.

Our study on a sufficient sample in ethnically homogeneous groups, using international diagnostic criteria, will make a certain contribution to the search for genetic associations of iRPL in independent human populations.

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