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POPULATION CHARACTERISTICS OF GENES ASSOCIATED WITH IDIOPATHIC RECURRENT MISCARRIAGE IN THE KAZAKH POPULATION

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Abstract

Background. Miscarriage is relevant medical and social problem, according to WHO its frequency is 20% and has no tendency to decrease, despite effective modern methods of diagnosis and treatment. The aim was to study alleles and single nucleotide polymorphisms (SNP) population frequencies of the coagulation system and cardiovascular system genes, associated with idiopathic recurrent pregnancy loss (iRPL) development as a risk factor in an ethnically homogeneous Kazakh population: MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A), ITGB (Leu33Pro), PLANH1 (5G / 4G); GPIa (C807T), AGTR1 (A1166C), ACE (I / D), eNOS (Glu298Asp).

Materials and Methods. The genomic database was analysed based on the results of genotyping of 700 conditionally healthy individuals of Kazakh nationality ~2.5 million SNPs using OmniChip 2.5 M Illumina chips at the DECODE Iceland Genomic Center as part of the joint implementation of the project "Genetic Studies of Preeclampsia in Populations of Central Asia and Europe" (InterPregGen) within the 7th Framework Programme of the European Commission under Grant Agreement No. 282540.

Results. The Kazakh population occupies an intermediate position between Europe and Asia populations described in project 1000 genomes by genes of the coagulation and cardiovascular systems. Distribution frequency analysis of the studied genotypes in the Kazakh population showed their correspondence to Hardy-Weinberg equilibrium for the majority of the studied polymorphisms ($p>0.05$), significant deviations from the expected heterozygosity demonstrated by the polymorphisms A1298C MTHFR gene ($p<0.002$) and A1166C AGTR1 gene ($p<0,0001$).

Conclusion. Coagulation and cardiovascular systems genes studied polymorphisms can be considered as possible genetic factors for the development of iRPL, due to the high frequency of this pathology in different populations, and its significant contribution to reproduction and fertility indicators. Conduct further studies to determine their significance in the development of iRPL in the Kazakh population.

Keywords: *single nucleotide polymorphisms, genotypes, idiopathic recurrent pregnancy loss.*

Резюме

ПОПУЛЯЦИОННЫЕ ХАРАКТЕРИСТИКИ ГЕНОВ, АССОЦИИРОВАННЫХ С ИДИОПАТИЧЕСКИМ ПРИВЫЧНЫМ НЕВЫНАШИВАНИЕМ БЕРЕМЕННОСТИ В КАЗАХСКОЙ ПОПУЛЯЦИИ

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Цель исследования: изучить популяционные особенности частота ллелейи генотипов полиморфных вариантов генов свертывающей и кардиоваскулярной системы: 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), предположительно ассоциированных с развитием идиопатической формы привычного невынашивания беременности в этнически однородной популяции казахов.

Материалы и методы. Проведен анализ геномной базы данных по результатам генотипирования 1800 условно здоровых лиц казахской национальности ~2,5 млн SNP с использованием чипов OmniChip 2,5 M Illumina в Геномном центре DECODE Iceland в рамках совместного выполнения проекта «Генетические исследования преэклампсии в популяциях Центральной Азии и Европы» (InterPregGen) 7 рамочной программы Европейской Комиссии по Грантовому соглашению №. 282540.

Результаты. Казахская популяция по генам свертывающей и кардиоваскулярной системы при сравнении с другими популяциями мира занимает промежуточное положение между описанными в проекте 1000 геномов популяциями Европы и Азии. Анализ частотного распределения исследованных генотипов в популяции казахов показал их соответствие равновесию Харди-Вайнберга для большинства изученных полиморфизмов (p>0,05), достоверные отклонения от ожидаемой гетерозиготности продемонстрировали полиморфизмы - A1298C гена MTHFR (P<0,002) и A1166C гена AGTR1 (P<0,0001).

Выводы. В связи с высокой частотой идиопатической формы привычного невынашивания беременности (иПНБ) в популяциях человека, ее значимым вкладом в показатели репродукции и рождаемости, изученные полиморфизмы генов свертывающей и кардиоваскулярной системы можно рассматривать как возможные генетические факторы развития данной патологии, провести дальнейшие исследования для определения их значимости в развитии иПНБ в казахской популяции.

Ключевые слова: *полиморфизм генов, генотипы, идиопатическая форма привычного невынашивания беременности.*

Түйіндеме

ҚАЗАҚ ПОПУЛЯЦИЯСЫНДАҒЫ ИДИОПАТИЯЛЫҚ ҚАЙТАЛАНАТЫН ТҮСІКПЕН БАЙЛАНЫСТЫ ГЕНДЕРДІҢ ПОПУЛЯЦИЯЛЫҚ СИПАТТАМАСЫ

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Зерттеудің мақсаты: популяция ерекшеліктерін зерттеу коагуляциялық және кардиоваскулярлық жүйе гендерінің полиморфты нұсқаларының генотиптерінің ллелия жиілігі: 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), болжам бойынша идиопатиялық форманың дамуымен байланысты.

Материалдар мен әдістер. "Орталық Азия мен Еуропа популяцияларындағы преэклампсияны генетикалық зерттеу" (Interpreggen) жобасын бірлесіп орындау шеңберінде decode Iceland геномдық орталығында OmniChip 2,5 M illumina чиптерін пайдалана отырып, 1800 шартты дені сау қазақ ұлтының ~2,5 млн SNP генотиптеу нәтижелері бойынша геномдық деректер базасына талдау жүргізілді. 282540.

Нәтижелер. Қазақ популяциясы әлемнің басқа популяцияларымен салыстырғанда ұю және кардиоваскулярлық жүйенің геномдары жобада сипатталған Еуропа мен Азияның 1000 геномы арасында аралық орынды алады. Зерттелген генотиптердің қазақтар популяциясында жиіліктік таралуын талдау олардың зерттелген полиморфизмдердің көпшілігі үшін Харди-Вайнберг тепе - теңдігіне сәйкестігін көрсетті (p>0,05), күтілетін гетерозиготалықтан сенімді ауытқулар MTHFR генінің-a1298c полиморфизмдерін көрсетті (P<0,002) және AGTR1 генінің A1166C (P<0,0001).

Қорытындылар. Адам популяцияларында жүктіліктің әдеттегі түсік түсіруінің идиопатиялық түрінің жоғары жиілігіне, оның репродукция және туу көрсеткіштеріне елеулі үлесіне, генді ұю және кардиоваскулярлық жүйенің зерттелген полиморфизмдеріне байланысты осы патологияны дамытудың ықтимал генетикалық факторлары ретінде қарастыруға, олардың қазақ популяциясындағы жтсб дамуындағы маңыздылығын анықтау үшін одан әрі зерттеулер жүргізуге болады.

Түйін сөздер*: гендік полиморфизм, генотиптер, әдеттің идиопатиялық түріжүктілікжүктілікжүктілік.*

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Introduction

According to WHO, miscarriage is a relevant medical and social problem frequency is 20% and does not tend to decrease, despite effective modern methods of diagnosis and treatment [27].

Recurrent pregnancy loss (further RPL), classically defined as two or more spontaneous miscarriages up to 20 weeks of pregnancy, is a heterogeneous disorder affecting up to 3% of couples in the reproductive period [9,18].

S.Vedishchev et al. (2013) - frequency of spontaneous abortion in Russia is high - from 15 to 23% of all recorded pregnancies, with about 80% of reproductive losses occurring in the first trimester [2]. According to N.M. Mamedalieva frequency of miscarriage ranges from 22% to 31% of all recorded pregnancies in Kazakhstan [3].

Despite numerous scientific studies of possible RPL causes, such as fetal chromosomal abnormalities, infectious agents, adverse environmental factors, bad habits, anatomical defects, thrombophilic disorders, etc., the etiology of RPL (up to 50% of cases) remains unclear [8,11,17,21]. Nowadays, RPL doesn't have an understandable etiology and effective therapy, which require etiopathogenesis study, considered idiopathic RPL (further iRPL).

As known, the leading cause of RPL is the genetic factor. The concept of RPL genetic causes meant the presence of chromosomal abnormalities (quantitative or structural violations of the karyotype) in both spouses with a history of miscarriages and abortions. Currently, new high-tech molecular diagnostic methods are applied; the concept of a genetic factor includes the study of predisposition genes.

According to many authors, RPL can be considered a multifactorial disease. A combination of genetic and environmental factors needs to evaluate in each specific case of RPL [1,15,16,27].

Multifactorial diseases are determined by a whole group of "predisposition" genes [1,15,16,27]. The relationship between genetic variants and their functions attracts the attention of many researchers, as it allows more accurately to determine the clinical significance of each specific polymorphism.

We selected statistically significant SNP polymorphisms for independent replicative genotyping in an ethnically homogeneous Kazakh population according to Genome-wide association studies (GWAS) and meta-studies of the RPL in world databases [http://www.ncbi.nlm.nih.gov; http://asia.ensembl.org/index.html; www.ncbi.nlm.nih.gov/gap; www.genome.gov; http://hapmap.ncbi.nlm.nih.gov/index.html.en; www.1000 genomes.org], QC control (p<5x10-8 cluster plot inspection, HWEtest, etc.).

The Aim was to study the population characteristics of the allelic and genotypic distribution of potentially significant polymorphisms of genes predisposing to iRPL in coagulation and cardiovascular systems (MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G) FGB (G455A), ITGB3 (Leu33Pro), AGTR1 (A1166C), ACE (I/D), GPIa (C807T), PLANH1 (5G/4G); eNOS (Glu298Asp)) in ethnically homogeneous Kazakh population.

Materials and Methods.

The study was approved by the bioethical committee of the Scientific Center of Obstetrics, Gynecology, and Perinatology (Almaty, Kazakhstan). All subjects were consulted about the objectives of the project and signed informed consent in its participation.

The material was DNA isolated from the peripheral blood of the recruited population control group, is represented by 700 conditionally healthy individuals of Kazakh nationality. Criteria for selection in the control group are ethnicity - Kazakhs, including grandparents; age 18 years and older, the subject's ability to make an independent decision on consent to participate in the project. DNA is stored in the Miras biobank of the Scientific Center for Obstetrics, Gynecology, and Perinatology (SCOGP).

Molecular genetic studies. The genomic database was analysed based on the results of genotyping of 700 conditionally healthy individuals of Kazakh nationality ~2.5 million SNPs using OmniChip 2.5 M Illumina chips at the DECODE Iceland Genomic Center as part of the joint implementation of the project “Genetic Studies of Preeclampsia in Populations of Central Asia and Europe” (InterPregGen) within the 7th Framework Programme of the European Commission under Grant Agreement No. 282540.

Statistical analysis. Statistical significance tests and analysis of the nonparametric criterion $\chi 2$ were performed using the PLINK software [20]. Hardy-Weinberg balance law score was calculated using the HWEtest function of the PLINK program. In all cases, the results are considered statistically significant at the level of $P<0.05$.

Results.

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

Table 2 shows the frequencies of genotypes and alleles of the coagulation and cardiovascular system genes in the Kazakh population. Table 3 presents the comparative analysis of the allelic frequencies of the studied genes in various world populations, according to GWAS data possibly associated with iRPL. Allele frequencies for previously studied populations are from the Project 1000 Genomes Phase III [www.genome.gov].

Table 1.

Description of genes polymorphisms of the coagulation and cardiovascular system.

№	Gene	Chromosome	SNP Identifier	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

Table 2.

Frequencies of genotypes and alleles of the genes of the coagulation and cardiovascular system in the Kazakh population.

MTHFR (rs1801133) C677T					
Allele/genotype	C	T	CC	CT	TT
n (%)	984 (70.3%)	416 (29.7%)	354 (50.6%)	276 (39.4%)	70 (10.0%)
MTHFR (rs1801131) A1298C					
Allele/genotype	A	C	AA	AC	CC
n (%)	930 (66.4%)	470 (33.6%)	327 (46.7%)	276 (39.4%)	97 (13.9%)
MTRR (rs1801394) A66G					
Allele/genotype	A	G	AA	GA	GG
n (%)	751 (53.6%)	649 (46.4%)	202 (28.9%)	347 (49.6%)	151 (21.6%)
MTR (rs1805087) A2756G					
Allele/genotype	A	G	AA	GA	GG
n (%)	1136 (81.1%)	264 (18.9%)	459 (65.6%)	218 (31.1%)	23 (3.3%)
F5 (rs6025) A506G					
Allele/genotype	G	A	GG	GA	AA
n (%)	1386 (99.0%)	14 (1.0%)	687 (98.1%)	12 (1.7%)	1 (0.1%)
F2 (rs1799963) G20210A					
Allele/genotype	G	A	GG	GA	AA
n (%)	1397 (99.8%)	3 (0.2%)	697 (99.6%)	3 (0.4%)	0
AGTR1 (rs5186) A1166C					
Allele/genotype	A	C	AA	AC	CC
n (%)	1121 (80.1%)	279 (19.9%)	477 (68.1%)	167 (23.9%)	56 (8.0%)
FGB (rs4220) G455A					
Allele/genotype	G	A	GG	AG	AA
n (%)	1197 (85.5%0	203 (14.5%)	515 (73.6%)	167 (23.9%)	18 (2.6%)
GPIa (rs1126643) C807T					
Allele/genotype	C	T	CC	CT	TT
n (%)	939 (67.1%)	461 (32.9%)	322 (46.0%)	295 (42.1%)	83 (11.9%)
PLANH1 (rs7242) 5G/4G					
Allele/genotype	5G	4G	5G5G	4G5G	4G4G
n (%)	705 (50.4%)	695 (49.6%)	184 (26.3%)	337 (48.1%)	179 (25.6%)
ITGB3 (rs5918) Leu33Pro					
Allele/genotype	L	P	LL	LP	PP
n (%)	1287 (91.9%)	113 (8.1%)	594 (84.9%)	99 (14.1%)	7 (1.0%)
ACE (rs4340) I/D					
Allele/genotype	I	D	II	ID	DD
n (%)	809 (57.8%)	591 (42.2%)	245 (35.0%)	319 (45.6%)	136 (19.4%)
eNOS (rs1799983) Glu298Asp					
Allele/genotype	G	A	GG	AG	AA
n (%)	856 (61.1%)	544 (38.9%)	257 (36.7%)	342 (48.9%)	101 (14.4%)

Table 3. **Comparative analysis of the frequency of genotypes and alleles, possibly predisposing to iRPL in the Kazakh population and populations of the world.**

Population	N	MAF	χ2	p
Coagulation and cardiovascular systems genes				
MTHFR (rs1801133) C677T				
Kazakhstan	700	0.30		
Europe**	503	0.37	6.45*	0.012
East Asia**	504	0.30	0.00	0.989
South Asia**	489	0.12	52.90*	<0.001
MTHFR (rs180113)1 A1298C				
Kazakhstan	700	0.34		
Europe**	503	0.31	1.19	0.277
East Asia**	504	0.22	20.42*	<0.001
South Asia**	489	0.42	7.73*	0.006
MTRR (rs1801394) A66G				
Kazakhstan	700	0.46		
Europe**	503	0.52	4.20*	0.041
East Asia**	504	0.26	49.98*	<0.001
South Asia**	489	0.53	5.59*	0.019
MTR (rs1805087) A2756G				
Kazakhstan	700	0.19		
Europe**	503	0.17	0.71	0.399
East Asia**	504	0.11	14.55*	<0.001
South Asia**	489	0.32	26.05*	<0.001
F5 (rs6025) A506G				
Kazakhstan	700	0.01		
Europe**	503	0.012	0.10	0.750
East Asia**	504	0	5.07*	0.025
South Asia**	489	0.011	0.001	0.970
F2 (rs1799963) G20210A				
Kazakhstan	700	0.002		
Europe**	503	0.008	3.01	0.083
East Asia**	504	0	0.72	0.396
South Asia**	489	0	0.70	0.404
FGB (rs4220) G455A				
Kazakhstan	700	0.15		
Europe**	503	0.20	5.32*	0.022
East Asia**	504	0.22	9.82*	0.002
South Asia**	489	0.14	0.28	0.599
ITGB3 (rs5918) Leu33Pro				
Kazakhstan	700	0.08		
Europe**	503	0.13	7.84*	0.006
East Asia**	504	0.009	29.92*	<0.001
South Asia**	489	0.11	3.18	0.075
PLANH1 (rs7242) 5G/4G				
Kazakhstan	700	0.50		
Europe**	503	0.45	3.01	0.083
East Asia**	504	0.45	2.89	0.090
South Asia**	489	0.40	11.40*	<0.001
AGTR1 (rs5186) A1166C				
Kazakhstan	700	0.20		
Europe**	503	0.27	8.65*	0.004
East Asia**	504	0.06	47.69*	<0.001
South Asia**	489	0.07	39.23*	<0.001
ACE (rs4340) I/D				
Kazakhstan	700	0.42		
Germany**	127	0.51	3.69	0.055
China**	132	0.19	24.98*	<0.001
Korea**	126	0.52	4.68*	0.031

Continuation of Table 3.

Population	N	MAF	χ2	p
Coagulation and cardiovascular systems genes				
GPIa (rs1126643) C807T				
Kazakhstan	700	0.33		
Europe**	503	0.40	6.16*	0.014
East Asia**	504	0.28	3.46	0.063
South Asia**	489	0.33	0.001	0.979
eNOS3 (rs1799983) Glu298Asp				
Kazakhstan	700	0.39		
Europe**	503	0.34	3.15	0.077
East Asia**	504	0.13	96.85*	<0.001
South Asia**	489	0.17	66.59*	<0.001

*Note: N - number of DNA samples; MAF - frequency of the minor allele; χ2 - Chi-square test; P - statistical significance; * - differences are statistically significant (p <0.05);**- www.1000 genomes.org*

Population frequency carriage of the unfavorable T allele of the MTHFR gene (C677T) in the study sample is 0.30 (29.7%), which is significantly higher (p <0.001) in the Kazakh population compared to the similar indicator for South Asia – 0.12, but significantly lower compared to Europe – 0.37 (p <0.012). No significant differences with the population frequency of the MTHFR allele T gene (C677T) in the East Asian population are not detected (p> 0.05).

The frequency of the C allele of the MTHFR gene (A1298C) in the studied Kazakh population is 0,34 (33.6%), which do not show any differences from the European population (p> 0.05), but exceeded similar indicator of the East Asian population – 0.22 (p <0.001). A significantly minor allele is found more often in the South Asian population – 0.42 (p <0.006). The Kazakh population occupies an intermediate position between South and East Asia in terms of the frequency of the MTHFR gene (C677T and A1298C alleles).

The frequencies of the MTRR (A66G) and MTR (A2756G) alleles in the studied Kazakh population are 0.46 (46.4%) and 0.19 (18.9%), respectively. Minor alleles of the MTRR and MTR genes are significantly lower than in South Asia – 0.53 and 0.32 (p <0.01) and significantly higher than the frequencies in East Asia (p <0.001).

Tables 2 and 3 demonstrate that the allele frequencies of the F2 (G20210A) and F5 (A506G) genes in the Kazakh population turned out to be very low - 0.002 (0.2%) and 0.01 (1.0%), respectively, which does not significantly differ from similar indicators in Europe, East and South Asia (P>0.05).

The frequency of the FGB gene minor A allele (G455A) in the Kazakhs is 0.15 (14,5%), which is no different from its frequency in South Asia, but lower than the similar frequency in Europe – 0.20 (p < 0.022) and East Asia 0.22 (p <0.002).

P alleles frequency of the ITGB3 gene (Leu33Pro) in the studied Kazakh population is 0.08 (8.1%), which does not show a significantly differ from South Asia (p>0.05), but exceeds similar indicator in East Asia (p <0.001) and Europe (p <0.006) populations.

The minor allele frequency of the PLANH1 gene (5G/4G) in the Kazakh population is 0.50 (49.6%), which is no different from Europe and East Asia, but significantly exceeds its frequency in South Asia (0.40) (P <0.001) populations.

The prevalence of deletion D frequency in the ACE gene (I/D) is 0.42 (42.2%), significantly exceeding a similar indicator in Chinese – 0.19 (p <0.001), lower than its frequency in Korea – 0.52 (p <0.03).

The occurrence of C allele frequency in the AGTR1 gene (A1166C) is 0,20 (19,9%), which is significantly higher than its frequency in populations of East and South Asia, slightly exceeds similar indicator in Europe (0.27; p <0.001).

The population frequency of T allele GPIa gene (C807T) carriage does not differ from the corresponding frequencies of East and South Asia, but it is significantly lower than that of Europe (p <0.014).

The frequency carriage of the A allele of the eNOS3 gene Glu298Asp polymorphism in the Kazakh population is 0.39 (38.9%), this is comparable with Europeans, but it is significantly higher than its population frequency in East Asian and South Asian countries (p <0.001).

Hardy-Weinberg equilibrium of coagulation and cardiovascular system genes in the Kazakh population. In medical genetics, the Hardy – Weinberg law makes it possible to assess the population risk of genetically determined diseases because each population has its allele pool and different frequencies of unfavorable alleles [8,21].

Frequency distribution of alleles and genotypes for coagulation and cardiovascular systems genes in the Kazakh population: MTHFR (C677T, A1298C), MTR (A2756G), MTRR (A66G), F5 (A506G), F2 (G20210A), FGB (G455A), ITGB3 (AGTR1Pro) (A1166C), ACE (I/D), GPIa (C807T), PLANH1 (5G / 4G), eNOS3 (Glu298Asp), obtained as a result of statistical processing in the PLINK program using the HWEtest function, are presented in Table 4.

According to table 4, the observed and expected heterozygosity distribution of genotypes is under Hardy-Weinberg equilibrium (p> 0.05) for all the studied polymorphisms in the Kazakh population, except folate metabolism gene MTHFR A1298C polymorphism (rs1801131) (P <0.002), cardiovascular system gene AGTR1 A1166C polymorphism (rs5186) (P <0.0001). It may be due to the insufficient sample size with a high level of population heterozygosity and the historical patterns of the Kazakh population formation. Our results confirm the effectiveness of the study to identify microevolutionary changes in an ethnically homogeneous Kazakh population.

Table 4.

Distribution correspondence of genotypes to Hardy-Weinberg equilibrium for coagulation and cardiovascular systems genes in the Kazakh population (700 Kazakhs).

Gene name	Chromosome	SNP Identifier	Position	Hardy-Weinberg equilibrium		
				H_o	H_e	P
1	2	3	4	5		
Coagulation and cardiovascular systems genes						
MTR A2756G	1	rs1805087	7870973	0.311	0.306	0.712
MTHFR A1298C	1	rs1801131	11854476	0.394	0.446	0.002
MTHFR C677T	1	rs1801133	11856378	0.394	0.418	0.147
MTRR A66G	1	rs1801394	237048500	0.496	0.49763	0.940
F5 A506G	1	rs6025	169519049	0.017	0.020	0.064
F2 G20210A	11	rs1799963	46761055	0.004	0.004	1.0
AGTR1 A1166C	3	rs5186	148742201	0.239	0.319	<0.0001
FGB G455A	4	rs4220	155491759	0.239	0.248	0.289
GPLaC807T	5	rs1126643	52347369	0.421	0.442	0.231
PLANH1 5G/4G	7	rs7242	100781445	0.481	0.500	0.326
ITGB3 Leu33Pro	17	rs5918	47283364	0.141	0.148	0.202
ACE I/D	17	rs4340	61565892	0.456	0.488	0.088
eNOS Glu298Asp	7	rs1799983	46761055	0.489	0.475	0.475
H_o – observed heterozygosity, H_e – expected heterozygosity; P – statistical significance						

Comparative analysis of the studied polymorphisms frequencies showed that the Kazakh population occupies an intermediate position between Europe and Asia populations described in the project 1000 genomes in terms of the most polymorphic loci frequencies of coagulation and cardiovascular systems 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).

Discussion

The discussed question is the legitimacy of the studied genetic polymorphisms choice based on a 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. Violations of this process at all stages can lead to abortion, which influenced the choice of specific maternal genomic polymorphisms involved in implantation and placentation processes, its include genes for hemostatic system proteins, platelet receptors, proteins involved in the pathogenesis of endothelial dysfunction, in blood pressure regulation [22,23,25].

According to the literature, genes are most fully described as associated with developing immunotolerance and inflammation, as well as changes in maternal metabolism and blood coagulation. Almost 90 different gene polymorphisms were studied, most of which showed a low relationship with the development of iRPL, some significant polymorphisms were not confirmed or showed contradictory results in subsequent replicative studies in other populations [1,12,15,15].

Thrombophilia makes a significant contribution to iRPL predisposition [13,19] due to thrombocytosis, increased platelet aggregation, level of blood coagulation factors activity, due to fibrinolytic inhibitors excess [4]. The most significant for increasing the genetic risk of thrombophilia in the genesis of RPL are FV mutations – Leiden factor (G1691A), prothrombin FII G20210A gene, variants of the MTHFR C667T genotypes encoding the enzyme methylenetetrahydrofolate reductase with low activity [22,23,25] and tissue plasminogen activator inhibitor type I PAI-1 4G/5G [7,26]. However, these associations may not be observed with iRPL, which requires further study of new predisposing factors, including other blood coagulation and cardiovascular systems genes [6,23,25]. However, numerous studies linking these genes with the development of iRPL are very controversial [4, 4,19,23,26].

It should be noted that the literature data are contradictory on coagulation and cardiovascular systems genes possibly associated with iRPL, due to several objective reasons: the lack of clear definitions of iRPL, complexity of recruiting, therefore the small sample size; lack of replicative studies in ethnically homogeneous populations [1,4,5,6,10,14,19,24].

Outcome

For the first time, the population features of genotypes and alleles distribution frequency of coagulation and cardiovascular systems genes were studied, showing a high genetic heterogeneity in the population group of Kazakhs, numbering 700 people. The obtained results reflect the characteristics of the Kazakh population structure, formed as a result of complex evolutionary and migration processes due to the mid-geographical location between populations of Asia and Europe.

We expected that the Kazakh population does not differ in frequency of minor alleles polymorphisms of FII and FV genes from previously studied world populations, which is due to their influence on the vital functions of blood coagulation and reflects their involvement in natural

selection processes. The highest allelic frequencies of unfavorable alleles polymorphisms were found for PLANH1, MTRR, and ACE genes (49.6%; 46.4%, and 42.2%, respectively).

iRPL is a stressful state for a married couple who cannot obtain information about the cause of miscarriages and, accordingly, are deprived of effective etiopathogenetic therapy. Due to the high frequency and lack of reliable data on iRPL, it becomes necessary to find genetic markers to predict the development of the disease. It is necessary to conduct a replicative study in an ethnically homogeneous population of Kazakhs with clear recruiting criteria, selection of etiopathogenetic polymorphisms of iRPL.

Ethical endorsement

All procedures conducted in studies involving people comply with the standards of the bioethical committee of the Scientific Center of Obstetrics, Gynecology, and Perinatology (Almaty, Kazakhstan) with the Ethical and Helsinki Declaration of 1964 and its subsequent amendments or comparable ethical standards.

Conflict of interest: *No conflict of interest is declared.*

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Literature:

1. *Беспалова О.Н.* Генетика невынашивания беременности // Журнал акушерства и женских болезней. 2007. LVI (1). С. 81-95. ISSN 1684–0461

2. *Ведищев С.И., Прокопов А.Ю., Жабина Ю.В., Османов Э.М.* Современные представления о причинах невынашивания беременности // Вестник ТГУ. 2013. 18(4). С. 1309-1312. ISSN 1810-0198.

3. *Мамедалиева Н.М.* Достижения и перспективы решения проблемы невынашивания беременности в Казахстане // Актуальные вопросы акушерства, гинекологии и перинатологии. 2012. 2(3). С. 123-1303.

4. *Al-Astal M.G., Sharif F.A.* Beta-fibrinogen (-455 G/A) and Integrin beta-3 (PLA1/A2) polymorphisms and recurrent pregnancy loss in Gaza strip-Palestine // International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2014. 3(1). P.134-138. DOI: 10.5455/2320-1770.ijrcog20140326

5. *Al-Mukaynizi F.B., AlKhuriji A., Babay Z., Addar M., AlDaihan S., Alanazi M., Warsy A.S.* Lack of Association between Angiotensin Converting Enzyme I/D Polymorphism and Unexplained Recurrent Miscarriage in Saudi Arabia // J. Med. Biochem. 2016. 35(2). P, 166–173 doi:10.1515/jomb-2015-0020

6. *Arias-Sosa L.A., Acosta I.D., Lucena-Quevedo E., Moreno-Ortiz H., Esteban-Pérez C., Forero-Castro M.* Genetic and epigenetic variations associated with idiopathic recurrent pregnancy loss // Journal Assisted Reproduction and Genetics. 2018. 35(3). P.355-366. doi:10.1007/s10815-017-1108-y

7. *Barlik M., Seremak-Mrozikiewicz A., Drews K., Klejewski A., Kurzawińska G., Łowicki Z., Wolski H.* Correlation between factor VII and PAI-1 genetic variants and recurrent miscarriage // Ginekologia Polska Journal. 2016. 87(7). P.504-509. doi: 10.5603/GP.2016.0034.

8. Branch DW, Gibson M, Silver RM. Clinical practice. Recurrent miscarriage. // N Engl J Med. 2010. 363(18). P. 740-7. doi: 10.1056/NEJMcp1005330.

9. *Christiansen O.B., Mathiesen O., Lauritsen J.G., Grunnet N.* Idiopathic recurrent spontaneous abortion. Evidence of a familial predisposition // Acta. Obstet. Gynecol. Scand. 1990. 69 (7-8). P.597–601. PMID:2094140 DOI:10.3109/00016349009028702

10. *Daher S., Mattar R., Gueuvoghlanian-Silva B.Y., Torloni M.R.* Genetic polymorphisms and recurrent spontaneous abortions: an overview of current knowledge // Am. J. Reprod. Immunol. 2012. 67(4). P.341-347. doi: 10.1111/j.1600-0897.2012.01123.x.

11. *Jivraj S., Anstie B., Cheong Y.-C., Fairlie F.M., Laird S.M., Li T.C.* Obstetric and neonatal outcome in women with a history of recurrent miscarriage: a cohort study // Hum Reprod. 2001. 16(1). P.102–106 doi: 10.1093/humrep/16.1.102.

12. *Hirschhorn J.N., Gajdos Z.K.* Genome-wide association studies: results from the first few years and potential implications for clinical medicine // Annual review of medicine. 2011. 62(1). P.11–24. DOI: 10.1146/annurev.med.091708.162036

13. *Lee G.S., Park J.C., Rhee J.H., Kim J.I.* Etiologic characteristics and index pregnancy outcomes of recurrent pregnancy losses in Korean women // Obstetrics and Gynecology Science. 2016. 59(5). P.379-387. doi: 10.5468/ogs.2016.59.5.379

14. *López-Jiménez J.J., Porras-Dorantes Á., Juárez-Vázquez C.I., García-Ortiz J.E., Fuentes-Chávez C.A., Lara-Navarro I.J., Jaloma-Cruz A.R.* Molecular thrombophilic profile in Mexican patients with idiopathic recurrent pregnancy loss // Genetics and Molecular Research. 2016. 15(4). doi: 10.4238/gmr.15048728.

15. *Manning A.K., Hivert M.F., Scott R.A., Grimsby J.L., Bouatia-Naji N., Chen H., et.al.* A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance // Nature genetics. 2012. 44. P.659–669. DOI: 10.1038/ng.2274

16. *McCarthy M.I., Hirschhorn J.N.* Genome-wide association studies: past, present and future // Human molecular genetics. 2008. 17(R2). P.100–101. doi: 10.1093/hmg/ddn298.

17. *Ogasawara M., Aoki K., Okada S., Suzumori K.* Embryonic karyotype of abortuses in relation to the number of previous miscarriages // Fertil. Steril 2000. 73(2). P.300–304. DOI:10.1016/s0015-0282(99)00495-1

18. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion // Fertility and Sterility 2013. 99(1). P.63. doi: 10.1016/j.fertnstert.2012.09.023

19. *Pereza N., Ostojić S., Kapović M., Peterlin B.* Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion // Fertility and Sterility. 2017. 107(1). P.150-159.e2. doi: 10.1016/j.fertnstert.2016.10.007.

20. *Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A.R, Bender D. et. al.* PLINK: a tool set for whole-genome association and population-based linkage analyses // Am J Hum Genet 2007. 81(3). P.559-75. doi: 10,1086/519795

21. *Rai R., Regan L.* Recurrent miscarriage // Lancet 2006. 368 (9535). P.601–11. DOI: 10.1016/S0140-6736(06)69204-0

22. *Rodger M.A., Betancourt M.T., Clark P., Lindqvist P.G., Dizon-Townson D., Said J. et.al.* The association of factor V Leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: a systematic review and meta-analysis of prospective cohort studies // PLoS Med. 2010. 7(6): e1000292. doi: 10.1371/journal.pmed.1000292.

23. *Silver R.M., Zhao Y., Spong C.Y., Sibai B., Wendel G.Jr., Wenstrom K. et. al.* Prothrombin gene G20210A mutation and obstetric complications // Obstet. Gynecol. 2010. 115(1). P.14-20. doi: 10.1097/AOG.0b013e3181c88918.

24. *Su M.T., Lin S.H., Chen Y.C.* Genetic association studies of angiogenesis- and vasoconstriction-related genes in women with recurrent pregnancy loss: a systematic review and meta-analysis // Hum. Reprod. Update. 2011. 17(6). P.803–812. doi: 10.1093/humupd/dmr027. PMID: 21642294

25. *Toth B., Vocke F., Rogenhofer N., Friese K., Thaler C.J., Lohse P.* Paternal thrombophilic gene mutations are not associated with recurrent miscarriage // Am. J. Reprod. Immunol. 2008. 60(4). P.325-332. doi: 10.1111/j.1600-0897.2008.00630.x.

26. *Wolski H., Barlik M., Drews K., Klejewski A., Kurzawińska G., Marcin Ożarowski M. et.al.* Contribution of inherited thrombophilia to recurrent miscarriage in the Polish population // Ginekology Polsky Journal. 2017. 88(7). P. 385-392. doi: 10.5603/GP.a2017.0072.

27. World Health Organization (WHO) URL: https://www.who.int/data/gho/data/themes/maternal-and-reproductive-health [Electronic resource] (accessed 16.10.2019).

References: [1-3]

1. Bespalova O.N. Genetika nevy`nashivaniya beremennosti [Genetics of miscarriage]. *Zhurnal akusherstva i zhenskikh boleznei* [Journal of obstetrics and women's diseases]. 2007. LVI (1). pp. 81-95. ISSN 1684–0461 [in Russian]

2. Vedishhev S.I., Prokopov A.Yu., Zhabina Yu.V., Osmanov E`.M. Sovremenny`e predstavleniya o prichinakh nevy`nashivaniya beremennosti [Modern ideas about the causes of miscarriage]. Vestnik Tomskogo Gosudarstvennogo universiteta [Bulletin of Tomsk State University]. 2013. 18(4). pp. 1309-1312. ISSN 1810-0198. [in Russian]

3. Mamedalieva NM. Dostizheniya i perspektivy` resheniya problemy` nevy`nashivaniya beremennosti v Kazakhstane [*Achievements and prospects for solving the problem of miscarriage in Kazakhstan*]. *Aktual`ny`e voprosy` akusherstva, ginekologii i perinatologii* [*Topical issues of obstetrics, gynecology and perinatology*]. 2012. 2(3). pp. 123-1303. [in Russian]

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