Original Article

Genes of Inflammation and Placental Function GWAS Associated with Idiopathic Recurrent Miscarriage in the Kazakh Population

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Abstract

Background: The loss of two or more pregnancies is considered recurrent miscarriage (RM). One of the causes of this pathology is the occurrence of mutations both in pleiotropic and pathway-specific regulators and in structural genes. The simplest type of such mutations is single nucleotide polymorphisms. **Aims:** The aim of the study is to study the relationship between gene polymorphisms of anti- and pro-inflammatory cytokines – interferon-gamma (T874A), interleukin (IL1B) (C3954T), IL6 (G572C) and IL10 (G1082A); placental function, apoptosis and angiogenesis – apolipoprotein C-III (APOC3) (G5163C), kinase insert domain receptor (A1719T, G1192A), P53 (Arg72Pro) and signal transducer and activator of transcription 3 (STAT3) (C1697G) with the development of idiopathic RM (iRM) in the Kazakh population. **Settings and Design:** This was a case–control study. **Materials and Methods:** Molecular genetic studies were performed by TaqMan using a single site-specific amplification and real-time genotyping method in 302 women with iRM and 300 with normal reproduction. DNA isolation from the biomaterial was carried out using kits containing binding magnetic particles. Both samples were analysed for alleles and genotypes for the studied polymorphisms. **Statistical Analysis Used:** For statistical data processing, Pearson's criterion, confidence interval (CI) and probability value were taken into account. **Results:** It was found that the carriage of unfavourable genotypes (G/C, C/C) for the G5163C polymorphism of the APOC3 gene increases the risk of developing iRM by three times (odds ratio = 3.0; 95% CI = 2.24–4.07). Other studied polymorphisms in the genes of ILs, interferon, P53 proapoptotic protein, kinase domain receptor and STAT3 transcription activator were not associated with RM. **Conclusion:** Significant associations of APOC3 gene genotypes with the development of iRM in the Kazakh population indicate the involvement of the placental system, which is realised by vascularisation defects and defective embryo implantation and leads to early pregnancy termination.

Keywords: *Angiogenesis, apoptosis, Genetics, genotype, pathology*

Introduction

According to the American College of Obstetricians and Gynecologists and the American Society for Reproductive Medicine Practice Committee, in almost 45%–50% of women, it is not possible to determine the cause of spontaneous miscarriage, and they form the group of 'unexplained' or idiopathic miscarriage. Usually, recurrent miscarriage (RM), that is, the termination of three or more pregnancies in a row, has a multiple aetiology. The diagnosis of idiopathic

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RM (iRM) is established in the presence of appropriate criteria for RM after the exclusion of genetic, anatomical, endocrine, immunological and haemostatic factors. The frequency of iRM in the population of women with RM is 50%–75%.[1]

A favourable outcome of pregnancy depends on the immunological maternal tolerance to the tissues of the embryo and the spectrum, and the level of cytokines produced, which favourably affects the development of the placenta and foetal growth. A T helper (Th1)/Th2 paradigm has been proposed to describe altered inflammatory events associated with early pregnancy, in which a Th1-type response mediated by interleukin-2 (IL2), IL12 and interferon-γ is considered essential for early implantation, while further progression of pregnancy depends on the contribution of regulatory or anti-inflammatory Th2-type cytokines, in particular IL10.[2] It has been shown that during normal pregnancy, there is an increased production of Th2 cytokines, whereas pregnancies with unfavourable outcomes are usually associated with a high content of Th1 cytokines. Clearly, during a normal pregnancy, an adequate balance of pro-inflammatory and anti-inflammatory cytokines is critical for a successful pregnancy. Higher levels of pro-inflammatory cytokines such as interferon gamma (IFNG), IL1B and IL6 have been shown to lead to pregnancy loss and anti-inflammatory cytokines such as IL10 and transforming growth factor (TNF-β) are considered essential for pregnancy progression.[3]

IL10 is an important immune inhibitory and anti-inflammatory molecule that inhibits T-cell proliferation, selectively suppresses Th1-mediated cellular responses and plays an important role in Th2-dependent immune responses.[4,5] A number of studies have been published, in which a significant correlation of polymorphisms of the IL10 gene with the level of cytokine production and the pathogenesis of recurrent pregnancy loss (RPL) was found. IL10, produced by cytotrophoblasts and decidual T-cells, protects the embryonic-placental border by reducing the secretion of cytokines by Th1 cells and macrophages. The relationship of IL10 gene polymorphisms with RM has been studied by many researchers: Finnish researchers were unable to determine the relationship between the G>A polymorphism in the promoter (−1082) and the risk of RM. A more systematic study of the IL10 gene promoter analysed together with the −592C/A, 819C/T and −1082A/G polymorphisms in strong LD linkage, in 350 women with RM and 200 with normal reproduction, found a significant contribution of −592A/819T/1082A haplotype into RM development (odds ratio [OR]: 4.01, 95% confidence intervals [CIs]: 1.83–7.95).[6,7]

IL6 is a multifunctional cytokine that is involved in inflammatory responses and T-cell differentiation. IL6, which is widely present in female reproductive organs and gestational tissues, plays a particularly important role in embryo implantation and placental development.[8] The protective effect of carrying GG genotypes at −634C>G IL6 promoter polymorphism (rs1800796) was found in the Japanese population, which was confirmed in the Chinese sample for the heterozygous (CG) genotype (OR: 0.61; 95% CI: 0.493–0.765; $P < 0.001$) and mutant (GG) genotype (OR: 0.41; 95% CI: 0.251–0.684; $P = 0.001$). Studies of the relationship between RM and polymorphisms of the genes of individual cytokines with the risk of miscarriage in Chinese found a significant contribution for the mutant (CC) genotype of the IL1B gene (OR: 1.38; 95% CI: 1.039–1.824; $P = 0.026$) in the development of RM. IL1B-511 T>C polymorphism may be an important risk factor for RM.[9]

The aim of the study was to study the relationship between gene polymorphisms of anti- and pro-inflammatory cytokines – IFNG (T874A), IL1B (C3954T), IL6 (G572C) and IL10 (G1082A); placental function, apoptosis and angiogenesis – apolipoprotein C-III (APOC3) (G5163C), kinase insert domain receptor (KDR) (A1719T, G1192A), P53 (Arg72Pro) and signal transducer and activator of transcription 3 (STAT3) (C1697G) with the development of iRM in the Kazakh population.

Materials and Methods

The study was conducted by a prospective case–control method in the Polyclinic Department of the Scientific Centre of Obstetrics, Gynecology and Perinatology, the 'Centre for Molecular Medicine' medical centre. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All participants in the study gave informed consent to the use of their blood samples and anamnestic data; permission from the ethics committee of the Scientific Centre of Obstetrics, Gynecology and Perinatology to conduct these studies was received. The main group with iRM consisted of 302 women of Kazakh nationality, age 18–45 years and who had two or more miscarriages before 12 weeks of gestation. The control group is represented by 300 Kazakh women with normal reproductive function, having at least one child, without indications of spontaneous miscarriages. The recruiting criteria for the main group with iRM included: belonging to the Kazakh nationality through maternal and paternal

grandparents, age 18–45 years, the presence of 2 or earlier spontaneous miscarriages and the presence of pregnancies were confirmed by ultrasound data and pregnancy hormones.

Criteria for exclusion from the project

luteal phase disorders in the results of endometrial biopsy, anatomical anomalies of the uterus, diagnosed by hysterosalpingography, hysteroscopy or sonohysteroscopy, carriage of balanced chromosomal abnormalities by karyotyping of both spouses, the presence of antiphospholipid syndrome, confirmed by the analysis of anti-beta2-glycoprotein I (IgGorIgM) antibodies, anti-cardiolipin (IgGorIgM) antibodies, 'lupus' anticoagulant; multiple pregnancies confirmed by ultrasound, the presence of sexually transmitted infections confirmed by two different analyses of various biological materials (enzyme-linked immunosorbent assay, polymerase chain reaction [PCR]), dysfunction of the thyroid gland by analysis of thyroid-stimulating hormone (TSH) and thyroid antibodies. DNA extraction was performed by separation of magnetic polyvinyl alcohol (M-PVA) magnetic particles on a Prepito automated analyser (PerkinElmer) for nucleic acid isolation Chemagic Prepito using the Prepito DNA Cyto Pure reagent kit. Molecular genetic studies were carried out by TaqMan by the method of a single site-specific amplification and real-time genotyping (real-time PCR) using test systems for molecular genetic studies. Statistical significance tests and χ^2 analysis were performed using PLINK, STATA13 software. Differences in allelic and genotypic frequencies were assessed using the χ^2 test with OR (OR: 95% CI).

Molecular genetic studies were performed using TaqMan using a single site-specific amplification and real-time genotyping method in 302 women with iRM and 300 with normal reproduction. DNA isolation from the biomaterial was carried out using kits containing binding magnetic particles. Both samples were analysed for alleles and genotypes for the studied polymorphisms. For statistical data processing, Pearson's criterion, CI and probability value were taken into account. It was found that the carriage of unfavourable genotypes (G/C, C/C) for the G5163C polymorphism of the APOC3 gene increases the risk of developing iRM by three times (OR = 3.0; 95% CI = 2.24–4.07). Other studied polymorphisms in the genes of ILs, interferon, p53 proapoptotic protein, kinase domain receptor and STAT3 transcription activator were not associated with RM. Significant associations of APOC3 gene genotypes with the development of iRM in the Kazakh population indicate the involvement of the placental system, which is realised by vascularisation defects, defective embryo implantation and leads to early pregnancy termination.

Results

Comparative analysis of allelic and genotypic frequencies in the main group with iRM and the control group with normal reproduction is presented in Table 1.

As can be seen from Table 1, the frequencies of alleles and genotypes in the compared groups did not differ significantly ($P > 0.05$), with the exception of the allelic and genotypic frequencies of the APOC3 (rs5128) G5163C gene polymorphism. Statistical analysis using PLINK involves calculating associations based on various models. Table 2 presents the results of a comparative analysis of the associations of the studied polymorphisms with iRM, based on the use of several models at a significance threshold of $P = 0.05$ for multiple tests performed on the sample data, including genotypic, additive, allelic, dominant and recessive models.

Of particular note are the frequencies of affected – the main group of patients with iRM, UNAFF (control group) for each test. The TEST column explains which test is used (GENO – basic genotype, TREND – additive test, DOM – dominant test and REC – recessive test). The calculation of associations based on various models, based on [Table 2], showed the most significant associations of iPNP, which imply a specific relationship between genotype and phenotype, including statistically highly significant differences in genotypic, additive (trend), general recessive and dominant models also for the placental function gene APOC3 (rs5128, G5163C) ($P < 0.00001$).

The absence of statistically significant differences in the compared groups by genes was because the clinical diagnosis of iRM excludes the presence of antiphospholipid syndrome, confirmed by the analysis of anti-beta2-glycoprotein I (IgGorIgM) antibodies, anticardiolipin (IgGorIgM) antibodies and 'lupus' anticoagulant were the criteria for exclusion from recruiting, and the results obtained confirm the 'purity' and high quality of recruiting of women with iRM in the analysed sample. The choice of cytokine polymorphisms IL1B T3954C, IL6 C634G, IL10 A1082G and IFNG A874T for the study was due to the large contribution of Th1 cytokines to the development of RM, their highly reliable values according to the results published by GWAS.[9]

IL10, which exhibits dual (inhibitory and stimulatory) immunological functions and therefore cannot be unambiguously classified as Th1 or Th2, was included to study the contribution of anti-inflammatory cytokines to the development of iRM. As evidenced by the materials in Table 1, there was no significant association of the four

Table 1: Frequencies of alleles and genotypes in the main group with idiopathic recurrent miscarriage and the control group

SNP polymorphism Allele/genotype	Absolute number (frequency)		χ^2 (*P*)	OR (95% CI)
	Main group	Control group		
Genes of the anti-inflammatory system				
IFNG (rs2069727) T874A				
T	472 (0.78)	450 (0.75)	1.18 (>0.05)	0.84 (0.64–1.1)
A	132 (0.22)	150 (0.25)		
TT	181 (0.6)	168 (0.56)		
AT	110 (0.36)	117 (0.39)		
AA	11 (0.04)	15 (0.05)		
IL1B (rs1143634) C3954T				
C	496 (0.82)	474 (0.79)	2.06 (>0.05)	0.82 (0.62–1.09)
T	108 (0.18)	126 (0.21)		
CC	203 (0.67)	184 (0.61)		
CT	89 (0.29)	104 (0.35)		
TT	10 (0.03)	12 (0.04)		
IL6 (rs1800796) G572C				
G	382 (0.63)	376 (0.63)	0.07 (>0.05)	0.98 (0.77–1.23)
C	222 (0.37)	224 (0.37)		
GG	126 (0.42)	118 (0.39)		
GC	131 (0.43)	140 (0.47)		
CC	45 (0.15)	42 (0.14)		
IL10 (rs1800896) G1082A				
G	468 (0.77)	458 (0.76)	0.16 (>0.05)	0.94 (0.72–1.23)
A	136 (0.23)	142 (0.24)		
GG	180 (0.6)	180 (0.6)		
AG	107 (0.35)	98 (0.33)		
AA	15 (0.05)	22 (0.07)		
Genes for placental function				
KDR (rs1870377) A1719T				
T	446 (0.74)	428 (0.71)	0.95 (>0.05)	0.88 (0.68–1.14)
A	158 (0.26)	172 (0.29)		
TT	163 (0.54)	151 (0.5)		
AT	120 (0.4)	126 (0.42)		
AA	19 (0.06)	23 (0.08)		
KDR (rs2305948) G1192A				
G	538 (0.89)	536 (0.89)	0.02 (>0.05)	1.02 (0.71–1.48)
A	66 (0.11)	64 (0.11)		
GG	238 (0.79)	238 (0.79)		
AG	61 (0.2)	59 (0.2)		
AA	3 (0.01)	3 (0.01)		
APOC3 (rs5128) G5163C				
G	424 (0.70)	526 (0.88)	53.1 (<0.001*)	3.02 (2.24–4.07)
C	180 (0.3)	74 (0.13)		
GG	143 (0.47)	230 (0.77)		
GC	139 (0.46)	65 (0.22)		
CC	20 (0.07)	5 (0.02)		
P53 (rs1042522) Arg72Pro				
A	432 (0.71)	436 (0.73)	0.19 (>0.05)	1.06 (0.82–1.36)
P	172 (0.29)	164 (0.28)		
AA	154 (0.51)	158 (0.52)		
AP	123 (0.41)	119 (0.4)		
PP	25 (0.08)	23 (0.08)		

Contd...

Table 1: Contd...

SNP polymorphism Allele/genotype	Absolute number (frequency)		χ^2 (*P*)	OR (95% CI)
	Main group	Control group		
Genes for placental function				
STAT3 (rs1053023) C1697G				
C	438 (0.73)	448 (0.75)	0.61 (>0.05)	1.11 (0.86–1.44)
G	166 (0.27)	152 (0.25)		
CC	163 (0.54)	168 (0.56)		
CG	113 (0.37)	112 (0.37)		
GG	26 (0.09)	20 (0.07)		

*Significant differences $P<0.05$ were revealed. OR=Odds ratio, CI=Confidence interval, INFG=Interferon gamma, IL=Interleukin, KDR=Kinase insert domain receptor, APCO3=Apolipoprotein C-III, P53=Protein 53, STAT3=Signal transducer and activator of transcription 3

studied polymorphisms of cytokine genes with the risk of developing iRM in the Kazakh population ($P > 0.05$), which is confirmed by low values of OR = 0.83–0.97, at 95% CI. Although the polymorphisms of the pro- and anti-inflammatory cytokine systems included in this work have been previously investigated due to their association with the risk of RM in a number of populations, the results do not contradict the scientific literature and are consistent with previously published studies that reject this contribution.[10]

As can be seen from Table 2, the possible contribution of the placental function genes KDR (rs2305948, G1192A), KDR (rs1870377, A1719AT), STAT3 (rs1053023, C1697G), eNOS3 (rs1799983, Glu298Asp), P53 (rs1042522, Arg72Pro), and APOC3 (rs5128, G5163C) in the development of iRM in the Kazakh population. The choice of these genes is due to their important role in the regulation of implantation, foetal development and full-fledged placental circulation, as well as a significant contribution to the aetiopathogenesis of RM according to the results of GWAS analyses. In the available scientific literature, studies of the association of these polymorphisms with iRM according to the results of GWAS have not been published. There were no significant differences in allelic and genotypic frequencies of KDR (rs2305948, G1192A), KDR (rs1870377, A1719T), STAT3 (rs1053023, C1697G) and P53 (rs1042522, Arg72Pro) polymorphisms between patients with iRM and control women ($P > 0.05$).

As evidenced by the materials of Table 1, highly significant differences in the frequency of alleles and genotypes in the iRM group and the control group were obtained for the rs5128 G5163C polymorphism of the APOC3 placental function gene (apolipoprotein C). The frequency of the unfavourable C allele in the iRM group was 0.30, which significantly exceeded the similar indicators in the group of women with normal reproduction –0.13 ($\chi^2 = 53.1$; $P < 0.001$). Statistically significant differences were obtained in a higher frequency of the homozygous unfavourable CC genotype in the main group –0.07, whereas in the control group, it was three times less common –0.02 ($P < 0.001$). Table 1 also indicates that the homozygous CC genotype of the APOC3 gene increased the risk of idiopathic RPL by three times (OR = 3.02; 95% CI: 2.24–4.07; $P < 0.001$), which suggests that the rs5128 G5163C polymorphism of the apolipoprotein APOC3 gene may be associated with the risk of developing iRM.

Discussion

Single-nucleotide polymorphism (SNP) IL17A correlates with iRM. In this study, SNPs were determined by PCR followed by restriction analysis (PCR-RFLP, EcoNI endonuclease).[11] In women with miscarriage and in the control group, a statistically significant difference ($P = 0.001$) was found between the frequency of GG, GA and AA genotypes, although the difference in allelic frequency was not significant. At the same time, the IL17A gene polymorphism did not affect its expression. In particular, in heterozygotes of the AG genotype, the risk of miscarriage decreased by 1.5 times, and in the homozygote of the AA genotype, it increased by 6 times. At the same time, the potential risk of foetal loss increased in the recessive model (AA vs. GA + GG) and significantly decreased in the homozygous model (GG vs. AA). Obviously, SNP plays a potential protective role for pregnancy in the AA genotype and poses a threat of pregnancy failure for the genotype (GA + GG). IL33 plays a significant role in the processes of rejection since its content in the blood increases significantly in cases of miscarriage. The IL33 gene is localised on the short arm of chromosome 9 (9p24.1). It belongs to the IL1 family of cytokines and activates T-helper-2 and mast cells.[12] Kamrani *et al*. showed a correlation between SNPs in the IL33 gene and RM.[13] In particular, an increased frequency of occurrence of the G allele and the GA genotype in the rs16924159 gene was annotated amongst individuals with iRM.

In chorionic samples of patients with repeated pregnancy loss, overexpression of the proapoptotic FAS and

Table 2: Comparative analysis of the associations of the studied polymorphisms

Gene name	Chromosome	SNP	Position	Test	AFF	UNAFF	χ^2	Df	*P*
Genes of the anti-inflammatory system									
IFNG T874A	12	rs2069727	68548223	GENO	11/110/181	15/117/168	1.309	2.0	0.5197
IFNG T874A	12	rs2069727	68548223	TREND	132/472	147/453	1.258	1.0	0.2621
IFNG T874A	12	rs2069727	68548223	ALLELIC	132/472	147/453	1.183	1.0	0.2767
IFNG T874A	12	rs2069727	68548223	DOM	121/181	132/168	0.9559	1.0	0.3282
IFNG T874A	12	rs2069727	68548223	REC	11/291	15/285	0.6712	1.0	0.4126
IL1B C3954T	2	rs1143634	113590390	GENO	10/89/203	12/104/184	2.274	2.0	0.3208
IL1B C3954T	2	rs1143634	113590390	TREND	109/495	128/472	2.086	1.0	0.1486
IL1B C3954T	2	rs1143634	113590390	ALLELIC	109/495	128/472	2.057	1.0	0.1515
IL1B C3954T	2	rs1143634	113590390	DOM	99/203	116/184	2.27	1.0	0.1319
IL1B C3954T	2	rs1143634	113590390	REC	10/292	12/288	0.2028	1.0	0.6525
IL6 G572C	7	rs1800796	22766246	GENO	45/131/126	42/140/118	0.658	2.0	0.7196
IL6 G572C	7	rs1800796	22766246	TREND	221/383	224/376	0.06915	1.0	0.7926
IL6 G572C	7	rs1800796	22766246	ALLELIC	221/383	224/376	0.0715	1.0	0.7892
IL6 G572C	7	rs1800796	22766246	DOM	176/126	182/118	0.3562	1.0	0.5506
IL6 G572C	7	rs1800796	22766246	REC	45/257	42/258	0.09875	1.0	0.7533
IL10 G1082A	1	rs1800896	206946897	GENO	15/107/180	22/98/180	1.713	2.0	0.4247
IL10 G1082A	1	rs1800896	206946897	TREND	137/467	142/458	0.157	1.0	0.6919
IL10 G1082A	1	rs1800896	206946897	ALLELIC	137/467	142/458	0.1639	1.0	0.6856
IL10 G1082A	1	rs1800896	206946897	DOM	122/180	120/180	0.009885	1.0	0.9208
IL10 G1082A	1	rs1800896	206946897	REC	15/287	22/278	1.461	1.0	0.2268
Genes for placental function									
KDR A1719T	4	rs1870377	55972974	GENO	19/120/163	23/126/151	0.9793	2.0	0.6129
KDR A1719T	4	rs1870377	55972974	TREND	158/446	172/428	0.9777	1.0	0.3228
KDR A1719T	4	rs1870377	55972974	ALLELIC	158/446	172/428	0.9514	1.0	0.3294
KDR A1719T	4	rs1870377	55972974	DOM	139/163	149/151	0.7992	1.0	0.3713
KDR A1719T	4	rs1870377	55972974	REC	19/283	23/277	0.4386	1.0	0.5078
KDR G1192A	4	rs2305948	55979558	GENO	3/61/238	3/59/238	0.021	2.0	0.886
KDR G1192A	4	rs2305948	55979558	TREND	67/537	65/535	0.02119	1.0	0.8843
KDR G1192A	4	rs2305948	55979558	ALLELIC	67/537	65/535	0.02075	1.0	0.8855
KDR G1192A	4	rs2305948	55979558	DOM	64/238	62/238	0.025	1.0	0.875
KDR G1192A	4	rs2305948	55979558	REC	3/299	3/297	0	1.0	0.994
APOC3 G5163C	11	rs5128	116832924	GENO	20/139/143	5/65/230	56.13	2.0	6.481E-13
APOC3 G5163C	11	rs5128	116832924	TREND	179/425	75/525	54.06	1.0	1.941E-13
APOC3 G5163C	11	rs5128	116832924	ALLELIC	179/425	75/525	53.1	1.0	3.176E-13
APOC3 G5163C	11	rs5128	116832924	DOM	159/143	70/230	54.88	1.0	1.284E-13
APOC3 G5163C	11	rs5128	116832924	REC	20/282	5/295	9.286	1.0	0.002309
P53 Arg72Pro	17	rs1042522	7579472	GENO	25/123/154	23/119/158	0.1941	2.0	0.9075
P53 Arg72Pro	17	rs1042522	7579472	TREND	173/431	165/435	0.1937	1.0	0.6599
P53 Arg72Pro	17	rs1042522	7579472	ALLELIC	173/431	165/435	0.1945	1.0	0.6592
P53 Arg72Pro	17	rs1042522	7579472	DOM	148/154	142/158	0.1688	1.0	0.6812
P53 Arg72Pro	17	rs1042522	7579472	REC	25/277	23/277	0.07669	1.0	0.7818
STAT3 C1697G	17	rs1053023	40466092	GENO	26/113/163	20/112/168	0.8559	2.0	0.6518
STAT3 C1697G	17	rs1053023	40466092	TREND	165/439	152/448	0.5896	1.0	0.4426
STAT3 C1697G	17	rs1053023	40466092	ALLELIC	165/439	152/448	0.6112	1.0	0.4344
STAT3 C1697G	17	rs1053023	40466092	DOM	139/163	132/168	0.2497	1.0	0.6173
STAT3 C1697G	17	rs1053023	40466092	REC	26/276	20/280	0.8048	1.0	0.3697

INFG=Interferon gamma, IL=Interleukin, KDR=Kinase insert domain receptor, APCO3=Apolipoprotein C-III, P53=Protein 53, STAT3=Signal transducer and activator of transcription 3, SNP=Single-nucleotide polymorphism, AFF=Affected, UNAFF=Unaffected

FAS-L genes was established, but no changes in BCL-2 expression were detected. The SNP FAS-L-844T/C in the promoter region changes the affinity of the promoter for the C/EBP beta enhancer protein.[14] It was shown that SNPs in the sequences of the above genes correlate with the phenomenon of miscarriage.[15] In particular, BAX-248GA is independently associated with iRM protection. The homozygous BAX-248AA

genotype is more common in women with RM. As for FAS, FAS-670A/G and FAS-1377G/A polymorphisms are associated with the probability of foetal rejection. For these SNPs, the FAS-670GG/1377AA genotype was at the highest risk of rejection. SNP FAS-L and BCL-2-938C/A did not correlate with iRM but only affected the level of messenger RNA expression.

SNPs that disrupt the normal course of pregnancy are not limited to genes of immunoregulatory functions. Peng *et al*. proved that SNPs in the genes responsible for haemostasis also affect successful gestation.[16] These include ANXA5, the product of which is annexin A5. It is a calcium-dependent placental anticoagulant protein that provides blood supply to the embryo. The lack of ANXA5 causes thrombosis in the vessels during pregnancy, so this protein is an anticoagulant. The six SNPs in the ANXA5 promoter are conventionally divided into two haplotypes: M1 and M2. The M1 haplotype includes two consecutive SNPs: T-422C, rs28651243 and A-448C, rs28717001. The M2 haplotype includes G-467A, rs112782763 and G-373A, rs113588187. In carriers of the M2 haplotype, the expression of ANXA5 is inhibited, which leads to the appearance of disorders related to thrombosis. A similar mechanism is triggered in the presence of antibodies to ANXA5 and antibodies to the phospholipid (aPl). The latter inhibits the action of annexin due to direct binding to this protein and a decrease in the expression level of its gene. For the treatment of patients with the M2 haplotype, low-molecular weight heparin is usually used.

The results of studies conducted in various populations on the association of cytokine polymorphisms of pro- and anti-inflammatory genesis with RM turned out to be few and contradictory, and iRM was practically not studied. In this regard, the results of the published meta-analyses by Azzawie deserve special attention, which can help clarify the problems of the influence of cytokine and RM polymorphisms.[17] This meta-analysis, which evaluated the effect of the TNFa, IFNG, IL1B, Il6 and IL10 genes on RM, showed that IL1B polymorphisms (−31T, OR: 2.12, CI: 1.04–4.33) in two studies and IL6 (−634G, OR: 0.22, 95% CI: 0.09–0.57) showed significant results in one study. Placental function, apoptosis and angiogenesis genes KDR (rs2305948, G1192A and rs1870377, A1719T), STAT3 (rs1053023, C1697G), P53 (rs1042522, Arg72Pro) and APOC3 (rs5128, G5163C). Normal embryonic development in early pregnancy is associated with trophoblastic proliferation and adequate angiogenesis after implantation. Dysfunction of the cytotrophoblast leads to an imbalance of cellular differentiation by disrupting angiogenesis and apoptosis and can lead to spontaneous abortion. Genes that regulate implantation and development of the foetus and placenta and are responsible for placental function are considered candidate RM genes. A good placental circulation is a key factor for the successful prolongation of pregnancy during implantation and development of embryos and foetuses.

APOC3 gene – apolipoprotein C3, polymorphism 3238C>G (rs5128) APOC3 plays an important role in lipid metabolism in the placenta and is considered a candidate gene for the development of RM. Unfavourable polymorphisms of the APOC3 and APOE genes contribute to the deposition of triglycerides in the vascular endothelium, narrowing of the vascular lumen and the formation of primary placental insufficiency and ineffective implantation. In the process of studying the causes of primary placental insufficiency, the interest of researchers was attracted by the G5163C (rs5128) polymorphism of the AROS3 gene, which is caused by a 28 nucleotide substitution of cytosine for guanine at position 5163, which leads to inhibition of lipoprotein lipase, an increase in protein expression and, as a result, an increase in the level of lipoproteins.[18] A study of modern GWAS associations has shown that APOC3 gene polymorphisms are amongst the strongest genetic determinants of plasma lipid concentrations.[19] However, a number of studies on the association of APOC3 rs5128 polymorphism and the risk of developing RM do not confirm this relationship.[20] According to the results of the analysis of GWAS and meta-studies of RM in the world databases Ensembl, 1000 genomes of quality control ($P < 5 \times 10–8$, cluster plot inspection, HWE test), statistically significant 9 SNPs of polymorphisms were selected for independent replicative genotyping in an ethnically homogeneous population of Kazakhs.

Conclusions

This study found a significant association between the APOC3 gene polymorphism rs5128 and risk of RM in Kazakh women. No associations were observed between RM and SNPs in cytokine, apoptosis or angiogenesis genes. Further research is needed to confirm the APOC3 finding in other populations and explore its biological mechanism. Additional gene variants related to inflammation, apoptosis and coagulation should also be investigated for their potential contribution to recurrent pregnancy loss.

Authors contribution

GB: conceptualization, methodology, data curation, writing-original draft preparation. AM: visualization, investigation, and supervision. GS, GU, and AS: software, validation, writing-reviewing, and editing. All authors read and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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