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Identifying key genetic markers linked to hemostatic changes in first trimester pregnancies among women with hyperproliferative uterine disorders

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Hyperproliferative uterine diseases (HUD) are a significant global health concern due to their high prevalence, potential for malignant transformation, and adverse effects on reproductive health. Women with HUD frequently exhibit a hypercoagulation state, increasing their risk of cardiovascular complications. Recent researches highlight the influence of genetic variants in folate cycle genes and hemostasis genes on hemostatic function. However, the relationship between these genetic markers and hemostatic function in women with HUD remains underexplored.
The objective: **to investigate the association between variants of folate metabolism genes and the hemostatic system in pregnant women with HUD, focusing on hemostatic parameters during the complicated course of the I trimester.**
Materials and methods**. The main group of the study included 75 pregnant women 24–46 years old with various HUD. The control group consisted of 60 women of similar age who had one or more children and did not have HUD. The following indices were studied: fibrinogen, plasma fibrin, activated partial thromboplastin time, prothrombin time, international normalized ratio, prothrombin activity, thrombocytes. Genotyping for the gene variants *MTHFR*, *MTR*, *MTRR*, *F5*, *F2*, *FGB*, *PAI-1*, *ITGA2*, and *ITGB3* was carried out for all participants.**
Results**. The TT genotype of rs1801133 in the *MTHFR* gene was significantly associated with an increased risk of HUD. Interactions between folate cycle genes modulated pregnancy risk, a lower risk was observed in the absence of gene function-reducing variants. Significant correlations were found between the following gene variants: rs1801133 of the *MTHFR* gene and prothrombin time (r_s = –0.55, p = 0.022); rs1800787 of the *FGB* gene and fibrinogen level (r_s = 0.40, p = 0.034); rs1126643 of the *ITGA2* gene and platelet level (r_s = 0.70, p = 0.037); rs5918 of the *ITGB3* gene and activated partial thromboplastin time (r_s = 0.68, p = 0.045). The connection of the variant rs1801133 of the MTHFR gene with the increased risk of complicated pregnancy, in particular the risk of threatened miscarriage in the I trimester in women with HUD was established.**
Conclusions**. Identifying genetic predisposition to hemostasis disorders in patients with HUD may serve as a theoretical basis for developing personalized prevention and treatment management.**
Keywords: *gene, FGB, hemostesiogram, hyperproliferative uterine disorders, pregnant women of reproductive age, ITGA2, ITGB3, MTHFR.*

Визначення ключових генетичних маркерів, пов'язаних зі змінами гемостазу в першому триместрі вагітності у жінок із гіперпроліферативними захворюваннями матки

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Гіперпроліферативні захворювання матки (ГПЗМ) викликають серйозне занепокоєння у сфері охорони здоров'я через їх високу поширеність, потенційну злоякісну трансформацію та несприятливий вплив на репродуктивне здоров'я. Жінки з ГПЗМ часто демонструють стан гіперкоагуляції, що підвищує ризик серцево-судинних ускладнень. Останні дослідження наголошують на впливі варіантів генів фолатного циклу та генів гемостазу на стан системи гемостазу. Однак зв'язок між цими генетичними маркерами та системою гемостазу в жінок із ГПЗМ залишається недостатньо вивченим.
Мета дослідження: дослідити зв'язок варіантів генів фолатного обміну та системи гемостазу у вагітних із гіперпроліферативними захворюваннями матки та гемостазіологічними показниками при ускладненому перебігу I триместру вагітності.
Матеріали та методи. До основної групи дослідження було залучено 75 вагітних віком 24–46 років із різними ГПЗМ. Контрольну групу становили 60 жінок відповідного віку, що мають 1 та більше дітей, без наявності ГПЗМ. Проведено визначення показників: фібриноген, фібрин плазми, активований частковий тромбопластиновий час, протромбіновий час, міжнародне нормалізоване відношення, активність протромбіну, тромбоцити. Для всіх учасників було проведено генотипування варіантів генів *MTHFR*, *MTR*, *MTRR*, *F5*, *F2*, *FGB*, *PAI-1*, *ITGA2* та *ITGB3*.
Результати. Встановлено, що генотип ТТ за варіантом rs1801133 гена *MTHFR* був асоційований із підвищеним ризиком ГПЗМ. Взаємодія між генами фолатного циклу модулювала ризик, причому менший ризик спостерігався за відсутності варіантів, що знижують функцію гена. Виявлено значущі кореляційні зв'язки між такими варіантами генів: rs1801133 гена *MTHFR* та протромбіновим часом (r_s = –0,55, p = 0,022); rs1800787 гена *FGB* та рівнем фібриногену (r_s = 0,40, p = 0,034); rs1126643 гена *ITGA2* та рівнем тромбоцитів (r_s = 0,70, p = 0,037); rs5918 гена *ITGB3* та активованим

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частковим тромбопластиновим часом (r_s = 0,68, p = 0,045). Встановлено зв'язок варіанта rs1801133 гена *MTHFR* із підвищеним ризиком ускладненої вагітності, зокрема ризиком загрози викидня у I триместрі в жінок із ГПЗМ.
Висновки. З'ясування генетичної схильності до порушень у системі гемостазу в пацієнток із ГПЗМ може стати теоретичним підґрунтям до розробки персоналізованої стратегії профілактики і лікування.
Ключові слова: *ген, FGB, гемостазіограма, гіперпроліферативні захворювання матки, вагітні жінки репродуктивного віку, ITGA2, ITGB3, MTHFR.*

The issue of hyperproliferative uterine diseases (HUD) in women is a significant concern worldwide. This relevance stems from their high prevalence, potential for malignant transformation, substantial treatment costs, and adverse effects on reproductive health, which can even lead to disability. For instance, hysterectomy for benign tumors is one of the most common gynecological procedures [1]. Research has indicated that women with HUD face an increased risk of various adverse obstetric outcomes, including complicated pregnancies, premature births, miscarriages, and infertility [2, 3]. This highlights the critical nature of addressing hyperproliferative uterine diseases from both social and economic perspectives.

It is well-established that chronic inflammation plays a significant role in the development of HUD. A variety of cytokines, chemokines, growth factors, and prostaglandins are involved in the pathogenesis of HUD [4]. Furthermore, these inflammatory mediators not only contribute to the progression of abnormal placentation but can also be produced by the hyperproliferative uterine tissue itself [4].

Inflammation is closely linked to disruptions in the blood coagulation and fibrinolytic systems. Consequently, researchers are actively investigating hemostatic markers in women with HUD to assess their potential as diagnostic indicators and to guide treatment decisions. Studies have demonstrated that women with HUD frequently exhibit a hypercoagulation state, which significantly elevates their risk of cardiovascular complications. This is particularly evident in women with conditions such as adenomyosis and uterine leiomyoma [5–11].

An individual's genetic profile plays a crucial role in determining the state of their hemostatic system. The most studied are variants of genes encoding folate cycle components (*MTHFR*, *MTRR*, *MTR*) and hemostasis genes (*F2*, *F5*, *FGB*, *PAI-1*, *ITGA2*, *ITGB3*).

The *MTHFR* gene encodes methylenetetrahydrofolate reductase, a key enzyme in the folate cycle. Among the variants of the *MTHFR* gene, the most significant are rs1801133 and rs1801131 (synonymous names – C677T and A1298C, respectively), which affect the activity of the enzyme [12]. The *MTR* gene encodes 5-methyltetrahydrofolate-homocysteine methyltransferase. The variant rs1805087 (or A2756G) can affect the regulation of homocysteine and DNA methylation [13]. The *MTRR* gene encodes another important enzyme of the folate cycle, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, which functions as an activation partner of 5-methyltetrahydrofolate-homocysteine methyltransferase [14]. According to the researchers, the presence of the rs1801394 (or A66G) variant in the *MTRR* gene is accompanied by a decrease in affinity for 5-methyltetrahydrofolate-homocysteine methyltransferase [15].

The *F5* gene encodes coagulation factor V, which is an important component of the coagulation cascade. The rs6025 (or G1601A) variant of the *F5* gene causes coagulation factor V to become resistant to activated protein C [16]. The *F2* gene encodes coagulation factor II (prothrombin), which is important for hemostasis and thrombosis. It is assumed that the rs1799963 (or G20210A) variant is associated with an increase in plasma prothrombin levels [17]. The *FGB* gene encodes the β-chain of fibrinogen, which is involved in blood clotting and thrombus formation. The variants rs1800787 and rs1800790 (synonymously referred to as C148T and G455A, respectively) are associated with increased plasma fibrinogen concentrations and may affect the characteristics of a fibrin clot [18, 19]. The *PAI-1* gene encodes_a plasminogen activator inhibitor-1, which is an important component of the hemostatic system and is an important marker in inflammatory processes. The rs1799768 (or 4G/5G) variant is associated with high serum levels of the *PAI-1* gene product [20]. The *ITGA2* gene encodes the alpha subunit of integrin α2β1 (also known as GP Ia-IIa), which is a transmembrane receptor for many matrix and non-matrix molecules [21]. It has been shown that this receptor plays an important role in physiological and pathological processes of inflammatory response, immune response, and thrombosis [22]. Integrin α2β1 is the main collagen receptor on platelets [22]. The presence of the rs1126643 (or C807T) variant is associated with increased receptor expression on the surface of some blood cells, in particular platelets [23]. The *ITGB3* gene encodes the β3 subunit of the platelet-derived fibrinogen receptor αIIbβ3, which mediates platelet aggregation [24]. Among the variants of the *ITGB3* gene, the most studied is rs5918 (or T1565C), which leads to changes in the affinity and avidity of the receptor [25].

Despite extensive research on the genetic components influencing the hemostatic system in women with HUD, there are still limited studies exploring the relationship between genetic markers and hemostatic function in this population.

The objective: to investigate the association between variants of folate metabolism genes and the hemostatic system in pregnant women with HUD, focusing on hemostatic parameters during the complicated course of the first trimester.

MATERIALS AND METHODS

The main group of the study included 75 pregnant women with HUD: uterine endometriosis (adenomyosis) (N80.0, according to the International Classification of Diseases, 10th Revision), uterine leiomyoma (D25), type 4–7 according to the classification of the International Federation of Gynecology and Obstetrics (FIGO), endometrial polyps (N84.0), glandular endometrial hyperplasia (N85.0). Patients with adenomatous endometrial hyperplasia (N85.1) were not included in the study. HUD in the examined pregnant women was detected before pregnancy

using ultrasound examination, laparoscopy and hysteroscopy. Endometrial polyps and glandular endometrial hyperplasia were removed before pregnancy during laparoscopy. Ultrasonographic examination of pregnant women was performed on an Esaote Biomedica (Italy) AU 4 Idea device.

The patients underwent treatment at the Department of Family Planning and Surgical Rehabilitation of Women's Reproductive Function and the Department of Prevention and Treatment of Purulent and Inflammatory Diseases in Obstetrics or were registered at the State Institution "Ukrainian center of maternity and childhood of the national academy of medical sciences of Ukraine" in the first trimester of pregnancy. The women were aged 24 to 46 years, the average age of the examined subjects in the main group was 32.4 ± 4.5 years, the control group was 33.6 ± 5.2 years.

Among the 75 pregnant women with HUD, uterine leiomyoma was identified in 39 patients (52.0%), adenomyosis in 23 (30.7%), endometrial polyps in 13 (17.3%), and endometrial hyperplasia in 20 (26.7%). Additionally, 46 women (61.3%) presented with a combination of these pathologies. The number of myomatous nodes varied from 2 to 6, with a mean of 3.21 ± 0.56, while their sizes ranged from 0.5 to 50 mm, averaging 21.4 ± 1.6 mm. All uterine leiomyomas were diagnosed before pregnancy, and the most frequent localization was type 5 and 6 according to FIGO (in 24 (61.5%) patients), type 4 was detected in 11 (28.2%) women, type 7 in 4 (10.3%) patients with uterine leiomyoma. Six women (15.4%) experienced an increase in node size of 15–20% by 11–13 weeks of gestation. Uterine endometriosis in the study group was primarily diffuse or mixed in type. The control group consisted of 60 women of similar age who had one or more children and did not have HUD.

We analyzed the obstetric history to determine the proportion of early reproductive losses in the examined women. We defined early reproductive losses as a missed abortion (O02.1), spontaneous abortion (miscarriage) (O03), recurrent pregnancy loss (N96), and ectopic pregnancy (O00) (Table 1).

Early pregnancy loss was noted in 24 (32%) women of the main group and in 3 (5%) women of the control group

Table 1

Consequences of previous pregnancies and labors in the examined pregnant women, abs. number (%)

Consequences of previous pregnancies and labors	Study groups	
	Main (n = 75)	Control (n = 60)
Artificial abortion (surgical)	16 (21.3)*	6 (10.0)
Medical abortion	11 (14.7)*	4 (6.7)
Spontaneous miscarriage (abrasion)	8 (10.7)*	1 (1.6)
Missed abortion	7 (9.3)*	–
Recurrent pregnancy loss	3 (4.0)	–
Ectopic pregnancy	6 (8.0)*	2 (3.3)
Antenatal fetal death in history	1 (1.3)	–
Term delivery	12 (16.0)*	51 (85.0)
Preterm labor with a live fetus	4 (5.3)	2 (3.3)

Note: * – the difference is significant relative to the control group ($p < 0.05$).

($p < 0.05$). Combined effects of previous pregnancies were observed in 20 (26.7%) women in the main group (abortion and ectopic pregnancy in 11 (14.7%) women, abortion and missed abortion in 7 (9.3%) women, spontaneous miscarriage and preterm labor in 2 (2.7%) women). In the first trimester, 66 (88.0%) women in the main group had a threat of pregnancy termination.

The hematologic study was conducted at the clinical diagnostic laboratory of the State Institution "Ukrainian center of maternity and childhood of the national academy of medical sciences of Ukraine". Hemostatic parameters were analyzed using an automatic analyzer, the "BIO-KSEL-6100" (Poland). The following hemostasiogram parameters were assessed: fibrinogen level, plasma fibrin, activated partial thromboplastin time (APTT), prothrombin time (PT), international normalized ratio (INR), and prothrombin activity (Quick method). The analysis was performed between 6 and 12 weeks of pregnancy.

All study participants underwent a comprehensive molecular genetic study for the following gene variants: *MTHFR* (rs1801133, rs1801131), *MTR* (rs1805087), *MTRR* (rs1801394), *F5* (rs6025), *F2* (rs1799963), *FGB* (rs1800787, rs1800790), *PAI-1* (rs1799768), *ITGA2* (rs1126643), *ITGB3* (rs5918). The molecular genetic study was performed in the molecular genetic laboratory of the SI "Reference-center for Molecular Diagnostic of Public Health Ministry of Ukraine" using polymerase chain reaction (PCR), allele-specific PCR, and PCR followed by restriction fragment length polymorphism analysis according to previously published protocols [26–29].

The study was conducted in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the Committee on Bioethics and Deontology of State Institution "Ukrainian center of maternity and childhood of the national academy of medical sciences of Ukraine" for all participants (Protocol No. 5 of June 24, 2020). Informed consent was obtained from all individuals included in this study.

The statistical analysis of the study results was performed using the SPSS Statistics v27 software package. The data were processed using variation and statistical analysis, with the mean (M) and standard deviation (σ) reported. The frequency analysis of nominal (qualitative) variables was conducted using contingency tables with the assessment of significance by Pearson's χ^2 criterion. The distribution of the hemostasis parameters was tested for normality using the Kolmogorov – Smirnov test. Comparisons of the studied parameters was performed using the Mann – Whitney U test. Spearman correlation analysis was performed between the studied indicators and genotypes. For correlation purposes, genotypes were coded as follows: homozygous for the major allele = 0, heterozygous = 1, and homozygous for the minor allele = 2. Differences wuth $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In the examined women of the main group, the indicators did not exceed the reference values, but significant differences were found in comparison with the coagulogram data of pregnant women in the control group (Table 2).

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Table 2

Hemostatic parameters in the examined pregnant women

Hemostasis Indicators	Study groups		Reference Values
	Main (n = 75)	Control (n = 60)	
Prothrombin time, sec	14.70 ± 0.64* 10.9–15.5	11.10 ± 0.32 10.8–13.4	11–13.5
Fibrinogen, g/l	4.8 ± 0.2* 1.9–6.8	2.9 ± 0.2 2.1–3.6	2–4
Activated partial thromboplastin time, sec	34.9 ± 2.1* 21–38	26.3 ± 1.3 20–33	19–37
International normalized ratio	1.09 ± 0.03 0.84–1.46	0.96 ± 0.02 0.86–1.23	0.82–1.22
Fibrin, mcg/ml	21.4 ± 1.3* (8–25)	12.5 ± 1.1 (8–15)	8–16
Quick prothrombin time method, %	129.4 ± 7.6* (75–132)	96.5 ± 2.3 (70–125)	70–130

Note: * – the difference is significant relative to the control group ($p < 0.05$).

Table 3

Distribution of genotypes by variants of folate cycle and hemostasis system genes in the study groups

Gene (variant)	Genotype	Study groups		p	χ^2
		Main (n = 75), %	Control (n = 60), %		
MTHFR (rs1801133)	CC	28 (37.3)	28 (46.7)	0.034	4.48
	CT	33 (44.0)	29 (48.3)		
	TT	14 (18.7)*	3 (5.0)*		
MTHFR (rs1801131)	AA	37 (49.4)	24 (40.0)	0.28	1.17
	AC	28 (37.3)	24 (40.0)		
	CC	10 (13.3)	12 (20.0)		
MTRR (rs1801394)	AA	22 (29.3)	13 (21.7)	0.30	1.09
	AG	33 (44.0)	26 (43.3)		
	GG	20 (26.7)	21 (35.0)		
MTR (rs1805087)	AA	44 (58.7)	39 (65.0)	0.12	2.37
	AG	30 (40.0)	16 (26.7)		
	GG	1 (1.3)	5 (8.3)		
PAI-1 (rs1799768)	5G/5G	13 (17.3)	12 (20.0)	0.40	0.72
	5G/4G	38 (50.7)	26 (43.3)		
	4G/4G	24 (32.0)	22 (36.7)		
FV (rs6025)	GG	72 (96.0)	56 (93.3)	0.76	0.09
	GA	3 (4.0)	4 (6.7)		
	AA	0 (0.0)	0 (0.0)		
FII (rs1799963)	GG	72 (96.0)	60 (100.0)	0.33	0.96
	GA	3 (4.0)	0 (0.0)		
	AA	0 (0.0)	0 (0.0)		
FGB (rs1800787)	CC	38 (50.7)	33 (55.0)	0.58	0.30
	CT	26 (34.7)	21 (35.0)		
	TT	11 (14.6)	6 (10.0)		
FGB (rs1800787)	GG	39 (52.0)	31 (51.7)	0.58	0.30
	GA	25 (33.3)	23 (38.3)		
	AA	11 (14.7)	6 (10.0)		
ITGA2 (rs1126643)	CC	22 (29.3)	19 (31.7)	0.33	0.56
	CT	40 (53.4)	29 (48.3)		
	TT	13 (17.3)	12 (20.0)		
ITGB3 (rs5918)	TT	53 (70.7)	44 (73.3)	0.70	0.15
	TC	21 (28.0)	15 (25.0)		
	CC	1 (1.3)	1 (1.7)		

Note: * – the difference is significant relative to the control group ($p < 0.05$).

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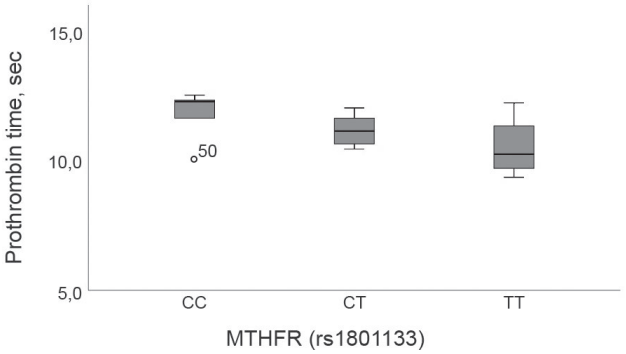

Fig. 1. Prothrombin time in pregnant women of the main group depending on the rs1801133 variant of the MTHFR gene

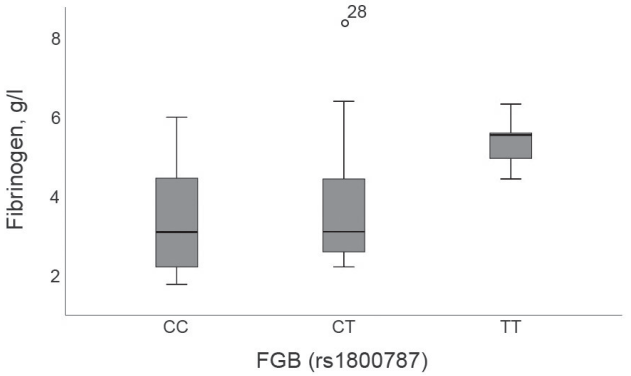

Fig. 2. Fibrinogen levels in pregnant women of the main group depending on the rs1800787 variant of the FGB gene

Pregnant women in the main group had a significantly elevated APTT, which characterizes the internal mechanism of blood coagulation and can be prolonged during anticoagulation and fibrinolytic therapy, as well as in various thrombophilias, in deficiency of blood coagulation factors, etc. The INR in the main group ranged from 0.85 to 1.46, with an average of 1.09 ± 0.03, in the control group – from 0.87 to 1.23, with an average of 0.96 ± 0.02, $p > 0.05$. The absence of significant differences in pregnant women with HUD compared to the control group may indicate the preservation of compensatory mechanisms in the first trimester of pregnancy.

Our results indicating abnormalities in systemic hemostasis (fibrin, fibrinogen, APTT, prothrombin time, and prothrombin activity according to Quick) demonstrate hypercoagulability. Obviously, these data are consistent with the fact that 66 (88%) of the subjects in the first trimester had a threat of pregnancy termination.

The results of genotyping, reflecting the distribution of genotypes by the studied gene variants in the study groups, are presented in Table 3.

In patients of the main study group, a significant increase in the frequency of the TT genotype at the rs1801133 variant of the *MTHFR* gene was noted, which may indicate an association of this gene variant with both the risk of developing HUD and the features of the course of the first trimester of pregnancy in the presence of HUD, that is, an association with complications. The data obtained with regard to the distribution of genotypes for other variants of the studied genes did not differ significantly when comparing both groups.

To analyze the possible influence of intergenic interaction, we analyzed the distribution of frequencies of genotype combinations by gene variants in pregnant women of the main group and control group. Statistically significant differences were identified for genes encoding components of the folate cycle. In particular, women in the control group had a higher frequency of the combination of homozygous genotypes CC (rs1801133 of the *MTHFR* gene)/AA (rs1801394 of the *MTRR* gene)/AA (rs1805087 of the *MTR* gene) – 13.3 vs. 2.7% ($\chi^2 = 4.08$, $p = 0.04$).

We also conducted a correlation analysis between the genotypes of pregnant women in the main study group and hemostasiogram parameters. Significant correlations were determined only for some gene variants: rs1801133 of the *MTHFR* gene and prothrombin time ($r_s = -0.55$, $p = 0.022$); rs1800787 of the *FGB* gene and fibrinogen level ($r_s = 0.40$, $p = 0.034$); rs1126643 of the *ITGA2* gene and platelet level ($r_s = 0.70$, $p = 0.037$); rs5918 of the *ITGB3* gene and APTT ($r_s = 0.68$, $p = 0.045$).

In pregnant women of the main group with the TT genotype at the rs1801133 variant of the *MTHFR* gene, lower prothrombin time values were noted (Fig. 1). In women of the main group, the highest value of prothrombin time was observed in the CC genotype – 12.4 [11.3; 12.5] sec, and the lowest – 10.3 [9.6; 11.9] sec – in the TT genotype. It should be noted that all these indicators are within the range of reference values.

The highest levels of fibrinogen were observed in patients with the TT genotype (5.6 [4.7; 6.0] g/l) at the rs1800787 variant of the *FGB* gene (Fig. 2). It is well known that fibrinogen plays an important role in hemostasis, in particular in blood clotting. During pregnancy, fibrinogen levels can rise to 6 g/L and this is normal.

We found a positive significant correlation between the genotypes for the rs1126643 variant of the *ITGA2* gene and platelet count (Fig. 3). In pregnant women of the main group with the TT genotype, the median platelet count was 409.5 [387.0; 432.2] * 10^9/L (Fig. 3). It should be noted that this value slightly exceeds the upper limit of the reference values.

APTT is also an important indicator characterizing the state of the hemostatic system. In pregnant women of the main study group with the CC genotype at the rs5918 variant of the *ITGB3* gene, an increase in APTT to 36.0 s was determined (Fig. 4).

In line with our aim, we determined the genotype frequencies of genes involved in folate metabolism and the hemostatic system in pregnant women with HUD. Despite existing discrepancies in research findings, folate metabolism genes and associated hereditary thrombophilia are recognized as significant contributors to gynecological pathologies and obstetric complications. This analysis revealed an increase in the frequency of the TT genotype at the rs1801133 variant of the *MTHFR* gene in pregnant women of the main group, indicating an association of this genotype with an increased risk of complicated pregnancy in the first trimester in women with HUD. It should be noted that similar results were obtained by other research groups. In particular, Shen et al. in their retrospective cohort study involving 2,411 pregnant women reported an

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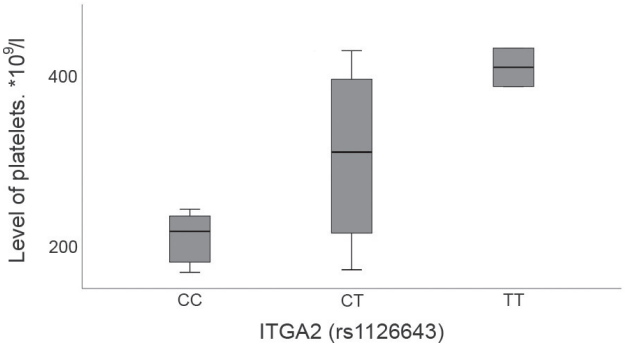

Fig. 3. Platelet counts in pregnant women of the main group depending on the rs1126643 variant of the ITGA2 gene

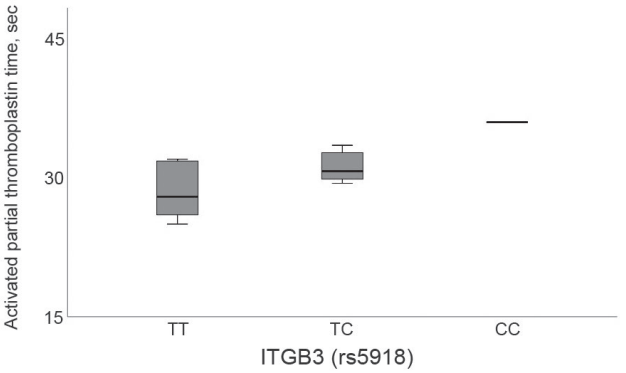

Fig. 4. APTT in pregnant women of the main group depending on the rs5918 variant of the ITGB3 gene

association of the rs1801133 variant of the *MTHFR* gene with the incidence of uterine fibroids [30]. A research team led by G. Delli Carpini showed that the TT genotype for the rs1801133 variant of the *MTHFR* gene is a risk factor for endometriosis [31]. The authors explained this effect of the rs1801133 variant by its influence on the mechanisms associated with epigenetic modifications.

We have also established the significance of intergenic interaction for the studied genes of folate metabolism in the first trimester of pregnancy in patients with HUD. In particular, we have determined the association of the presence of a combination of wild-type homozygotes for the *MTHFR*, *MTRR* and *MTR* genes with a reduced risk of complicated pregnancy in the first trimester in patients with HUD. It should be noted that there is very little data on this query in the scientific literature. Most likely, this is due to the fact that large-scale studies have not yet been conducted to determine the role of intergenic interaction of the studied folate metabolism genes in the course of pregnancy in patients with HUD. Although, as the results of some studies show, combinations of variants of folate metabolism genes modify the risk of hyperhomocysteinemia in reproductive disorders [29]. In this study, homocysteine levels were not determined, but the potential effect of the *MTHFR* genetic variant on HUD and complicated pregnancy is likely to be realized through hyperhomocysteinemia and hemostatic disorders, which was also proven in above-mentioned study [29, 32–34].

A significant correlation has been identified between the rs1801133 variant of the *MTHFR* gene and the duration of prothrombin time. It has also been previously noted that the presence of the rs1801133 variant of the *MTHFR* gene leads to a decrease in enzyme activity, which in turn leads to an increase in homocysteine levels [29]. On the other hand, it has been experimentally shown that homocysteine can affect the extrinsic pathway of blood coagulation activation [35]. In particular, regression analysis has shown a relationship between high plasma homocysteine levels and low prothrombin time. Therefore, we believe that it is the increased levels of homocysteine in the presence of the TT genotype in patients (not measured in this study) that can explain the reduction in prothrombin time.

The association of the rs1800787 variant of the *FGB* gene with elevated fibrinogen levels has also been determined. Similar results were obtained by Imran et al. who determined that the mutant genotypes (CT and TT) and the T allele were significantly associated with hyperfibrinogenemia [36]. However, the fibrinogen levels in this study did not exceed the reference limits at the time of the examination. However, it has been shown that women with various forms of HUD have a tendency to have elevated fibrinogen levels – hyperfibrinogenemia [5, 37]. The absence of elevated fibrinogen levels, but the detected association does not exclude that women with mutant genotypes are at greater risk of hyperfibrinogenemia during pregnancy.

We have shown that the rs1126643 variant of the *ITGA2* gene has a strong positive correlation with platelet count. Patients with the major SS genotype had lower platelet counts, and patients with the TT genotype, on the contrary, had higher platelet counts. A similar result was shown by Laine et al. [38]. In their study, it was determined that the presence of the C allele at the rs1126643 variant of the *ITGA2* gene was associated with a decrease in platelet count [38]. It is worth mentioning that α2β1, the end product of the *ITGA2* gene, mediates platelet functions during blood coagulation, but is not directly involved in regulating platelet count. Therefore, at present, we cannot provide mechanisms that would explain the effect we have identified.

The positive correlation between the rs5918 variant of the *ITGB3* gene and APTT, an important indicator in characterizing the intrinsic coagulation pathway, is also noteworthy. We found one study with a similar analysis design, conducted by Xiang et al. in which no significant effect of the rs5918 variant and APTT was found [39]. This may be due to the fact that the study by Xiang et al. involved healthy volunteers. In our opinion, high APTT and the presence of a correlation with the rs5918 variant may be associated with complex interactions in the blood coagulation system in pregnant women with HUD. In general, the correlations we identified indicate that pregnant women with HUD are at risk of developing hemostatic disorders during further gestation. Therefore, further large-scale studies in this area are needed to confirm the identified correlations and evaluate them for possible use in clinical practice.

CONCLUSIONS

We determined the association of the rs1801133 variant of the *MTHFR* gene with an increased risk of

complicated pregnancy, specifically the risk of threatened miscarriage, in the first trimester in women with HUD. Significant intergenic interactions of folate cycle genes were found to modulate the risk of complicated pregnancy, reducing it in the absence of variants associated with a decrease in gene functional activity. A significant correlation between the studied variants of the *MTHFR, FGB, ITGA2, ITGB3* genes and the levels of prothrombin time, fibrinogen, platelets and APTT in the first trimester of pregnancy was determined in women with HUD. Further study of the identified correlations will contribute to the formation of a personalized strategy for assessing genetic risk and developing appropriate preventive measures.

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