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Genetic-epigenetic aspects of infertility in women with long-COVID-19

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The aim of the study is to determine the genetic and epigenetic features of infertile patients with long-term COVID-19 in order to clarify the risk factors for the failure of assisted reproductive technologies (ART) use.

Materials and methods. The *MTHFR* (C677T, A1298C), *RFC-1* (G80A) and *BHMT* (G742A) gene polymorphisms and the methylation of *ESR1* gene was performed. 40 patients (main group) with infertility due to long-term COVID-19 were examined: the subgroup 1 — 25 women in whom the use of ART was ineffective, the subgroup 2 — 15 patients with effective use of ART. The control was taken from literary sources: for *RFC* (G80A), *MTHFR* (C677T) and *MTHFR* (A1298C) polymorphisms — 35 Ukrainian women without infertility and reproductive losses; for *BHMT* (G742A) — 60 people of the Ukrainian population. The methods of variational statistics were used, in particular the Fisher test with a significance level of $p<0.05$.

Results. Patients of the main group have a higher frequency of the homozygous genotype of the *MTHFR* gene polymorphism (C677T) on the mutant T allele (20.0% vs. 3.2%; $p<0.05$). When ART is unsuccessful, the TT genotype is 4 times greater (28.0% vs. 6.7%; $p<0.05$). A study by genotypes of *MTHFR* polymorphism (A1298C) did not reveal a significant difference. Patients of the main group `have a higher frequency of the mutant allele A of the *RFC* gene (G80A) (80.0% vs. 51.4%; $p<0.05$). No significant difference was found depending on the success rate of ART. The frequency of *BHMT* gene polymorphism (G742A) in the main group did not differ from that in the Ukrainian population, however, in the case of unsuccessful ART, it was observed less often and only in the heterozygous variant (40.0% vs. 66.6%; $p<0.05$). The analysis of pairwise intergenic interaction revealed the highest frequency of the combination of AAGA for the *MTHFR* (A1298C)+*BHMT* (G742A) pair — 35.0% and GAAA for the *RFC* (G80A)+*MTHFR* (A1298C) pair — 30.0% in the main group. Hypermethylation of the promoter region of the *ESR1* gene is observed in 20 (50.0%) patients of the main group: in 17 (68.0%) women of the subgroup 1 versus 3 (20.0%) women of the subgroup 2 ($p<0.05$).

Conclusions. The genetic and epigenetic conditioning of the success of ART programs in infertility associated with long-term COVID-19 has been revealed, which opens up new diagnostic and therapeutic opportunities for identifying factors predisposing to unsuccessful ART treatment and increasing the effectiveness of such treatment by correcting disorders of folic acid metabolism, hyperhomocysteinemia and the estrogens receptor apparatus.

The research was carried out in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Local Ethics Committee of the institution mentioned in the work. Informed consent of the women was obtained for the research.

The authors declare no conflict of interest.

Keywords: long-COVID-19, infertility, assisted reproductive technologies, folate and homocysteine metabolism genes, DNA methylation.

Генетико-епігенетичні аспекти неплідля у жінок з лонг-COVID-19

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Мета — визначити генетичні та епігенетичні особливості неплідних пацієнток із лонг-COVID-19 для уточнення факторів ризику неуспішності застосування допоміжних репродуктивних технологій (ДРТ)

Матеріали та методи. Досліджено поліморфізми генів *MTHFR* (C677T, A1298C), *RFC-1* (G80A) та *BHMT* (G742A) і метилювання гена *ESR1*. Обстежено 40 пацієнток (основна група) із неплідддям із лонг-COVID-19: до підгрупи 1 залучено 25 жінок, в яких застосування допоміжних репродуктивних технологій (ДРТ) було неефективним, до підгрупи 2 залучено 15 пацієнток із ефективним застосуванням ДРТ. Контроль взято з літературних джерел: для поліморфізмів *RFC* (G80A), *MTHFR* (C677T) та *MTHFR* (A1298C) 35 українських жінок без неплідддя і репродуктивних втрат; для *BHMT* (G742A) — 60 осіб української популяції.

Застосовано методи варіаційної статистики, зокрема, критерій Фішера з рівнем значущості $p<0,05$.

Результати. У пацієнток основної групи частота гомозиготного генотипу поліморфізму *MTHFR* (C677T) була вищою за мутантною алеллю Т (20,0% проти 3,2%; $p<0,05$). У разі неуспішності ДРТ частота генотипу ТТ була вищою в 4 рази (28,0% проти 6,7%; $p<0,05$). За генотипами поліморфізму *MTHFR* (A1298C) не виявлено достовірної різниці. В основній групі була вищою частота мутантної алелі А поліморфізму *RFC* (G80A) (80,0% проти 51,4%; $p<0,05$). Достовірної різниці залежно від успішності ДРТ не встановлено. Частота поліморфізму гена *BHMT* (G742A) в основній групі не відрізнялася від загальноукраїнської популяції, проте в разі неуспішності ДРТ він відзначався рідше і лише в гетерозиготному варіанті (40,0% проти 66,6%; $p<0,05$). Аналіз попарної міжгенної взаємодії виявив в основній групі найвищу частоту комбінації AAGA для пари *MTHFR* (A1298C)+*BHMT* (G742A) — 35,0% та GAAA для пари *RFC (G80A)+MTHFR* (A1298C) — 30,0%. Гіперметилювання промоторної ділянки гена *ESR1* спостерігалося у 20 (50,0%) пацієнток основної групи: у 17 (68,0%) жінок підгрупи 1 проти 3 (20,0%) жінок підгрупи 2 ($p<0,05$).

Висновки. Виявлена генетична та епігенетична обумовленість успішності програм ДРТ при неплідді, асоційованому з лонг-COVID-19, що відкриває нові діагностично-лікувальні можливості з виявлення факторів схильності до неуспішного лікування ДРТ та підвищення ефективності такого лікування шляхом корекції порушень метаболізму фолієвої кислоти, гіпергомоцистеїнемії та рецепторного апарату естрогенів.

Дослідження виконано згідно з принципами Гельсінської декларації. Протокол дослідження ухвалено Локальним етичним комітетом зазначеної в роботі установи. На проведення досліджень отримано інформовану згоду жінок.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: лонг-COVID-19, неплідддя, допоміжні репродуктивні технології, гени фолатного та гомоцистеїнового обміну, метилювання ДНК.

Folic acid metabolism affects ovarian function, implantation, embryogenesis and the entire pregnancy process. In addition to the well-established effect on the incidence of neural tube defects, there has been an association between low folic acid levels and high homocysteine concentrations, on the one hand, and recurrent miscarriages and other pregnancy complications, on the other. In infertile women undergoing in vitro fertilisation (IVF) / intracytoplasmic sperm injection (ICSI) treatment, a correlation was found between the concentration of folic acid in the plasma and the frequency of pregnancy [23], other researchers found that the administration of folic acid and iodine was associated with a reduction in the time to conception [12].

Folic acid deficiency may be caused by dietary or genetic factors and may impair the function of metabolic pathways such as amino acid metabolism, purine and pyrimidine synthesis, and methylation of nucleic acids, proteins, and lipids, leading to elevated homocysteine concentrations. Some of the side effects of folic acid deficiency and homocysteine accumulation include insufficient cell division and production of inflammatory cytokines, can impair fertility and lead to pregnancy complications, as these processes are involved in oocyte development, endometrial receptivity preparation, embryo implantation and pregnancy. In folliculogenesis, hyperhomocysteinemia can activate apoptosis, leading to follicular atresia and affecting oocyte maturity and embryo quality cultured *in vitro* [4].

5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which serves as a methyl donor in the remethylation of homocysteine to methionine. Mutations in *MTHFR* are associated with many developmental abnormalities and pregnancy loss. Previous studies have shown that *MTHFR* is expressed in human oocytes and preimplantation embryos. Moreover, an increased frequency of the *MTHFR* 677TT mutant genotype was demonstrated in women who did not undergo at least four IVF cycles, and another study showed that women with the MTHFR 1298CC mutant genotype who underwent IVF were less likely to become pregnant than patients with the 1298AA genotype wild type The study shows that the number and maturity of the obtained oocytes can be associated with the *MTHFR* C677T and A1298C polymorphisms [4].

Regenerated folate carrier-1 (RFC-1) is a well-characterized cell membrane folate transporter with high affinity for physiological folate. The genes encoding these proteins contain polymorphisms that can reduce enzymatic activity and impair the entry of folic acid into the cell and are associated with the development of pathological processes, congenital malformations [15].

One-carbon metabolism plays an important role in complex and important metabolic pathways, as hundreds of intracellular transmethylation reactions, including DNA methylation and DNA synthesis, have been implicated in carcinogenesis and processes closely related to homocysteine (Hcy) metabolism, the elevation of which is an important risk marker for occurrence of adverse events. Vitamins, in particular folic acid (B_9) and other B vitamins, such as B_6 and B_{12}, are important cofactors of one-carbon metabolism, facilitating the remethylation of homocysteine, thereby preventing the increase in the concentration of this metabolite [22].

S-adenosylmethionine (SAM) is the main methyl donor in the cell. It is involved in numerous cellular reactions, including DNA methylation and phosphatidylcholine synthesis, as well as reactions involving neurotransmitters, creatine, carnitine, and antioxidants (such as glutathione and taurine). Methionine, betaine, choline, and 5-methyltetrahydrofolate (5-MTHF) are important dietary sources of labile methyl groups in mammalian cells. SAM is produced from methionine by *methionine adenosyltransferase* (MAT). Methionine is either obtained from the diet or formed from homocysteine via methionine synthase or betaine homocysteine methyltransferase (BHMT). The BHMT pathway is particularly active in the liver and kidney, which are the main organs that store large amounts of betaine. During methyl group donation, SAM is converted to S-adenosylhomocysteine (SAH), a potent competitive inhibitor of many methyltransferases [16].

The *BHMT* gene encodes the production of the enzyme betaine-homocysteine-methyltransferase (BHMT). The BHMT enzyme is involved in the transfer of methyl groups with the formation of methionine from homocysteine. The human *BHMT* polymorphism is thought to produce an enzyme with a higher affinity for homocysteine than the wild type. This polymorphism may play a crucial role in homocysteine homeostasis. The 677 C→T mutation in the *MTHFR* gene has been shown to contribute to increased Hcy and

is a genetic risk factor for diseases associated with hyperhomocysteinemia [14].

MTHFR is a key regulatory enzyme in the one-carbon cycle. This enzyme is required for the metabolism of methionine, folic acid, and RNA, as well as for the production of proteins, DNA, and RNA. MTHFR catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to its active form, 5-methyltetrahydrofolate, which is a cosubstrate for the remethylation of homocysteine to methionine. Numerous variants of the *MTHFR* gene were known, among which the most extensively studied variant is C677T. The C677T polymorphism, which results in the conversion of valine to alanine at codon 222, is associated with reduced activity and increased thermolability of the enzyme. Violation of MTHFR efficiency is associated with an increase in the level of homocysteine, which can contribute to an increase in the production of reactive oxygen species and the development of oxidative stress [25].

Vascular endothelium plays an important role in maintaining vascular homeostasis by regulating vascular tone, inflammation, and cell growth. Endothelial dysfunction caused by hyperhomocysteinemia can trigger inflammation, apoptosis and subsequent formation of atherosclerotic lesions. Studies involving cell cultures and animal models have shown that Hcy impairs the ability of endothelial cells to produce nitric oxide and prostacyclin, which are potent endogenous vasodilators, and there is evidence that Hcy induces inflammation in endothelial cells, leading to the release of inflammatory cytokines, including interleukin-6, interleukin-8 and tumor necrosis factor-α. In addition, it was shown that Hcy induces apoptosis in endothelial cells, causes atherosclerotic changes in blood vessels [10].

The metabolism of folic acid, betaine, choline and methionine is interconnected, and the deficiency of one of the substances can be partially compensated. Deficiency of dietary methyl donors can cause metabolic and functional disorders. Metabolic changes in the cell are reflected by increased levels of homocysteine, disruption of energy and lipid metabolism, as well as dysregulation of DNA methylation and protein synthesis.

In addition to the connection of disorders of folic acid metabolism, one-carbon metabolism, hyperhomocysteinemia, which are regulated genetically, with the reproductive function of a woman, the results of assisted reproductive technologies (ART) programs [18], some researchers have established the connection of these disorders with the severity of the course of COVID-19, thromboembolic complications [9,19,24].

Abnormal increase or decrease in DNA methylation promotes or is a marker of cancer formation, also associated with neurological, immunological diseases, atherosclerosis and osteoporosis [5].

There is increasing evidence that epigenetics plays a key role in the pathogenesis of endometriosis. Epigenetic modifications include dynamic and reversible changes in chromatin structure that affect gene expression in a heritable manner. Characteristic features of epigenetic gene regulation are DNA methylation (hypo- and hypermethylation), histone modifications, and miRNA production, which leads to the expression or suppression of specific proteins. Some researchers provide data on the role of epigenetic factors in the etiopathogenesis of endometriosis and associated infertility [2]. A direct correlation with the expression of genes influencing the process of implantation in eutopic endometrium of women with endometriosis was revealed. Susceptibility and decidualization of the endometrium depend on hormonally regulated molecular processes. Reaction signaling pathways of estradiol and progesterone are regulated in the epithelial and stromal parts of the endometrium.

Functional dysregulation of steroid hormone signaling in endometriosis, such as increased E2-induced cell proliferation, inflammation, and progesterone resistance, appears to play an important role in impaired endometrial receptivity in these patients. The shift toward estrogen dominance promotes inflammation, angiogenesis, cell proliferation, and immunosuppression. Progesterone receptor expression levels are lower in women with endometriosis-associated infertility, while estrogen receptor 1 (ESR-1) levels are increased in the mid-secretory phase endometrium of these women compared to controls [17].

The aim of the study is to determine the genetic and epigenetic features of infertile patients with long-term COVID-19 in order to clarify the risk factors for the failure of assisted reproductive technologies (ART) use.

Materials and methods of the study

The genetic and epigenetic examination included 40 patients (the main group) who applied to the clinic of reproductive technologies for infertility treatment and who showed signs of «long-COVID». The patients of the main group were

divided into 2 subgroups: the subgroup 1 — 25 women in whom the use of ART was ineffective, the subgroup 2 — 16 patients with effective treatment of infertility with the help of ART. Controls were taken from literary sources: for *RFC* (G80A), *MTHFR* (C677T) and *MTHFR* (A1298C) gene polymorphisms, Ukrainian women without infertility and reproductive losses [20], n=35; for the *BHMT* polymorphism (G742A) — the Ukrainian population [7], n=60.

Molecular genetic analysis of *MTHFR* (C677T, rs1801133; A1298C, rs1801131), *RFC-1* (G80A, rs1051266) and *BHMT* (G742A, rs3733890) genes was performed using routine molecular genetic methods (allele-specific polymerase chain reaction and polymerase chain reaction with length of restriction fragments polymorphism analysis) [21].

The methylation status of the promoter region of the *ESR1* gene was determined using the molecular genetic method [6]. The result of determining the methylation status of the promoter region of the *ESR1* gene was taken into account depending on the number of hypermethylated alleles: in the heterozygous state — Met/UnMet, and in the absence of methylation — UnMet/UnMet.

The obtained data were processed by the methods of variational statistics accepted in medicine, using Fisher's angular transformation for indicators represented by frequencies, with a critical significance level of $p<0.05$. The Microsoft Excel statistical analysis package was used.

The study was carried out in accordance with the main provisions of GCP ICH and the Declaration of Helsinki, agreed with the ethics committee of the Ivano-Frankivsk National Medical University and the ethics and academic integrity commission of the Shupyk National University of Health Care of Ukraine. All studies were carried out after receiving the patient's informed consent for diagnosis and treatment. The work is a fragment of the SRW «Improving tactics of preconception counseling and management of early pregnancy of women with reproductive health disorders».

Research results and discussion

Studies of gene polymorphisms that regulate folic acid metabolism (*RFC* and *MTHFR*) and *BHMT* revealed the following (Table 1). In the women of the main group, the study of the MTHFR polymorphism (C677T) revealed a significantly higher frequency of the homozygous genotype for the mutant T allele (20.0% vs. 3.2% in the control, $p<0.05$). The analysis of the result depending on the success of ART programs demonstrated the identity of the distributions in the subgroup 2 (successful ART) and the controls, while in the case of ART failure, the proportion of women with the homozygous genotype for the wild allele C was significantly lower (24.0% vs. 53.3% in the subgroup 2, $p<0.05$) and a 4 times greater share of patients with the TT genotype (28.0% vs. 6.7%, $p<0.05$). This polymorphism has been proven to be associated with a deficiency of folic acid and other vitamins of group B, which leads to various pathological conditions, in particular in the reproductive sphere, developmental anomalies, and pregnancy loss.

A study by genotypes of another *MTHFR* polymorphism (A1298C) did not reveal a significant difference in the distributions between groups, although the proportion of the heterozygous variant of AS is slightly higher in the subgroup 1.

As for the study of the polymorphism of the reduced carrier folate-1 *RFC* gene (G80A), which can reduce the enzymatic activity of RFC and impair the entry of folic acid into the cell, the frequency of mutant allele A is significantly higher in women of the main group (80.0% vs. 51.4% in the control, $p<0.05$). No significant difference was found in the distributions of the subgroups 1and 2, although the proportion of women with a mutant allele is slightly higher in the case of unsuccessful ART.

The polymorphism of the *BHMT* gene (G742A), which is associated with a decrease in the level of homocysteine and elimination of folic acid deficiency, occurred with the same frequency both in the main group and in the control, however, in the case of unsuccessful ART, it was observed significantly less often only in the heterozygous variant (40.0% versus 66.6%, $p<0.05$).

In addition to the influence of separate gene polymorphisms on the occurrence of pathological conditions, the influence of combinations of polymorphisms of several genes is also studied, in particular, domestic authors studied the role of intergenic interactions in the development of early reproductive losses and infertility in married couples [20]. The study of pairwise intergenic interaction revealed in the main group the highest frequency of the combination of AAGA for the *MTHFR* (A1298C)+*BHMT* (G742A) pair — 35.0% and GAAA for the *RFC* (G80A)+*MTHFR* (A1298C) pair — 30.0% (Fig.). Further, more detailed studies are needed to substantiate the role of intergenic interaction in the occurrence of infertility associated with long-COVID-19,

Table 1

Distribution of women in groups by genotypes of polymorphisms of genes regulating folic acid metabolism (RFC and MTHFR) and betaine-homocysteine-methyltransferase (BHMT)

Polymorphism	Genotype	Main group, n=40	Subgroup 1, n=25	Subgroup 2, n=15	Control[1]
MTHFR (C677T)	CC	14 (35.0)	6 (24.0)#	8 (53.3)	17 (46.6)
	CT	18 (45.0)	12 (48.0)	6 (40.0)	17 (48.6)
	TT	8 (20.0)*	7 (28.0)#	1 (6.7)	1 (3.2)
MTHFR (A1298C)	AA	24 (60.0)	14 (56.0)	10 (66.7)	18 (51.4)
	AC	16 (40.0)	11 (44.0)	5 (33.3)	14(40)
	CC	–	–	–	3 (8.6)
RFC-1 (G80A)	GG	8 (20.0)*	4 (16.0)	4 (26.7)	17 (48.6)
	GA	18 (45.0)	9 (36.0)	6 (40.0)	10 (28.6)
	AA	14 (35.0)	12 (48.0)	5 (33.3)	8 (22.9)
BHMT (G742A)	GG	20 (50.0)	15 (60.0)#	5 (33.3)	27 (45.0)
	GA	18 (45.0)	10 (40.0)	8 (53.3)	30 (50.0)
	AA	2 (5.0)	–	2 (13.3)	3 (5.0)

Notes: 1 — the control is taken from literary sources: for RFC (G80A), MTHFR (C677T) and MTHFR (A1298C) gene polymorphisms Ukrainian women without infertility and reproductive losses [20], n=35; for the BHMT (G742A) polymorphism, the Ukrainian population [7], n=60; * — the difference is significant compared to control patients (p<0.05); # — the difference is significant in relation to patients of the subgroup 2 (p<0.05).

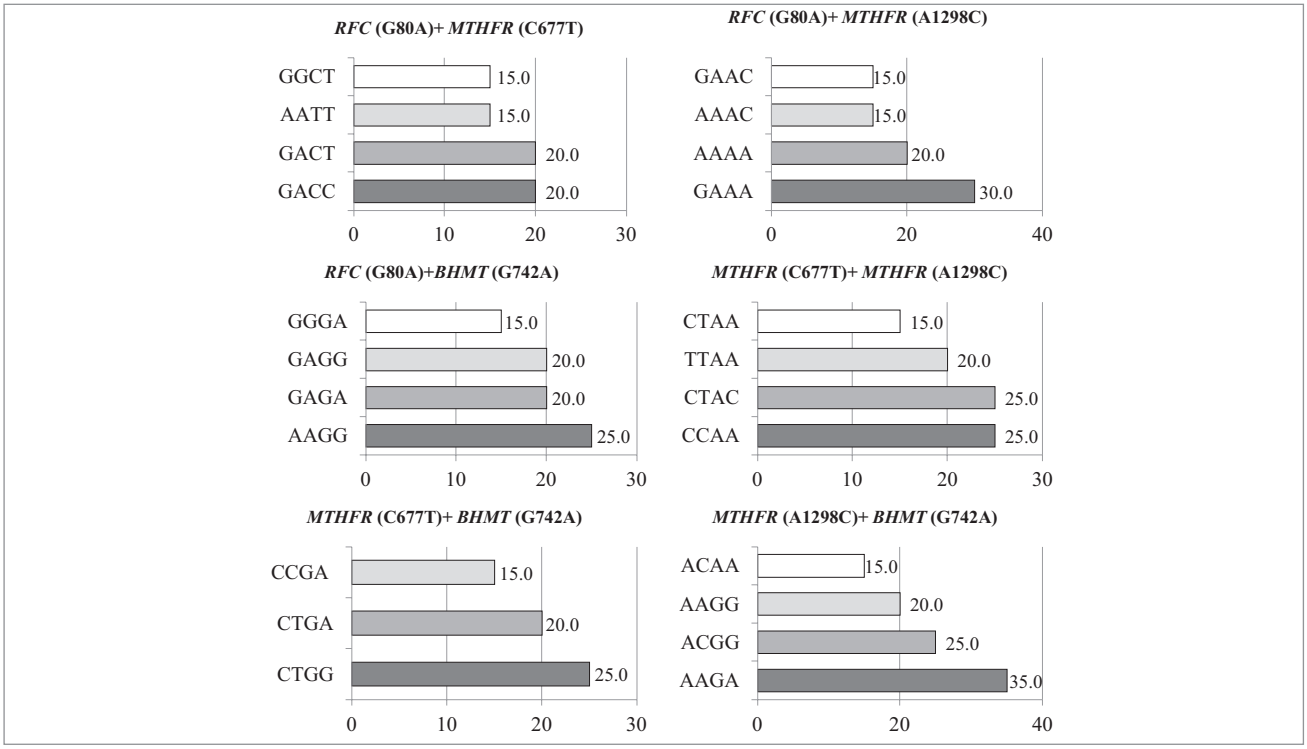

Fig. Variants of intergenic interaction were identified for the studied polymorphisms of genes regulating folic acid metabolism (RFC and MTHFR) and betaine-homocysteine-methyltransferase (BHMT), %

the effectiveness of ART programs in its treatment, and the identification of combinations that may serve as appropriate biological markers of risk.

Metabolic changes due to the presence of certain polymorphisms can lead to dysregulation of protein methylation, and as a result, changes in the expression of relevant substances and interrelated pathological conditions, in particular, infertility, implantation disorders. Biomarkers of DNA methylation are considered extremely promising in the context of human health [3].

According to a few studies by domestic and foreign authors, hypermethylation of the promoter region of the estrogen receptor α gene (*ESR1*) is associated with pathological conditions of the female reproductive sphere (oncological diseases, leiomyoma of the uterus [11], endometriosis [1,13], sleep problems in older women [8].

Table 2

The interaction of genetic (polymorphisms of metabolism genes *MTHFR, RFC* and *BHMT*) and epigenetic (methylation of the promoter region of the ESR1 gene) factors in patients with infertility and long-term COVID-19

Polymorphism	Genotype	Hypermethylation Met/UnMet, n=20	Absence of hypermethylation UnMet/UnMet, n=20
RFC (G80A)	GG	8 (40.0)	6 (30.0)
	GA	8 (40.0)	10 (50.0)
	AA	4 (20.0)	4 (20.0)
MTHFR (C677T)	CC	10 (50.0)*	4 (20.0)
	CT	6 (30.0)*	12 (60.0)
	TT	4 (20.0)	4 (20.0)
MTHFR (A1298C)	AA	14 (70.0)	10 (50.0)
	AC	6 (30.0)	10 (50.0)
	CC	–	–
BHMT (G742A)	GG	2 (10.0)	–
	GA	12 (60.0)*	6 (30.0)
	AA	6 (30.0)*	14 (70.0)
MTHFR (C677T)+ *BHMT* (G742A)	CTGA	6 (30.0)	2 (10.0)
	CTGG	–	10 (50.0)

Note: * — the difference is significant in the presence/absence of hypermethylation of the promoter region of the ESR1 gene ($p<0.05$).

According to our data, hypermethylation of the promoter region of the *ESR1* gene was detected in 20 (50.0%) patients of the main group. In 17 (68.0%) women of the subgroup 1 versus 3 (20.0%) women of the subgroup 2 ($p<0.05$).

Taking into account the influence of one-carbon and folate metabolism on DNA methylation processes, the connections of epigenetic changes (methylation of the promoter region of the ESR1 gene) with genetic factors (gene polymorphisms responsible for metabolic processes) were analyzed, the results are presented in Table 2. No differences were found depending on the presence/absence of hypermethylation of the *RFC* (G80A) and *MTHFR* (A1298C) gene polymorphisms. However, according to the received data. with hypermethylation of the promoter region of the *ESR1* gene, the homozygous wild allele C genotype of the *MTHFR* gene polymorphism (C677T) was significantly more frequent: 50.0% vs. 20.0% ($p<0.05$) and the wild allele G of the *BHMT* gene polymorphism (G742A): 70.0% versus 30.0% ($p<0.05$), mainly due to the heterozygous genotype: 60.0% versus 30.0% ($p<0.05$).

The analysis of the combined effect of *MTHFR* (C677T)+*BHMT* (G742A) gene polymorphisms on the methylation of the promoter region of the *ESR1* gene showed that with hypermethylation, the combination of heterozygous genotypes of these polymorphisms was 3 times more common, and no combination of the heterozygous variant of the *MTHFR* gene polymorphism (C677T) with the homozygous for the mutant allele G of the *BHMT* gene polymorphism (G742A) was observed at all, although in the absence of hypermethylation, this genotype occurred in 50.0% of cases.

Conclusion

Therefore, in patients with infertility and long-term COVID-19 symptoms, the frequency of the homozygous genotype of the *MTHFR* gene polymorphism (C677T) on the mutant allele T is higher (20.0% vs. 3.2%, $p<0.05$). When ART is unsuccessful, the proportion of patients with the TT genotype homozygous for the mutant allele is 4 times greater (28.0% vs. 6.7%, $p<0.05$). A study by genotypes of another *MTHFR* polymorphism (A1298C) did not reveal a significant difference.

Patients with infertility and long-term COVID-19 symptoms have a higher frequency of the mutant allele A of the RFC gene (G80A) (80.0% vs. 51.4% in controls, $p<0.05$). No significant difference was found depending on the success of ART, although the proportion of women with a mutant allele is slightly higher when ART is unsuccessful.

The frequency of polymorphism of the *BHMT* gene (G742A) in women with infertility and symptoms of long-term COVID-19 did not differ from that in the Ukrainian population, however, in the

case of unsuccessful ART, it was observed significantly less often and only in the heterozygous variant (40.0% vs. 66.6%, p<0.05).

The analysis of pairwise intergenic interaction revealed the highest frequency of the combination AAAA for the *MTHFR* (A1298C)+*BHMT* (G742A) pair — 35.0% and GAAA for the *RFC* (G80A)+ *MTHFR* (A1298C) pair – 30.0% in patients with infertility and long-term COVID-19, which requires further studies of their clinical significance.

Hypermethylation of the promoter region of the *ESR1* gene is observed in 20 (50.0%) patients with infertility and long-term COVID-19. Moreover, the vast majority of 17 (85.0%) of these cases are associated with unsuccessful attempts to use ART methods in the treatment of infertility, with the effectiveness of ART methods, the frequency of hypermethylation was 20.0% versus 68.0% (p<0.05).

The analysis of the relationship between epigenetic changes and genetic factors showed that with hypermethylation of the promoter region of the *ESR1* gene, the homozygous wild allele C genotype of the *MTHFR* gene polymorphism (C677T) is significantly more common: 50.0% versus 20.0% (p<0.05) and the wild allele G of the *BHMT* gene polymorphism (G742A): 70.0% vs. 30.0% (p<0.05), mainly due to the heterozygous genotype: 60.0% vs. 30.0% (p<0.05). With hypermethylation, the combination of heterozygous genotypes of the *MTHFR* (C677T)+*BHMT* (G742A) gene polymorphisms is observed 3 times more often, and the combination of the heterozygous variant of the *MTHFR* gene polymorphism (C677T) with the homozygous mutant allele G of the *BHMT* gene polymorphism (G742A) is not observed at all, although in the absence hypermethylation, this genotype occurs in 50.0% of cases.

Thus, genetic and epigenetic conditioning of the success of ART programs in infertility associated with long-term COVID-19 has been revealed, which opens up new diagnostic and therapeutic opportunities for identifying factors predisposing to unsuccessful ART treatment and increasing the effectiveness of such treatment by correcting disorders of folic acid metabolism, hyperhomocysteinemia and the estrogens receptor apparatus.

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