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ESR1 GENE VARIANTS AFFECT *FSHR*-DEPENDED RISK OF FIBROCYSTIC MASTOPATHY IN INFERTILE WOMEN

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***Background*: The infertile women have an increased risk of developing benign and malignant tumors, in particular, breast cancer. Most studies have examined the role of gene variants in the risk of developing breast cancer, but there is little evidence of genetic risk factors for benign tumors. *Aim*: To assess the combined genetic risk of developing mastopathy in women with *FSHR* (rs6165, rs6166) and *ESR1* (rs9340799, rs2234693) gene variants. *Materials and Methods*: The study included 87 infertile women (45 with concomitant fibrocystic mastopathy and 42 without mastopathy). *Results*: For rs9340799 and rs2234693 variants of the *ESR1* gene, we did not find any significant differences in the distribution of genotypes in infertile women with or without mastopathy. In patients with mastopathy, there was a reliable increase in the frequency of 307Ala/Ala and 680Ser/Ser genotypes of *FSHR* gene ($\chi^2 = 6.39$, $p = 0.012$, OR = 4.49 (1.48–13.65)) as compared to patients without mastopathy. In the presence of 307Thr/Thr and 680Asn/Asn genotypes of the *FSHR* gene, a 4.88-fold reduction of mastopathy risk ($\chi^2 = 8.06$, $p = 0.005$, OR = 0.21(0.07–0.59)) was observed. The frequency of the *FSHR* and the *ESR1* genotypes combinations — 307Thr/Thr+680Asn/Asn+351AG+397TC was significantly decreased in patients with mastopathy. *Conclusions*: Our study did not find an association of *ESR1* gene variants with the risk of developing of mastopathy in infertile women although heterozygous variants of the *ESR1* gene enhanced the "protective" effect of *FSHR* gene variants and reduced the risk of mastopathy.**

***Key Words*: mastopathy, infertility, *FSHR*, *ESR1*.**

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The results of several large-scale retrospective cohort studies have confirmed that infertile women have an increased risk of developing benign and malignant tumors, in particular, of breast localization [1–4]. This is attributed primarily to the hormonal imbalance, in particular, of sex hormones in a woman's body, and possibly to the effects of infertility treatment. The candidate genes that can directly and indirectly affect the functional state of the breast have been already identified [5–7]. However, most studies have examined the role of gene variants in the risk of developing breast cancer, but there is little evidence of genetic risk factors for benign tumors. One previous study reported that *ESR1* gene variants modulated the risk of developing both malignant and benign breast tumors in women, but their association with impaired fertility was not investigated [8]. Moreover, we found the association between *FSHR* gene variants with a risk of infertility and mastopathy [9]. Given the elucidation of the influence of these genes on reproductive processes [10], including replenishment of the follicular reserve [11], and the ability to select optimal protocols for *in vitro* fertilization [12], the aim of this study was to assess the combined genetic risk of developing mastopathy in women with the *ESR1* (rs9340799, rs2234693) and *FSHR* (rs6165, rs6166) gene variants.

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Abbreviations used: FSH – follicle-stimulating hormone; OR – odds ratio.

MATERIALS AND METHODS

The study included 87 women with reproductive disorders (long-term infertility over 7 years and infertility that occurred after early reproductive losses). Of these, 45 (51.7%) were women with concomitant fibrocystic mastopathy (code ICD-10 — N60.1.) — group 1, and 42 (48.3%) were women without mastopathy — group 2. Exclusion criteria were as follows: age over 48 years, chronic inflammatory infectious diseases, uterine abnormalities, and cancer. Informed consent was obtained from each participant included in the study. The study was approved by the Ethics Committee of the State Institution "Institute of Pediatrics, Obstetrics and Gynecology of the NAMS of Ukraine".

The *ESR1* (rs9340799, rs2234693) and *FSHR* (rs6165, rs6166) gene variants were determined using the molecular genetic methods according to previously published protocols [8–10].

Statistical data processing was conducted using Microsoft Excel Pro Plus 2016 and SPSS v.27. Genotype and allele frequencies in the study and comparison groups were compared using the χ^2 test. The association of variants of the studied genes with the risk of mastopathy was examined by calculating the odds ratio (OR) within 95% of the confidence interval (CI). Differences were considered reliable for all types of analysis at a significance level (p) less than 0.05.

RESULTS AND DISCUSSION

Previously we have analyzed the influence of *FSHR* gene variants on the risk of mastopathy and demonstrated that the patients of group 1 had significantly increased frequency of 307Ala/Ala and 680Ser/Ser genotypes in the *FSHR* gene ($\chi^2 = 6.39$, $p = 0.012$, OR = 4.49 (1.48–13.65)) as compared to patients of group 2. The risk of mastopathy in infertile women with this genotype increased almost by 4.5 times [9]. In the presence of 307Thr/Thr and 680Asn/Asn genotypes of the *FSHR* gene, a reduced risk of mastopathy 4.88 times was observed ($\chi^2 = 8.06$, $p = 0.005$, OR = 0.21 (0.07–0.59)). It should be noted that the significantly influential variants of the *FSHR* gene, which we have identified, are characterized by the linked nature of inheritance.

The molecular genetic analysis in the same study groups revealed no differences in the distribution of genotype frequencies for rs9340799 and rs2234693 variants of the *ESR1* gene in the groups of women with reproductive disorders with or without mastopathy (Figure). Therefore, *ESR1* gene variants does not seem to affect the risk of developing of mastopathy in the studied group of infertile women.

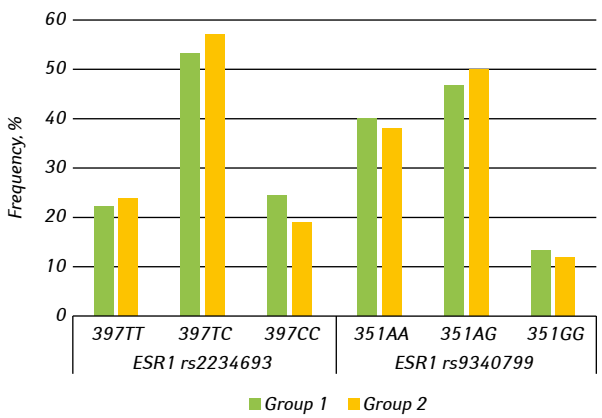

Figure. The frequencies of genotypes of the *ESR1* gene in the comparison groups

Table. The frequencies of identified combinations of *ESR1* and *FSHR* genotypes in the comparison groups

FSHR gene variants	*ESR1* gene variants		Group 1 (n = 45)	Group 2 (n = 42)
rs6165	rs2234693	rs9340799		
307Ala/Ala	397TT	351AA	5 (11.1%)	4 (9.5%)
	397TC	351AA	2 (4.4%)	1 (2.4%)
		351AG	5 (11.1%)	0 (0.0%)
		351GG	1 (2.2%)	0 (0.0%)
	397CC	351AG	2 (4.4%)	0 (0.0%)
		351GG	2 (4.4%)	0 (0.0%)
307Ala/Thr	397TT	351AA	4 (8.9%)	4 (9.5%)
	397TC	351AA	2 (4.4%)	1 (2.4%)
		351AG	9 (20.0%)	9 (21.4%)
		351GG	0 (0.0%)	1 (2.4%)
	397CC	351AA	1 (2.2%)	0 (0.0%)
		351AG	3 (6.7%)	0 (0.0%)
		351GG	3 (6.7%)	4 (9.5%)
307Thr/Thr	397TT	351AA	1 (2.2%)	2 (4.8%)
	397TC	351AA	3 (6.7%)	3 (7.1%)
		351AG	**2 (4.4%)**	**9 (21.4%)**
	397CC	351AA	0 (0.0%)	1 (2.4%)
		351AG	0 (0.0%)	3 (7.1%)

Note: Only in combination given in bold, the difference between two groups was significant ($p < 0.05$).

To elucidate the possible interaction between *ESR1* and *FSHR* genotypes and the possible modifying effect of the *ESR1* gene on the *FSHR*-depended risk of mastopathy, we investigated the effect of their combinations in infertile women on the risk of developing mastopathy. The data are summarized in the Table.

We found a significant decrease in the frequency of combination of genotypes 307Thr/Thr+680Asn/Asn+351AG+397TC in terms of the *FSHR* and *ESR1* genes in the group of women with reproductive disorders with mastopathy as compared to those without mastopathy (4.4% *vs* 21.4%, $\chi^2 = 4.43$, $p = 0.036$, OR = 0.17 (0.03–0.84)). Accordingly, in patients of group 1 as compared to group 2, we observed an increased frequency of detection of the combination of genotypes 307Ala/Ala+680Ser/Ser+351AG+397TC in terms of the *FSHR* and *ESR1* (11.1% *vs* 0.0%, $\chi^2 = 3.11$, $p = 0.078$) due to the linked nature of inheritance, but the differences were not significant. Thus, heterozygous variants of the *ESR1* gene enhance the "protective" effect 307Thr/Thr+680Asn/Asn variants of *FSHR* gene to the development of mastopathy in infertile women (5.86-fold risk reduction).

Recently, there has been an increased interest in determining the factors that lead to the development of benign breast tumors and their role in increasing the risk of breast cancer [13, 14]. A special place in these studies belongs to women with reproductive disorders, in particular, infertility, because hormonal imbalance and hormone therapy used in the treatment of these diseases are predictors of breast cancer. The results of a cross-sectional study conducted by Lundberg *et al*. [15] showed that women with a history of infertility have increased mammographic density.

In our previous study, we found that the presence of homozygous 307Ala/Ala and 680Ser/Ser genotypes of *FSHR* gene was associated with the development of mastopathy in infertile women (they were found in almost 40% of infertile patients) as in their presence the risk of mastopathy increased by 4.5 times [9]. However, these variants of the *FSHR* gene, in particular the presence of the 680Ser allele, are known to be associated with a decreased sensitivity to follicle-stimulating hormone (FSH) and are due to higher levels of serum FSH [11, 16]. On the other hand, the results of studies indicate the role of FSH in the development and progression of benign and malignant neoplasms of the breast [17, 18]. Meanwhile, our results confirm the findings of the above studies.

Our study of these variants of the *FSHR* gene indicates the possibility of developing pharmacogenetic tests that will be used in the treatment of infertile women. This is clearly confirmed by the existing data that patients with homozygous 680Ser/Ser genotype have a 2-fold increase in the risk of resistance to clomiphene citrate [19], which, on the one hand, is effec-

tive in the treatment of some forms of infertility, and on the other hand, may increase the risk of breast cancer [20]. Therefore, the use of this pharmacogenetic test will allow us to develop protocols for personalized prevention and treatment, as well as to optimize the purpose of clomiphene citrate, taking into account genetic characteristics.

We did not find significant differences for the studied variants of *ESR1* gene in infertile women. However, our previous studies have shown an association between these variants of *ESR1* gene with infertility in Ukrainian married couples. This discrepancy can be explained by different study designs, including the fact that currently, the study group included only infertile women who have frequencies of genotypes for variants in terms of *ESR1* gene that differ from the population ones [10].

For infertile women, in whom the markers of genetic risk have been identified, active prophylactic measures may be recommended to reduce the risk of developing benign and malignant breast tumors. In particular, the use of herbal preparations and vitamins that can help normalize hormonal balance is feasible [21–22].

Our study did not find an association of *ESR1* gene variants with the risk of developing of mastopathy in infertile women. But heterozygous variants of the *ESR1* gene enhanced the protective effect of *FSHR* gene variants and reduced the risk of mastopathy.

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ВАРІАНТИ ГЕНА *ESR1* ВПЛИВАЮТЬ НА *FSHR*-ЗАЛЕЖНИЙ РИЗИК РОЗВИТКУ МАСТОПАТІЇ У БЕЗПЛІДНИХ ЖІНОК

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***Стан питання*:** У жінок з безпліддям відмічають підвищений ризик розвитку доброякісних та злоякісних новоутворень, зокрема, локалізованих у молочній залозі. Більшість досліджень присвячені вивченню ролі варіантів генів в ризику розвитку раку молочної залози, але практично відсутні дані про генетичні чинники ризику виникнення доброякісних новоутворень. ***Мета*:** Оцінка комбінованого генетичного ризику розвитку мастопатії у жінок з варіантами генів *ESR1* (rs9340799, rs2234693) та *FSHR* (rs6165, rs6166) для формування стратегії персоналізованих клінічних рішень при лікуванні безпліддя. ***Матеріали та методи*:** До дослідження було залучено 87 жінок із безпліддям. Із них — 45 (51.7%) жінок із супутньою фіброзно-кістозною мастопатією та 42 (48.3%) жінки без мастопатії. ***Результати*:** Для варіантів rs9340799 та rs2234693 гена *ESR1* нами не було виявлено жодних вірогідних відмінностей у розподілі генотипів у безплідних жінок двох груп порівняння. У пацієнток з мастопатією було відмічено вірогідне підвищення частоти генотипів 307Ala/Ala та 680Ser/Ser гена *FSHR* (χ^2 = 6,39, p = 0,012, OR = 4,49 (1,48–13,65)) порівняно з особами без мастопатії. А за наявності генотипів 307Thr/Thr та 680Asn/Asn гена *FSHR* було виявлено зниження ризику розвитку мастопатії у 4,88 раза (χ^2 = 8,06, p = 0,005, OR = 0,21 (0,07–0,59)). Частота поєднання генотипів за генами *FSHR* та *ESR1* -307Thr/Thr+680Asn/Asn+351AG+397TC була значущо знижена у пацієнтів з мастопатією порівняно з пацієнтами без мастопатії (4,4% проти 21,4%, χ^2 = 4,43, p = 0,036, OR = 0,17 (0,03–0,84)). ***Висновки*:** У нашому дослідженні не було виявлено асоціацій варіантів гена *ESR1* з ризиком розвитку мастопатії у жінок з безпліддям. Але гетерозиготні варіанти гена *ESR1* посилювали "протективний" ефект варіантів гена *FSHR* і знижували ризик розвитку мастопатії.

***Ключові слова*:** мастопатія, безпліддя, *FSHR*, *ESR1*.