Abstract

I. M. Lukavenko,
Sumy State University, Sumy, Ukraine

PVUII POLYMORPHISM IN THE ESTROGEN RECEPTOR ALPHA GENE AS AN INDICATOR FOR SURGICAL TREATMENT IN PATIENTS WITH BENIGN MAMMARY DYSPLASIA

The paper presents the results of studying the relationship between the genetic characteristics of the individual and the phenotypic manifestations of benign mammary dysplasia. Data were provided on the role of PvuII polymorphism in the development of breast tissue proliferation through the mechanisms of EsRα overexpression; this can be used as a marker for surgical treatment necessity. The objective of the work was to develop criteria for the diagnosis of proliferative benign mammary dysplasia on the basis of immunohistochemical and molecular genetic studies to substantiate the indications for surgical treatment.

Materials and methods. The study involved 84 patients: 66 (78.6%) subjects from Sumy and 18 (78.6%) subjects – from the Sumy region. The mean age of the subjects was (32.3 ± 1.1) years, with the range of 16–62 years. Among the subjects, 82 (97.6%) were women with BMD and 2 (2.4%) were men who suffered from nodular gynecomastia. The burdened history of breast cancer in close relatives was reported in 33 (39.3%) individuals. Apart from a profound assessment of history data, the clinical course of the disease and comorbidities were studied. Instrumental and laboratory tests were performed. The morphological and immunohistochemical features of dissected tissues, as well as genetic differences of patients, were studied. By age, the subjects were divided into three groups: the first group (under 21 years) included 15 (17.8%) individuals, the second group (22–39 years) – 43 individuals (51.2%), the third group (over 40 years) – 26 individuals (31.0%).

Results. The frequency of allelic variants of the EsRα gene PvuII polymorphism in patients with a proliferative form of benign mammary dysplasia was distributed as follows: T/T genotype - 27.4%, T/C genotype – 51.2%, C/C genotype - 21.4%. The most significant clinical predictors in patients with proliferative benign mammary dysplasia were: mastodynia ($\chi^2 = 11.444$; P = 0.003), decreased BMI of up to (21.17 ± 1.06) kg/m^2 (F = 5.020; P = 0.009), prolonged menstruation of up to (5.67 ± 0.30) days (F = 3.017; P = 0.055). A group of patients whose mammary cells do not have estrogen receptors was identified. Since prescription of antiestrogens as a means of prevention in patients of this group will not be effective, such patients should be offered surgery as an option for further atypia prevention.

Conclusions. Additional studies of EsRα expression and the pathological C-allele of the EsRα gene PvuII polymorphism have been found to play an important role as criteria for the diagnosis of proliferative benign mammary dysplasia that substantiate indications for surgical treatment. The specificity of the histological structure of tissue, the features of the cell receptor apparatus, and genetic predictors are important indicators for understanding the causes and mechanisms of proliferation in BMD. The calculated results indicate that BMDs begin to develop against the background of retained menstrual cycle and reproductive function, which indicates a crucial role of local estradiol receptors status in breast tissue in the development of proliferation foci in BMD. Hormone imbalance contributes to morphofunctional changeover. The results of the study will serve as the basis for identifying patients prone to the development of BMD proliferative forms and their timely surgical treatment to prevent the development of malignancy.

Keywords: benign mammary dysplasia, PvuII polymorphism in the estrogen receptor α gene, tissue proliferation.

Corresponding author: *i.lukavenko@med.sumdu.edu.ua*

Резюме

І. М. Лукавенко,
Сумський державний університет, м. Суми, Україна

ПОЛІМОРФІЗМ PVUII ГЕНА РЕЦЕПТОРА ЕСТРАДІОЛУ АЛЬФА ЯК ПОКАЗНИК ДО ХІРУРГІЧНОГО ЛІКУВАННЯ ХВОРИХ НА ДОБРОЯКІСНУ ДИСПЛАЗІЮ МОЛОЧНИХ ЗАЛОЗ

У роботі представлені результати вирішення задачі стосовно зв'язку між генетичними особливостями індивідуума та фенотипічними проявами доброякісної дисплазії молочної залози. Наведені дані щодо ролі поліморфізму PvuII у розвитку проліферації тканини молочної залози через механізми гіперекспресії EsRα, що можна використовувати як критерій необхідності хірургічного лікування. Мета роботи полягала в розробці критеріїв діагностики проліферативної доброякісної дисплазії молочної залози на основі імуногістохімічних та молекулярно-генетичних досліджень із метою обґрунтування показань до хірургічного лікування.

Матеріали і методи. Спостереженням було охоплено 84 пацієнти: 66 (78,6 %) осіб м. Сум та 18 (78,6 %) – Сумської області. Середній вік обстежених становив (32,3 ± 1,1) роки і був у межах 16 – 62 років. Серед досліджених – 82 (97,6 %) жінки з ДДМЗ та 2 (2,4 %) чоловіки, які страждали на гінекомастію з вузлоутворенням. Обтяжений анамнез на РМЗ у близьких родичів мали 33 (39,3 %) особи. Крім поглиблених даних щодо анамнезу, вивчали особливості клінічного перебігу та супутніх захворювань. Проводили інструментальні та лабораторні дослідження. Вивчалися морфологічні та ІГХ-особливості видалених тканин, а також генетичні відмінності пацієнтів. За віком досліджені були розділені на три групи: до першої групи (до 21 року) ввійшло 15 (17,8 %) осіб, до другої (22–39 років) – 43 (51,2 %), до третьої (старших за 40 років) – 26 (31,0 %) досліджених.

Результати. Показано, що частота алельних варіантів гена EsRα за поліморфізмом PvuII у пацієнтів із проліферативною формою доброякісної дисплазії молочної залози розподілилася так: генотип Т/Т – 27,4 %, генотип Т/С – 51,2 %, генотип С/С – 21,4 %. Установ-

лено, що найбільш значущими клінічними предикторами у хворих на проліферативні форми доброякісної дисплазії молочної залози визначаються: мастодинія (χ^2 = 11,444; P = 0,003), зменшення ІМТ до (21,17 ± 1,06) кг/м^2 (F = 5,020; P = 0,009), подовжена менструація до (5,67 ± 0,30) дня (F = 3,017; P = 0,055). Визначена група пацієнтів, клітини молочної залози яких не мають рецепторів до естрогену. Зроблено висновок про те, що оскільки призначення антиестрогенів як засобу профілактики пацієнтам цієї групи не дасть бажаного результату, таким хворим необхідно запропонувати інший варіант профілактики подальшої атипії – хірургічний.

Висновки. Установлено, що додаткові дослідження експресії EsRα та наявності патологічного С-алеля за поліморфізмом PvuII гена EsRα відіграють важливу роль як критерії діагностики проліферативної доброякісної дисплазії молочної залози для обґрунтування показань до хірургічного лікування. Показано, що специфіка гістологічної будови тканини, особливостей рецепторного апарату клітини та генетичних предикторів виступає важливим показником у розумінні причин та механізмів проліферації при ДДМЗ. Розрахункові результати вказують на те, що ДДМЗ починають формуватися на тлі збереженого менструального циклу та репродуктивної функції, що говорить про вирішальну роль у розвитку осередків проліферації при ДДМЗ стану локальних рецепторів естрадіолу в тканині молочної залози. Дисбаланс гормонів сприяє морфофункціональній перебудові. Результати роботи будуть покладені в основу виявлення пацієнток, схильних до розвитку проліферативних форм ДДМЗ та їх своєчасного хірургічного лікування для запобігання розвитку малігнізації.

Ключові слова: доброякісна дисплазія молочної залози, поліморфізм PvuII гена рецептора естрадіолу альфа, проліферація тканини.

Автор, відповідальний за листування: *i.lukavenko@med.sumdu.edu.ua*

Introduction

Pre-cancerous mammary conditions have recently become widespread both in Ukraine and around the world. The incidence of benign mammary dysplasia (BMD) and its proliferative forms, which are the background for breast cancer (BC), among women of reproductive age, equals 70–95%. The main etiological factor that provokes changes in mammary glands is considered to be estrogen imbalance. Thus, under the influence of steroid hormones, the proliferative activity of mammary epithelial cells is manifested. Hyperestrogenism plays a significant role in the development of mastopathy and breast cancer [1, 2]. In the practice of a mammologist, there are many cases when pre-cancerous pathological changes in mammary glands are due to the level of expression of hormone receptors rather than to their circulating levels. However, information on the localization of these receptors in a mammary gland has been insufficiently studied and relates mainly to breast cancer studies [3–5].

Diagnostic studies of mammary glands today are focused on focal neoplasm detection. However, in 56% of subjects, atypical mammary hyperplasia proved to occur without nodulation. Hence, the frequency of errors with cytological diagnosis in patients with benign mammary tumors reaches 7%, and the diagnostic value of puncture fails in 18.6% [6].

Recently, some studies have been performed on the influence of genetic factors, namely simple single nucleotide polymorphisms, on the human phenotype. Some information is available on the influence of single polymorphisms in the estrogen alpha gene (EsRα) on the development of mammary gland proliferation [7–9]. But information on the functional influence of individual polymorphisms is limited. A study of the PvuII polymorphism in the EsRα gene and

provision of information on the features of estrogen-positive or estrogen-negative status of breast cancer may contribute to the effectiveness of estrogen therapy.

A study of allelic polymorphism along with clinical and laboratory findings in individuals with pre-cancerous conditions in the breast will provide useful information for the diagnosis, prevention, and treatment. The issue of diagnosis of pre-cancerous mammary gland conditions as factors of high risk for breast cancer remains relevant.

Given the growing incidence of proliferative forms of mastopathy in recent decades and the young age of those suffering from these processes, it remains essential to find mechanisms for early diagnosis and more relevant criteria for assessing pre-cancerous conditions [10]. However, traditional invasive methods for clinical and instrumental assessments and morphological changes evaluation are not informative enough, and their use during pregnancy and lactation is limited. Therefore, the search for molecular genetic predictors of pre-cancerous conditions is becoming increasingly important, which determines the relevance of this problem.

The objective of the work was to develop criteria for the diagnosis of proliferative benign mammary dysplasia on the basis of immunohistochemical and molecular genetic studies to substantiate the indications for surgical treatment.

Materials and methods

The study and subject assessments were conducted in compliance with the main provisions of the European Convention of Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on Ethical Principles for Medical Research Involving Human Subjects and the Order of the Ministry of Health of Ukraine No. 616 dated 03.08.2012. All patients signed the written consent form for taking part in the study and genetic analysis.

The subjects were examined on an outpatient basis by a surgeon working under license conditions (license AG No. 600519), were operated at the clinical sites of the Department of Surgery with a Course of Pediatric Surgery and Urology, in particular at the Sumy Regional Oncology Center; morphological material was studied at the Center for Pathomorphological Research of the Department of Pathological Anatomy of Sumy State University; molecular genetic research was

conducted at the molecular-genetic research laboratory of SSU.

All patients were treated surgically. The inclusion criteria were the following: multiple primary tumors in one organ; multiple primary tumors in different organs; bilateral primary tumors in paired organs; multifocal lesions within one organ; early-onset cancer (up to 21 years); ≥ 1 close relative with the same type of tumor; ≥ 2 relatives with the same type of tumor; ≥ 2 relatives with one localization tumors; ≥ 2 relatives with tumors belonging to familial cancer; ≥ 2 relatives with a rare form of cancer; ≥ 3 relatives in two generations with tumors of one localization.

The exclusion criteria were: non-proliferative changes in mammary glands; no signs of genetic predisposition to breast diseases; refusal of a patient to participate in the study.

Mammary gland tissue and blood were examined. For histological examination, the material was taken during surgery and placed in a container with formalin. Blood was drawn from a peripheral vein before or after surgery according to standard methods and stored at -20 °C.

Modern highly informative research methods were used in the study, involving reagents and equipment from leading manufacturers of laboratory and diagnostic equipment.

During breast examination, we determined size, symmetry, the condition of the nipple-areolar complex and regional lymph nodes, mammary glands discharge, if any. All patients had their clinical and biochemical blood parameters determined and blood tests for estradiol performed. For mammary glands discharge, a cytological study was carried out.

Estradiol was studied by analyzing a combination of a competitive enzyme-linked immunosorbent assay and fluorescent determination of reaction products; blood plasma analysis was performed.

The study involved 66 (78.6%) subjects from Sumy and 18 (78.6%) subjects – from the Sumy region. The mean age of the subjects was (32.3 ± 1.1) years, with the range of 16–62 years. Among the subjects, 82 (97.6%) were women with BMD and 2 (2.4%) were men who suffered from nodular gynecomastia. The burdened history of breast cancer in close relatives was reported in 33 (39.3%) individuals.

Apart from a profound assessment of history data, the clinical course of the disease and comorbidities were studied. Instrumental and

laboratory tests were performed. The morphological and immunohistochemical features of dissected tissues, as well as genetic differences of patients, were studied. By age, the subjects were divided into three groups: the first group (under 21 years) included 15 (17.8%) individuals, the second group (22–39 years) – 43 individuals (51.2%), the third group (over 40 years) – 26 individuals (31.0%).

The analysis of anthropometric data showed no deviations in the subjects: the average body weight was (59.4 ± 1.2) kg, the average height – (166.6 ± 0.63) cm, and BMI – (21.41 ± 0, 42) kg/m^2. Duration of the underlying disease (BMD) averaged (2.65 ± 0.20) years. The average size of lesions was (22.5 ± 1.23) mm.

Forty-four subjects (53.4%) were diagnosed with one mammary neoplasm, 34 (40.5%) – with two neoplasms, and 6 (7.1%) – with three lesions. Neoplasms were resected in all subjects, followed by histological or immunohistochemical study. Thus, 74.6% of patients with a proliferative form of BMD were operated on for multifocal mammary lesions.

Given the influence of hormones, particularly estradiol, on the development of BMD, special attention was paid to the analysis of obstetric and gynecological history. Thus, menarche in the subjects occurred on average at (13.30 ± 0.18) years. The duration of menstrual cycle was (27.81 ± 0.35) days. 28 (33.3%) women reported a history of abortions. 41 (48.8%) subjects had chronic diseases of the uterus and uterine appendages: adnexitis, menstrual disorders, ovarian cysts; 42 patients (52.0%) had a history of parturition and lactation.

Ultrasound and mammography were the primary and standard procedures for diagnosing BMD. Retrospective analysis of ultrasound and mammography data was performed on the individual basis for each tumor. Ultrasound examination was performed using Toshiba Nemio XG SSA-580A ultrasound diagnostic system (Japan) (multifrequency linear sensor with a frequency of 6–12 MHz). Mammography was performed with a Hologic Lorad M-IV with a Kodak Direct View Classic CR digitizer (USA).

One hundred thirty-four mammary neoplasms obtained from 84 operated patients were studied. In 72 (53.7%) cases, the samples subjected to morphological examination were resected from one organ, and in 62 (46.3%) cases, neoplasms were resected in patients with bilateral breast lesions. The average time of preclinical observation of the subjects was (2.78 ± 0.161) years. The average size of a lesion was (22.29 ± 1.02) mm.

Distribution of BMD morphological samples by age demonstrated that most of the surgical specimens resected for BMD – 94 (70.2%) – were taken from young patients (up to 40 years); 40 (29.8%) morphological samples were taken from the patients over 40 years of age. A significant number of morphological samples was due to multiple mammary lesions.

BMD samples with stage 3–4 proliferative activity and metaplasia and samples with a tendency to atypical changes were observed in 86 (64.2%) subjects. Neoplasms with indolent stage 1–2 proliferative activity were a minority – 48 (35.8%) cases.

Venous blood was taken from the patients under sterile conditions into monovettes (2.7 ml) containing ethylenediaminetetraacetic acid (EDTA) potassium salt (11.7 mM) as an anticoagulant ("Sarstedt," Germany); after that, the blood was frozen and stored at a temperature -20°C.

DNA was extracted from undiluted blood using DIAtom DNA Prep 100 kit («Isogene», Russia). This method was based on the use of guanidine isocyanate lysis reagent intended for cell lysis, solubilization of cellular debris, and also for denaturation of cellular nucleases. With lysis reagent, DNA was actively absorbed with NucleoS™ silica solution; then, DNA was easily washed from proteins and salts by spirit solution. Further, the DNA was extracted from the sorbent and transferred to sterile DNA- and RNA-free microtubes. The obtained DNA was directly used for polymerase chain reaction (PCR). The kit helps to extract high-molecular-weight DNA from fresh biological material (40–50 kilobase pairs of high purity (OD$_{260/280нм}$ 1.6–2.0)). Pure DNA yield from 100 mcL of whole blood equaled 3–5 mcg.

PvuII (international name rs2234693) is a polymorphism in intron 1, at position 152.163.335, nucleotide substitution at position 1943 T> C; it was determined by polymerase chain reaction (PCR) followed by the analysis of restriction fragment length polymorphism (PCR-RFLP). The promoter region of the gene was amplified using a pair of specific primers synthesized by "Metabion" (Germany): a direct one (sense) -5 'CACACATCACCATTCTCAGC 3' and a reverse one (antisense) – 5` TCTAGACCACACTCAGGGTCTC 3'.

Prior to the analysis, the clinical associations of genotype distribution for the studied PvuII

polymorphism were adjusted in accordance with Hardy–Weinberg equilibrium.

Terms of Hardy's law: the population of diploid organisms is so large that random fluctuations in gene frequencies can be ignored; no new mutations of the studied gene in the population; no migration of individuals in the population; in the population, there is a selection by the genotypes studied; in the case of a dichotomous polymorphism, the frequencies of genotypes in the population remain constant from generation to generation and comply with the Hardy-Weinberg ratio:

p2 + 2pq + q2 = 1,

where *p* and *q* – frequencies of the corresponding alleles.

Analysis of allele and genotype distribution for the PvuII polymorphism in the EsRα gene located on chromosome 6 (6q25.1) was performed. All studied genotypes complied with the Hardy-Weinberg law.

When analyzing the distribution of genotype variants for PvuII polymorphism in the EsRα gene, the following results were obtained: genotype T/T – 36 (26.9%), genotype T/C – 71 (53.0%), genotype C/C – 27 (20.1%). It was found that depending on the receptor status of tumors, the correlation of the major allele homozygotes (T/T), heterozygotes (T/C) and the minor allele homozygotes (C/C) was 4

(5.4%), 43 (58.1%) and 27 (36.5%), respectively, for samples with EsRα-; for EsRα+ samples, the corresponding values were 32 (53.3%,) 28 (46.7%) and 0 (0%).

Study results and discussion

Using statistical analysis methods, the correlation was studied between the clinical course of BMD and the main risk factors for the disease, as well as the influence of EsRα expression level in breast tissue and allelic distribution of the PvuII polymorphism in the EsRα gene.

The data in Table 1 indicate that body weight in patients with proliferative forms of BMD is significantly different (F = 8.050; P = 0.001) and depends on the genotype of the PvuII polymorphism in the EsRα gene. For example, bodyweight in women with T/T genotype was (66.40 ± 2.97) kg, with genotype T/C – (55.94 ± 1.06) kg, with genotype C/C – (58.89 ± 2.42) kg. The major allele homozygotes (T/T) have been shown to have significantly higher body weight than T/C genotype heterozygous carriers and the minor allele homozygotes (C/C). In patients with proliferative forms of BMD with different genotypes by the PvuII polymorphism, BMI and shoe size showed a significant difference (F = 5.020; P = 0.009 and F = 4.756; P = 0.011, respectively).

Table 1 – Anthropometric parameters in BMD patients depending on the genotype variant for the Pvull polymorphism in the EsRα gene (M ± m)

Parameter	T/T	T/C	C/C	F	P
	(n = 23)	(n = 43)	(n = 18)		
Body weight, kg	66.40 ± 2.97	55.94 ± 1.06	58.89 ± 2.42	8.050	0.001
Height, cm	168.35 ± 1.08	165.37 ± 0.81	167.28 ± 1.69	2.202	0.117
BMI, kg/m^2	23.41 ± 1.01	20.45 ± 0.35	21.17 ± 1.06	5.020	0.009
Shoe Size (eur)	38.26 ± 0.29	37.33 ± 0.151	37.50 ± 0.31	4.756	0.011
Height of the glandular part of the breast (mm)	12.52 ± 0.65	13.98 ± 0.76	13.44 ± 0.70	0.849	0.431
Height of the fibroglandular part of the breast (mm)	19.31 ± 1.50	18.37 ± 0.82	19.28 ± 1.30	0.252	0.778

In Tables 1–7, the following notation is used: n – number of subjects; k – number of morphological samples; EsRα- – estrogen receptor-negative samples; EsRα+ – estrogen receptor-positive samples.

The data in Tables 2 and 3 show that all patients were divided into three age groups. The first group (under 21 years) included 15 (17.8%) individuals; the second group (22–39 years) – 43 individuals (51.2%), the third group (over 40 years) – 26 individuals (31.0%), respectively. Statistical analysis and comparison performed in each group

by receptor status of EsRα expression in BMD tumors, depending on the age of patients, showed that in the first group (under 21 years), EsRα-negative samples were found in 43.5% of cases, while EsRα-receptor-positive samples were observed in 56.5% of cases. In the second group, receptor-negative samples were observed in 39.4%, and receptor-positive samples – in 60.6%. In the third group (over 40 years), receptor-negative samples were found in 55.0%, and receptor-positive samples – in 45.0%.

Table 2 – Frequency of EsRα expression in different age group patients with BMD

EsRα expression	Age of patients, k (%)		
	Up to 21	22–29 years	More than 40
EsRα-	10 (43.5)	28 (39.4)	22 (55.0)
EsRα+	13 (56.5)	43 (60.6)	18 (45.0)
Total	23 (100)	71 (100)	40 (100)

$\chi^2 = 2.525$; P = 0.283

The C/C variant was 2.3 times more frequent than the T/T variant. In the third group, the T/C heterozygous variant was observed in 46.2% of cases, which was 1.2 times more often than the T/T variant (38.5%), and 3.0 times more often than the C/C variant.

The analysis of the results showed that the receptor status of BMD tumors did not depend on patients' age ($\chi^2 = 2.525$; P = 0.283). In addition, the distribution of EsRα gene allelic variants for the PvuII polymorphism did not differ significantly among the age groups ($\chi^2 = 2.620$; P = 0.623). Thus, we can state that among the studied patients, no statistically significant relationship was found between the receptor status features and the PvuII polymorphism depending on age.

Depending on the site of BMD tumors, patients were divided into two groups: patients operated on for unilateral breast lesion – 54 (64.29%) subjects, and patients operated on for bilateral lesion – 30 (35.71%) subjects, respectively (Table 4). The table shows that in C/C homozygotes the studied PvuII

polymorphism did not differ statistically from that among the major allele homozygotes (T/T) and did not lead to a specific localization of BMD foci. The studied group of patients was found to have no statistical correlation between unilateral or bilateral breast lesions and the studied polymorphism ($\chi^2 = 1.281$; P = 0.527).

Table 3 – Frequency of allelic variants of the EsRα gene PvuII polymorphism in different age group patients with BMD

Genotype	Age of patients, n (%)		
	Up to 21	22–39 years	More than 40
T/T	3 (20.0)	10 (23.3)	10 (38.5)
T/C	8 (53.3)	23 (53.4)	12 (46.2)
C/C	4 (26.7)	10 (23.3)	4 (15.3)
Total	15 (100)	43 (100)	26 (100)

$\chi^2 = 2.620$; P = 0.623

Due to the fact that the level of estradiol plays an essential role in the functioning of the mammary glands, all patients were tested for the level of estradiol in the blood. The latter was increased in 10 (11.9%) patients. The distribution of EsRα gene allelic variants for the PvuII polymorphism did not differ significantly between patients with physiological estradiol levels in the blood and patients with hyperestrogenism ($\chi^2 = 0.409$; P = 0.815). Thus, among the patients we operated on, the pathological genotype (C/C) of the EsRα gene PvuII polymorphism did not lead to hyperestrogenism.

Table 4 – Frequency of allelic variants of the EsRα gene PvuII polymorphism in patients with unilateral and bilateral lesions and patients with different levels of estradiol in the blood

Genotype	Unilateral lesion, n (%)	Bilateral lesion, n (%)	Normal level, n (%)	Increased level, n (%)
T/T	17 (31.5)	6 (20.0)	21 (28.4)	2 (20.0)
T/C	26 (48.1)	17 (56.7)	37 (50.0)	6 (60.0)
C/C	11 (20.4)	7 (23.3)	16 (21.6)	2 (20.0)
Total	54 (100)	30 (100)	74 (100)	10 (100)

$\chi^2 = 1.281$; P = 0.527 | | | $\chi^2 = 0.409$; P = 0.815 | |

Studies were conducted on the association between gynecological diseases among operated patients and allelic variants of the EsRα gene PvuII polymorphism (Table 5). Groups of patients with pre-existing gynecological condition and patients without it were almost equal in number: 41 (48.8%) and 43 (51.2%). The P-index by Pearson's χ^2 test was 0.306. Therefore, the C/C genotype of the

EsRα gene PvuII polymorphism was not associated with pre-existing gynecological diseases.

The distribution of patients operated for BMD depending on the receptor status of resected neoplasms was analyzed. When comparing morphological samples by EsRα receptor status, no significant difference was found between the patients with pre-existing gynecological diseases

and the patients without them: P-value determined by Pearson's χ^2 test was equal 0.783. Thus, there is no correlation between the EsRα receptor status of mammary tumors and gynecological diseases. The presence of mastodynia depending on allelic variants of the EsRα gene PvuII polymorphism, was also studied (Table 6).

Table 5 – Frequency of allelic variants of the EsRα gene PvuII polymorphism depending on concomitant gynecological diseases

Genotype	Existing gynecological disease, n (%)	No existing gynecological disease, n (%)
T/T	13 (31.7)	10 (23.2)
T/C	22 (53.7)	21 (48.8)
C/C	6 (14.6)	12 (12.0)
Total	41 (100)	43 (100)
$\chi^2 = 2.368$; P = 0.306		

The T/T genotype tended to be observed in women who did not have breast pain and edema, while the C/C genotype was associated with the development of mastodynia. Among the examined patients who had complaints of mastodynia, the correlation of the major allele homozygotes (T/T), heterozygotes (T/C) and the minor allele homozygotes (C/C) was 20.7, 48.3, and 31.0%, respectively. In patients who did not report mastodynia, the distribution was different: T/T – 42.3%, T/C – 57.7%, C/C – 0%. The P-value determined by Pearson's χ^2 test was 0.003, which indicated a significant difference in the distribution of allelic variants of the EsRα gene for the PvuII polymorphism in patients depending on the presence of mastodynia.

Given the influence of genetic characteristics on the development of various diseases, we found it important to study patients' family history. The examined patients were divided into two groups according to the presence of BC in close relatives. Thus, the first group (with unremarkable family history) included 51 (60.7%) patients.

Conclusions

1. Additional studies of EsRα expression and the pathological C-allele of the EsRα gene PvuII polymorphism have been found to play an important role as criteria for the diagnosis of proliferative benign mammary dysplasia that substantiate indications for surgical treatment.

2. The specificity of the histological structure

Table 6 – The correlation betwen allelic PvuII polymorphism in the EsRα gene and mastodynia in patients with proliferative BMD

Genotype	No mastodynia, n (%)	Existing mastodynia, n (%)
T/T	11 (42.3)	12 (20.7)
T/C	15 (57.7)	28 (48.3)
C/C	0 (0)	18 (31.0)
Total	26 (100)	58 (100)
$\chi^2 = 11.444$; P = 0.003		

The second group (burdened family history) included 33 (39.3%) patients. The results obtained for the distribution of patients by the genotype of the studied polymorphism depending on family history of breast cancer are presented in Table 7.

Table 7 – Frequency of allelic variants of the EsRα gene PvuII polymorphism in the operated patients depending on the family history of breast cancer

Genotype	Unremarkable family history, n (%)	Burdened family history, n (%)
T/T	15 (29.4)	8 (24.2)
T/C	23 (45.1)	20 (60.6)
C/C	13 (25.5)	5 (15.2)
Total	51 (100)	33 (100)
$\chi^2 = 2.136$; P = 0.344		

The frequency of different allelic variants of the EsRα gene for the PvuII polymorphism in the studied groups did not differ significantly. The difference in the distribution of subjects with different allelic variants of the gene was insignificant ($\chi^2 = 2.136$; P = 0.344). Thus, the pathological allele (C/C) of the PvuII polymorphism in the EsRα gene was not associated with a burdened family history of breast cancer in the studied patients.

of tissue, the features of the cell receptor apparatus and genetic predictors are important indicators for understanding the causes and mechanisms of proliferation in BMD.

3. The calculated results indicate that BMDs begin to develop against the background of retained menstrual cycle and reproductive function, which indicates a crucial role of local estradiol receptors

status in breast tissue in the development of proliferation foci in BMD. Hormone imbalance contributes to morphofunctional changeover.

4. The results of the study will serve as the basis for identifying patients prone to the development of BMD proliferative forms and their timely surgical treatment to prevent the development of malignancy.

Prospects for future research

Prospects for further research are related to the issue of surgical treatment advisability in patients with benign proliferative dysplasia of the mammary glands and prevention of malignancy based on the results of molecular genetic methods.

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Conflict of interest

The authors declare no conflict of interest.

Відомості про авторів

Лукавенко Іван Михайлович, к. мед. н., асистент кафедри хірургії, травматології, ортопедії та фтизіатрії, Сумський державний університет, вул. Римського-Корсакова, 2, м. Суми, Україна (e-mail: i.lukavenko@med.sumdu.edu.ua , тел.: 095-535-43-84).