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BRIEF COMMUNICATION

Contribution of *BRCA1* 5382insC mutation to triplene-gative and luminal types of breast cancer in Ukraine

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Abstract

Purpose The gene BRCA1 plays a key role in DNA repair in breast and ovarian cell lines and this is considered one of target tumor suppressor genes in same line of cancers. The 5382insC mutation is among the most frequently detected in patients (Eastern Europe) with triple-negative breast cancer (TNBC). In Ukraine, there is not enough awareness of necessity to test patients with TNBC for BRCA1 mutations. That is why this group of patients is not well-studied, even through is known the mutation may affect the course of disease.

Methods The biological samples of 408 female patients were analyzed of the 5382insC mutation in BRCA1. We compared the frequency of the 5382insC mutation in BRCA1 gene observed in Ukraine with known frequencies in other countries.

Results For patients with TNBC, BRCA1 mutations frequency was 11.3%, while in patients with luminal types of breast cancers, the frequency was 2.8%. Prevalence of 5382insC among TNBC patients reported in this study was not different from those in Tunisia, Poland, Russia, and Bulgaria, but was higher than in Australia and Germany.

Conclusion The BRCA1 c.5382 mutation rate was recorded for the first time for TNBC patients in a Ukrainian population. The results presented in this study underscore the importance of this genetic testing of mutations in patients with TNBC. Our study supports BRCA1/2 genetic testing for all women diagnosed with TNBC, regardless of the age of onset or family history of cancer and not only for women diagnosed with TNBC at <60y.o., as guidelines recommend.

Keywords 5382insC mutation of *BRCA1* · Triple-negative breast cancer · Luminal breast cancer · Ukraine

Introduction

Breast cancer is the most common form of cancer among women worldwide. Ukraine shows similar incidence rates as the rest of the world, according to the National Cancer Registry of Ukraine 2019–20, where 138,509 new cases of malignant neoplasms were registered in 2019. Among them, breast cancer accounted for 14,855 cases (14,720 in women and 135 in men) [1].

Breast cancer 1 (*BRCA1) gene* (17q21, chromosome 17: base pairs 43,044,294 – 43,125,482) encodes a 1,863-amino acid protein and is composed of 24 exons. BRCA1 carries the N-terminal region with zinc binding RING finger domain, which is essential for BRCA1-BARD1 (BRCA1 Associated RING Domain protein 1) interaction and formation of E3 ubiquitin ligase complex. The *C* terminus hosts two BRCT (BRCA1 C-terminal) phosphopeptide-binding

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domains that mediate interaction of BRCA1 with key partner proteins such as CtIP (C-terminal binding protein 1 (CtBP1) interacting protein), BRCA1 A Complex Subunit (ABRAXAS), and BRCA1 interacting protein C-terminal helicase 1 (BRIP1) [2–4]. It is reported that the central part of *BRCA1*, at 11–13 exons, is highly variable [5]. More than 1600 mutations have been identified for *BRCA1*. Some of these genetic alterations are mutations which occur with high frequency in isolated groups and are referred to as founder mutations. Among these there are the 185delAG mutation in RING and BRCT domains reported for Ashkenazi population and 5382insC (also known as 5266dupC or 5385insC) that we reported for Scandinavia or northern Russia [6–8]. 5382insC appears to be the most common mutation among Eastern European patients with breast or ovarian cancer [9–13]. The 5382insC mutation rate in unselected breast cancer patients in Ukraine is reported at 4,7%, in ovarian cancer patients – 5,9% [10, 14].

Triple-negative breast cancer (TNBC) is one of four known molecular subtypes of breast cancer. Tumors of this subtype are characterized as estrogen receptor (ER)-negative and progesterone receptor (PR)-negative and feature underexpression of human epidermal growth factor receptor 2 (HER2). According to historical data, triple-negative tumors account for 10–20% of all breast cancers [15–17]. There is no data of breast cancer cases distributed by molecular biological subtypes in Ukraine at present.

BRCA1 is an essential breast cancer predisposition gene for TNBC [18]. Two studies of unselected TNBC cases in USA have shown that 9–14% overall and ~20% of cases diagnosed under age 50 harbor germline *BRCA1* mutations [19]. Furthermore, *BRCA1* is responsible for 34% of hereditary TNBC development [20]. The presence of mutations in *BRCA1*gene makes the prognosis of breast cancer worse and necessitates the modification of treatment [21]. At the same time, patients with *BRCA1* positive TNBC have significantly improved prognosis with respect to chemotherapy, relative to non-carriers. [22–25].

The frequencies and spectrum of mutations in the *BRCA1* gene in TNBC patients have been characterized for a number of populations and have ethnic features [26]. According to the Triple-Negative Breast Cancer Consortium, the overall mutation rate in the *BRCA1* gene varies from 11,2% (102/913) in the USA to 3,5% (3/87) in Finland [27]. In total, in a study with a large sample of 1824 TNBC patients from five countries (USA, Finland, UK, Greece and Germany) one of the most frequently detected mutations was 5382insC which occurred at a rate of 1% (19/1824)[27]. However, the frequency of this mutation in patients with TNBC is characterized only for a small number of countries and often with small sample sizes: e.g. Germany 3,8% (11/291), Tunisia 6% (2/33), Poland 14,5% (18/124), Australia 0,2% (1/439), Bulgaria 5% (1/20), and Russia 31% (5/16) [28–33]. The frequency of the 5382insC founder mutation varies among countries from complete absence [34] to as high as 31% and therefore needs to be studied on case-by-case basis.

Modern oncology has made significant progress in treatments, which was achieved by effective action on known targets (i.e. ER, PR, HER2). TNBC has no known targets for treatment, which is one of the biggest problems in oncology. Approximately 75% of TNBCs express basal markers (cytokeratin 5, cytokeratin 14 and epidermal growth factor receptor), whereas 15–20% of basal tumors are not triple-negative [35]. TNBC is usually represented by low-grade invasive carcinoma, younger age of patients compared to luminal subtypes, high proliferative activity and rapid tumor growth. All this makes triple-negative tumors more aggressive with worse prognoses. TNBC patients respond better to neoadjuvant chemotherapy, an indicator of this is higher pathologic complete response (pCR) rates compared to other subtypes [36, 37]. The problem with patients who have not received pCR is a poor prognosis and a higher risk of chemotherapy resistance [36]. There are genetically determinate forms of breast cancer, with mutations in BRCA genes as the most common abnormalities, which significantly affect the prognosis, but also the epigenetic effects associated with these genes, and the overall course of the disease [37–40].

The aim of this study was to estimate the frequency of 5382insC *BRCA1* mutation in patients with TNBC and to perform comparative analysis with mutation frequencies in patients with luminal subtypes of breast cancer in Ukraine.

Materials and methods

Study Participants

The study involved 408 female patients, 338 of whom had clinically diagnosed breast cancer, including 124 patients with TNBC, 214 patients with luminal types of breast cancer and 70 people without breast cancer. Information about ER, PR, HER2 tumors and other information was obtained from the clinical records and accessed with the patients' permission. Expression of steroid hormones was considered positive at > 1% (with additional threshold at > 10%). HER2 was determined as negative, 0 or positive by immunohistochemical assay. When there was a questionable result of HER2 (2+), a fluorescent in situ hybridization was performed for cross-confirmation.

Biological samples were collected at the Kyiv City Clinical Oncology Center (136 patients), National Cancer Institute (Ukraine) (63 patients), and the State Institution National Research Center for Radiation medicine of NAMS (139 patients) of Ukraine between 2015 and 2019. All patients provided written informed consent in accordance with the Declaration of Helsinki. The study was

approved by the local ethics committee (Committee on Bioethics: Bogomolets National Medical University, Kyiv (# 0120U100871)). Information about the age of onset of the disease was available for 328 patients. Among patients with TNBC, 23 displayed early onset (at or before 40 years old), and 92 had an onset after 40 years of age. Among patients with luminal types of breast cancer, 58 showed early onset while 155 had an onset after 40 years of age. All patients resided in Ukraine and identified as Ukrainians.

Analysis of the 5382insC mutation in *BRCA1*

Genomic DNA was isolated from dried blood drops or from peripheral blood using multiple different commercially available kits: Quick-DNA^{TM}Universal Kit (ZymoResearch, USA), DNA extraction kit DNA-SORB-B (AmpliSense, Russian Federation) and NeoPrep^{100} DNA Magnet (NeoGene, Ukraine). The *BRCA1* mutation 5382insC was detected by mutagenically separated PCR (MS-PCR) using published specific primers or Real-Time PCR (RT-PCR). The method used depended on the laboratory in which the testing took place. In MS-PCR we used three primers as described by Chan et al. (1999) with minor optimization. The amplification assay was performed in a volume of 20 µl (2 µl of 10 × PCR buffer (10 × DreamTaq Buffer, «Thermo Scientific», USA), 2 µl of 2 mm dNTP («Thermo Scientific», USA), 2 µl of 2.5 mM MgCl2 («Thermo Scientific», USA), 0.4 µl of 20 mm primers, 1 unit of Taq polymerase («Thermo Scientific», USA), 10 µl of distilled water) and 3 µl of genomic DNA. Reaction scheme: 95 °C for 3 min; 40 cycles: 94 °C for 30 s; 52 °C for 30 s; 72 °C for 30 s; elongation 72 °C 4 min. Visualization of products was performed in 3% agarose gel. Each sample was analyzed in duplicate. Blood samples for real-time PCR analysis were collected into Vacutest tubes with K3-EDTA as an anti-coagulant («Vacutest Kima», Italy). The BRCA kit («DNA Technologia», Russian Federation) was used according to the manufacturer's instructions to detect the specific mutation. Real-time PCR was performed using the 7500 Real-Time PCR System («Applied Biosystems», USA).

Statistical analysis

Age differences between groups was assessed with a Mann–Whitney test (U). Fisher's exact test (F) was used to compare frequencies of the mutation between various cancer types and different populations. A p-value below 0.05 indicated statistical significance.

Results

We analyzed the *BRCA1* mutation 5382insC in 338 patients with breast cancer. Among them, 124 patients were with TNBC and 214 patients with luminal types of breast cancer. Twenty cases of mutation were detected, which makes the mutation prevalence (frequency) 5.9%. For patients with TNBC, a total of 14 *BRCA1* mutations were reported (frequency 11.3%), while in patients with luminal types of breast cancers the frequency was 2.8%. Therefore, the frequency of *BRCA1* mutations in patients with TNBC is significantly higher than in patients with hormone-dependent types of breast cancer (F, $p = 0.0041$). We found no *BRCA1* mutations in the control cohort of patients without breast cancer.

Data on the age of onset of the disease was available for 328 patients. The mean age was 49.26 ± 0.72 years. The mean age of patients with TNBC was 50 ± 1.23 years ($N = 12$), whereas with luminal types of breast cancer, the mean age was 48.86 ± 0.88 y ($N = 6$). The age of onset for TNBC mutation carriers did not differ from that of luminal types breast cancer carriers (47 vs 36; Mann-Whitney U-test, $p < 0.12114$). The age of patients with TNBC carrying the *BRCA1* mutation 5382insC did not differ from that of patients without mutations (U, $p < 0.285$). The mutation rate in TNBC patients with early onset (up to 40 years) was 13.04% (3/23), whereas with onset after 40 years—9.7% (9/92). There was no difference between the frequency of studied mutations in patients with TNBC with either early or late onset of the disease (F, $p < 0.6957$). The frequency of mutations with hormone-dependent cancer with early onset was 6.9% (4/58), while with late onset—1.29% (2/155), reaching the threshold of significance (F, $p < 0.0553$).

Information about the stage of the disease was only available for five carriers of mutations with TNBC—all of them were diagnosed with stage 1 or 2 breast cancer. Carriers of mutations with luminal types of breast cancer were at stage 2 or 3. Information about family history was available for four cases of TNBC and four cases of luminal types. Among the carriers TNBC mutations, in two cases there was a family history of cancer, while there was no family history of cancer for two other cases. Among the carriers of mutations with luminal types of breast cancer, there was a family history of cysts or cancer for three of the four cases.

Discussion

This study evaluated the prevalence of the 5382insC (c.5266dupC) *BRCA1* mutation in Ukrainian patients with breast cancer. We focused on the frequency of this type of *BRCA1* mutation in patients with TNBC and compared it with the frequency of luminal subtypes of breast cancer.

The 5382insC *BRCA1* mutation was detected in 11.3% of patients with TNBC, in contrast with 2.8% among patients with luminal types of breast cancer. Therefore, this mutation is more common in TNBC cases compared to other types of breast cancer (F, $p < 0.0041$), which corresponds to what has previously been reported in the literature [41, 42]. Data on the association of 5382insC *BRCA1* mutation with different types of breast cancer are limited. However, a Polish study found that patients with 5382insC *BRCA1* mutation had more frequent TNBC (61.7% vs 15.0%)[43]. Other studies have shown that, in general, mutations in the gene *BRCA1* are specific for TNBC [28, 41, 44]. In addition, one of the molecular features of TNBC when compared with luminal types of breast cancer is the inactivation of *BRCA1* in tumor cells [45]. Therefore, germline mutations in this gene may frequently contribute to the development of this type of pathology.

Comparison of the frequency of the 5382insC mutation in *BRCA1* gene observed in Ukraine with known frequencies in other countries showed no significant difference with the indicators in Tunisia (6%), in Poland (14,5%), in Russia (31%), in Bulgaria (5%) (F, $p > 0{,}05$)[29, 32, 34, 35]. However, the frequency of mutations in Ukraine is higher than in Australia (0.2%) and Germany (3.8%), (F, $p < 0{,}05$)[28, 29]. But, it should be noted that data on the frequency of mutations in TNBC patients are only available for a limited number of countries or for a small number of patients cohorts within a particular country, which complicates comparisons and does not allow definitive conclusions to be drawn. In addition, in many countries, the analysis showed the absence of this mutation in the samples analyzed, which does not permit accurate frequency determination of this mutation. It has been suggested that the frequency of 5383insC mutation in *BRCA1* in TNBC is < 0.1% in Korea, < 1.5% in Japan, < 0.6% in USA, < 0.95% in Spain, < 2% in Italy (Sardinia), < 2.3 in Palestine (Table 1), which is significantly lower than that in Ukraine.

The data regarding the age of patients with the 5382insC mutation is very scarce in the literature. Age data available only for *BRCA1* mutations in general. However, in a study from Poland in which most of the detected mutations were exactly 5382insC (18/30), *BRCA1/2* mutation carriers the average age was statistically lower for TNBC diagnosis compared to nonmutation patients (41 vs 47 years, respectively) [30]. Other studies also show that carriers of such mutations have a higher risk of developing TNBC at a young age [19, 27]. According to data from the Triple-Negative Breast Cancer Consortium, the mean age of carriers of the mutation in the BRCA1 gene was lower (44 y.o.) than of non-carriers [27]. The median age was 46 and 49 for the *BRCA*-positive and -negative patients respectively. Our results partially support this argument as women in Ukraine aged 40–49 with TNBC are being characterized by the presence of mutations more frequently, and the average age of mutation carriers approached 47 vs 50 yrs for patients with TNBC without the mutation. Our data concerning the lower age of patients from Ukraine does not suggest an additional risk of TNBC due to the presence of mutations. However, given lower incidence rates within available cohort sizes, such questions related to genetic factors in TNBC patients requires further research.

Some TNBC patients with *BRCA1* mutations reported having family members with breast (50%; $p < 0.001$) and ovarian cancers (18%; $p < 0.001$) suggesting the importance of family history [27]. In an Australian cohort, 59% of the mutation-positive patients did not have a family history of breast or ovarian cancer [31]. In Japan, the prevalence of

Table 1 Frequency of 5383insC mutation in *BRCA1* gene in TNBC patients from different countries

Country	No of samples analyzed	No of mutations detected	Frequency, %	*P**	References
Ukraine	115	12	11.3		This study
Poland	124	18	14.5	0.4425	[30]
Tunisia	33	2	6	0.7362	[27]
Australia	**439**	**1**	**0.2**	**< 0.00001**	[29]
Korea	999	0	< 0.1		[34]
Germany	**291**	**11**	**3.8**	**0.0193**	[28]
Bulgaria	20	1	5	0.6939	[33]
Russia	16	5	31	0.0691	[32]
Japan	65	0	< 1.5		[46]
USA	182	0	< 0.6		[47]
Spain	105	0	< 0.95		[48]
Italy (Sardinia)	49	0	< 2		[49]
Palestine	44	0	< 2.3		[50]

*p-value for Fisher's exact test; Values in which the mutation frequency differs from our data in are highlighted in bold

germline *BRCA1/2* mutations among patients with a family history was 41.4% (12/29) [46]. Given the limited nature of our data set, it is difficult to draw conclusions concerning family history in carriers and non-carriers of the mutation. However, in four cases of mutation carriers, at least two of the patients (50%) had knowledge of similar cancer incidences within their family.

Today, chemotherapy is the only option for systemic treatment for TNBC patients, yet there are many unresolved issues in the protocols. Anthracycline and taxane-based therapies are primary in the treatment of TNBC patients. *BRCA 1/2* mutation carriers with TNBC are currently treated the same as patients with sporadic TNBC, but the response to treatment in these patients is different. The treatment is usually supplemented with a platinum-based chemotherapy, which has shown promising results in the neoadjuvant and metastatic settings [51].

There are interesting data concerning the relationship between *BRCA1* 5382insC mutation in TNBC patients with sensitivity to chemotherapy. The research of Maksimenko J. et al. showed that positive *BRCA1* mutation status significantly reduces the risk of distant recurrence and breast cancer-specific mortality. The authors explain this by the higher sensitivity to chemotherapy in such patients, because tumor cells with *BRCA1* mutations have a defective homologous recombination repair pathway that predisposes a high sensitivity to DNA-damaging agents [22].

There are, however, reports showing contradictory conclusions. Bayraktar S. reported that *BRCA* status does not adversely impact survival outcomes in patients with TNBC moreover, *BRCA* carriers tended to have a decreased risk of breast cancer recurrence and death. The outcomes in this study were similar between *BRCA* carriers and non-carriers, most of whom received anthracycline–taxane-containing chemotherapies, suggesting that TNBCs with *BRCA* mutation were as sensitive to conventional chemotherapy regimens as other high-grade TNBCs [52]. This suggests that the problem requires further study with more detailed consideration of various factors that can affect the outcome of chemotherapy in patients. Therefore, it is extremely important to test patients with TNBC for the presence of mutations in *BRCA1*.

Currently, the most promising treatment of TNBC is a targeted therapy by poly (ADP-ribose) polymerase (PARP) inhibitors. Poly (ADP-ribose) polymerase enzymes play an important role in DNA damage repair. Cancers with defects in DNA repair, such as *BRCA1/2*-related breast cancers, are targets for inhibition with PARPI [53].

In 2018, the FDA approved olaparib and talazoparib for treating advanced HER2-negative breast cancer in patients with a *BRCA1/2* mutation. Olaparib approval was based on data from the OlympiAD Phase III trial (NCT02000622), which showed a potential overall survival (OS) benefit among patients with no prior chemotherapy for metastatic breast cancer, but there was no statistically significant improvement in OS with olaparib compared to the control group [54]. Talazoparib was approved in view of results published after EMBRACA Phase III trial (NCT01945775) that showed an increase of median progression-free survival by 46% in BRCA1/2-mutated HER2-negative locally advanced or metastatic breast cancer patients with previous chemotherapy including an anthracycline and/or taxane [55].

Conclusion

The *BRCA1* c.5382 mutation rate was recorded for the first time for TNBC patients in a Ukrainian population. The results presented in this study underscore the importance of genetic testing of mutations in the *BRCA1* gene in patients with TNBC, a previously under-researched group, as these mutations may affect the course of disease and response to treatment. Currently, guidelines recommend *BRCA1/2* genetic testing for women diagnosed with TNBC at < 60y.o.; however, our study supports genetic testing for all women diagnosed with TNBC, regardless of the age of onset or family history of cancer.

This is the first step toward targeted treatment of TNBC patients with *BRCA1* mutation c.5382 thus creating opportunities to improve current cancer therapy for this germline mutation. At this time, the contribution and associated risks of mutations in most genes is not yet well studied for all Ukrainian ethnicities, but a gradual increase in the number of clinical trials to understand molecular and immunological aspects of *BRCA1* mutation c.5382 in TNBC patients may lead to more meaningful clinical benefits.

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Data availability The datasets generated during and analyed during the current study are not publicly available due to principles of ethics and medical confidentiality but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki and approved by the local ethics committee (Committee on Bioethics: Bogomolets National Medical University, Kyiv (# 0120U100871).

Informed consent Informed consent was obtained from all individual participants included in the study.

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