

# Genetic predisposition in female patients with triple-negative breast cancer

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**Abstract.** Triple-negative breast cancer (TNBC) is an immunohistochemical tumor type characterized by the absence of estrogen, progesterone and human epidermal growth factor receptors. Its complexity renders it difficult to select an effective therapy. TNBC accounts for ~15% of all breast cancer cases in the Caucasian population and 35% among African American women; it is most common in women <40 years of age. The present study examined the spectrum and frequency of germline mutations in genes with a high and moderate penetrance in a cohort of women with TNBC. Molecular analysis was performed with a multigene panel of 94 genes for cancer predisposition using next-generation sequencing. The mean age of the TNBC cohort at the time of diagnosis was 44 years, 14 years younger than the mean age of the non-TNBC cohort (58 years). The results revealed a high frequency (41.2%) of pathogenic/likely pathogenic variants in susceptible genes in women with TNBC. Pathogenic germline variants in BRCA1/2 were found in 32% of the women (70% of pathogenic variants detected), and alterations in other predisposing genes (FANCM, CDKN2A and BLM) were found in 9% of the women. The data of all the patients with TNBC studied revealed a family history of cancer in 30% of the cases; most frequently, this involved relatives with breast cancer (11.8%). In the present study, the most frequent variant detected (in 11.8% of patients with TNBC) was a pathogenic variant in the BRCA1 gene (c.5266dup). The recommendations of genetic

counselors for patients with a pathogenic BRCA1/2 germline variant followed accepted prevention and risk reduction guidelines. By contrast, in the case of a detected pathogenic germline variant in genes with a moderate or low penetrance, the recommendations depended on the assessment of genetic counselors based on age at diagnosis, family history, the gene affected and the pathogenic variant.

## Introduction

Breast cancer remains the most frequently diagnosed type of cancer among women worldwide, accounting for 31% of all cancer cases (1). The situation is similar in Bulgaria, with 26.8% of all cancer cases (<https://www.sbaloncology.bg/index.php/bg/>). Despite the improvement in diagnostic techniques and treatment, breast cancer is still the most common cause of cancer death in women (2). Breast cancer is genetically and clinically heterogeneous and has several subtypes. The most widely used classification is from an immunohistochemical perspective and is based on the expression of the following hormone receptors: Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). According to the latter, four subtypes of BC are widely recognized: i) Luminal A (presence of ER and/or PR and absence of HER2, and a low expression of the proliferation marker, Ki-67, <20%); ii) luminal B (presence of ER and sometimes PR, and the absence of HER2 and a high expression of Ki-67, >20%); iii) HER2 subtype (high expression of HER2, and the absence of ER and PR); iv) triple-negative breast cancer (TNBC) (absence of ER, PR and HER2) (3).

TNBC accounts for ~15% of all BC cases among Caucasian women and 35% in African American women (4); it is most common in women <40 years of age (3). This breast cancer subtype is more likely associated with a larger tumor size, a poorly differentiated histology and a higher incidence of lymph node metastases, leading to more aggressive behavior and poor outcomes (4). As with other breast cancer types, TNBC exhibits heterogeneity in histology, patterns of dissemination, response to therapy and outcomes. The majority of cases of TNBC have been shown to be invasive ductal carcinomas, although other histologies are possible, with the 5-year survival rates ranging

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from 100% in patients with medullary tumors, to 56% in those with metaplastic TNBC (5).

A number of risk factors have been found to be associated with TNBC, which are not associated with an increased risk of other cancer subtypes, such as an older age at menarche and first pregnancy, higher parity, lack of breastfeeding and a higher body mass index (6). A strong association has been found between TNBC and carrier status of a germline (inherited) mutation in the highly penetrant genes BRCA1 and BRCA2. Of note, 70-90% of BRCA1 carriers and 16-23% of BRCA2 carriers have been found to have TNBC (7). By contrast, among women with TNBC, the frequency of BRCA1 and BRCA2 germline mutations is lower, at ~35% (range, 9-100%) and 8% (2-12%), respectively (5). Germline mutations in BRCA1/2 account for 15% of TNBC cases. Recently, the advent of next-generation sequencing (NGS) has enabled simultaneous multigene panel testing, revealing that patients with TNBC could have been carriers of germline mutations in other breast cancer predisposition genes that are distinct from BRCA1/2, other high-penetrance genes (such as PALB2, TP53, PTEN and STK11) and moderate-penetrant (such as RAD51D, ATM and CDKN2A) (8).

Genetic counseling and recommendations for prophylactic measures for women and their relatives differ in cases of a carrier status of a germline mutation in predisposition genes with a high or moderate penetrance. Evidence-based guidelines for BRCA1/2 germline mutations are well-established worldwide. By contrast, recommendations for germline mutations in other predisposition genes depend on the age of diagnosis, family history, affected genes and genetic variants.

The present study investigated the spectrum and frequency of germline mutations in genes with a high and moderate penetrance in a cohort of Bulgarian women with TNBC.

## Patients and methods

**Patients.** The study population was selected from the Cancer Registry of Dr. Georgi Stranski University Hospital-Pleven, registered from January, 2009 to December, 2013 and from January, 2019 to December, 2020. All living patients were contacted, informed about the aim of the study, and invited to participate. The Medical University-Pleven Ethics and Research Committees approved the study. The patients who accepted the invitation visited the Centre of Medical Genetics of Dr. Georgi Stranski University Hospital. A genetic counselor prepared and filled out a questionnaire after interviewing each participant. The questionnaire provided information about the patient's age, menarche and menstrual history, childbearing history, breastfeeding, menopause, use of oral contraceptives, menopausal hormone therapy, diet, alcohol consumption, smoking status, previous benign breast disease or other cancers, and a family history of breast or other cancers. A pedigree including at least three generations was prepared for each patient, defined as a proband (the first in a family treated for a genetic disorder).

**Germline pathogenic variant detection.** Blood samples (in EDTA tubes), ~5 ml, were collected from all patients interviewed without selection criteria and after obtaining informed consent. Genomic DNA was isolated from each

blood sample using the MagCore Genomic DNA Whole Blood kit (Ref: MGB400-02, RBC Bioscience) according to the manufacturer's protocol.

The genetic testing of patients was performed using NGS. The Trusight Cancer Sequencing Panel (Illumina®) was used for library preparation. The pan-hereditary cancer panel contained oligoprobes for 94 genes and 284 SNPs associated with an increased cancer predisposition (9). Qualifying libraries were sequenced on the Illumina NextSeq 550 platform using a 2x150 bp configuration. Reads were aligned to the human reference genome hg19. Data output files (gVCF) were imported into BaseSpace Variant Interpreter (Illumina®). Custom filters were created to improve variant annotation and interpretation, including a minimum read depth of 20x per variant and excluding silent variants. The five-tier terminology system of the American College of Medical Genetics and Genomics (ACMG) was used for variant classification (10), including pathogenic (P), likely pathogenic (LP), variant of unknown clinical significance (VUS), likely benign (LB) and benign (B). Variants automatically annotated using the software were manually checked in the major human genome databases: ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>), dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>) and Ensembl (<https://www.ensembl.org/index.html>).

## Results

The present study included 202 women with histologically confirmed breast cancer. Of these, 34 patients had TNBC, accounting for 16.8% of all the breast cancer cases. The focus of the present study was the TNBC cohort. The mean age at diagnosis of the TNBC cohort was 44 years, 14 years lower than the mean age of the non-TNBC cohort (58 years). In addition, ~44% of the TNBC cases were diagnosed before the age of 40. The analysis of the clinical and histologic characteristics of the patients with TNBC revealed that the left breast was most commonly affected in the studied patients (65%), and the most common histological type was ductal invasive carcinoma (82%). Of note, ~40% of the tumors were high-grade (G3). In ~30% of cases with TNBC, there was a family history of oncologic disease, most frequently breast cancer, in the patients' relatives (11.8%). The detailed clinical, familial and histological characteristics of the studied patients are presented in Table I.

The frequency of pathogenic/likely pathogenic variants in susceptibility genes in women with TNBC was 41.2% (14/34) (Table I), with germline pathogenic variants in BRCA1/2 affecting 32% of the women (70% of pathogenic variants detected) and alterations in other predisposing genes, affecting 9% of women. The average age at diagnosis for all women carrying germline mutations was 40 years (range, 29-61 years). Among the women diagnosed at an age >40 years, no carriers of mutations in BRCA1 and other predisposing genes were found; however, a pathogenic variant in BRCA2 in one patient (2.9%) diagnosed at the age of 61 years was found. Of the patients with ductal invasive type breast cancer (n=28), 43% (n=12) were germline mutation carriers in predisposing genes. Of the poorly differentiated tumors (n=13), 38.5% (n=5) occurred in women with a BRCA1 germline mutation.

Table I. Main clinical, familial, histological and genetic characteristics of the patients with TNBC in the present study.

Characteristic	No. of women (%)	Pathogenic/likely pathogenic variant in BRCA1, no. of women (%)	Pathogenic/likely pathogenic variant in BRCA2, no. of women (%)	Pathogenic/likely pathogenic variant in other genes, no. of women (%)
Total no. of patients	34 (100)	10 (29.4)	1 (2.9)	3 (8.8)
Personal history of TNBC				
Age at diagnosis, years				
≤35	6 (17.6)	4 (11.58)	0	0
35-40	9 (26.5)	3 (8.8)	0	2 (5.9)
41-50	11 (32.4)	3 (8.8)	0	1 (2.9)
>50	8 (23.5)	0	1 (2.9)	0
Family history <sup>a</sup>				
Breast cancer	4 (11.8)	3 (8.8)	1 (2.9)	0
Ovarian	1 (2.9)	1 (2.9)	0	0
Pancreatic	1 (2.9)	1 (2.9)	0	0
Endometrial	1 (2.9)	1 (2.9)	0	0
Colorectal	3 (8.8)	1 (2.9)	0	2 (5.9)
Without a family history	24 (70.6)	3 (8.8)	0	1 (2.9)
Clinical characteristics				
Localization				
Left breast	22 (64.7)	7 (20.6)	0	1 (2.9)
Right breast	12 (35.3)	3 (8.8)	1 (2.9)	2 (5.9)
Histological type				
Ductal	28 (82.4)	8 (23.5)	1 (2.9)	3 (8.8)
Lobular	1 (2.9)	1 (2.9)	0	0
Ducto-lobular	2 (5.9)	1 (2.9)	0	0
Mucinous	1 (2.9)	0	0	0
Medullary	1 (2.9)	0	0	0
Lymphoepitelioma-like	1 (2.9)	0	0	0
Grade				
Well differentiated (Low-grade) G1	0	0	0	0
Moderately differentiated (intermediate-grade) G2	8 (23.5)	1 (2.9)	1 (2.9)	1 (2.9)
Poorly differentiated (high-grade) G3	13 (38.2)	5 (14.7)	0	0
Not defined	13 (38.2)	4 (11.8)	0	2 (5.9)

<sup>a</sup>First-, second-, or third-degree relatives.

The most frequently affected gene was BRCA1 (29.4% of all TNBC cases), and the most frequently found pathogenic variant was c.5266dup (n=4, 11.8% of all TNBC cases). The affected non-BRCA predisposing genes were FANCM, CDKN2A and BLM. The detailed characteristics of the genetic variants in the women with TNBC are presented in Table II.

The limitations of the study are, first, the small number of patients with BC studied and, second, that the genetic analysis performed is informative only for small genetic defects but not for large genomic rearrangements.

## Discussion

The TNBC subtype is defined by the absence of ER, PR and HER2, and is associated with a poor prognosis (4). One of the main goals of researchers in the field of breast cancer is a better understanding of the etiology and pathogenesis of TNBC, as these patients do not benefit from endocrine or anti-HER2 therapy and require specific therapeutic strategies (11). To the best of our knowledge, this is the first study on the genetic etiology of TNBC in Bulgarian women using NGS technology and a multigene panel.

Table II. Detailed characteristics of the detected pathogenic/likely pathogenic genetic variants in the patients with TNBC.

Case no.	Gene	Variant	Clinical significance/ according to ACMG
2-1	FANCM	Frameshift Indels NM_020937.3 <b>c.1139_1140del</b> p (Arg380IlefsTer14) Exon: 6/23	Likely pathogenic
11-1	BRCA1	Frameshift Indels NM_007294.3 <b>c.5266dup</b> p (Gln1756ProfsTer74) Exon: 19/23	Pathogenic
21-1	BRCA1	Missense NM_007294.3 <b>c.181T&gt;G</b> p (Cys61Gly) Exon: 4/23	Pathogenic
58-1	BRCA1	Frameshift Indels NM_007294.3 <b>c.5266dup</b> p (Gln1756ProfsTer74) Exon: 19/23	Pathogenic
86-1	BRCA1	Inframe deletion NM_007294.3 <b>c.5062_5064del</b> p (Val1688del) Exon: 16/23	Pathogenic
87-1	BRCA1	Frameshift Indels NM_007294.3 <b>c.5266dup</b> p (Gln1756ProfsTer74) Exon: 19/23	Pathogenic
94-1	BRCA1	Frameshift Indels NM_007294.3 <b>c.2019del</b> p (Glu673AspfsTer28) Exon: 10/23	Pathogenic
95-1	BRCA1	Inframe deletion NM_007294.3 <b>c.5062_5064del</b> p (Val1688del) Exon: 16/23	Pathogenic
2-2	BRCA1	Frameshift Indels NM_007294.3 <b>c.5266dup</b> p (Gln1756ProfsTer74) Exon: 19/23	Pathogenic
9-2	BRCA1	Frameshift Indels NM_007294.3 <b>c.2019del</b> p (Glu673AspfsTer28) Exon: 10/23	Pathogenic
10-5	BRCA1	Splice acceptor NM_007294.3 <b>c.5333-1G&gt;A</b> Exon:	Pathogenic
48-6	BRCA2	Frameshift Indels NM_000059.3 <b>c.3975_3978dup</b> p (Ala1327CysfsTer4) Exon: 11/27	Pathogenic
85-6	CDKN2A	Missense NM_000077.4 <b>c.71G&gt;C</b> p.(Arg24Pro) Exon: 1/3	Pathogenic
23-7	BLM	Stop gained NM_000057.3 <b>c.1642C&gt;T</b> p.(Gln548Ter) Exon: 7/22	Pathogenic

The pathogenic variants are indicated in bold font.

The TNBC phenotype has been reported to account for 12-24% of all breast cancers (12). The present study found TNBC with a frequency of 16.8% (34/202), consistent with a previous study on the Bulgarian population (13). An earlier age at diagnosis characterizes patients with TNBC, as well as poorly differentiated, often heterogeneous and more aggressive tumors (14). The median age at diagnosis of patients with TNBC in the present study was 44 years, ~14 years younger than the median age for the non-TNBC cohort (58 years). In other studies, the mean age at diagnosis for TNBC cases is usually >50 years (5,15,16); thus, the TNBC cohort was younger. Nevertheless, the present study demonstrated the same incidence of TNBC in women up to 40 years of age (~44%) as reported in large-scale studies (4,17).

In the present study, the frequency of pathogenic/likely pathogenic variants in susceptibility genes in women with TNBC was 41.2% (14/34). The most commonly altered gene was BRCA1, affecting 32% of women (70% of detected pathogenic variants). In 9% of the patients, a germline mutation was found in other predisposing genes (FANCM, CDKN2A and BLM). The average age at diagnosis of all women carrying germline mutations in susceptibility genes was 40 (range, 29-61 years). Among the women diagnosed at >40 years of

age, no carriers of mutations were found in BRCA1 and other predisposing genes. However, a pathogenic variant of BRCA2 was found in 1 patient (2.9%) diagnosed at age 61 years of age. The carrier rate of pathogenic variants in susceptibility genes found was high compared to other European populations (reported frequency, 15-19%) (18), although with a value similar to that found in African American populations (reported frequency, 39%) (18,19-21). There are three possible explanations for these carriership rates. First, the study group consisted of ~80% of patients diagnosed with TNBC at a young age (<50 years), and the mean age at diagnosis was 44 years, whereas in the majority of studies, the mean age at diagnosis was 50 years. Second, the presence of mutations was established through a very qualified immunohistochemical evaluation of the receptor status. In addition, the group studied was relatively small (34 women). The carrier frequency found herein was even higher in the group of women diagnosed prior to the age of 40 (frequency of 60%), and in the group of women diagnosed prior to the age of 35, it was 75%.

The high proportion of carriers of pathogenic/likely pathogenic variants in TNBC predisposition genes found in the present study points to the need for clinicians and genetic counselors to strictly adhere to the NCCN (National

Comprehensive Cancer Network) (22) recommendations that genetic testing for predisposition to breast cancer should be performed in all women diagnosed with TNBC <60 years of age.

The most common histological type of tumor detected in the present study was infiltrating ductal carcinoma, with a prevalence of 82%. Other histological types, such as mucinous, medullary, lymph epithelioma-like and lobular, were also found. The results of the present study support the conclusions of other studies that TNBC encompasses a broad spectrum of histologic types and is a highly heterogeneous group (14,23). The tumors of the patients in the present study were mostly high-grade (G3), with 40% of poorly differentiated tumors occurring in women with a BRCA1 germline mutation. This fact supports the findings of other studies that high-grade TNBC is highly likely to have a BRCA1 germline mutation (an essential tool for choosing the most effective therapy) (24).

From the summarized family data of all the patients with TNBC in the present study, oncologic disease was found in the family history in 30% of the cases, most frequently relatives with breast cancer (11.8%). This result is consistent with the data from the literature, demonstrating an overall frequency of familial cases with TNBC ranging from 13 to 19% (25), with a higher prevalence in women <50 years of age (26), as was the case in the present study, which mainly included patients diagnosed at a young age. Of all women with familial breast cancer, BRCA1/2 mutations were found in 60% of the patients, confirming the importance of genealogical analysis as part of genetic counseling, aiming to determine the appropriateness of genetic testing and personalize the risk for a patient and her relatives.

The most common variant detected in the present study in women with TNBC (11.8%) was the pathogenic variant, c.5266dup, in the BRCA1 gene; this finding is in agreement with the data from the literature for other European populations (25), and data from a previously published study on the Bulgarian population (13). The variant c.5266dup (p.Gln1756Profs\*74) is an insertion of one nucleotide in exon 19 of BRCA1 mRNA (c.5266dupC), resulting in a frameshift at codon 1756. This results in a premature stop of protein synthesis after 74 amino acids, leading to a truncated and non-functional BRCA1 protein. The variant has been described in a number of patients with breast and/or ovarian cancer (27,28), pancreatic cancer (29) and prostate cancer (30). This variant has been described with a founder effect for the Ashkenazi Jewish population (31). A high frequency has also been reported in Poland and Eastern Europe (31-33). This mutation is also the most common among Bulgarian populations (13). The variant is associated with a 67-89% risk of developing breast cancer and a 33-42% risk of developing ovarian cancer (22). The frequency of other pathogenic BRCA1 variants (c.2019del, c.5062\_5064del, c.181T > G, c.5333-1G > A) was lower: 6% (n=2), 6% (n=2), 3% (n=1) and 3% (n=1), respectively. The c.2019del and c.5062\_5064del variants in Bulgarian populations have not been reported in the literature (13).

The pathogenic variant (c.3975\_3978dup) was detected in BRCA2 in a patient diagnosed with TNBC at age 61 with a family history of early breast cancer. The variant c.3975\_3978dup p.(Ala1327CysfsTer4) is pathogenic as it produces a premature stop codon (p.Ala1327Cysfs\*4) in the

BRCA2 gene. This results in an absent or defective protein product that loses function. The variant was found in a population database of healthy individuals with a very low incidence (rs764689249, ExAC 0.002%). The variant has been found in patients with breast cancer (34,35) and families with familial breast and ovarian cancer (35,36).

The recommendations of genetic counsellors in all cases with pathogenic germline variants in BRCA1/2 (as highly penetrant genes) follow the widely accepted guidelines for prevention and risk reduction (22,37,38).

In 9% (n=2) of all the patients with TNBC in the present study, a germline mutation was detected in other predisposition genes (FANCM, CDKN2A and BLM).

A likely pathogenic variant c.1139\_1140del p.(Arg380IlefsTer14) was detected in the FANCM gene in case 2-1, diagnosed with TNBC at 38 years of age. The variant is a deletion of two nucleotides, leading to a shift in the reading frame of the coding sequence and generating a stop signal after 14 amino acids. Since the variant is located in the 6th (of 23 exons of the gene), translation of the protein chain is terminated early, resulting in a severely truncated protein that most likely has no functional activity. The discovered variant is novel and has not been previously described in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and HGMD (<https://www.hgmd.cf.ac.uk/ac/index.php>). According to the ACMG criteria, it is likely pathogenic (10). FANCM is the most conserved protein involved in a mechanism responsible for DNA repair, known as the Fanconi anemia mechanism (39). The primary function of this mechanism is to activate DNA repair by directing processes to stalled replication forks. The FANCM protein has translocase and endonuclease activity, and its function is essential for the repair of misfolded structures at the replication fork, thus playing the role of a tumor suppressor protein in the cell (40,41).

Fanconi anemia is a genetically heterogeneous, autosomal recessive (biallelic inactivation of the gene is required to cause the disease) disorder characterized by chromosomal instability, hypersensitivity to DNA-damaging agents and abnormalities in the DNA repair system (42). The clinical picture includes disruptions in the development of major organs and systems, the early development of bone marrow failure (in the first decade of life), and a high risk of developing oncologic disease. Carrying a monoallelic mutation in any Fanconi anemia gene is associated with an increased risk of developing various oncologic diseases. A total of 15 genes [FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG (XRCC9), FANCI, FANCI (BRIP1), FANCL, FANCM, FANCN (PALB2), FANCO (RAD51C) and FANCP (SLX4)] are involved in the etiology of Fanconi anemia, with BRCA2, BRIP1, PALB2 and RAD51C exhibiting high penetrance. When a monoallelic mutation is present in these genes, there is a high risk of developing breast cancer. Data from other studies have demonstrated a strong association of variants resulting in a truncated and nonfunctional protein with a triple-negative receptor immunophenotype of the tumor and a better prognosis of such patients following radiotherapy, as the transmission of pathogenic variants in Fanconi anemia genes is associated with a greater sensitivity to radiotherapy (43). In this case, the genetic counselor recommends an annual

MRI scan of the breast and an annual ultrasound scan of the ovaries in combination with a test for a tumor marker (CA-125).

The variant found in codon 24 of the CDKN2A gene (p16INK4a) of patient 85-6 (diagnosed with TNBC at 38 years of age) results in the replacement of the amino acid arginine, which is basic and polar, with proline, which is neutral and nonpolar. The variant occurs at a low frequency in the population database of healthy individuals (rs104894097, gnomAD 0.004%). The variant has been reported in patients with pancreatic cancer and in patients with multiple primary melanomas (44,45). Experimental studies have demonstrated that the variant in CDKN2A (p16INK4a) disrupts protein function (46,47). For these reasons, the variant found in the patient was considered pathogenic. An association of malignant melanoma and laryngeal cancer in families carrying a pathogenic variant in CDKN2A has been reported in the literature (44,45), and as regards breast cancer, mutations in this gene are considered to be of low penetrance (with a low risk for BC) (48). In this case, the genetic counselor recommended an annual MRI scan of the breast, an annual abdominal ultrasound scan, and a full-body dermatologic examination.

The variant c.1642C > T (p.Q548X) in the BLM gene found in patient 23-7 (diagnosed with TNBC at the age of 46 years) has been described in the literature as homozygous or compound heterozygous (two different mutations but a homozygous recessive state) in individuals with Bloom syndrome (49). Bloom syndrome is an autosomal recessive inherited disorder, presenting with severe prenatal and postnatal growth retardation, decreased immune response, reduced sensitivity to sunlight, insulin resistance and a high risk of developing various malignancies at a young age (50). The detected c.1642C > T variant has also been described in the heterozygous state in patients with prostate, breast, ovarian and colorectal cancers. A previous meta-analysis found that carrying this pathogenic variant in the BLM gene was associated with a 2- to 5-fold higher risk of developing breast cancer (51); i.e., BLM is a gene with moderate penetrance. The variant has been described with a founder effect for the Slavic population (52). It is a nonsense mutation that produces a premature stop signal at position 548 in the BLM protein. In this case, the genetic counselor recommended an annual MRI scan of the breast and an annual ultrasound scan of the ovaries combined with a tumor marker test (CA-125).

In conclusion, the results of the present study demonstrated that even though the most frequently affected susceptibility gene in TNBC in Bulgaria is BRCA1, pathogenic variants were found in other predisposition genes. A multigene panel approach for genetic testing appears to be more efficient, not only for selecting the most appropriate therapy, but also for personalizing the risk for other cancer localizations and recommending prophylactic measures in the patient carrier, but also in the relatives at risk (found to be carriers of the same pathogenic variant).

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## Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the ClinVar repository (<https://www.ncbi.nlm.nih.gov/clinvar/submitters/507074/>).

## Authors' contributions

SLP and DDD identified the patients. ZBK, SLP, DDD and KSK collected the clinical and biological data. ZBK and SEN initiated the molecular analysis. SLP and DDD were responsible for the diagnosis and treatment of the patients. ZBK conducted the data analysis. ZBK, SLP and KSK were involved in the writing and revision of the manuscript. SEN and DDD reviewed and revised the manuscript. ZBK and SLP confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

The Medical University-Pleven Ethics and Research Committees approved the study. Informed consent was obtained from all patients.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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