

PREVALENCE OF DELETERIOUS MUTATIONS AMONG PATIENTS WITH BREAST CANCER REFERRED FOR MULTIGENE PANEL TESTING IN A ROMANIAN POPULATION

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Abstract

Aim. Multigene panel testing for Hereditary Breast and Ovarian Cancer (HBOC) using next generation sequencing is becoming more common in medical care.

We report our experience regarding deleterious mutations of high and moderate-risk breast cancer genes (*BRCA1/2*, *TP53*, *STK11*, *CDH1*, *PTEN*, *PALB2*, *CHEK2*, *ATM*), as well as more recently identified cancer genes, many of which have increased risk but less well-defined penetrance.

Methods. Genetic testing was performed in 130 consecutive cases with breast cancer referred to our clinic for surgical evaluation and who met the 2016 National Comprehensive Cancer Network (NCCN) criteria for genetic testing.

Results. 82 patients had pathogenic/likely pathogenic mutations and VUS mutations, and 48 were negative; 36 of the pathogenic mutations were in the high-risk genes and 16 were in the moderate risk genes and only 5 cases in the intermediary risk group.

From the VUS mutation group 21 cases were in the intermediary risk group, 9 cases were in the moderate risk group and only 7 cases in high risk group.

The most frequent *BRCA1* variant was *c.3607C>T* (7 cases) followed by *c.5266dupC* and *c.4035delA* (each in 4 cases). Regarding *BRCA2* mutations we identified *c.9371A>T* and *c.8755-1G>A* in 6 cases and we diagnosed VUS mutations in 3 cases.

Conclusion. Our study identified 2 mutations in the *BRCA1* gene that are less common in the Romanian population, *c.3607C>T* and *c.4035delA*. Both variants had particular molecular phenotypes, *c.3607C>T* variant respecting the triple negative pattern of *BRCA1* breast cancer while *c.4035delA* were Luminal B HER positive.

Keywords: *BRCA1/2*, hereditary breast and ovarian cancer, next generation sequencing hereditary cancer panels

Introduction

In the last decade, we witnessed an accelerated progress in understanding and also in the diagnosis and treatment of patients with hereditary cancer, studies demonstrating the importance and usefulness of genetic

testing.

About 30% of breast cancers are caused by a family predisposition, but only 10% of the cases are due to the inheritance of a mutation in a major gene involved in the occurrence of this pathology [1].

Until recently, genetic diagnosis of hereditary breast and ovarian cancer (HBOC) was based mainly on determining mutations in the *BRCA1/-2* genes. Along with

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the US Supreme Court's decision which held that human genetic material cannot be patented, and after introduction of Next Generation Sequencing (NGS) [2] this has changed. Furthermore genetic testing for HBOC has seen an even greater publicity in May 2013, when Angelina Jolie announced that she had undergone prophylactic bilateral mastectomy because she was a *BRCA1* mutation carrier [3].

The aim of these genetic tests is to create the opportunity for multidisciplinary and individualized treatment for each patient, depending on the mutation they carried [4]. It also creates the possibility of genetic counseling to the relatives of these patients about the risk of developing breast cancer or another type of cancer caused by the inheritance of certain mutations [1,4].

Along with the technological progress and the use of NGS on a larger scale, based on the clonal amplification and multiple parallel sequencing, it was possible to investigate more genes in a shorter time and with lower costs [5,6]. Moreover, this new method increased the sensitivity for detecting individual mutations that occur less frequently or have a lower penetrance [2,7].

Another advantage of NGS technology is that it made possible to identify some mutation carriers originating from families with hereditary cancer, but with atypical or particular phenotypes [7].

Unfortunately, a major deficiency of using multigene test panels is the deterioration of the clinical genetic conduct [2] and also the lack of interpretation of some mutations in clinical context [7]. Furthermore, for some mutations we don't have specific guidelines regarding genetic counseling or prophylactic surgery in mutation carriers or for their relatives [7].

Mutations in genes with high penetrance such as *PTEN*, *CDH1*, *STK11* and *TP53* [8-11] or in genes with moderate penetrance like *PALB2*, *CHEK2*, *ATM* [8,12-14] are known to be responsible for familial forms of breast cancer. Less attention has been paid to mutations in genes with a lower contribution in the occurrence of breast cancer, such as those responsible for Lynch syndrome (*MLH1*, *MSH2*, *MSH6* and *PMS2*), or other rare genes like *BARD1*, *MUTYH*, *RAD50* etc. [15,16], but this has changed during the past years.

The objective of our study is to report our experience from the first 130 consecutive patients with breast cancer referred to our service for multipanel genetic testing, providing data on the frequency of pathogenic genetic variants by gene.

Material and method

Ethics

This study was conducted with the approval of the ethics committee of Iuliu Hațieganu University of Medicine and Pharmacy “, Cluj-Napoca (no.369 / 14.10.2016). We obtained the informed consent of all patients at the first medical evaluation.

Patients

Were enrolled in the study 130 consecutive patients diagnosed with breast cancer who presented for oncological examination to Oncosurg Surgical Oncology Clinic of Cluj-Napoca between January 2015 and June 2017 and met the 2016 National Comprehensive Cancer Network (NCCN) criteria for genetic testing [4]. 48 patients were excluded due to the negative result on genetic testing.

All patients had a prior Tru-Cut biopsy to confirm the diagnosis and a complete histopathological report.

Genetic testing

Each patient was approached for genetic testing after the diagnosis. Genomic DNA was prepared and analyzed as described in our previous research [17].

The genes were grouped into three risk categories based on penetrance data:

- High penetrance and high risk: *BRCA1*, *BRCA2*, *TP53*, *CDH1*, *STK11*, *PTEN*
- Moderate penetrance and high risk: *ATM*, *CHEK2*, *PALB2*
- Increased penetrance but less well-defined risk: *BARD1*, *BLM*, *BRIP1*, *FAM175A*, *MEN1*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *PMS2*, *RAD50*, *RAD51C*, *RAD51D*, *XRCC2*.

Results

During the study, from the 130 patients with breast cancer which were eligible for genetic testing, 82 were included in the study. In the 82 patients tested we identified 93 mutations from the multigene panel (56 pathogenic variants and 37 VUS variants) – Table I, Figure 1.

The most frequent *BRCA1* mutation was *c.3607C>T* (28%), followed by *c.5266dupC* (16%) and *c.4035delA* (16%) and *c.181T>G* (8%) variant (Table I, Figure 2).

Regarding *BRCA2* gene mutations *c.9371A>T* and *c.8755-1G>A* variants were found each in 27.27% of cases. 3 VUS mutations have been identified in the *BRCA2* gene (27.27%) - Figure 3.

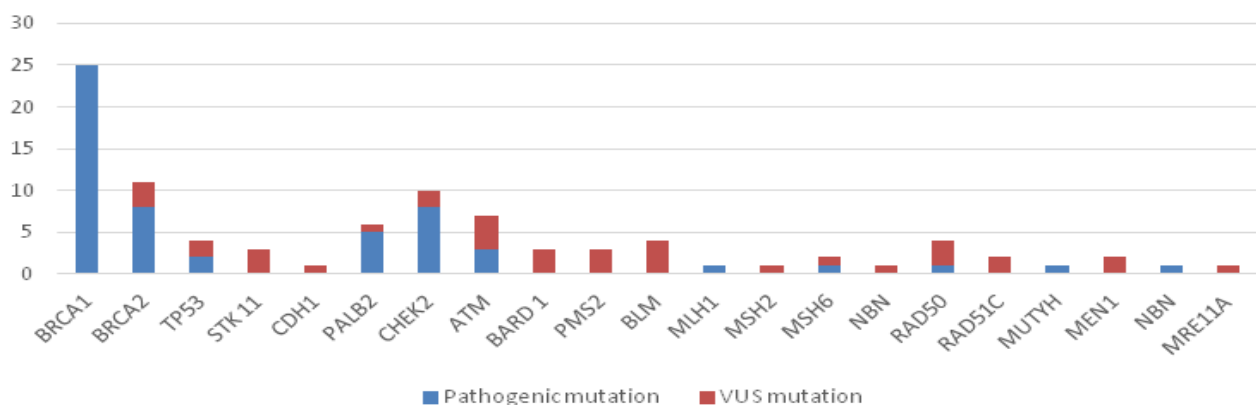
Mutations in the additional genes were more often VUS variants, in only 5 cases being pathogenic (Figure 4a and 4b).

The majority of VUS variants were from the patients with rare mutations, while in the *BRCA1* mutation group we did not identify any VUS variants (Table I, Figure 1).

Nine patients had 2 mutations (*BRCA1c.2241dupC* + *MEN1 c.777G>A*; *BRCA1 c.135-2A>G* + *MUTYH c.158-3C>T*; *BRCA1 c.843_846delCTCA* + *CHEK2 c.470T>C*; *BRCA2 c.9371A>T* + *ATM c.2735G>A*; *CHEK2 c.1232G>A* + *RAD50 c900G>A*; *c.470T>C* + *BLM c.4076+4T>G*; *MSH6 c.2136delG* + *MUTYH c.1187G>A*; *NBN 657_661delACAAA* + *MRE11A c.1091G>A*; *RAD50 c.1663A>G* + *RAD51 c.790G>A*) and one patient had 3 mutations (*CHEK2 1283C>T* + *BLM c.1642C>T* + *ATM c.4768C>T*).

Table I. Distribution of mutations in the studied group.

High penetrance breast genes			
Gene	Cases	Pathogenic Mutation	VUS mutation
<i>BRCA1</i>	25	<i>c.3607C>T</i> (7), <i>c.5266dupC</i> (4), <i>c.181T>G</i> (2), <i>c.4035delA</i> , <i>c.3726 C>T</i> , <i>c.2241dupC</i> , <i>c.135-2A>G</i> , <i>c.1789G>A</i> , <i>c.737delT</i> , <i>c.3700_3704delGTAAA</i> , <i>c.843_846delCTCA</i> , <i>c.4035delA</i> (3), <i>c5329_5330insC</i>	-
<i>BRCA2</i>	11	<i>c.9371A>T</i> (3), <i>c.8755-1G>A</i> (3), <i>c.1528G>T</i> , <i>c.8695 C>T</i>	<i>c.3547G>C</i> , <i>c.3562A>G</i> (2)
<i>TP53</i>	4	<i>c.469G>T</i> (2)	<i>c.847C>T</i> , <i>c.480G>A</i>
<i>STK11</i>	3	-	<i>c.1225C>T</i> (3)
<i>CDH1</i>	1	-	<i>c.1840A>G</i>
Moderate penetrance breast genes			
Gene	Cases	Pathogenic Mutation	VUS mutation
<i>PALB2</i>	6	<i>c.93dupA</i> (2), <i>c.3549C>G</i> , <i>c.509_510delGA</i> , <i>c.79G>T</i>	<i>c.2461A>T</i>
<i>CHEK2</i>	10	<i>c.470T>C</i> (5), <i>c.1232G>A</i> (2), <i>1283C>T</i>	<i>c.1521T>C</i> , <i>c.1313A>T</i>
<i>ATM</i>	7	<i>c.7630-2A>C</i> , <i>c.2250G>A</i> , <i>c.1564_1565delGA</i>	<i>c.9077T>G</i> , <i>c8734A>G</i> , <i>c.2735G>A</i> , <i>c.4768C>T</i>
Additional genes			
Gene	Cases	Pathogenic Mutation	VUS mutation
<i>BARD 1</i>	3	-	<i>c.1333G>A</i> , <i>c.2282G>A</i> , <i>c.2640del15</i>
<i>PMS2</i>	3	-	<i>c.620G>A</i> , <i>c.2012C>T</i> , <i>c.852A>G</i>
<i>BLM</i>	4	-	<i>c.3014T>C</i> , <i>c.3879A>G</i> , <i>c.4076+4T>G</i> , <i>c.1642C>T</i>
<i>MLH1</i>	1	<i>c.2041G>A</i>	-
<i>MSH2</i>	1	-	<i>c.1597C>G</i>
<i>MSH6</i>	2	<i>c.2136delG</i>	<i>c.1068T>G</i>
<i>NBN</i>	1	-	<i>c.511A>G</i>
<i>RAD 50</i>	4	<i>c.2165dupA</i>	<i>c.785T>G</i> , <i>c900G>A</i> , <i>c.1663A>G</i>
<i>Rad51C</i>	2	-	<i>c.790G>A</i> (2)
<i>MUTYH</i>	2	<i>c.1187G>A</i>	<i>c.158-3C>T</i>
<i>MEN1</i>	1	-	<i>c.777G>A</i>
<i>NBN</i>	1	<i>c657_661delACAAA</i>	-
<i>MRE11A</i>	1	-	<i>c.1091G>A</i>

**Figure 1.** Classification of mutations depending on the risk of pathogeny.

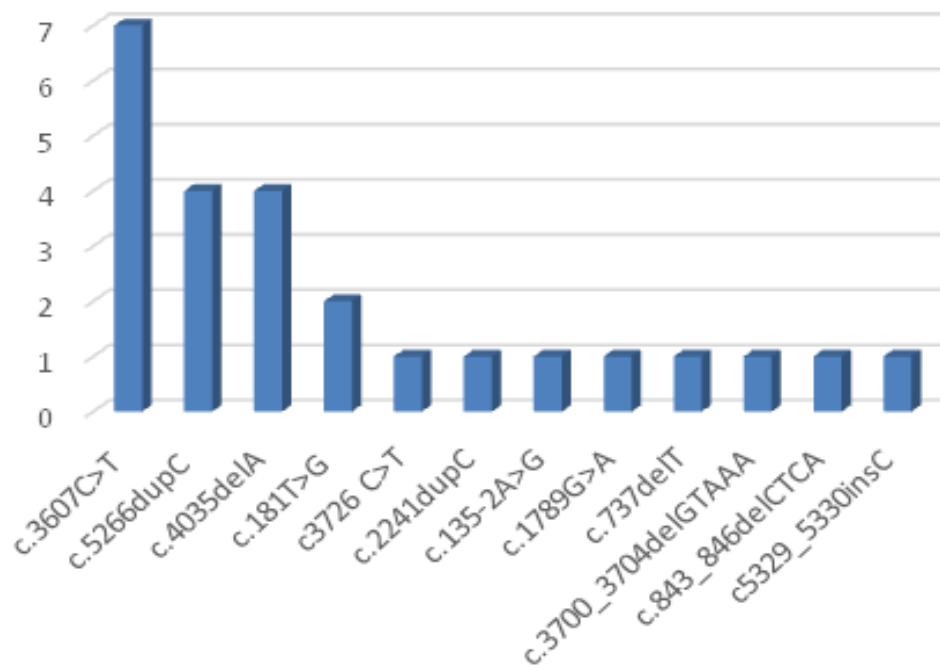


Figure 2. Distribution of *BRCA1* variants.

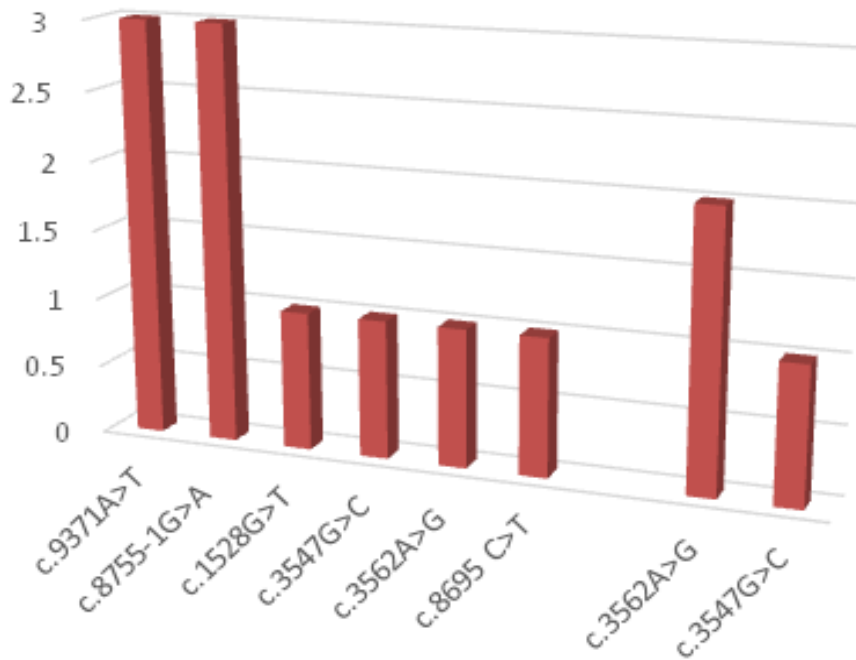
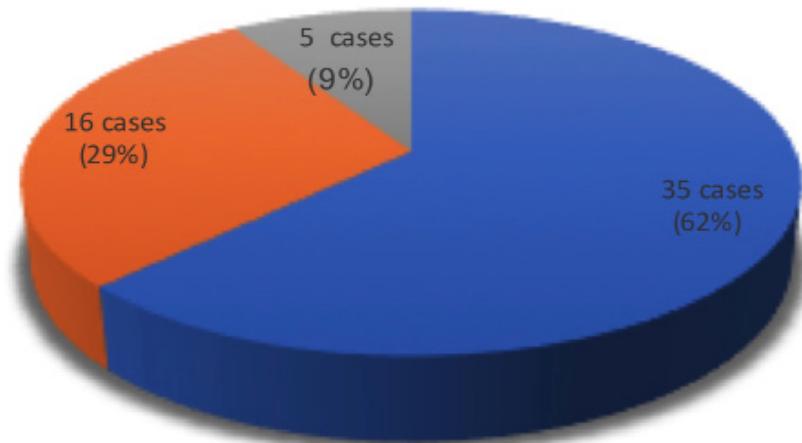
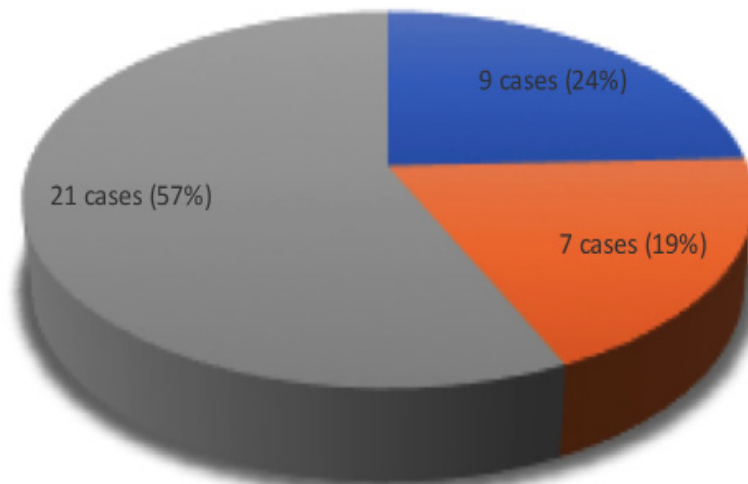


Figure 3. Distribution of *BRCA2* variants.



- High penetrant gene mutations (BRCA1,2,TP53)
- Moderate penetrant gene mutations (PALB2,CHEK2,ATM)
- Other mutations

a



- High penetrant gene mutations (BRCA2,TP53, STK11, CDH1)
- Moderate penetrant gene mutations (PALB2,CHEK2,ATM)
- Other mutations

b

Figure 4. Distribution of mutations according to penetrance: a- Pathogenic mutations; b- VUS mutations.

Discussion

BRCA1

The most common *BRCA1* mutations in our study were *c.3607C>T* (7 cases), *c.5266dupC* (4 cases), *c.4035delA* (4 cases) and *c.181T>G* (2 cases).

BRCA1 c.3607C>T (p.Arg1203)*, exon 11 - This sequence change creates a premature translational stop signal at codon 1203 (*p.Arg1203**), causing the absence or disruption of protein synthesis [18]. *BRCA1 c.3607C>T* variant was diagnosed in 7 patients and was not reported until now in Romanian population, being the most frequent in the study group, all the patients originating from northwestern towns of Romania [17].

In 6 cases we identified the association of this variant with triple negative breast cancer (TNBC), which is in accordance with the general immunohistochemical phenotype of *BRCA1* mutations [17].

*BRCA1 c.5266dupC (p.Gln1756Profs*74)*, exon 20 - This mutation determines the insertion of one nucleotide (cytosine), creating a novel STOP codon after 74 amino acid residues and therefore will produce a truncated and a non-functional protein. This variant has been described as being the most prevalent mutation in the north-eastern Romania population, in the only two studies conducted so far in the *BRCA1* mutation carriers from this region [19,20].

BRCA1 c.5266dupC is one of the *BRCA1* founder mutations encountered in Hebrews and is most common in Polish population. This is due to the expulsion of Jews from countries like England, Spain, Portugal, Germany and Italy, around 1650, Poland totaling nowadays about 30% of Hebrew population worldwide [21]. In Polish population the *c.5266dupC BRCA1* mutation is commonly associated with ovarian cancer [22], only one patient in our study from the four mutation carriers had a personal history of ovarian cancer, the breast cancer being diagnosed four years later.

C.5266dupC mutation is more commonly encountered in Russian population and in the Nordic countries near Russia (Estonia Lithuania, Latvia) [21], where it is considered to have originated, but an increased incidence of this variant is also found in countries bordering with Romania, like Hungary [23], Serbia and Montenegro [24].

BRCA1 c.4035delA variant is described as the third most common mutation in Polish people and alongside *c.5266dupC* and *c.181T>G* they're representing 90% of pathogenic mutations in this population. Its origin seems to be more of Baltic origin, rather than Slavic, being described with an increased frequency among Estonian population [25].

In our study, we identified this mutation in 4 patients, of which 3 were relatives (mother and 2 daughters). What is more interesting is that all patients had as immunohistochemical characteristic the overexpression of HER2, being known that most *BRCA1* mutations are triple negative.

BRCA1 c.181T>G (p.Cys61Gly), exon 5 - This mutation causes a sequence change that replaces cysteine

with glycine at codon 61 of the *BRCA1* protein causing a disruption in protein function.

This variant is the most common mutation in the *BRCA1* gene in the Austrian population [26] and 2nd after *c.5266dupC* variant in Czech [27], Slovenia [28], Hungary [29] and Polish population [30]. In our study, 2 patients were diagnosed with breast cancer and *BRCA1 c.181T>G* variant both of Hungarian ethnicity, mother and daughter.

For the rest of *BRCA1* mutations found in one case each in our study (*c.3726 C>T*, *c.2241dupC*, *c.135-2A>G*, *c.1789G>A*, *c.737delT*, *c.3700_3704delGTAAA*, *c.843_846delCTCA*, *c5329_5330insC*) there is limited data available.

BRCA2

We found 11 *BRCA2* variants, eight pathogenic and likely pathogenic (*c.9371A>T* (3 cases), *c.8755-1G>A* (3 cases), *c.1528G>T* (1 case) and *c.8695 C>T* (1 case)) and 3 VUS mutations.

BRCA2 c.9371A>T (p.Asn3124Ile), exon 25 - is a mutation caused by a single base substitution, asparagine with isoleucine at position 3124, this change leading to an alteration in the protein synthesis of *BRCA2* gene [31]. This variant is a rare but deleterious mutation in *BRCA2* protein synthesis, described as associated with an increased risk of breast and ovarian cancer, and is considered more likely to be pathogenic [30,32].

BRCA2 c.8755-1G>A, intron 21- This mutation results in an incorrect mismatching of the mRNA causing a reading alteration and a non-functional protein synthesis after this point [33]. The pathogenesis of this variant is still a subject of debate, but most studies suggest that it is most likely pathogenic [27,33].

BRCA2 c.8695C>T (p.Gln2899Ter) - is a rare variant described in literature as pathogenic and mentioned in Romanian population in a recent study [34].

BRCA2 c.1528G>T (p.Glu510Ter; p.Glu510Glu) is a rare pathogenic mutation in the *BRCA2* gene described in the population of Slovenia and present in our study in one patient [35].

TP53

The two patients with *TP53* mutation had both *c.469G>T* variants. This sequence change replaces Valine with Phenylalanine at codon 157 of the *TP53* protein (*p.Val157Phe*).

The valine residue is highly conserved and there is little difference between the physicochemical properties of Valine and Phenylalanine, but this sequence change was reported in literature in breast and liver cancer patients [36].

PALB2

Among the patients with *PALB2* mutations we identified five pathogenic variants: *c.93dupA* (2 cases), *c.3549C>G*, *c.509_510delGA*, *c.79G>T*.

*PALB2 c.93dupA (p.Leu32Thrfs*11)* – This sequence change inserts 1 nucleotide in exon 2 of the *PALB2* mRNA (*c.93dupA*), causing a frameshift at codon 32. This creates a premature translational stop signal 11 amino acid residues later and causes the production of a modified protein.

This variant has been described in literature in a patient with triple negative breast cancer [37]; in our study, only 1 patient was diagnosed with this molecular form, the 2nd being diagnosed with LUMINAL B HER negative type.

PALB2 c.3549C>G (p.Tyr1183)* – This sequence change results in the replacement of Tyrosine with a terminal codon at position 1183 of the *PALB2* protein, resulting in a truncated *PALB2* protein missing the last three amino acids [38]. This variant has been reported in literature for individuals people affected by breast cancer, pancreatic cancer and Fanconi anemia and it is considered pathogenic[39].

*PALB2 c.509_510delGA (p.Arg170Ilefs*14)* – This mutation is caused by the deletion of 2 base pairs at codon 509 of the *PALB2* gene. It is a frame-shift mutation that will result in the creation of an unrecognized protein after amino acid 170 and a new stop codon after 14 amino acids, causing the absence or an abnormal protein synthesis [40]. This mutation is recognized as pathogenic, being identified primarily in the Polish population [30,40], where it is found at a higher frequency compared to the rest of Europe.

PALB2 c.79G>T (p.Glu29)* – This variant causes Glutamate to be replaced by a terminal codon at position 29 resulting in the modification of the *PALB2* protein synthesis. For this reason, this variant it is considered to be more likely pathogenic and it is associated with breast and vulvar cancer and most international guidelines recommend testing this mutation [41].

CHEK2

We diagnosed 10 CHEK2 mutations, 8 pathogenic (*c.470T>C* (5 cases), *c.1232G>A* (2 cases), *1283C>T* (1 case), and 2 VUS cases.

CHEK2 c.470T>C (p.Ile157Thr) – This sequence is due to the replacement of Isoleucine with Threonine at codon 157. This change leads to the synthesis of a modified CHEK 2 protein, which is associated with increased risk of lobular breast cancer (OR=1.48-1.58) [42], but our study has not confirmed this observation because the 3 patients were diagnosed with invasive ductal carcinoma (Table 2).

CHEK2 1283C>T (p.Ser428Phe) - This sequence is due to the replacement of Serine with Phenylalanine at codon 428, causing changes in protein kinase domain [43]. This mutation is known to be a pathogenic and associated with breast and prostate cancer, especially in the Ashkenazy Jews who have a twofold increased risk for developing breast cancer.

CHEK2 c.1232G>A (p.Trp411)* - This sequence change creates a premature translational stop signal at codon 411 of the CHEK2 protein causing absent or abnormal

protein product.

ATM

ATM c.7630-2A>C – This sequence change occurs in 2 nucleotides before exon 52 of the ATM gene. This mutation determines the synthesis of a truncated protein, and literature data associates this variant with a higher breast cancer risk [44].

ATM c.2250G>A – This variant occurs at the last base of exon 14, a position that is highly conserved in the human genome. Although it does not result in an amino acid substitution, it has been demonstrated to affect splicing of the mRNA, specifically in frame skipping of the entire exon 14 [45]. This variation is cited in literature as pathogenic and the carriers of this mutation are at increased risk for breast cancer [46].

ATM c.1564_1565delGA - This sequence change deletes 2 nucleotides in exon 10 of the ATM gene, causing a frameshift after codon 522 and the creation of a premature translational stop signal 43 amino acid residues later - *p.(Glu522Ilefs*43)* [47].

For high penetrance mutations but with less well-defined risk data on the association of breast cancer is reduced, *RAD50 c.2165dupA (p.Glu723Glyfs*5)* [48], *MSH6 c.2136delG (p.Asp713Ilefs*23)* [49], *MUTYH c.1187G>A (p.Gly396Asp)* [50], *MLH1 c.2041G>A (p.Ala681Thr)* [51] and *NBN c657_661delACAAA* [52], this association being described only in isolated cases.

Conclusion

Our study has successfully identified 2 mutations in the *BRCA1* gene that are less common in the Romanian population, *c.3607C>T* and *c.4035delA*. Furthermore, the two pathogenic variants were associated with different histological and molecular phenotypes. The *c.3607C>T* variant was associated with triple negative breast cancer (negative ER, PR and HER2), respecting the general phenotype of patients with *BRCA1* mutations while variant *c.4035delA* was associated with HER positive, hormone-negative forms, an atypical element for patients with *BRCA1* mutations.

For the rest of the genes, the information could be useful for establishing specific variants of these mutations in the Romanian population and thus for their targeted identification and thus to a decrease of costs and a reduced time for obtaining results.

The absence of VUS mutations in patients in the *BRCA1* group is most likely due to the fact that mutations in the *BRCA1* gene were more extensively studied compared to those of other genes. This could be also the explanation for the numerous VUS mutations identified in the group of moderate penetrance and intermediate risk genes, where larger studies are missing.

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