BRCA1/2 pathogenic variants in triple-negative *versus* luminal-like breast cancers: genotype–phenotype correlation in a cohort of 531 patients

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Abstract

Background: Several available data suggest the association between specific molecular subtypes and *BRCA1/2* mutational status. Previous investigations showed the association between *BRCA1/2* pathogenic variants (PVs) in specific genomic regions and phenotypic variations of cancer relative risk, while the role of PV type and location in determining the breast cancer (BC) phenotypic features remains still unclear. The aim of this research was to describe the germline *BRCA1/2* PVs in triple-negative breast cancer (TNBC) *versus* luminal-like BC and their potential leverage on BC phenotype.

Patients & methods: We retrospectively collected and analyzed all clinical information of 531 patients with BC genetically tested for germline *BRCA1/2* PVs by Next-Generation Sequencing analysis at University Hospital Policlinico "P. Giaccone" of Palermo (Sicily) from January 2016 to February 2020.

Results: Our results corroborate the evidence that *BRCA1*-related tumors often have a profile which resembles the TNBC subtype, whereas *BRCA2*-associated tumors have a profile that resembles luminal-like BC, especially the Luminal B subtype. Interestingly, our findings suggest that the PVs identified in TNBC were not largely overlapping with those in luminal-like tumors. Differences in the frequency of two PVs potentially associated with different molecular tumor subtypes were observed. *BRCA1*-633delC was detected with relatively higher prevalence in patients with TNBC, whereas *BRCA2*-1466delT was found mainly in Luminal B tumors, but in no TNBC patient.

Conclusion: Future studies examining the type and location of *BRCA1/2* PVs within different molecular subtypes are required to verify our hypothesis and could provide an interesting insight into the complex topic of genotype–phenotype correlations. Additionally, a more in-depth understanding of the potential correlations between *BRCA* PVs and clinical and phenotypic features of hereditary BC syndrome patients could be the key to develop better strategies of prevention and surveillance in *BRCA*-positive carriers without disease.

Keywords: *BRCA1*, *BRCA2*, breast cancer, genetic testing, germline pathogenic variants, luminal-like breast cancer, triple-negative breast cancer

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Introduction

The critical role of *BRCA1* and *BRCA2* genes in breast cancer (BC) and ovarian cancer (OC) was discovered for the first time in 1994 and 1995.[1,2] Since then, the characteristics of *BRCA1/2*-related BCs have been well investigated. The recent diffusion of high-throughput next-generation sequencing technologies has provided a deep insight into

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the genetics and molecular biology of these tumors,[3] shedding light on their biological heterogeneity. Over the years, the eligibility criteria for *BRCA1/2* genetic testing have been expanded and updated.[4,5] *BRCA1* and *2*, in addition to being considered indicators for cancer risk assessment, have become also biomarkers of predictive utility. Several other genes involved in the homologous recombination (HR) pathway have been evaluated,[6–10] and the knowledge on *BRCA*-mediated tumor phenotypes continues to evolve. In this context, marked by great progress and change in the field of genetic testing, the study and characterization of pathogenic variants (PVs) in *BRCA1/2* genes are becoming increasingly important for BC and OC screening and prevention.

Germline PVs detected in *BRCA1/2* genes are the primary causes of inherited breast tumors and confer an approximately 70% of lifetime risk in mutation carriers.[11,12] PVs are detected in 5–10% of all women with BC, and the prevalence increases to 10–20% in triple-negative breast cancer (TNBC).[13,14] TNBC is a particularly proliferative and aggressive subtype because the absence of actionable targets results in poor prognosis and unfavorable outcomes for the patients.[15,16]

Several available data suggest the association between specific molecular subtypes and *BRCA1/2* mutational status. *BRCA1/2* PV carriers have been shown to harbor TNBC more frequently, and this association is strongest of all in *BRCA1*-related BC.[17,18]

Studies about the molecular and clinicopathological characteristics of patients with *BRCA1*- *versus* *BRCA2*-related BC showed significant and distinct features. Approximately 70% of BCs developing in *BRCA1* PV carriers are TNBCs, which tend to have higher histological grade than *BRCA2* PV carriers, and are most frequently invasive ductal carcinomas. Conversely, approximately 75% of BCs in *BRCA2* PV carriers are estrogen-receptor (ER)-positive, more often Luminal B. ER-positive BCs which develop in *BRCA* PV carriers are lobular or ductal type, and have higher histologic grade than sporadic ER-positive BCs.[19,20]

These differences highlight the heterogeneity in BC biology and molecular phenotype among tumors related to different germline *BRCA1/2* PVs. Although the molecular and clinicopathological characteristics of *BRCA1/2*-related BCs have been well investigated, the available studies on the genotype–phenotype correlations are predominantly based on the involvement of the *BRCA1* or *BRCA2* gene. To date, the role of PV type and its location in determining the BC phenotypic features still remains unclear. Previous investigations showed the association between *BRCA1/2* PVs in specific genomic regions and phenotypic variations of cancer relative risks.[21] Further and deeper information on *BRCA* PVs could help us better understand the impact of genotype on breast tumor phenotype. Finally, some studies revealed the existence of a close correlation between specific *BRCA1/2* PVs and variations of BC and OC relative risks, by identifying specific putative breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) located on the coding DNA sequences of *BRCA1* and *BRCA2* genes.[21,22]

Based on a Breast Cancer BRCA System database retrospectively collected at University Hospital Policlinico "P. Giaccone" of Palermo, the aim of this research was to describe the type and gene location of germline *BRCA1/2* PVs in TNBC versus other BC molecular subtypes (Luminal A, Luminal B and HER2-enriched) and their potential leverage on BC phenotype.

Patients and methods

Study population

We carried out a retrospective cohort study at the "Sicilian Regional Center for the Prevention, Diagnosis and Treatment of Rare and Heredo-Familial Tumors" of the Section of Medical Oncology of University Hospital Policlinico "P. Giaccone" of Palermo. We have collected and analyzed all information regarding all women diagnosed with primary BC who underwent to germline *BRCA1/2* genetic testing from January 2016 to February 2020.

Genetic counseling was performed by a multidisciplinary team consisting mainly of an oncologist, a geneticist and a psychologist. Information about personal and familial history of cancer, family geographical origin, age of cancer diagnosis, histological tumor subtype, molecular phenotype and disease stages (I–IV), was anonymously recorded for all patients who previously provided a written informed consent. The study (G-Land 2017, approval number: 01-03-2017) was approved by the ethical committee (Comitato Etico Palermo 1) of the University-affiliated

hospital A.O.U.P. "P. Giaccone" of Palermo. The ER, progesterone-receptor (PgR), HER2-receptor status (HER2), Ki67 status, and histological grade (Grades I, II, and III) of the primary tumors were reported in local pathology testing of diagnostic core biopsies or tumor resections for clinical use. Based on histological grade and biomarker expression, invasive tumors were categorized as Luminal A-like (LA = ER/PR+ and HER2–, histological grade 1 or 2), Luminal B-like (LB = ER/PR+ and HER2+, or ER/PR+, HER2–, and grade 3), HER2 enriched (E) (ER/PR– and HER2+) or triple negative (TN = ER–, PR– and HER2–).[23]

The patients were selected for mutational screening based on probability rate of carrying PV assessed by the BRCAPRO genetic risk prediction model[24] and according to the previously established criteria by the Italian Association of Medical Oncology (AIOM) (https://www.aiom.it/linee-guida-aiom-neoplasie-della-mammella-2019/). These criteria are based on personal and family history and age of cancer onset, in order to identify individuals at high risk of harboring a PV in the Hereditary Breast and Ovarian Cancer (HBOC) predisposition genes. The AIOM every year updates its guidelines for identifying the individuals who should receive *BRCA* genetic testing, and in 2016 AIOM included in the population to be genetically tested woman with TNBC <60 years, regardless of family history.

The following criteria were used to select patients to be genetically tested for germline *BRCA1/2* PVs: (i) Personal history of (a) male with BC; (b) women with BC and OC; (c) woman with BC <36 years; (d) woman with TNBC <60 years; (e) woman with bilateral BC <50 years; (ii) Personal history of BC <50 years and at least one first-degree relative with: (a) BC <50 years; (b) non-mucinous and non-borderline OC at any age; (c) bilateral BC; (d) male BC; (iii) Personal history of BC >50 years and family history of BC or OC in two or more relatives who have a first-degree relationship with each other (including one who has a first-degree relationship with her); (iv) Family history of known pathogenic variant in a predisposing gene (https://www.aiom.it/linee-guida-aiom-neoplasie-della-mammella-2019/).

The *BRCA* test result was considered informative when a pathogenic or likely pathogenic variant was identified in an individual. Conversely, *BRCA* test result was considered not informative when no pathogenic or likely pathogenic variant was identified but its presence could not be excluded, or a variant of uncertain significance (VUS) to which it was not possible to attribute a risk value was detected.

Patients harboring a germline PV in *BRCA1/2* genes were directed to enhanced screening programs and/or risk-reducing surgical strategies by a professional with expertise in cancer genetics. Targeted *BRCA1/2* testing was proposed and extended to the first-degree family members of *BRCA*-variant patients, after providing informed consent.

Sample collection and next-generation sequencing analysis for BRCA1/2 *genes*

Peripheral blood samples were collected from BC patients. Genomic DNA was isolated from the peripheral blood using the DNeasy® Blood Kit (QIAGEN, Hilden, Germany), quantified by Qubit®3.0 fluorometer (Thermofisher Scientific, Waltham, MA, USA) and its quality was assessed by using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). We used 4 ng of DNA to prepare the barcoded library using *BRCA* Screen kit (4bases SA) that has allowed us to investigate all the exons of *BRCA1* (NM_007300.3) and *BRCA2* (NM_000059.3) genes. The kit consists of three multiplex PCR primer pools. We used 20 ng of DNA per primer pool for multiplex PCR amplification, followed by ligation of a barcode and purification with Agentcourt AMPureXP reagent (Beckman Coulter, Beverly, MA, USA). The quantity and the quality of prepared libraries were evaluated using Qubit®3.0 fluorometer (Thermofisher Scientific, Waltham, MA, USA) and Agilent 2100 Bioanalyzer on-chip electrophoresis (Agilent Technologies, Santa Clara, CA), respectively, as previously described.[25] Subsequently, libraries were mixed in an equimolar ratio and emulsion PCR was performed with the Ion OneTouch OT2 System (Thermofisher Scientific, Waltham, MA, USA) using Ion 520 & Ion 530 Kit-OT2 (Thermofisher Scientific, Waltham, MA, USA). Finally, sequencing was performed with Ion 520 Chip (Thermofisher Scientific, Waltham, MA, USA) using Ion Torrent S5 (Thermofisher Scientific, Waltham, MA, USA) instrument. The sequencing data were analyzed with Amplicon Suite (SmartSeq s.r.l., Novara, Italy) and Ion Reporter Software v.5.12 (Thermofisher Scientific, Waltham, MA, USA).

Sanger sequencing

Pathogenic variants of *BRCA1/2* genes were confirmed by Sanger sequencing using a BigDye Therminator 3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA, USA) and read through the 3130×l Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), according to the manufacturers' protocols.

CNV analysis by multiplex ligation-dependent probe amplification analysis

The presence of large genomic rearrangements (LGRs) was additionally tested by Multiplex ligation-dependent probe amplification (MLPA), using the SALSA MLPA probemix P002-C2 for *BRCA1* gene and SALSA MLPA Probemix P090 for *BRCA2* gene according to the manufacturer's instructions (MRC–Holland, Amsterdam, the Netherlands). Probe amplification products were analyzed by capillary electrophoresis using ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Results were analyzed by GeneMapper™ Software Version 3.5 (Applied Biosystems, Foster City, CA, USA) to determine peak heights and areas, and fragment sizes in base pairs (bp), as described previously.[26,27] Positive results were confirmed with an additional analysis using the same kit on a second blood sample.

Genetic variant classification

The *BRCA* genetic variants were screened based on the classification criteria developed by the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium (https://enigmaconsortium.org/) and according to IARC recommendations,[28] using a system of division into five classes: benign (class I), likely benign (class II), VUS (class III), likely pathogenic (class IV), and pathogenic (class V). Several databases, such as ClinVar, *BRCA* Exchange, LOVD, were used for the search and classification of *BRCA* variants. The positions of the variants on *BRCA1* and *BRCA2* genes were obtained and graphically represented using the informatic tool Mutation Mapper-cBioPortal for Cancer Genomics.[29,30]

The *BRCA* PVs identified in *BRCA*-positive carriers were named according to the systematic nomenclature of The Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/),[31] and to the recommendations for the description of sequence variants established by the Human Genome Variation Society (HGVS). HGVS nomenclature was authorized by the HGVS, Human Variome Project, and the Human Genome Organization.[32]

Statistical analysis

Clinicopathological variables and prevalence of *BRCA1/2* PVs were evaluated for each subgroup of patients. The comparison between subgroups was made with Fisher's Exact test. p-values <0.05 were considered significant.

Statistical analyses were conducted using IBM SPSS Statistics for Windows Version 23.0 (IBM Corporation, Armonk, NY, USA).

Results

Distribution of molecular subtypes

Between 1 January 2016 and 28 February 2020, 531 patients with BC who met eligibility criteria for *BRCA1/2* gene testing were included in a retrospective analysis. Mutational screening was offered at the "Regional Center for the prevention, diagnosis and treatment of rare and heredo-familial tumors of adults" of the Section of Medical Oncology of the University Hospital Policlinico "P. Giaccone" of Palermo, according to national guidelines (see section Patients and Methods). All women were tested for *BRCA1* and *BRCA2* germline PVs, after appropriate genetic counseling.

The distribution of BC molecular subtypes involved 125 (23.5%) Luminal A (LA), 223 (42%) Luminal B (LB), 29 (5.5%) HER2-enriched (HER2E), and 154 (29%) TNBC.

Breast cancer patients with BRCA1/2 pathogenic variants

In total, 83 out of 531 BC patients (15.6%) resulted positive for *BRCA1/2* PVs; 39 (47%) were *BRCA1*-positive, 43 (51.8%) were *BRCA2*-positive, and one patient (1.2%) showed double heterozygosity for *BRCA1* and *BRCA2* PVs. Among *BRCA1*-positive patients, 28 (71.9%) had a TNBC, 10 (25.6%) a LB tumor, one (2.5%) HER2E and none LA. Among *BRCA2*-positive tumors, 29 (67.5%) were LB, six (13.9%) TNBC, six (13.9%) LA and two (4.7%) HER2E (Figure 1).

Tumors from patients with *BRCA1* PVs were predominantly TNBCs ($p=0.0001$) and tumors with *BRCA2* PVs were mainly LB/HER2-negative ($p=0.0014$) (Table 1).

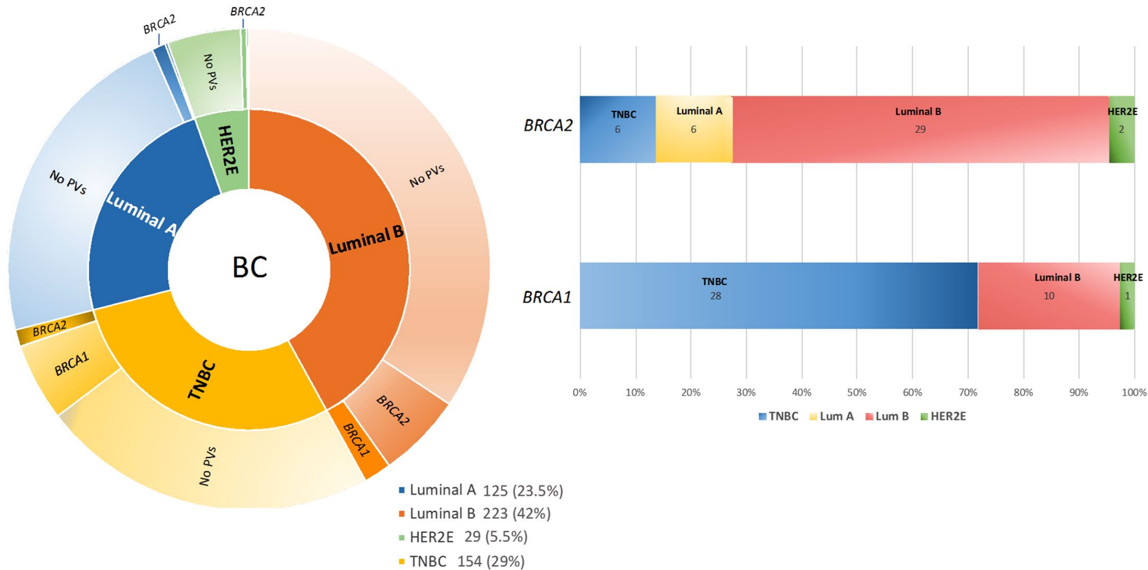

Figure 1. Number of breast cancer patients genetically tested for *BRCA1/2* PVs (Jan 2016–Feb 2020).
(a) Distribution of molecular subtypes in the study population; (b) Prevalence of molecular subtypes in *BRCA1*- and *BRCA2*-positive breast cancer patients. The numbers reported inside each box indicate the patient number, excluding the LB breast cancer patient showing double heterozygosity for *BRCA1* and *BRCA2* PVs.

Type and gene location of pathogenic variants of triple-negative versus *luminal-like breast cancers*

Our study was also aimed to evaluate the typology and gene location of germline *BRCA1* and *BRCA2* PVs in triple-negative versus luminal-like BCs, in order to investigate potential associations between specific PVs and tumor phenotype.

Based on the classification criteria developed by the ENIGMA consortium (https://enigmaconsortium.org/) and according to the IARC recommendations,[28] mutational analysis revealed that 45 PVs were present in a total of 83 patients with *BRCA1/2*-related BC. Mutational screening showed that 23 (51.1%) out of 45 observed PVs were detected in TNBCs, 18 of which were in *BRCA1* gene and five in *BRCA2* gene (Table 2), whereas 33 PVs were found in luminal-like BCs, eight of which in *BRCA1* and 25 in *BRCA2*, and, finally, three were observed in HER2E BCs, one of which in *BRCA1* and two in *BRCA2* (Table 3). Fourteen of 45 total PVs are reported two folds in Tables 2 and 3, beacause they were observed in different molecular subtypes of BC.

The most frequent PV identified in most of the analyzed *BRCA1*-positive TNBCs is named *BRCA1*-633delC (HGVS nomenclature: c.514del; p.Gln172fs) and involves the deletion of one nucleotide containing a cytosine (C) in *BRCA1* exon 8, which causes a frameshift, resulting in the substitution of the amino acid glutamine with asparagine at codon 172, creation of a premature stop codon at position 62 of the new reading frame, and formation of a truncated or absent BRCA1 protein.[33,34] This alteration was detected in five Sicilian families, involving a total of 15 PV carriers (five probands and 10 family members). The second most recurrent *BRCA1* PV associated with TNBCs and found in three families for a total of 10 PV carriers (three probands and seven family members) is named *BRCA1*-4023G>T (HGVS nomenclature: c.3904G>T; p.Glu1302Ter). This variant involves the substitution of one nucleotide containing a guanine (G) with another containing a thymine (T) in the *BRCA1* exon 11, causing the change of glutamic acid with a premature stop codon at codon 1302, resulting in the formation of a truncated BRCA1 protein.[35] In general, most PVs detected in the *BRCA1* gene of TNBC patients showed a low prevalence. Also, the few identified *BRCA2* PVs were detected with a lower frequency in TNBC patients. Therefore, no association between specific *BRCA1/2* PVs and TNBC in Sicilian study population was observed (Figure 2).

Concerning the distribution of *BRCA1/2* PVs in patients with luminal-like BC, the most frequent PV, named *BRCA2*-1466delT (HGVS

Table 1. *BRCA1/2* PV detection rate in Luminal A, Luminal B, HER2-enriched and TNBC patients.

	Total	*BRCA1*	*BRCA2*	DH *BRCA1-BRCA2 PVs*	Absence of PVs	*p*-value *
Luminal A	125	0 (0%)	6 (4.8%)	0 (0%)	119 (95.2%)	$p = 0.213$
Luminal B	223	10 (4.5%)	29 (13%)	1 (0.5%)	183 (82%)	$p = 0.0014$
HER2E	29	1 (3.4%)	2 (6.8%)	0 (0%)	26 (89.8%)	$p = 1.00$
TNBC	154	28 (18.2%)	6 (3.9%)	0 (0%)	120 (77.9%)	$p = 0.0001$

*Comparison *BRCA1* PV *versus BRCA2* PV *versus BRCA1/2* w.t.
DH, Double Heterozygosity; HER2E, Her2-enriched; TNBC, Triple Negative Breast Cancer.

nomenclature: c.1238del; p.Leu413fs), was observed in the *BRCA2* gene of LB molecular subtypes and detected in a total of 15 PV carriers (eight probands and seven family members). This variant involves the deletion of one nucleotide containing a thymine (T) in the *BRCA2* exon 10, which causes a frameshift resulting in the change of a Leucine with a Histidine at codon 413, formation of a premature termination codon and loss of the normal protein function.[36]

Interestingly, the most common Sicilian founder variant named *BRCA1*-5083del19 (HGVS nomenclature: c.4964_4982del; p.Ser1655fs)[27] showed a low prevalence both in TNBCs (6%) and luminal-like BCs (7.5% in LB tumors), as this PV was detected only in two and three families, respectively.

In general, most of the PVs found in patients with luminal-like BC are localized on *BRCA2* gene. No *BRCA1* PV was observed in patients harboring LA molecular subtypes. In the same way as TNBC patients, most PVs detected in *BRCA1* and *BRCA2* genes of LA and LB molecular subtypes showed a low prevalence in the Sicilian population, suggesting the absence of a significant association between specific *BRCA1/2* PVs and luminal-like tumors (Figures 3 and 4).

As regards the gene location of *BRCA1* and *BRCA2* PVs detected in TNBC patients of the Sicilian study cohort, most PVs have been shown to be localized inside three hypothetical cluster regions present in the BRCA1 protein structure which include the RING domain at the N-terminus, region encoded by exon 11, and BRCT domain near the C-terminus (Supplemental material Figure 1). Ten (55.5%) out of 18 *BRCA1* PVs were detected in exon 11 of TNBC patients (nucleotides: 916-4023; codons: 267-1302), whereas four were in the sequence corresponding to the BRCT repeats (nucleotides: 5083-5382; codons: 1655-1756) and only two in the RING domain (nucleotide: 185; codon: 23). More than half (11) of *BRCA1* PVs were frameshift mutations, whereas three were nonsense and three missense. Only a LGR involving a deletion (c.-232_4675del) ranging from exon 1 to exon 15 of *BRCA1* gene was detected in one TNBC patient. Almost all *BRCA2* PVs (four) observed in TNBCs were frameshift mutations distributed along the entire gene sequence (Supplemental material Figure 1).

Although *BRCA1* PVs are poorly represented in patients with luminal-like BC, these few variants are equally distributed into three putative cluster regions containing the RING domain, region encoded by exon 11, and BRCT domain, as already observed in TNBC patients. Conversely, most PVs observed in patients with luminal-like BC were mainly localized inside three other putative cluster regions present in the BRCA2 protein structure, which include the PALB2 binding site at the N-terminus, BRC repeats (located within the exon 11), and DNA binding helical domain near the C-terminus (Supplemental material Figure 2). Six (24%) out of 25 *BRCA2* PVs were detected in exon 11 (mainly within the BRC repeats) of patients with luminal-like BC (nucleotides: 3036-6352; codons: 938-2042), whereas three were in exon 10 (nucleotides: 1466-2070; codons: 413-615) and three in the DNA binding helical domain (nucleotides: 7909-9481; codons: 2561-3085). More than a third (nine) of *BRCA2* PVs were frameshift mutations, seven were intronic variants (IVS), whereas five were nonsense and four missense. Half of the *BRCA1* PVs (four) observed in LB cancer patients were frameshift mutations distributed along the entire gene sequence (Supplemental material Figure 2).

Table 2. *BRCA1/2* pathogenic variants in TNBCs.

TNBC						
Gene	**Type of PV**	**HGVS Nomenclature**	**BIC Nomenclature**	**Protein change**	**No. families**	**No. PV carriers (patients and family members)**
BRCA1	Deletion	c.514del	633delC	p.Gln172fs	5 (14.5%)	15
BRCA1	SNV	c.3904G>T	4023G>T	p.Glu1302Ter	3 (9.1%)	10
BRCA1	Duplication	c.5266dupC	5382insC	p.Gln1756Profs	3 (9.1%)	5
BRCA1	Deletion	c.4964_4982del	5083del19	p.Ser1655fs	2 (6%)	4
BRCA1	SNV	c.3400G>T	3519G>T	p.Glu1134Ter	2 (6%)	2
BRCA1	Deletion	c.798_799del	916delTT	p.Ser267fs	1 (2.9%)	9
BRCA1	Deletion	c.1360_1361del	1479delAG	p.Glu453_ Ser454insTer	1 (2.9%)	7
BRCA1	Deletion	c.3228_3229del	3347delAG	p.Gly1077fs	1 (2.9%)	6
BRCA1	Deletion	c.1531del	/	/	1 (2.9%)	6
BRCA1	Deletion	c.5030_5033del	5147del4	p.Thr1677fs	1 (2.9%)	4
BRCA1	Duplication	c.66dupA	185insA	p.Glu23Argfs	1 (2.9%)	3
BRCA1	SNV	c.5123C>A	5242C>A	p.Ala1708Glu	1 (2.9%)	1
BRCA1	Deletion	c.3266del	3385delT	p.Leu1089fs	1 (2.9%)	1
BRCA1	Deletion	c.3599_3600del	3718delAG	p.Gln1200Argfs	1 (2.9%)	1
BRCA1	Deletion	c.882del	1001delA	p.Asp295fs	1 (2.9%)	1
BRCA1	SNV	c.2722G>T	2841G>T	p.Glu908Ter	1 (2.9%)	1
BRCA1	Deletion	c.66_67del	185_186delAG	p.Glu23fs	1 (2.9%)	1
BRCA1	LGR	c.-232_4675del	/	/	1 (2.9%)	1
BRCA2	Deletion	c.5851_5854del	6076del4	p.Ser1951fs	2 (6%)	4
BRCA2	SNV	c.8954-15T>G	/	/	1 (2.9%)	4
BRCA2	Deletion	c.1238del	1466delT	p.Leu413fs	1 (2.9%)	1
BRCA2	Deletion	c.9455_9456del	9683delAG	p.Glu3152fs	1 (2.9%)	1
BRCA2	Deletion	c.6082_6086del	6310del5	p.Glu2028fs	1 (2.9%)	1

Abbreviations: LGR, large genomic rearrangement; PV, pathogenic variant; SNV, single nucleotide variant.

Association between BRCA1/2 pathogenic variants and clinical variables

In the TNBC subgroup, the vast majority (82.4%) of *BRCA*-carriers were premenopausal at BC diagnosis (before the age of 50 years), with mean age of 43.7 years (median: 43). A statistically significant difference in mean age between *BRCA1* and *BRCA2* PV carriers is evident. The mean age at BC diagnosis of *BRCA1* PV carriers was 41.7 years (median: 42; range: 28–58 years), 52.8 years (median 52.5; range: 42–62 years) for *BRCA2* PV carriers, and 48.2 years (median 48;

Table 3. *BRCA1/2* pathogenic variants in luminal-like and HER2E patients.

Luminal B						
Gene	**Type of PV**	**HGVS Nomenclature**	**BIC Nomenclature**	**Protein change**	**No. families**	**No. PV carriers (patients and family members)**
BRCA1	Deletion	c.4964_4982del	5083del19	p.Ser1655fs	3 (7.5%)	7
BRCA1	Deletion	c.514del	633delC	p.Gln172fs	2 (5%)	2
BRCA1	SNV	c.2722G>T	2841G>T	p.Glu908Ter	1 (2.4%)	2
BRCA1	SNV	c.5096G>A	5215G>A	p.Arg1699Gln	1 (2.4%)	1
BRCA1	Deletion	c.3228_3229del	3347delAG	p.Gly1077fs	1 (2.4%)	1
BRCA1	Deletion	c.66_67del	185_186delAG	p.Glu23fs	1 (2.4%)	1
BRCA1	SNV	c.3904G>T	4023G>T	p.Glu1302Ter	1 (2.4%)	1
BRCA2	Deletion	c.1238del	1466delT	p.Leu413fs	8 (19.5%)	15
BRCA2	Deletion	c.9026_9030del	9254del5	p.Tyr3009fs	2 (5%)	4
BRCA2	Deletion	c.6082_6086del	6310del5	p.Glu2028fs	2 (5%)	4
BRCA2	SNV	c.476-2A>G	IVS5-2A>G	/	2 (5%)	4
BRCA2	Duplication	c.9253dup	9481insA	p.Thr3085Asnfs	2 (5%)	2
BRCA2	SNV	c.631G>A	859G>A	p.Val211Ile	1 (2.4%)	3
BRCA2	Deletion	c.5851_5854del	6076del4	p.Ser1951fs	1 (2.4%)	3
BRCA2	SNV	c.8754+4A>G	IVS21+4A>G	/	1 (2.4%)	3
BRCA2	SNV	c.8632+2T>C	/	/	1 (2.4%)	2
BRCA2	SNV	c.6124C>T	6352C>T	p.Gln2042Ter	1 (2.4%)	2
BRCA2	SNV	c.7681C>T	7909C>T	p.Gln2561Ter	1 (2.4%)	2
BRCA2	Deletion	c.2808_2811del	3036del4	p.Ala938Profs	1 (2.4%)	2
BRCA2	Duplication	c.1842dup	2070insT	p.Asn615Terfs	1 (2.4%)	1
BRCA2	SNV	c.7007G>A	7235G>A	p.Arg2336His	1 (2.4%)	1
BRCA2	Deletion	c.1472del	1700delC	p.Thr491Ilefs18	1 (2.4%)	1
BRCA2	SNV	c.396T>A	624T>A	p.Cys132Ter	1 (2.4%)	1
BRCA2	Deletion	c.5595_5596del	5823delAT	p.Phe1866fs	1 (2.4%)	1
BRCA2	SNV	c.8487+1G>A	IVS19+1G>A	/	1 (2.4%)	1
BRCA1/ BRCA2	SNV	c.181T>G*	300T>G*	p.Cys61Gly	1 (2.4%)	4
	SNV	c.8331+2T>C*	IVS18+2T>C*	/		

(Continued)

Table 3. (Continued)

Luminal A						
Gene	**Type of PV**	**HGVS Nomenclature**	**BIC Nomenclature**	**Protein change**	**No. families**	**No. PV carriers (patients and family members)**
BRCA2	SNV	c.631G>A	859G>A	p.Val211Ile	1 (16.67%)	3
BRCA2	SNV	c.8487+1G>A	IVS19+1G>A	/	1 (16.67%)	2
BRCA2	SNV	c.93G>A	321G>A	p.Trp31Ter	1 (16.67%)	1
BRCA2	SNV	c.7007G>A	7235G>A	p.Arg2336His	1 (16.67%)	1
BRCA2	Duplication	c.5073dup	5301insA	p.Trp1692Metfs	1 (16.67%)	1
BRCA2	SNV	c.8754+4A>G	IVS21+4A>G	/	1 (16.67%)	1
HER2E						
Gene	**Type of PV**	**HGVS Nomenclature**	**BIC Nomenclature**	**Protein change**	**No. families**	**No. PV carriers (patients and family members)**
BRCA1	Duplication	c.5266dupC	5382insC	p.Gln1756Profs	1 (33.3%)	2
BRCA2	Deletion	c.5073del	5301delA	p.Lys1691fs	1 (33.3%)	1
BRCA2	Deletion	c.7679-7680del	7907delTT	p.Phe2560fs	1 (33.3%)	1

Abbreviations: PV, Pathogenic Variant; SNV, Single Nucleotide Variant.
*These PVs are present in one proband showing double heterozygosity for *BRCA1* and *BRCA2* PVs.

range: 30–70 years) for subjects with no *BRCA1/2* PV. On average, patients with TNBC and *BRCA1* PVs developed BC 6.45 years earlier than non-carrier individuals ($p < 0.001$), and 11.1 years earlier than *BRCA2* PV carriers ($p < 0.001$) (Figure 5a). Prevalence of PVs was 12/34 (35.3%) in the age group ⩽40 years, 16/34 (47.1%) in age range of 41–50 years, 5/34 (14.7%) in 51–60 years, and 1/34 (2.9%) in subjects with age >60 years (Table 4).

In the luminal-like subgroup, the mean age at BC diagnosis for *BRCA*-positive carriers was 43.75 years (median 40), 39.1 years (median: 36.5; range: 31–55 years) for *BRCA1* PV carriers, and 45.1 years (median: 41; range: 26–82 years) for *BRCA2* PV carriers. Individuals with no *BRCA1/2* PV showed mean age at BC diagnosis of 45.7 years, with a wider range (21–84 years; median: 44.5). On average, patients with luminal-like *BRCA1*-PVs developed BC 6.6 years earlier than non-carrier individuals ($p = 0.0538$), and 6 years earlier than *BRCA2* PV carriers ($p = 0.78$) (Figure 5b).

Prevalence of PVs was 27/46 (58%) in the age group ⩽40 years, 9/46 (20%) in age range of 41–50 years, 6/46 (13%) in 51–60 years, and 4/46 (9%) in subjects with age >60 years (Table 4).

Significant clinicopathological differences between *BRCA* PV carriers and non-carrier BC patients were observed (Table 4). In the luminal-like subgroup, *BRCA*-positive BCs were more likely associated to lower ER ($p = 0.001$) and PR expression ($p = 0.007$), and were more frequently HER2-negative ($p = 0.048$). *BRCA* PV carriers had a high proliferation rate (Ki-67%; $p = < 0.001$) and higher histological grade (Grade III *versus* I/II) than non-carriers ($p = < 0.001$). In either subgroup, TNBCs and luminal-like tumors, patients with *BRCA* PV more likely had an axillary nodal involvement ($p = 0.002$ and $p = 0.016$, respectively), while no significant differences were observed in tumor size (T) ($p = 0.802$ and $p = 0.920$, respectively).

All TNBC and most luminal-like BC patients showed ductal histotype, without statistically

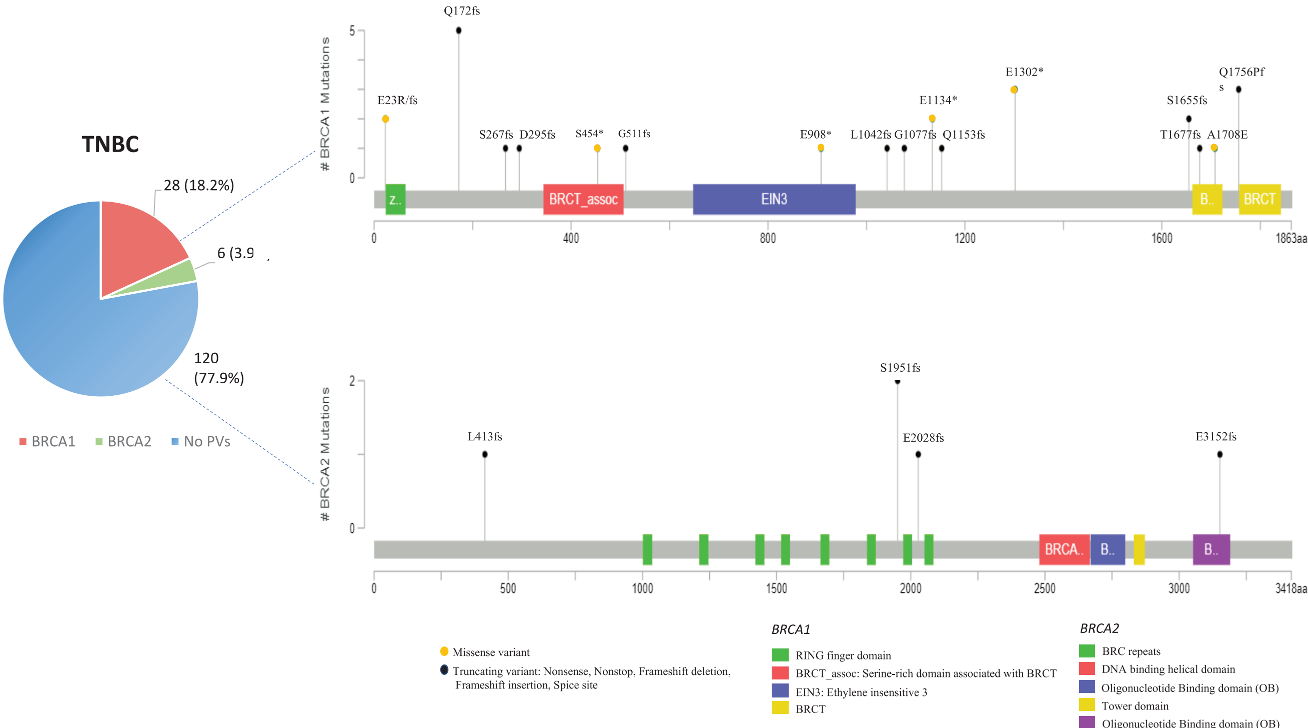

Figure 2. Lollipop plots showing the distribution and frequency of *BRCA1* and *BRCA2* PVs identified in TNBC patients. The plots were obtained by the informatic tool Mutation Mapper-cBioPortal for Cancer Genomics (GenBank Reference BRCA1: NM_007294 and GenBank Reference BRCA2: NM_000059). The Intronic Variant Sequences (IVS) are not shown in the lollipop plots. The lollipop height indicates the frequency of *BRCA1/2* PVs in different molecular subgroups of our study cohort.

significant differences between *BRCA* PV carriers and non-carriers ($p=0.337$ and $p=0.7$, respectively).

Contralateral breast tumors occurred in 96 (19.1%) patients: five (14.7%) out of 34 *BRCA1/2* PV carriers with TNBC, 13 (28.2%) out of 46 *BRCA1/2* PV carriers with luminal-like BC, and 78 (18.4%) out of 422 *BRCA*-negative patients, including 12 (10%) out of 120 TNBCs and 66 (21.8%) out of 302 luminal-like tumors. Controlateral tumors in *BRCA1/2*-positive TNBC patients were diagnosed at a younger age (50 years) than non-carriers (56 years) ($p=0.033$). In patients with luminal-like BC, the difference between median age of *BRCA*-carriers and non-carriers was lower (52 *versus* 53 years).

Median time to contralateral BC was 10 years in *BRCA*-positive TNBC patients and 6.5 years in *BRCA*-negative TNBC patients ($p=0.389$). In the luminal-like subgroup, the median time between the first and second tumor was shorter both in *BRCA*-positive (4 years) and *BRCA*-negative patients (3 years) ($p=0.465$). Overall, the median time of onset of bilateral tumors was lower in luminal-like than TNBC patients. Clinicopathological characteristics of Triple-Negative and Luminal-like BC patients are presented in Table 4.

Discussion

Breast cancer is a heterogeneous disease at the genetic, histological, molecular and clinical level, showing a wide variability in the prognosis, treatment and patient outcomes. This heterogeneous nature is highlighted from recent advances in genetic and genomic fields. In recent years, an increasing amount of new information on germline PVs in cancer susceptibility genes has been collected,[37] determining a substantial increase in the request and indication of genetic testing for cancer risk assessment[27] and requiring physicians to integrate this information into strategies of prevention, surveillance and treatment decision making.[38]

Germline *BRCA1/2* PVs confer high risk of developing BC, increased more than fourfold compared with the general population. Multiple

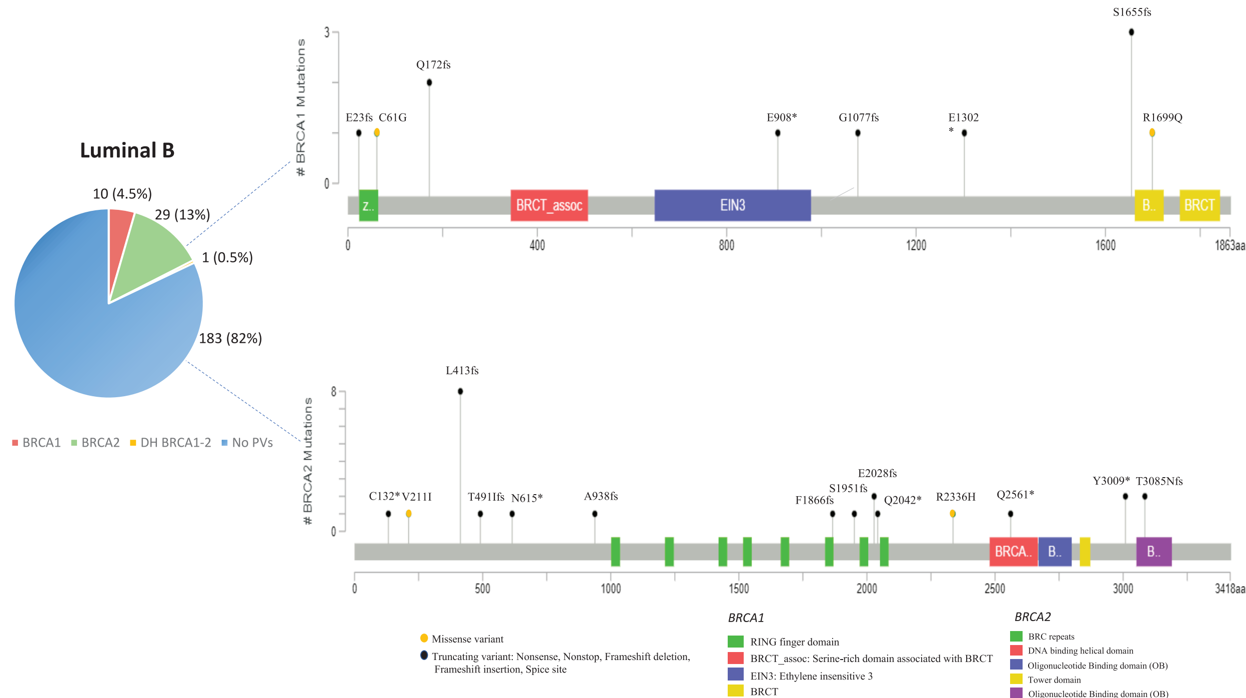

Figure 3. Lollipop plots showing the distribution and frequency of *BRCA1* and *BRCA2* PVs identified in luminal B breast cancer patients. The plots were obtained by the informatic tool Mutation Mapper-cBioPortal for Cancer Genomics (GenBank Reference BRCA1: NM_007294 and GenBank Reference BRCA2: NM_000059). The Intronic Variant Sequences (IVS) are not shown in the lollipop plots. The lollipop height indicates the frequency of BRCA1/2 PVs in different molecular subgroups of our study cohort.

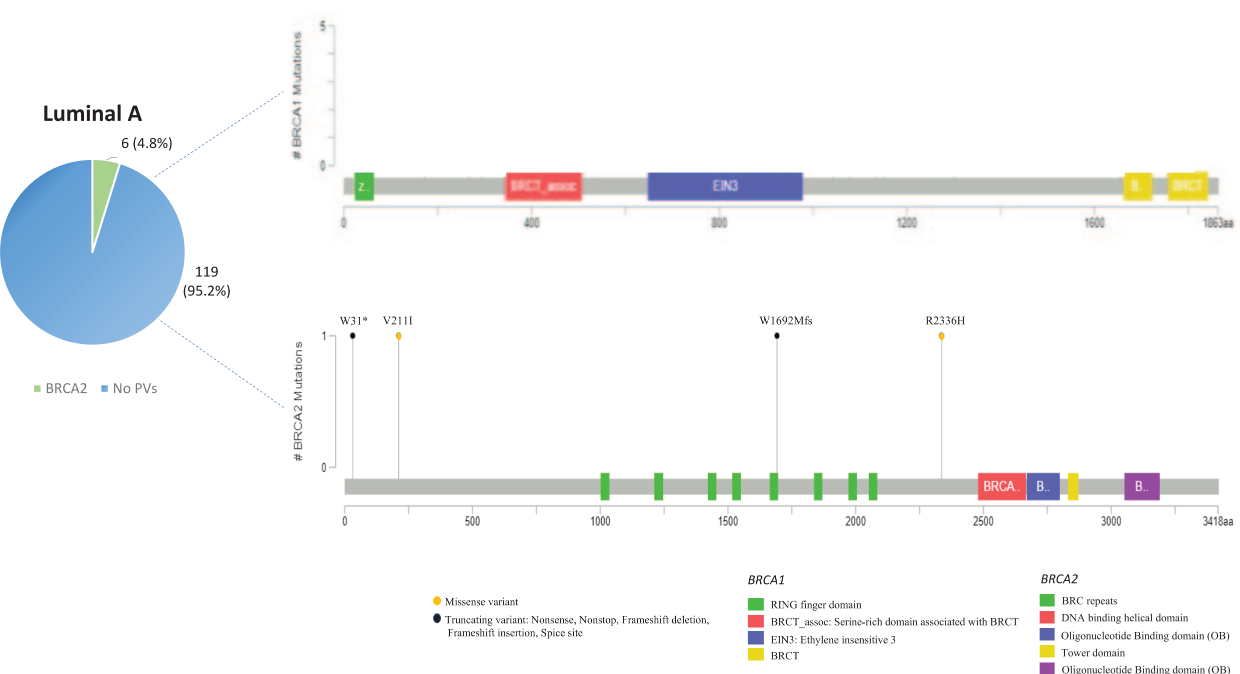

Figure 4. Lollipop plots showing the distribution and frequency of *BRCA1* and *BRCA2* PVs identified in luminal A breast cancer patients. The plots were obtained by the informatic tool Mutation Mapper-cBioPortal for Cancer Genomics (GenBank Reference BRCA1: NM_007294 and GenBank Reference BRCA2: NM_000059). The Intronic Variant Sequences (IVS) are not shown in the lollipop plots. The lollipop height indicates the frequency of BRCA1/2 PVs in different molecular subgroups of our study cohort.

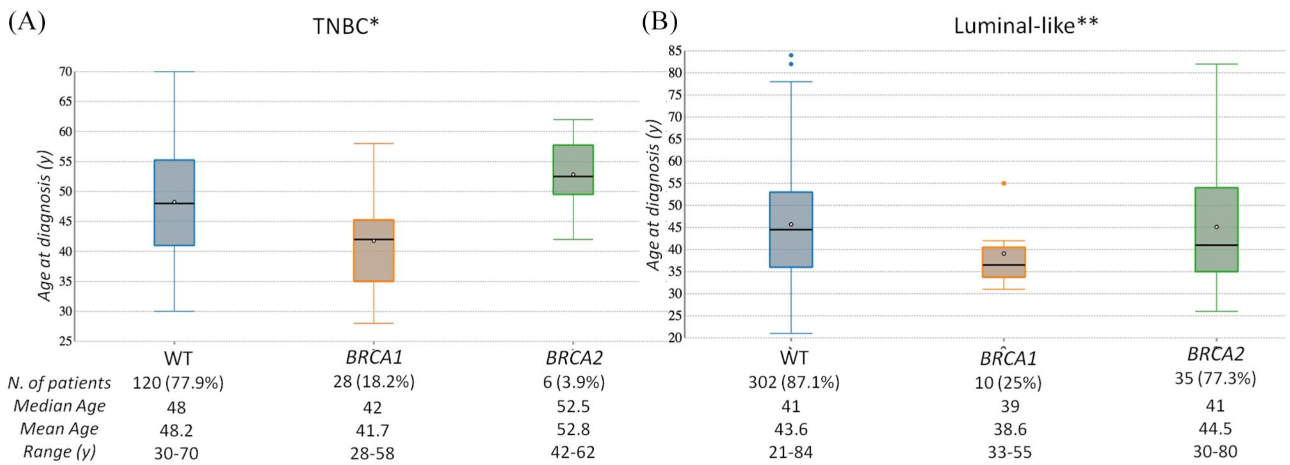

Figure 5. Boxplots showing difference in age at diagnosis among women without *BRCA1/2* PVs *versus* women with *BRCA1* or *BRCA2* PV. (a) TNBC subgroup; (b) Luminal-like BC subgroup.
*WT *versus* BRCA1 $p < 0.001$; WT *versus* BRCA2 $p = 0.26$; BRCA1 *versus* BRCA2 $p < 0.001$; **WT *versus* BRCA1 $p = 0.053$; WT *versus* BRCA2 $p = 0.94$; BRCA1 *versus* BRCA2 $p = 0.78$.

Table 4. Baseline characteristics and clinicopathological information of Triple-Negative and Luminal-like BC patients.

	TNBC*		**Luminal-like****		***p-value***	****p-value***
	WT	***BRCA1/2***	**WT**	***BRCA1/2***		
Number of patients (502)	120 (77.9%)	34 (22.1%)	302 (86.8%)	46 (13.2%)	-	-
Age at diagnosis (y):						
Median	48	43	41	40	0.013	0.308
Mean	48.25	43.7	43.6	42.8		
Range	30–70	28–62	21–84	30–80		
Age groups (y)						
⩽ 40	30 (25%)	12 (35.3%)	140 (46.3%)	27 (58%)	0.135	0.580
41–50	43 (35.8%)	16 (47.1%)	86 (28.4%)	9 (20%)		
51–60	36 (30%)	5 (14.7%)	49 (16.2%)	6 (13%)		
>60	11 (9.2%)	1 (2.9%)	27 (9.1%)	4 (9%)		
Histological Subtype						
Ductal	119 (99.2%)	33 (97%)	237 (78.5%)	36 (78.2%)	0.337	0.700
Lobular	0 (%)	0 (%)	32 (10.6%)	7 (15.2%)		
Others	1 (0.8%)	1 (3%)	30 (9.9%)	3 (6.6%)		
unknown	\	\	3 (1%)	\		
ER (%)						
⩽20			14 (4.6%)	8 (17.4%)	-	0.001

(Continued)

Table 4. (Continued)

	TNBC*		Luminal-like**		**p-value*	***p-value*
	WT	***BRCA1/2***	**WT**	***BRCA1/2***		
>20			267 (88.5%)	34 (73.9%)		
unknown	\	\	21 (6.9%)	4 (8.7%)		
PR (%)						
⩽20			60 (19.9%)	19 (41.3%)	-	0.007
>20			211 (69.9%)	23 (50%)		
unknown	\	\	31 (10.2%)	4 (8.7%)		
HER2 (%)						
pos	\	\	63 (20.9%)	4 (8.7%)	-	0.048
neg			220 (72.8%)	42 (91.3%)		
unknown			19 (6.3%)	/		
Ki-67 (%)						
<20	10 (8.3%)	2 (5.9%)	105 (34.8%)	6 (13%)	0.854	<0.001
20–50	34 (28.4%)	9 (26.5%)	120 (39.7%)	21 (45.7%)		
>50	76 (63.3%)	23 (67.6	39 (12.9%)	15 (32.6%)		
unknown	\	\	38 (12.6%)	4 (8.7%)		
Histological grade						
G1	4 (3.3)	1 (2.9%)	43 (14.3%)	1 (2.2%)	0.882	<0.001
G2	18 (15%)	4 (11.8%)	141 (46.7%)	14 (30.4%)		
G3	98 (81.7%)	29 (85.3%)	84 (27.8%)	26 (56.5%)		
unknown	\	\	34 (11.2%)	5 (10.9%)		
Tumor size (T)						
T1	74 (61.7%)	18 (53%)	142 (47.1%)	19 (41.3%)	0.802	0.920
T2	34 (28.3%)	12 (35.3%)	77 (25.6%)	10 (21.7%)		
T3	10 (8.3%)	3 (8.8%)	5 (1.7%)	/		
T4	2 (1.7%)	1 (2.9%)	4 (1.4%)	1 (2.2%)		
unknown	\	\	73 (24.2%)	16 (34.8%)		
Axillary nodal involvement (N)						
N0	88 (73.3%)	13 (38.2%)	126 (41.7%)	16 (34.8%)	0.002	0.016
N1	22 (18.3%)	14 (41.3%)	66 (21.9%)	10 (21.7%)		
N2	8 (6.7%)	6 (17.6%)	13 (4.3%)	2 (4.4%)		
N3	2 (1.7%)	1 (2.9%)	4 (1.3%)	4 (8.7%)		

(Continued)

Table 4. (Continued)

	TNBC*		Luminal-like**		*p-value*	***p-value*
	WT	*BRCA1/2*	WT	*BRCA1/2*		
unknown	\	\	93 (30.8%)	14 (30.4%)		
Bilateral						
Yes	12 (10%)	5 (14.7%)	66 (21.8%)	13 (28.2%)	0.439	0.425
No	108 (90%)	29 (85.3%)	236 (78.2%)	33 (71.8%)		
Median age at diagnosis (y)						
Primary tumor	48	40	48	41	0.033	0.0474
Secondary tumor	56	50	53	52		
Time between 1st and 2nd tumors (y)						
Median	6.5	10	3	4	0.389	0.465

*Comparison TNBC WT *versus BRCA1/2;* **Comparison luminal-like WT *versus BRCA1/2.*

germline or somatic mutations in other genes involved in homologous recombination deficiency (HRD),[6] such as *PALB2, CHEK2, ATM, RAD51, ATR, CHK1* and *WEE1*, have been recently observed, but at relatively low frequencies, occurring in 4–6% of BC patients, and with lower lifetime risk than *BRCA1/2* PVs.[39–41] However, the evidence regarding other BC susceptibility genes is still limited and additional studies are needed to better define their role. Therefore, despite variations in the prevalence among different ethnic groups and geographical zones,[42–44] inherited PVs in *BRCA1/2* genes are confirmed as the most frequent in BC. Integrating all genetic knowledge into surveillance and prevention strategies and patient care is the crucial aim of physicians.

The germline PVs in *BRCA1/2* genes are associated with an increased risk of developing cancer for each molecular subtype of BC, defined by estrogen, progesterone and HER2 receptor status. However, it was demonstrated that *BRCA1*-related tumors have often a profile which resembles the TNBC subtype, whereas *BRCA2*-associated tumors have a profile that resembles luminal B or, less frequently, luminal A tumor subtypes.[45] These differences point to a heterogeneity in BC biology and molecular phenotype among tumors related to different germline *BRCA1* and *BRCA2* PVs.[19]

Previous research has indicated that structural and functional changes of mutated proteins caused by different *BRCA1* PVs are not identical and can lead to various tumor phenotypes.[22] However, several studies were mainly focused on the impact of PVs located in different exons of the *BRCA1* or *BRCA2* genes and on phenotypic variations of cancer relative risks.[46,47] Rebbeck *et al.*[21] investigated whether the type and location of *BRCA1/2* PVs were associated with the variation in BC and OC risk, showing that patients carrying *BRCA1* PVs within exon 11 had different disease phenotypes than patients carrying *BRCA1* PVs in other gene loci. Similarly, different PVs in specific genomic regions were associated with variability in BC and OC risk.[21,47–49] Also murine models of different mutations in *BRCA1/2* suggested that a genotype–phenotype correlation exist.[49,50] However, how molecular phenotypes differ by type, function and location of *BRCA1/2* PVs has not been fully investigated.

In this study, we screened 531 patients with BC for germline PVs in *BRCA1/2* genes according to national guidelines. We detected 45 *BRCA1/2* PVs in 83 BC patients. TNBC has been shown to be the molecular subgroup where *BRCA1/2* PVs are found more frequently (22.1%), followed by Luminal B tumors (18%), whereas *BRCA1/2* alterations are less frequent in HER2E (10.2%) and Luminal A (4.8%) BC patients. We confirmed a significant association between TNBC and *BRCA1* PVs and between Luminal B tumors and *BRCA2* PVs.

Confirming the previous findings, in our patient cohort, breast tumors with *BRCA* PVs occur in younger women. *BRCA1* PV carriers, in addition to a higher predisposition toward the onset of TNBC, developed BC earlier than *BRCA2* PV carriers and non-carrier individuals, and the difference in age at diagnosis between *BRCA1* and *BRCA2* PV carriers is greater in TNBC patients.

The findings in this study indicate that tumors present in *BRCA1/2* PV carriers were differentially associated with several prognostic factors compared with non-carriers. The tumors in *BRCA1/2*-positive patients showed a higher proportion of Ki67-positive cells, a higher histological grade and an axillary nodal involvement. In the luminal-like subgroup, *BRCA*-positive BCs were more likely associated with low ER, PR and HER2 expression. Furthermore, differences were observed between PV carriers and non-carriers in the presence of bilateral tumors. In *BRCA*-positive patients, controlateral BC was more common and had a lower median time to second tumor development in luminal-like BC compared with TNBC and *BRCA*-negative patients.

Concerning the variant type, although no significant association between specific *BRCA1/2* PVs and TNBC or luminal-like tumors in the study cohort was observed, a phenotypic variation of BC in patients with different *BRCA1/2* PV type seems to be detectable. Most of the TNBC-associated *BRCA1* PVs and luminal-like BC-associated *BRCA2* PVs were frameshift mutations for both molecular subtypes. However, a significant percentage of pathogenic IVS was detected in *BRCA2* gene of luminal-like BC patients. Interestingly, differences in the frequency of two PVs potentially associated with TNBC and luminal-like tumors, respectively, were observed. *BRCA1*-633delC was detected with higher prevalence in TNBC patients (five families, including 15 PV carriers) and only in two families with LB tumors, whereas *BRCA2*-1466delT was found in eight families (including 15 PV carriers) with LB tumors, but in no TNBC patient.

The *BRCA1*-633delC emerged as a PV type related to TNBC diagnosed at younger age and featuring poor prognostic factors, such as high proliferation rate and nuclear grade.

The *BRCA2*-1466delT was more likely associated to HER2-negative BC with higher ER expression (range 70–95%), in patients who carried a high proportion of bilateral breast tumors.[51] In addition, as *BRCA1*-633delC has been infrequently observed in other Italian regions or in the world, this PV could be further investigated for a possible founder effect specific for the Sicilian population.[27]

Understanding the mutational background underlying the phenotype of each tumor may have not only prognostic, but also preventive and therapeutic implications. The four surrogate intrinsic subtypes are the most important criteria for clinical decisions and imply distinct treatment approaches. Systemic therapies are routinely selected through a few well-established biomarkers of response, including tumor ER and PgR expression, and amplification or overexpression of tumor HER2. Although advances in molecular profiling and genetic expression studies have identified different subtypes of TNBC, making it increasingly heterogeneous, primary TNBC continues to be typically treated as a single disease, due to the absence of specific drivers.[52]

In TNBC, chemotherapy is the standard treatment option and typically involves the use of anthracycline and taxane. For *BRCA*-associated TNBC, the platinum-based agents and PARP inhibitors, such as olaparib and talazoparib, showed a particular efficacy.[53]

We hypothesized that also among BRCA-related TNBCs there is a marked genetic heterogeneity, which could define the phenotype of BCs associated with mutations. This could affect the natural history of these tumors and make them potential candidates for different treatment options.

In our cohort, the patients with BRCA1-633delC were found to be less chemosensitive than those harboring BRCA1-4023G>T and BRCA1-5382insC, who showed, instead, a more chemosensitive and prolonged survival benefit. Our observations should be interpreted cautiously due to the limited number of patients in each subgroup. Nevertheless, our investigation suggests that chemosensitivity in TNBC patients may widely vary in the same molecular phenotype of the tumor. Conversely, no correlation between different responses to treatment and different mutation type/localization was observed in patients with luminal-like tumor.

BRCA testing, that was previously used solely to predict the risk of future cancers and drive

surgical treatments, could acquire, in the future, an additional significance for treatment response and resistance.

Referring to the work by Rebbeck and colleagues,[21] we tried to identify the regions for which the variant site could define a possible genotype/phenotypic effect related to TNBC risk or luminal-like tumors in the Sicilian population. In particular, we have observed that most of the *BRCA1* PVs (55.5%) and *BRCA2* PVs (24%) detected in TNBC and luminal-like patients, respectively, were located within exon 11, which represents the majority of the coding sequence of both genes and is generally considered a "coldspot" for missense PVs.[54] In addition to the region encoded by exon 11, other two BRCA1 protein regions, RING domain at the N-terminus and BRCT domain, seem to be involved, to a lesser extent, in the TNBC risk, confirming the crucial role in tumor suppression played by these structural components.[54,55] Our data did not allow us to define new regions other than those already known in the literature, such as the OCCRs or BCCRs. The heterogeneous distribution of PVs and their low prevalence in TNBC patients could reflect the genetic heterogeneity of the Sicilian population, probably determined by the colonization of this island of Mediterranean Sea by many and different peoples throughout history.

Although this study adds significant and useful information to the current knowledge in the field, it does however, show some potential limitations. Our study is a retrospective analysis of BC patients who were referred to the genetic counseling service for testing of the *BRCA1* and *BRCA2* genes. Thus, the *BRCA* non-carrier control group may not be a fair representation of sporadic cancers. In addition, our results need to be confirmed by future studies, which prospectively test for *BRCA1/2* mutations in BC patients, in order to minimize the possibility of selection bias.

In conclusion, our results corroborate the evidence that *BRCA1*-related tumors have often a profile which resembles the TNBC subtype, whereas *BRCA2*-associated tumors have a profile that resembles luminal-like BCs, especially the luminal B tumor subtypes.

Previous studies showed the association between type and location of *BRCA1/2* PVs and phenotypic variations of cancer relative risks. The findings from this study suggest that, although no clear association between specific *BRCA1/2* PVs and TNBC or luminal-like tumors was observed, the pathogenic variants identified in TNBC were not largely overlapping with those detected in luminal-like tumors. Future studies examining the type and location of *BRCA1/2* PVs within the molecular subtype are required to verify this hypothesis, and could offer an interesting insight into the complex topic of genotype–phenotype correlations.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Author contributions

Conceptualization, L.I., D.F., A.R. and V.B.; genetic counselling D.F., V.C.; sample collection and gene testing, M.B., D.C., A.F., N.B., and A.P.; data curation and analysis, L.I., D.F., C.B., S.C., G.B., L.R.C, M.L., and S.C; writing L.I. and D.F.; supervision, A.R., G.B. and V.B. All authors have read and agreed to the published version of the manuscript.

Supplemental material

Supplemental material for this article is available online.

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