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ANNALS OF ONCOLOGY DRIVING INNOVATION IN ONCOLOGY

ORIGINAL ARTICLE

Association between type and location of germline *BRCA1/2* pathogenic or likely pathogenic variants with phenotype and prognosis in young patients with breast cancer: results from an international cohort study

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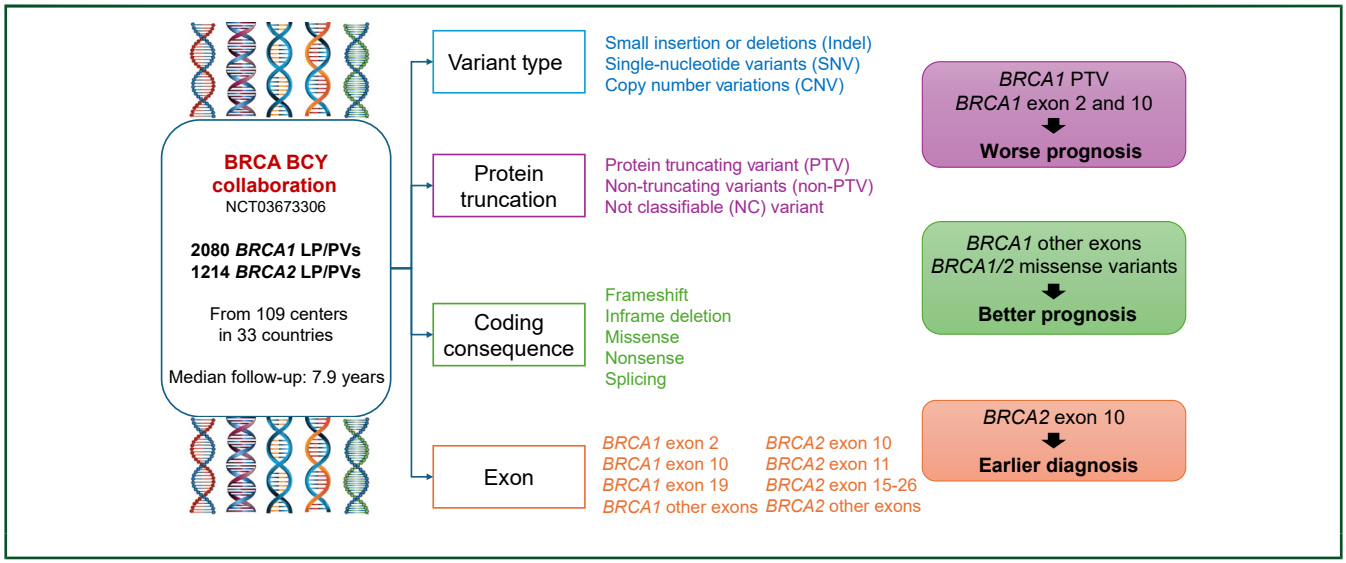
GRAPHICAL ABSTRACT



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Background: The clinical implications of specific pathogenic and likely pathogenic variant (LP/PV) types and locations in the *BRCA1 or BRCA2* tumor-suppressor genes remain to be elucidated.

Patients and methods: The BRCA BCY Collaboration (NCT03673306) is an international, multicenter, hospital-based, retrospective cohort study that included *BRCA* carriers diagnosed with invasive breast cancer at the age of $\leq$40 years between January 2000 and December 2020. In this analysis, only patients with detailed available information on LP/PVs in the *BRCA* genes were included. Clinicopathological features and survival outcomes [disease-free survival (DFS) and overall survival (OS)] were investigated according to LP/PV type [insertion-deletion (indel) versus single-nucleotide variants versus copy number variations; truncating versus non-truncating LP/PVs; frameshift versus nonsense versus splicing versus missense LP/PVs] and location (exon involved and protein domain).

Results: Out of 5660 patients from 109 centers worldwide, 3294 were eligible for the present analysis (2080 *BRCA1* and 1214 *BRCA2*). The distribution of LP/PV types showed no meaningful associations with baseline clinicopathological features. BRCA1 protein-truncating variants were associated with worse OS compared with non-truncating variants [hazard ratio (HR) 2.00, 95% confidence interval (CI) 1.17-3.41]. A similar, though non-significant, trend was observed for *BRCA2*. Missense variants were linked to better OS for both *BRCA1* (HR 0.48, 95% CI 0.28-0.84) and *BRCA2* carriers (HR 0.17, CI 0.03-0.96). Regarding variant location, *BRCA1* LP/PVs outside exons 2, 10, and 19 were associated with improved OS. In *BRCA2*, LP/PVs located in exons 15-26 and other regions were linked to worse DFS compared with those in exon 10, with no significant differences in OS.

Conclusions: This study advances our understanding of the influence of specific types of *BRCA* LP/PVs on breast cancer characteristics and outcomes. A deeper understanding of these variant-specific features will drive future research and support the development of tailored clinical strategies based on individual *BRCA* variant.

Key words: hereditary breast cancer, *BRCA1*, *BRCA2*, variant type, protein-truncating variant, overall survival

INTRODUCTION

BRCA1 and *BRCA2* are tumor-suppressor genes encoding for multidomain proteins, involved in the error-free repair of DNA double-strand breaks (DSBs) through homologous recombination.[1] *BRCA1* also plays a role in cell-cycle regulation, as well as participating in non-homologous end-joining and single-strand annealing.[1]

The *BRCA1* gene encompasses 24 exons, 22 of which encode a protein with multiple domains including a RING finger at its N-terminal region, two nuclear localization signals (NLS), a coiled-coil domain, and two *BRCA1* C-terminal (BRCT) domains.[2] The *BRCA2* gene consists of 27 exons coding for a protein with four main regions: the N-terminal fragment, the central region containing eight BRC

repeats that recruit RAD51 at DSB sites (RAD51-binding domain), the DNA-binding domain (DBD) located in the C-terminal region with an alpha helical domain followed by three oligonucleotide/oligosaccharide-binding (OB) folds, and the NLS located at the extreme C-terminal region.[3,4]

To date, thousands of sequence variants in the *BRCA* genes have been identified, with only a few subsets recognized as likely pathogenic or pathogenic variants (LP/PVs). The distribution of these LP/PVs varies greatly in frequency and types among different populations.[5] Most known *BRCA1* and *BRCA2* LP/PVs are small insertions or deletions (indels), followed by single-nucleotide variants (SNVs). The most frequent consequence of indels is a disruption of the reading frame, namely frameshift variants, usually resulting in premature stop codons. The consequent transcription termination can trigger nonsense-mediated messenger RNA decay or produce truncated, non-functional proteins.[6] Conversely, the substitution of one nucleotide for another can result in the replacement of one amino acid with a different one, known as missense variants, or the introduction of stop codons, known as nonsense variants.[7] The pathogenicity of less common alterations, such as splicing variants, can impact structure, expression, or function of the encoded protein.[8] Finally, variations in the number of copies of a particular DNA segment [copy number variations (CNVs)] are rare, accounting for ∼5% of all LP/PVs and being more prevalent in *BRCA1* versus *BRCA2*.[9]

LP/PVs in the *BRCA1 or BRCA2* genes significantly increase the risk of developing breast cancer, ovarian cancer, and other malignancies.[10] Particularly, clinicopathological features of breast cancer may be influenced by the specific gene involved, with *BRCA1* more frequently associated with triple-negative breast cancer and *BRCA2* with hormone receptor-positive tumors.[11-13] Evidence also suggests that the clinical implications of these LP/PVs may vary depending on the specific variant type or location within *BRCA1* and *BRCA2* genes. Previous studies showed that LP/PVs occurring in distinct regions of *BRCA1* or *BRCA2* genes confer varying levels of risk of developing breast or ovarian cancer.[14,15] Moreover, variant types that result in partially functional BRCA1 or BRCA2 proteins might confer reduced cancer risk and lower tumor aggressiveness compared with LP/PVs leading to transcript decay or protein truncation.[16-18] Additionally, specific *BRCA1* or *BRCA2* variant types have been shown to influence treatment responses in patients with ovarian cancer, especially to platinum-based chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors.[19,20] However, no evidence exists in this regard for patients with breast cancer.

Identifying associations between the type of genetic variant and specific breast cancer characteristics, such as age at onset or tumor aggressiveness, may contribute to optimizing preventive strategies, including the intensification of surveillance protocols or the anticipation of risk-reducing surgical interventions. Furthermore, determining the prognostic significance of variant type or location could aid in refining therapeutic approaches, guiding decisions on treatment escalation or de-escalation. On these grounds, the present study aimed to investigate the association between specific type and location of *BRCA1* or *BRCA2* LP/PVs with clinicopathological features and survival outcome in a global population of *BRCA* carriers diagnosed with breast cancer at age ≤40 years.

PATIENTS AND METHODS

Study design and participants

This is an international, multicenter, hospital-based, retrospective cohort study including patients diagnosed with invasive breast cancer between January 2000 and December 2020, who were ≤40 years and known to harbor germline LP/PVs in *BRCA1* and/or *BRCA2* genes.[21,22] Main exclusion criteria included a history of non-invasive breast cancer, diagnosis of ovarian cancer or other malignancies with no history of invasive breast cancer, or variants of uncertain clinical significance (VUS). For this specific analysis, patients for whom the specific variant was not provided and patients with stage IV *de novo* disease, unclassifiable variants, unknown uptake or timing of risk-reducing surgeries, multiple *BRCA1* and *BRCA2* LP/PVs, and previous LP/PVs reclassified as VUS were also excluded.

Clinicopathological characteristics of patients were collected, including country of enrollment, year at breast cancer diagnosis, age at diagnosis, body mass index, timing of genetic testing, *BRCA* LP/PVs, tumor histology, grade, stage at diagnosis, hormone receptor status, human epidermal growth factor receptor 2 status, type of breast and axillary surgery, risk-reducing surgeries, systemic anticancer treatments, and survival outcomes.

The study was conducted in accordance with the Declaration of Helsinki. The Institut Jules Bordet (Brussels, Belgium) sponsored the study and acted as the central ethics committee. The study also obtained ethical approval from local, regional, or national institutional review boards/ethics committees of the participating centers if requested by local regulations. The first (AT and EB) and last (CDA and ML) authors guarantee for the accuracy and completeness of the data and analyses.

The STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement was followed to report this work.[23]

The study is registered at ClinicalTrials.gov (NCT03673306).

Variant classification

BRCA LP/PVs were categorized as SNVs when a single-nucleotide change occurred, indels when one or more bases were lost or duplicated, and CNVs when one-copy gains and losses of single or multiple exons occurred in the coding sequence.

The nomenclature of LP/PVs was harmonized to the standards of the International Human Genome Variation Society (HGVS) for both SNVs and indels.[24,25] CNVs were reported with no exact genomic breakpoints allowing for comparable data across centers and over time. The annotation and

classification of LP/PVs were verified by consulting supporting evidence from the literature, public databases (ENIGMA, https://enigmaconsortium.org/; BRCA Exchange, https://brcaexchange.org; ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/; and LOVD, https://www.lovd.nl/), and annotation tools such as VarSome[26] and Franklin by Genoox (https://franklin.genoox.com). LP/PVs were further categorized according to ClinGene Curation Resource[27] and the American College of Medical Genetics and Genomics (ACMG) classification.[28]

Genetic information for LP/PVs was compiled, including coding consequence, exon position, affected protein domain, and whether the genetic defect was predicted to truncate the coding sequence of the gene, resulting in a shorter version of the protein (protein truncation).

The coding consequences were summarized as nonsense and missense variants (indicating a stop codon or a different codon), inframe deletions (deletion of triplets preserving the gene's reading frame), frameshifts (shifting and disrupting the reading frame), and splicing (if variants are predicted to disrupt the RNA splicing).

LP/PVs were defined as protein-truncating variants if SNVs or indels were predicted to introduce premature stop codons (nonsense variants) or to disrupt the reading frame of the transcript (frameshift variants). When protein truncation could not be reliably predicted, the variant classification was considered not classifiable, indicating that the data were not evaluable rather than missing.

The affected exon was determined by the position of each variant in the coding sequence, according to the transcripts from the Matched Annotation from the NCBI and EMBL-EBI (MANE) collaboration[29]: NM_007294.4 for *BRCA1* and NM_000059.4 for *BRCA2*. The current exon 10 in the MANE transcript of *BRCA1* is often reported as exon 11 in prior literature. This miscalculation happened because over time different transcript models and different databases were used, employing different counting schemes for the 5′ untranslated exons (1a, 1b, and sometimes 2) and the tiny exon 4 (21 bp long). Moreover, the *BRCA1* transcript was sometimes numbered starting from exon 2 because exon 1 is non-coding and does not contribute to the protein sequence. However, in the current MANE reference transcript (NM_007294.4), which is the reviewed major transcript, *BRCA1* is described as having 24 exons, named exon 1 to exon 24, with numbering beginning at the first exon regardless of whether it codes for protein. The RefSeq/MANE Select transcript NM_007294.4 is the current standardized reference used for exon numbering and variant annotation according to the European Molecular Genetics Quality Network (EMQN) and HGVS guidelines.[30,31]

Protein-level positions were inferred based on the DNA-level information. BRCA1 and BRCA2 protein domains were considered if well characterized in the literature.[2,4,32,33] In the absence of a protein domain associated to the position of the variant, 'unknown' was assigned, whereas in the presence of more than one domain involved, 'multiple domains' or 'whole protein' was used.

Objectives

The aim of the present study was to investigate the role of specific type and location of *BRCA* LP/PV on the clinical features and outcomes of breast cancer in young *BRCA* carriers. Clinical features and survival outcomes were separately investigated for patients with *BRCA1* and *BRCA2* LP/PVs, according to variant category (indels versus SNVs versus CNVs), protein truncation (protein-truncating versus protein non-truncating versus unclassified variants), coding consequence (frameshift versus nonsense versus splicing versus missense versus inframe deletion variants), and LP/PV location (exon and protein domain involved).

Statistical analysis

Descriptive statistics were used to summarize continuous and categorical variables. Continuous variables were reported as median and interquartile range (IQR), while categorical variables as frequencies and percentages. The associations between LP/PV types and clinicopathological features were analyzed using chi-square test or Kruskal—Wallis—Wilcoxon rank-sum test, for categorical or continuous variables as appropriate. The median follow-up in the entire cohort and according to the LP/PV type was computed using the reverse Kaplan—Meier method.

To investigate the association between LP/PV types and survival outcomes, disease-free survival (DFS) and overall survival (OS) were considered as endpoints. DFS was defined as the time from diagnosis until locoregional recurrence, distant metastases, new contralateral or ipsilateral breast cancer, second primary malignancy, or death from any cause. OS was defined as the time from diagnosis until death from any cause.[34] DFS and OS event rates were computed as the ratio between the total number of events and the total observation time. Unadjusted and adjusted Cox models were used to estimate the association between each specific type and location of *BRCA* LP/PV and survival outcomes. Adjusted models included country and year at breast cancer diagnosis as stratification factors and the uptake of risk-reducing surgeries (risk-reducing mastectomy and risk-reducing salpingo-oophorectomy) as time-dependent covariates. Furthermore, to account for potential immortal time bias (i.e. patients had to survive to undergo *BRCA* testing), both unadjusted and adjusted survival models where observation times were left truncated at the time of *BRCA* testing were carried out. Considering this potential bias, results of the adjusted Cox model with left truncation were reported as the primary analysis in the manuscript, and the Kaplan—Meier method, accounting for left truncation, was utilized to plot the survival curves. In the figures, a P value <0.05 indicates that at least one comparison among the groups is statistically significant.

For the comparison of the location of variants in different exons, for *BRCA1*, the three exons with the highest frequency of LP/PVs (exon 2, exon 10, and exon 19) were compared with each other and other exons. Specifically, exon 2 contributes to the coding of the RING domain,

exon 10 to the NLS and the coiled-coil domains, and exon 19 to the BRCT domains. For *BRCA2*, the two exons with the highest frequency of LP/PVs (exon 10 and exon 11) and exons 15-26 were considered for the comparative analysis. In this gene, exons 10 and 11 encode the BRC repeats, whereas exons 15-26 encode the DBD.

For the comparison of different protein domains, only patients with known domain and protein non-truncating LP/PVs were included. For the comparison of different exons and type of LP/PVs in terms of frameshift versus nonsense versus splicing versus missense versus inframe deletion variants, patients with CNVs were excluded.

For the comparison of frameshift versus inframe deletion versus nonsense versus splicing versus missense LP/PVs, and for the comparison of exons, Cox models were repeated by shifting the reference group in order to identify any significant comparison.

Considering the explorative nature of the analysis, no adjustment for multiple tests was carried out. All reported *P* values are two-sided, and $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using SAS software, version 9.4 (SAS Institute Inc., Cary, NC), and R software, version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

Table 1. Distribution of LP/PV type and location in *BRCA1* and *BRCA* genes

	BRCA1 $n = 2080$ (%)	*BRCA2* $n = 1214$ (%)	Total $n = 3294$ (%)
Variant type			
Indel	1248 (60.0)	772 (63.6)	2020 (61.3)
SNV	667 (32.1)	409 (33.7)	1076 (32.7)
CNV	165 (7.9) (143 OCDel-22 OCDup)	33 (2.7) (32 OCDel-1 OCDup)	198 (6.0) (175 OCDel-23 OCDup)
Protein truncation			
Yes	1514 (72.8)	1007 (83.0)	2521 (76.5)
No	227 (10.9)	50 (4.1)	277 (8.4)
NC	339 (16.3)	157 (12.9)	496 (15.1)
Coding consequence[a]	$n = 1915$ (%)	$n = 1181$ (%)	$n = 3096$ (%)
Frameshift	1158 (60.5)	710 (60.1)	1868 (60.3)
Inframe deletion	14 (0.7)	2 (0.2)	16 (0.5)
Missense	213 (11.1)	48 (4.1)	261 (8.4)
Nonsense	356 (18.6)	299 (25.3)	655 (21.2)
Splicing	174 (9.1)	122 (10.3)	296 (9.6)
Exon[a]	$n = 1915$ (%)	$n = 1181$ (%)	$n = 3096$ (%)
Exon 2	243 (12.7)	—	243 (7.8)
Exon 10	623 (32.5)	—	623 (20.1)
Exon 19	346 (18.1)	—	346 (11.2)
Other exons	703 (36.7)	—	703 (22.7)
Exon 10	—	107 (9.1)	107 (3.5)
Exon 11	—	564 (47.8)	564 (18.2)
Exon 15-26	—	312 (26.4)	312 (10.1)
Other exons	—	198 (16.8)	198 (6.4)
Protein domain[b]	$n = 227$ (%)	$n = 50$ (%)	$n = 277$ (%)
RING domain	117 (51.5)	—	117 (42.2)
BRCT domain	108 (47.6)	—	108 (39.0)
OB domain	—	44 (88.0)	44 (15.9)
BRC domain	—	2 (4.0)	2 (0.7)
Alpha helix	—	1 (2.0)	1 (0.4)
Unknown	2 (0.9)	3 (6.0)	5 (1.8)

BRCT, *BRCA1* C-terminal; CNV, copy number variations; indel, small deletions or insertions; NC, not classifiable; OB, oligonucleotide/oligosaccharide-binding; OCDel, one-copy deletion; OCDup, one-copy duplication; SNV, single-nucleotide variants.
[a]Only in patients without CNV.
[b]Only in patients with no protein truncation.

RESULTS

Patient population

From a cohort of 5660 patients enrolled across 109 centers globally in the primary study, 3294 patients were included in the present analysis (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2025.11.004). Among them, 2080 harbored a *BRCA1* LP/PV, while 1214 harbored a *BRCA2* LP/PV. The distribution of LP/PV type and location within the *BRCA1* and *BRCA2* genes is detailed in Table 1. The baseline characteristics of patients included in the present study are summarized in Table 2. Overall, the median follow-up was 7.9 (IQR 4.5-12.7) years for *BRCA1* carriers and 7.9 (IQR 4.5-13.1) years for *BRCA2* carriers.

The most common LP/PVs (observed in at least 10 patients), along with their prevalence and geographic distribution, are included in Supplementary Tables S1 and S2, available at https://doi.org/10.1016/j.annonc.2025.11.004.

Variant type (indel, SNV, and CNV)

In *BRCA1* carriers, the distribution of variant types differed by geographic region; no other meaningful association between baseline clinicopathological variables and LP/PV types was observed (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS events and the rate of OS events for *BRCA1* patients are detailed in Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2025.11.004. No difference in DFS was found according to the type of LP/PV (Figure 1A and Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2025.11.004). Accordingly, no significant difference in OS was observed among SNV, CNV, and indel variants (Figure 1B and Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2025.11.004).

In *BRCA2* carriers, the distribution of variant types differed by geographic region; no other meaningful association between baseline clinicopathological variables and LP/PV types was observed (Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS events and the rate of OS events for *BRCA2* patients are detailed in Supplementary Table S8, available at https://doi.org/10.1016/j.annonc.2025.11.004. No difference in DFS (Figure 1C) or in OS (Figure 1D and Supplementary Tables S9 and S10, available at https://doi.org/10.1016/j.annonc.2025.11.004) was found according to the type of LP/PV.

Protein truncation (protein-truncating variants and non-truncating variants)

In *BRCA1* carriers, the distribution of protein-truncating variants differed according to geographic region. No other meaningful associations between baseline clinicopathological variables and protein truncation were detected (Supplementary Table S11, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS

Table 2. Baseline characteristics of patients

	BRCA1 $n = 2080$ (%)	*BRCA2* $n = 1214$ (%)	Total $n = 3294$ (%)
Patients' characteristics			
Region			
Central/South America	153 (7.4)	70 (5.8)	223 (6.8)
Australia/Oceania	105 (5.1)	74 (6.1)	179 (5.4)
Northern Europe	269 (12.9)	179 (14.7)	448 (13.6)
Eastern Europe	189 (9.1)	59 (4.9)	248 (7.5)
North America	255 (12.3)	131 (10.8)	386 (11.7)
Southern Europe	781 (37.5)	534 (44.0)	1315 (39.9)
Asia	325 (15.6)	164 (13.5)	489 (14.9)
Africa	3 (0.1)	3 (0.2)	6 (0.2)
Year of diagnosis			
2000-2004	269 (12.9)	173 (14.3)	442 (13.4)
2005-2008	321 (15.4)	180 (14.8)	501 (15.2)
2009-2012	419 (20.1)	222 (18.3)	641 (19.5)
2013-2016	477 (22.9)	283 (23.3)	760 (23.1)
2017-2020	594 (28.6)	356 (29.3)	950 (28.8)
Median age (years) at diagnosis (IQR)	35.0 (31.0-37.0)	35.0 (32.0-38.0)	35.0 (31.0-38.0)
Age at diagnosis, years			
≤30	478 (23.0)	190 (15.7)	668 (20.3)
31-35	716 (34.4)	446 (36.7)	1162 (35.3)
36-40	886 (42.6)	578 (47.6)	1464 (44.4)
Median BMI at diagnosis (IQR)	23.0 (20.6-26.2)	22.8 (20.3-25.9)	23.0 (20.4-26.0)
BMI			
Underweight	108 (5.2)	71 (5.8)	179 (5.4)
Normal weight	1059 (50.9)	658 (54.2)	1717 (52.1)
Overweight	423 (20.3)	216 (17.8)	639 (19.4)
Obese	201 (9.7)	105 (8.7)	306 (9.3)
Unknown	289 (13.9)	164 (13.5)	453 (13.8)
Median time (years) to *BRCA* test (IQR)	0.4 (0.1-2.2)	0.7 (0.1-3.3)	0.5 (0.1-2.6)
Timing of *BRCA* testing[a]			
Test before diagnosis	227 (10.9)	90 (7.4)	317 (9.6)
Test at diagnosis	851 (40.9)	446 (36.7)	1297 (39.4)
Test after diagnosis	919 (44.2)	638 (52.6)	1557 (47.3)
Unknown date of *BRCA* testing	83 (4.0)	40 (3.3)	123 (3.7)
Tumor characteristics			
Tumor histology			
Ductal	1775 (85.3)	988 (81.4)	2763 (83.9)
Other	286 (13.8)	217 (17.9)	503 (15.3)
Unknown	19 (0.9)	9 (0.7)	28 (0.9)
Tumor grade			
G1	21 (1.0)	45 (3.7)	66 (2.0)
G2	304 (14.6)	476 (39.2)	780 (23.7)
G3	1634 (78.6)	628 (51.7)	2262 (68.7)
Unknown	121 (5.8)	65 (5.4)	186 (5.6)
Tumor size			
T0	3 (0.1)	0 (0.0)	3 (0.1)
T1	741 (35.6)	470 (38.7)	1211 (36.8)
T2	988 (47.5)	526 (43.3)	1514 (46.0)
T3/T4	271 (13.0)	169 (13.9)	440 (13.4)
Unknown	77 (3.7)	49 (4.0)	126 (3.8)
Nodal status			
N0	1173 (56.4)	478 (39.4)	1651 (50.1)
N1	647 (31.1)	472 (38.9)	1119 (34.0)
N2/N3	207 (10.0)	217 (17.9)	424 (12.9)
Unknown	53 (2.5)	47 (3.9)	100 (3.0)
Hormone receptor status			
Negative	1534 (73.8)	189 (15.6)	1723 (52.3)
Positive	536 (25.8)	1021 (84.1)	1557 (47.3)
Unknown	10 (0.5)	4 (0.3)	14 (0.4)
HER2 status			
Negative	1907 (91.7)	1031 (84.9)	2938 (89.2)
Positive	100 (4.8)	136 (11.2)	236 (7.2)
Unknown	73 (3.5)	47 (3.9)	120 (3.6)

Continued

Table 2. Continued

	BRCA1 $n = 2080$ (%)	*BRCA2* $n = 1214$ (%)	Total $n = 3294$ (%)
Treatment			
Breast surgery			
None	6 (0.3)	5 (0.4)	11 (0.3)
Breast conserving	864 (41.5)	354 (29.2)	1218 (37.0)
Mastectomy	1201 (57.7)	850 (70.0)	2051 (62.3)
Unknown	9 (0.4)	5 (0.4)	14 (0.4)
Axillary surgery			
No axillary surgery	46 (2.2)	39 (3.2)	85 (2.6)
Sentinel node biopsy only	971 (46.7)	420 (34.6)	1391 (42.2)
Axillary dissection	1031 (49.6)	741 (61.0)	1772 (53.8)
Unknown	32 (1.5)	14 (1.1)	46 (1.4)
Use of CT			
No	86 (4.1)	132 (10.9)	218 (6.6)
Yes	1981 (95.2)	1068 (88.0)	3049 (92.6)
Unknown	13 (0.6)	14 (1.1)	27 (0.8)
Type of CT[b]			
Anthra + taxane	1473 (74.4)	744 (69.7)	2217 (72.7)
Anthra only	300 (15.1)	179 (16.8)	479 (15.7)
Other	208 (10.5)	145 (13.6)	353 (11.6)
Use of platinum[c]			
No	1023 (73.2)	105 (69.5)	1128 (72.9)
Yes	317 (22.7)	38 (25.2)	355 (22.9)
Unknown	57 (4.1)	8 (5.3)	65 (4.2)
Use of ET[d]			
No	51 (9.5)	25 (2.4)	76 (4.9)
Yes	477 (89.0)	982 (96.2)	1459 (93.7)
Unknown	8 (1.5)	14 (1.4)	22 (1.4)
Type of ET[e]			
Tamoxifen alone	165 (34.6)	307 (31.3)	472 (32.4)
Tamoxifen + LHRHa	131 (27.5)	259 (26.4)	390 (26.7)
LHRHa alone	16 (3.4)	16 (1.6)	32 (2.2)
AI + LHRHa	90 (18.9)	210 (21.4)	300 (20.6)
Tamoxifen → AI	61 (12.8)	166 (16.9)	227 (15.6)
Other	7 (1.5)	15 (1.5)	22 (1.5)
Unknown	7 (1.5)	9 (0.9)	16 (1.1)

AI, aromatase inhibitor; BMI, body mass index; CT, chemotherapy; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; IQR, interquartile range; LHRHa, luteinizing hormone-releasing hormone analogue.

[a]Test before diagnosis, carried out any time up to 2 months before the diagnosis of breast cancer; test at diagnosis, carried out from 2 months before and up to 6 months after the diagnosis of breast cancer; test after diagnosis, carried out >6 months after the diagnosis of breast cancer.

[b]Only patients with use of chemotherapy.

[c]Only patients with use of chemotherapy and triple-negative breast cancer.

[d]Only patients with hormone receptor-positive tumors.

[e]Only patients with hormone receptor-positive tumors treated with ET.

events and the rate of OS events for *BRCA1* patients are detailed in Supplementary Table S12, available at https://doi.org/10.1016/j.annonc.2025.11.004. No difference in DFS was found according to protein truncation (Figure 2A and Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2025.11.004). Nonetheless, patients with *BRCA1* protein-truncating variants showed worse OS than patients with non-truncating variants [hazard ratio (HR) 2.00, 95% confidence interval (CI) 1.17-3.41; Figure 2B and Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2025.11.004].

In *BRCA2* carriers, a different distribution of protein-truncating variants was observed according to geographic region. No other meaningful associations between baseline clinicopathological variables and protein truncation were observed (Supplementary Table S13, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS events and the rate of OS events for *BRCA 2* patients are detailed in Supplementary Table S14, available at https://doi.org/10.1016/j.annonc.2025.11.004. Protein-truncating variants were associated with numerically worse DFS, without statistical significance (Figure 2C and Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2025.11.004). Patients with *BRCA2* protein-truncating variants exhibited numerically worse OS than those with non-truncating variants (HR 6.27, 95% CI 0.86-45.87; Figure 2D and Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.2025.11.004), although not statistically significant.

Coding consequence (frameshift, inframe deletion, missense, nonsense, splicing variants)

In *BRCA1* carriers, a different distribution of coding consequences was observed according to geographic region. No other meaningful associations between baseline clinicopathological variables and coding consequence were detected (Supplementary Table S15, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first

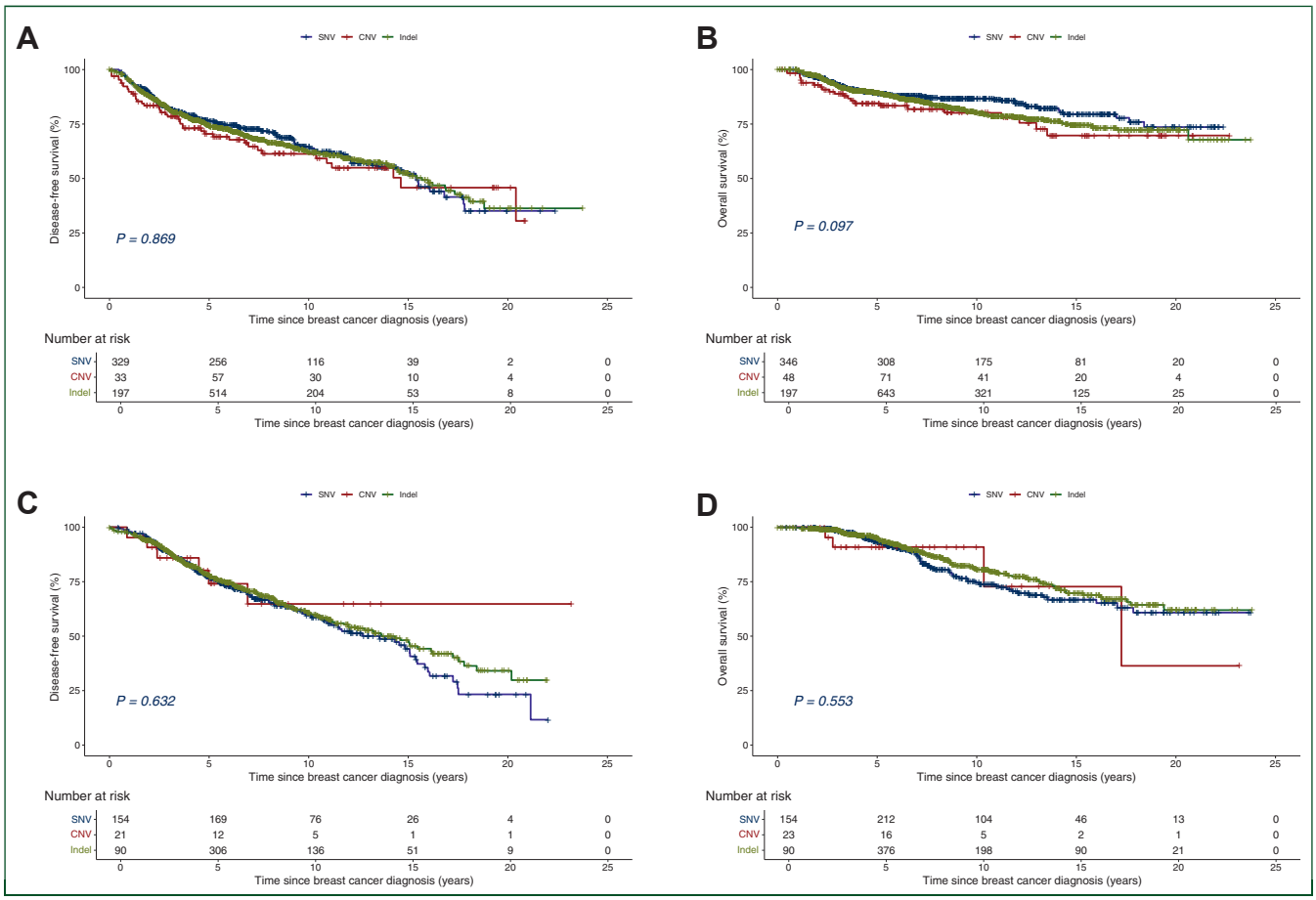

Figure 1. Disease-free survival (DFS) and overall survival (OS) according to variant type. (A) DFS in *BRCA1* carriers. (B) OS in *BRCA1* carriers. (C) DFS in *BRCA2* carriers. (D) OS in *BRCA2* carriers.
CNV, copy number variations; indel, small deletions or insertions; SNV, single-nucleotide variants.

DFS events and the rate of OS events for *BRCA1* patients are detailed in Supplementary Table S16, available at https://doi.org/10.1016/j.annonc.2025.11.004. No differences in DFS were identified according to coding consequence (Figure 3A and Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2025.11.004). Nevertheless, BRCA1 missense variants were associated with better OS compared with frameshift variants (HR 0.48, 95% CI 0.28-0.84; Figure 3B and Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2025.11.004).

In *BRCA2* carriers, a different distribution of coding consequences was observed according to geographic region. No other meaningful associations between baseline clinicopathological variables and coding consequence were detected (Supplementary Table S17, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS events and the rate of OS events for *BRCA2* patients are detailed in Supplementary Table S18, available at https://doi.org/10.1016/j.annonc.2025.11.004. No statistically significant differences in DFS were observed according to coding consequence (Figure 3C and Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2025.11.004). Nevertheless, *BRCA2* missense variants were associated with better OS compared with splicing variants (HR 0.12, 95% CI 0.02-0.90; Figure 3D and Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.2025.11.004).

Variant location (exon and protein domain)

In *BRCA1* carriers, a different distribution of exons involved was observed according to geographic region. No other meaningful association between baseline clinicopathological variables and exons was identified (Supplementary Table S19, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS events and the rate of OS events for *BRCA1* patients are detailed in Supplementary Table S20, available at https://doi.org/10.1016/j.annonc.2025.11.004. No differences in DFS were observed based on variant location (Figure 4A and Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2025.11.004). However, LP/PVs located outside exons 2, 10, and 19 were associated with better OS compared with patients with variants in exon 2 (HR 0.56, 95% CI 0.34-0.94) and exon 10 (HR 0.53, 95% CI 0.38-0.75; Figure 4B and Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2025.11.004).

In *BRCA2* carriers, patients with LP/PV located in exon 10 had earlier diagnosis ($\leq$30 years) compared with patients with variants in exon 11, exons 15-26, and other exons

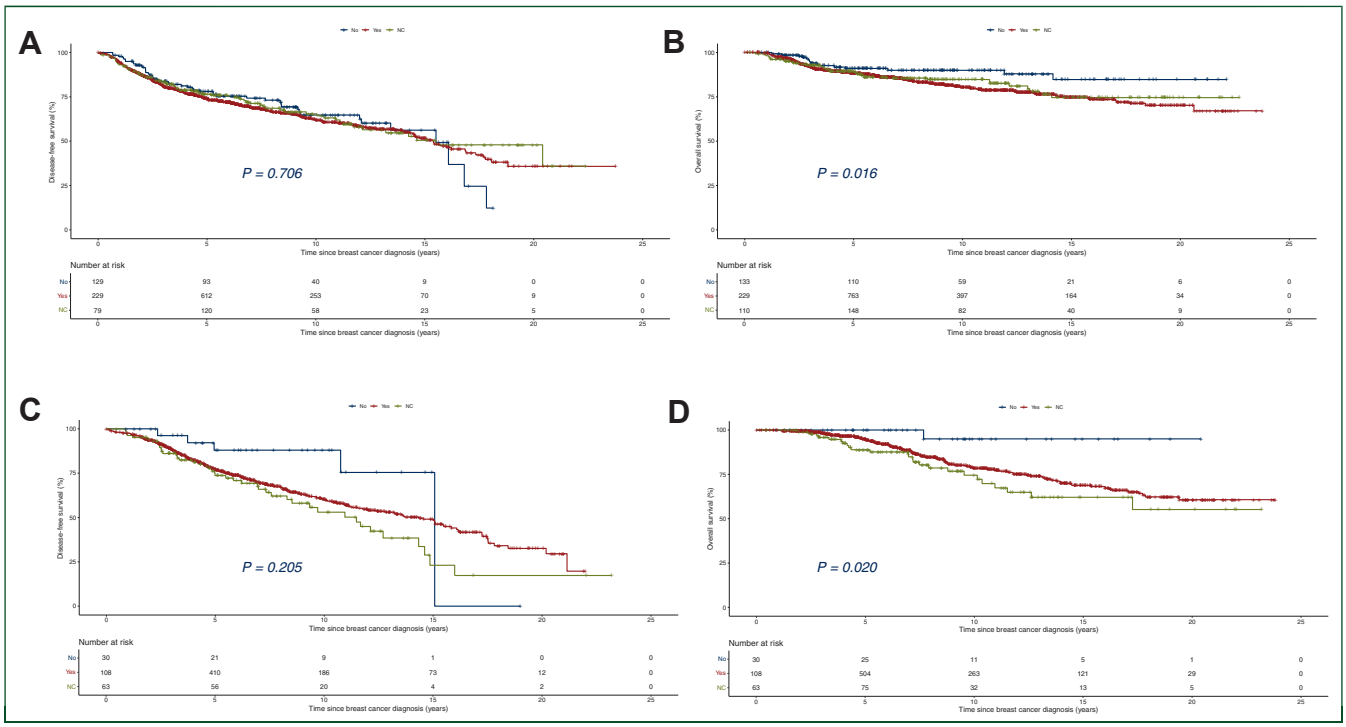

Figure 2. Disease-free survival (DFS) and overall survival (OS) according to protein truncation. (A) DFS in *BRCA1* carriers. (B) OS in *BRCA1* carriers. (C) DFS in *BRCA2* carriers. (D) OS in *BRCA2* carriers.
NC, not classifiable.

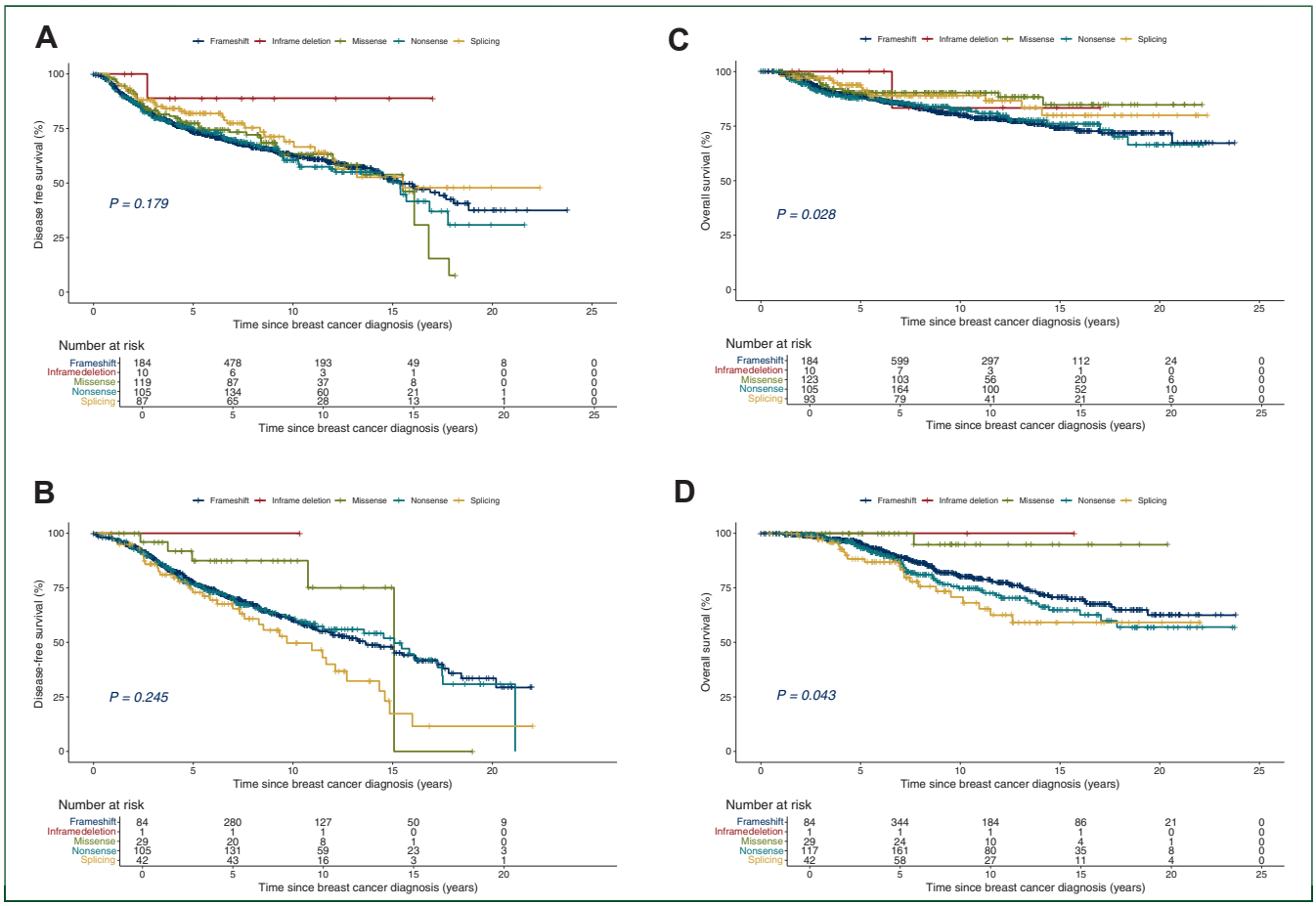

Figure 3. Disease-free survival (DFS) and overall survival (OS) according to coding consequence. (A) DFS in *BRCA1* carriers. (B) OS in *BRCA1* carriers. (C) DFS in *BRCA2* carriers. (D) OS in *BRCA2* carriers.

(26.2% versus 13.7%, 15.4%, 17.2%, respectively) (Supplementary Table S21, available at https://doi.org/10.1016/j.annonc.2025.11.004). The rate and type of first DFS events and the rate of OS events for *BRCA2* patients are detailed in Supplementary Table S22, available at https://doi.org/10.1016/j.annonc.2025.11.004.

Patients with LP/PV in exons 15-26 showed worse DFS compared with those with LP/PV in exon 10 (HR 1.74, 95% CI 1.02-2.98). Patients with LP/PV in the 'other exons' showed worse DFS compared with those with LP/PV in exon 10 (HR 1.91, 95% CI 1.09-3.35; Figure 4C and Supplementary Table S9, available at https://doi.org/10.1016/j.annonc.2025.11.004). No differences in OS were observed according to the exon involved (Figure 4D and Supplementary Table S10, available at https://doi.org/10.1016/j.annonc.2025.11.004).

Considering patients with only non-truncating variants, no differences in terms of baseline characteristics, DFS, or OS (Supplementary Tables S5, S6, S23, and S24, available at https://doi.org/10.1016/j.annonc.2025.11.004) were detected according to the most represented protein domains of *BRCA1* (BRCT domain versus RING domain). Among *BRCA2* carriers, most of the patients harbored LP/PVs in the OB domain (88%); therefore, no comparisons were carried out.

DISCUSSION

In this global study, the specific type and location of *BRCA* LP/PV seemed not to influence tumor clinicopathological features, although they might have a prognostic role in a cohort of *BRCA* carriers affected by early-onset breast cancer.

The distribution of variant type among patients included in the present study is consistent with that observed in prior breast cancer studies,[35] with the majority being indel, followed by SNVs. Regarding the coding consequences, the majority of LP/PVs were frameshift variants in both the *BRCA1* and *BRCA2* genes, with protein-truncating variants being the most common in this study population. Furthermore, as previously described,[36] in this study the majority of LP/PVs were located in *BRCA1* exon 10 (formerly exon 11) and *BRCA2* exon 11, which are the largest exons of both *BRCA* genes. Overall, the most frequent variants were the founder mutations *BRCA1* c.5266dup, *BRCA1* c.68_69del, *BRCA2* c.5946del, and *BRCA2* c.2808_2811del, along with *BRCA1* c.181T>G and *BRCA2* c.6275_6276del.[37] *BRCA1* c.5266dup, likely originating from Northern Europe 1500-1800 years ago and then entering the Ashkenazi Jewish population more recently, was the most common variant, particularly among carriers from South and East Europe and Israel.[38] *BRCA1* c.68_69del was enriched in

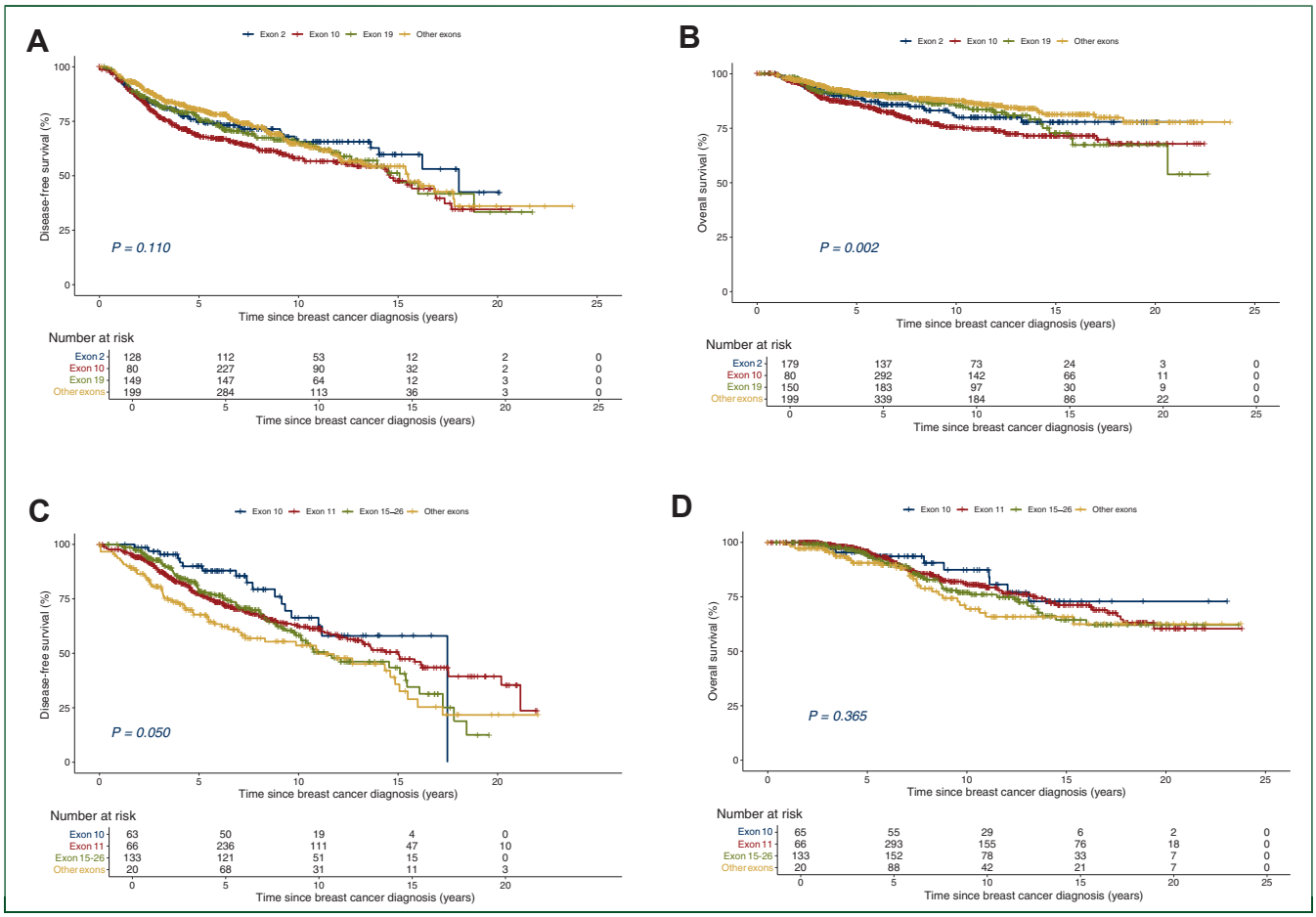

Figure 4. Disease-free survival (DFS) and overall survival (OS) according to exon involved. (A) DFS in *BRCA1* carriers. (B) OS in *BRCA1* carriers. (C) DFS in *BRCA2* carriers. (D) OS in *BRCA2* carriers.

North America and Asia, reflecting both Ashkenazi Jewish-associated haplotypes and independent origins in populations such as Pakistanis and Malaysians.[39-43] *BRCA1* c.181T>G, predominantly observed in South and East Europe, supports its classification as an Eastern European founder variant.[44] *BRCA2* c.5946del was consistent with its role as a founder variant in Ashkenazi Jewish and Northern European populations, including Iceland,[37,45,46] whereas *BRCA2* c.2808_2811del showed a recurrent mutational origin across geographically distant populations.[47-49] Finally, *BRCA2* c.6275_6276del, previously described in Western populations,[37] was highly represented in Asian carriers in our dataset. Notably, we found significantly different distributions of variant type and location across ethnic and geographic groups. This could be partially attributable to population characteristics such as founder effects, but it can also be influenced by the wide heterogeneity in the methods of genetic testing available in each country. It is noteworthy that this study was conducted on a population diagnosed with breast cancer between 2000 and 2020; during this period, the methods for variant detection have significantly evolved, shifting from 'hotspot' searches to increasingly larger panels, and the detection of CNVs has spread only in the last decade.

In this large cohort of *BRCA* carriers with breast cancer, variant type (indel, SNV, CNV) seemed not to be associated with differences in prognosis (DFS or OS). Prior studies showed that *BRCA* CNVs were correlated to a higher breast cancer risk versus non-CNV LP/PVs (i.e. small variants such as nonsense, missense, and frameshift).[50] Moreover, in a small cohort of young women with triple-negative breast cancer, *BRCA1* CNVs were associated with superior OS rates compared with other LP/PVs.[51] Nevertheless, to date, no other data on their impact on breast cancer phenotype and outcome have been documented.

To the best of our knowledge, this is the first study describing a possible correlation between BRCA protein truncation and worse survival in patients with breast cancer. Indeed, the present study showed that patients with protein-truncating variants of *BRCA1* gene display worse OS compared with patients with non-truncating variants. Interestingly, despite comparable overall DFS, patients with *BRCA1* truncating variants showed a higher rate of distant relapses and second primary tumors compared with non-truncating variants, which might have impacted OS. On the other hand, the divergence between DFS and OS among *BRCA1* carriers might also suggest a possible role of *BRCA1* truncating variants in influencing other determinants of life expectancy, such as other health conditions. Nonetheless, data on causes of death other than breast cancer were not collected in the present study; therefore, further research is needed to confirm this hypothesis.

Interestingly, the present study showed that patients with missense variants display better OS compared with patients with frameshift variants in *BRCA1* gene and better OS compared with patients with splicing variants in *BRCA2* gene. In these cases as well, absolute DFS was comparable; however, patients with *BRCA1* missense LP/PVs showed a lower rate of distant relapses compared with those with *BRCA1* frameshift LP/PVs. Similarly, *BRCA2* missense LP/PVs were associated with lower rates of both locoregional and distant relapses compared with *BRCA2* splicing LP/PVs. The favorable prognosis associated with missense variants was already described by Corso et al.,[17] who found that missense and splicing LP/PVs of *BRCA1/BRCA2* were independently associated with a lower incidence of locoregional recurrence after primary surgery, when compared with indels, nonsense LP/PVs, and structural variants in a multivariable analysis. However, in this previous research, no difference in clinicopathological features or in OS was observed.[17]

Patients with LP/PVs located in exon 10 of *BRCA2* exhibited a higher rate of early-onset diagnosis ($\leq$30 years of age) than patients with variants in the other exons and these patients also showed better DFS compared with those with variants in most of the other exons. Additionally, patients with LP/PVs located in *BRCA1* exon 2 and exon 10 had worse OS compared with patients with variants in most of the other exons. In this case as well, poorer OS is associated with a higher rate of distant relapses. Studies investigating the relationship between LP/PV location and clinicopathological characteristics or prognosis in breast cancer remain limited. Contrary to the data described here, Rebbeck et al.[14] found that LP/PVs in *BRCA1* exon 10 (formerly exon 11) and *BRCA2* exon 11 were associated with earlier ages at breast and ovarian cancer diagnosis. On the other hand, Bayraktar et al.[52] did not find any difference in age at breast cancer diagnosis and phenotypic features of breast cancer by ethnicity or variant location. Bayraktar et al.[52] also found no significant differences in OS in relation to variant location (exons 2, 11, and 20 among *BRCA1* carriers and 10, 11, and 25 among *BRCA2* carriers). Nevertheless, when the OS was analyzed according to three combined exon groups, the 10-year OS estimates were significantly better for *BRCA2* carriers with LP/PVs in exon 11 and with LP/PVs in exons 1-10, compared with LP/PVs in exons 12-25. Further research is needed in order to confirm and better understand the role of variant position in the prognosis of these patients.

Several limitations of this study must be considered when interpreting these results. Firstly, this retrospective study lacked a control cohort of healthy *BRCA* carriers. The study population specifically included patients diagnosed with breast cancer at a young age, excluding patients with diagnosis of ovarian cancer or other malignancies with no history of invasive breast cancer; therefore, these results may not be generalizable to the entire population of *BRCA* carriers. Additionally, family clustering was not collected in this study; therefore, although being relatively unlikely due to the special population included, this cohort might include relatives with the same variant who could also share other clinicopathological features and prognostic factors. Moreover, survival outcomes might have also been influenced by factors that were not included in our investigation (i.e. lifestyle, environmental factors, or

comorbidities), particularly given the regional variation observed in the distribution of *BRCA* variants. Data on treatments at relapse or in the metastatic setting were also not available. However, it is important to note that patients were enrolled between 2000 and 2020, a period during which Cyclin-dependent kinase 4/6 inhibitors, PARP inhibitors, immune checkpoint inhibitors, and antibody—drug conjugates were not yet standard of care in the treatment of patients with early-stage disease. Finally, no correction for multiple testing was applied with subsequent potential increased risk of false-positive findings.

In conclusion, this analysis enhances our understanding of the influence of the specific types of *BRCA1/BRCA2* LP/PVs on the characteristics and outcomes of breast cancer in young women. Specifically, this study identified young *BRCA* carriers affected by breast cancer who may have a better prognosis (*BRCA1* and *BRCA2* missense LP/PVs) and a worse prognosis (*BRCA1* protein-truncating variants, *BRCA1* exons 2 and 10). Notably, the predicted functional consequence, rather than the variant type *per se*, appears to hold greater biological relevance with respect to its impact on gene function and subsequent phenotypic outcomes. A deeper understanding of these variant-specific features will drive future research and support the development of tailored clinical strategies based on individual *BRCA1 or BRCA2* LP/PV profiles.

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DISCLOSURE

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DATA SHARING

Deidentified individual patient data, data dictionary, and statistical analysis plan will be available for 5 years after publication upon reasonable request to ML (matteo.lambertini@unige.it), after proper revision of the data transfer agreement of each participating center and if ultimately allowed by local ethics committees.

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