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Original Research

Male breast cancer risk associated with pathogenic variants in genes other than *BRCA1/2*: an Italian case-control study

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Abstract ***Background:*** Germline pathogenic variants (PVs) in *BRCA1/2* genes are associated with breast cancer (BC) risk in both women and men. Multigene panel testing is being increasingly used for BC risk assessment, allowing the identification of PVs in genes other than *BRCA1/2*. While data on actionable PVs in other cancer susceptibility genes are now available in female BC, reliable data are still lacking in male BC (MBC). This study aimed to provide the patterns, prevalence and risk estimates associated with PVs in non-*BRCA1/2* genes for MBC in order to improve BC prevention for male patients.

Methods: We performed a large case-control study in the Italian population, including 767 *BRCA1*/2-negative MBCs and 1349 male controls, all screened using a custom 50 cancer gene panel.

Results: PVs in genes other than *BRCA1/2* were significantly more frequent in MBCs compared with controls (4.8% vs 1.8%, respectively) and associated with a threefold increased MBC risk (OR: 3.48, 95% CI: 1.88–6.44; $p < 0.0001$). PV carriers were more likely to have personal ($p = 0.03$) and family ($p = 0.02$) history of cancers, not limited to BC. *PALB2* PVs were associated with a sevenfold increased MBC risk (OR: 7.28, 95% CI: 1.17–45.52; $p = 0.034$), and *ATM* PVs with a fivefold increased MBC risk (OR: 4.79, 95% CI: 1.12–20.56; $p = 0.035$).

Conclusions: This study highlights the role of *PALB2* and *ATM* PVs in MBC susceptibility and provides risk estimates at population level. These data may help in the implementation of multigene panel testing in MBC patients and inform gender-specific BC risk management and decision making for patients and their families.

1. Introduction

It is well established that germline pathogenic and likely pathogenic variants (PVs), herein collectively termed PVs, in *BRCA1* and *BRCA2* are associated with increased risk of developing breast cancer (BC) in both sexes, although gender-specific differences emerged [1]. The cumulative BC risk at age 80 years is about 70% for female *BRCA1* and *BRCA2* PV carriers, whereas it is around 4% and 0.4% for male *BRCA2* and *BRCA1* PV carriers, respectively [2,3].

In the last years, the wide use of next-generation sequencing (NGS) multigene panel testing has allowed the identification of PVs in BC genes other than *BRCA1/2* [4,5], and studies have been performed to estimate their prevalence and associated risk in female BC (FBC) [6,7]. Recently, two large case-control studies, performed in FBC patients unselected for family history, provided accurate and consistent estimates of BC risk associated with PVs in *ATM, CHEK2* and *PALB2* [8,9]. PVs in other BC predisposing genes, such as *BARD1*, *RAD51C* and *RAD51D*, were associated with risk of specific BC subtypes, that is, triple negative BC [8]. Thus, female carriers of PVs in those established BC risk genes could now benefit from individualised BC risk management [10].

On the other hand, a small number of studies applied multigene panel testing to investigate other genes associated with male BC (MBC) predisposition [11–17]. *ATM, CHEK2* and *PALB2* were proposed as MBC risk genes with an aggregate PVs prevalence of about 1–6% across studies [11,13,16,18]. Nonetheless, for most of the genes included in the multigene panels, evidence of association with MBC risk, as well as accurate risk estimates, are currently not available. Thus, the missing hereditary component of MBC needs to be further investigated in order to better define the set of genes associated with MBC and provide reliable risk estimates, to inform screening strategies for male patients.

In this context, performing case-control studies using cases not selected for family history and controls from the same geographical area is crucial to obtain precise estimates of MBC risk associated with PVs, as demonstrated by studies performed in FBC [8,9].

The aim of this study was to evaluate the associations between germline PVs in genes other than *BRCA1/2* and MBC risk. We performed a comprehensive multigene panel testing on a large and well-characterised series of Italian MBC cases and controls to provide the patterns and prevalence of PVs in 50 cancer-related genes, and carried out a case-control study to assess MBC risk estimates for PV carriers.

2. Methods

2.1. Study population

An observational, retrospective, case-control study including 2116 men was set up to evaluate the associations between germline PVs in genes other than *BRCA1/2* and MBC risk (Supplementary Table S1, Supplementary Fig. S1).

A series of 767 *BRCA1/2*-negative MBC cases, older than 18 years, unselected for cancer family history and age at diagnosis, were included in the study. Cases were enrolled from 17 Italian Research Centers participating in the Italian multicenter study on MBC, starting from 2010, and included 503 cases that had previously been described [11, 19, 20]. For all cases, the main clinical–pathologic characteristics were collected as previously reported [11].

A series of 1349 male individuals older than 18 years, without personal history of cancer, were included as control population. Most controls (86%) were recruited among blood donors or randomly selected from municipality lists, as in our previous population-based case-control studies [21]; a smaller percentage (14%) were men enrolled under research or clinical protocols allowing for germline testing in healthy individuals, specifically unaffected/non-blood related relatives of probands with hereditary diseases. Controls were recruited in the same period and geographical area of the MBC cases and, at enrolment, were untested for *BRCA1/2* PVs and their mean age was 48.4 years (range 18–88).

Whole exome sequencing data from a cohort of 814 Italian healthy male controls, enrolled in non-oncological clinical studies [22], was interrogated to further address particularly debated genes, such as *CHEK2*.

Blood samples or DNA from peripheral blood leucocytes were collected for all study participants. DNA from blood samples was extracted using ReliaPrep Blood gDNA Miniprep System, according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA).

The study was approved by the Local Ethical Committee (Sapienza University of Rome, Prot. 669/17), and an informed consent for using information and biological samples for research purposes was obtained from all participants to the study.

2.2. NGS analysis and variant classification

A custom multigene panel, sequencing all exons and flanking intronic sequences of 50 cancer genes, was specifically designed [11] (Supplementary Table S2). Targeted genomic regions were prepared in paired-end libraries using the Illumina DNA Prep with Enrichment kit (Illumina, San Diego, California, USA). Subsequent sequencing and bioinformatic analysis were performed on the Illumina MiniSeq platform, as previously reported [11].

Variants were named according to Human Genome Variation Society nomenclature (https://www.hgvs.org/) and annotated using the Matched Annotation from NCBI and EMBL-EBI transcript [23].

Heterozygous variants with minor allele frequency lower than 1% were retained, and were classified as PVs, variants of uncertain significance (VUS), or benign/likely benign [24]. Variants were classified as pathogenic if loss-of-function (nonsense, frameshift, splicing ± 1 or 2) and/or if reported as pathogenic or likely pathogenic in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). All PVs detected by NGS were validated by double-stranded Sanger sequencing (primer sequences are available upon request).

Variants were classified as VUS if reported as VUS by VARSOME online tool (https://varsome.com/) and/or in ClinVar. After classification, VUS were stratified using REVEL, a method for predicting the pathogenicity of variants based on *in silico* tools, giving each variant a score between 0 (benign) and 1 (pathogenic) [25]. A REVEL score ≥ 0.6 was considered as the cut-off to classify VUS with a higher risk of having a deleterious effect, as reported in previous studies [26]. VUS with a REVEL score ≥0.6 were considered as prioritised VUS, but not included as PVs in the case-control study.

All variants identified in our previous study [11] have been rechecked using the methods described above, in order to update their pathogenic status according to the most recent data.

MUTYH variants were not reported as they were all described in a previous case-control study [27].

2.3. Statistical analysis

To assess MBC risk associated with PVs, a case-control study was performed by multivariate logistic regression analysis. MBC risk was assessed by the odds ratio (OR) and its corresponding 95% confidence interval (CI). In the logistic regression, the case/control status was the outcome, and the presence of PVs (overall and in each analysed gene) was the exposure variable. To address potential sources of bias, all analyses were adjusted for age at diagnosis/enrolment for cases and controls respectively, and for centre of origin. In case of zero PVs among either MBC cases or controls, penalised maximum likelihood (also known as Firth's) logistic regression was performed.

Clinical history and pathologic characteristics were compared between PV carriers and non-carriers. Chi-square test, Fisher exact test and Mann–Whitney test were used where appropriate. A p value ≤ 0.05 was considered statistically significant. All statistical analyses were performed with StataSE v17 statistical software.

Table 1
Clinical–pathologic characteristics of male breast cancers (MBCs) analysed, overall and by pathogenic variant (PV) carrier status.

Characteristic[a]	Overall		PV carriers		PV non-carriers		p value[b]
	N = 767	%	N = 37	%	N = 730	%	
Median age at diagnosis (range)	64.0 (21–91)		60.0 (36–85)		64.0(21–91)		0.053
Contralateral BC	13	1.7	0	0.0	13	1.8	1
Family history of BC/OC							
Negative	542	70.7	25	67.6	517	70.8	0.7
Positive	225	29.3	12	32.4	213	29.2	
Family history other than BC/OC							
Negative	430	56.1	14	37.8	416	57.0	0.02
Positive	337	43.9	23	62.2	314	43.0	
Personal history other than BC							
Negative	622	81.1	25	67.6	597	81.8	0.03
Positive	145	18.9	12	32.4	133	18.2	
Tumour histotype							
Invasive ductal carcinoma	527	81.8	31	91.2	496	81.1	0.5
In situ ductal carcinoma	49	7.6	1	2.9	48	7.9	
Lobular carcinoma	8	1.3	0	0.0	8	1.5	
Other	60	9.3	2	5.9	58	9.5	
TNM stage							
0–1	296	54.7	13	59.1	283	54.6	0.8
2	156	28.8	5	22.7	151	29.0	
3	76	14.0	4	18.2	72	13.9	
4	13	2.5	0	0.0	13	2.5	
Histologic grade							
1	63	11.7	3	12.5	60	11.9	0.3
2	322	60.1	11	45.8	311	60.6	
3	151	28.2	10	41.7	141	27.5	
Lymph node status							
Negative	332	61.7	15	65.2	317	61.4	0.7
Positive	206	38.3	8	34.8	198	38.6	
ER status[c]							
Negative	25	4.2	3	9.7	22	4.0	0.1
Positive	575	95.8	28	90.3	547	96.0	
PR status[c]							
Negative	57	9.8	1	3.4	56	10.1	0.2
Positive	524	90.2	28	96.6	496	89.9	
HER2 status[c]							
Negative	381	80.2	22	84.6	359	80.0	0.6
Positive	94	19.8	4	15.4	90	20.0	
Ki67/MIB1 status[c]							
Low	228	49.1	13	50.0	215	49.1	0.9
High	236	50.9	13	50.0	223	50.9	

BC, breast cancer; OC, ovarian cancer; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.
[a] Some data for each pathologic characteristic are not available.
[b] p-values from Fisher exact test for categorical variables and Mann–Whitney test for continuous variables.
[c] Cut-offs used: ER/PR positive if > 1% of positive nuclei; HER2 positive if 3+ by immunohistochemistry or 2+ if amplified by fluorescence in situ hybridisation; Ki67/MIB1 high if > 20% of positive nuclei.

3. Results

3.1. Clinical–pathologic characteristics of MBC cases

The clinical–pathologic features of the 767 *BRCA1/2*-negative MBCs, overall and stratified by PV carrier status in other genes, are summarised in Table 1. Overall median age at first BC diagnosis was 64 years (range 21–91). First-degree family history of breast and/or ovarian cancer (BC/OC) was reported in 29.3% of MBCs and first-degree family history of cancer other than BC/OC in 43.9% of MBCs. Personal history of other cancers, mostly prostate, colorectal and bladder cancers, was reported in 18.9% of cases.

The majority of MBCs were invasive ductal carcinomas (81.8%), at early stage (54.7%), moderate grade (60.1%), negative lymph node status (61.7%), oestrogen and progesterone receptor positive (95.8% and 90.2%, respectively) and HER2 negative (80.2%) status.

PV carriers (37 out of 767 MBCs, 4.8%) were more likely to have family history of cancers other than BC/OC ($p = 0.02$) and personal history of other cancers besides BC ($p = 0.03$), compared with non-carriers (730 out of 767 MBCs, 95.2%). A trend toward a significantly

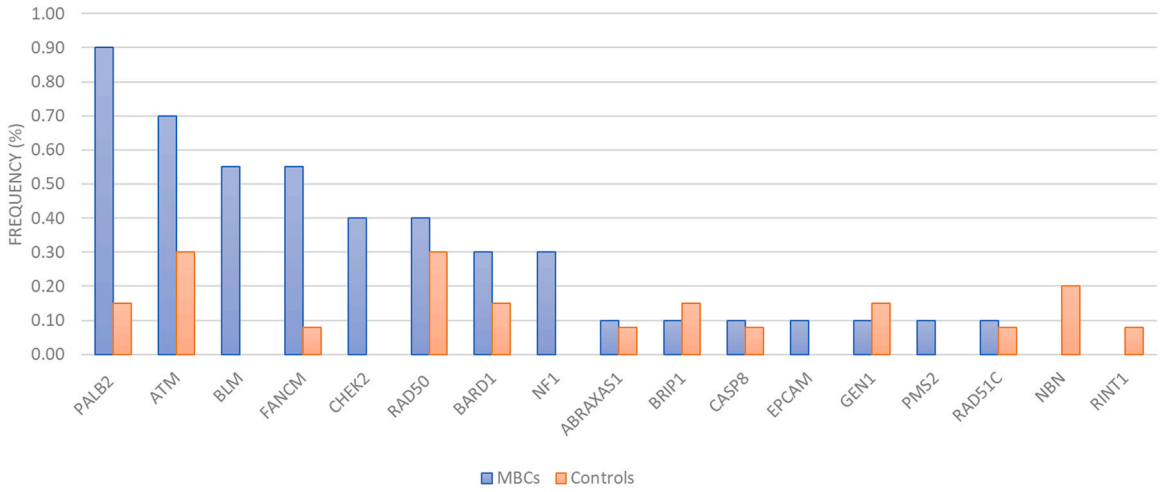

Fig. 1. Distribution and frequency of pathogenic variants (PVs) identified in 767 male breast cancers (MBCs) and 1338 male controls.

younger age at diagnosis for PV carriers compared with non-carriers emerged (p = 0.053). No statistically significant differences emerged between carriers and non-carriers with regards to other characteristics (Table 1).

3.2. Pattern and prevalence of germline variants in MBC cases and controls

Multigene panel testing performed in 767 *BRCA1/2*-negative MBCs showed that 37 (4.8%) MBCs were carriers of PVs in 15 genes including *PALB2, ATM, BLM, FANCM, CHEK2, RAD50, BARD1, NF1, ABRAXAS1, BRIP1, CASP8, EPCAM, GEN1, PMS2* and *RAD51C* (Figure 1; Table 2; Supplementary Table S3).

Multigene panel screening of the 1349 controls showed that 35 (2.6%) healthy controls were PV carriers.

In particular, 11 (0.8%) controls were *BRCA1/2* PV carriers (seven *BRCA1* and four *BRCA2*) and 24 (1.8%) controls were carriers of PVs in genes other than *BRCA1/2*, including *PALB2, ATM, FANCM, RAD50, BARD1, ABRAXAS1, BRIP1, CASP8, GEN1, RAD51C, NBN* and *RINT1* (Figure 1; Table 2; Supplementary Table S4).

A total of 213 and 245 VUS were identified in MBCs and healthy controls, respectively. After VUS classification, a prioritisation of those with a higher risk of

Table 2
Pathogenic variants (PVs) distribution by gene in 767 male breast cancers (MBCs) and 1338 male controls and MBC risk estimates.

Gene	MBC cases No. PV carriers (%)	Controls No. PV carriers (%)	OR (95% CI)[a]	p value[a]
Overall	37 (4.80)	24 (1.80)	3.48 (1.88–6.44)	< 0.0001
PALB2	7 (0.90)	2 (0.15)	7.28 (1.17–45.52)	0.034
ATM	5 (0.70)	4 (0.30)	4.79 (1.12–20.56)	0.035
BLM	4 (0.55)	0 (0.00)	-	-
FANCM	4 (0.55)	1 (0.08)	8.11 (0.65–101.23)	0.10
CHEK2	3 (0.40)	0 (0.00)	-	-
RAD50	3 (0.40)	4 (0.30)	1.23 (0.22–6.90)	0.81
BARD1	2 (0.30)	2 (0.15)	1.24 (0.13–11.90)	0.85
NF1	2 (0.30)	0 (0.00)	-	-
ABRAXAS1	1 (0.10)	1 (0.08)	2.16 (0.12–37.75)	0.60
BRIP1	1 (0.10)	2 (0.15)	0.81 (0.04–15.11)	0.89
CASP8	1 (0.10)	1 (0.08)	1.02 (0.06–16.41)	0.98
EPCAM	1 (0.10)	0 (0.00)	-	-
GEN1	1 (0.10)	2 (0.15)	2.02 (0.2–22.7)	0.57
PMS2	1 (0.10)	0 (0.00)	-	-
RAD51C	1 (0.10)	1 (0.08)	1.02 (0.02–59.64)	0.99
NBN	0 (0.00)	3 (0.20)	-	-
RINT1	0 (0.00)	1 (0.08)	-	-

[a] Odds ratios (OR) with 95% confidence interval (CI) and P-values from multivariate logistic regression analysis, adjusted for age at diagnosis/enrolment and for centre of origin. In case of zero PVs, penalised maximum likelihood was performed (Supplementary Table S6).

being pathogenic was performed using a REVEL score > 0.6. A total of 112 and 131 VUS were reclassified as prioritised VUS in 101(13.2%) MBCs and 129 (9.6%) controls, respectively (Chi-square p = 0.01) (Supplementary Table S5; Supplementary Fig. S2). The majority of prioritised VUS were found in *ATM*, *CHEK2* and *BRCA2* (Supplementary Table S5; Supplementary Fig. S2).

3.3. Gene-specific MBC risk estimates

The case-control association analysis was performed including 767 MBCs and 1338 controls, all tested negative for *BRCA1/2* PVs. As shown in Table 2, overall PVs in genes other than *BRCA1/2* were associated with an OR of 3.48 (95% CI: 1.88–6.44; p < 0.0001), *PALB2* PVs were associated with an approximately sevenfold increased MBC risk (OR: 7.28, 95% CI: 1.17–45.52; p = 0.034) and *ATM* PVs with an approximately fivefold increased MBC risk (OR: 4.79, 95% CI: 1.12–20.56; p = 0.035).

For genes in which zero PVs were detected among either MBC cases or controls, penalised maximum likelihood was performed (Supplementary Table S6). Results from this analysis showed no statistically significant results, except for *CHEK2* (Supplementary Table S6).

In order to further assess the debated association of *CHEK2* PVs and MBC risk, we increased the study population by interrogating an additional dataset of 814 exomes from healthy male controls. *CHEK2* PVs were identified in 6 controls (0.7%), none heterozygous for the *CHEK2* c.1100delC variant (Supplementary Table S7). The case-control analysis, including a total of 767 MBCs and 2152 controls, showed no association between *CHEK2* PVs and MBC risk (Supplementary Table S8).

4. Discussion

In this study, we performed a multigene panel testing on a series of 767 *BRCA1*/2 PVnegative MBC cases and 1349 healthy male controls and provided the patterns, prevalence and MBC risk estimates associated with PVs in non-*BRCA1/2* genes. To the best of our knowledge, this is the largest series of unselected *BRCA1/2* PV negative MBCs, collected in a single country and analysed by multigene panel testing [11–17,28–30].

The aggregate prevalence of PVs in MBC cases, identified in 15 of the 50 analysed genes, was 4.8%. Thus, about 5% of MBC cases are carriers of germline PVs in genes other than *BRCA1* and *BRCA2*, which together account for about 15% of MBC cases, and individually for up to 2% and 13% of MBC cases, respectively [11,12].

PALB2 emerged as the most common mutated gene (about 1% of MBCs), followed by *ATM*. These results are in line with PV prevalence reported in previous studies on unselected MBC patients but, as expected, lower than those reported in series enriched for familial MBC cases [12,16–18,28,31].

In agreement with previous evidence [11,17,32], PV carriers were more likely to have personal history of cancers other than breast, and cancer family history, not limited to BC and OC. These results suggest that performing multigene panel testing in MBC patients with personal and family history of multiple cancers may be instrumental to identify PVs in genes other than *BRCA1/2*, with important implications in cancer surveillance and prevention for the affected men and their relatives.

To date, the majority of existing studies have been performed on cohorts of MBC cases, while case-control analyses have used online datasets as control population [11–18,32]. Differently, this study provides population-specific MBC risk estimates associated with PVs in a case-control setting including a large cohort of male controls, previously untested for *BRCA1/2* PVs, enrolled in the same geographic areas of cases.

As the multigene panel used included *BRCA1/2* genes, we were able to identify population controls carrying *BRCA1/2* PVs, documenting that 0.8% of male controls were carriers of *BRCA1/2* PVs. Literature data showed that the frequency of male *BRCA1/2* PV carriers in the general population is about 0.6%, consistent with our results [33]. To the best of our knowledge, this is the first study providing the percentage of *BRCA1/2* PVs in the Italian male population. These epidemiological data could be used for further studies on the impact of *BRCA1/2* PVs in healthy men.

The case-control study here performed showed that, as expected, the aggregate prevalence of PVs was significantly higher in MBC cases than in controls, with PV carriers having about threefold increased MBC risk, consistent with a moderate penetrance.

Our data showed that *PALB2* PVs confer an approximately sevenfold increased risk of developing MBC in the Italian population, in line with previous studies reporting OR values ranging from 6 to 14, depending on the study design and population analysed [12,16,34]. Notably, the magnitude of BC ORs is reportedly higher in unselected men than in unselected women [8]. Overall, our findings confirmed *PALB2* as a main MBC susceptibility gene, together with *BRCA2* and *BRCA1*. Notably, *PALB2* risk estimates for MBC are comparable, if not higher, to those recently reported for *BRCA1* [2], thus highlighting the importance of including *PALB2* in screening and surveillance protocols for male patients.

ATM is a well-established moderate-risk gene in FBC, with ORs ranging from 2 to 5 [35], while data on MBC are still scarce [11, 16, 35]. In our study, *ATM* PVs were associated with MBC risk with an OR of 4.8. Although the OR may be overestimated by the small

number of PVs identified, our results indicate that *ATM* may be considered a moderate-risk gene in MBC.

The role of *CHEK2* in MBC susceptibility has been, notoriously, conflicting [11,12,16,17,36]. Indeed, the contribution of *CHEK2* PVs, and in particular the *CHEK2* c.1100delC variant, in MBC predisposition varies by ethnic group and from country to country [36–40]. The present study supports previous data indicating that *CHEK2* PVs do not play a relevant role in MBC genetic predisposition in the Italian population [36]. Our results highlight the importance of performing population-specific association studies, since the prevalence and the penetrance of PVs in specific genes may not overlap in all populations.

In this study, no other gene emerged as significantly associated with MBC risk, in line with large studies in women indicating as core BC susceptibility genes *ATM*, *CHEK2*, and *PALB2*, in addition to *BRCA1/2* [8,9].

Association of PVs with BC outcomes is critical for improving BC prevention strategies. It is not clearly established as to whether *ATM*, *CHEK2*, or *PALB2* PVs have prognostic implications [41]. Currently, we are collecting overall survival data for cases with PVs and, based on available data, male *PALB2* PV carriers seem to have a poor outcome, consistent with findings in women reporting a reduced 10-year survival for *PALB2* PV carriers compared with non-carriers [42]. The impact of PVs on survival, as well as the efficacy of eventual surveillance protocols developed for male PV carriers, needs to be addressed in further studies.

One of the issues raised by multigene panel testing is the high number of VUS detected, which still represents a challenge in the clinical management [43]. Using *in silico* predictors to assess VUS pathogenicity allows for the assessment of a large number of variants, to select those deserving further investigation, possibly including functional studies. Many *in silico* tools have been developed, but there is currently no consensus in the clinical setting, thus representing a possible limitation. Here, we used REVEL score to prioritise the large number of VUS identified in MBC cases and controls, as REVEL is consistently reported to outperform others *in silico* meta-predictors for clinical variant classification, in accordance with current classification guidelines [44]. Notably, the frequency of VUS with a high probability of pathogenicity (REVEL score ≥ 0.6) was significantly higher in MBC cases than in controls, suggesting a possible clinical relevance of some VUS that might be reclassified as PVs by further studies.

The study has some limitations. First, this is an observational and retrospective case-control study, thus not allowing for evaluation of outcomes, interventions, and absolute risk calculations.

One of the main issues of concern with a case-control study is the selection of an appropriate control group. In this study, controls were all men recruited in the same period and geographical areas of the MBC cases; however, they were significantly younger than cases and collected from heterogeneous source types. To address these possible sources of bias, all analyses were adjusted for age and centre of origin. Unfortunately, we were not able to collect other data from the population controls, including family history of cancer, thus not allowing additional sensitivity analyses.

Despite the large series of MBC cases and controls, due to the rarity of the disease and of the PVs, we could not assess MBC risk in some of the analysed genes or for single specific variants. We cannot exclude that, at least for some genes, the study may be underpowered to detect a significant association with MBC risk, or that PVs in these genes may be associated with specific MBC subtypes, as suggested [8,9,19]. In addition, information on tumour characteristics is missing for some cases. As the genotype–phenotype correlation may be different for each gene, a larger number of carriers are needed in order to identify specific pathological characteristics as potential predictors of PVs for each gene. Moreover, the presence of pathogenic alterations other than gene variants (i.e. large rearrangements and copy number variants) was not evaluated. Thus, data on the prevalence of pathogenic alterations in some genes may be underestimated. Further collaborative studies are needed to address these issues.

5. Conclusions

Overall, the results of this large case-control study show that PVs in genes other than *BRCA1/2* account for about 5% of MBCs, and that *PALB2* and *ATM* PVs are associated with increased MBC risk. These data may help in the implementation of multigene panel testing in male patients and inform gender-specific BC risk management and prevention strategies for patients and their families.

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CRediT authorship contribution statement

Conceptualization: L.O. Methodology: V.S. Validation: A.Bu., G.C., V.V. Formal Analysis: A.Bu., G.C.,V.S. Investigation: A.Bu., G.C., V.V., V.S. Resources: C.C.,

A.Br., M.T., B.B., D.C., A.C., G.G., V.G., S.M., L.M., M.M., P.P., P.R., A.R., M.G.T., D.T., A.V., I.Z., D.P. Data curation: A.Bu, G.C., V.S. Writing – original draft: A.Bu., G.C., V.V., V.S., L.O. Writing – Review and Editing: all authors. Visualization: A.Bu, G.C., V.S. Supervision: L.O. Project Administration: L.O. Funding acquisition: M.T., G.G., L.O. All authors approved the study for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.04.022.

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