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EPIDEMIOLOGY

Association of low-penetrance alleles with male breast cancer risk and clinicopathological characteristics: results from a multicenter study in Italy

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Abstract It is well-known that male breast cancer (MBC) susceptibility is mainly due to high-penetrance *BRCA1/2* mutations. Here, we investigated whether common low-penetrance breast cancer (BC) susceptibility alleles may influence MBC risk in Italian population and whether variant alleles may be associated with specific clinicopathological features of MBCs. In the frame of the Italian Multicenter Study on MBC, we genotyped 413 MBCs and 745 age-matched male controls at 9 SNPs annotating known BC susceptibility loci. By multivariate logistic regression models, we found a significant increased MBC risk for 3 SNPs, in particular, with codominant models, for rs2046210/*ESR1* (OR = 1.71; 95 % CI: 1.43–2.05; $p = 0.0001$), rs3803662/*TOX3* (OR = 1.59; 95 % CI: 1.32–1.92; $p = 0.0001$), and rs2981582/*FGFR2* (OR = 1.26; 95 % CI: 1.05–1.50; $p = 0.013$). Furthermore, we showed that the prevalence of the risk genotypes of *ESR1* tended to be higher in ER− tumors ($p = 0.062$). In a case–case multivariate analysis, a statistically significant association between *ESR1* and ER− tumors was found (OR = 1.88; 95 % CI: 1.03–3.49; $p = 0.039$). Overall, our data, based on a large and well-characterized MBC series, support the hypothesis that common low-penetrance BC susceptibility alleles play a role in MBC susceptibility and, interestingly, indicate that *ESR1* is associated with a distinct tumor subtype defined by ER-negative status.

Keywords Male breast cancer · *BRCA1/2* · Low-penetrance breast cancer alleles · SNPs · Clinicopathological characteristics · ER/PR status

Introduction

Breast cancer (BC) does affect not only women but also men, although rarely. In Western countries, male breast

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cancer (MBC) makes up less than 1 % of all BCs and less than 1 % of all cancers in men [1, 2]. Its incidence is estimated at 1.1 per 100,000 men a year [3]; however, recent epidemiologic studies suggest that the incidence of MBC is increasing by 1.1 % yearly [1, 2]. In Italy, incidence rates, standardized on the European population, are about 1.2 new cases per 100,000 men per year and around 450 men with BC are estimated to be diagnosed in 2011 [4].

Similar to female BC (FBC), MBC is likely to be caused by the concurrent effects of different risk factors, including hormonal, environmental, and genetic risk factors [5]. Family history of BC and personal history of cancer are frequently observed in MBC patients [6], thus pointing to a relevant genetic component in MBC predisposition. Mutations in the two major high-penetrance BC genes, *BRCA1* and, mainly, *BRCA2*, play the most relevant role in MBC susceptibility [6, 7]. Rare variants in moderate-penetrance BC susceptibility genes, including *CHEK2, BRIP1*, *PALB2,* and *RAD51C*, may also play a role in MBC, although at lower extent [7]. We recently reported that only a small proportion (about 14 %) of MBCs is accounted by high- and moderate penetrance BC susceptibility genes in Italy [8–10]. Thus, much of the genetic contribution to MBC risk remains to be elucidated.

According to the "polygenic" model, the genetic susceptibility to BC can be attributed to the combined effects of common low-penetrance susceptibility alleles, each conferring a small effect on BC risk and explaining a high proportion of cancers that may arise in a genetically susceptible minority [11, 12]. Genome-wide association studies (GWAS) have identified associations between single nucleotide polymorphisms (SNPs), acting as common low-penetrance variant alleles, and BC risk in women [13–21]. An involvement of some of the SNPs found to be associated with FBC has been suggested in MBC susceptibility [22], and by GWAS we could recently identify a novel common variant associated with MBC [23].

Recent studies show that common low-penetrance BC susceptibility alleles may modify the risk associated with *BRCA1* and *BRCA2* mutations in FBC, thus indicating a role of low penetrance alleles as modulators of the risk conferred by high-penetrance BC susceptibility genes [24]. At present, there are no data whether low-penetrance BC susceptibility alleles may modulate BC in *BRCA1/2* male carriers.

In addition, there is evidence that the increased risk associated with several of the loci identified to date shows BC subtype specificity in FBC [25–28]. Notably, associations with most of the susceptibility loci appear to be stronger for estrogen receptor-positive (ER+) rather than for ER-negative (ER−) BCs [25]. In particular, variants in *CASP8*, 5p12, *FGFR2* show the strongest evidence of association with ER+ tumors whereas variants in 2q35, *MAP3K1, LSP1, TOX3* are associated with both ER+ and ER− disease [25, 27]. On the other hand, variants in *ESR1* and 19p13 show evidence of an association primarily with ER− BCs [20, 29–31]. At present, there are no data on possible correlations between clinicopathological characteristics and common low-penetrance BC susceptibility alleles in MBC.

Compared with FBC, MBC more often displays ER+ and progesterone receptor-positive (PR+) status [3]. We recently showed that *BRCA2*-related MBCs represent a subgroup of tumors with a peculiar phenotype characterized by high tumor grade, the absence of PR expression and HER2 positive status [10], thus providing evidence that high-penetrance genetic factors can also influence tumor type in MBC. This raises the possibility that other susceptibility loci may also be associated with specific subtypes of MBC.

Here, taking advantage of the ongoing Italian Multicenter Study on MBC, we conducted a case–control study of a large series of well-characterized MBC cases and matched controls to evaluate the impact of common low-penetrance BC alleles in Italian MBC patients and to assess whether common BC alleles may be associated with specific clinicopathological features of MBCs.

Materials and methods

Study population

The study was performed comparing a series of 413 MBC patients and 745 healthy age-matched male controls. All subjects were recruited from ten Italian Investigation Centers distributed across the country in the frame of the ongoing Italian Multicenter Study on MBC [10]. Information collected

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for each MBC case included: age at diagnosis, family and personal history of cancer, recurrence of the disease, *BRCA1/2* mutation status, tumor histological type, grade (G), node (N) status, ER, PR, Ki-67, and HER2 expression. Control samples were obtained from individuals enrolled under research or clinical protocols and from blood donors. All control individuals were Caucasians and residing in the same areas of cases. In addition, 198 unaffected male *BRCA1/2* carriers were included into the study as a separate control group. The study was approved by the local ethical committees.

Blood collection and DNA extraction/genotyping

From each study participant blood or DNA samples were obtained. DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Blood mini kit (Qiagen, Venlo, The Netherlands), following manufacturer instructions. DNA samples were quantified using NanoDrop 1000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

MBC cases and controls were genotyped by allelic-discrimination real-time PCR with TaqMan probes in ABI 7500 fast real-time PCR instrument (Life Technologies, Carlsbad, California, USA) at 2q35 rs13387042, *CASP8* rs1045485, 5p12 rs10941679, *MAP3K1* rs889312, *ESR1* rs2046210, *FGFR2* rs2981582, *LSP1* rs3817198, *TOX3* rs3803662, and 19p13 rs2363956 by commercially available assays from Life Technologies (Carlsbad, California, USA), according to the manufacturer's instruction.

Statistical analyses

The genotype frequencies for each individual DNA polymorphism were evaluated in both series of cases and controls, and deviations from Hardy–Weinberg equilibrium in controls were assessed by a Chi-square test with one degree of freedom.

Considering a minor allele frequency of 11 % (lower value in our control's series) and a dominant model, with a case–control ratio of 1:1.8 (413 cases and 756 controls), we could identify an odds ratio (OR) of 1.59 with a power of 90 % and $\alpha = 0.05$.

Chi-square test was used to evaluate the difference of specific parameters between different groups of cases. Logistic regression models, adjusted for enrollment center and age of participants, were performed to assess the association between each DNA polymorphism and overall MBC risk by ORs and their 95 % confidence intervals (CIs). For each gene, a specific model was used to evaluate separately the effect of the heterozygous genotype and of the homozygous variant. In each model, the common homozygote genotype (in the control population) was considered as the reference category. We also evaluated the MBC risk by separate logistic regression models based on different inheritance model: dominant, recessive, and multiplicative codominant effect.

To evaluate the potential associations between genetic susceptibility and specific MBC clinicopathological characteristics, multinomial regression logistic analyses, including the enrollment center and age of participants, were performed by stratifying MBC cases according to ER/PR combined status and tumor subtypes. The analyses were carried out using three different models based on dominant, recessive, or codominant effect.

A p value <0.05 was considered statistically significant. All the analyses were performed using SAS (SAS/STAT version 9.1) statistical program.

Results

The clinicopathological features of the 413 MBC included in this study are summarized in Table 1. Age at first MBC diagnosis ranged between 22 and 90 years, with a mean age of 61.3 years (SD 11.8). 160 cases (38.7 %) reported a family history (FH) of breast and/or ovarian cancer in first-degree relatives and 62 cases (15 %) a personal history of other cancers. 17 cases (4.1 %) reported a contralateral BC. With regard to *BRCA1/2* mutation status, 3 MBCs carried *BRCA1* (0.7 %) and 49 *BRCA2* mutations (11.9 %). Male breast tumors were more often invasive ductal carcinomas (85.5 %), G2 (56.4 %) and with stage I–II of the disease (77.7 %), 57.7 % of the cases showed negative node status (N−) and 54.8 % low proliferative activity (Ki-67 low). As shown in Table 2, the majority of tumors were ER+ (91.9 %), PR+ (84.7 %) and HER2− (75 %); 83 % of MBCs were ER+/PR+ tumors and 69.6 % were Luminal A (ER and/or PR+, HER2−) subtype.

All MBC cases and controls were genotyped at nine SNPs (rs13387042/2q35, rs1045485/*CASP8*, rs10941679/5p12, rs889312/*MAP3K1*, rs2046210/*ESR1*, rs2981582/*FGFR2*, rs3817198/*LSP1*, rs3803662/*TOX3*, and rs2363956/19p13) annotating the strongest BC associated loci that showed association with tumor subtype defined by hormone receptor status. Genotype distribution was consistent with Hardy–Weinberg equilibrium among controls.

Estimates for the association between the nine SNPs evaluated and overall BC risk are shown in Table 3. When we compared 413 MBC cases with 745 age-matched male controls, statistically significant associations emerged by separate logistic regression models, adjusted for center of enrollment and age of participants, between overall MBC risk and three SNPs: rs2046210/*ESR1*, rs2981582/*FGFR2*, and rs3803662/*TOX3*. In particular, based on a codominant model, the following ORs were found: *ESR1* OR = 1.71 (95 % CI: 1.43–2.05; $p = 0.0001$), *TOX3* OR = 1.59 (95 %

Table 1 Clinicopathological characteristics of the 413 MBC cases

Characteristics	*N*	%
Age at diagnosis		
<50	68	16.5
50–70	254	61.5
>70	91	22.0
First degree family history of breast/ovarian cancer		
Negative	253	61.3
Positive	160	38.7
Personal history of other cancers		
Negative	351	85.0
Positive	62	15.0
Contralateral BC		
No	396	95.9
Yes	17	4.1
BRCA1/2 mutation status		
BRCA1 mutation positive	3	0.7
BRCA2 mutation positive	49	11.9
BRCA1/2 wild-type	361	87.4
Histology[a]		
Invasive ductal carcinoma	283	85.5
In situ ductal carcinoma	26	7.9
Medullary carcinoma	2	0.6
Lobular carcinoma	4	1.2
Other	16	4.8
Grading[a]		
1	37	12.4
2	168	56.4
3	93	31.2
Stage[a]		
I	104	41.4
II	91	36.3
III	48	19.1
IV	8	3.2
Node status[a]		
Negative	162	57.7
Positive	119	42.3
Ki-67[a]		
Low	131	54.8
High	108	45.2

[a] Some data for each characteristic are not available

Table 2 Receptor status and tumor subtypes of the 413 MBC cases

Characteristics[a]	*N*	%
ER		
Negative	26	8.1
Positive	295	91.9
PR		
Negative	49	15.3
Positive	271	84.7
HER2		
Negative	183	75.0
Positive	61	25.0
ER/PR		
−/−	21	6.6
−/+	5	1.6
+/−	28	8.8
+/+	266	83.0
Subtype		
Luminal A	167	69.6
Luminal B	56	23.3
HER2+	5	2.1
Triple negative	12	5.0

Luminal A ER and/or PR+, HER2−, *Luminal B* ER and/or PR+, HER2+, *Her2+* ER−, PR−, HER2+, *Triple negative* ER−, PR−, HER2−

[a] Some data for each characteristic are not available

CI: 1.32–1.92; $p = 0.0001$), and *FGFR2* OR = 1.26 (95 % CI: 1.05–1.50; $p = 0.013$). Similar results also emerged by separate regression analyses based on dominant or recessive models for *ESR1* ($p = 0.0001$ in both cases) and *TOX3* ($p = 0.0001$ in both cases), while for *FGFR2* we found a significant effect ($p = 0.03$) with the dominant model and a borderline effect ($p = 0.058$) with the recessive model (data not shown).

We also evaluated the association between the nine SNPs and overall MBC risk after exclusion of the 52 MBC carriers of germ-line *BRCA1/2* mutations and obtained the same significant associations with the three above mentioned SNPs. In particular, we found a significant effect for *ESR1* ($p = 0.0001$), *TOX3* ($p = 0.0001$), and *FGFR2* ($p = 0.004$) with codominant models (data not shown). We then compared the 52 *BRCA1/2* carriers in the MBC series with the additional control group of 198 unaffected male *BRCA1/2* carriers, a statistically significant association ($p = 0.02$; OR = 2.51; 95 % CI 1.16–5.43) between increased MBC risk and *ESR1* risk genotype emerged (data not shown).

We further analyzed the distribution of the three SNPs associated with overall MBC risk according to tumor receptor status in the series of MBC cases (Table 4). The prevalence of the risk genotypes of *ESR1* tended to be higher in ER− subgroup ($p_{\text{Chi-square}} = 0.062$). As shown in Table 4, a statistically significant association between *ESR1* and ER− tumors emerged in a case–case multivariate analysis adjusted for center of enrollment and age of patients (OR = 1.88; 95 % CI: 1.03–3.49; $p = 0.039$). When tumors were classified according to the co-expression of ER and PR status, the prevalence of the risk genotypes of *ESR1* tended to be higher in ER−/PR− tumors versus ER+/PR+ tumors ($p_{\text{Chi-square}} = 0.11$). As

Table 3 Distribution of 413 cases and 745 controls according to genotype frequencies[a] and MBC risk estimates for selected susceptibility SNPs[b]

SNP	Chromosome	Gene	Genotype	Cases *N* (%)	Controls *N* (%)	OR (95 % CI)	*p* value[c]
rs13387042	2q35		GG	83 (20.1)	163 (21.9)	1	
			GA	189 (45.8)	366 (49.1)	0.96 (0.69–1.34)	0.82
			AA	141 (34.1)	216 (29.0)	1.13 (0.94–1.34)	0.19
			Codominant model			1.14 (0.96–1.37)	0.14
rs1045485	2q33.1	*CASP8*	GG	324 (78.6)	587 (78.8)	1	
			GC	82 (19.9)	150 (20.1)	1.01 (0.74–1.39)	0.93
			CC	6 (1.5)	8 (1.1)	1.27 (0.72–2.24)	0.41
			Codominant model			1.06 (0.80–1.41)	0.68
rs10941679	5p12		AA	226 (54.9)	427 (57.3)	1	
			AG	160 (38.8)	273 (36.6)	1.13 (0.87–1.48)	0.37
			GG	26 (6.3)	57 (6.1)	1.09 (0.83–1.42)	0.55
			Codominant model			1.11 (0.90–1.36)	0.33
rs889312	5q11.2	*MAP3K1*	AA	183 (44.3)	371 (49.8)	1	
			AC	200 (48.4)	318 (42.7)	1.26 (0.97–1.64)	*0.08*
			CC	30 (7.3)	56 (7.5)	1.10 (0.85–1.41)	0.47
			Codominant model			1.17 (0.96–1.43)	0.11
rs2046210	6q25.1	*ESR1*	CC	109 (26.5)	324 (43.5)	1	
			CT	212 (51.5)	318 (42.7)	**1.94 (1.45–2.59)**	**0.0001**
			TT	91 (22.0)	103 (13.8)	**1.72 (1.42–2.08)**	**0.0001**
			Codominant model			**1.71 (1.43–2.05)**	**0.0001**
rs2981582	10q26.13	*FGFR2*	GG	110 (26.6)	245 (32.9)	1	
			GA	205 (49.6)	361 (48.5)	1.28 (0.95–1.72)	*0.10*
			AA	98 (23.8)	139 (18.7)	**1.26 (1.05–1.51)**	**0.013**
			Codominant model			**1.26 (1.05–1.50)**	**0.013**
rs3817198	11p15.5	*LSP1*	TT	186 (45.1)	331 (44.4)	1	
			TC	174 (42.2)	334 (44.8)	0.90 (0.69–1.18)	0.46
			CC	52 (12.7)	80 (10.8)	1.09 (0.88–1.35)	0.43
			Codominant model			1.02 (0.85–1.23)	0.83
rs3803662	16q12.1	*TOX3*	CC	143 (34.7)	352 (47.2)	1	
			CT	195 (47.3)	323 (43.4)	**1.45 (1.10–1.91)**	**0.008**
			TT	74 (18.0)	70 (9.4)	**1.65 (1.35–2.01)**	**0.0001**
			Codominant model			**1.59 (1.32–1.92)**	**0.0001**
rs2363956	19p13		GG	112 (27.2)	223 (29.9)	1	
			GT	213 (51.7)	374 (50.2)	1.13 (0.84–1.52)	0.41
			TT	87 (21.1)	148 (19.9)	1.07 (0.89–1.28)	0.49
			Codominant model			1.07 (0.89–1.28)	0.46

[a] Some genotypes were not available for the whole series of cases and controls

[b] ORs and 95 % CI for specific genotypes and codominant model were calculated using separate logistic regression models adjusted for enrollment's center and age of study subjects

[c] *p* values <0.05 in bold text; *p* values 0.05–0.10 in italics

shown in Table 5, in a case–case analysis using a polynomial regression approach including terms for center of enrollment, age of patients and genotype (codominant model), this association failed to reach the level of statistical significance (OR = 1.90; 95 % CI: 0.98–3.71; $p = 0.057$). No significant differences in the distribution of the risk genotypes of *FGFR2* and *TOX3* according to combined ER/PR receptor status were found.

No significant differences in the distribution of the risk genotype of *ESR1* were observed in the case series classified by Luminal A (ER and/or PR+, HER2), Luminal B (ER and/or PR+, HER2+), HER2+ (ER−, PR−, HER2+), and triple negative (ER−, PR−, HER2−) tumor subtypes (Supplementary Table 1).

Although detailed subgroup analyses were precluded by small numbers, it is interesting to note that the HER2+

Table 4 Distribution of the three SNPs associated with overall MBC risk in the case's series according to ER, PR, and HER2 status, and *p* values of the association with specific MBC subtypes

	ER		PR		HER2	
	− ($n = 26$) *N* (%)	+ ($n = 295$) *N* (%)	− ($n = 49$) *N* (%)	+ ($n = 271$) *N* (%)	− ($n = 183$) *N* (%)	+ ($n = 61$) *N* (%)
ESR1						
CC	4 (15.4)	82 (27.8)	10 (20.4)	76 (28.0)	47 (25.7)	15 (24.6)
CT	12 (46.1)	155 (52.5)	26 (53.1)	140 (51.7)	97 (53.0)	33 (54.1)
TT	10 (38.5)	58 (19.7)	13 (26.5)	55 (20.3)	39 (21.3)	13 (21.3)
p value[a]	**0.039**		0.25		0.98	
FGFR2						
GG	5 (19.2)	78 (26.4)	11 (22.4)	72 (26.6)	39 (21.3)	17 (27.9)
GA	12 (46.2)	146 (49.5)	27 (55.2)	130 (48.0)	94 (51.4)	26 (42.6)
AA	9 (34.6)	71 (24.1)	11 (22.4)	69 (25.4)	50 (27.3)	18 (29.5)
p value[a]	0.26		0.96		0.74	
TOX3						
CC	9 (34.6)	94 (31.9)	18 (36.7)	85 (31.3)	55 (30.1)	23 (37.7)
CT	11 (42.3)	147 (49.8)	20 (40.8)	137 (50.6)	97 (53.0)	27 (44.3)
TT	6 (23.1)	54 (18.3)	11 (22.5)	49 (18.1)	31 (16.9)	11 (18.0)
p value[a]	0.89		0.92		0.49	

[a] *p* value from separate logistic regression models including terms for enrollment's center, age of patients and genotype (codominant). *p* values <0.05 in bold text

Table 5 Distribution of the three SNPs associated with overall MBC risk in the case's series according to combined ER/PR status, and *p* values of the association with specific MBC risk

	ER+/PR+ ($n = 266$) *N* (%)	ER−/PR− ($n = 21$) *N* (%)	ER−/PR+ ($n = 5$) *N* (%)	ER+/PR− ($n = 28$) *N* (%)
ESR1				
CC	75 (28.2)	3 (14.3)	1 (20.0)	7 (25.0)
CT	138 (51.9)	10 (47.6)	2 (40.0)	16 (57.1)
TT	53 (19.9)	8 (38.1)	3 (40.0)	5 (7.9)
p value[a]		*0.057*	0.39	0.99
FGFR2				
GG	70 (26.3)	3 (14.3)	2 (40.0)	8 (28.6)
GA	128 (48.1)	10 (47.6)	2 (40.0)	17 (60.7)
AA	68 (25.6)	8 (38.1)	1 (20.0)	3 (10.7)
p value[a]		0.14	0.54	0.19
TOX3				
CC	84 (31.6)	8 (38.1)	1 (20.0)	10 (35.7)
CT	135 (50.8)	9 (42.9)	2 (40.0)	11 (39.3)
TT	47 (17.6)	4 (19.0)	2 (40.0)	7 (25.0)
p value[a]		0.72	0.30	0.82

[a] *p* value from multinomial regression logistic models including terms for enrollment's center, age of patients and genotype (codominant). The reference category is the group of cases "ER+/PR+" in each model. *p* values 0.05–0.10 in italics

MBC subtype (five cases) showed the highest percentage (60 %) of the risk genotype of *ESR1*, and that three out of these five MBCs harbored a *BRCA2* mutation. In a case–case analysis using a polynomial regression approach, including terms for center of enrollment, age of patients, and genotype (co-dominant model), this association was not significant ($p = 0.067$). No significant differences in the distribution of the risk genotypes of *FGFR2* and *TOX3* according to cancer subtype was observed.

Finally, we evaluated the distribution of *ESR1*, *FGFR2,* and *TOX3* genotypes in the MBC series according to selected tumor characteristics, such as grade, stage, nodal involvement, and proliferative activity, and no significant differences in genotype distribution emerged (Supplementary Table 2).

Discussion

We have shown that common low-penetrance BC susceptibility alleles modulate MBC risk in the Italian population examined in this study, and, interestingly, that these alleles are associated with increased risk of specific breast tumor subtypes.

Three of the nine SNPs genotyped in our series (rs2046210/*ESR1*, rs2981582/*FGFR2* and rs3803662/*TOX3*) showed statistically significant associations with overall MBC risk. In

particular, *ESR1* and *TOX3* showed the strongest association. Notably, we recently reported a strong association for *TOX3* and *ESR1* with increased MBC risk in a large collaborative series [23]. In particular, *TOX3* reached a genome wide-significance and showed an association stronger in males than that in females, while *ESR1* was validated in the same series [22, 23]. In contrast, *FGFR2* did not emerge in such analysis.

Added value of the present study is that all MBC cases analyzed here were characterized for *BRCA1/2* mutation status, including 52 MBC *BRCA1/2* carriers, and that we could examine a control series of 198 unaffected male *BRCA1/2* carriers. This allowed us to show that low-penetrance alleles, associated with increased risk of BC in the general population, may also modify the risk of developing BC in male *BRCA1/2* mutation carriers. At present, there are no data whether low-penetrance susceptibility alleles may modulate BC risk in male *BRCA1/2* carriers. Notably, we showed that rs2046210/*ESR1* was associated with increased MBC risk in analyses restricted to *BRCA1/2* male carriers, suggesting that it may act as a genetic modifier of *BRCA* genes in MBC. Although based on a relatively small series, our data suggest that common BC susceptibility alleles might modulate the risk of BC in *BRCA* male carriers, as known in female carriers [24]. Large collaborative studies on *BRCA1/2* male carriers are needed to validate our results.

Furthermore, our well-characterized MBC series enabled us to carry out a more detailed analysis revealing that the relative risk associated with common genetic variants may also be linked to clinically important characteristics of tumors defined by hormonal receptor status. At present, there are no data on possible correlations between clinicopathological characteristics and common low-penetrance BC susceptibility alleles in MBC. On the other hand, in FBC, there is increasing evidence that associations between common variants and BC risk could vary by clinically important tumor characteristics, mainly by ER expression [25–31]. We found that *ESR1* was significantly associated with the risk of ER− BCs in males. Intriguingly, *ESR1* locus has also been associated with bone mineral density, a phenotype that is affected by estrogens [32]. Interestingly, a history of bone fractures has been reported to be associated with increased MBC risk, possibly because of alterations in the bio-available ratio of estrogen to testosterone [33].

It is noteworthy that *ESR1* has been recently associated with the risk of triple negative BCs in women [29]. While we did not observe this association in our MBC series, on the other hand, we could observe that the highest percentage of the risk genotype of *ESR1* was present in the small subgroup of MBCs represented by HER2+ (ER−, PR− HER2+) tumor subtype. Intriguingly, we have recently shown that HER2+ subtype was associated with *BRCA2* mutations in MBC [10]. Although based on small number of cases, these results may suggest that the observed associations between susceptibility loci and ER/PR status may reflect underlying associations with particular molecular profiles.

Overall, when combined with data in FBC, our results suggest that *ESR1* may be specifically associated with ER− BCs. Since *ESR1* gene encodes estrogen receptor α (ERα) that regulates signal transduction of estrogens, it is tempting to speculate that inherited variation may affect *ESR1* expression and promote formation of ER− tumors. Compared to ER+ BCs, ER− BCs are quite infrequent, thus our results may shed some light on the genetic predisposition to this rare BC subtype that represents a challenge in the clinical setting.

Although we had overall a large sample size, a potential limitation of our study was that information on tumor characteristics was not available for all cases. In addition, these data had been predominantly abstracted from medical records, rather than being obtained through a standardized pathology review. Missing data are likely to be independent of susceptibility loci, and thus would tend to underestimate associations rather than lead to spurious associations. However, the strong associations with ER status suggest that this effect was minimal, at least for the features examined here.

Overall, our data, based on a large and well-characterized MBC series, add to the accumulating evidence that common low-penetrance BC susceptibility alleles, particularly at *ESR1* and *TOX3* loci, play a role in MBC susceptibility and, interestingly, indicate that *ESR1* may be associated with distinct tumor subtypes defined by ER negative status in men.

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Conflict of interest The authors declare that they have no conflict of interest.

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