Novel and known genetic variants for male breast cancer risk at 8q24.21, 9p21.3, 11q13.3 and 14q24.1: Results from a multicenter study in Italy

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Abstract
Increasing evidence indicates that common genetic variants may contribute to the heritable risk of breast cancer (BC). In this study, we investigated whether single nucleotide polymorphisms (SNPs), within the 8q24.21 multi-cancer susceptibility region and within

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1. Introduction

Male breast cancer (MBC) is a rare disease representing less than 1% of all breast cancers (BCs) and less than 1% of all cancers in men [1]. About 20% of MBC patients show positive family history of BC and about 20% develop a second non-breast tumour, in particular prostate and colon cancer [2]. These observations point to a relevant genetic component in MBC predisposition.

Mutations in high-penetrance BC genes, *BRCA1* and, more frequently, *BRCA2*, and in moderate-penetrance genes, such as *CHEK2* and *PALB2*, have a relevant role in MBC susceptibility [3]. However, only about 10–15% of all MBCs are accounted for by mutations in these genes, thus, much of the genetic contribution to MBC risk still remains to be elucidated.

Growing evidence indicates that the genetic susceptibility to cancer can be attributed to the combined effects of low-penetrance susceptibility single nucleotide polymorphisms (SNPs) [4,5]. A large number of SNPs, identified through Genome Wide Association Studies (GWAS) or candidate gene approach, have been associated with many types of cancer, including BC [6,7].

Multiple GWAS have identified SNPs within 8q24.21 region that are linked to susceptibility for different types of cancer [8–15]. Five distinct 8q24.21 sub-regions have emerged, displaying patterns of association that appear to be specific for breast, colorectal and prostate cancer [16,17]. In particular, three regions were associated with prostate cancer only, one with prostate and colorectal cancer and one with prostate and breast cancer [18]. The 8q24.21 susceptibility region has been described as a gene-desert because of the lacking of annotated protein-coding genes [19]. However, the c-MYC oncogene, which is located 300 kb telomeric, seems the most likely candidate to be functionally linked to the susceptibility conferred by the 8q24.21 SNPs and there is evidence that its expression may be affected by SNPs within 8q24.21 region [17,20].

To date, a large number of studies have been performed to investigate low-penetrance genetic susceptibility in female BC (FBC), and susceptibility alleles have been reported in about 70 loci widespread in the genome [21]. By contrast, only a few studies addressed the role of low-penetrance alleles in MBC susceptibility [22–24].

Two SNPs, rs1314913 in *RAD51B* gene and rs3803662 near *TOX3* gene, were found to be associated with MBC risk by GWAS. In particular, rs1314913 was found specifically associated with increased BC risk in men, whereas rs3803662 was found associated with increased BC risk also in women [23]. Furthermore, by gene candidate approach *ESR1* locus was found to be associated with BC risk in men, and, in particular, with increased risk in oestrogen receptor (ER) negative MBC cases and in male *BRCA1*/*2* mutation carriers [22,24].

Based on the observation that MBC cases may frequently develop additional non-breast tumours, in this study we explored the possibility that the 8q24.21 multi-cancer susceptibility region may have a role in MBC risk. In addition, we aimed to evaluate whether other common low-penetrance susceptibility alleles, recently identified and associated with BC risk in women [15,25,26], may also influence BC risk in men. Finally, we explored possible associations between SNPs and clinical-pathologic characteristics of MBC, in order to provide further insight into the biological basis of BC.
in men, and eventually improve both the identification of men at risk to develop this rare disease and the personalised clinical management of MBC patients.

2. Patients and methods

2.1. Study population

The study was performed comparing a series of 386 MBC cases, including 50 BRCA1/2 mutation carriers, and 908 healthy male controls. An additional control series of 197 healthy male BRCA1/2 mutation carriers was collected and analysed.

Cases, together with their clinical-pathologic characteristics, were recruited in the frame of the ongoing Italian Multicenter Study on MBC, as previously described [24,27]. Overall, mean age at first BC diagnosis was 61.3 years (SD 11.8); 152 cases (39.4%) reported first-degree family history of breast and/or ovarian cancer and 61 cases (15.8%) had a personal history of other cancers (16 prostate, 9 colorectal and 6 bladder cancers). Regarding BRCA1/2 mutation status, 2 cases (0.6%) carried BRCA1 and 48 (12.4%) BRCA2 mutations. The majority of male breast tumours were invasive ductal carcinomas (85.8%), ER+ (92.7%), progesterone receptor (PR)+ (86.3%) and HER2− (75.2%).

Controls were obtained from individuals enrolled under research or clinical protocols and from blood donors. All controls were residing in the same areas of cases.

The study was approved by Local Ethics Committee (“Sapienza” University of Rome, Prot. 264/12).

2.2. Blood collection and DNA extraction/genotyping

From each of the 1491 participants, blood or DNA samples were obtained. DNA was extracted and quantified as already described [24]. MBC cases and controls were genotyped by using MALDI-TOF spectrophotometric mass determination of allele specific primer extension products with Sequenom’s MassARRAY platform and iPLEX technology (Sequenom, San Diego, USA). Optimal amplification and extension primers for use in a multiplex format were designed. A total of 19 susceptibility SNPs within 8q24.21 region and 10 candidate BC susceptibility SNPs were genotyped (Supplementary Table 1). MALDI-TOF mass analysis was performed on a MassARRAY Compact Analyzer. Data were analysed with Sequenom’s MassARRAY Typer software. Genotyping calls were viewed in call cluster plots, and peak intensities were reviewed in each respective sample spectrum.

2.3. 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 13% (lower value in our control’s series) and a dominant model, with a case–control ratio of 1:2.3 (386 cases and 908 controls), we could identify an odds ratio (OR) of 1.53 with a power of 90% and \( z = 0.05 \). Chi-square test was used to evaluate the potential association between genotypes of the 29 SNPs and the presence of the disease. Linear logistic regression models, adjusted for enrolment centre and age of participants, were performed to assess the association between each SNP found potentially associated with the disease and overall MBC risk by ORs and their 95% confidence intervals (CIs). For each SNP, a specific model was used to evaluate separately the effect of the heterozygous genotype and of the rare homozygous genotype. In each model, the common homozygote genotype (in the control population) was considered as the reference category. We also evaluated MBC risk by multiplicative co-dominant model, as estimate of the per-allele effect.

An additional step-wise analysis applied to a linear logistic regression in which all the SNPs appeared as main effects was performed.

Chi-square test and logistic regression models were also performed in a case-case analysis in order to evaluate the difference of specific parameters between different groups of cases and the potential associations between genetic susceptibility and specific MBC subgroups.

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

3. Results

The whole series of 1491 subjects was genotyped at 29 SNPs, including 19 SNPs in 8q24.21 region and 10 candidate BC susceptibility SNPs across the genome. Genotype distribution was consistent with Hardy–Weinberg equilibrium among controls for all SNPs analysed.

A statistically significant difference in the distribution of genotypes in 386 MBC cases and 908 healthy male controls (Supplementary Table 2) emerged for five SNPs: rs1562430 \((p < 0.0001)\) and rs445114 \((p = 0.01)\) both within the breast/prostate cancer sub-region in 8q24.21, and rs1011970/9p21.3 \((p = 0.009)\), rs614367/11q13.3 \((p = 0.008)\) and rs1314913/14q24.1 \((p < 0.0001)\).

As shown in Table 1, statistically significant differences in the proportion of cases with MBC risk and these five SNPs emerged by linear logistic regression models. Compared with the reference genotype, the following ORs for the rare homozygous were found for the 8q24.21 SNPs: rs1562430 OR = 0.52 (95% CI: 0.34–0.79; \( p = 0.002)\) and rs445114 OR = 1.57 (95% CI: 1.05–2.34; \( p = 0.026\). Of
these two SNPs, rs445114 showed no independent association with MBC risk after adjustment for rs1562430 (data not shown). The following ORs for the rare homozygous were found for the other candidate BC susceptibility SNPs: rs1011970/9p21.3 OR = 2.38 (95% CI: 1.22–4.64; \(p=0.011\)), rs614367/11q13.3 OR = 2.57 (95% CI: 1.19–5.54; \(p=0.016\)), and rs1314913/14q24.1 OR = 3.10 (95% CI: 1.84–5.22; \(p<0.0001\)).

By using a stepwise approach in which all 29 SNPs were included, rs1562430/8q24.21 (OR for rare homozygous = 0.54, 95% CI: 0.35–0.83; \(p=0.0043\)) and rs1314913/14q24.1 (OR for rare homozygous = 3.00, 95% CI: 1.78–5.07; \(p<0.0001\)) emerged as the SNPs more strongly associated with MBC risk.

We then restricted the analysis to male BRCA1/2 mutation carriers, by comparing 50 BRCA1/2 mutation positive MBC cases with a control group of 197 unaffected BRCA1/2 carriers (Supplementary Table 3). Differences in the distribution of genotypes between affected and unaffected BRCA1/2 carriers emerged for rs1314913/14q24.1 (\(p=0.017\)). A statistically significant association between rs1314913/14q24.1 risk genotype and increased MBC risk in BRCA1/2 carriers emerged (OR for rare homozygous = 3.22; 95% CI 1.02–10.17; \(p=0.046\). OR for co-dominant model = 1.69; 95% CI 1.02–2.78; \(p=0.041\)).

We further analysed the distribution of the five SNPs associated with overall MBC risk in the series of MBC cases, according to clinical-pathologic features including hormone receptors (ER/PR) and HER2 status (Table 2).

4. Discussion

In this study, we investigated 29 cancer-associated SNPs for their associations with BC risk in men.

Five out of the 29 SNPs analysed were found associated with MBC risk: rs1562430 and rs445114, both within the 8q24.21 region, and rs1011970/9p21.3, rs614367/11q13.3 and rs1314913/14q24.1. By stepwise analysis, rs1562430/8q24.21 and rs1314913/14q24.1 showed the strongest association with MBC risk.

Our results provide the first evidence that the 8q24.21 region is associated with MBC risk. The 8q24.21 region harbours five distinct susceptibility sub-regions including three associated with prostate cancer, one with prostate and colorectal cancer and one with prostate and...
breast cancer [18]. To date, studies investigating BC susceptibility focused on SNPs located within the prostate and breast cancer sub-region. Within this sub-region, two SNPs rs1562430 and rs13281615, were found associated with BC risk in women [10,15]. Of these two SNPs, located in the same linkage disequilibrium block, rs1562430 T allele (the common allele) showed a more significant association with increased FBC risk than the correlated rs13281615 G allele [15]. Recently, rs13281615 was examined for its association with BC risk also in men, but no significant association emerged [22,23]. In this study we investigated all the five 8q24.21 susceptibility sub-regions and genotyped 19 cancer-associated SNPs. Our results showed that two SNPs, rs1562430 and rs445114, both located in the 8q24.21 breast and prostate cancer sub-region, were associated with MBC risk, with rs1562430 showing a stronger and independent association. It is noteworthy that rs445114 was previously found as a prostate cancer specific susceptibility SNP [14]. Male breast and prostate cancers are hormonally driven diseases, and share biological similarities and risk factors [28]. Intriguingly, prostate cancer is the more frequent second malignancy that affects MBC patients [29]. Thus, the 8q24.21 breast and prostate cancer sub-region may have a pleiotropic effect, being involved in susceptibility of both MBC and prostate cancer. On the other hand, based on our results, a role of prostate-specific susceptibility sub-regions in MBC risk may be excluded. Studies of larger series of cases affected by both breast and prostate are needed to further investigate these hypotheses.

In this study, we also showed that other common BC susceptibility SNPs across the genome (rs1011970/9p21.3, rs614367/11q13.3 and rs1314913/14q24.1) were associated with MBC susceptibility in our population. These results are in agreement with and corroborate findings from a previous GWAS study on MBC from different populations [23].

The rs1011970/9p21.3 lies in a block that includes CDKN2A and CDKN2B, genes that are frequently mutated or deleted in several human tumours. CDKN2A germline mutations predispose to malignant melanoma and pancreatic cancer. Intriguingly, these two types of cancer are often found in families with MBC, particularly in families whose BC susceptibility is associated with BRCA2 mutations [30].

The rs614367/11q13.3 lies in gene-desert region. Fine-scale mapping of this region has identified a candidate causal variant that is a regulatory element targeting cyclin D1 (CCND1) gene and that may be associated with low CCND1 protein levels in tumours [31]. CCND1 somatic alterations, such as copy number gain, are very frequent in MBC, and seem to be correlated with poor prognosis [32].

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chromosome/Gene</th>
<th>ER (n: 22)</th>
<th>PR (n: 279)</th>
<th>HER2 (n: 170)</th>
<th>p-Value</th>
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<td>114 (44.0)</td>
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<td></td>
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<td>48 (18.5)</td>
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<td>178 (69.0)</td>
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<td></td>
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<td>CT (12)</td>
<td>70 (25.2)</td>
<td>68 (26.4)</td>
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<td>15 (5.3)</td>
<td>12 (4.6)</td>
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<td>134 (48.0)</td>
<td>125 (48.3)</td>
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</table>

Abbreviations: SNP, Single Nucleotide Polymorphism; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

*p-Values < 0.05 in bold text.

Table 2
Distribution of the five SNPs associated with overall (MBC) risk in the cases series according to ER, PR and HER2 status, and p values of the association with specific MBC subtypes.
The rs1314913/14q24.1, located in intron 7 of RAD51B gene, was previously identified as a specific MBC susceptibility allele by GWAS [23] and in our study showed the strongest association with increased MBC risk.

In the present study, we also tested possible associations between the identified MBC susceptibility SNPs and main clinical-pathologic characteristics. Differences in the distribution of rs614367/11q13.3 genotypes according to ER status and of rs1011970/9p21.3 according to HER2 status emerged.

The frequency of rs614367/11q13.3 risk genotype tended to be higher in ER+ cases. In agreement with our results, rs614367 was associated with ER+/PR+ FBCs, consistently with the role of CCND1 as a mediator of oestrogen-induced cell proliferation [33].

In our MBC series rs1011970/9p21.3 risk genotype was associated with HER2+ disease. The association of rs1011970 with HER2 status was not previously evaluated in FBC [33].

ER/PR and HER2 status have been recognised as having a role as important prognostic factors and as predictive markers for the response to treatment with endocrine and trastuzumab therapy. Thus, identification of susceptibility SNPs with subtype-specific associations may improve our knowledge on the genetic predisposition to important MBC subtypes that may have prognostic and predictive value in the clinical setting.

There is growing evidence that low-penetrance alleles, associated with increased risk of BC in the general population, may also modify the risk of developing BC in BRCA1/2 mutation carriers [34]. In this study, we were able to compare MBC cases with BRCA1/2 mutations with a control series of unaffected male BRCA1/2 mutation carriers. By analyses restricted to BRCA1/2 carriers, we provided the first evidence that rs1314913/14q24.1 was associated with MBC risk, suggesting that it may act as a modifier locus of the risk conferred by BRCA1/2 mutations in men. Further studies in larger populations are needed to validate our findings.

Although we had overall a large sample size compared to other MBC series thus far analysed, the power of the study may be modest in order to identify smaller risk effects. Moreover, information on tumour characteristics was not available for all cases, and these data had been predominantly abstracted from medical records, rather than being obtained through a standardised pathology review. Thus, some associations may be underestimated.

In conclusion, we showed for the first time that common low-penetrance BC susceptibility alleles within 8q24.21 region, and in particular, rs1562430, play a role in MBC susceptibility. Moreover, we validated three BC susceptibility SNPs, including the MBC-specific SNP rs1314913, in the Italian population and provided the first evidence that this SNP may act as a risk modifier locus in male BRCA1/2 carriers. Overall, our present findings add new data to the accumulating evidence that common low-penetrance susceptibility alleles play a relevant role in MBC susceptibility.

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Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2015.07.020.

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