

The *CASP8* rs3834129 polymorphism and breast cancer risk in *BRCA1* mutation carriers.

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Abstract

Purpose. The rs3834129 polymorphism, in the promoter of CASP8 gene, has been recently reported as associated with breast cancer risk in the general population, with the minor allele del having a protective effect. Some of the genetic variants found associated with breast cancer risk were reported as risk modifiers in individuals with mutations in *BRCA1* and *BRCA2* genes. Here, we tested the effect of the rs3834129 del allele on breast cancer risk in *BRCA* mutation carriers. **Methods.** The rs3834129 was genotyped in a total of 1,207 Italian female *BRCA* mutation carriers. Of these, 740 carried a *BRCA1* mutation and 467 a *BRCA2* mutation. Overall, 699 were affected with breast cancer and 508 were unaffected. **Results.** When considering class 1 (loss-of-function) *BRCA* mutations, hazard ratios estimated by weighted multivariable Cox regression model, for individuals with at least one copy of the del allele, were 1.46 (95% confidence interval (CI): 1.08 1.99) for *BRCA1* and *BRCA2* mutation carriers combined, 1.74 (95% CI: 1.24 2.46) for *BRCA1* mutation carriers, and 1.09 (95% CI: 0.66 1.80) for *BRCA2* mutation carriers. **Conclusions.** These results suggest that the minor allele del of rs3834129 is associated under a dominant model with increased breast cancer risk in carriers of *BRCA1* mutations but not in carriers of *BRCA2* mutations.

Keywords

Breast cancer risk; CASP8; rs3834129; polymorphism; *BRCA* mutation carriers.

Introduction

Caspases are apical positive mediators of apoptosis. Apoptosis is a key process in maintaining cell proliferation and controlling cancer development. For this reason, germ-line variations of caspase encoding genes have been studied with respect to cancer risk. The common polymorphism rs3834129, consisting of a six-nucleotide (AGTAAG) insertion/deletion in the promoter of the CASP8 gene at nucleotides 657_652, has been recently investigated in association with breast cancer risk. The first study, carried out in unselected Chinese breast cancer cases, suggested that the minor allele del (also referred to as 652 6N del) is protective versus breast cancer, with an odds ratio (OR) of heterozygotes 0.65 (95% confidence interval, CI: 0.54 0.78) and the OR of homozygotes for the del allele 0.50 (95% CI: 0.34 0.74) [1]. These findings, however, were not confirmed in each of three subsequent studies in Caucasian unselected breast cancer cases and controls [2-4]. We performed a fourth study in Italian familial breast cancer cases with no mutations in BRCA genes. This study indicated a lack of association between rs3834129 and breast cancer risk, but a case-only analysis showed a significant increasing trend for the del/del genotype frequency with later age of breast cancer diagnosis [5]. More recently, two concomitant meta-analyses corroborated the protective effect of the del allele versus breast cancer risk. The first was based on the four above-mentioned studies and estimated for the del allele carriers an overall OR of 0.94 of borderline significance (95% CI: 0.884 1.008). This analysis pointed out that each of the single studies was probably statistically underpowered to detect the protective effect [6]. The second meta-analysis combined three different case-control series, and the ORs for the heterozygous and for the homozygotes for the del allele were 0.95 (95% CI: 0.83 1.08) and 0.82 (95% CI: 0.70 0.95), respectively [7]. Thus, it appears that in the general population the rs3834129 polymorphism is associated with breast cancer risk and that the minor allele del is acting as a protective factor.

It has been shown that most of the genetic variants associated with breast cancer risk identified in the general population by genome-wide association studies are also modifiers of breast cancer risk in carriers of mutations in *BRCA1* or *BRCA2* genes [8,9]. In the present study, we investigated if the rs3834129 polymorphism modifies breast cancer risk testing a large series of Italian female carriers of mutations in *BRCA* genes.

Material and Methods

Study populations

BRCA gene mutation carriers were originally recruited at 8 different cancer genetic clinics participating in the Consorzio degli Studi Italiani sul Tumore Ereditario alla Mammella (CONSIT TEAM; Consortium of Italian Studies on Hereditary Breast Cancer). The CONSIT TEAM was established in 2009 as a national initiative to identify and study genetic modifiers of breast cancer risk and as a collaborative group to studies promoted by the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) [10]. Eligibility criteria for the CONSIT TEAM were those adopted by CIMBA. All individuals included in this study were female carriers of a mutation in either *BRCA1* or *BRCA2*, classified as a deleterious according to widely recognized criteria (Breast Cancer Information Core, <http://research.nhgri.nih.gov/bic/>). All individuals were enrolled at host institutions under research or clinical protocols approved by the local Ethic Committees and were ≥ 18 years old at recruitment. Clinical information collected included data of birth, *BRCA* mutation description and type (according to functional effect), age at last follow-up, age at breast or ovarian cancer diagnosis, and age at bilateral prophylactic mastectomy. Related individuals were identified by a unique family identifier.

Genotyping

Genotyping of rs3834129 was performed at Consortium for Genomic Technologies (Cogentech), Milan by direct sequencing (primer sequences and PCR protocol are available upon request). Deviation from Hardy-Weinberg equilibrium (HWE) was evaluated as a quality-control check in single individuals randomly selected from each family using Pearson chi-squared test.

Statistical analysis

The association of rs3834129 with breast cancer risk was assessed by estimating hazard ratios (HR) and their corresponding 95% CI using weighted multivariable Cox proportional hazards regression with robust estimates of variance, by clustering the observations on the family identity number. This approach allowed correcting for the bias in HR and variance estimates related to the fact that *BRCA1* and *BRCA2* mutation carriers are not randomly sampled with respect to their disease status and that they could belong to the same family. A detailed description of this approach has been published elsewhere [11,12]. Individuals were classified according to their age at the first of the following events: breast cancer diagnosis, ovarian cancer diagnosis, bilateral prophylactic mastectomy or last observation. Only individuals censored at breast cancer diagnosis were considered affected, while the other were considered unaffected. Weights were assigned separately for *BRCA1* and *BRCA2* mutation carriers according to their disease status and by age interval (<25, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, >70 years), such that the weighted observed incidences in the sample agree with established estimates for mutation carriers [13].

We considered log-additive, dominant and “three genotypes” models. Additional covariates included in each model were: center at which *BRCA* mutation carriers were ascertained and year of birth, as previously reported (11).

The latter, grouped in 5 classes (<1940, 1940–1949, 1950–1959, 1960–1969, 1970), was included to account for possible cohort effect. We evaluated the goodness of fit of the data to the different models by using the Akaike's information criterion (AIC) [14]. All statistical analyses were performed with the SAS software (Version 9.2.; SAS Institute Inc. Cary, NC).

Results

Overall 1,241 *BRCA1* and *BRCA2* mutation carriers were contributed by the collaborating centers (Table 1). Genotyping failed for 18 samples. Thirteen women who self-reported as “non-Caucasian” and 3 women who carried mutations in both *BRCA1* and *BRCA2* were excluded from the study. Thus, 1,207 individuals remained included. Of these, 740 carried a *BRCA1* mutation and 467 a *BRCA2* mutation. These individuals were censored at the age of the first of the following events: breast cancer diagnosis (n = 699), last observation (n = 318), ovarian cancer diagnosis (n = 173), and bilateral prophylactic mastectomy (n = 17).

Genotypic frequencies of rs3834129 and weighted multivariable Cox model results are shown in Table 2. The genotypic frequencies were consistent with Hardy-Weinberg equilibrium. There was evidence for association with breast cancer risk for *BRCA1* mutation carriers but not for *BRCA2* mutation carriers. Each copy of the minor allele del conferred an HR of 1.23 (95% CI: 1.02 1.49) and of 0.92 (95% CI: 0.66 1.29) in the two groups, respectively. However, by resorting to a “three genotypes” model, the estimated risk for heterozygotes was higher than the estimated risk for homozygotes for the risk allele del, suggesting that its effect was not consistent with a log-additive model. Using the AIC as overall measure of the goodness of fit of the data to different models, the dominant model was selected. Under this parsimonious model, the HR for carriers with at least one copy of the del allele was 1.52 (95% CI: 1.14 2.02) for

BRCA1 mutation carriers, and 1.35 (95% CI: 1.04 1.76) for *BRCA1* and *BRCA2* mutation carriers combined.

We pursued with an additional analysis and classified BRCA mutation according to their expected functional effects. Mutations of class 1 were those potentially causing loss-of-function and thus haplo-insufficiency; mutations of class 2 those potentially causing stable mutant protein with possible dominant negative effect [9]. Mutations were classified as of class 1 or class 2 as indicated in the database available to CIMBA collaborating groups at the CIMBA web site (<http://www.srl.cam.ac.uk/consortia/cimba/members/login.html>). Carriers of class 1 mutations were approximately 68% in *BRCA1* mutation carriers and approximately 89% in *BRCA2* mutation carriers. Genotypic frequencies of rs3834129 and weighted multivariable Cox model results for carriers of class 1 mutations are shown in Table 3. The number of class 2 mutation carriers was too small to justify separate analysis. Again, the dominant model resulted the most appropriate, and HR individuals with at least one copy of the del allele was 1.74 (95% CI: 1.24 2.46) for *BRCA1* mutation carriers, and 1.46 (95% CI: 1.08 1.99) for *BRCA1* and *BRCA2* mutation carriers combined.

Discussion

Our study showed that the del allele of the rs3834129 polymorphism increases breast cancer risk in *BRCA1* mutation carriers indicating an opposite effect with respect to that observed in the general population. This apparent discrepancy could be explained by the different pathological characteristics of breast cancers arising in women from the general population (and in *BRCA2* mutation carriers) compared to those of *BRCA1*-associated cancers. In fact, while the majority of *BRCA1* breast cancers have a basal-like phenotype — clinically identified as the absence of estrogen receptor (ER), progesterone receptor (PR) and HER2 protein expression (so-called “triple negative”) —

cancers of the general population and of *BRCA2* mutation carriers are often ER-positive and PR-positive. Moreover, *BRCA1* cancers, in contrast with sporadic ones, are high-grade and highly proliferative, have a higher frequency of *TP53* somatic mutations, and are associated with poor prognosis [reviewed in 15]. It has been shown that susceptibility alleles at different loci, including *FGFR2*, *TNRC9*, 5p12, 2q35 and 8q24, are associated with a significant increased risk for ER-positive breast cancers, but have no or a significantly smaller effect on ER-negative breast cancer risk [16]. This indicates that genetic factors can be differentially associated with the risk for specific breast cancer subtypes. Consistently, a few single nucleotide polymorphisms (SNPs) identified in the general population as breast cancer low-penetrance alleles have been shown to increase breast cancer risk in *BRCA2*, but not *BRCA1* mutation carriers [8,9].

To our knowledge, this is the first report of a breast cancer associated allele with opposite effect in the general population compared to *BRCA1* mutation carriers. Further analyses are necessary to confirm the association of the rs3834129 del allele with breast cancer ER status and to verify if this association, that we observed in Italian at risk women, is present also in other populations. It has been shown that the del allele destroys a binding element in the *CASP8* promoter for the stimulatory protein 1 (Sp1) and causes a reduced expression of the caspase-8 protein that decreases apoptosis of antitumor T lymphocytes [1]. Consequently, it was postulated that this could affect antitumor immune response and ultimately decrease cancer susceptibility. On the other hand, *BRCA1*-associated breast cancers show an increased proliferation activity, as measured by mitotic count, significantly higher than that observed in sporadic cancers and in cancers from *BRCA2* mutation carriers [17]. Thus, it might be speculated that in individuals who are particularly prone to develop highly replicating tumors a reduced apoptosis response leads to a less efficient mechanism of control of cancer progression and to increased risk.

In conclusion, our study suggested that rs3834129 is a risk modifier in *BRCA1* mutation carriers. In particular, the del allele is associated with an

increased breast cancer risk under a dominant model. Moreover, the risk increase appeared to be higher in carrier of mutations causing haplo-insufficiency.

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Tables

TABLE 1. List of centers participating to CONSIT TEAM with numbers of *BRCA1* and *BRCA2* mutation carriers contributed. Mutation analysis in *BRCA1* and *BRCA2* was done at the center at which the samples were recruited with the exception of samples from Istituto Nazionale Tumori and European Institute of Oncology that were tested at Consortium for Genomic Technologies (Cogentech) Milan.

Center	<i>BRCA1</i> mutation carriers	<i>BRCA2</i> mutation carriers	Total of carriers (%)
Fondazione IRCCS Istituto Nazionale dei Tumori, Milano	349	152	501 (40.4)
Dip. di Genetica, Biologia e Biochimica, Università di Torino	100	69	169 (13.6)
Istituto Europeo di Oncologia, Milano	95	73	168 (13.5)
Centro Riferimento Oncologico, Aviano	52	66	118 (9.5)
Dip. di Medicina Sperimentale, Sapienza Università di Roma	61	44	105 (8.5)
Sezione di Genetica Medica, Università di Firenze	59	30	89 (7.2)
Istituto Nazionale per la Ricerca sul Cancro, Genova	37	24	61 (4.9)
Laboratorio di Immunologia, Istituto Nazionale Tumori, Roma	14	16	30 (2.4)
All	767	474	1,241 (100)

TABLE 2. Genotype frequencies of rs3834129 by *BRCA* mutation and disease status, and hazard ratio estimates in all carriers of *BRCA* mutations.

<i>BRCA</i> group	Genotype	Unaffected (%)	Affected (%)	HR ^a	95% CI ^b	<i>P</i> -value
<i>BRCA1</i> and <i>BRCA2</i> (N=1,207)	nor/nor	177 (34.8)	208 (29.8)	1.00		
	nor/del	224 (44.1)	346 (49.5)	1.40	1.06–1.85	0.018
	del/del	107 (21.1)	145 (20.7)	1.26	0.90–1.77	0.176
	del vs nor (per allele)			1.15	0.96–1.38	0.121
	Dominant (del)			1.35	1.04–1.76	0.023
<i>BRCA1</i> (N = 740)	nor/nor	125 (35.5)	105 (27.1)	1.00		
	nor/del	152 (43.2)	193 (49.7)	1.56	1.16–2.13	0.003
	del/del	75 (21.3)	90 (23.2)	1.42	1.00–2.03	0.052
	del vs nor (per allele)			1.23	1.02–1.49	0.033
	Dominant (del)			1.52	1.14–2.02	0.004
<i>BRCA2</i> (N = 467)	nor/nor	52 (33.3)	103 (33.1)	1.00		
	nor/del	72 (46.2)	153 (49.2)	1.09	0.66–1.78	0.746
	del/del	32 (20.5)	55 (17.7)	0.80	0.42–1.55	0.516
	del vs nor (per allele)			0.92	0.66–1.29	0.619
	Dominant (del)			1.00	0.62–1.60	0.988

^aHR, hazard ratio (adjusted for center and year of birth); ^bCI, confidence interval
nor/nor, common homozygotes; nor/del, heterozygotes; del/del, rare homozygotes

TABLE 3. Genotype frequencies of rs3834129 by *BRCA* mutation and disease status, and hazard ratio estimates in carriers of *BRCA* loss-of-function (class 1) mutations.

<i>BRCA</i> group	Genotype	Unaffected (%)	Affected (%)	HR ^a	95% CI ^b	<i>P</i> -value
<i>BRCA1</i> and <i>BRCA2</i> (N=920)	nor/nor	143 (36.8)	156 (29.4)	1.00		
	nor/del	165 (42.4)	265 (49.9)	1.56	1.12–2.15	0.008
	del/del	81 (20.8)	110 (20.7)	1.29	0.87–1.93	0.208
	del vs nor (per allele)			1.18	0.95–1.46	0.138
	Dominant (del)			1.46	1.08–1.99	0.015
<i>BRCA1</i> (N = 504)	nor/nor	94 (38.4)	69 (26.6)	1.00		
	nor/del	100 (40.8)	128 (49.4)	1.83	1.27–2.64	0.001
	del/del	51 (20.8)	62 (23.9)	1.60	1.03–2.48	0.035
	del vs nor (per allele)			1.33	1.05–1.69	0.019
	Dominant (del)			1.74	1.24–2.46	0.002
<i>BRCA2</i> (N = 416)	nor/nor	49 (34.0)	87 (32.0)	1.00		
	nor/del	65 (45.1)	137 (50.4)	1.24	0.73–2.10	0.419
	del/del	30 (20.8)	48 (17.6)	0.79	0.40–1.57	0.506
	del vs nor (per allele)			0.92	0.65–1.32	0.662
	Dominant (del)			1.09	0.66–1.80	0.738

^aHR, hazard ratio (adjusted for center and year of birth); ^bCI, confidence interval
nor/nor, common homozygotes; nor/del, heterozygotes; del/del, rare homozygotes