Disease family history and modification of breast cancer risk in common *BRCA2* variants

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Abstract. A number of *BRCA1* and *BRCA2* polymorphisms have been extensively studied in order to test their association with breast cancer risk. Subsequently, discordant results were reported. In the present study, the genotypes of one *BRCA1* (Q356R) and three *BRCA2* (203G>A, N372H, IVS21-66T>C) common variants were evaluated in a series of 252 breast cancer patients, 155 age-matched controls and analysed in relation to family history (low- or high-risk) and *BRCA1/2* mutation status. A complete analysis of the *BRCA1/2* coding regions was performed on the 217 women from high-risk families and 44 *BRCA1/2* mutation carriers were identifed. According to a dominant inheritance model, the *BRCA2* IVS21-66T>C variant showed a 1.79-fold (95% CI, 1.16-2.78; P=0.009) increased breast cancer risk for the overall series. The *BRCA2* N372H polymorphism was associated with a 2.29-fold (95% CI, 1.16-4.49; P=0.016) increased risk in the subgroup of high-risk families with no *BRCA1/2* mutations. Conversely, the *BRCA1* Q356R and *BRCA2* 203G>A polymorphisms did not show any significant associations with breast cancer risk. In conclusion, the analysis of some *BRCA2* variants could help to identify women at a higher risk of developing breast cancer who could be candidates for chemoprevention protocols.

Introduction

Germline mutations in *BRCA1* and *BRCA2* are the highest predisposing factors for hereditary breast and ovarian cancer (HBOC). Many sequence variants retain uncertain clinical relevance and their classification into high- or low-risk categories remains a relevant problem in clinical genetics. However, important information can be gained through epidemiological observations such as familial disease co-segregation and the extent of family disease history, amino acid conservation studies and functional assays.

A large number of unclassified *BRCA1/2* variants have been tested for their contribution to breast cancer risk (1,2). At least six *BRCA1* polymorphisms, P871L (*1186A*>C), IVS11-141C>A (*4209*-141C>*A*), K1183R (*3668A*>G), S1436S (*4427C*>T), S1613G (*4956A*>G) and IVS18+66A>G (*5272*+66A>*G*), have been reported to show close pairwise linkage disequilibrium, defining two major haplotype blocks. However, no significant differences in their distribution have been found between breast cancer patients and healthy populations (3,4). The widely studied *BRCA1* Q356R (*1186A*>G) variant has not been found to be in linkage disequilibrium with any of the polymorphisms cited above (3). Indeed, the R356 allele has been reported to have either a putative protective effect (4) or no association with risk whatsoever (5,6). Moreover, a large haplotype-based case-control study of 28 *BRCA1* polymorphisms did not report any associations with breast cancer risk for any of the variants tested (7).

An analysis of the common *BRCA2* variants in sporadic breast cancers performed by the same authors found modest associations for homozygous carriers in three blocks of co-segregating haplotypes (8). A borderline association of 203G>A (5'-UTR -26G>*A*) with cancer risk has been reported in sporadic breast cancer patients (9), however, it was not confirmed in a subgroup of *BRCA1* mutation carriers (10). A 1.3-1.5-fold breast cancer risk has also been reported for the *BRCA2* N372H (*1342A*>C) variant in carriers of the HH genotype (9,11), although these results have not been confirmed in larger population studies (10,12-14).

In the present study, we analyzed the influence of *BRCA1/2* polymorphisms on breast cancer risk in a case series retrospectively selected from genetic counseling programmes.

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In particular, we examined one *BRCA1* (Q356R) and three *BRCA2* (203G>A, N372H, IVS21-66T>C) polymorphisms that were frequent in our population and which have been highlighted by other authors who also analysed their possible association with family disease history in relation to *BRCA1/2* mutation status.

Materials and methods

Patients. Breast cancer patients and healthy age-matched females were recruited from breast screening programmes at the Cancer Prevention Units of Morgagni-Pierantoni Hospital (Forlì) and Santa Maria delle Croci Hospital (Ravenna), and from the National Cancer Institute (Bari, Italy). All women completed a questionnaire on family disease history and gave written informed consent for *BRCA1/2* testing in accordance with an in-house protocol for the genetic study and early diagnosis of hereditary breast and ovarian cancer (17). Families were classified as 'high-risk' when three or more cases of breast or ovarian cancer were documented, or when one of these tumors occurred in a family member under 36 years of age, or in the controlateral breast, or in a male member of the family. In all other situations, families were classified as 'low-risk'. Only one case or control was used from each single family to avoid study bias. Peripheral blood was analysed from 252 women with breast cancer (192 from high-risk and 60 from low-risk families) and 155 age-matched controls (95 from high-risk, and 60 from low-risk families). The study was approved by the Local Ethics Committee of each centre taking part (17).

BRCA1/2 genotyping. Total genomic DNA was extracted from peripheral blood using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) and genotyped for the *BRCA1* Q356R (*1186A>G*) and *BRCA2* 203G>A (5'-UTR *-26G>A*), N372H (*1342A>C*) and IVS21-66T>C (*8983-66T>C*) polymorphisms by PCR amplification and sequencing using the following primers: Q356R: B1.ex11F1 (forward) 5'-CCTCCAAGGTG TATGAAGTA-3' and B1.ex11R1 (reverse) 5'-GAGGTAGA TGAATATTCTG-3'; 203G>A: B2.ex2F (forward) 5'-CAGC GCTTCTGAGTTTTACCT-3' and B2.ex2R (reverse) 5'-GCA ACACTGTGACGTACTGG-3'; N372H: B2.ex10F1 (forward) 5'-CAGCGCTTCTGAGTTTTACCT-3' and B2.ex10R1 (reverse) 5'-TCTTGCAGTAAAGCAGGCAA-3'; IVS21-66T>C: B2.ex22F (forward) 5'-GGGCATTAGTAG TGGATTTTGC-3' and B2.ex22R (reverse) 5'-GCAAAAT CCACTACTAATGCCC-3'.

Complete *BRCA1/2* mutation analysis was carried out on 217 women from high-risk families who met our stringent selection criteria. The coding regions of *BRCA1* (exons 2-24) and *BRCA2* (exons 2-27), including flanking introns, were amplified by standard PCR (all primer sequences and PCR reaction conditions are available upon request from the authors). Sequencing of PCR products was performed using the Big dye terminator cycle sequencing kit on an Applied Biosystems 3100 Avant genetic analyzer (Applied Biosystems, Foster City, CA).

Statistical methods. Observed and expected genotype frequencies were assessed under the assumption of the Hardy-Weinberg equilibrium (HWE) (18). A two-sided Fisher's exact test was performed to evaluate differences in the genotype distributions. Age-adjusted odds ratios (ORs) and their 95% confidence intervals (CI) were analysed by unconditional logistic regression. Variability between age classes (10-year age intervals: >30, 31-40, 41-50, 51-60, >60) was analysed by a χ^2 test (4 df). All analyses were performed using the SAS Statistical Analysis System Software (version 8; SAS Institute, Cary, NC). Statistical significance for all tests was taken as $P<0.05$.

Results

In the overall series, no significant differences in genotype distributions between patients and controls were observed for the *BRCA1* Q356R, the *BRCA2* 203G>A, or the N372H variants (Table I). In contrast, for IVS21-66T>C, significantly higher frequencies of the TC (P=0.02) and CC genotypes (P=0.04) and -66C allele (P=0.02) were observed in patients than in healthy women. Moreover, no significant differences between low- and high-risk families were observed for any of the four variants tested (results not shown). Conversely, a breakdown analysis performed within high-risk families showed, for the N372H variant, a statistically higher frequency of the NH genotype (P=0.004) and H372 allele (P=0.045) in patients without *BRCA1/2* mutations (Table I).

An analysis of the breast cancer risk associated with the four variants, according to different inheritance models, was carried out in the overall series and in the subgroup of women from high-risk families without *BRCA1/2* mutations (Table II). In the overall series, using a dominant model, IVS21-66T>C was associated with a 1.79-fold increased risk of breast cancer (95% CI, 1.16-2.78; P=0.009) and was not dependent on family risk or *BRCA1/2* mutation status. For N372H, no association was found between allelic distributions and breast cancer risk in the overall series for any of the inheritance models used. Conversely, this variant was associated with an increased risk of 2.29-fold (95% CI, 1.16-4.49; P=0.016) in *BRCA1/2* mutation non-carriers, according to dominant inheritance.

Discussion

Germline mutations in the *BRCA* pathway highly predispose an individual to breast and/or ovarian cancer (16). A number of common *BRCA1/2* variants have been investigated in order to ascertain their relationship with breast cancer risk. Although numerous studies have been performed on different unselected breast and ovarian cancer populations (4,9,12,13), on subgroups of high-risk patients defined on the basis of family history (3,11) and *BRCA1/2* mutation status (10,11), results are not in complete agreement.

In the present case-control study, we investigated the association between four common *BRCA1/2* variants and breast cancer risk in relation to family disease history and *BRCA1/2* mutation status. For the *BRCA1* Q356R and *BRCA2* 203A variants, rare allele frequencies were similar to those reported elsewhere, and we confirmed the lack of association with breast cancer risk (3,4,9,10,19).

With regard to the *BRCA2* IVS21-66T>C intronic polymorphism, our results show, for the first time, evidence of an apparent association with breast cancer risk that is

Table I. *BRCA1/2* common variant genotype distributions in breast cancer cases and controls in the overall series and high-risk non-carrier subgroup.

| | Overall series | | | | | | High-risk *BRCA1/2* non-carriers | | | | | |
| | Cases (252) | | Controls (155) | | Fisher's Test | | Cases (90) | | Controls (69) | | Fisher's Test | |
	n	%	n	%	χ^2	P	n	%	n	%	χ^2	P
BRCA1 Q356R												
QQ	210	83	46	77	Ref.		78	87	54	78	Ref.	
QR	40	16	14	21	1.71	n.s.	10	11	14	20	1.85	n.s.
RR	2	1	0	2	0.40	n.s.	2	2	1	2	0.001	n.s.
Q356 allele		0.09		0.12	2.70	n.s.		0.08		0.12	0.92	n.s.
BRCA2 203G>A												
GG	128	51	38	51	Ref.		45	50	35	51	Ref.	
GA	108	43	19	43	0.001	n.s.	39	43	30	43	0.001	n.s.
AA	16	6	3	6	0.001	n.s.	6	7	4	6	0.001	n.s.
203A allele		0.28		0.27	0.001	n.s.		0.28		0.27	0.001	n.s.
BRCA2 IVS21-66T>C												
TT	62	24	16	37	Ref.		25	28	26	38	Ref.	
TC	125	50	32	43	4.91	0.02	42	47	30	43	0.70	n.s.
CC	65	26	12	20	4.31	0.04	23	25	13	19	1.33	n.s.
-66C allele		0.51		0.42	5.43	0.02		0.49		0.41	1.85	n.s.
BRCA2 N372H												
NN	127	50	34	58	Ref.		39	43	45	65	Ref.	
NH	111	44	25	35	2.52	n.s.	43	48	17	25	8.09	0.004
HH	14	6	1	7	0.08	n.s.	8	9	7	10	0.04	n.s.
H372 allele		0.28		0.25	0.46	n.s.		0.33		0.22	3.60	0.045

n.s., not significant.

Table II. Breast cancer risk associated with the *BRCA1/2* common variant genotypes according to different inheritance models in the overall series and high-risk *BRCA1/2* non-carrier subgroup.

| | Overall series | | High-risk *BRCA1/2* non-carriers | |
	Adj OR[a] (95% CI)	P	Adj OR (95% CI)	P
BRCA1 Q356R				
Dominant: (QR + RR)/QQ	0.67 (0.40-1.11)	0.117	0.40 (0.16-0.98)	0.046
Co-dominant: RR/QQ	0.29 (0.05-1.79)	0.182	0.44 (0.04-5.29)	0.514
R356 allele multiplicative risk	0.66 (0.41-1.04)	0.076	0.46 (0.20-1.02)	0.055
BRCA2 203G>A				
Dominant: (GA + AA)/GG	1.00 (0.67-1.50)	1.000	0.83 (0.43-1.63)	0.594
Co-dominant: AA/GG	1.02 (0.42-2.47)	0.966	0.63 (0.15-2.68)	0.536
203A allele multiplicative risk	1.01 (0.73-1.39)	0.966	0.84 (0.50-1.42)	0.525
BRCA2 IVS21-66T>C				
Dominant: (TC + CC)/TT	1.79 (1.16-2.78)	0.009	1.43 (0.71-2.88)	0.320
Co-dominant: CC/TT	2.05 (1.15-3.65)	0.014	2.13 (0.81-5.59)	0.125
-66C allele multiplicative risk	1.45 (1.09-1.94)	0.011	1.43 (0.89-2.29)	0.134
BRCA2 N372H				
Dominant: (NH + HH)/NN	1.25 (0.83-1.89)	0.277	2.29 (1.16-4.49)	0.016
Co-dominant: HH/NN	0.82 (0.36-1.86)	0.633	1.36 (0.43-4.28)	0.596
H372 allele multiplicative risk	1.10 (0.79-1.52)	0.578	1.65 (0.97-2.80)	0.066

[a]ORs were adjusted for age at diagnosis for cases and by age at invitation to the study for controls.

independent of family disease history and *BRCA1/2* mutation status. A large number of intronic variants are thought to be too distant from the protein coding regions to affect normal function. However, the tightly linked intronic *ATM* IVS22-77T>C and IVS48+238C>G polymorphisms, in the homozygote state, have been associated with an increased breast cancer risk (20). Non-coding regions may contain sequences, particularly in close proximity to open reading frames, which are important for post-transcriptional modification or regulatory functions involved in gene expression (21). Indeed, several intronic splice donor/acceptor consensus site variants have been shown to affect heteronuclear RNA (hnRNA) processing, leading to the loss of functional *BRCA* proteins (22). We submitted the *BRCA2* exon 22 sequence and flanking intronic regions to SpliceSiteFinder (http://www.genet.sickkids.on.ca/~ali.splicesitefinder.html). The algorithm used to calculate the donor/acceptor sequence scores is based on the model by Shapiro and Senapathy (23). However, the base pair change variant at position 8983-66T>C (IVS21-66T>C) was not predicted to disrupt the exon 22 donor site. Although our preliminary data showing an association between IVS21-66T>C and breast cancer risk is an interesting observation, its influence on cancer phenotypes is uncertain and needs to be evaluated further through functional studies.

In contrast to IVS21-66T>C, the N372H variant was not linked to breast cancer risk in the overall series, however it appeared to be associated with the subgroup of women from high-risk families not carrying any *BRCA1/2* mutations (P=0.016). Other authors have reported the HH genotype to be associated with a modest increase in breast cancer risk (9,11), but pooled analyses from large collaborative studies have not confirmed these initial findings (10,14). These discordant data could, to a great extent, be due to the characteristics of the case series selected in terms of, for example, family risk classification and age of disease onset (15,16). Another possible explanation is that, since these variants usually have a very low penetrance, their effect on cancer risk could easily be masked by other genetic factors. Although ours is a relatively small series compared to those considered in other studies, we can hypothesize that in the absence of deleterious *BRCA1/2* mutations, common *BRCA1/2* variants such as N372H and other low-penetrance genes, may have a cumulative effect on breast cancer risk in families with a strong disease history.

In conclusion, our preliminary results seem to suggest that, within high-risk families, analysis of the N372H variant could identify a subgroup of women with a more than 2-fold higher risk of developing breast cancer, which is not dependent on the presence of *BRCA1/2* mutations. Our study also shows the need to re-assess the methods of patient selection, taking into account genetic factors other than deleterious *BRCA1/2* mutations.

Acknowledgements

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