Short Communication

Common *BRCA2* Variants and Modification of Breast and Ovarian Cancer Risk in *BRCA1* Mutation Carriers

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**Abstract**

The HH genotype of the nonconservative amino acid substitution polymorphism N372H in the *BRCA2* gene was reported to be associated with a 1.3- to 1.5-fold increase in risk of both breast and ovarian cancer. As these studies concerned sporadic cancer cases, we investigated whether N372H and another common variant located in the 5′-untranslated region (203G > A) of the *BRCA2* gene modify breast or ovarian cancer risk in *BRCA1* mutation carriers. The study includes 778 women carrying a *BRCA1* germ-line mutation belonging to 403 families. The two *BRCA2* variants were analyzed by the TaqMan allelic discrimination technique. Genotypes were analyzed by disease-free survival analysis using a Cox proportional hazards model. We found no evidence of a significant modification of breast cancer penetrance in *BRCA1* mutation carriers by either polymorphism. In respect of ovarian cancer risk, we also saw no effect with the N372H variant but we did observe a borderline association with the 5′-untranslated region 203A allele (hazard ratio, 1.43; CI, 1.01-2.00). In contrast to the result of Healey et al. on newborn females and adult female controls, we found no departure from Hardy-Weinberg equilibrium in the distribution of N372H alleles for our female *BRCA1* carriers. We conclude that if these single-nucleotide polymorphisms do modify the risk of cancer in *BRCA1* mutation carriers, their effects are not significantly larger than that of N372H previously observed in the general population. (Cancer Epidemiol Biomarkers Prev 2005;14(1):265–7)

**Introduction**

Germ-line mutations in the *BRCA1* and *BRCA2* genes strongly predispose heterozygous carriers to breast and ovarian cancer. Their penetrance is highly variable both between and within *BRCA1* and *BRCA2* mutation carrier families. Such variations suggest that cancer risk associated with *BRCA* mutations can be modified by lifestyle, environmental, and/or genetic factors (see ref. 1 for a review). Both *BRCA1* and *BRCA2* are involved in detection and repair of DNA double-strand breaks in response to DNA damage (2). The effectiveness of these repair processes in *BRCA1* mutation carriers could be compromised by the presence of polymorphisms in the wild-type *BRCA1* allele found to modify ovarian cancer risk in *BRCA1* carriers (3). We hypothesize that this may also be the case for *BRCA2* acting in these same pathways. Several studies have analyzed the effect of the two most common *BRCA2* variants on the risk of breast and ovarian cancer in the general population: a polymorphism in the 5′-untranslated region (203G > A, rare allele frequency 0.28) and one in the coding region (1342A > C/N372H, rare allele frequency 0.26; refs. 4-8). N372H is the unique *BRCA2* variant that results in an amino acid change and has a rare allele frequency greater than 10%. It is not known if the 203G > A and N372H variants have any functional consequences, although for N372H the substitution of a basic amino acid (asparagine) by a small neutral amino acid (histidine) may be expected to affect the structure and function of *BRCA2*. N372H lies within a region of *BRCA2* (residues 290-453) that has been shown to interact with the histone acetyltransferase P/CAF to transcriptionally activate other genes (9).

The HH genotype of N372H in the *BRCA2* gene was reported to be associated with a 1.3-fold increased risk of breast cancer penetrance in *BRCA1* mutation carriers by either polymorphism. In respect of ovarian cancer risk, we also saw no effect with the N372H variant but we did observe a borderline association with the 5′-untranslated region 203A allele (hazard ratio, 1.43; CI, 1.01-2.00). In contrast to the result of Healey et al. on newborn females and adult female controls, we found no departure from Hardy-Weinberg equilibrium in the distribution of N372H alleles for our female *BRCA1* carriers. We conclude that if these single-nucleotide polymorphisms do modify the risk of cancer in *BRCA1* mutation carriers, their effects are not significantly larger than that of N372H previously observed in the general population. (Cancer Epidemiol Biomarkers Prev 2005;14(1):265–7)
breast cancer from a large combined analysis of five case-control studies of Northern European women (3,459 cases and 3,014 controls; ref. 4). A similar magnitude of increased risk was found for the 372HH genotype in case-control study of breast cancer in Australian women (5) and in kin-cohort study of breast cancer in the relatives of breast cancer patients in the cohort of the U.S. radiologic technologists (6). A similar effect of the 372HH genotype was reported on the risk of epithelial ovarian cancer in British and Australian women (7). However, a Japanese study involving 149 cases of sporadic breast cancer and 154 controls did not show any effect of this variant, although the power of this study to detect such associations is low because of the small numbers included (8). As these studies concerned sporadic breast or ovarian cancer cases, we sought to examine whether these BRCA2 variants modify breast and ovarian cancer risk in BRCA1 mutation carriers. N372H and 203G > A were genotyped in our cohort of 778 BRCA1 mutation carriers. Although we had insufficient power to detect the slightly elevated risk for homozygous HH subjects (see statistical analysis part of materials and methods), we undertook this study suspecting that the risk associated with the BRCA2 372HH genotype might be stronger in a population of BRCA1 carriers, in whom the BRCA pathway is already compromised, as compared with that in the general population.

Materials and Methods

Subjects. All cases in this study were included after giving informed consent. The study includes 778 women belonging to 403 families recruited and identified as carriers of BRCA1 germ-line mutations in the frame work of research and counseling programs on hereditary breast and ovarian cancer in France, United States, Canada, and Greece. Carriers were selected from families with a strong familial history of breast-ovarian cancer at first for genetic linkage studies and later in the context of diagnostic screening for BRCA1/2 mutations. Several common mutation-screening techniques were used, including fluorescent sequencing, heteroduplex analysis, and denaturing high-performance liquid chromatography, to cover all the BRCA1 coding and flanking regions. Of these 778 women, 113 have been diagnosed with ovarian cancer, 407 with breast cancer, 78 with breast-ovarian cancer, and 180 were cancer-free at the time of last follow-up. The mean age of diagnosis was 40 years for a first breast cancer (20-76 years) and 47 years for a first ovarian cancer (22-75 years). Information available on study subjects included clinical characteristics, date of birth, age at last follow-up exam or age at death, age at diagnosis of breast and/or ovarian cancer, age at prophylactic surgery (oophorectomy or mastectomy), and parity.

BRCA2 Genotyping. Two polymorphisms in the BRCA2 gene, 203G > A and 1342A > C/N372H, were typed by the TaqMan allelic discrimination technique (Applied Biosystems, ABI, Foster City, CA). The primers and the TaqMan probes were selected with the Primer Express and Oligo Design Software version 2.0.0 (Applied Biosystems). We carried out a standard PCR on lymphocyte DNA (10 ng) using forward primers 5'-CTCAGTCACATAAAAGGGAAT for 203G > A and 5'-CCACATTGGAAGCTACATG for N372H plus reverse primers 5'-ACACTGTGACGTACTGGGTTTT for 203G > A and 5'-TGTAAGCTGAAATCCTGCC for N372H (7.5 µmol each). Products were amplified using 1 unit of Taq DNA polymerase (Invitrogen, Carlsbad, CA) in 30 µL volumes. The PCR conditions were 5 minutes at 94°C, 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds followed by a 7-minute extension at 72°C in a Perkin-Elmer Cetus model 9600 thermal cycler. This PCR gives two amplicons, 516 bp for N372H and 256 bp for 203G > A.

TaqMan PCRs (10 µL in 96-well plates) were done using the initial PCR reactions as template (0.5 µL of a 10× dilution), 1× TaqMan universal PCR master mix, forward (5'-GTAAGTG CAATTGGCTCTCTGTTT for 203G > A and 5'-TGGAAC CAAATGATACCTGATCCAT for N372H) and reverse (5' AAAATTTGGCCTTCTCTTTGGA for 203G > A and 5'- CAACTCCTTGAGATTGTGACCT for N372H) nested primers (900 nmol/L). 5' FAM/3' TAMRA labeled probe (ITTCAGACTTTAATTTACCAAACTTTGGA for 203A and ATTCAGATTGCAAATGAGCCCTTTG for N372H/1342A) and 5'TET/3' Tamra labeled probe (TTTCAAATTATTTCAACAGTTGGAG for 203G and TCAAATGTCACACTGACATGACAGC for N372H/ 1342C; 200 nmol/L, MWG Biotech AG, Ebersberg, Germany). Amplification conditions were 95°C for 10 minutes followed by 35 cycles of 92°C for 15 seconds and 60°C for 1 minute. Genotypes were assigned using the Allelic Discrimination Sequence Detection Software of the ABI PRISM 7900HT Sequence Detector. After the first 96-well plate was analyzed, the corresponding amplicons of nine individuals carrying the three possible genotypes for each variant were sequenced (ABI 3100) to confirm the genotyping calls and provide genotype controls. These controls plus “no template” controls were then included in each subsequent 96-well plate.

Statistical Analysis. The data were analyzed by disease-free survival analysis using a Cox proportional hazards model with the STATA statistical analysis package (STATA Corporation, College Station, TX). For the estimation of cancer risk, the women were followed until the diagnosis of breast or ovarian cancer. Patients were censored at age of first malignancy (if they had multiple breast or ovarian tumors), age of bilateral prophylactic mastectomy (for the analysis of breast cancer risk) or oophorectomy, other cancer diagnosis, last follow-up exam, or death. Robust variance analysis based on family membership was undertaken to account for the fact that a number of the women studied were related to one another. The estimation of linkage disequilibrium between N372H and 203G > A polymorphisms was performed by the program 2LD. Tests of Hardy-Weinberg equilibrium used standard χ2 methods. To evaluate the power of the study to detect an effect of given magnitude, simulation of carrier status for each individual in our sample was done using an S-plus program (v. 3.4 MathSoft International19), keeping the age at censure and disease end points fixed. For each value of the hazard ratio (HR) associated with carrier status, 2000 replicates of the data were generated and evaluated. The estimated power was the proportion of replicates in which the null hypothesis of HR = 1 was rejected (assuming α = 0.05). Given the size and composition of our sample as well as the frequency of N372H polymorphism, there is 80% power to detect a HR = 1.62 effect of the HH genotype on breast cancer risk.

Results

Rare allele frequencies of the BRCA2 203G > A and N372H polymorphisms in unrelated individuals of our series of BRCA1 carriers were found to be similar (0.25 and 0.29, respectively) to those reported in population controls by Healey et al. (4). These authors proposed an effect of N372H on fetal survival in a sex-dependent manner as newborn females showed an excess of heterozygotes and reverse of homozygotes, whereas the opposite was observed in newborn males. Auranen et al. (7) reported a slight but nonsignificant

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19 AC Antoniou, personal communication.
Table 1. Breast and ovarian cancer risk in BRCA1 mutation carriers in relation to BRCA2 polymorphisms

<table>
<thead>
<tr>
<th>BRCA2 sequence variants</th>
<th>Breast cancer</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95% CI]</td>
<td>HR [95% CI]</td>
</tr>
<tr>
<td>203G &gt; A/5'-untranslated region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(AG + AA)/GG</td>
<td>0.92 [0.75-1.12]</td>
<td>1.43 [1.01-2.00]</td>
</tr>
<tr>
<td>AA/CG</td>
<td>0.85 [0.57-1.27]</td>
<td>1.32 [0.68-2.54]</td>
</tr>
<tr>
<td>1342A &gt; C/N372H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH + HH)/NN</td>
<td>1.02 [0.84-1.23]</td>
<td>0.81 [0.57-1.16]</td>
</tr>
<tr>
<td>HH/NN</td>
<td>0.90 [0.63-1.28]</td>
<td>0.74 [0.40-1.35]</td>
</tr>
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</table>

departure from Hardy-Weinberg equilibrium due to an excess of heterozygotes in controls. They also found a marginal difference in genotype distributions between cases and controls due to a higher prevalence of HH homozygotes among ovarian cancer cases (odds ratio, 1.36; 95% CI, 1.04-1.77). In contrast to these results, we found no deviation from Hardy-Weinberg equilibrium in the distribution of N372H alleles for our female BRCA1 carriers. The estimation of linkage disequilibrium indicates that the two rare alleles, 203A and 372H, are in strong negative linkage disequilibrium ($D' = -0.892, P = 0.000$), and thus for the most part are present on different haplotypes.

Our study shows no evidence that either 203G > A or N372H affects the risk of breast cancer in BRCA1 mutation carriers (Table 1). However, we observed a borderline significant association of the 5'-untranslated region variant with risk of ovarian cancer (HR, 1.43; CI, 1.01-2.00). Adjustment for year of birth and parity, known modifiers of breast and ovarian cancer risk, did not significantly alter the HR estimates.

Discussion

Our aim for this study was to examine whether the two most common variants in the BRCA2 gene (203G > A and N372H) modify breast and ovarian cancer risk in female BRCA1 mutation carriers. We found no evidence to suggest that either polymorphism modified the risk of breast cancer, or that N372H had any effect on ovarian cancer risk. For the 5'-untranslated region 203A allele we observed an association with a small increase in ovarian cancer risk (HR, 1.43; CI, 1.01-2.00). Due to the small sample size of ovarian cancers (N = 113) and the marginal significance, we feel that this tentatively positive result requires testing in a larger sample series. Healey et al. (4) reported a reduction in the risk of breast cancer in the general population for the 203A allele in a preliminary study, but they did not confirm this result in a larger study.

These authors as well as several other groups have shown an elevation of risk for breast cancer (4-6) and ovarian cancer (7) of about 1.3 to 1.5 for the 372H homozygous carriers in the general population. Our study lacks the statistical power to detect an equivalent modifying effect of the 372H genotype. We have 80% power to detect a HR = 1.62 effect of the HH genotype on breast cancer risk in our sample. Our hypothesis at the outset of the study was that as the BRCA pathway is already disturbed in a population of BRCA1 carriers, the 372HH genotype might be a stronger modifier of cancer risk in BRCA1 carriers compared with that in the general population.

Thus, studies with a larger number of BRCA1 mutation carriers need to be done to more definitively evaluate the 203G > A and N372H variants of BRCA2 as modifiers of BRCA1-associated cancer risk. Most likely this would require a large collaborative study as few research groups individually possess the necessary sample size. Indeed, we have needed the cooperation of 17 research groups from four countries to obtain a set of nearly 800 BRCA1 mutation carriers. However, our results lead us to conclude that if these single-nucleotide polymorphisms do modify the risk of cancer in BRCA1 mutation carriers, their effects are not significantly larger than those observed in the general population.

The functional consequences of these variants remain to be established. N372H is the only frequent polymorphism in the BRCA2 gene resulting in an amino acid change and this nonconservative substitution may be expected to affect the structure and function of BRCA2. As N372 is in the N-terminal region of BRCA2 that interacts with the histone acetyltransferase P/CAF to activate transcription (9), it would be interesting to examine whether 372H affects this interaction. We are currently analyzing the effect of polymorphisms in other genes involved in DNA repair. Discovery of genetic modifiers of cancer risks associated with mutated BRCA1 and BRCA2 should help refine individual risk estimates and aid in our understanding of the function of these genes. Many of these modifiers may themselves be moderate- to low-penetrance predisposing genes in the general population.

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References

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