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Association of the Variants \textit{CASP8} D302H and \textit{CASP10} V410I with Breast and Ovarian Cancer Risk in \textit{BRCA1} and \textit{BRCA2} Mutation Carriers

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Running Head: CASP8 D302H and CASP10 V410I in BRCA1/2 Mutation Carriers
Abstract

Background: The genes caspase-8 (CASP8) and caspase-10 (CASP10) functionally cooperate and play a key role in the initiation of apoptosis. Suppression of apoptosis is one of the major mechanisms underlying the origin and progression of cancer. Previous case-control studies have indicated that the polymorphisms CASP8 D302H and CASP10 V410I are associated with a reduced risk of breast cancer in the general population.

Methods: In order to evaluate whether the CASP8 D302H (CASP10 V410I) polymorphisms modify breast or ovarian cancer risk in BRCA1 and BRCA2 mutation carriers, we analyzed 7,353 (7,227) subjects of white European origin provided by 19 (18) study groups that participate in the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). A weighted cohort approach was used to estimate hazard ratios (HRs) and 95% confidence intervals (95%CIs).

Results: The minor allele of CASP8 D302H was significantly associated with a reduced risk of breast cancer (per allele HR 0.85, 95%CI 0.76-0.97, p-trend=0.011) and ovarian cancer (per allele HR 0.69, 95%CI 0.53-0.89, p-trend=0.004) for BRCA1 but not for BRCA2 mutation carriers. The CASP10 V410I polymorphism was not associated with breast or ovarian cancer risk for BRCA1 or BRCA2 mutation carriers.

Conclusions: CASP8 D302H decreases breast and ovarian cancer risk for BRCA1 mutation carriers but not for BRCA2 mutation carriers.

Impact: The combined application of these and other recently identified genetic risk modifiers could in the future allow better individual risk calculation and could aid in the individualized counselling and decision making with respect to preventive options in BRCA1 mutation carriers.
**Introduction**

Caspase-8 encoded by the *CASP8* gene (OMIM 601763; Chromosome 2q33) is an apical caspase involved in receptor-induced apoptosis. Caspase-10, encoded by the *CASP10* gene (OMIM 601762; Chromosome 2q33) adjacent to *CASP8* in the human genome, cooperates with caspase-8 in the transduction of death-receptor mediated apoptotic signals. In addition to deregulated cell proliferation, suppression of apoptosis is one of the major mechanisms underlying the origin and progression of cancer (1-3). Although occurring at low frequencies, inactivating mutations in *CASP8* as well as *CASP10* have been identified in different tumor types (4-6). In addition, several coding polymorphisms in selected apoptotic genes, including *CASP8* and *CASP10*, have been investigated with regard to their effect on cancer risk (7-16). Three independent studies found significant associations between the coding variant D302H in *CASP8* (rs1045485) and reduced sporadic breast cancer risk (7, 9, 13). In addition, the largest of these studies performed on 16,423 cases and 17,109 controls within the Breast Cancer Association Consortium, revealed no associations between the variant and age of onset, hormone receptor status, grade, stage or history of breast cancer in first-degree female relatives (13).

In case-control studies on patients selected for a family history of breast cancer, Frank et al. investigated the effect of the two coding polymorphisms *CASP8* D302H (rs1045485) and *CASP10* V410I (rs13010627) on breast cancer risk (9, 10). While there was no significant association between the *CASP8* 302H allele and breast cancer risk, carriers of the *CASP10* 410I allele were at a significantly reduced risk of breast cancer (odds ratio, OR=0.62, 95% confidence interval, 95%CI=0.43-0.88, \( p_{\text{trend}}=0.0039 \)). The breast cancer risk was found to be even lower for individuals who also carried the *CASP8* H302 allele (OR=0.35, \( p_{\text{trend}}=0.007 \)).
The associations between these variants and cancer risk for \textit{BRCA1} and \textit{BRCA2} mutation carriers have not been previously investigated. In the present paper we used data from the Consortium of Investigator of Modifiers of BRCA1/2 (CIMBA) to evaluate the associations the \textit{CASP8} D302H and \textit{CASP10} V410I variants and breast and ovarian cancer risk for \textit{BRCA1} and \textit{BRCA2} mutation carriers.
Materials and Methods

Study population. Female *BRCA1* and *BRCA2* mutation carriers over the age of 18 years were identified through 19 clinical and population-based research groups mainly from Europe, North America and Australia. This work was done within the framework of the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA). CIMBA is an international collaboration which was established in 2005 to conduct collaborative analyses of genetic polymorphisms as modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers using sample sizes large enough to provide sufficient power to detect even moderate effects (17). Recruitment of subjects was approved by institutional review boards or ethics committees at all sites. Eligibility was restricted to individuals who were carriers of clearly pathogenic mutations according to defined criteria (http://research.nhgri.nih.gov/rojects/bic/) (18). Phenotype data included year of birth, *BRCA* mutation description, ethnicity, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis, age at bilateral prophylactic mastectomy, and age at bilateral prophylactic oophorectomy.

Genotyping. Samples from seven centres were genotyped at the Queensland Institute of Medical Research for the *CASP8* and *CASP10* SNPs using the iPLEX technology. The remaining centres used TaqMan allelic discrimination assays (13, 19). The TaqMan PCR primers were 5’-ACCACGACCTTTGAAGAGCTT-3’ (forward) and 5’-GTGGTCCATGAGTGGTAGATTTTCA-3’ (reverse) for the *CASP8* analysis and 5’-GGCCTGCCAAGGTGAAGAG-3’ (forward) and 5’-GCCTGCTCAGGGTTCAGA-3’ (reverse) for the *CASP10* analysis. The probe for the *CASP8* C allele was FAM-5’-CCCCACCATGACTG-3’ and for the *CASP8* G allele was VIC-5’-CCCCACGATGACTG-3’. For the *CASP10* analysis the probe for the A allele was FAM-5’-CAGCCTTCCATATCC-3’ and for the G allele was VIC-5’-CAGCCTTCCGTATCC-3’. All of the rare *CASP8*
homozygotes (CC) were confirmed by sequencing using the following primers: 5’-GTGCTCTCCAGCTGTGGTC-3’ (forward) and 5’-CCAGTGAACTGACATGTCAGC-3’ (reverse).

The DKFZ samples were genotyped using PCR-based RFLP analyses. CASP8 D302H was genotyped using newly designed 5’-GCTTTGACCACGACCTTTGAAG-3’ (forward) and 5’-GTTACTGTGGTCCATGAGTTGGTAGAT-3’ (reverse) primers. The reaction was set up in 10 µl containing 25 ng genomic DNA, 1x PCR buffer (Qiagen, Hilden, Germany), 3.5 mM MgCl₂, 0.2 µM of each primer, 200 µM of each dNTP (Promega, Mannheim, Germany), and 0.4 U HotStarTaq DNA polymerase (Qiagen, Hilden, Germany). After an initial denaturation step for 15 min at 95°C, 35 cycles of PCR reactions consisting of 1 min. at 94°C, 1 min. at 62°C and 1 min. at 72°C were carried out, which were followed by a final extension step for 10 min at 72°C. PCR products were digested with 4 U Hsp92II (Promega GmbH, Mannheim, Germany) and separated on a 3.5% agarose gel containing ethidium bromide (Sigma-Aldrich, Steinheim, Germany) and scored by UV visualization. Sizes of the labelled fragments were 102 bp and 12 bp for the G allele and 52 bp, 50 bp and 12 bp for the C allele. CASP10 V410I was also genotyped with PCR-based RFLP analyses using newly designed 5’-GATCATGTCTCCTCAGAGCAGC-3’ (forward) and 5’-AAGTGGGTGCCTGCTCAG-3’ (reverse) primers. PCR reactions and conditions were as for the CASP8 D302H analysis, with the exception of using 2.5 mM MgCl₂ and amplifying by 35 cycles of 30 sec. at 94°C, 30 sec. at 56°C and 30 sec. at 72°C. PCR products were digested with 2.5 U BfuI (Fermentas, St. Leon, Germany), separated on a 2.5% agarose gel containing ethidium bromide (Sigma-Aldrich, Steinheim, Germany) and scored by UV visualization. Fragment sizes were 112 bp and 34 bp for the G allele and 146 bp for the A allele.

To ensure consistency in genotyping across studies, all genotyping centres had to adhere to the CIMBA genotyping quality control criteria: for each study site, a call rate of over 95%
after exclusion of samples that failed amplification of both SNPs, was required. At least 2% of the samples had to be included in duplicate and every plate had to be composed of no template controls and random mixture of affected and unaffected carriers. The concordance among duplicates had to be at least 98%. To further validate the accuracy of genotyping across centres, all centres were required to genotype 95 DNA samples from a standard test plate (Coriell Institute, Camden, New Jersey, USA) for the CASP8 D302H SNP. Details of the CIMBA quality control guidelines are publicly available (http://www.srl.cam.ac.uk/consortia/cimba/eligibility/eligibility.html).

**Patient selection:** Of 8,890 cases genotyped for CASP8 we excluded 218 cases because they failed genotyping quality control, 35 because they were ascertained by more than one study group, 71 because of insufficient documentation of pathogenic BRCA mutations, 17 because date of birth was not available, nine because of missing data on age at last observation or age under 18 years, 202 because of non-Caucasian origin and 985 because of significant deviation from Hardy-Weinberg-Equilibrium in two study groups. The remaining 7,353 cases were analysed as described below.

Of 7,842 cases genotyped for CASP10 we excluded 246 cases because they failed genotyping quality control, 35 because they were ascertained by more than one study group, 73 because of insufficient documentation of pathogenic BRCA mutations, 17 because date of birth was not available, 9 because of missing data on age at last observation or because they were under 18 years old and 235 because of non-caucasian origin. The remaining 7,227 cases were analysed as described below.

**Statistical analysis.** Deviations of observed from expected genotype frequencies under the Hardy-Weinberg equilibrium were assessed among unrelated carriers within each contributing
study group using an exact test. Study groups showing a significant deviations of $p<0.005$ were excluded from further analysis. As a result, data from two study groups were excluded from the $\text{CASP8 D302H}$ analysis.

The associations between $\text{CASP8 D302H}$ or $\text{CASP10 V410I}$ genotype and breast or ovarian cancer risk data were analyzed within a Cox proportional hazards framework. To adjust for the non-random sampling of mutation carriers with respect to their disease status, we analysed the data using a weighted cohort approach as described elsewhere (20, 21). This involves assigning differential weights to affected and unaffected mutation carriers such that the breast or ovarian cancer incidence rates observed in the study population are consistent with established $\text{BRCA1}$ and $\text{BRCA2}$ incidences (22). Subjects were followed from birth until the event of interest (first breast or ovarian cancer) or time of censoring. When analyzing breast cancer as the event of interest, subjects without breast cancer were censored at bilateral mastectomy, ovarian cancer, or last observation, whichever occurred first. When ovarian cancer was analyzed, subjects without ovarian cancer were censored at bilateral oophorectomy, first breast cancer, or last observation, whichever occurred first. Follow up time was censored at age 80 years old. The associations were evaluated by modelling the per-allele hazard ratio (one degree-of-freedom) and by fitting models with separate HR for the heterozygote and homozygote carriers of the minor allele (two degree-of-freedom model).

Analyses were carried out separately for $\text{BRCA1}$ and $\text{BRCA2}$ mutations carriers (carriers of compound $\text{BRCA1}$ and $\text{BRCA2}$ mutations were included in the BRCA1 subgroup). Analyses were stratified by study group, country of residence (because some study groups contributed samples from more than one country) and year of birth (grouped into $<1930$, $1930-1939$, $1940-1949$, $1950-1959$, $1960+$). To allow for the non-independence among members of the same family, a robust variance approach was used to estimate the standard errors associated with the parameter estimates (23). The heterogeneity of hazard ratios among study groups
were tested using Cochran's Q test based on inverse variance weights. P-values equal to or smaller than 0.05 were considered statistically significant. All analyses were carried out using SPSS 15.0.1.1 (SPSS Inc.) and R 2.9.1 (The R Foundation for Statistical Computing) using the survival package version 2.35-4.
Results

A total of 7,353 and 7,227 Caucasian subjects could be analyzed for the association of \textit{CASP8} D302H and \textit{CASP10} V410I with age of onset of breast cancer or ovarian cancer. The cohort comprised 7,782 different individuals, of which 6,798 were genotyped for both SNPs, 555 for only \textit{CASP8} D302H, and 429 for only \textit{CASP10} V410I.

Tables 1 and 2 give the number of carriers by study, genotype and disease status. The overall minor allele frequencies were 0.12 (range 0.04 and 0.16) for \textit{CASP8} D302H and 0.07 (range 0.05 and 0.09) for \textit{CASP10} V410I. The mean age at breast cancer was 42 and 44 years for \textit{BRCA1} and \textit{BRCA2} mutation carriers, respectively, and the corresponding mean ages of ovarian cancer were 49 and 56 years. Table 3 shows the distribution of genotypes by SNP, mutated BRCA gene and disease status.

Tables 4 and 5 summarize the results of the association analysis. A significant association was found for \textit{BRCA1} mutation carriers (p$_{2df}$=0.028, p$_{trend}$=0.011), but not for \textit{BRCA2} mutation carriers (p$_{2df}$=0.794, p$_{trend}$=0.550). Within the \textit{BRCA1} mutation carriers, each copy of the minor allele was estimated to confer a hazard ratio (HR$_{\text{per-rare-allele}}$) of 0.85 (95%CI 0.76-0.97). There was also a significant association between \textit{CASP8} D302H and ovarian cancer risk for \textit{BRCA1} mutation carriers (p$_{2df}$=0.008, p$_{trend}$=0.004), but not in the \textit{BRCA2} subgroup (p$_{2df}$=0.718, p$_{trend}$=0.398). As with breast cancer, the effect of the rare allele was protective for ovarian cancer for \textit{BRCA1} mutation carriers (HR$_{\text{per-rare-allele}}$ 0.69, 95%CI 0.53-0.89).

Based on the two degree of freedom test, there were no significant associations between \textit{CASP10} V410I genotype and the risk of breast or ovarian cancer. However, the per-allele effect on ovarian cancer within the \textit{BRCA2} carriers was marginally significant (p$_{trend}$=0.045) with a HR$_{\text{per-rare-allele}}$ of 1.78 (95%CI 1.01-3.12).
Figure 1 shows the study-specific estimates of the per-allele hazard ratio for the two analyses in which significant associations were detected. There was no significant heterogeneity between the study-specific estimates for the association of CASP8 D302H with breast cancer risk in BRCA1 carriers (p_{heterogeneity}=0.712). However, there was a marginally significant heterogeneity for the association of CASP8 D302H with ovarian cancer risk in BRCA1 carriers (p_{heterogeneity}=0.048). For one study group (MAYO), the estimate of the hazard ratio was significantly different and in the opposite direction compared to the overall CIMBA estimate (HR=6.10, 95%CI 1.95-19.12), suggesting that the observed heterogeneity is caused by this study group. We therefore repeated the association analysis without the data from the MAYO study group. The overall association remains significant with a similar hazard ratio (HR_{per-rare-allele} 0.67, 95%CI 0.51-0.86, p_{2df}=0.003, p_{trend}=0.002), but heterogeneity among study-groups is no longer present (p_{heterogeneity}=0.625).

An analysis of the combined per-allele effects of the two SNPs in a multiplicative (log-additive) model revealed no qualitative changes in the results regarding the main effects, i.e. the risk reducing association between CASP8 D302H and breast and ovarian cancer in BRCA1 carriers was still significant. There were no significant interactions between CASP8 and CASP10. However, the marginally significant per-allele effect of CASP10 on ovarian cancer within the BRCA2 carriers was not seen in this combined analysis.
Discussion

In this study, we pooled data from 19 study groups worldwide to investigate the association of \textit{CASP8} D302H (\textit{CASP10} V410I) genotypes with breast and ovarian cancer risk in over 7,000 Caucasian carriers of deleterious mutations in \textit{BRCA1} or \textit{BRCA2}.

We found that the minor allele of \textit{CASP8} D302H was associated with a reduced risk of breast cancer (per allele HR 0.85, 95%CI 0.76-0.97) for \textit{BRCA1} but not for \textit{BRCA2} mutation carriers. Previously published case-control studies in unselected breast cancer cases also described a significantly decreased risk of breast cancer in carriers of the \textit{CASP8} 302H allele with an OR of 0.83-0.89 (7, 13). Thus, the per-allele HR estimate for \textit{BRCA1} mutation carriers in our study was consistent with the magnitude of the association in the general population. A recent paper by Palanca Suela et al. reported on an OR of 0.40 of \textit{CASP8} D302H for breast cancer in a combined analysis of both \textit{BRCA1} and \textit{BRCA2} mutation carriers (24). This is partly consistent with our finding for \textit{BRCA1} mutation carriers but not for \textit{BRCA2}, although they had not investigated the associations separately in \textit{BRCA1} and \textit{BRCA2} mutation carriers, and their study included only 186 \textit{BRCA1} and 204 \textit{BRCA2} mutation carriers.

The finding that \textit{CASP8} D302H is not a risk modifier in both \textit{BRCA1} and \textit{BRCA2} carriers is consistent with previous findings for other SNPs, which also showed associations for one subgroup only (25). The differential association patterns between \textit{BRCA1} and \textit{BRCA2} subgroups might be explained by the fact that these groups represent pathologically distinct entities. In our study we could not analyze whether there are differences in the association by estrogen (ER) or progesterone receptor (PR) status because such data were not available at the time of analysis. Cox et al. did not find significant differences in the \textit{CAPS8} rs1045485 risk association by ER or PR status of the tumors, however, the statistical power to detect such a
difference might have been too low given the weak effect and the relatively low minor allele frequency. Larger studies will aim to clarify the associations with different disease subtypes in the general population.

Our study was reasonably powered to detect an allele with a minor allele frequency of 0.13 and a per-allele relative risk (RR) of 0.88 (which are the MAF and association magnitude for CASP8 D302H observed in the general population) at a significance level of 0.05 for BRCA1 mutation carriers (power=70%), but the power was lower for BRCA2 mutation carriers (power=37%). Larger studies are needed to elucidate the role of this polymorphism in breast cancer for BRCA2 mutation carriers.

Recent haplotype analysis of the CASP8 locus provided evidence that the D302H variant is not the functionally relevant variant because a haplotype including the D302H variant could be determined that conferred a higher risk than the variant alone. This suggests that the variant cosegregates with one or even several causative variants (26).

This is the first report on a risk-reducing effect of the CASP8 D302H variant for ovarian cancer in BRCA1 mutation carriers (per allele HR 0.69, 95%CI 0.53-0.89). In contrast, there was no association between the variant and ovarian cancer in a large study comprising more than 4,600 unselected ovarian cancer cases (27), although the effect estimate was in same direction as in our study. The reason for the lack of association in the general population is not clear. Furthermore, the authors could not find significant associations with different disease subtypes. As indicated above, we could not evaluate in our study whether the association varies with disease subtype. The observed association in our study could potentially reflect a BRCA1 specific effect, but future studies with larger numbers of BRCA1
mutation carriers with ovarian cancer should aim to evaluate this further. Palanca Suela et al. also reported a lack of association of ovarian cancer in BRCA mutation carriers with the D302H variant (24). However, this may be due to the small study sample size as their cohort comprised only 182 affected mutation carriers without stating the exact number of patients diagnosed with breast or ovarian cancer. Latif et al. found that the D302H variant is associated with a significantly reduced risk of ovarian cancer in a case-control study including 101 women from families with familial ovarian cancer who were BRCA1/2 negative (OR=0.52, 95%CI 0.30-0.88) (28). However, they could not detect any association in 52 women from BRCA1/2 positive families, probably because of the small sample size.

The localization of the CASP10 V410I polymorphism five amino acids upstream of the proenzyme-cleavage site and seven amino acids downstream of the active-site motif rendered it an interesting SNP as well (29). In a case-control study of 511 familial breast cancer cases who did not carry BRCA1 and BRCA2 mutations and 547 controls, carriers of the CASP10 410I allele had a significantly reduced risk of breast cancer (10). However, a large study in more than 30,000 breast cancer cases could not confirm the association of V401I with breast cancer risk in the general population (30). This is in line with our results of no association between CASP10 V410I and breast cancer risk in BRCA1 and BRCA2 mutation carriers.

The present study is the largest of its kind in BRCA1/2 mutation carriers and provides significant evidence of association between CASP8 D302H variant and breast and ovarian cancer in BRCA1 but not in BRCA2 mutation carriers. A per-allele HR of 0.85 would result in a risk difference in the absolute risk of breast cancer of approximately 11% between the common and rare homozygotes by age of 80 years. Based on the present association, this polymorphism is estimated to account for only 0.4% of the genetic variability in breast cancer
risk for BRCA1 mutation carriers (31). Thus, the clinical utility of this single variant is limited. However, several other loci which were originally suggested as genetic risk factors in genome-wide association studies, were recently found by CIMBA to function as risk modifiers in $BRCA1$ and $BRCA2$ mutation carriers (31, 32). As further genetic modifiers of risk are identified for $BRCA1$ mutation carriers, the $CASP8$ variant in combination with others may be informative for risk assessment purposes. Furthermore, additional consideration of non-genetic risk factors may improve individualized risk prediction.
Table 1. Numbers of carriers included in the analysis (by study group and genotype)

| Study group |  | CASP8 D302H |  |  |  | CASPI0 V410I |  |  |  |  |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|             | Total GG | Total GC | Total CC | MAF | Total GG | Total GA | Total AA | MAF |           |           |           |           |           |
| CNIO        | 365      | 295      | 66       | 4   | 0.10     | 370       | 332      | 37   | 1   | 0.05       |           |           |           |
| DKFZ        | 98       | 76       | 20       | 2   | 0.12     | 98        | 84       | 12   | 2   | 0.08       |           |           |           |
| DNA HEBON   | 420      | 335      | 83       | 2   | 0.10     | 481       | 416      | 63   | 2   | 0.07       |           |           |           |
| EMBRACE     | 1081     | 819      | 251      | 11  | 0.13     | 1086      | 930      | 150  | 6   | 0.07       |           |           |           |
| FCCC        | 132      | 108      | 23       | 1   | 0.09     | 137       | 121      | 16   | 0   | 0.06       |           |           |           |
| GEMO        | 1270     | 968      | 266      | 36  | 0.13     | 753       | 657      | 92   | 4   | 0.07       |           |           |           |
| GC-HBOC     | 856      | 646      | 196      | 14  | 0.13     | 855       | 737      | 113  | 5   | 0.07       |           |           |           |
| HCCS        | 168      | 135      | 29       | 4   | 0.11     | 168       | 151      | 14   | 3   | 0.06       |           |           |           |
| HEBCS       | 184      | 141      | 39       | 4   | 0.13     | 184       | 161      | 23   | 0   | 0.06       |           |           |           |
| INHERIT     | 154      | 124      | 30       | 0   | 0.10     | 154       | 134      | 19   | 1   | 0.07       |           |           |           |
| kConFab     | 767      | 579      | 170      | 18  | 0.13     | 764       | 635      | 125  | 4   | 0.09       |           |           |           |
| MAYO        | 160      | 128      | 31       | 1   | 0.10     | 162       | 142      | 19   | 1   | 0.06       |           |           |           |
| NCI         | 188      | 148      | 37       | 3   | 0.11     | 195       | 170      | 24   | 1   | 0.07       |           |           |           |
| OCGN        | -        | -        | -        | -   | -        | 306       | 263      | 43   | 0   | 0.07       |           |           |           |
| PBCS        | 81       | 63       | 16       | 2   | 0.12     | 84        | 75       | 9    | 0   | 0.05       |           |           |           |
| SMC         | 345      | 317      | 28       | 0   | 0.04     | 334       | 304      | 29   | 1   | 0.05       |           |           |           |
| SWE-BRCA    | 537      | 411      | 119      | 7   | 0.12     | 551       | 478      | 70   | 3   | 0.07       |           |           |           |
| ModSQuaD    | 173      | 122      | 47       | 4   | 0.16     | 174       | 154      | 18   | 2   | 0.06       |           |           |           |
| UPENN       | 374      | 304      | 67       | 3   | 0.10     | 371       | 327      | 43   | 1   | 0.06       |           |           |           |
| **Total**   | **7353** | **5719** | **1518** | **116** | **0.12** | **7227** | **6271** | **919** | **37** | **0.07** |           |           |           |
Table 2. Characteristics of study subjects genotyped for the CASP8 D302H and CASP10 V410I polymorphisms

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CASP8 D302H</th>
<th>CASP10 V410I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>BRCA1</td>
</tr>
<tr>
<td>Carriers</td>
<td>7353</td>
<td>4844</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no.</td>
<td>3302</td>
<td>2241</td>
</tr>
<tr>
<td>age at censure, mean ±SD</td>
<td>43.0 ±12.6</td>
<td>42.7 ±12.5</td>
</tr>
<tr>
<td>affected</td>
<td>4051</td>
<td>2603</td>
</tr>
<tr>
<td>age at event, mean ±SD</td>
<td>42.0 ±9.7</td>
<td>40.8 ±9.4</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no.</td>
<td>6628</td>
<td>4268</td>
</tr>
<tr>
<td>age at censure, mean ±SD</td>
<td>41.6 ±10.9</td>
<td>40.6 ±10.7</td>
</tr>
<tr>
<td>affected</td>
<td>725</td>
<td>576</td>
</tr>
<tr>
<td>age at event, mean ±SD</td>
<td>50.6 ±9.9</td>
<td>49.4 ±9.5</td>
</tr>
</tbody>
</table>

Table 3. Genotype distributions for the CASP8 D302H and CASP10 V410I polymorphisms

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CASP8 D302H</th>
<th>CASP10 V410I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>GG</td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not affected</td>
<td>4844</td>
<td>3784</td>
</tr>
<tr>
<td>affected</td>
<td>2241</td>
<td>1725</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not affected</td>
<td>6628</td>
<td>4268</td>
</tr>
<tr>
<td>affected</td>
<td>725</td>
<td>576</td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not affected</td>
<td>1935</td>
<td>535</td>
</tr>
<tr>
<td>affected</td>
<td>1101</td>
<td>288</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not affected</td>
<td>2360</td>
<td>1816</td>
</tr>
<tr>
<td>affected</td>
<td>149</td>
<td>119</td>
</tr>
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</table>
Table 4. Association of \textit{CASP8} D302H with breast and ovarian cancer risk

<table>
<thead>
<tr>
<th>\textit{CASP8} D302H</th>
<th>hazard ratio (95%CI)</th>
<th>p-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per rare allele</td>
<td>heterozygotes$^a$</td>
</tr>
<tr>
<td><strong>Breast cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{BRCA1}</td>
<td>0.85 (0.76-0.97)</td>
<td>0.83 (0.72-0.95)</td>
</tr>
<tr>
<td>\textit{BRCA2}</td>
<td>1.06 (0.88-1.27)</td>
<td>1.04 (0.84-1.30)</td>
</tr>
<tr>
<td><strong>Ovarian cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{BRCA1}</td>
<td>0.69 (0.53-0.89)</td>
<td>0.73 (0.55-0.98)</td>
</tr>
<tr>
<td>\textit{BRCA2}</td>
<td>1.27 (0.73-2.23)</td>
<td>1.25 (0.67-2.34)</td>
</tr>
</tbody>
</table>

$^a$ versus common homozygotes  
$^b$ 2df test

Table 5. Association of \textit{CASP10} V410I with breast and ovarian cancer risk

<table>
<thead>
<tr>
<th>\textit{CASP10} V410I</th>
<th>hazard ratio (95%CI)</th>
<th>p-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per rare allele</td>
<td>heterozygotes$^a$</td>
</tr>
<tr>
<td><strong>Breast cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{BRCA1}</td>
<td>0.98 (0.84-1.15)</td>
<td>0.94 (0.80-1.10)</td>
</tr>
<tr>
<td>\textit{BRCA2}</td>
<td>1.03 (0.80-1.31)</td>
<td>1.06 (0.82-1.38)</td>
</tr>
<tr>
<td><strong>Ovarian cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{BRCA1}</td>
<td>1.12 (0.83-1.52)</td>
<td>1.08 (0.78-1.52)</td>
</tr>
<tr>
<td>\textit{BRCA2}</td>
<td>1.78 (1.01-3.12)</td>
<td>1.60 (0.85-2.98)</td>
</tr>
</tbody>
</table>

$^a$ versus common homozygotes  
$^b$ 2df test
**Figure 1.** Forest plots of the study-specific estimates of the per-allele hazard ratios for *CASP8* D302H in *BRCA1* mutation carriers (endpoints breast and ovarian cancer). The area of the squares is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals. The vertical dashed line indicates the overall effect estimate.
Competing Interests

No competing interests were identified among the authors.

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Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)


*Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers study (GEMO)*

The GEMO study (Cancer Genetics Network "Groupe Génétique et Cancer", Fédération Nationale des Centres de Lutte Contre le Cancer, France) is supported by the Ligue Nationale Contre le Cancer, Association for International Cancer Research Grant AICR-07-0454 and the Association "Le cancer du sein, parlons-en!" Award. LB is supported by Association for
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*National Cancer Institute study (NCI)*

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MoDSquaD

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*Interdisciplinary Health Research International Team Breast Cancer susceptibility (INHERIT)*

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*Ontario Cancer Genetics Network Study (OCGN)*

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Pisa Breast Cancer Study (PBCS)
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References


