BRCA1 Wild-Type Allele Modifies Risk of Ovarian Cancer in Carriers of BRCA1 Germ-Line Mutations

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Abstract

Strong inter- and intrafamilial variation of penetrance of breast and ovarian cancer is observed in BRCA1 mutation carriers. The wild-type copy of the BRCA1 gene is a plausible candidate as a cancer risk modifier given that the residual function corresponding to the intact BRCA1 allele may influence the process of tumor formation in BRCA1 carriers. Indeed, growing evidence is now becoming available on impaired reparation of double-strand DNA breaks in cells heterozygous for BRCA1 mutations, implying an enhanced mutability of BRCA1+/− cells. To determine whether certain variant forms of the wild-type BRCA1 allele are implicated in variation of the BRCA1-related cancer risk, their effect was studied in a panel of 591 women with BRCA1 germ-line mutations. We found that BRCA1 carriers with the wild-type BRCA1 copy bearing a common Gly1038 variant were at increased risk of ovarian cancer (hazards ratio, 1.50; 95% confidence interval, 1.03–2.19). The results of our study imply that a quite significant proportion of the interindividual variability in ovarian cancer penetrance in BRCA1 carriers may be explained by a common BRCA1 Gly1038 wild-type allele, given its high frequency (0.27).

Introduction

Carriers of mutations in the BRCA1 and BRCA2 genes have considerably increased susceptibility to develop breast and ovarian cancer as compared with the general population. Strong inter- and intrafamilial variation of penetrance of these cancers is observed in mutation carriers. Substantially different BRCA1 penetrance estimates have been reported depending on the method of family ascertainment: lifetime cancer risk of about 80% for breast cancer and 60% for ovarian cancer have been found in the studies of highly selected families with multiple cancer cases, whereas in population-based studies, these estimates appeared to be much lower (40–50% for breast cancer, 20–40% for ovarian cancer; Refs. 1–6). These differences suggest that genetic and/or nongenetic factors have influence on BRCA1-associated cancer risk. Several such factors have been suggested already (7). A number of hormonal aspects, mainly associated with a woman’s reproductive life, e.g., pregnancy, oral contraceptives use, breastfeeding, oophorectomy, were found to modify breast and ovarian cancer expression in mutation carriers (8–12). An effect of genetic modifiers was reported as well. The presence of at least one rare allele of the variable number of tandem repeats (VNTR) polymorphism in the downstream region of the HRAS1 proto-oncogene was found to be associated with an elevated risk of ovarian cancer in women carrying BRCA1 mutations (13). The progesterone receptor variant characterized by an intronic Alu insertion confers an increased ovarian cancer risk in BRCA carriers who never used oral contraceptives (14). The risk of breast cancer was increased in those carriers with at least one long allele (≥28 CAG repeats) as compared with that with shorter alleles in the genes coding for the androgen receptor and the AIB1 steroid hormone receptor coactivator (15, 16). However, these modifier effects were not examined or were not replicated in independent studies (17).

Although not yet considered, the wild-type copy of the BRCA1 gene appears to be a plausible candidate for cancer risk...
modifier in BRCA1 mutation carriers given that the residual function corresponding to the intact BRCA1 allele may influence the process of tumor formation in BRCA1 carriers (18). This hypothesis is mainly based on the growing evidence of impaired repair of double-strand DNA breaks in cells heterozygous for BRCA1 mutations, implying an enhanced mutability of BRCA1 "+/-" cells (19–22). To determine whether certain variant forms of the wild-type BRCA1 allele are implicated in variation of the BRCA1-related cancer risk, their effect was studied in a panel of women with BRCA1 germ-line mutations.

Materials and Methods

Study Participants. The study included 591 women belonging to 282 different families recruited and identified as carriers of BRCA1 germ-line mutations in the framework of research and counseling programs on hereditary breast and ovarian cancer in 15 centers in France, the United States, Canada, and Greece. Of these 591 women, 119 have been diagnosed with ovarian cancer, 272 with breast cancer, 51 with both breast and ovarian cancer, and 149 women as breast- and ovarian-cancer-free at the time of the last follow-up. Information available on study subjects included clinical characteristics, date of birth, current age or age at death, age at diagnosis of breast and/or ovarian cancer, age at prophylactic surgery (oophorectomy or mastectomy), and parity (Table 1).

BRCA1 Genotyping. The samples were genotyped for four coding bi-allelic single nucleotide BRCA1 polymorphisms: A1186G/Gln356Arg, G2196A/Asp693Asn, A3232G/Glu1038Gly, and D17S1323/Ser1613Gly. DNA samples were available for 568 study participants. For 23 affected women whose samples were lacking, the BRCA1 genotype was established using DNA of their children and husband. Heteroduplex analysis, denaturing gradient gel electrophoresis (DGGE), denaturing high-precision liquid chromatography (DHPLC), enzymatic digestion (AlwNiI for A1186G, Hsp92II for G2196A, and NlaIV for A3232G and A4956G) and sequencing (ABI 3100; Applied Biosystems) were used for genotyping of the BRCA1 polymorphisms. These procedures were performed as described elsewhere (23–25). For each of the four BRCA1 polymorphisms studied, the corresponding BRCA1 amplicons of five individuals were sequenced (ABI 3100; Applied Biosystems), confirming the genotyping accuracy of the procedures used.

Identification of Polymorphic Variant Carried on BRCA1 Wild-Type Allele. In individuals heterozygous for the typed BRCA1 polymorphisms, it was necessary to determine which polymorphic variant was carried on the wild-type BRCA1 copy. This was done using genotype information in homozygous relatives when available. In four families with 5382insC-ter1828 the common mutual origin was established using haplotype data on several microsatellite markers surrounding the BRCA1 locus (D17S1185, D17S1320, D17S1321, D17S855, D17S1322, D17S1323, D17S1327, D17S1326, and D17S1325), permitting the phase identification of the BRCA1 polymorphisms in carriers in these families. BRCA1 PCR fragments encompassing both polymorphism and mutation sites were cloned in 41 mutation carriers from families in which only heterozygotes for the BRCA1 polymorphisms studied were detected. The cloning was performed with the use of the TOPO TA Cloning kit (Invitrogen) according to the manufacturer’s instructions. Cloned DNA was PCR amplified and sequenced (ABI 3100; Applied Biosystems). All of the carriers in whom the phase of the BRCA1 polymorphisms could not be determined by the above methods were excluded from the analysis. The proportion of such individuals was very similar in the groups of breast cancer patients, ovarian cancer patients, and unaffected carriers, varying between 0.012 and 0.014 for Gln356Arg, Arg693Asn, and between 0.033 and 0.051 for the Gln1038Gly and the Ser1613Gly polymorphisms.

Results

Five hundred ninety-one female BRCA1 mutation carriers have been included in the study. Clinical characteristics, year of birth, and parity of carriers are presented in Table 1. The samples were genotyped for four coding BRCA1 SNPs. Two of them (A3232G/Glu1038Gly and A4956G/Ser1613Gly) have been reported to be in strong linkage disequilibrium with each other, as well as with at least six other polymorphisms in the BRCA1 gene, the frequency of the rare allele being about 0.30 (26, 27). The other two BRCA1 polymorphisms tested were

Table 1: BRCA1 mutation carriers included in the study

<table>
<thead>
<tr>
<th>BRCA1 carriers:</th>
<th>591 (388)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at diagnosis (yr)</td>
<td>47.5 (47.2)</td>
</tr>
<tr>
<td>Mean year of birth</td>
<td>1941 (1945)</td>
</tr>
<tr>
<td>Mean parity</td>
<td>2.8 (2.4)</td>
</tr>
<tr>
<td>Breast (BR/ )</td>
<td>51 (43)</td>
</tr>
<tr>
<td>Ovarian (OV) cancer</td>
<td>272 (175)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>149 (86)</td>
</tr>
<tr>
<td>Breast- and ovarian-cancer free</td>
<td>43.9* (44.5)*</td>
</tr>
</tbody>
</table>

* In bold, total of BRCA1 carriers; in parentheses, BRCA1 carriers independent for segregation of the wild-type BRCA1 allele.

a Mean current age.
A1186G/Gln356Arg and G2196A/Asp693Asn, with reported frequencies of the rare allele of about 0.06 (26, 27). Thus the analyzed SNPs included all of the known *BRCA1* polymorphisms with a frequency greater than 0.05, Glu1038Gly and Ser1613Gly having been chosen as tag SNPs corresponding to the above mentioned linkage disequilibrium block.

Analysis of each of the *BRCA1* wild-type allele variants, considered independently, suggested an association of Gly1038 with an increased risk of ovarian cancer (HR, 1.39; 95% CI, 0.98–1.95). Results on the other variants tested were not significant. To account for the fact that a number of the analyzed subjects were related to one another, the survival analysis was undertaken in a group of carriers independent for segregation of the wild-type *BRCA1* allele. Because an excess of sharing of the wild-type *BRCA1* allele among female *BRCA1* carriers concerns only sisters and not other relationships (e.g., mother-daughter), the group of independent individuals included one carrier per sister-set selected according to the following priority criteria: (a) ovarian cancer case diagnosed at the youngest age; (b) the oldest unaffected carrier; and (c) breast cancer case diagnosed at the oldest age. This resulted in the selection of 388 carriers (Table 1). The effect of Gly1038 allele on ovarian cancer risk appeared to be stronger in independent carriers as compared with the total set of carriers (HR, 1.50; 95% CI, 1.03–2.19; Table 2). The age-specific ovarian cancer-free survival for *BRCA1* mutation carriers with either the Gln356 or the G1038 allele is presented in Fig. 1. The examination of this survival curve indicated that the effect of the *BRCA1* Gly1038 wild-type allele was essentially limited to the set of women aged 50 or more, an observation implying age and/or reproductive hormones contribution to the Gly1038-mediated variation of ovarian cancer risk.

To refine the evaluation of the relationship between *BRCA1* polymorphisms and breast and ovarian cancer risk, we examined the effect of common *BRCA1* haplotypes formed by the polymorphisms studied. This could be explored in the sample of 293 of 388 independent individuals for which complete four-locus wild-type-phased haplotypes were available. Allele frequencies of the *BRCA1* sequence variants and linkage disequilibrium coefficients (D’) were estimated using our data on the wild-type *BRCA1* allele in these independent *BRCA1* carriers (Table 3; Ref. 28). The allelic frequencies of each of the four polymorphisms were similar to those reported in population controls by Durocher et al. (26) and Dunning et al. (27). Ten SNP haplotypes were observed, five of them being very rare. Their composition and estimated frequencies are given in Table 4. Not unexpectedly Gln356/Asp693/Gly1038/Gly1613 and Gln356/Asn693/Gly1038/Gly1613 haplotypes were found to confer an increased ovarian cancer risk (HR = 1.66, 95% CI, 1.05–2.63 and HR = 1.82, 95% CI, 0.82–4.01, respectively) because most of Gly1038 are carried on these two haplotypes (Table 4). Compared with the results of Gly1038 on its own, its effect was similar in the context of common Gln356/Gly1038/Gly1613-carrying haplotypes, suggesting that the modulation of ovarian cancer risk is attributable to the Gly1038 variant alone, rather than a specific combination of the four polymorphisms.

The modifier effects detected in this analysis were also estimated while adjusting for year of birth and for parity, because these variables have been reported in previous studies to influence breast and ovarian cancer risk in *BRCA* carriers (8, 9). The magnitude of the estimated effect of Gly1038 wild-type allele on ovarian cancer risk was slightly reduced by adjustment for year of birth and for parity (HR = 1.35, 95% CI, 0.88–2.08).

A recent analysis of Thompson and Easton (29) using the largest series of *BRCA1* breast/ovarian cancer families analyzed thus far for genotype/phenotype correlation, provided evidence that *BRCA1* truncating mutations located in the central portion of the coding sequence (nucleotides 2401–4190) are associated with 30% reduction of breast cancer risk compared with mutations located outside of this region. This study also detected less ovarian cancers among carriers of 3’ mutations (nucleotides 4190–5711) than among those with mutations in the remaining part of the gene. We evaluated the effect of mutation position on breast and ovarian cancer risk in our sample and found no significant trend. The Gly1038-associated increase of ovarian cancer risk was similar in the groups of carriers of *BRCA1* mutations in the 3’ region and in the rest of the gene (data not shown).

### Table 2  Effect of *BRCA1* wild-type allele variants on breast and ovarian cancer penetrance in 388 independent *BRCA1* mutation carriers

<table>
<thead>
<tr>
<th><em>BRCA1</em> wild-type allele variant</th>
<th>Breast cancer HR (95% CI)</th>
<th>Ovarian cancer HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg356</td>
<td>0.78 (0.45–1.33)</td>
<td>0.82 (0.40–1.68)</td>
</tr>
<tr>
<td>Asn693</td>
<td>0.99 (0.60–1.66)</td>
<td>1.18 (0.57–2.43)</td>
</tr>
<tr>
<td>Gly1038</td>
<td>0.82 (0.60–1.12)</td>
<td>1.50 (1.03–2.19)</td>
</tr>
<tr>
<td>Gly1613</td>
<td>0.88 (0.65–1.18)</td>
<td>1.38 (0.93–2.03)</td>
</tr>
</tbody>
</table>

### Discussion

Our findings suggest that certain alleles of the wild-type copy of the *BRCA1* gene modify the risk of ovarian cancer in women whose other *BRCA1* copy is inactivated by a mutation. The difficulty of collecting samples from *BRCA1* carriers affected with ovarian cancer, given the poor survival of these patients, didn’t allow us to perform our analysis on a very large set. Because of the rapidly growing number of newly identified *BRCA1* mutation carriers, it will become easier in the future to gather samples to verify, in an independent set of carriers, the effect of the *BRCA1* Gly1038 wild-type allele on ovarian cancer risk detected in our series. The biological rationale underlying this effect might be related to certain interactions of *BRCA1* with its multiple cellular partners, reflecting the variety of the *BRCA1* functions, and is expected in particular to be related to the role of *BRCA1* in DNA damage-sensing mechanisms. Although these mechanisms have been primarily studied in *BRCA1* nullizygous cells (reviewed in Ref. 18), growing evidence is becoming available on the impaired repair of double-strand DNA breaks in cells heterozygous for *BRCA1* mutations, implying an enhanced mutability of *BRCA1* cells. An increased radio-sensitivity and high level of micronuclei formation were observed in *BRCA1*+/- lymphoblasts, lymphocytes, or fibroblasts (19–21). The study of Baldeyron et al. (22) demonstrated a reduced fidelity of double-strand breaks end-joining in *BRCA1*+/- cells compared with controls. Thus, the specific function of the protein encoded by the second, intact copy of the *BRCA1* gene appears to be critical for accelerating or slowing down the accumulation of unrepaired DNA damage and chromedly Gly356/Gln356/BRCA1+/- cells. Therefore, functional variations between different polymorphic forms of the wild-type *BRCA1* are likely to exist and would be expected to influence cancer penetrance in *BRCA1* carriers.

This postulate might appear to contradict the tumor-suppressor two-hit model supported by the loss of the *BRCA1* wild-type allele observed in most of the *BRCA1*-associated tumors studied (30, 31). However, the timing of this event
remains uncertain. It seems very plausible that BRCA1/del/BRCA1 cells accumulating DNA anomalies would be prone to acquire for their survival a mutation(s) in checkpoint gene(s) rather than loss of the second BRCA1 allele (18). Although no convincing data are available on the chronology of key genetic events in cancer-prone tissues in BRCA1 carriers, for BRCA2 heterozygotes, there is evidence of inactivation of the BRCA2 wild-type copy late in tumor progression (32).

Examples of inherited diseases of which the major gene penetrance is modified by the wild-type allele are being reported in the literature. Such a modifier effect has been described in the case of the dominant inherited disorder of heme biosynthesis, erythropoietic protoporphyria, caused by mutations in the FECH gene resulting in ferrochelatase deficiency (33). The intronic variant IVS3–48C of the wild-type FECH (allelic frequency, 0.11), associated with diminished expression because of increased efficiency of abnormal splicing was found to boost clinical expression of the disease. The severity of another hematological disorder, hereditary elliptocytosis, is also augmented by the presence in trans of the common Val1857/IVS45–12T/IVS46–12A allele of -spectrin in heterozygote -spectrin mutation carriers (34, 35). This polymorphic allele produces transcripts with partially skipped exon 46 coding for a motif involved in the formation of spectrin dimers, resulting in under-representation of wild-type spectrin dimers in erythrocyte membrane.

It is difficult to speculate about specific mechanisms of modulation of ovarian cancer risk by the wild-type BRCA1 Gly1038-carrying allele because no data are available on its functional evaluation. However, it is tempting to hypothesize that this allele might have tissue-dependent functional particularities (regarding transcript or protein stability, alternative splicing, interaction with tissue-specific partners, and so forth) rendering BRCA1/del ovarian, but not breast cells more susceptible to malignant transformation. The fact that the effect of the Gly1038 allele is observed in BRCA1 carriers older than age 50 years suggests that certain latency period of DNA error accumulation might be necessary for the modifier effect to

Table 3: Linkage disequilibrium and allele frequency of BRCA1 polymorphisms studied

<table>
<thead>
<tr>
<th>Polymorphism: residue, nucleotide</th>
<th>Allele frequencies</th>
<th>Linkage disequilibrium coefficients (D')</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Asp693Asn G2196A</td>
</tr>
<tr>
<td>Gln356Arg A1186G</td>
<td>0.95/0.05</td>
<td>-1.00</td>
</tr>
<tr>
<td>Asp693Asn G2196A</td>
<td>0.96/0.04</td>
<td>0.57</td>
</tr>
<tr>
<td>Glu1038Gly A3232G</td>
<td>0.73/0.27</td>
<td>0.88</td>
</tr>
<tr>
<td>Ser1613Gly A4956G</td>
<td>0.69/0.31</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Estimation based on our data on BRCA1 wild-type allele in 293 BRCA1 independent carriers for which the BRCA1 wild-type haplotypes composed of all four polymorphisms were determined.

Table 4: Effect of BRCA1 wild-type allele haplotypes on breast and ovarian cancer penetrance in 293 independent BRCA1 mutation carriers

<table>
<thead>
<tr>
<th>BRCA1 wild-type allele haplotype</th>
<th>Haplotype frequency</th>
<th>Breast cancer HR (95% CI)</th>
<th>Ovarian cancer HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>356/693/1038/1613</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Asp/Glu/Ser</td>
<td>0.540</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gln/Asp/Gly/Gly</td>
<td>0.209</td>
<td>1.66 (1.05-2.63)</td>
<td></td>
</tr>
<tr>
<td>Gln/Asp/Glu/Gly</td>
<td>0.088</td>
<td>0.87 (0.37-2.03)</td>
<td></td>
</tr>
<tr>
<td>Gln/Asn/Gly/Gly</td>
<td>0.060</td>
<td>1.82 (0.82-4.01)</td>
<td></td>
</tr>
<tr>
<td>Arg/Asp/Glu/Ser</td>
<td>0.047</td>
<td>1.02 (0.46-2.24)</td>
<td></td>
</tr>
<tr>
<td>Gln/Asp/Gly/Ser</td>
<td>0.019</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gln/Asp/Gly/Ser</td>
<td>0.019</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gln/Glu/Asp/Ser</td>
<td>0.009</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gln/Asp/Glu/Ser</td>
<td>0.005</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gln/Glu/Asp/Ser</td>
<td>0.005</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Estimation based on our data on BRCA1 wild-type allele in 293 BRCA1 independent carriers for which the BRCA1 wild-type haplotypes composed of all four polymorphisms were determined.

b Reference haplotype.

c NA, not analyzed.
become detectable. An alternative to this speculation is that the postmenopausal hormonal background might favor manifestation of variations of the BRCA1 function in estrogen-signaling pathways (36, 37).

Several studies compared the frequency of this BRCA1 allele between sporadic breast or ovarian cancer cases and controls; however, no statistically meaningful difference could be detected (26, 27, 38). These results suggest that the cancer risk associated with the Gly1038 allele might be minor and hardly detectable in the relatively small and heterogeneous samples studied (26, 38). Alternatively, this BRCA1 allele might have no effect in the absence of an inactivating mutation in the other BRCA1 copy (24). In conclusion, the results of our study imply that a quite significant proportion of the interindividual variability in ovarian cancer penetrance in BRCA1 carriers may be explained by a common BRCA1 Gly1038 wild-type allele, given its high frequency (0.27).

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References


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