**The HRAS1 Minisatellite Locus and Risk of Ovarian Cancer**

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**Abstract**

Approximately 10% of ovarian cancers are due to mutations in highly penetrant inherited cancer susceptibility genes. The highly polymorphic HRAS1 minisatellite locus, located just downstream from the proto-oncogene H-ras-1 on chromosome 11p, consists of four common progenitor alleles and several dozen rare alleles, which apparently derive from mutations of the progenitors. Mutant alleles of this locus represent a major risk factor for cancers of the breast, colorectum, and bladder, and it was found that BRCA1 mutation carriers with at least one rare HRAS1 allele have a greater risk of ovarian cancer than BRCA1 carriers with only common HRAS1 alleles. There are no conclusive studies of HRAS1 alleles in sporadic epithelial ovarian cancer.

A case-control study of HRAS1 alleles was performed on DNA from 136 Caucasian patients with ovarian cancer and 108 cancer-free controls using conventional (Southern blot) and PCR-based methods to determine the frequency of rare HRAS1 alleles. Odds ratios (ORs) were estimated using unconditional logistic regression methods. A single degree of freedom test was used to assess the significance of linear trend across categories of increasing exposure.

A statistically significant association between rare HRAS1 alleles and risk of ovarian cancer was observed [OR, 1.70; 95% confidence interval (CI), 1.03–2.80; P = 0.04]. Having only one rare allele was associated with a relative risk of 1.66 (95% CI, 0.91–3.01), whereas having two rare alleles increased the relative risk to 2.86 (95% CI, 0.75–10.94; trend P = 0.03). Analysis of HRAS1 allele types by the age of the case at diagnosis revealed that younger cases (<45 years) had a borderline statistically significant increased association with rare HRAS1 alleles compared to older cases (≥60 years; OR, 1.89; 95% CI, 0.90–3.98; P = 0.09).

Rare HRAS1 alleles contribute to ovarian cancer predisposition in the general population. Thus, the HRAS1-variable number of tandem repeats locus may function as a modifier of ovarian cancer risk in both sporadic and hereditary ovarian cancer.

**Introduction**

Ovarian cancer is the fifth leading cause of cancer death among women residing in the United States. Between 1991 and 1995, an average of 14.9 per 100,000 incident cases of ovarian cancer were diagnosed in the United States, with mortality from the disease at 7.7 per 100,000 (1). Because the majority (61%) of these cancers are diagnosed when the patient has advanced stage disease (International Federation of Gynecology and Obstetrics stage III or IV), only about half of the women diagnosed with ovarian cancer live more than 5 years (1, 2). Major risk factors for the disease include increasing age, Caucasian race, nulliparity, use of oral contraceptives, history of infertility, and family history of ovarian cancer. Approximately 10% of ovarian cancers overall are due to inherited mutations in cancer susceptibility genes. Germline BRCA1 and BRCA2 mutations have been implicated in the majority of hereditary ovarian cancer cases. Mutations in mismatch repair genes (associated with hereditary non-polyposis colorectal cancer syndrome) are implicated in a minority of cases.

It is estimated that women who carry BRCA1 mutations have a 27–63% lifetime risk of developing ovarian cancer (3–5). Some variation in penetrance of BRCA1 may be related to the highly polymorphic HRAS1 minisatellite locus (6). This locus, located just downstream from the proto-oncogene H-ras-1 on chromosome 11p, consists of four common progenitor alleles and several dozen rare alleles, which apparently derive from mutations of the progenitors (7). Mutant alleles of this locus represent a major risk factor for several common types of cancer (8, 9). In a study conducted by Phelan et al. (6), it was found that BRCA1 mutation carriers with at least one rare HRAS1-VNTR allele have more than double the risk of ovarian cancer than BRCA1 carriers with only common HRAS1 alleles. Previous studies have implicated a clear association between rare HRAS1 alleles and cancers of the breast, colorectum, and bladder (9). There are no conclusive studies of HRAS1-VNTR alleles in sporadic epithelial ovarian cancer (10).

To understand how the HRAS1 minisatellite locus may relate to sporadic ovarian cancer, a case-control study using conventional (Southern blot) and PCR-based methods was performed. DNA from 136 Caucasian patients with ovarian cancer and 108 cancer-free controls were compared to determine the frequency of rare HRAS1 alleles.

**Patients and Methods**

Peripheral blood and/or tumor tissue was obtained from 136 Caucasian cases with histologically confirmed epithelial ovarian cancer and from 108 age- and race-matched cancer-free controls. Clinical data collected on each case included tumor histology, grade, and stage. Both cases and controls were recruited from the New England Medical Center or Massachusetts General Hospital. Controls were derived from the benign gynecology or general medicine services at either of these institutions and were restricted to Caucasian women with intact ovaries who had no personal history of cancer (except basal cell carcinoma). Prior to collection, each participating subject provided her informed consent. Study procedures were approved by the respective Institutional Review Boards in accordance with assurances approved by the United States Department of Health and Human Services.

**Laboratory Methods**

DNA was extracted from blood (n = 96), tissue (n = 16), or both (n = 24) by conventional techniques (organic extraction, Ref. 11). DNAs were allelotyped for the HRAS1-VNTR polymorphism at 11p15.5. All cases and controls were scored by PCR amplification as described previously (12). In addition, 84 (34.6%) samples were also scored by Southern blot-based methods. For 16 cases (11.8%), only tumor tissue was available for analysis. In the tumor...
A2.1 allele is 1 repeat unit larger than a2.)

Assess the significance of linear trend across categories of increasing exposure, regression methods (13). Ninety-five % CIs for the OR were estimated using the method proposed by Prentice (14). Rare alleles were designated by decimals with respect to the smallest common allele (e.g., a1.15 is a rare allele 15 repeat units larger than a1, whereas an a1.0 allele is identical to a1). In the PCR/automated sequencer-based analyses, the nomenclature was modified. Rare alleles were designated by an integer (e.g., a1, a2, a3, or a4) and other alleles (rare) were designated by decimals with respect to the closest smaller common allele (e.g., a1.1, a1.2 for alleles larger than a1 but smaller than a2, and a2.1, and a2.2 for alleles larger than a2 but smaller than a3). In the PCR/automated sequencer-based analyses, genotypes were determined by comparison of the TET-labeled Tumor tissue included. In all 24 cases, we observed complete concordance between blood and tumor genotypes.

Assignment of genotypes by Southern methodology was based on the original system reported by Kronir et al. (9), in which common alleles were designated by an integer (i.e., a1, a2, a3, or a4) and other alleles (rare) were designated by decimals with respect to the closest smaller common allele (e.g., a1.1, a1.2 for alleles larger than a1 but smaller than a2, and a2.1, a2.2 for alleles larger than a2 but smaller than a3). In the PCR/automated sequencer-based analyses, genotypes were determined by comparison of the TET-labeled patient DNAs to the FAM reference ladder and GS2500 molecular weight marker. Using this methodology, single VNTR repeat resolution was achieved for all common and rare alleles. Because of the increased sensitivity afforded by the PCR-based analyses, the nomenclature was modified. Rare alleles were named with respect to the smaller common allele nearest in size separated by a decimal (i.e., a1.15 is a rare allele 15 repeat units larger than a1, whereas an a2.1 allele is 1 repeat unit larger than a2.)

Statistical Analysis. ORs were estimated using unconditional logistic regression methods (13). Ninety-five % CIs for the OR were estimated using the logarithm of the OR and its SE. A single degree of freedom test was used to assess the significance of linear trend across categories of increasing exposure, where relevant.

Results

The mean age of the cases was 53.2 (SD = 13.3), whereas the mean age of the controls was 52.5 (SD = 15.4). Table 1 summarizes the clinical characteristics of the ovarian cancer cases. No associations were found between rare HRAS1 alleles and tumor stage or histology. Table 2 summarizes the frequency of rare and common HRAS1 alleles among both cases and controls. Analyses of these data revealed a statistically significant association between rare HRAS1 alleles and risk of ovarian cancer (OR, 1.76; 95% CI, 1.08–2.87; Table 2). The rate of rare HRAS1 alleles in the cases was 21.5%, compared to only 13.4% in the cancer-free controls. When the 15 eligible cases with only tumor tissue were excluded from the analyses, the results were unchanged (results not shown).

Analyses of the number of rare alleles in individual cases and controls revealed that having only one rare allele was associated with a relative risk of 1.66 (95% CI, 0.91–3.01), whereas having two rare alleles increased the relative risk to 2.86 (95% CI, 0.75–10.94; trend P = 0.03; Table 3). Table 4 summarizes HRAS1 allele types by the age of the case at diagnosis. In these analyses, it was found that younger cases (<45 years) had a borderline statistically significant increased association with rare HRAS1 alleles compared to older cases (≥60 years; OR, 1.89; 95% CI, 0.90–3.98; trend P = 0.09).

Discussion

A case-control study was performed using both Southern blot and PCR-based methods to score HRAS1 alleles in DNA from 136 patients with sporadic epithelial ovarian cancer and 108 cancer-free controls. These data suggest a statistically significant (P = 0.04) increased incidence of rare HRAS1 alleles in Caucasian ovarian cancer patients (20.8%) compared to age- and race-matched cancer-free controls (13.4%). These results indicate that rare HRAS1-VNTR alleles contribute to an ovarian cancer predisposition and that they parallel the allele distributions seen in the large published series (9) with other common cancers, such as breast, bladder, and colorectal cancer. The current study also showed a statistically significant increased association between ovarian cancer and the number of rare HRAS1 alleles (0 versus 1 versus 2; trend P = 0.03). In addition, it was found that younger cases (<45 years) were almost twice as likely to have a rare HRAS1 alleles compared to older cases (≥60 years; OR, 1.89; 95% CI, 0.90–3.98; trend P = 0.09).

The nature of the phenomenon underlying the association between HRAS1 and ovarian cancer is not known. However, preliminary functional analysis of the HRAS1-VNTRs has shown associated transcriptional regulatory protein binding (at least four members of the rel/nuclear factor kB family bind to the HRAS1 minisatellite; Ref. 14). Furthermore, specific alleles have demonstrated enhancer or suppressor activity in vitro (15). This suggests a potential biological basis for the association of rare HRAS1 alleles and cancer. There are numerous genes near HRAS1. Our own studies have demonstrated the existence of a cluster of genes in the vicinity of HRAS1 on chromosome 11p15.5 (16). HRCI, identified just 30 kb upstream of HRAS1, is a candidate tumor suppressor gene with features of the helix-loop-helix/leucine zipper gene class. Perhaps the HRAS1-VNTR influences the expression of these nearby genes.

Table 1 Tumor characteristics in cases with invasive epithelial ovarian cancer

<table>
<thead>
<tr>
<th>Histology</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>54</td>
</tr>
<tr>
<td>Clear cell</td>
<td>20</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>33</td>
</tr>
<tr>
<td>Mixed Epithelial</td>
<td>9</td>
</tr>
<tr>
<td>Mucinous</td>
<td>7</td>
</tr>
<tr>
<td>Small Cell</td>
<td>1</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>7</td>
</tr>
<tr>
<td>Not specified</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>23</td>
</tr>
<tr>
<td>II</td>
<td>11</td>
</tr>
<tr>
<td>III</td>
<td>71</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
</tr>
<tr>
<td>Unknown</td>
<td>14</td>
</tr>
</tbody>
</table>

| Total | 135         |

Table 2 Frequency of HRAS1 alleles and risk of epithelial ovarian cancer (based on 270 case alleles and 216 control alleles)

<table>
<thead>
<tr>
<th>HRAS1</th>
<th>a1 (%)</th>
<th>a2 (%)</th>
<th>a3 (%)</th>
<th>a4 (%)</th>
<th>Rare alleles (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>131 (0.61)</td>
<td>24 (0.11)</td>
<td>19 (0.09)</td>
<td>13 (0.06)</td>
<td>29 (0.13)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>Case patients</td>
<td>135 (0.50)</td>
<td>38 (0.14)</td>
<td>20 (0.07)</td>
<td>19 (0.07)</td>
<td>58 (0.22)</td>
<td>1.76 (1.08–2.87)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Tumor tissue included.

Table 3 Univariate ORs and 95% CIs for epithelial ovarian cancer associated with HRAS1 rare alleles (based on 135 cases and 108 controls)

<table>
<thead>
<tr>
<th>HRAS1</th>
<th>No. of cases (%)</th>
<th>No. of controls (%)</th>
<th>OR (95% CI)</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common alleles only</td>
<td>86 (0.64)</td>
<td>82 (0.76)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>One rare allele one common allele</td>
<td>40 (0.30)</td>
<td>23 (0.21)</td>
<td>1.66 (0.91–3.01)</td>
<td></td>
</tr>
<tr>
<td>Two rare alleles</td>
<td>9 (0.07)</td>
<td>3 (0.03)</td>
<td>2.86 (0.75–10.94)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Tumor tissue included.
Recent studies of genetic factors involved in susceptibility to insulin-dependent diabetes mellitus demonstrate the importance of population-based association studies, and lend support to the concept that biological significance might be attached to variations in a minisatellite locus. Specific variants of a VNTR locus adjacent to the insulin gene have been implicated in insulin-dependent diabetes mellitus, and other studies have ascribed negative regulatory activity to specific the insulin gene 5′-VNTR alleles (17).

Finally, mutations of the HRAS1-VNTR may be a marker for a propensity to mutations in similar sequences elsewhere in the genome. The mutator phenotype associated with alterations of the MSH2 gene family in hereditary nonpolyposis colorectal cancer syndrome is an example of such a phenomenon. Proliferating cell nuclear antigen is a candidate gene for VNTR instability because it destabilizes such sequences in yeast (18).

Previous studies have indicated relative stability of HRAS1 allelotypes in tumor tissue compared to blood (8). In a subset of 24 cases for which both blood and tissue were available, we observed no discordance between blood and tumor genotypes. The only evidence of somatic genetic instability in the VNTR in previous studies is allelic deletions resulting in LOH (8). In the 16 cases in which only tissue was available, tests were performed to rule out LOH. The single case in which LOH occurred was excluded from all analyses.

The use of a PCR-based methodology with fluorescent primers and size fractionations and detection on an automated sequencer appears to afford significantly better resolution of allele sizes (12). Among the subsets in this series that were typed by both methods, there was complete concordance for most cases, although the new methodology showed a greater sensitivity with a modest increase in rare HRAS1 alleles (approximately 8%) among both cases and controls.

In contrast with the current report, a single survey by O'Briant et al. (10) did not find a statistically significant association between HRAS1 rare alleles and risk of ovarian cancer. This study was based on 42 ovarian cancer cases compared to 76 age- and race-matched controls and included both African-American and Caucasian participants. Results of this study were not stratified by race. Racial variation in the frequency of rare alleles has been documented (19). Because of the small sample size in the study by O'Briant et al. (10), there was very likely insufficient power to discern a statistically significant association between rare alleles and risk of ovarian cancer among the Caucasian subgroup.

It is important to note that the cases in this series do not represent a population-based series, and therefore the generalizability of these results may be limited. The use of hospital-based controls with benign gynecological conditions may also limit the generalizability of these results. Because many of the gynecological patients seen at the New England Medical Center and Massachusetts General Hospital were referred to these hospitals for clinical trial protocols, there may be some referral bias toward a younger subset of ovarian cancer patients.

In summary, variations in the HRAS1-VNTR are associated with apparently sporadic ovarian cancer risk in the general population. Along with previous work (6) indicating an influence on penetrance of ovarian cancer in BRCA1 mutation-bearing families, this work supports the contention that the HRAS1-VNTR locus may function as a modifier of ovarian cancer risk in both sporadic and hereditary ovarian cancer.

Acknowledgments

We thank Dr. Donna Smith, Ellie Kelly, Barbara Gregoire, and Sharon Kelly for their assistance in the care of these patients and collection of clinical data and samples and Shidong Liao for excellent technical assistance.

References


Table 4 Univariate ORs and 95% CIs for epithelial ovarian cancer associated with age (based on 270 case alleles)*

<table>
<thead>
<tr>
<th>Age at diagnosis (yr)</th>
<th>a1</th>
<th>a2</th>
<th>a3</th>
<th>a4</th>
<th>Rare alleles</th>
<th>OR (95% CI)</th>
<th>Trend P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45</td>
<td>31 (0.46)</td>
<td>9 (0.13)</td>
<td>5 (0.07)</td>
<td>4 (0.06)</td>
<td>19 (0.28)</td>
<td>1.89 (0.90–3.98)</td>
<td>0.09</td>
</tr>
<tr>
<td>45–59</td>
<td>48 (0.47)</td>
<td>18 (0.18)</td>
<td>5 (0.05)</td>
<td>9 (0.09)</td>
<td>22 (0.22)</td>
<td>1.34 (0.66–2.71)</td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>56 (0.56)</td>
<td>11 (0.11)</td>
<td>10 (0.10)</td>
<td>6 (0.06)</td>
<td>17 (0.17)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
</tbody>
</table>

* Tumor tissue included.

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