The Androgen Receptor and Genetic Susceptibility to Ovarian Cancer: Results from a Case Series

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Abstract

Our objectives were to test whether polymorphic variation in the (CAG)ₙ repeat of the androgen receptor (AR) gene affects penetrance of germ-line BRCA mutations for ovarian cancer or age of diagnosis for ovarian cancer. Using a case-series study design, 179 consecutive Ashkenazi Jewish ovarian cancer patients were genotyped for AR repeat length and BRCA mutation status. There was no association between AR repeat length and presence of a BRCA mutation. However, ovarian cancer patients from both groups (with or without BRCA mutation) who carried a short AR allele were diagnosed an average of 7.2 (95% confidence interval, 2.3–12.1) years earlier than patients who did not carry a short allele (P = 0.004). These data suggest that AR allele length affects age of diagnosis of ovarian cancer, irrespective of BRCA mutation status.

Introduction

Epithelial ovarian cancer is the leading cause of mortality from gynecological cancers (1). Established risk factors for developing ovarian cancer include genetic, hormonal, and environmental influences (2), with the large majority of autosomal dominant genetic predisposition to ovarian cancer conferred by mutations in the BRCA1 or BRCA2 genes (reviewed in Ref. 3). The penetrance of BRCA mutations for ovarian cancer is incomplete, however, with estimates of lifetime risk ranging from 14 to 63%, depending on the gene, the mutation, and the population studied (4–7). Hormonal and additional genetic factors are presumed to affect BRCA penetrance, but few such modifiers have been identified to date (8, 9).

The AR gene represents a plausible candidate genetic modifier of risk for ovarian cancer. Substantial evidence supports the existence of a physiological interaction between androgen and the ovarian surface epithelium, as well as the possible role of this interaction in ovarian neoplasia. Androgen stimulates the growth of rodent ovarian epithelial cells in vivo, leading to benign ovarian neoplasms (10). Furthermore, ovarian cancer patients have higher levels of circulating androgen prior to their cancer diagnosis than women without cancer (11). Additionally, the majority of ovarian cancers express AR (12, 13) and ovarian cancer cell growth is inhibited in vitro by antiandrogens (14). Few data have been gathered regarding the specific role of the AR as a direct mediator of steroid-dependent tumorigenesis in the ovarian epithelium.

Exon 1 of the AR gene contains a polymorphic trinucleotide repeat, (CAG)ₙ, with the normal variation in repeat length ranging from 11 to 31 trinucleotide units (15). The transcriptional transactivation function of the AR protein in vitro correlates inversely with length of the polyglutamine tract encoded by this repeat (16, 17). Consistent with this functional relationship between repeat length and receptor activity are the observations that shorter repeat length is associated with an increased risk of prostate cancer as well as an earlier age of diagnosis (18, 19). The germ-line expansion of this repeat is causal for the spinal and bulbar muscular atrophy syndrome, which includes androgen insensitivity (20). More recently, a case-control study found that long AR alleles are associated with an increased risk and a younger age of breast cancer diagnosis in BRCA1 heterozygotes (21), a result consistent with the inhibitory effect of androgen on the proliferation of breast cancer cells (22, 23). These observations prompted us to test the hypotheses that polymorphic variation in the AR gene modifies penetrance of BRCA mutations for ovarian cancer, or affects the age of diagnosis for ovarian cancer generally.

Materials and Methods

Study Population. One-hundred seventy-nine consecutive Ashkenazi Jewish patients with pathologically confirmed invasive epithelial ovarian cancer from the Memorial Sloan-Kettering Cancer Center over a 12-year period were identified. Our experience at this institution is that >95% of self-described Jews are of Eastern/Central European descent (Ashkenazi), and that any bias introduced through this study design is presumed to be small. Relevant clinical and pathological data were collected for all of the patients and attached to tissue specimens obtained from the Department of Pathology; the tissue samples and associated clinical data were then anonymized. Using a case-series (or case-case) study design, the subjects in this retrospective cohort were genotyped with regard to the three deleterious founder mutations in BRCA genes that exist in this population. Genotyping was performed on nonmalignant tissue associated with each case. Those patients with BRCA mutations (n = 85) were categorized as hereditary and those without (n = 94) as sporadic. The statistical validity of the case-series study design in evaluating gene-environment or gene-gene associations has been demonstrated elsewhere (24). This study was approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center.

Laboratory Methods. After pathological review to confirm the diagnosis of invasive epithelial ovarian cancer, genomic DNA was isolated from tissues using standard procedures (25). Genotyping for germ-line mutations in BRCA1 (185delAG and 5382 insC) and BRCA2 (6174delT) was accomplished as described previously (26). Genotyping for the AR repeat was accomplished using the PCR primers 5′-TCCAGAATCTGGTTCAGAGCGTGC-3′ (forward) and 5′-GCTGTAAGGGTGTCTGGTCCAT-3′ (reverse) to generate a product of mean length, 280 bp. Each PCR was carried out in a volume of 10 μl containing 50 ng of genomic DNA, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM each dNTP, 0.8 μM each primer, and 1 unit of Taq polymerase (Perkin-Elmer). One primer was end-labeled with [γ-³²P]ATP using T4 polynucleotide kinase. Thirty-five PCR cycles were performed, each consisting of 20 s at 95°C, 20 s at 64°C, and 30 s at 72°C, followed by a 7-min extension at 72°C. The PCR products were processed by diluting 1:1 in denaturing loading buffer (95% formamide, 10 mM NaOH, 0.05% xylene cyanol FF, and 0.05% bromphenol blue), heated at 95°C for 5 min, and placed on ice. Electrophoresis of 5.5 μl of this sample was carried out in 6% polyacrylamide gels containing 7.0 M urea in Tris-borate EDTA buffer for 5 h at 80 W. The gels were dried and exposed to Hyperfilm MP autoradiography film (Amersham) for 36 h.

After autoradiography, polyacrylamide gel sections containing PCR products of varying lengths were excised and suspended in 40 μl of H₂O for 2 h at 4°C.
Two µL of eluted DNA were used as a template for PCR amplification under conditions identical to those described above, except that radiolabeled ATP was excluded. Each PCR product was electrophoresed in its entirety in NuSieve 3:1 agarose (FMC BioProducts), visualized with ethidium bromide, excised from the gel, and purified using a Qiaex II gel extraction kit (Qiagen). Two ng of each DNA template were subjected to sequence analysis using the Thermo Sequenase radiolabeled terminator cycle sequencing kit (USB Corp.) and the primer, 5’-AGAGGCAGGC-AGGCACACACCTC-3’. After the sequencing reactions, 4 µL of stop solution were added to 7 µL of each termination reaction and heated to 70°C for 10 min. Electrophoresis of 5.5 µL was carried out in 6% polyacrylamide gels containing 7.0 M urea in Tris-borate EDTA buffer. After electrophoresis at 80 W for 2.5 h, gels were dried and subjected to autoradiography as above.

To determine the length of the CAG repeat in each allele, DNA samples with known repeat lengths, as determined by direct sequence analysis, were ordered in a fixed sequence on both sides of the unknown DNA samples and electrophoresed as described above (Fig. 1).

**Statistical Analyses.** The distributions of the shorter, longer, and average AR allele lengths in the hereditary and sporadic ovarian cancer groups were compared as continuous variables with Student’s t test. Relationships between allele length and age were assessed with the Pearson correlation coefficient. Linear regression analysis was used to determine the strength of association between the age at diagnosis and AR allele length. Length was also analyzed as a dichotomous variable, and significance was assessed with the χ² test or the Fisher’s exact test, where appropriate. Statistical significance was determined according to the conventional P of less than 0.05. Odds ratios were calculated for significant associations. All of the analyses were two-sided and performed using the SPSS statistical software (SPSS, Inc.).

**Results**

Eighty-five patients with one of three founder mutations in BRCA1 or BRCA2 (hereditary group) and 94 patients without a BRCA mutation (sporadic group) were included for study. The mean age of the women was 55 ± 11 (range, 30–79) and 64 ± 12 (range, 25–87) years for the hereditary and sporadic groups, respectively. The clinical and pathological features of these cases are reported elsewhere (26). There were no significant differences in the number of repeats on the shorter or longer allele between the hereditary and sporadic case groups (Table 1). The mean repeat length (in terms of CAG units) was 18.9 (95% CI, 18.3–19.4) for the hereditary group and 19.3 (95% CI, 18.7–19.9) for the sporadic group (P = 0.27). Results were similar when repeat length was analyzed as a continuous or dichotomous variable. The sample size under investigation was associated with 80% power to detect a difference in average allele length of 1.2 trinucleotide repeat units.

In regard to age at diagnosis, initial analysis revealed a positive correlation between average AR allele length and age at diagnosis for all of the ovarian cancer cases, both hereditary and sporadic. For a one-unit increase in average allele length, the age at diagnosis was increased by 0.7 year (95% CI, 0.01–1.35; P = 0.046). Based on this association between average AR allele length and age at diagnosis, we examined the relationship between individual AR allele length and age of ovarian cancer diagnosis. The initial dichotomous analysis revealed a weak association between the presence of an allele with the median number of repeats (≤19), and age at diagnosis (P = 0.16). However, for each allele 19 repeat units or less, the average age at diagnosis decreased by 2.8 years (95% CI, 0.4–5.3; P = 0.023). The data in Table 2 demonstrate that the relationship between allele length and age at diagnosis can be affected by the modeling of allele lengths.

To test whether shorter allele length cutoff points were associated with an even greater difference in age at diagnosis, the association between age at diagnosis and the presence of a short allele, here defined as less than 15 repeat units, was examined. Patients with at least one short allele were diagnosed with ovarian cancer an average of 7.2 years earlier than patients with only long alleles (95% CI, 2.3–12.1; P = 0.004). For patients diagnosed earlier than age 45, the odds ratio associated with the presence of a short allele was 3.5 (95% CI, 1.3–9.3; P = 0.015), and was 3.6 (95% CI, 1.6–8.2; P = 0.001) for patients diagnosed earlier than age 55.

**Discussion**

In this case-series analysis, no evidence was found for an association between length of the polymorphic AR trinucleotide repeat and germ-line BRCA mutation status in ovarian cancer patients; thus, the hypothesis that AR acts as a modifier of BRCA penetrance for ovarian cancer was rejected. These findings contrast with those from a recent report suggesting that AR alleles with longer repeat lengths may increase BRCA penetrance for breast cancer (26). It is noteworthy that among all of the candidate genetic modifiers of BRCA penetrance for breast or ovarian cancer for which a positive association has been reported, none has been found to affect penetrance for both tumor types. Rare alleles of the HRAS1 locus increase BRCA1 penetrance for ovarian but not for breast cancer (8), and the APC I1307K allele increases penetrance of BRCA mutations for breast but not for ovarian cancer (27, 28). The data reported herein add to the growing body of

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**Table 1**  
Age and AR allele length in study population

<table>
<thead>
<tr>
<th></th>
<th>Hereditary (n = 85)</th>
<th>Sporadic (n = 94)</th>
<th>Total (n = 179)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55.5 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.9 ± 12.3</td>
<td>59.9 ± 12.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Short allele&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.0 ± 3.4</td>
<td>17.5 ± 3.2</td>
<td>17.2 ± 3.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Long allele</td>
<td>20.7 ± 3.0</td>
<td>21.2 ± 3.5</td>
<td>21.0 ± 3.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Average of alleles</td>
<td>18.9 ± 2.7</td>
<td>19.3 ± 2.8</td>
<td>19.1 ± 2.8</td>
<td>0.27</td>
</tr>
</tbody>
</table>

<sup>a</sup> In years.  
<sup>b</sup> Mean ± SD.  
<sup>c</sup> Allele length expressed as number of repeat units.
as recently demonstrated for the relationship exists, it is likely to involve a modest relative risk, such rather, we demonstrated that the average age of ovarian cancer diagnosis between germ-line polymorphic variation in the is associated with an earlier age of diagnosis for ovarian cancer. BRCA penetrance for breast and ovarian cancer.

AR

hormone-related tumor types, for which a relationship between the distribution has been established for lengths at which function is meaningfully altered, possibly reflecting a continuum of protein activity in relation to allele length, as observed in vitro (16, 17). In support of this concept are data derived from the study of two other hormone-related tumor types, for which a relationship between the age of diagnosis and AR repeat length has been demonstrated. For prostate cancer, a shorter AR allele length is associated with an earlier age of diagnosis (19), consistent with the stimulatory effect of androgen on cell proliferation in this organ. For breast cancer, a longer AR allele length is associated with an earlier age of diagnosis (21), consistent with an inhibitory effect of androgen on breast epithelial proliferation.

The data reported here for ovarian cancer, in which a shorter allele length is associated with an earlier age of diagnosis, are consistent with a substantial body of literature supporting a hypothetical model in which the risk of ovarian cancer is increased by factors associated with excess androgenic stimulation of ovarian epithelial cells (29). Using the case-series study design, we were not able to assess the risk conferred by short AR alleles in developing ovarian cancer per se; rather, we demonstrated that the average age of ovarian cancer diagnosis was significantly younger in women with at least one short AR allele, regardless of hereditary or sporadic classification. A case-control analysis will be required to examine the potential role of short AR alleles in genetic predisposition to ovarian cancer. If such a relationship exists, it is likely to involve a modest relative risk, such as recently demonstrated for the HRAS1 locus and ovarian cancer (30), placing AR in the “low penetrance” category of cancer predisposition genes.

References


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