Short Communication

The HRAS1 Variable Number of Tandem Repeats and Risk of Breast Cancer

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Abstract

Rare alleles at the HRAS1 variable number of tandem repeats (VNTRs) locus have been implicated in breast cancer risk. We assessed the association of rare HRAS1 alleles and breast cancer in a case-control study nested within the Nurses’ Health Study cohort. Using PCR-based methods, 717 incident breast cancer cases and 798 controls were genotyped for the HRAS1 VNTRs. The prevalence of the rare alleles in breast cancer cases was not different compared with controls (10.7 versus 12.0%, respectively; P = 0.45, two-sided Cochran-Mantel-Haenzel χ² test). There was no evidence that women heterozygous (multivariate odds ratio, 0.97; 95% confidence interval, 0.73–1.27) or homozygous (multivariate odds ratio, 0.83; 95% confidence interval, 0.32–2.14) for rare alleles were at an increased risk of breast cancer or that a positive gene-dose effect existed. The results did not vary by menopausal status. Although as a group the rare alleles were not associated with breast cancer, one class of rare alleles between the common alleles of a3 and a4 was associated with a significantly increased risk. These results suggest that there is no overall association between rare alleles of the HRAS1 VNTR and breast cancer.

Introduction

Rare alleles of a HRAS1 minisatellite are suspected to be associated with risk of a number of cancers, including breast cancer (1). The HRAS1 proto-oncogene contains a variable number of tandem repeats (VNTRs) 1 kb downstream of the polyadenylation site. This minisatellite is highly polymorphic, composed of a 28-bp unit, which is repeated in tandem 26–110 times (2, 3). There are four common alleles, reported to make up 94% of the alleles in Caucasian populations, and >30 rare alleles (2). The function of the VNTRs with respect to cancer risk is unclear, although it is hypothesized to function as a transcriptional regulator (4).

A meta-analysis of eight studies examining the relationship between this HRAS1 VNTR and breast cancer reported a >2-fold increased risk among rare allele carriers (5). Based upon this analysis of 694 cases and 937 controls, this polymorphism could explain up to 9.2% of breast cancers, more than BRCA1 or BRCA2 (5, 6). However, these earlier studies have suffered from a number of limitations, including technical genotyping problems and the potential for selection bias, warranting additional investigations of this minisatellite and breast cancer (7). A more recent population-based case-control study, using a similar PCR-based genotyping technique, reported no association between rare alleles and breast cancer among women under the age of 40 years (8).

Materials and Methods

We examined the role of this HRAS1 minisatellite and breast cancer in the Nurses’ Health Study. Detailed information on the nested case-control study population has been described previously (9). This study was approved by the Committee on Human Subjects at Brigham and Women’s Hospital. This nested case-control study consists of 717 pathologically confirmed incident breast cancer cases and 798 controls.

HRAS1 genotyping was conducted using PCR/automated sequencer-based methods described by Ding et al. (3). Four common alleles have been previously identified as a1, a2, a3, and a4, having repeats of 30, 46, 68, and 84 units, respectively. The genotyping method used in this study is able to accurately assess repeat lengths to a single unit. Previous methods, relying on restriction enzyme digestion and Southern blotting, are unable to resolve allele lengths with the same accuracy (10). It is unlikely that older methods could have resolved alleles especially at higher molecular weights, primarily those close in size to the common a3 and a4 alleles. To maintain exposure classifications, which are consistent with and comparable with the previous body of literature, we have categorized alleles that are one repeat unit greater or smaller than the a3 and a4 alleles as common alleles of these two classes, respectively.

Original classification schemes of the HRAS1 alleles combined all rare alleles together, treating them as one homogeneous group, which may not necessarily be the case. The group of rare alleles encompasses a diverse collection of alleles with a wide range of repeat lengths (26–96 repeat units). Recent studies have demonstrated that rare alleles of the HRAS1 VNTRs are derived from the nearest common allele (3, 11, 12) and that there is sequence variation with respect to the repeat units (3). To assess if these rare alleles confer different risks depending on its progenitor, we have also categorized rare alleles as unique classes based upon their lengths in relation to the com-
Table 1 describes the risk of breast cancer by HRAS1 allele classes and genotypes (raw data are available on line). Overall, the rare alleles were not associated with an increased risk of breast cancer (multivariate odds ratio, 0.95; 95% confidence interval, 0.75–1.21). There was evidence that the rare allele class between common alleles a3 and a4 was associated with an increased risk of breast cancer (multivariate odds ratio, 3.06; 95% confidence interval, 1.14–8.21). There was no evidence that women heterozygous or homozygous for rare alleles were at an increased risk of breast cancer or that a positive gene-dose effect existed (Table 1). The results did not vary by menopausal status.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Repeat numbers</th>
<th>Case alleles</th>
<th>Control alleles</th>
<th>Odds ratio (95% confidence interval)</th>
<th>Multivariate odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common a1</td>
<td>30</td>
<td>842</td>
<td>918</td>
<td>1.00 (Ref)</td>
<td>1.00 (Ref)</td>
</tr>
<tr>
<td>Common a2</td>
<td>46</td>
<td>159</td>
<td>171</td>
<td>1.03 (0.82–1.31)</td>
<td>1.10 (0.86–1.41)</td>
</tr>
<tr>
<td>Common a3</td>
<td>67–69</td>
<td>150</td>
<td>168</td>
<td>1.00 (0.79–1.27)</td>
<td>1.07 (0.83–1.37)</td>
</tr>
<tr>
<td>Common a4</td>
<td>83–85</td>
<td>130</td>
<td>148</td>
<td>0.99 (0.76–1.28)</td>
<td>1.05 (0.80–1.37)</td>
</tr>
<tr>
<td>Rare &lt; a1</td>
<td>26–29</td>
<td>18</td>
<td>24</td>
<td>0.77 (0.41–1.42)</td>
<td>0.73 (0.38–1.41)</td>
</tr>
<tr>
<td>Rare a1–a2</td>
<td>31–45</td>
<td>87</td>
<td>125</td>
<td>0.80 (0.60–1.07)</td>
<td>0.88 (0.64–1.19)</td>
</tr>
<tr>
<td>Rare a2–a3</td>
<td>47–66</td>
<td>28</td>
<td>30</td>
<td>1.13 (0.66–1.93)</td>
<td>1.26 (0.72–2.20)</td>
</tr>
<tr>
<td>Rare a3–a4</td>
<td>70–82</td>
<td>17</td>
<td>7</td>
<td>3.60 (1.38–9.38)</td>
<td>3.06 (1.14–8.21)</td>
</tr>
<tr>
<td>Rare &gt; a4</td>
<td>86–96</td>
<td>3</td>
<td>5</td>
<td>0.47 (0.09–2.45)</td>
<td>0.37 (0.06–2.19)</td>
</tr>
</tbody>
</table>

**Table 1.** Association between HRAS1 variable number of tandem repeat alleles and genotypes and breast cancer in the Nurses’ Health Study, 1989–1996

- a Conditional logistic regression estimates adjusted for matching variables: age; menopausal status; postmenopausal hormone use; date of blood draw; time of blood draw; and fasting status at blood draw.
- b Conditional logistic regression estimates adjusted for matching variables and age at menarche (<12, 12, 13, >13 years), age at menopause (±45, 46–50, 51–60 years), first degree family history of breast cancer (yes/no), history of benign breast disease (yes/no), weight gain since age 18 (±5, ±5 to <20, ≥20 kg), body mass index at age 18 (continuous), age at first birth/parity (nulliparous, one to four children age at first birth ≤24 years, five or more children age at first birth ≤24 years, five or more children age at first birth ≥24 years), and duration of postmenopausal hormone use (premenopausal, never, past user <5 years duration, past user ≥5 years duration, current user <5 years duration, current user ≥5 years duration).
- c Premenopausal and postmenopausal women do not add to the total number of women because women with uncertain menopausal status at diagnosis were excluded.
- d Unconditional logistic regression adjusted for the matching variables.
- e Unconditional logistic regression adjusted for the matching variables and body mass index at age 18 years, weight gain since age 18 years, age at menarche, history of benign breast disease, first degree family history, and age at first birth/parity.
- f Unconditional logistic regression adjusted for the matching factors, the factors above, age at menopause, and duration of postmenopausal hormone use.

Common alleles. Table 1 indicates the categories of allele groups and the repeat lengths they encompass.

Odds ratios and 95% confidence intervals were calculated using conditional and unconditional logistic regression. Interactions between genotype and breast cancer risk factors were assessed by including interaction terms between genotype and risk factors in multivariate models. Statistical significance was assessed by conducting likelihood ratio tests. All statistical tests are two sided.

**Results**

The frequency of rare HRAS1 alleles was not significantly different between cases and controls (10.7 versus 12.0%, \( P = 0.45 \)). There was no evidence that genotypes deviated from Hardy-Weinberg equilibrium among the controls (\( \chi^2 \) goodness of fit, \( P = 0.89 \)). The frequency of rare alleles among the controls is comparable with the reported frequency (13%) using the same genotyping method in a United States Caucasian population (13).

**Internet address:** http://www.channing.harvard.edu/nhs/pub.html#hras2003.
HRAS1 vary by race and ethnicity (14, 15). In this case-control study, 7 women self-reported themselves as African American, 2 as Hispanic, and 2 Asian with rare allele frequencies of 14.3, 75, and 0%, respectively. Analyses excluding these women did not change the interpretation of the results.

We evaluated the relationship between HRAS1 rare alleles and breast cancer according to established risk factors. There was no significant interaction between rare alleles and body mass index (P = 0.71), parity (P = 0.54), age at first birth (P = 0.40), age at menarche (P = 0.10), age at menopause (P = 0.36), family history of breast cancer (P = 0.59), postmenopausal hormone use (P = 0.11), or benign breast disease (P = 0.73).

It has been suggested that HRAS1 rare alleles may be associated with hormone receptor positivity, primarily among black and younger women (14). In case-only analyses, we did not find an association between HRAS1 rare alleles and hormone receptor status (data are available on line); in fact, rare alleles were nonsignificantly associated with hormone receptor positivity in premenopausal women.7

Discussion

In this prospective nested case-control study, rare HRAS1 alleles as a group were not associated with breast cancer risk. Only rare HRAS1 alleles between the two largest common alleles, a3 and a4, were significantly associated with risk of breast cancer. Although expansions leading to large minisatellites are most often the ones associated with human disease (16), both large and small rare HRAS1 alleles had previously been associated with breast cancer in prevalent case-control studies, suggesting that the observed association with this class of alleles is likely due to chance.

Recent studies using PCR with polyacrylamide gel methods have been able to resolve alleles to a single repeat unit. Studies using this genotyping method have estimated the frequency of rare alleles among controls to be ∼12%, nearly two to three times greater than original studies had indicated (8, 13). Earlier studies, relying on Southern blot methods and visual sizing of alleles, were unable to resolve alleles as definitively as the newer automated methods. Firgaira et al. (8) used similar genotyping methods to the ones used in this study and reported no association of rare alleles with premenopausal breast cancer. Also, a study of rare HRAS1 allele sharing in siblings with breast cancer that uses PCR/acrylamide typing methods shows no evidence of linkage of the HRAS1 locus to breast cancer risk.8

The likely misclassification of alleles in earlier studies may explain the discrepancy in study results between those using newer genotyping techniques and earlier studies. The general lack of evidence for incident breast cancer risk in the present study may also be due to a potential bias of case ascertainment in previous studies with prevalent cases, if rare allele carrier status was associated with longer survival. Alternatively, given that all prevalence studies were conducted at tertiary academic medical centers, rare alleles may be associated with later stage and/or higher grade cancers. Whatever the basis for this discrepancy, however, our results indicate that rare HRAS1 alleles will not serve reliably as a genetic marker for incident breast cancer risk.

References


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