We investigated the frequency of the HER2 Val655Ile polymorphism in a British-based case–control study. Patients with breast cancer (n = 315) were selected who had an age at disease onset of under 40 years, a family history of breast cancer irrespective of age at onset, or bilateral breast cancer irrespective of family history of breast cancer or age at onset. Women with incident cases of ovarian carcinoma (n = 314) were ascertained from those undergoing primary surgery for the disease in hospitals in southeast England from 1993 through 1998. Control subjects (n = 256) were all white female volunteers who either were staff members at the Princess Anne Hospital (n = 125) or were patients at the Princess Anne Hospital being treated for non-neoplastic disease conditions (n = 131). The mean age of the women in the breast cancer, ovarian cancer, and control groups were 38, 62, and 39 years, respectively. The Val655Ile polymorphism was analyzed by use of an allele-specific polymerase chain reaction (PCR). The PCR used red and green fluorescently labeled forward primers (5’-CAGCCCTCTGACGTCCATCG-3’ and 5’-CAGCCCTCTGACGTCCATCA-3’) and a common reverse primer (5’-TGCGGAGACTGCTGCAGGAAAC-GGA-3’). The alleles were separated by electrophoresis through 6% denaturing polyacrylamide gels and analyzed with a scanning laser fluorescence imager. Genotyping was confirmed for 100 patients with a PCR and restriction fragment length polymorphism assay described previously (7).

The frequency of the valine allele was similar to that reported by Ameyaw et al. (7), and the genotype frequencies among the cancer and control groups were not statistically significantly different from those expected from Hardy–Weinberg equilibrium. We did not observe any statistically significant differences in the valine allele frequency or in the genotype distribution in the cancer groups compared with the control group (Table 1). When the ovarian cancers were stratified according to histologic subtype, stage, and grade, no statistically significant differences were observed (data not shown). We conclude that the HER2 valine allele does not represent a breast or ovarian cancer risk allele, at least in a British population.

The association of the valine allele with breast cancer risk reported by Xie et al. (6) was strongest among women diagnosed with breast cancer under 45 years of age. We studied predominantly patients with early-onset breast cancer (mean age = 38 years), so that a difference in the age distribution is unlikely to offer an explanation for the discrepancy. Whether the association reported by Xie et al. reflects a true ethnic variation in the penetrance of the valine allele or a type I statistical error warrants further investigation.

**References**

5. Papewalis J, Nikitin AYu, Rajewsky MF. G to A polymorphism at amino acid codon 655 of HER2, a growth factor receptor 2 (HER2) is a transmembrane glycoprotein with tyrosine kinase activity and, among other functions, controls cellular proliferation. Overexpression of HER2 has been observed in many solid tumors, including 20%–30% of breast cancers (1) and 19%–59.4% of epithelial ovarian cancers (2), and is a predictive marker for response in breast cancer (3,4).

A valine-to-isoleucine polymorphism at codon 655 (Val655Ile) (5) was reported in the Journal (6) to be associated with an increased risk of breast cancer among a Chinese population. Subsequently, Ameyaw et al. (7) reported in the Journal that the distribution of the polymorphism varied considerably between ethnic groups, with the valine allele being detected in 20% of Caucasians but absent from an African population. They raised the possibility that any influence of the valine allele on cancer susceptibility may vary considerably between different ethnic populations.

Table 1. Summary of genotyping for the valine-655 to isoleucine (Val655Ile) polymorphism in U.K. subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>No. with specified genotype (%)</th>
<th>Valine allele frequency</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ile/Ile</td>
<td>Val/Ile</td>
<td>Val/Val</td>
</tr>
<tr>
<td>Control</td>
<td>256</td>
<td>138 (53.9)</td>
<td>101 (39.4)</td>
<td>17 (6.6)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>315</td>
<td>190 (60.3)</td>
<td>109 (34.6)</td>
<td>16 (5.1)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>314</td>
<td>183 (58.3)</td>
<td>107 (34.1)</td>
<td>24 (7.6)</td>
</tr>
</tbody>
</table>

*P values were calculated by combining valine homozygotes and valine/isoleucine heterozygotes and using Fisher’s exact test (two-sided).


NOTES

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RESPONSE

We read with interest the letter by Drs. Baxter and Campbell regarding the association between HER2 genetic polymorphism and breast cancer risk. Drs. Baxter and Campbell found in a hospital-based, case-control study conducted among British women that genotype frequencies of the HER2 gene at codon 655 (Ile to Val) were similar between case patients with breast or ovarian cancer and control subjects. This finding was inconsistent with our finding in a population-based, case-control study conducted among Chinese women (1). In that study, we found that the Val/Val genotype was associated with an elevated risk of breast cancer, particularly among younger women (1).

We concur with Drs. Baxter and Campbell that the reported association between the valine allele and breast cancer risk warrants further investigation. As with any association studies of gene polymorphisms and cancer risk, we cannot rule out the possibility of type I error and population stratification effect on our study results. Ameyaw et al. (2) reported recently a large interracial variation in the allele frequency of the HER2 gene. Such a variation is unlikely to exist in our study population, because more than 95% of Shanghai residents belong to a single ethnic group (Han Chinese). As demonstrated by Wacholder et al. (3), population stratification is unlikely to bias substantially the results from a well-designed case-control study of genetic factors, particularly the one conducted in a relatively homogenous population with a similar ethnic background and cancer risk.

Although our reported study of HER2 polymorphism and breast cancer risk was conducted with a prior hypothesis, we do have some concern about the possibility of type I error. To evaluate this possibility, we recently completed a small case-control study to examine the association between HER2 polymorphism and the amplification of this gene in breast cancer tissue samples with the technique of fluorescence in situ hybridization. Paraffin-embedded tissue sections were obtained from 134 breast cancer patients, including 65 with the Ile/Ile genotype, 56 with the Ile/Val genotype, and 13 with the Val/Val genotype. Tissue-section slides were hybridized with DNA probes for the HER2 gene and the centromere of chromosome 17, and 100 cells in each sample were scored for the signals of these two markers. Any tumor tissue sample with an average oncogene amplification ratio of 2.0 or higher for the signal counts of the HER2 gene over the centromere of chromosome 17 was considered to be HER2 amplified. The amplification of the HER2 gene was found in seven (53.8%) of the 13 case patients with the Val/Val genotype, 24 (42.9%) of the 56 case patients with the Ile/Val genotype, and 21 (32.3%) of the 65 case patients with the Ile/Ile genotype (two-sided trend test, \( P = .095 \)). Although this study did not directly evaluate the functional importance of the polymorphism and the association was only of borderline statistical significance, the direction of the association was consistent with the finding reported from our early study for the association between HER2 polymorphism and breast cancer risk.

Recently, we also evaluated the association between HER2 polymorphism and breast cancer risk in a small case-control study conducted within a cohort of postmenopausal women from the state of Iowa (4). Included in the study were 154 case patients with breast cancer diagnosed among cohort members from 1992 through 1994 and 325 cohort members who were free of cancer during the study period. Blood samples from these subjects were obtained and assayed for the Val\(^{655}\)Ile polymorphism of the HER2 gene by use of the polymerase chain reaction and restriction fragment length polymorphism described previously (1). The frequencies of the Ile/Ile, Ile/Val, and Val/Val genotypes were 58.4%, 38.3%, and 3.3%, respectively, in case patients and 56.9%, 38.8%, and 4.3%, respectively, in control subjects (test for difference, \( P = .84 \)). These genotype frequencies were close to those reported by Drs. Baxter and Campbell, suggesting no association between HER2 polymorphism and breast cancer risk among postmenopausal Iowa women.

The reasons for the inconsistent results from studies conducted among Chinese and Caucasian women are not clear, and well-designed larger studies will be needed. It is possible that the Val\(^{655}\)Ile polymorphism per se may not be functionally significant. As we indicated in our original article (1), this polymorphism might be in linkage disequilibrium with the functional allele(s) at other site(s) in the Chinese population and a closely linked nonfunctional allele may also be useful in identifying high-risk women for breast cancer prevention. We agree that it is premature to conclude on the association between HER2 genetic polymorphism and breast cancer risk based on the limited number of studies conducted thus far. Currently, we are in the process of genotyping more case patients and control subjects for the polymorphism of the HER2 gene and will evaluate the association of this polymorphism with breast cancer in a larger study.

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REFERENCES


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