Chenevix-Trench et al. (1) reported two ATM gene alterations associated with a risk of breast cancer in multiple-case families. The T7271G mutation was detected in one patient with breast cancer of 525 examined who were unselected for a family history of breast cancer and in four more affected family members (1). This transversion was previously found in two families with mild ataxia-telangiectasia (A-T) and a history of breast cancer (2) and in a sporadic T-cell leukemia (3). ATM IVS10-6T→G was not found in 262 unselected patients with breast cancer but was found in two of 76 index patients from multiple-case families with breast cancer (1) and, previously, in patients with A-T and in patients with breast cancer (4,5). Expression and activity analyses of ATM in heterozygous cell lines indicated a dominant negative effect for both mutations (1), a mechanism distinct from that previously proposed for ATM-mediated tumorigenesis in a sporadic T-cell leukemia (3), where the mutation pattern and the lack of wild-type alleles in most mutated samples were consistent with ATM acting as a tumor suppressor gene.

We examined germline DNA from patients with breast cancer and from control subjects in two European populations for the presence of both mutations (Table 1). The samples were ascertained as described (6,7). Genotyping was carried out by polymerase chain reaction, as reported (1), except for the forward primer [5'-TGAAAAGAGC-CAAAaAGGAAAG], the underlined nucleotide was guanine in (1)] in the T7271G assay. The amplified products and positive control DNA samples carrying T7271G (3) and IVS10-6T→G (a gift from T. Dörk, Medical School, Hannover, Germany) were digested with restriction enzymes (1) and separated on agarose gels.

Although we did not detect the T7271G mutation among 612 patients, the IVS10-6T→G mutation was identified in one (0.3%) of 339 unselected Swedish patients and one (0.7%) of 138 selected Swedish familial patients without a detectable BRCA1/BRCA2 mutation (Table 1). We also found one (1.1%) such individual among 88 Czech female blood donors. The overall frequency of the IVS10-6G allele was 0.1%. No homozygote was found for either mutation. We also examined tumor DNA from the familial patient for allelic imbalance at the IVS10-6T→G polymorphism but found no loss of heterozygosity (LOH), consistent with the proposed dominant negative effect of the mutation. No DNA sample was available from the remaining family members.

If the loss of ATM alleles confers a detectable risk of breast cancer, then one would expect to see enrichment for ATM mutation/inactivation in tumor DNA samples with LOH at 11q22–q23. We selected 42 tumor DNA samples with LOH at 11q22–q23 from a larger number of paired normal and breast cancer DNA samples by use of polymorphic markers at and around ATM (D11S2179, D11S1778, D11S1391, D11S1347, D11S1345, D11S922, and D11S1318). We analyzed this material for mutations in exons 50–66 with single-strand conformation polymorphism, as described (3). No mutation was observed, including T7271G and IVS10-6T→G.

Additional studies in independent populations will help clarify the role of

<table>
<thead>
<tr>
<th>Population</th>
<th>ATM T7271G, No. detected/total No.</th>
<th>ATM IVS10-6T→G, No. detected/total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swedish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case patients</td>
<td>0/340</td>
<td>2/477 (0.4)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0/469</td>
<td></td>
</tr>
<tr>
<td>Czech</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case patients</td>
<td>0/272</td>
<td>0/291 (0.0)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>1/88 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case patients</td>
<td>0/612</td>
<td>2/768 (0.3)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>1/557 (0.2)</td>
<td></td>
</tr>
</tbody>
</table>
the two mutations as breast cancer risk modifiers, but we still need to reconcile a clear predominance of frame shift mutations among A-T alleles [2, 3] and references therein] and a putative lack of chain-terminating heterozygous mutations in the germline of patients with breast cancer in the context of original epidemiologic studies indicating an increased risk of breast cancer among A-T heterozygotes. In addition, more accurate proportions of mutations altering the correct splicing of ATM and dominant negative mutations remain to be shown in A-T.

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REFERENCES


NOTES

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RESPONSE

Lei et al. have determined the frequency of two ATM mutations in a large number of unselected and familial breast cancer case patients, and random control subjects, from Sweden and the Czech Republic. The IVS10–6→G mutation was identified in two case patients of a total of 768 case patients (one of whom had a family history of breast cancer) and one control subject of a total of 557 control subjects, confirming the fact that it is a relatively common mutation in Caucasians. Although it is interesting to note that the IVS10–6→G mutation occurs in the Swedish and Czech populations, this observation does not contribute to the estimation of the penetrance of the IVS10–6→G mutation, because no family members of the affected case patients were available for genotyping, and the personal and family breast cancer history of the control carrier is not known. However, these data emphasize that to estimate penetrance with any precision, it is necessary to carry out such studies in multiple-case families in which many DNA samples are available from unaffected and affected family members or in a large population-based series of case families with similar DNA sampling. If the variant is likely to confer only low risks for breast cancer, well-characterized control subjects will also need to be studied.

The authors also use single-strand conformation polymorphism analysis to examine 42 tumors for ATM mutations that were selected for having loss of heterozygosity (LOH) around the ATM locus, and they found no somatic mutations. However, if breast cancer-causing ATM mutations act as dominant negative mutations, as our functional data on T7271G and IVS10–6T→G suggest (1), then this null result is not surprising. Mutation analysis of breast tumors without LOH might uncover somatic mutations although, of course, for reasons that are not clear, such mutations are exceedingly rare in BRCA1 and BRCA2 genes and would, therefore, not necessarily be expected in ATM (2). As Lei et al. suggest, the mechanism of action of ATM mutations may be different in T-cell leukemia, where both alleles appear to be inactivated (3), compared with breast tumors where a dominant negative effect on BRCA1 phosphorylation may be critical.

Finally, we thank Lei et al. for pointing out the error in our published primer sequence.

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