

ABSTRACT

In order to explore the possible role of E-cadherin in familial cancer, 19 familial breast cancer patients, whose tumours demonstrated loss of heterozygosity (LOH) at the E-cadherin locus, were screened for germline mutations. No pathogenic germline alterations were detected in these individuals. However, a somatic mutation was found (49-2A→C) in one of the tumours. This tumour showed a pattern of both ductal and lobular histology. Another 10 families with cases of breast, gastric and colon cancer were also screened for germline mutations, and no mutations were found. A missense mutation in exon 12 of E-cadherin (1774G→A; Ala592Thr) was previously found in one family with diffuse gastric cancer, and colon and breast cancer. An allelic association study was performed to determine whether the Ala592Thr alteration predisposes to breast cancer. In total, we studied 484 familial breast cancer patients, 614 sporadic breast cancer patients and 497 control individuals. The frequencies of this alteration were similar in these groups. However, a correlation between the Ala592Thr alteration and ductal comedo-type tumour was seen. These results, together with previously reported studies, indicate that germline mutations and, more commonly, somatic mutations in E-cadherin may have an influence on the behaviour of the tumours, rather than predispose to breast cancer.

INTRODUCTION

Synopsis Introduction: E-cadherin (CDH1) is expressed on the cell surface in most epithelial tissues, and evidence is rapidly accumulating that the mammalian E-cadherin gene product plays a role in epithelial tumourigenesis. Germline mutations in E-cadherin have been described in families with early-onset diffuse gastric cancer, and loss of function of this gene has been implicated in the pathogenesis of early-onset colorectal and breast cancers. Despite the fact that E-cadherin expression is decreased in 50% of invasive ductal carcinoma, mutations in the coding sequence of E-cadherin have not been observed in this type of breast cancer. The role of E-cadherin mutation in development of hereditary gastric cancer has been shown, but its role in predisposing to breast cancer is still unproved. In order to further explore the involvement of E-cadherin in breast cancer, we examined all 16 exons of E-cadherin in 31 familial breast cancer patients, in whom BRCA1 and BRCA2 had been excluded as predisposing genes. In a previous study of a family with diffuse gastric cancer (Fig. 1a) (Salahshor S, et al, manuscript submitted), we identified an E-cadherin germline mutation that cosegregated with the disease. This missense mutation in exon 12 (Ala592Thr) was also detected in the index patient's mother, who had ductal breast cancer. In an attempt to clarify a possible role for the Ala592Thr alteration in predisposing to breast cancer, we screened for this specific alteration in different series of breast cancer patients and control individuals. In total, 1328 patients with sporadic or familial breast cancer and 497 control individuals were analyzed for this specific alteration. **Materials and method:** Nineteen probands with familial breast cancer who demonstrated LOH at chromosome 16q, and 12 patients from 10 families with breast, gastric and colorectal cancers were screened for germline mutations in the coding sequences of E-cadherin. Nine tumours from the 19 breast cancer cases were also screened for somatic mutations. The frequencies of the 1774G→A variant (Ala592Thr) were determined in DNA extracted from the blood of 358 unrelated probands with familial breast cancer, 214 unrelated early-onset breast

cancer patients (age of onset <41 years), 126 unrelated BRCA1 or BRCA2 carriers, and 604 unselected breast cancer patients and 497 control individuals, who were considered to represent the general population in Sweden and Norway. Two markers D16S7/p79-2-23 and APRT/HUAP15, which both map to 16q24.3, were used to identify LOH, as in a previous study of allelic loss at 16q in familial tumours. Single-stranded conformation polymorphism (SSCP) and denaturing high-performance liquid chromatography (DHPLC; Transgenomic, Santa Clara, CA, USA) were used to screen 16 exons of the E-cadherin gene for the presence of alterations (Table 1). We used the same primers as described by Berx et al and Salahshor et al (manuscript submitted), but with some modification. Samples that exhibited aberrant bands on the SSCP gel or an altered DHPLC pattern were sequenced by reamplifying the corresponding exon. Sequencing was performed either manually using the ThermoSequensase (Amersham Pharmacia Biotech, Uppsala, Sweden) kit, or by 377 ABI automated sequencer using the Taq-Cycle sequencing BigDye Terminator kit (Applied Biosystems Inc ABI, Stockholm, Sweden), according to conditions recommended by the manufacturers. The Ala592Thr variant frequency was detected using specific polymerase chain reaction (PCR)-restriction fragment length polymorphism tests (Salahshor S, et al, manuscript submitted). Results: No pathogenic mutations were identified in the 12 patients from families with documented breast, gastric or colon cancer. We also searched for germline alterations in 19 individuals with familial breast cancer who showed LOH at the E-cadherin locus in their tumours. Nine tumours from these 19 patients were also tested for somatic alterations in the E-cadherin gene. One somatic mutation (49-2A→C) was found in one of the lobular cancer cases (Fig. 2). One previously reported common polymorphism in E-cadherin was found in exon 13 at codon 692 (GCC→GCT). In a previous study in one family with familial diffuse gastric cancer and colon cancer (Fig. 1a) (Salahshor S, et al, manuscript submitted), we found a germline missense mutation in exon 12 (Ala592Thr) of E-cadherin. This alteration cosegregated with diffuse gastric cancer and colorectal cancer in this family, although the penetrance was not complete. The alteration was present in the index patient's mother, who had ductal breast cancer of comedo type at the age of 65 years (Fig. 1a, II:12). In order to investigate whether this alteration was associated with an increased risk for breast cancer, we used a specific restriction enzyme digestion/PCR test to detect the variant, in an allelic association study in familial and sporadic breast cancer cases, as well as control samples that represented the general population. We found the mutation in two out of 358 (0.56%) non-BRCA1/BRCA2 carrier women with familial breast cancer (families 205 and 2027). In family 205 (Fig. 1b), one woman with ductal comedo-type breast cancer at age 75 years had the Ala592Thr variant. The other available case from this family (Figure 1b, II:2) tested negative, and the histology report was not available. In family 2027 (Fig. 1c), one woman with a breast cancer of ductal comedo type at age 37 years showed the Ala592Thr variant. Her two sisters, who had breast cancer at ages 45 and 51 years, did not. One of the sisters had lobular breast cancer, whereas the histopathological information from the third case was unavailable for study. Because the variant did not segregate with the disease, it is not likely that this alteration predisposes to breast cancer in either of these two families. We also screened 126 BRCA1 or BRCA2 carriers from different families, and found the mutation in one breast cancer patient (family 4056) with a BRCA1 germline mutation (delC2594). It was possible to obtain a sample from her sister who also carried the BRCA1 mutation, and she was found to share the Ala592Thr variant (Fig. 1d). Both tumours were of ductal comedo type. Because both of these tumours were caused by a germline BRCA1 mutation, the E-cadherin variant was not

likely to have predisposed to breast cancer. Among the 604 sporadic breast cancer cases, five were found to carry the Ala592Thr alteration (0.83%; Table 2). One of the tumours was of lobular type, and the other four were of ductal type. One of the ductal tumours was of comedo type, and the tumour type of the other three was not known. Four cases with Ala592Thr alteration were also found among the 497 control individuals (0.80%). The alteration was not found among any of the 214 early-onset breast cancer patients. Discussion: So far, E-cadherin germline mutations have been reported in 17 diffuse gastric cancer families. The frequency of E-cadherin germline mutations in breast cancer reported thus far is low. Mutations reported in E-cadherin in lobular breast cancer patients are in most cases tumour restricted, and not germline alterations. In the present study, we did not find any pathogenic alteration in 10 families that included patients with breast, gastric or colon cancer. We conclude that, although germline E-cadherin mutations are sometimes found in familial gastric and colon cancer, they are not frequently involved in families in which gastric cancer appears to segregate as a part of an inherited predisposition to primary breast cancer. The frequency of Ala592Thr alteration, which was first identified in one family with familial diffuse gastric cancer plus one case of colon and one case of breast cancer (Fig. 1a), was similar in the various groups studied here (Table 2). This finding does not support an effect of this alteration in predisposing to breast cancer in general. Thus, available data indicate that germline E-cadherin mutations do not constitute a major risk factor for breast cancer. However, other studies have indicated that somatic alterations could have an impact on the phenotype in lobular breast cancer. The fact that many tumours with the alteration (Ala592Thr) in the present study were of the ductal comedo type may indicate a genetic basis for the phenotypic divergence caused by this germline E-cadherin alteration. Although lobular breast carcinoma and ductal breast carcinoma of comedo type are different from each other histologically, neither of these cancers are associated with good prognosis. However, individual prognosis with breast cancer is assessed mainly by grading rather than by histological type. In summary, the present study, together with previously reported data, suggests that a germline mutation in E-cadherin is not a major risk factor for breast cancer. However, germline, and more often somatic mutations in this gene could have an impact on phenotypic divergence and prognosis, including growth pattern of the tumours, such as in lobular and perhaps ductal comedo-type breast cancer. In addition, other genetic alterations or epigenetic changes in the E-cadherin gene may have an impact on the metastatic behaviour of the cancer cells, and thereby on the clinical outcome. Introduction E-cadherin (CDH1) is expressed on the cell surface in most epithelial tissues, and evidence is rapidly accumulating that the mammalian E-cadherin gene product plays a role in epithelial tumorigenesis. Loss of function in E-cadherin and/or other collaborating proteins contributes to increased proliferation, invasion and metastasis in many malignant epithelial tumours. Mutations in E-cadherin, which encodes a transmembrane protein, have been described in cancers of the endometrium and ovary, signet ring cell carcinoma of the stomach, the diffuse sclerosing variant of papillary thyroid carcinoma, invasive lobular breast cancer, and diffuse and mixed gastric cancer. E-cadherin germline mutations have been found in early-onset hereditary diffuse gastric cancer, and such mutations have even been implicated as risk factors for early-onset breast and colon cancers. Inactivating mutations and decreased expression of E-cadherin have been reported in invasive lobular breast carcinomas, which demonstrate involvement of E-cadherin alteration in sporadic lobular breast cancer. Lobular carcinoma in situ is known to be a risk factor for breast cancer, but thus far constitutional E-cadherin mutations have not been identified in patients with lobular

carcinoma in situ. In sporadic breast cancer, a correlation between loss of E-cadherin expression and metastatic behaviour has been reported. Despite the fact that E-cadherin expression is decreased in 50% of invasive ductal carcinomas, mutations in the coding sequence of E-cadherin have not been observed in this type of breast cancer. A role of E-cadherin mutation in development of hereditary gastric cancer has been shown, but its role in predisposing to breast cancer is still unproved. In order to further explore the involvement of E-cadherin in breast cancer, we examined all 16 exons of E-cadherin in 31 familial breast cancer patients in whom involvement of BRCA1 and BRCA2 had been excluded. Twelve of these breast cancer patients (from 10 families) had, besides a family history of breast cancer, a family history of gastric or colorectal cancer. The remaining 19 familial breast cancer patients were identified as having LOH at 16q in their tumour. LOH at 16q occurs frequently in sporadic and in familial breast cancer. It has also been shown to correlate with distant metastasis. In the familial breast cancer patients in whom LOH at 16q was identified, E-cadherin was suggested to be a candidate predisposing tumour suppressor gene, and the aim of the present study was to elucidate this relation. In a previous study in a family with diffuse gastric and colon cancer (Salahshor S, et al, manuscript submitted), we found an E-cadherin germline mutation that cosegregated with the disease. This missense mutation in exon 12 (Ala592Thr) was also detected in the index patient's mother, who had ductal breast cancer. In an attempt to clarify a possible role of the Ala592Thr alteration in predisposing to breast cancer, we screened for this specific alteration in different series of breast cancer patients and control individuals. In total, 1328 patients with sporadic or familial breast cancer and 497 control individuals were tested for this specific alteration.

CONCLUSION

This present study, together with previously reported data, suggests that a germline mutation in E-cadherin is not a major risk factor for breast cancer. However, germline, and more often somatic mutations in this gene probably have an impact on phenotypic divergence and prognosis, including growth patterns of tumours, such as in lobular and perhaps ductal comedo breast cancers. In addition, other genetic alterations or epigenetic events at the E-cadherin gene may have an impact on the metastatic behaviour of the cancer cells, and thereby on the clinical outcome. Abbreviations DHPLC = denaturing high-performance liquid chromatography; LOH = loss of heterozygosity; PCR = polymerase chain reaction; SSCP = single-stranded conformation polymorphism.