Report

Loss of heterozygosity at BRCA2 in a ductal carcinoma in situ and three invasive breast carcinomas in a family with a germline BRCA2 mutation

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Key words: BRCA2, breast cancer, DCIS, hereditary cancer, LOH

Summary

We have examined a family with a germline BRCA2 mutation in three cases of invasive breast cancer and one case of ductal carcinoma in situ (DCIS). Loss of heterozygosity (LOH) has been demonstrated at the BRCA2 locus in all cases. This result may suggest that the germline mutation in BRCA2 is the initiating step of DCIS and support the theory that DCIS is a precursor of invasive breast carcinoma in hereditary breast cancer.

Results

Carriers of BRCA1 or BRCA2 germline mutations have a considerable increased risk of breast and ovarian cancer [1, 2]. Ductal carcinoma in situ (DCIS) is a non-invasive neoplastic lesion characterised by intraductal location with a high risk of progressing to invasive ductal carcinoma in sporadic cancer. In the invasive carcinoma DCIS may be erased or retained as part of the tumour. The role of DCIS in hereditary breast cancer is unclear.

The prevalence of germline BRCA1 and BRCA2 mutations in women with DCIS is significantly lower than in women with invasive breast cancer [3]. This might indicate that different mechanisms are involved in tumour development. However, in a prospective study of 67 women with genetic susceptibility to breast cancer prophylactically mastectomies were performed and a high prevalence of premalignant lesions inclusive DCIS was found [4]. This might indicate that DCIS is a precursor of invasive disease in hereditary breast cancer.

In the present study DCIS has been identified in a woman with a family history of breast cancer. The woman (Figure 1, II-2) was diagnosed with DCIS in the left breast 39 years old. Microcalcifications detected by mammography in a non-palpable lesion were removed by lumpectomy. Histological examination showed an 8 mm area of DCIS of solid and cribriform type with nuclear grade 2. Her two sisters and her father have been diagnosed with invasive breast cancer which all had foci of DCIS (Figure 1).

The woman with DCIS was examined for BRCA1 and BRCA2 mutations with the protein truncation test (PTT, Promega). A PCR fragment in exon 11 of BRCA2, which showed premature protein truncation in the PTT test, was sequenced. A single base deletion at position 6601 (codon 2125) causing frameshift and premature stop codon at position 2136 was detected. The mutation was also demonstrated in the father and the two sisters (Figure 1).

Inactivation of the wild type allele by somatic deletion (LOH) of the gene is a common observation in tumours from patients with germline mutations in a tumour suppressor gene. Therefore, we used LOH analysis to determine the involvement of BRCA2 in the development of DCIS. Six microsatellite markers were used for LOH analysis: D13S1226, D13S260, D13S1699, D13S1701, D13S171 and D13S267 (http://gdbwww.gdb.org). Polymerase chain reaction (PCR) reactions were
replicated at least twice, the PCR products were separated and visualised on an ABI-377 (Applied Biosystems) and LOH was detected in all four affected family members. The mother (Figure 1, I-2) was genotyped to determine the phase of the haplotypes to ensure that wild types were lost. LOH of the wild type BRCA2 gene was also detected by sequencing the fragment covering the mutation in the three daughters (this analysis was not conclusive for the father due to low quality of the DNA purified from paraffin sections). This is the best evidence for LOH in the BRCA2 gene since no useful intragenic microsatellite markers are available.

Discussion

The two hit hypothesis for tumour suppressor genes predict that LOH of the wild type allele in a carrier of a germline mutation will result in tumour development [5]. Several studies have indicated that LOH of BRCA1 and BRCA2 is the most common second hit in tumour development in hereditary breast and ovarian cancer [6–10]. In the present study we have demonstrated LOH in the DCIS as well as in three invasive tumours. These results might indicate that DCIS can be initiated by a BRCA2 mutation and support the theory that DCIS preceed development of invasive carcinoma in the breast in this family with hereditary breast cancer. Involvement of BRCA1 in development of an ovarian carcinoma in situ has previously been suggested by detection of LOH in BRCA1 in a family with a BRCA1 germline mutation [11]. The possibility that random LOH has occurred in these DCIS without being the cause of tumourgenesis is present. LOH in

Figure 1. LOH analysis in a family with one case of DCIS, and three cases of invasive breast cancer. (A) The physical position of the six microsatellite markers used in this study and BRCA2 locus on chromosome 13q are indicated. (B) Pedigree of the family. The haplotypes of the six microsatellite markers are denoted by the length of the PCR fragments in base pairs. The result of the analysis of loss of heterozygosity performed with microsatellite markers or sequencing of the mutation are also indicated: • – denotes LOH, ○ – LOH not detected, ø – not informative. Some markers were not informative because the quality of the tumour DNA extracted from paraffin was not sufficient for analysis with these markers.