High Incidence of Loss of Heterozygosity in Breast Tumors from Carriers of the BRCA2 999del5 Mutation

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ABSTRACT

Germ-line mutation in the BRCA2 gene confers an increased risk of breast cancer. An elevation of additional genetic defects in tumors of patients with germ-line mutation in the BRCA2 gene compared with sporadic breast tumors has been reported. To evaluate the nature of the difference, we did detailed mapping of chromosomes 1p, 3p, 6q, 11, 13q, 16q, 17, and 20q, using microsatellite markers. We found that the frequency of loss of heterozygosity was similar at some chromosomal regions in the BRCA2 999del5 and sporadic tumors but significantly different at others. These other includes chromosomal arms 3p, 6q, 11p, 11q, 13q, and 17p. Loss of heterozygosity mapping suggests that the same chromosome regions are involved in both tumor groups but at elevated frequencies in BRCA2 999del5 tumors. This higher frequency of genetic aberrations could pinpoint genes that selectively promote tumor progression in individuals predisposed to breast cancer due to the BRCA2 999del5 germ-line mutation. Accumulation of somatic genetic changes during tumor progression may follow a specific and more aggressive pathway of chromosome damage in these individuals.

INTRODUCTION

A complex set of genetic alterations is involved in the etiology of breast cancer. The detected genetic abnormalities in breast tumors are amplification of oncogenes (MYC, ERBB2, and CCND1), mutation of the tumor suppressor gene TP53, and LOH in chromosome 1, 3p, 6q, 7q, 8p, 11q, 13q, 16q, 17q, and 22q (reviewed in Refs. 1 and 2). The LOH may correspond to losses or inactivation of tumor suppressor genes. Furthermore, mutations of BRCA1 and BRCA2 genes have been implicated in inherited breast cancer (3–5). Somatic loss of the wild-type chromosome in tumors of BRCA1 and BRCA2 mutation carriers suggests that both alleles of the corresponding gene are inactivated in cancer, a pattern expected of a tumor suppressor gene (6–8). A germ-line mutation in the BRCA2 gene, termed 999del5, has been detected in Icelandic breast cancer families, and a common haplotype in the vicinity of the gene in these families suggests a founder effect (9–11). The BRCA2 999del5 germ-line mutation has been found in 8% of patients diagnosed with breast cancer in Iceland (11, 12). A genome-wide search for the chromosome changes in tumors of BRCA2 carriers has been done by CGH and demonstrated a higher frequency of aberrations at several chromosome arms compared with sporadic tumors (13). These results suggested a specific tumor progression pathway in patients predisposed to breast cancer due to a BRCA2 mutation. In this study, we have used microsatellite markers for more detailed mapping of somatic aberrations in breast tumors of BRCA2 999del5 carriers and compared changes in sporadic breast tumors at chromosome regions already shown to differ in the two groups by CGH (13). A profile of LOH has not been reported earlier on a set of tumors carrying a single specific mutation in BRCA1 or BRCA2 genes.

MATERIALS AND METHODS

Subjects. Breast cancer patients diagnosed in the years 1989–1997 were screened for the BRCA2 999del5 mutation. Male breast cancer was excluded from the study. Forty-six patients were positive for the BRCA2 999del5 mutation, of 541 unselected female patients analyzed. All tumors were primary invasive breast tumors, 487 of ductal and 54 of lobular histological type. A set of tumors negative for the BRCA2 999del5 mutation was used as the control group (number of tumors are given in Table 1). Part of this patient material has been used in our previous studies on LOH in human breast tumors at chromosome regions: 1p, 232 patients (14); 3p, 140 patients (15); 6q, 204 patients (16); 11, 116 patients (17); 13q, 139 patients (18); and 16q, 150 patients (19). These studies were done prior to the knowledge of the BRCA2 999del5 mutation, except the study on chromosome 13, where BRCA2 999del5 carriers were excluded.

Mutation and Microsatellite Marker Analysis. The BRCA2 999del5 mutation analysis is based on allele size difference (186-bp normal and 181-bp mutant allele) using forward (5'-ATGGATAAGGGGGACTA-3') and reverse (5'-AAACCTGAGATCCGGTG'T-3') primers in a PCR analysis (11). Ten chromosome arms were analyzed with microsatellite markers to score for LOH: 1p, 3p, 6q, 11q, 13q, 16q, 17p, and 20q. The rationale for the inclusion of these chromosome regions was based on previous results using CGH, showing significant difference in the frequency of aberration in BRCA2 tumors compared with controls (13). PCR primers used for the microsatellite marker analysis were (cytogenetic localization according to the Genome Data Base are given in parentheses): D1S243 (1p36.3); D1S468 (1p36.3); D1S214 (1p36.3); D1S228 (1p36.1); D1S507 (1p36.1); D1S436 (1p36.1); D1S233 (1p35); D1S201 (1p35); D1S496 (1p34.3); D1S197 (1p); D1S209 (1p32.1-p33); D1S16 (1p32.1-p33); D1S465 (1p); D1S207 (1p32.1-p33); D1S488 (1p32.1-p33); D1S167 (1p31.3); D1S435 (1p31.3); D1S188 (1p31.3); D1S424 (1p31.3); D1S236 (1p31.3); D1S221 (1p32.4-p22); D1S1067 (1p31.1-p14.3); D1S1234 (1p31.1-p14.2); D1S1300 (1p21.1-p14.2); D1S1233 (1p31.3-p21.1-p14.1); D1S1217 (1p31.4-p14.1); D1S1210 (1p31.4-p12); GLUT2 (2q26.2-q27); D2S216 (2q21-q22.1); D2S292 (2q22-q23); D2S311 (2q31-q25); D2S90 (2q31-q25.2); ESR (2q31.5); D2S305 (2q5.2-q27); D2S264 (2q5.2-q27); D2S261 (2q6.7); D1S902 (11p15.5); D1S907 (11p13); D1S905 (11p13-q13); D1S925 (11q23.3); D1S912 (11q23.2-p25); D1S969 (11q24.1-q25); D1S1717 (11q12.3); D1S1246 (11q12.3); D1S171 (13q23.3-q13); D1S1695 (13q12-q13); D1S1694 (13q12-q13); D1S14 (13q12-q14.2); D1S153 (13q14.1-q14.3); D1S176 (13q14.3); D1S160 (13q12.3-q2); D1S154 (13q21-q32); D1S153 (13q21-q32); D1S173 (13q23-q32); D1S1285 (13q34); D16S262 (16p21); D16S422 (16q24.2); D16S413 (16q24.3); D16S305 (16q24.3); TP53 (17p13.1); THRA (17q11.2-q12); D17S800 (17q12); D17S855 (17q11.2); D17S801 (17); D17S802 (17); D17S784 (17); D17S928 (17); D20S156 (20); D20S156 (20); D20S191 (20); D20S19 (20); D20S196 (20); D20S120 (20q13.1-q12.2); and D20S171 (20q13.2-13.3). Twenty-five ng of DNA samples were subjected to PCR analysis in a total volume of 25 μl. DynaZyme polymerase (Finzymes Oy, Espoo, Finland) was used in the buffer solution provided by the manufacturer with 100 μM of each deoxynucleotide triphosphate and 0.25 μM primers. After 5 min denaturation at 94°C, samples

Received 5/26/98; accepted 8/3/98.

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1This work was supported by the Research Council of Iceland, Nordic Cancer Union, Science Fund of the Icelandic Cancer Society, Research Fund of the University of Iceland, and the Memorial Fund of Berghora Magnusdottr and Jakob B. Bjarnason.

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3The abbreviations used are: LOH, loss of heterozygosity; CGH, comparative genomic hybridization.
**RESULTS**

In general, tumors from BRCA2 999del5 carriers showed a higher frequency of LOH than in controls. The frequency of LOH in cases varied from 47% (11p and 20q) to 92% (13q; Table 1). LOH detected at chromosomes 3p, 6q, 11p, 11q, 13q, and 17p was significantly higher in tumors from the carriers than in the control group (Table 1). The results of LOH analysis determined with individual markers at these chromosomes are shown in Fig. 1, as well as the P obtained from the statistical analysis.

The highest frequency of LOH at the 3p region in the BRCA2 999del5 tumors is detected by the D3S1210 marker (63%) and is significantly higher than in the control group (P < 0.001; Fig. 1A). This marker is located at the 3p12–p14.1 region. A significant difference was also detected with markers D3S1217 (located at 3p14.1–p14.2), D3S1067 (located at 3p14.3–p21.1), and D3S1300 and D3S1234 (located at 3p14.2–p21.1 within the FHIT gene). In tumors where both D3S1234 and D3S1300 were informative, a consistency of LOH was detected in most cases. Both markers showed LOH in 16 tumors (62%), both markers showed retention of heterozygosity in 8 tumors (31%), LOH with marker D3S1234 but not with marker D3S1300 was detected in two tumors (8%), and LOH with marker D3S1300 and retention of heterozygosity with marker D3S1234 was not detected in any tumor. In contrast, no significant difference was seen in LOH frequency between the groups detected by the marker GLUT2, mapping to 3q (Fig. 1A).

The results for chromosome 6q are shown in Fig. 1B. The frequency of LOH is significantly higher in the carriers at all markers tested, except for D6S262, the most centromeric marker. The majority of cases showing LOH have lost all or a large part of chromosome 6q, and a minority of cases show loss at a small region telomeric to 6p. LOH was detected in most cases. In tumors from the carriers, both markers showed retention of heterozygosity in 8 tumors (31%), LOH with marker D3S1234 but not with marker D3S1300 was detected in two tumors (8%), and LOH with marker D3S1300 and retention of heterozygosity with marker D3S1234 was not detected in any tumor. In contrast, no significant difference was seen in LOH frequency between the groups detected by the marker GLUT2, mapping to 3q (Fig. 1A).

The results for chromosome 11q are shown in Fig. 1C. The frequency of LOH in the carriers is significantly higher at all markers tested, except for D11S903, the most centromeric marker. The majority of cases showing LOH have lost all or a large part of chromosome 11q, and a minority of cases show loss at a small region telomeric to 11p. LOH was detected in most cases. In tumors from the carriers, both markers showed retention of heterozygosity in 8 tumors (31%), LOH with marker D11S907 and D11S903 was detected in two tumors (8%), and LOH with marker D11S903 and retention of heterozygosity with marker D11S907 was not detected in any tumor. In contrast, no significant difference was seen in LOH frequency between the groups detected by the marker GLUT2, mapping to 3q (Fig. 1A).

The results for chromosome 17q are shown in Fig. 1D. The frequency of LOH in the carriers is significantly higher at all markers tested, except for D17S298, the most centromeric marker. The majority of cases showing LOH have lost all or a large part of chromosome 17q, and a minority of cases show loss at a small region telomeric to 17p. LOH was detected in most cases. In tumors from the carriers, both markers showed retention of heterozygosity in 8 tumors (31%), LOH with marker D17S298 and retention of heterozygosity with marker D17S298 was not detected in any tumor. In contrast, no significant difference was seen in LOH frequency between the groups detected by the marker GLUT2, mapping to 3q (Fig. 1A).

The results for chromosome 20q are shown in Fig. 1E. The frequency of LOH in the carriers is significantly higher at all markers tested, except for D20S162, the most centromeric marker. The majority of cases showing LOH have lost all or a large part of chromosome 20q, and a minority of cases show loss at a small region telomeric to 20p. LOH was detected in most cases. In tumors from the carriers, both markers showed retention of heterozygosity in 8 tumors (31%), LOH with marker D20S162 and retention of heterozygosity with marker D20S162 was not detected in any tumor. In contrast, no significant difference was seen in LOH frequency between the groups detected by the marker GLUT2, mapping to 3q (Fig. 1A).
within the same tumor (Fig. 2B). Much higher LOH is detected at chromosome 17p in the mutation carriers compared with the control group, whereas the difference of LOH at chromosome 17q does not reach statistical significance (Fig. 1E).

The pattern of LOH at 13q is similar in cases and controls but different in some aspects (Fig. 1D). There is a highly significant elevation around the BRCA2 gene (markers D13S260 and D13S171) at 13q in carriers compared with controls. This difference diminishes telomeric to the D13S160 marker and does not reach statistical significance with markers D13S154, D13S173, and D13S285. All detected losses at 13q involved the wild-type chromosome. The loss is confirmed by loss of the 186-bp normal and retention of the 181-bp BRCA2 mutant PCR products. Only 3 of 39 tumors did not show loss of the wild-type chromosome 13q. Mapping results strongly suggest the BRCA2 gene to be the target of the genetic loss (Fig. 2C).

A trend toward larger aberration in the tumors of the BRCA2 999del5 compared with the sporadic tumors was noted. In tumors showing LOH at chromosome 6q, a major loss of large chromosome region was detected in 27% of sporadic tumors and in 68% of BRCA2 999del5 tumors (P = 0.001). Twenty-five percent of sporadic tumors with aberration at chromosome 11 show LOH at markers from both 11p and 11q, whereas 44% of chromosome 11 LOH-positive BRCA2 999del5 tumors show aberrations at both chromosome arms (not a statistically significant difference). Of tumors showing LOH at 13q, 21% in the sporadic group and 27% of the BRCA2 999del5 group showed total loss of chromosome 13 (not statistically significant). Furthermore, 35% of sporadic tumors and 82% of BRCA2 tumors with 13qLOH showed large deletion between markers D13S283 and D13S176 (Fig. 2C), a region of 44 cM (P < 0.001).

**DISCUSSION**

In this study, we report an elevation of LOH at several chromosome arms in tumors of BRCA2 999del5 carriers compared with tumors from individuals without this mutation. All of these regions have been implicated previously in sporadic breast cancer, but in most cases at lower frequency.

Elevation of 3p LOH in BRCA2 999del5 carriers is in line with our earlier finding in a study of sister pairs with breast cancer carrying the germ-line mutation (23). The affected region harbors several known genes, e.g., BAP1, located on chromosome 3p21.3. It has been shown recently to function in the BRCA1 growth-control pathway, and its protein product binds to the RING finger domain of the BRCA1 protein (24). Another gene of interest is the FHIT gene at 3p21.1-p14.2. Several reports have described abnormalities in the FHIT gene in breast carcinomas (25–27). Furthermore, the FHIT gene has been shown to suppress tumorigenicity of cancer cells (28). The FHIT gene encompasses the carcinogen-sensitive common fragile site, FRA3B (29). Another but less studied fragile site of potential interest with respect to the chromosome regions analyzed in this study is FRA6E at chromosome 6q26.

Earlier findings based on the loss of wild-type chromosome 13q suggested a strong selection of tumor cells with both alleles of the BRCA2 gene being mutated (6–7). Here we have shown that the loss at 13q involves the BRCA2 gene in the majority of tumors. The mutation status of the BRCA2 gene at the wild-type chromosome in the three tumors with retention at the BRCA2 locus is unknown, but somatic BRCA2 mutations are considered to be rarely found in breast tumors (30, 31). A highly significant elevation of LOH is detected in cases at all markers between D13S217 and D13S176. It includes
marker D3S153, located close to the RB1 tumor suppressor gene. This suggests that there might not only be an enhanced growth selection toward the loss of the BRCA2 gene but also for the RB1 gene or another tumor suppressor gene located at 13q.

The region at chromosome 17 that shows an significant elevation of LOH in BRCA2 999del5 tumors in comparison to sporadic tumors harbors the TP53 gene (Fig. 1E; Ref. 22). Knockout mouse experiments have suggested that p53 protein is accumulating in BRCA2-defective mice and that a cell cycle checkpoint mechanism is activated due to defective BRCA2 protein and corresponding DNA damage (32). It has been suggested that accumulation of p53 protein can reduce the malignant behavior of BRCA2-defective tumors due to cell cycle checkpoint activation (33). In only a minority of BRCA2 999del5 tumors showing LOH at the TP53 locus, TP53 mutation is detected in the remaining allele (22). A growth advantage of BRCA2-defective tumors may be enhanced if only one copy of the TP53 gene is deleted or the LOH target is another gene in the close vicinity of TP53.

The association of both BRCA1 and BRCA2 proteins with the RAD51 protein establishes a direct link between these proteins and the control of genomic integrity and stability because Rad51 is required for meiotic and mitotic recombination events and the repair of double-stranded DNA breaks (34, 35). Furthermore, fibroblasts from BRCA2 knockout mice show defects in DNA repair of double-stranded breaks (33). Defects in chromatin exchange during mitotic recombination result in numerous spontaneous chromosomal abnormalities in fibroblasts from BRCA2 knockout mice (36). Our results may reveal the inability of mutated BRCA2 to participate in the Rad51-mediated repair. A putative model of LOH selection might be that due to improper fidelity in mitotic recombination and corresponding DNA repair; the BRCA2 999del5 follows a more aggressive pathway of chromosome damage than tumors without this mutation, but in most cases the same chromosome regions are involved in both groups. Whether loss of additional genes is involved in the tumor progression of BRCA2 999del5 carriers compared with sporadic tumors remains unsolved.

In this study, we used microsatellite markers to detect somatic changes in breast tumors. The results from our study support the results of a CGH study comparing tumors from BRCA2 carriers with sporadic breast tumors, with a few exceptions (13). We detected a significant elevation of LOH at the 17p region, not detected in the CGH study, and although a general elevation of allelic imbalance in BRCA2 tumors versus sporadic ones is detected, the difference is not as high as according to CGH. This slight discrepancy might be explained by the different resolution power of the two methods. CGH is not sensitive to allelic losses that are caused by mechanisms other than large physical deletions. Indeed, the architecture of the LOH is not sensitive to allelic losses that are caused by mechanisms other than large physical deletions. Indeed, the architecture of the LOH is different in the two tumor groups at some chromosome regions, because the BRCA2 999del5 tumors show that larger regions are involved. In tumors showing loss at a given chromosome arm, specific regions can be preferably affected in the BRCA2 999del5 tumors in comparison with sporadic tumors. Our findings possibly pinpoint candidate loci for the search for genes that when inactivated promote tumor progression in individuals predisposed to breast cancer due to the BRCA2 999del5 mutation.


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