Consortium Study on 1280 Breast Carcinomas: Allelic Loss on Chromosome 17 Targets Subregions Associated with Family History and Clinical Parameters¹

Catherine M. Phelan, ² Åke Borg, Marguerite Cuny, David N. Crichton, Trausti Baldersson, Tone Ikdahl Andersen, Maria Adelaide Caligo, Rosette Lidereau, Annaika Lindblom, Susanne Seitz, David Kelsell, Ute Hamann, Pascale Rio, Steinunn Thorlacius, Janos Papp, Edith Olof, Yves-Jean Bignon, Siegfried Scherneck, Rosa Barkardottir, Anne-Lise Borresen-Dale, Jorunn Eyfjörd, Charles Theillet, Alastair M. Thompson, Peter Devilee, and Catharina Larsson

Department of Molecular Medicine, Endocrine Tumour Unit, Karolinska Hospital, S-17176 Stockholm, Sweden [C. M. P., A. L. C. L.]; Institute of Oncology, Lund University, S-22185 Lund, Sweden [A. B.]; Institute of Molecular Genetics of Montpellier, 34033 Montpellier, France [M. C. C. T.]; Department of Surgery, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland [D. N. C., A. M. T.]; Department of Pathology, University Hospital of Iceland, IS-121 Reykjavik, Iceland [T. B., R. B.]; Department of Genetics, Institute of Cancer Research, N-0310 Oslo, Norway [T. I. A., A.-L. B.-D.]; Department of Medical Genetics, Ulleval University Hospital, N-0315 Oslo, Norway [T. I. A.]; Institute of Pathology, University of Pisa, 56124 Pisa, Italy [M. A. C.]; Centre Rene Huguenin, F-92211 Saint-Cloud, France [R. L.]; Department of Tumour Genetics, Max Delbruck Centrum, 13122 Berlin, Germany [S. S., S. S.]; Imperial Cancer Research Fund, Herfordshire EN6 3LD, England [D. K.]; Deutsches Krebsforschungszentrum and Department of Molecular Genome Analysis, AG Molecular Genetics of Breast Cancer, D6912 Heidelberg, Germany [U. H.]; Centre Jean-Perrin, 36011 Clermont-Ferrand, France [P. R., Y.-J. B.]; Icelandic Cancer Society, IS-125 Reykjavik, Iceland [S. T., J. E.]; National Institute of Oncology, 1122 Budapest, Hungary [J. P. E. D.]; CRC Department of Pathology, Cambridge CB2 2QG, England [B. P.]; and Departments of Human Genetics and Pathology, University of Leiden, NL2333 Leiden, the Netherlands [P. D.]

ABSTRACT

The pattern of loss of heterozygosity (LOH) on chromosome 17 in human breast cancer is complicated and shows many different regions of loss. In an attempt to narrow down the relevant regions of LOH on chromosome 17, we have studied the deletion pattern and its association with clinical parameters in 1280 breast carcinoma-venous blood lymphocyte pairs. In total, 42 different chromosome 17 loci were investigated, and between 25 and 625 cases were analyzed at each locus. The frequency of LOH observed on the p arm was much higher than that observed on the q arm. The opposite effect was observed in 52 ovarian cancer cases investigated, with less LOH on 17p than on 17q. Patterns of loss consistent with interstitial and terminal deletions, as well as loss of either the p or q arm or monosomy 17 were observed. To determine whether loss at a particular locus may be associated with biological features of breast tumors, clinical data including age of onset, family history of breast cancer, tumor histopathology, tumor size, estrogen receptor (ER) status, and occurrence of lymph node or distant metastases were collected for each case. Overall, large-sized, ER-negative, lymph node-positive ductal tumors showed the highest frequencies of LOH, with ER-negative and ductal tumors showing LOH for markers along the majority of the chromosome. Eight regions of chromosome 17 appear to be associated with human breast cancer, two on 17p and six on 17q. These regions were not necessarily in the areas exhibiting the highest frequencies of LOH but were defined by interstitial and terminal deletions in multiple independent cases. Seven of these regions showed statistically significant differences in LOH associated with clinical parameters. These data strongly suggest that loci on chromosome 17 may determine aspects of tumor presentation and disease behavior in human breast cancer and pinpoint candidate tumor suppressor gene loci.

INTRODUCTION

Chromosome 17 aberrations are the most common genetic abnormality in human breast cancer and appear to be an early event in tumorigenesis (1). Numerous cytogenetic alterations involving chromosome 17 have been reported (2), including gain of copy number of this autosome (1). Cell transfection experiments in breast cancer cell lines suggest that a number of tumor suppressor genes are present and involved in breast cancer proliferation (3–5). Many studies investigating LOH³ on chromosome 17 have implicated a number of different regions on both arms, with the levels of LOH ranging from 22–75%. The region of chromosome 17 located between 17q11–q24 also harbors a number of proto-oncogenes, such as ERBB2, TOP2A, NGFR, and others (6). Comparative genome hybridization studies have shown gains in copy number of the 17q23–24 region. ERBB2 is amplified and/or overexpressed in approximately 20% of breast cancer cases and is considered an indicator of bad prognosis (1, 7).

On 17p, there are two main regions of LOH, one at the TP53 locus (17p13.1) and the more distal region in 17p13.3 (8–11). LOH on 17p is reported in around 50–60% of breast and ovarian cancer cases (12), whereas somatic mutations in the TP53 gene have been reported in 30–50% of cases with LOH (13, 14), implying the presence of alternative tumor suppressor genes (8). A second tumor suppressor gene more telomeric to TP53 has been cloned, referred to as OVA1. This gene has shown reduced expression in ovarian tumor tissue as compared to normal ovarian epithelia and some inactivating mutations in ovarian tumors (15, 16).

On 17q, there are several reported regions of common loss (11). These include a region from D17S250 to THRAI, a region from D17S776 to D17S579, including BRCA1, and a region distal to D17S733. Other studies have placed the first region on the proximal portion of 17q21 (including BRCA1), the second region centering at D17S74, and the third region on distal 17q25 at the D17S24 locus (17). The region of loss around D17S74 has also been specifically implicated in ovarian cancer (18), and the 17q25 region has been observed in a structural rearrangement in male breast cancer (19). In one study, the smallest common region defined on 17q was a 120-kb interval in the BRCA1 region (20). LOH at BRCA1 has been reported in the range of 30–70% (8, 11, 13, 15, 17, 21–30), yet this gene does not appear to be somatically mutated in human breast cancer (31), although it has been reported to have a loss of expression in a few cases of sporadic breast tumors (32) and is mutated in approximately 5–10% of ovarian cancer cases (33, 34).

Other genes in the BRCA1 region have revealed somatic breast cancer mutations. One such gene is the α-n-acetylglucosaminidase gene, shown to be mutated in three cases of sporadic breast cancer (25). Farther from the BRCA1 gene is the prohibitin gene, which has shown 5 of 125 (4%) somatic breast tumor mutations (35), and the MDC gene, which has shown rearrangements in 2 of 600 cases (0.3%; Ref. 36). However, given the level of LOH, many more mutations

Received 9/2/97; accepted 1/5/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ The abbreviations used are: LOH, loss of heterozygosity; ER, estrogen receptor.
would be expected in sporadic breast cancer. Thus, it would appear that the 17q tumor suppressor gene(s) that are responsible for the majority of the observed LOH have not yet been identified.

Currently, certain clinical features are used in predicting the prognosis of particular subsets of tumors (37). Increased tumor size, lymph node metastatic spread, metastasis to other tissues, and hormone receptor absence are considered as predictive of a worse prognosis. It is not known which specific genes/chromosomal events are responsible, and because chromosome 17 aberration appears to be an early event in breast tumorigenesis, it is essential to define any relationships involving imbalance of this chromosome and tumor clinical parameters.

In this multicenter study within the European Breast Cancer Linkage Consortium, we have investigated a very large panel of 1280 breast tumors for LOH on chromosome 17, so that we may determine (a) relevant regions of LOH on chromosome 17 in breast cancer, (b) the frequency of particular rearrangements in this cancer, and (c) associations between particular chromosome 17 regions of loss and specific clinicopathological features.

**MATERIALS AND METHODS**

**Patient Material.** Seventeen centers from 10 different countries participated in this study. Each center had their own collection of breast carcinoma-blood pairs. In total, 1280 women with breast carcinoma were studied; 1241 cases were primary tumors, 23 were recurrences in the same region, and 16 were metastases to the breast. Histopathological examination of tumor tissue was performed on adjacent tumor pieces to those studied, and tumor classification was made according to the WHO guidelines (38, 39). The following clinical information was collected for each case (if available): age of onset of primary tumor (≤50 years versus >50 years); presence/absence of a family history of breast cancer, defined as having/not having one or more first-degree relatives with breast cancer; tumor histopathological subtype; size of tumor (<2 cm, 2-5 cm, or >5 cm in diameter); ER status (positive versus negative according to local criteria); and occurrence of metastases to the lymph nodes or to other tissues (Table 1).

**LOH Analysis.** Each of the 17 centers performed the LOH analysis for each case with 2-23 markers; some of the LOH results have been published previously (10, 17, 22, 23, 40). General information on RFLP analysis and PCR methods is given below.

**RFLP Analysis.** Constitutional and tumor DNA was digested with the appropriate restriction enzymes (as described in HGM 11.0; Ref. 47), separated by electrophoresis, and Southern blotted onto nylon membranes. A total of 22 probes that identify RFLPs on chromosome 17 were used, and for two loci, TP53 and NME1, both RFLPs and microsatellite markers were available (Table 2; Fig. 1). Ten RFLP-based markers are localized to the p arm, 1 to the centromere, and the remaining 11 to the q arm (HGM 11.0; Ref. 47). Usually, the probe DNA was labeled with 32P using the random priming technique (41). The signal was visualized either by normal autoradiography or by using a PhosphoImager. Densitometry was carried out where appropriate.

**PCR-based LOH Analysis.** The 42 markers used in the LOH analysis are given in Table 2, and the order along the chromosome is shown in Fig. 1. Twenty-two markers were PCR-based microsatellite markers. Four markers were localized on the p arm, and the remaining 18 microsatellite repeat markers were localized on the q arm. The oligonucleotide primer sequences, allele sizes, and order of markers along the chromosome are according to the Génethon genetic linkage map (42) and the Genome Database. The PCR assays were performed as described previously.

**Statistical Analysis.** Statistical significance was determined using a χ² (2 X 2) test using the Instat2 statistical package (GraphPad). Where appropriate (values of 10 or less), the Yates correction was incorporated into the χ² value. With the use of 42 markers, Ps of < 0.05 (i.e., 1 in 20) would be expected due to chance. Therefore, only associations that meet a statistically significant value of P < 0.01 (i.e., 1 in 100 observations due to chance) were considered significant. Other associations, in which 0.01 < P < 0.05, were deemed inconclusive.

### RESULTS

There were 1280 breast carcinoma-blood pairs analyzed with between 2 and 23 chromosome 17 markers. In total, 42 different chromosome 17 loci were investigated, which were well spaced on the chromosome (Fig. 1). Thirteen loci were mapped to the p arm, 28 were localized to the q arm, and 1 was localized to the centromere (Table 2). One hundred sixteen cases were analyzed with 17q markers only, whereas 381 cases were analyzed with one 17p marker (usually TP53) and three or more 17q markers. The remaining samples were analyzed with three or more markers on each chromosome 17 arm.

Fifty-two ovarian tumors were also analyzed with two markers on 17p and four markers on 17q. LOH on 17p was observed in 38% of informative cases and on 17q in 65% of informative cases. The regions of common deletion were large due to the use of a limited number of markers.

**Frequencies of LOH and Narrowing of Target Regions.** LOH at one or more chromosome 17 loci was observed in 704 of 1258 (55%) cases, with 411 of 772 (53.2%) informative cases showing LOH with at least one 17p marker; 536 of 1140 (47.0%) informative cases showed LOH with at least one 17q marker, and 243 of 1258 (19%) of the breast tumors showed LOH with at least one marker on both the p and q arms. Of the 1258 informative cases, 262 (20%) showed no chromosome 17 loss or abnormality on either arm; however, in many of these cases, four or fewer markers were used in the analysis, and they may not have been informative. In general, the greater the number of chromosome 17 markers used, the more complicated the "zebra" pattern of allele loss.

The number of cases studied for each marker, the number of informative cases, and the number and percentage of cases that showed LOH are given in Table 2 and shown in Fig. 1. The percentage of LOH at each marker is calculated from the number of cases
terstitial deletions and terminal deletions. Multiple chromosome 17 aberrations observed in single tumors, as shown in Fig. 2 (case IG912). Numerous interstitial deletions were observed, and many were seen in multiple independent cases. Terminal deletions occurred at almost every marker, with 112 tumors showing clear terminal deletions. From the deletion pattern, nine relevant regions were identified, as illustrated in Fig. 2. These regions overlap with previously published areas of common deletion in breast. The nine regions are as follows and show the following percentages of LOH (Fig. 2): on 17p and centromere regions, 17pter-D17S55 (I), 45–61%; D17S31–TP53 (II), 45–52%; and D17S78–D17S250 (III), 10–52%; and on 17q, THRA1–D17S357 (IV), 30–36%; D17S791–HOX2 (V), 14–41%; D17S293–D17S75 (VI), 30–47%; ITGB3–D17S785 (VII), 27–35%; D17S20–TK1 (VIII), 0–27%; and D17S777–D17qter (IX), 25–31% (Table 2; Figs. 2 and 4).

Association between LOH and Clinical Parameters. Statistically significant associations between LOH at specific loci and particular clinical features were observed, and these are detailed in Table 3 and Fig. 3 (a–g) and summarized in Fig. 4. In general, early age of onset, large tumor size, ER negativity, and ductal histopathology were associated with higher frequencies of LOH.

Tumors of size greater than 2 cm revealed more LOH at most of the chromosome 17 markers, but the difference in LOH between them and smaller tumors was not significant (Fig. 3a). ER-negative tumors showed significantly more LOH than did ER-positive tumors at a number of loci along the chromosome (Table 3; Figs. 3b and 4). This

showing LOH/number of informative cases. The extent of LOH on the short arm of chromosome 17 in breast cancer ranged from 45 to 67%, whereas the extent of LOH on the long arm ranged from 26 to 37%, taking those markers into account for which more than 99 cases were assayed (Fig. 1, points joined by the boldface line). The number of cases studied for each marker varied from 25 to 625 (Fig. 1; n is the number of cases assayed for each marker). Some markers are more informative than others, with the microsatellite repeat markers (Table 2, bold print) generally being more informative than the RFLP markers.

Fifteen tumors showed LOH with at least four chromosome 17p markers, consistent with a pattern of loss of the entire short arm; 25 tumors showed a pattern consistent with loss of the entire long arm (i.e., LOH at a minimum of five markers); and approximately 70 (6%) tumors revealed LOH at all informative markers used. Forty-six of these tumors showed LOH at five or more informative markers along the entire chromosome, consistent with monosomy 17. These figures are probably conservative, and there are undoubtedly more cases with each aberration. However, only a small number of markers was used in the majority of cases (i.e., four markers on the whole chromosome), and given the zebra-like patterns of LOH of chromosome 17 in breast cancer, it is not possible to define the exact nature of the chromosome 17 abnormality in several cases.

Specific chromosome 17 aberrations observed included small interstitial deletions and terminal deletions. Multiple chromosome 17 aberrations were observed in single tumors, as shown in Fig. 2 (case IG912). Numerous interstitial deletions were observed, and many were seen in multiple independent cases. Terminal deletions occurred at almost every marker, with 112 tumors showing clear terminal deletions. From the deletion pattern, nine relevant regions were identified, as illustrated in Fig. 2. These regions overlap with previously published areas of common deletion in breast. The nine regions are as follows and show the following percentages of LOH (Fig. 2): on 17p and centromere regions, 17pter-D17S55 (I), 45–61%; D17S31–TP53 (II), 45–52%; and D17S78–D17S250 (III), 10–52%; and on 17q, THRA1–D17S357 (IV), 30–36%; D17S791–HOX2 (V), 14–41%; D17S293–D17S75 (VI), 30–47%; ITGB3–D17S785 (VII), 27–35%; D17S20–TK1 (VIII), 0–27%; and D17S777–D17qter (IX), 25–31% (Table 2; Figs. 2 and 4).

Association between LOH and Clinical Parameters. Statistically significant associations between LOH at specific loci and particular clinical features were observed, and these are detailed in Table 3 and Fig. 3 (a–g) and summarized in Fig. 4. In general, early age of onset, large tumor size, ER negativity, and ductal histopathology were associated with higher frequencies of LOH.

Tumors of size greater than 2 cm revealed more LOH at most of the chromosome 17 markers, but the difference in LOH between them and smaller tumors was not significant (Fig. 3a). ER-negative tumors showed significantly more LOH than did ER-positive tumors at a number of loci along the chromosome (Table 3; Figs. 3b and 4). This

showing LOH/number of informative cases. The extent of LOH on the short arm of chromosome 17 in breast cancer ranged from 45 to 67%, whereas the extent of LOH on the long arm ranged from 26 to 37%, taking those markers into account for which more than 99 cases were assayed (Fig. 1, points joined by the boldface line). The number of cases studied for each marker varied from 25 to 625 (Fig. 1; n is the number of cases assayed for each marker). Some markers are more informative than others, with the microsatellite repeat markers (Table 2, bold print) generally being more informative than the RFLP markers.

Fifteen tumors showed LOH with at least four chromosome 17p markers, consistent with a pattern of loss of the entire short arm; 25 tumors showed a pattern consistent with loss of the entire long arm (i.e., LOH at a minimum of five markers); and approximately 70 (6%) tumors revealed LOH at all informative markers used. Forty-six of these tumors showed LOH at five or more informative markers along the entire chromosome, consistent with monosomy 17. These figures are probably conservative, and there are undoubtedly more cases with each aberration. However, only a small number of markers was used in the majority of cases (i.e., four markers on the whole chromosome), and given the zebra-like patterns of LOH of chromosome 17 in breast cancer, it is not possible to define the exact nature of the chromosome 17 abnormality in several cases.

Specific chromosome 17 aberrations observed included small interstitial deletions and terminal deletions. Multiple chromosome 17
phenomenon was more clearly observed at central and terminal por
tions of 17q, with a potentially significant difference observed at
D17S784. Tumors from women with a family history of breast cancer
did show statistically significant increased LOH compared with spo-
radic cases at the marker D17S250 on the q arm (Fig. 3f, boldface). 
Surprisingly, there was little difference in the percentage of LOH at
TP53 or BRCA1 (D17S855; Fig. 3f).
Ductal tumors were the largest histological subtype (Table 1); the
majority of the remaining tumors were of the lobular subtype. Ductal
tumors revealed more chromosome 17 abnormalities than did the
lobular (and other) types, with statistically significant associations at
three loci (Table 3; Figs. 3g and 4).
On the basis of the interstitial and terminal deletions observed and
the results of the associations to clinical parameters, there appear to be
seven relevant regions (I, II, IV, and VI–IX) on chromosome 17 in
breast cancer, as shown in Fig. 4.

DISCUSSION
There are many genes on chromosome 17 that are involved in
breast cancer, as has been implicated by cell transfection and allelo-
typing studies (4, 5, 17), even apart from BRCA1 and TP53 (3). The
highly complicated zebra pattern of LOH has made the localization of
relevant genes difficult. In this study, we have attempted to more
precisely define the localization of such genes by investigating chro-
mosome 17 LOH data in 1280 breast tumors in a collaborative effort
within the European Breast Cancer Linkage Consortium. The majority
of cases in this study were analyzed with three or more markers on
each chromosomal arm. More than half (55%) of informative cases
showed LOH with at least one marker, with almost half of those
showing LOH on both chromosomal arms, which in itself strongly
implicates a number of regions on chromosome 17 in breast cancer.
In total, there were higher levels of LOH on the short arm (an average of
54%) than on the long arm (average of 31%; Table 2 and Fig. 1). We
also analyzed 52 ovarian cancers with markers on both arms. In
contrast with breast cancer, there was more LOH on the q arm
(average 65%) compared to the p arm (average 38%; data not shown).

The results suggest there are eight relevant breast cancer regions on
chromosome 17, two regions located on 17p and six regions on 17q.
These regions were defined by multiple cases of interstitial deletions
and terminal deletions (Figs. 2 and 4). The two regions on 17p include
TP53 (17p13.1) and a more telomeric region at 17p13.3 that includes
TP53 or BRCA1. The seven relevant regions (I, II, IV, and VI–IX) on
chromosome 17 in breast cancer, as shown in Fig. 4.

DISCUSSION
There are many genes on chromosome 17 that are involved in
breast cancer, as has been implicated by cell transfection and allelo-
typing studies (4, 5, 17), even apart from BRCA1 and TP53 (3). The
highly complicated zebra pattern of LOH has made the localization of
relevant genes difficult. In this study, we have attempted to more
precisely define the localization of such genes by investigating chro-
mosome 17 LOH data in 1280 breast tumors in a collaborative effort
within the European Breast Cancer Linkage Consortium. The majority
of cases in this study were analyzed with three or more markers on
each chromosomal arm. More than half (55%) of informative cases
showed LOH with at least one marker, with almost half of those
showing LOH on both chromosomal arms, which in itself strongly
implicates a number of regions on chromosome 17 in breast cancer.
In total, there were higher levels of LOH on the short arm (an average of
54%) than on the long arm (average of 31%; Table 2 and Fig. 1). We
also analyzed 52 ovarian cancers with markers on both arms. In
contrast with breast cancer, there was more LOH on the q arm
(average 65%) compared to the p arm (average 38%; data not shown).

The results suggest there are eight relevant breast cancer regions on
chromosome 17, two regions located on 17p and six regions on 17q.
These regions were defined by multiple cases of interstitial deletions
and terminal deletions (Figs. 2 and 4). The two regions on 17p include
TP53 (17p13.1) and a more telomeric region at 17p13.3 that includes
D17S75, D17S73, and D17S849. This latter region was defined by 17
independent observations of terminal deletion at D17S75 (thereby
removing the remainder of the terminus) and a further seven tumors
showing a smaller terminal deletion at the marker D17S73; the im-

Table 3 Statistically significant association between clinical features and LOH at particular chromosome 17 markers

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Marker</th>
<th>No. of cases with LOH/total no. of cases</th>
<th>No. of cases with LOH/total no. of cases</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER status</td>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td>111/233</td>
<td>81/128</td>
<td>8.119</td>
<td>0.0044</td>
</tr>
<tr>
<td></td>
<td>THRA1</td>
<td>43/182</td>
<td>33/81</td>
<td>7.99</td>
<td>0.0047</td>
</tr>
<tr>
<td></td>
<td>D17S800</td>
<td>13/67</td>
<td>12/23</td>
<td>9.176</td>
<td>0.0025</td>
</tr>
<tr>
<td></td>
<td>D17S855</td>
<td>33/116</td>
<td>28/57</td>
<td>7.157</td>
<td>0.0075</td>
</tr>
<tr>
<td></td>
<td>D17S579</td>
<td>47/185</td>
<td>44/106</td>
<td>8.131</td>
<td>0.0044</td>
</tr>
<tr>
<td></td>
<td>NM23</td>
<td>32/142</td>
<td>39/82</td>
<td>15.04</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>D17S754</td>
<td>57/247</td>
<td>57/123</td>
<td>20.801</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>D17S785</td>
<td>14/61</td>
<td>19/35</td>
<td>9.004</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>D17S4</td>
<td>46/211</td>
<td>42/91</td>
<td>18.261</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>D17S24</td>
<td>32/152</td>
<td>28/60</td>
<td>13.909</td>
<td>0.0002</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>14/89</td>
<td>21/89</td>
<td>8.697</td>
<td>0.0032</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D17S250</td>
<td>160/248</td>
<td>6/22</td>
<td>10.315</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td>196/343</td>
<td>13/41</td>
<td>9.522</td>
<td>0.0020</td>
</tr>
<tr>
<td></td>
<td>D17S574</td>
<td>110/346</td>
<td>3/42</td>
<td>9.862</td>
<td>0.0017</td>
</tr>
</tbody>
</table>

$^\circ$ $\chi^2$ values included Yates continuity correction.
Fig. 3. Comparison of percentage of chromosome 17 LOH for each clinical feature. a–g, left, order of chromosome 17 markers; bottom, percentage scale. Each clinical feature is divided into two subsets, and the level (percentage) of LOH for these subsets is compared. The number of cases assayed for the two subsets at each marker is shown in two columns. Statistically significant differences in LOH (i.e., P < 0.01) are shown with the corresponding $\chi^2$ values and $P$ (boldface). Other values, where 0.05 > $P$ > 0.01, are also shown (lightface). * the Yates continuity correction has been incorporated into these $\chi^2$ values. Horizontal line in each panel indicates the position of the centromere. a, the difference in LOH for tumors of ≤2 cm (○) versus tumors >2 cm in size (●). b, the differences in LOH for ER-positive tumors (○) versus ER-negative tumors (●). c, LOH differences for tumors that had metastasized to the lymph nodes (●) compared to those that had not metastasized to the lymph nodes (○). d, LOH differences for tumors with distant metastases (●) versus those without (○). e, LOH differences for cases with an age of onset ≤50 years (○) compared to those age >50 years (●). f, LOH in tumors with a family history of breast cancer (●) compared to those cases without (○). g, differences in LOH in ductal (●) versus lobular and other (○) cases.
portance of this region in breast and other tumors has been has been reported previously (43). Breast tumors with LOH at D17S55 have been associated with a higher risk of recurrence than those showing retention of this region (11). In this study, there was a statistically significant association with LOH in this region, as well as ductal histopathology (Table 3; Fig. 4) and a potentially significant association with positive family history ($\chi^2 = 5.697, P = 0.0170$; Fig. 3e). The second region on 17p incorporating the TP53 gene was implicated as a result of numerous independent interstitial deletions centered at TP53 and D17S786 and a significant association with ER negativity and ductal histology.

The six relevant regions on 17q (IV–IX) were also defined by multiple independent observations of interstitial and terminal deletions. Region III is not considered to be relevant, because the deletion defining this region was only seen in one tumor (Figs. 2 and 4) and did not show significant associations with any of the clinical parameters. In the region 17q22–qter, there appear to be five different regions of deletion (Fig. 4). There were several cases of interstitial deletion that spread from NM23 to D17S4 but retained the terminus, which suggests the presence of several genes in this area, and deletions of only the terminus indicate another more distal gene(s). Numerous terminal deletions were observed in single cases; however, they did not contribute to narrowing down regions of deletion.

Region IV contains the BRCA1 gene (D17S855 is within BRCA1). This region showed a strong association with ER negativity. An association of LOH at the BRCA1 region and ER negativity has been reported previously, specifically to the marker D17S579 (9, 11, 40, 44), which suggests that there may be some interplay between ER and BRCA1. ER-negative tumors showed significantly higher frequencies of LOH at almost all markers on chromosome 17 compared to ER-positive tumors, which correlates with this feature as an indicator of poor prognosis. The small interstitial deletions defining region V, centered around D17S806, were observed in multiple independent cases. However, there was no association between this region and a clinicopathological feature.

Region VI includes the metastasis-associated gene NM23. This region was significantly associated with ER negativity and ductal histology but not with either lymph node or distant metastasis. Previous studies have associated chromosome 17 imbalance and lymph node metastasis (2), and particularly LOH at D17S34 on 17p and NM23 on 17q had been associated with lymph node-positive tumors (9, 30). We did not observe such an association in this study. Distant metastasis at follow-up have previously been associated with chromosome 16q LOH in familial breast tumors (45). The genes on chromosome 17 may be involved at an earlier stage, as abnormalities have been identified in ductal carcinoma in situ (29). Alternatively, the lack of association with distant metastases may be due to patient selection. It is unusual to operate on the breast tumor of patients who already have distant metastasis, so there may be a selection against the inclusion of cases with this feature in this study.

Region VII includes the growth hormone (GH) gene. There were no significant associations with the region and any clinical feature. Regions VIII and IX showed associations with ER negativity and some potential associations with age of onset ($P = 0.0235$; Fig. 3e) and family history ($P = 0.0155$; Fig. 3f). Breast cancer cases age $\leq 50$ years revealed a higher frequency of LOH on chromosome 17 in general than did older women, but statistical significance was not reached for any marker in this study. This is in contrast to published data (9), in which more LOH was seen in postmenopausal women at loci on chromosome 17. Surprisingly, the differences in LOH for the age of onset and family history data were not centered around BRCA1 or TP53, which are both genes giving rise to earlier onset of hereditary breast cancer (TP53 to a lesser extent). Instead, there was more frequent LOH on the telomeric portions of both the p and q arms in sporadic tumors. However, tumors from women with a family history showed statistically significantly more LOH at D17S250 only. This suggests that different regions of chromosome 17 may be involved in sporadic versus familial breast cancer. There have been reports of familial breast tumors showing LOH at 17pter and 17qter (28), which was observed in quite small families that may not have been linked to BRCA1. A relevant region of chromosome 17 distal to BRCA1 is repeatedly deleted in ovarian carcinomas from cases with a family history of breast and ovarian cancer (27). These data suggest that there may be other genes on chromosome 17 involved in inherited breast and ovarian cancer that are not as penetrant as BRCA1 and TP53 (46) and that perhaps give rise to smaller breast cancer kindreds.

There was considerable variation in the levels of LOH for each marker between the different centers, which is probably due to variation between the different ethnic groups included in this study. This variation may account for the association between a particular clinical
parameter and multiple relevant regions on chromosome 17; e.g., histopathology is associated with three regions (Figs. 3f and 4).

The finding of a significant association was not likely to result from the number of informative tumors for a particular marker. A large number of tumors with data on lymph node metastasis was assayed with THRA1 (120 node-positive and 108 node-negative tumors) and D17S74 (198 node-positive and 208 node-negative tumors), yet showed similar frequencies of LOH in lymph node-positive and -negative cases (Fig. 36).

In conclusion, in the largest study of its kind in breast cancer, we have narrowed down eight relevant regions of chromosome 17 involved in breast cancer. These regions are defined by genetic abnormalities in multiple independent cases. Seven of these regions show significant differences in LOH with specific clinical features of breast tumors. We have also shown that chromosome 17 abnormalities as defined by LOH are associated with increased aggressiveness of breast tumors (larger size, ER-negative status, early age of onset, and ductal subtype). It is not clear, however, whether the chromosome 17 abnormalities may cause tumor aggressiveness or, alternatively, that chromosome 17 disintegration occurs as a consequence of tumor progression and development.

ACKNOWLEDGMENTS

We acknowledge all of the patients who participated in this study and the clinicians and surgeons who collected the blood samples and tumor tissues. We acknowledge the participation of the Breast Cancer Somatic Genetics Consortium (the Netherlands), including C. J. Cornelisse, M. Van Vliet, and N. Kuipers.

REFERENCES

REGIONS TARGETED BY CHROMOSOME 17 LOH IN BREAST CANCER


Consortium Study on 1280 Breast Carcinomas: Allelic Loss on Chromosome 17 Targets Subregions Associated with Family History and Clinical Parameters

Catherine M. Phelan, Åke Borg, Marguerite Cuny, et al.

*Cancer Res* 1998;58:1004-1012.

**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/58/5/1004

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.