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13. ABSTRACT

Genetic changes implicated in the etiology of breast cancer have been identified by the detection of loss of heterozygosity at specific loci. Our study utilizes a series of genetic polymorphisms detectable by the polymerase chain reaction (PCR) to look for changing patterns of LOH as breast cancer progresses from intraductal to invasive and then to metastatic disease. The initial phases of this work involved the identification of cases from the AFIP archives, the development of procedures for cutting cases, and microdissection of individual tumor components. In the past year, lysates from 116 breast carcinomas have been analyzed for LOH at 11 markers on chromosome 11p15, 5 on 17q, two on 3p, and two on 14q. Analysis of the 11p15 data localizes a common region of LOH to a region of approximately 9 megabases centered on the markers D11S1318 and D11S4046. LOH of chromosome 17 shows a more complex distribution which is being characterized through the use of multiple markers along this chromosome. LOH at the markers analyzed thus far is almost always seen in the intraductal carcinoma and maintained throughout the subsequent stages of tumor progression.

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TABLE OF CONTENTS

INTRODUCTION

The earliest events in the pathogenesis of breast cancer typically involve the loss of a normal growth regulatory mechanism by a ductal or lobular epithelial cell. Progression of the disease through the stages of intraductal proliferation to invasive carcinoma and then to metastatic disease appears to require additional alterations in growth regulatory pathways. A substantial body of evidence now supports the idea that these alterations in growth regulation result from genetic events such as point mutation, deletion, and gene amplification [1-4]. Our study aims to characterize genetic alterations in breast tumors at the various stages of tumor progression. If metastasis requires additional genetic events beyond those responsible for the intraductal and invasive components of the tumor, one should find genetic alterations in the metastasis that are not present in primary tumor. Alternatively, there may be certain genetic lesions which occur early in tumor development that can predispose a tumor to metastasize without the acquisition of additional genetic defects. The identification of such a lesion would provide an important prognostic indicator, because it would provide a means for predicting the likelihood of the development of metastatic disease in tumors identified at an early stage. The characterization of genetic changes present in individual tumor components thus offers the possibility of identifying new prognostic indicators as well as helping to elucidate the significance of genetic events to tumor progression.

The type of genetic analysis performed in our study is the amplification of polymorphic loci by the polymerase chain reaction (PCR) [5]. This technique permits the detection of loss of heterozygosity (LOH) in tumor specimens relative to normal tissue from the same patient. LOH at specific loci has been observed frequently in breast cancer. High frequency of LOH for a specific genetic marker is thought to imply the presence of a tumor suppressor gene at that locus [3, 4]. In certain cases (e.g., p53 on 17p, DCC on 18q), the loss of one copy of the tumor suppressor gene (LOH) is found in association with mutation of the remaining copy. In such cases, LOH indicates that both copies of the tumor suppressor gene have become inactivated, resulting in the loss of a normal growth regulatory pathway. The PCR methodology also permits the detection of gene amplification, assuming that amplification involves only one of the two copies of the gene present. In breast cancer, amplification of the HER2/neu oncogene is of particular interest because of potential prognostic implications [2].

The general strategy of our study involves the identification of a group of breast cancer cases from the AFIP archives followed by microdissection of the intraductal, infiltrating, and metastatic components present in each tumor, and analysis of each tumor component for LOH at multiple genetic loci. The results should help address questions such as when during tumor progression specific genetic lesions occur, and whether LOH at any particular locus has value in predicting the course of progression of an individual tumor. In addition, through the analysis of multiple closely linked markers, the boundaries of each region of LOH can be identified. Comparison of multiple cases showing interstitial deletions often demonstrates a narrow region where these deletions overlap one another. The

identification of such a region of overlap suggests the existence of a tumor suppressor gene in the common segment of overlapping LOH.

BODY

The analysis of LOH during breast cancer progression during the past year has focused on a group of 116 cases obtained from the AFIP archives. The methods developed during the first year of funding for microdissection, oligonucleotide design, and PCR to detect short tandem repeat polymorphisms continued to prove reliable and effective for generating the required data. A major focus of our effort has been the analysis of cases with interstitial deletions at 11p15, a locus suspected to harbor an as yet unidentified tumor suppressor gene. Additional loci examined include 17q, 3p, and 14q.

The 11p15 study involved the analysis of the 116 breast carcinomas for LOH at eleven loci spanning approximately nine megabases from 11p15.3 to 11p15.5. 47 (41%) of these cases demonstrated LOH at one or more of the markers. When present, LOH at 11p15 was always detected at the earliest stage of the tumor available for analysis. Of the 47 cases with LOH at 11p15, 19 showed LOH at all informative markers, while the remaining 28 yielded results consistent with interstitial deletions. Of these 28 cases, LOH was detected at a group of contiguous markers, suggesting loss of a single chromosomal region, in all but five cases. Case 41 retained heterozygosity for the three proximal markers D11S4124, D11S4181, and D11S1323, but showed LOH for the 11p15.3 marker ST5 as well as for the three telomeric markers D11S922, D11S1318, and TH. Case 101 showed LOH at the proximal markers ST5 and D11S988 and the distal marker D11S4046, but remained heterozygous at TH and D11S988. Three additional cases demonstrated LOH only at 11p15.3 marker, retaining heterozygosity for all informative 11p15.5 markers. These cases suggest the possibility of two regions of LOH on 11p15, one centered on the markers TH/D11S1318/D11S4046, and the second located more proximally, at 11p15.3-15.4. The data suggest localization for one tumor suppressor gene at 11p15 between D11S922 and D11S988, a chromosomal segment comprising approximately four megabases. The markers showing the highest sensitivity for detecting LOH were D11S1318 and D11S4046, suggesting that the tumor suppressor gene may be localized to the smaller region containing these markers.

Additional loci were chosen for analysis based on evidence for their importance in breast cancer. At the present time, the panel of cases has been analyzed for LOH at five markers on 17q, two on 3p, and two on 14q. For all markers tested to date, whenever LOH is observed in a tumor, it is seen at all stages of progression. Thus, our hypothesis that LOH at certain loci would characteristically appear only in later stages (infiltrating or metastatic tumor) of breast cancer progression has not been supported by data from the markers analyzed. It remains possible that examination of additional loci may yet reveal a locus at which LOH is seen only in the infiltrating or metastatic components of the disease.

Chromosome 17 contains several genes of importance to breast cancer, including

HER2, BRCA1, and NM23, all on 17q, and p53 on 17p. Although breast cancers are known to show frequent LOH of chromosome 17, it is not known whether this LOH reflects inactivation of a tumor suppressor gene, amplification of an oncogene (e.g., HER2), or some other pattern of genetic alteration. Our initial data, based on the 5 markers studied thus far, suggests a complex pattern of LOH that most commonly involves a segment of 17q involving the BRCA1 gene. However, BRCA1 is not thought to be involved in a high percentage of sporadic breast cancers, and other laboratories have suggested that a distinct tumor suppressor gene located in this region might be important in breast cancer. Since several genes at widely varying loci on chromosome 17 may play a role in breast cancer, we plan to expand our study to a panel of 15-20 markers spanning the full length of this chromosome. This larger study should reveal a picture of the overall dynamics of chromosome 17 alterations in breast cancer, and may help clarify the significance of LOH at the various segments of this chromosome.

Data presented at a conference in the past year suggested that LOH at 14q was predictive of a non-metastasizing phenotype. This report prompted us to examine this hypothesis with our own panel of cases. Our analysis demonstrated similar frequencies of LOH at 14q in metastasizing and non-metastasizing tumors. These results refute the hypothesis that LOH at 14q predicts a non-metastatic phenotype.

Finally, LOH data has been generated for two markers on 3p, one of which lies within the FHIT gene which work during the past year suggests may be abnormal in breast cancer. Analysis of this data is in its early stages.

CONCLUSIONS

The 11p15 study localizes the common region of overlap to a region similar to that observed by other laboratories, but localized to a smaller segment of the chromosome than in previous studies. Our results show that the markers D11S1318 and D11S4046 have the greatest sensitivity for detecting LOH in this region, and suggest that the tumor suppressor gene may lie close to these markers. Data from the 14q markers refute a hypothesis that LOH at this locus predicts a non-metastasizing phenotype. The results of the 17q markers suggest complex genetic alterations affecting this chromosome which need to be studied in more detail. The data as a whole demonstrate that LOH at 11p15, 14q, 17q, and 3p occurs in the intraductal stage of the tumor.

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High Density Polymorphism Mapping of Chromosome 11p15 in Breast Cancer Defines a Single Region of Loss of Heterozygosity Present in Early Stages of Tumor Development

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ABSTRACT

Loss of heterozygosity at chromosome 11p15 occurs with high frequency in multiple tumor types. This region is involved in the Beckwith-Wiedemann Syndrome (BWS) and has been shown directly to contain a tumor suppressing activity by chromosome transfer experiments. In this study we have mapped the region of LOH at 11p15 in a panel of 116 breast cancers using high density short tandem repeat polymorphisms. Our data are consistent with a single minimal region of overlap between the markers D11S4046 and D11S1758. Results from four cases suggest the possibility of a second important region at 11p15.3, but LOH at this region was never observed independently of LOH at the more distal markers. Microdissected specimens of intraductal, infiltrating, and metastatic tumor were analyzed to determine the stage of progression at which LOH at 11p15 occurs. LOH at 11p15 was always present in the intraductal component of the tumor and maintained throughout subsequent stages of progression. LOH at 11p15.5 showed no correlation with estrogen receptor or lymph node status, tumor size, histological grade, or long term survival. The results show that LOH at 11p15.5 in breast cancer occurs at an early stage of tumor development, and that the minimal common region of LOH includes the telomeric cluster of BWS breakpoints and chromosomal segments with tumor suppressor activity.

INTRODUCTION

Several lines of evidence suggest the existence of a tumor suppressor gene on chromosome band 11p15. This locus has been shown to demonstrate a high frequency of loss of heterozygosity (LOH) in multiple tumor types, including malignancies of the breast (1-7), lung (8-12), cervix (13), testis (14, 15), bladder (16), stomach (17), and pediatric tumors of the adrenal and liver (18, 19). This region has also been of interest because patients with the Beckwith-Wiedemann syndrome (BWS) often have chromosomal rearrangements involving 11p15 (20). This syndrome causes a characteristic pattern of hypertrophy of several organs and is associated with several childhood malignancies, including hepatoblastoma and Wilm's tumor. Eight BWS breakpoints have been cloned (21-23). The location of these breakpoints relative to several known genes is shown in Fig. 1. Five of the breakpoints are clustered in a region of 11p15.5 less than 400 kb in length which contains the candidate tumor suppressor gene p57Kip2 at its centromeric border. (24-27). The KVLQT1 gene, encoding a putative potassium channel, spans all five breakpoints in this region and is considered a candidate susceptibility gene for BWS (28). This region lies 100-200 kb centromeric to a locus that contains three very closely spaced genes: insulin, IGF2, and tyrosine hydoxylase (TH). Another candidate tumor suppressor, H19, lies less than 200 kb telomeric to IGF2. The remaining three BWS breakpoints occur within a 1.2 megabase region of band 11p15.3, approximately four megabases centromeric to the cluster of five breakpoints. The recently described tsg101 gene, which was found to be mutated in several breast carcinomas, localizes to 11p15.1, a locus centromeric to the region containing the BWS breakpoints and the common region of LOH identified in multiple tumor types (29). Within the region of about

six megabases containing the BWS breakpoints it is not known whether a single gene accounts for the BWS phenotype or, as has been proposed (24), if the translocations result in the abnormal regulation of several growth regulatory genes located at 11p15.

Additional interest in this region comes from studies in which growth inhibition or suppression of tumorigenicity has been demonstrated by the physical transfer of chromosomal segments into cultured cancer cells. By using this technique, a tumor suppressor activity for the cell line G401 has been mapped to 11p15.5 (30, 31). Using subchromosomal transferable fragments, a growth inhibitory activity for the rhabdomyosarcoma cell line RD was mapped to a similar region (32). Transfer of chromosome 11 into the breast cancer cell lines MDA-MB-435 and MCF7 has been shown to suppress metastasis (33) and tumorigenicity (34), respectively.

Although comparisons of interstitial deletions revealed by studies of LOH at multiple markers generally suggest a minimal region of overlap, some studies report two or three such regions within 11p15 (6, 8, 11, 18). There appears to be a close match, at least at the level of resolution of the markers tested, between the minimal regions of overlap in adult tumors, the chromosomal segment containing the BWS breakpoints, and the region demonstrating growth inhibitory activity in chromosome transfer experiments. However, it remains unclear whether the same gene or genes are involved in these three settings. The detection of multiple minimal regions of overlap in some studies raises the question of whether several tumor suppressor genes could be independently involved within this relatively short chromosomal segment. Finally, while some of the minimal regions of overlap identified by LOH analysis seem to be the same for different tumor types, several studies, in particular those addressing LOH in breast and lung carcinomas, have led to the suggestion that the

minimal regions of overlap for these two types of tumor might be distinct (11).

Recent research aimed at mapping the human genome has identified large numbers of well localized, highly polymorphic genetic markers that can be used to study LOH at a level of resolution not previously possible. In this study we used a panel of such markers to further refine the localization of the minimal region of overlap at 11p15 in breast cancer. In addition, we wished to define the point during breast cancer progression at which 11p15 LOH occurs. To address these questions, the intraductal, invasive, and metastatic components of the tumors studied were isolated by microdissection and tested individually for LOH. We chose a group of eight CA repeat markers from the Genethon panel (35) that span the segment of 11p15 containing the BWS breakpoints. For several of these markers, no LOH data has been previously published. We also studied our panel of tumors for LOH at polymorphic markers within the Tyrosine Hydroxylase (TH) gene, which lies within or at the border of a previously identified minimal region of overlap at 11p15.5, and within the ST5 (D11S837E) gene which maps within a group of potentially growth regulatory genes at 11p15.3, the region containing the more centromeric BWS breakpoints (36-38).

MATERIALS AND METHODS

Case selection. The material used in this study consisted of formalin fixed paraffin embedded tissue from the archives of the AFIP. The cases analyzed were selected from a group of approximately 1000 cases submitted to the AFIP between 1975 and 1982. These cases were selected for a study of clinical-pathological correlations and consisted of cases with a diagnosis of breast carcinoma selected in the order of accession from a subset for whom the social security number was available to facilitate determination of vital status. A subset of these cases was selected for LOH analysis on the basis of the availability of normal tissue and the presence of either intralobular or intraductal lesions which were believed by the initial observers to be separable by microdissection from invasive and metastatic components of the tumor. Of 116 cases, 66 (57%) were node negative, and 50 (43%) had dissectible intraductal components. Each tumor component present was isolated by microdissection from 12 μm sections which had been deparaffinized with Hemo-De (Fisher). Lysates were prepared from these tissue specimens by incubation in 200 μl of 10 mM Tris, pH 8.0/50 mM KCl/0.1 mM EDTA/0.5% Tween 20/100 μg/mL Proteinase K for 12-16 hr at 55°C followed by a 5 min incubation at 95°C to inactivate the protease. Insoluble material was pelleted by centrifugation for 5 min and the supernatant was used as the source of DNA template for PCR.

LOH Analysis. The markers used were taken from the Genethon panel (35, 39). The order assigned is based on the Genethon linkage data and physical mapping studies (40). The marker TH was placed proximal to D11S1318 in two previous studies of breast cancer (4, 6), but recent mapping data suggests the opposite order, (11, 41), as shown in Fig. 1.

PCR reactions were performed in the presence of one ^{32}P-end labeled primer. Products were resolved on 6% polyacrylamide/7 M urea gels and visualized by autoradiography. The ratio of alleles present was evaluated by visual inspection of an appropriately exposed autoradiogram, and a change in allelic ratio estimated to be greater than 50% between normal and tumor tissue was scored as LOH. Cases were considered uninformative if the normal tissue revealed homozygosity for that marker or if the normal or tumor tissue failed to amplify. Because nonamplifiable specimens were scored as uninformative, the percentage of cases reported as uninformative for each marker studied is greater than the percentage of homozygotes.

RESULTS

Allelic Loss of Chromosome 11p15 in Breast Cancer. 116 carcinomas of the breast were analyzed for LOH at ten loci spanning approximately six megabases from 11p15.3 to 11p15.5 (Fig.1). Of the 116 cases, 43 (37%) demonstrated LOH at one or more of the markers. Results for these cases are presented in Fig. 2. Of these 43 cases, 19 showed LOH at all informative markers. Of the remaining 24 cases, 20 yielded results consistent with loss of a continuous segment of the chromosome. Data from four cases (41, 93, 101, and 103) suggest two regions of LOH, one centered on the markers TH and D11S1318, and the second located more proximally, at 11p15.3-15.4. Cases 41 and 93 retained heterozygosity for the markers D11S4181 and D11S1323, but showed LOH for the 11p15.3 marker D11S837 as well as for several of the more telomeric markers. Cases 101 and 103 similarly demonstrated LOH at the proximal marker D11S837 and one of the distal markers, but retained heterozygosity at one of the intervening loci.

Examples of the various patterns of LOH observed are presented in Fig. 3. Case 6 shows LOH at all informative markers. Case 38 demonstrates LOH at the more telomeric of the markers analyzed, but shows retention of heterozygosity over the centromeric segment of the region analyzed. Case 93 is an example of a case with two regions of LOH separated by a region of retention of heterozygosity.

In this group of cases, microsatellite instability was observed only at D11S922, the most telomeric of the markers analyzed. Each of the three cases demonstrating instability at this locus (31,69, and 115) showed LOH at all other informative markers.

The sensitivity of each marker for detecting LOH at 11p15 is presented in Table 1.

Both TH and D11S1318 detected LOH in 100% of informative cases. The flanking markers on either side of this region showed reduced sensitivity, an effect that becomes especially apparent when only those cases with interstitial deletions are considered. In this group of cases, each of the three most proximal markers detected less than half of all informative cases with LOH.

Analysis of patterns of LOH in cases with interstitial deletions suggests a minimal region of overlap between D11S4046 and D11S1758. All three cases with retention of heterozygosity at D11S922 (#58, 72, and 120), as well as the three cases with microsatellite instability at this locus (#31, 69, and 115), demonstrated LOH at D11S4046. Two cases retained heterozygosity at D11S4046, providing evidence that the minimal region of overlap does not extend telomeric to this marker. At the centromeric border of this region, one case (#38) retained heterozygosity at D11S1758, and two (#38 and 101) at D11S4146, suggesting that these markers lie proximal to a minimal region of overlap.

LOH at 11p15 during breast cancer progression. Wherever possible, intraductal, infiltrating, and metastatic components of the tumor were isolated by microdissection and analyzed separately. Samples of intraductal carcinoma were available for analysis in 32 of the 43 cases (74%) showing LOH at 11p15.5. In each of these cases, LOH was present in the intraductal component of the cancer and maintained in the invasive and, when present, the metastatic components of the tumor.

Clinicopathological correlations. Clinical and pathological data on these cases were reviewed and analyzed for associations with LOH at 11p15. No relationship was found between LOH at this locus and estrogen receptor status, the presence of positive lymph nodes, S phase fraction, tumor size, histological grade, or long term survival.

DISCUSSION

We have analyzed a panel of 116 carcinomas of the breast for LOH at ten loci spanning a region of chromosome 11p15 approximately six megabases in length. LOH was identified in 43 (37%) cases, of which 24 provided evidence of interstitial deletions. The overall percentage showing LOH is comparable to the 35% previously reported by Winquist et al. (6) in breast cancer, who also noted that LOH at this locus is most often interstitial. Similar percentages of LOH have been reported in other tumors, including lung (43%, (11)), bladder (30%, (16)), stomach (41%, (17)), and testis (59%, (14)).

Our results are consistent with a simple model in which LOH at 11p15.5 reflects the loss of a single tumor suppressor gene. The most likely locus of this gene is defined by the minimal region of overlap bordered by the markers D11S4046 and D11S1758. As in other studies of similar size, the number of cases supporting this minimal region is relatively small: two cases with retention of heterozygosity at D11S4046 and one at D11S1758. The data more strongly support the localization of a minimal region of overlap between D11S922, with six cases retaining heterozygosity (including the three with microsatellite instability) and D11S988, with four cases showing retention of both alleles. It could be argued that the four cases that showed two regions of LOH separated by a region of retained heterozygosity provide evidence for a second significant region of LOH located more proximally. However, no case was identified with LOH exclusively in the more proximal region. Although several studies have reported occasional cases with LOH in two distinct regions of 11p15, none have described a case with LOH of only the proximal segment of this band (4, 6). These results suggest that the mechanism of LOH may be sufficiently complex to allow retention of

heterozygosity for some markers within a region of LOH. However, the alternative possibility, that LOH at 11p15.3 reflects the presence of a distinct tumor suppressor gene, cannot be excluded.

Our interpretation of the LOH data differs in some respects from those derived from previous studies. The results of Winqvist et al. (6) identified the markers TH and D11S988 as boundaries of a minimal region of overlap. However, data from only one case supported TH as the telomeric boundary. In contrast to our finding that LOH at D11S1318 occurred in 20 of 20 informative cases (100%), these investigators observed retention of heterozygosity at this locus in 5 cases with LOH at other 11p15.5 markers. Although the reasons for this apparently discrepant observation are unclear, the consequence is that we include the gene cluster containing insulin/IGF2/tyrosine hydoxylase and more telomeric sequences, extending to a point near the candidate tumor suppressor H19, which lies close to D11S4046, in our minimal region of overlap. The results of the previously reported study argued that the minimal region most likely excluded these genes. Data from another study (4) was interpreted as supporting two independent regions of LOH at 11p15.5, one between D11S1318 and D11S988, the other located distal to D11S1318. However, the evidence for a distinct distal region of LOH consisted of a single case with retention of heterozygosity at D11S1318 and LOH of both proximal and distal markers. As noted above, the mechanism by which LOH occurs may occasionally result in retention of heterozygosity for some markers internal to a larger region of LOH. In the absence of multiple cases with a distinct region of LOH distal to D11S1318, there appears to be little evidence to support the existence of a distinct tumor suppressor gene in this region.

Minimal regions of shared LOH have been described in several tumor types other than

breast cancer. In non-small cell lung cancer (NSCLC), one study (11) reported evidence for a telomeric region distal to TH, centered on D11S922, and a second region proximal to D11S988, centered on D11S909. The latter marker is close to D11S837, the most proximal marker used in the present study. As observed in other studies of LOH at 11p15.5, no cases demonstrated LOH exclusively at the more proximal locus. These results suggest that the 11p15.5 gene involved in NSCLC may be different from that involved in breast cancer. However, other studies support the involvement of the same chromosomal segment in multiple tumor types. Characterization of 100 carcinomas of the bladder suggested a minimal region of overlap between D11S922 and D11S569 (16), which includes the region defined by our study. A study of 13 hepatoblastomas suggested a significant region of LOH distal to the HBB locus (19). In an analysis of 60 adenocarcinomas of the stomach (17), most cases were found to have LOH of all informative markers, but two cases suggested a minimal region of overlap bordered by D11S1318 and D11S988.

Studies of chromosomal translocations in other human malignancies have mapped breakpoints within the common region of LOH at 11p15. One report localized a breakpoint approximately 60 kb centromeric to the TH gene (42) in a rhabdoid tumor. In another study an 11p15 breakpoint was cloned and found to represent a fusion between the Nup98 gene on 11p15 and the homeobox gene HOXA9 on chromosome 7 in a myeloid leukemia (43). Studies of BWS translocations have led to the identification of at least 11 genes within a 320 kb segment containing the more telomeric cluster of five breakpoints (24). None of these genes has been definitively shown to be responsible for BWS, although KVLQT1, which spans all five breakpoints, and p57Kip2, which may regulate cell growth, are considered strong candidates. This 320 kb segment maps to the proximal portion of the minimal region

of LOH defined by the present study, but extends only to a point approximately 100 kb centromeric to the insulin/TH/IGF2 gene cluster, which lies centrally within the region of LOH defined here.

In colon cancer, specific molecular alterations have been associated with the various stages in the progression of the tumor from a benign epithelial cell to a carcinoma. It might be expected that a similar sequence of events would occur in carcinoma of the breast. To examine the state of progression in which LOH at 11p15 occurs, we microdissected the available intraductal, infiltrating, and metastatic components of each tumor. For all cases with LOH at 11p15, LOH was detected in all tumor components analyzed. Furthermore, the ratio of the alleles in the intraductal tumors was similar to that seen in the other components, suggesting that the intraductal tumor is clonal with respect to LOH at 11p15. Deng et al. (44) have reported that LOH at several loci can occasionally be found in morphologically benign terminal duct lobular units (TDLU) adjacent to foci of intraductal carcinoma. In their series, only one of five cases with LOH at 11p15.5 was found to have LOH in the adjacent TDLU whereas chromosome 3p markers showed LOH with a much higher frequency in such morphologically normal specimens. This observation, together with our finding that LOH at this locus is always present in the intraductal carcinoma, suggests that LOH at 11p15.5 may be an event involved in the progression from morphologically benign epithelium to intraductal carcinoma.

A role for the relevant gene in this region in an early stage of carcinogenesis is consistent with the lack of correlation between LOH at 11p15.5 and measures of clinical outcome. Presumably, the significant genetic alteration at this locus occurs before the cell has acquired the aberrant growth characteristics necessary for invasion and metastasis.

Although some studies have reported associations between LOH at 11p15 and clinical parameters, one report has suggested that these correlations may actually reflect LOH at 11q, which is believed to contain a distinct tumor suppressor gene (6), and other investigators have also noted a lack of correlation between 11p15 LOH and clinical parameters (4).

FIGURE LEGENDS

Figure 1. Location of markers used in this study and candidate tumor suppressor genes. The upper map shows the position of several genes, including the candidate tumor suppressor genes H19 and KIP2, along the region of approximately 6 Mb analyzed in this study. The lower map shows the positions of the genetic markers used. The map positions of the Genethon markers are from the sex averaged Genethon Human Linkage Map (35). The distances between Genethon markers are indicated below the map in centiMorgans. The marker D11S837 (ST5 gene) has been localized within the centromeric group of BWS breakpoints (45). The locations of the markers D11S988 and TH are from the CHLC/GDB map. The upper and lower maps are shown in approximate alignment. The KVLQT1 gene occupies approximately 300 kb and encompasses the more telomeric group of BWS breakpoints (28). The TH gene is known to be closely linked to D11S1318, but the order of these two loci is not known with certainty. The H19 gene has been identified on a 100 kb bacterial artificial chromosome clone that also contains the marker D11S4046, suggesting close proximity of these loci in the genome.

Figure 2. Sublocalization of the minimal region of LOH at 11p15 in breast cancer. Results obtained with the 43 cases showing LOH with at least one marker are presented. Solid circles: LOH; Shaded circles: Retention of heterozygosity; Open circles: Uninformative (homozygous or nonamplifiable); M: Microsatellite instability. The solid bar to the right of the figure indicates the minimal shared region of LOH inferred from this data set.

Figure 3. Patterns of LOH at 11p15 in breast cancer. A. Case 6: LOH at all informative markers. B. Case 38: LOH at the distal group of markers, retention of heterozygosity at D11S1758, D11S4146, and D11S988. C. Case 93: LOH at proximal and distal markers with retention of heterozygosity between these two regions. N: normal tissue; T: tumor. Arrowheads indicate the allele lost in assays showing LOH.

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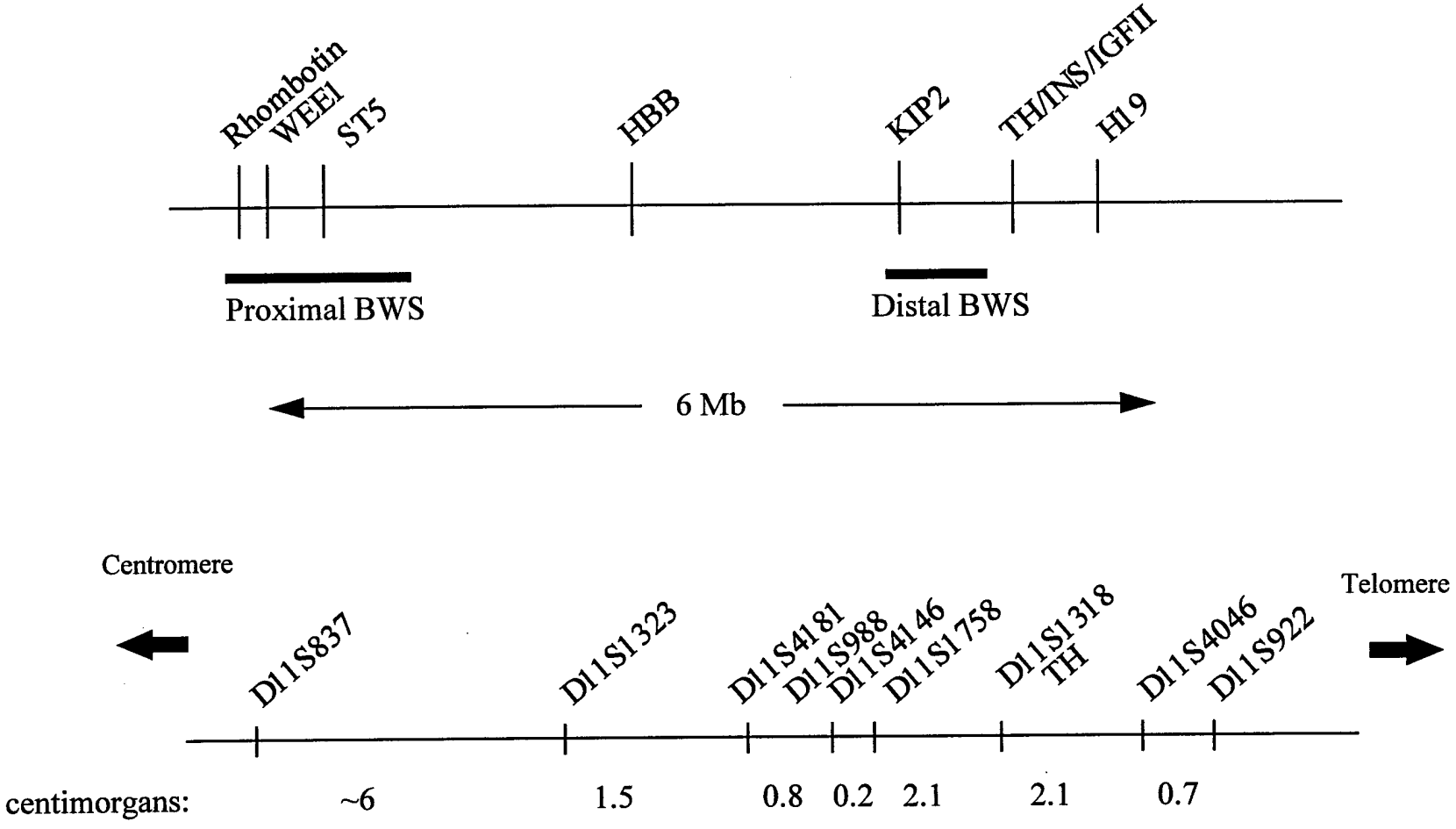
Table 1. Sensitivity of each marker for detection of LOH at 11p15.

	All Cases		Cases with Interstitial Deletions	
Locus	# LOH/#Informative	(%)	# LOH/#Informative	(%)
D11S922	23/29	(79%)	11/17	(65%)
D11S4046	24/26	(92%)	11/13	(85%)
TH	31/31	(100%)	18/18	(100%)
D11S1318	19/19	(100%)	11/11	(100%)
D11S1758	15/16	(79%)	6/7	(86%)
D11S4146	16/18	(89%)	7/9	(78%)
D11S988	18/22	(82%)	9/13	(69%)
D11S4181	14/22	(64%)	7/15	(47%)
D11S1323	10/14	(71%)	4/8	(50%)
D11S837	18/29	(62%)	9/20	(45%)


Rhombotin
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ST5
HBB
KIP2
TH/INS/IGFII
H19
Proximal BWS
Distal BWS
6 Mb
Centromere
Telomere
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D11S1323
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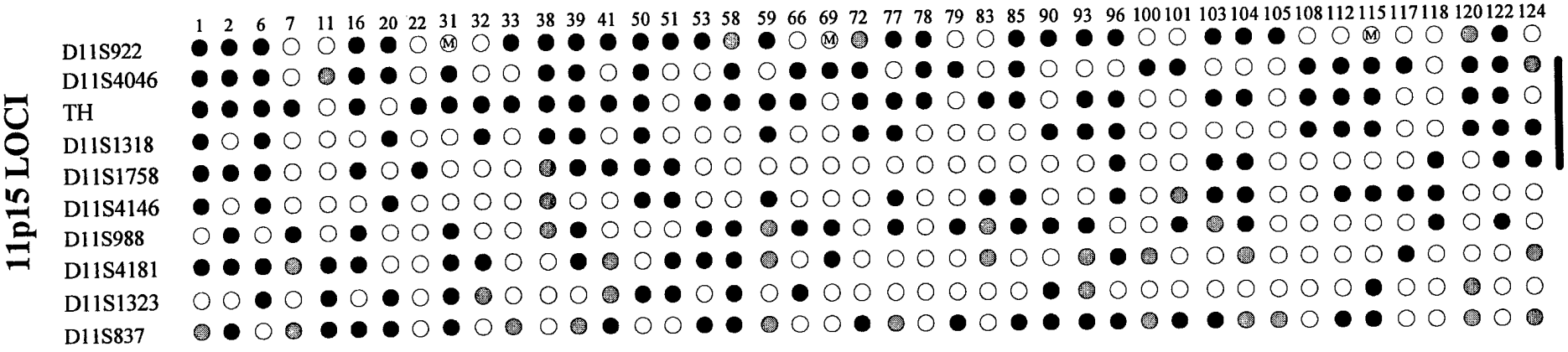

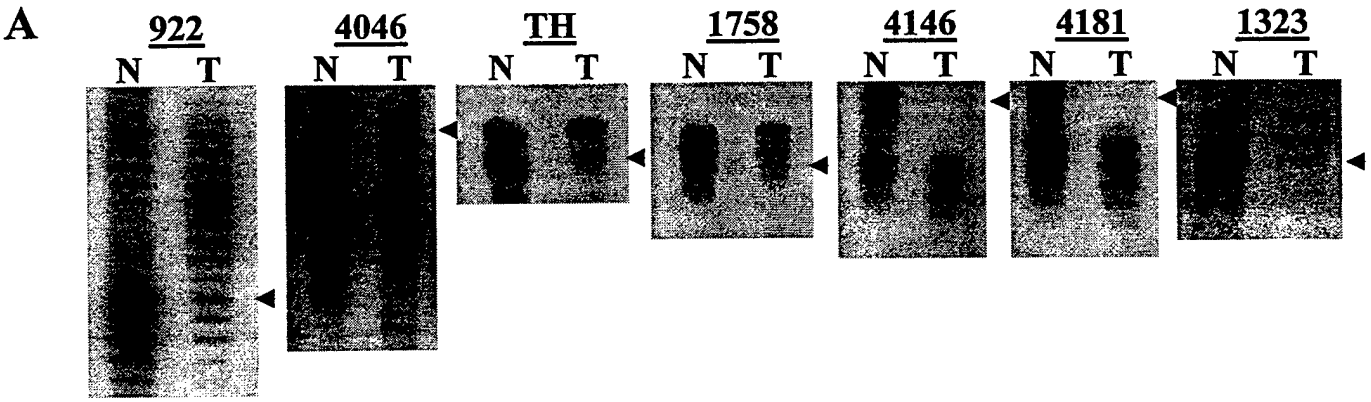
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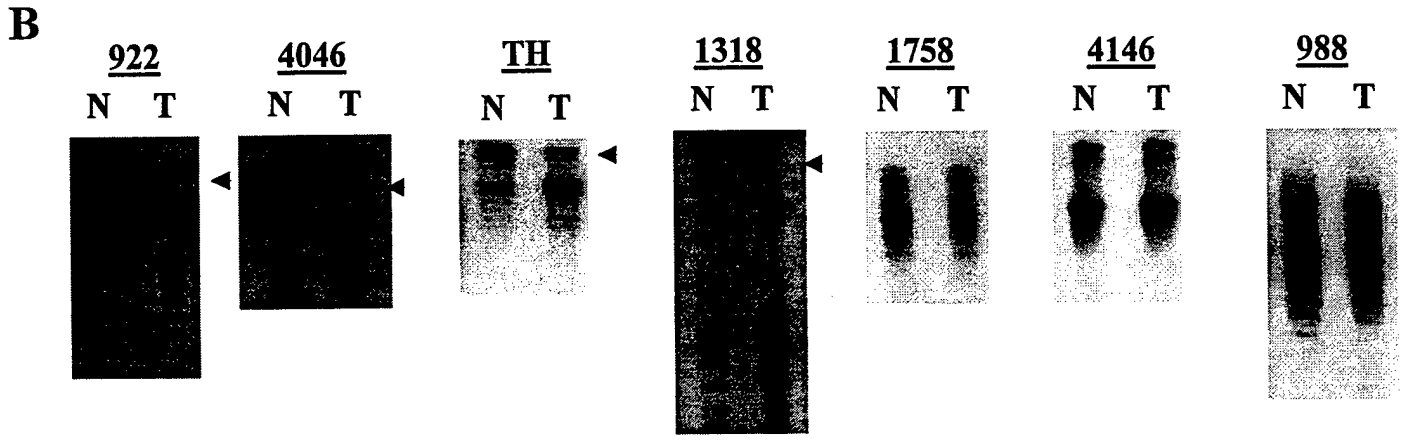
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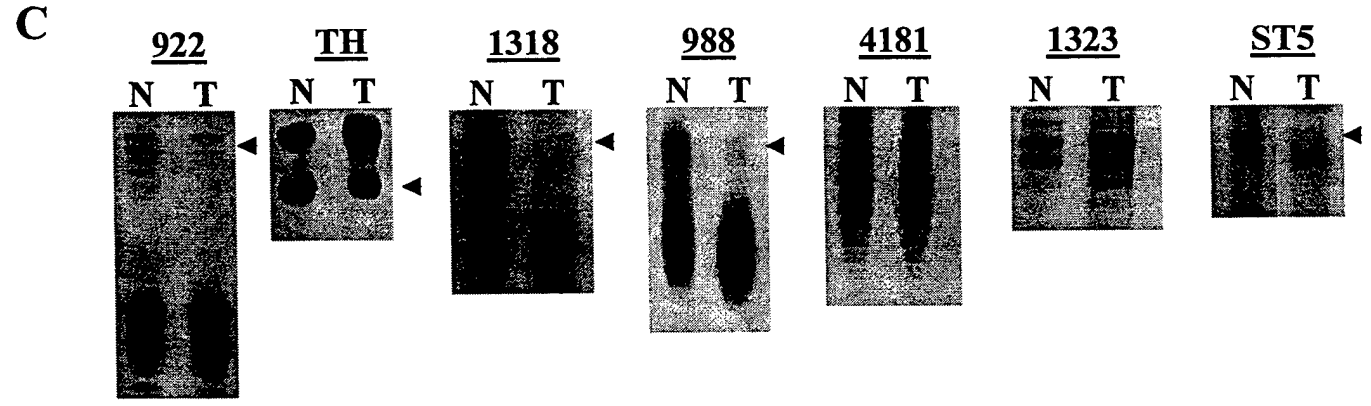
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