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Genetics

Loss of heterozygosity on chromosomes 17 and 18 in breast carcinoma: Two additional regions identified

(tumor-suppressor genes/primary breast carcinoma/restriction fragment length polymorphism/oncogenes)

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ABSTRACT **The loss of heterozygosity (LOH) at specific regions of the human genome in tumor DNA is recognized as evidence for a tumor-suppressor gene located within the corresponding region of the homologous chromosome. Restriction fragment length polymorphism analysis of a panel of primary human breast tumor DNAs has led to the identification of two additional regions on chromosomes 17q and 18q that frequently are affected by LOH. Deletions of each of these regions have a significant correlation with clinical parameters that are associated with aggressive breast carcinomas. Previous restriction fragment length polymorphism analysis of this panel of tumors has uncovered several other frequently occurring mutations. LOH on chromosome 18q frequently occurs in tumors with concomitant LOH of loci on chromosomes 17p and 11p. Similarly, tumors having LOH on 17q also have LOH on chromosomes 1p and 3p. This suggests that certain combinations of mutations may collaborate in the development and malignant progression of breast carcinomas.**

The genetic etiology of breast cancer appears, at least in part, to reflect an accumulation of mutations that are selected during tumor development. These mutations are thought to deregulate normal development of the mammary gland or to provide the affected cell with a selective growth advantage in the host (1–5). In breast cancer the most frequent type of tumor-associated mutation is the somatic loss of heterozygosity (LOH) at specific regions of the human genome (6). The initiation of these genes appears to be a consequence of a "two-hit" process (7–11). Commonly, one allele contains a point mutation or a small allelic deletion, while the second allele is lost by an interstitial deletion, chromosome loss, or aberrant mitotic recombinational event. Cytogenetic analysis of primary and metastatic breast tumors has demonstrated frequent genetic alterations involving chromosomes 1, 6, 7, and 11 (12). More recently, the use of recombinant DNA probes that detect restriction fragment length polymorphism (RFLP) has led to the identification of several chromosomal regions (chromosomes 1p, 1q, 3p, 11p, 13q14.1, 17p) that are frequently affected by LOH in primary breast tumor DNAs (6). These observations suggest that multiple tumor-suppressor genes may be involved in the etiology of breast carcinomas and raise the question of whether specific subgroups of tumors might be defined by the particular set of mutations that they contain. In this report we describe two new regions of the human genome on chromosomes 17 and 18 that are affected by LOH in primary breast tumor DNAs and demonstrate that they frequently occur in tumors containing other specific mutations.

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MATERIALS AND METHODS

Patients and Tumor Selection. Primary breast carcinomas and matching peripheral lymphocytes were collected at the Centre Rene Huguenin in Saint Cloud, France, from patients who had received no prior therapy. The distribution of clinical and pathological parameters associated with the tumors and patients has been published (13).

DNA Preparation and Southern Hybridization. Genomic DNA was extracted, and 10 μg was digested with the restriction enzyme of choice (14). The digested DNA was fractionated by agarose gel electrophoresis, transferred to Genatran 45 nylon membranes (Plasco, Woburn, MA), and baked for 2–3 hr at 80°C. The membranes were prehybridized and then hybridized with ^{32}P-labeled DNA probes made by the nick-translation (15) or random-primer (16) system. After hybridization, the membranes were washed under stringent conditions (15 mM NaCl/1.5 mM sodium citrate, pH 7, at 65°C for 20 min) and autoradiographed.

DNA Probes. The DNA probes used for chromosome 17, their probable chromosomal order (17, 18), and polymorphic restriction sites are as follows: p144-D6 (17p13.3; *Pst* I; ref. 19), pYNZ22 (17p13.3; *Bam*HI, *Pst* I, *Taq* I; refs. 20 and 21), p17H8 (17cen; *Eco*RI; ref. 22), pTHH59 (17q23–q25; *Eco*RI, *Pst* I, *Taq* I; ref. 23), and pRMU3 (17q23–q25; *Bam*HI; ref. 24). Evidence for homozygous deletions at 17p13 were sought with pHRp5.5 (25) (*Eco*RI digests) and php53B (26) (*Eco*RI and *Hin*dIII digests), which do not detect RFLPs. An α satellite DNA probe, p17H8, was used to detect centromeric changes of chromosome 17 with *Eco*RI-digested DNA.

The DNA probes used for chromosome 18, their probable chromosomal order (27, 28) and polymorphic restriction sites are as follows: L2.7 (18q11.2; *Pst* I; refs. 28 and 29), pB16 (18q21.3; *Eco*RI; ref. 30), pMS1-3 (18q; *Pst* I; ref. 31), OS-4 (18q21.3–qter; *Taq* I; refs. 32 and 33), and pERT25 (18q23; *Eco*RI, *Hin*dIII, *Pst* I, *Taq* I; ref. 34).

RESULTS

We have detected two independent regions on chromosome 17 that frequently are affected by LOH in the tumor DNAs (Fig. 1 and Table 1). On the short arm of chromosome 17, 48% (24 of 50) of the informative DNAs for pYNZ22 and 38% (9 of 24) of those informative for p144-D6 had LOH on 17p13 (Table 1). Tumor DNA 211 is an example of probable hemizygosity because of the loss of one chromosome 17 homologue, since each of the four probes detected LOH. In

Abbreviations: LOH, loss of heterozygosity; RFLP, restriction fragment length polymorphism; VNTR, variable number of tandem repeat.

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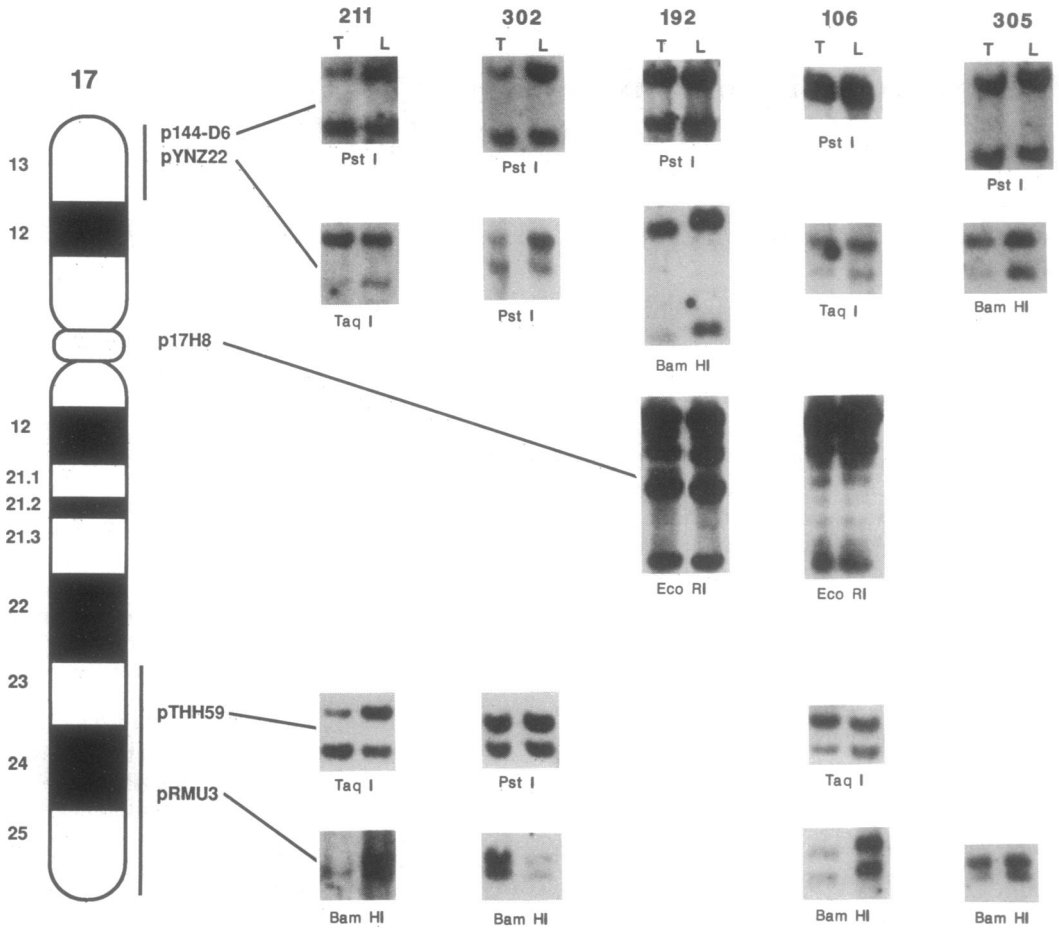

FIG. 1. Representative normal and tumor genotypes showing LOH on chromosome 17.

this and other cases, LOH at a particular allele is reflected as a decrease in signal intensity rather than as a complete loss of signal. This may reflect either contamination of the tumor biopsy material with normal stromal tissue or heterogeneity of tumor cells with respect to those that contain the mutation. In the case of tumor DNA 302, LOH is restricted to the loci defined by pYNZ22 and p144D-6 on chromosome 17p13. The affected region is further defined in tumor DNA 192, where LOH was detected at pYNZ22 but not at p144-D6 or p17H8. A high frequency of deletions on chromosome 17p also has been reported in other studies of human breast carcinoma DNAs (35–37). On the long arm of chromosome 17, we observed LOH in 29% of 42 informative DNAs for pTHH59 and 38% of 39 informative DNAs for pRMU3 (Table 1). LOH was detected in tumor DNA 305 with pRMU3 (chromosome 17q) and pYNZ22 (chromosome 17p) but not with p144-D6 (chromosome 17p). Because the tumor DNA was uninformative with the other probes, we could not determine whether LOH was the result of a nonreciprocal translocation, with the break point lying between the loci defined by p144-D6 and pYNZ22, or whether this tumor contained two independent regions of LOH on each arm of chromosome 17. However, in tumor DNA 106, LOH was detected with probe pTHH59, whereas heterozygosity was maintained at the more distal locus defined by pRMU3, the centromere, and the 17p chromosomal loci. In addition, 32% of the 37 tumors that were informative for at least one marker on both arms of chromosome 17 had LOH only at 17q23–qter. Although a similar study by Devilee *et al.* (36) reported 3 of 14 informative breast tumors with LOH on chromosome 17q, 2 of these were the result of the loss of one chromosome 17 homologue. However, our results are consistent with the presence of a previously unappreciated tumor-suppressor gene(s) on chromosome 17q that is a target for mutation during breast tumor development.

Table 1. Chromosome 17 allelic deletions

DNA probes		Tumors*, no. (% of total)		
Name	Locus†	Total	Informative	Deletions
p144-D6	*D17S32*	38	24 (63)	9 (38)
pYNZ22	*D17S30*	72	50 (69)	24 (48)
p17H8	*D17Z1*	47	24 (51)	6 (25)
pTHH59	*D17S4*	75	42 (56)	12 (29)
pRMU3	*D17S24*	70	39 (55)	15 (38)

Summary of the data for the chromosome 17 probes.
*Total number of tumors screened, number and percentage of the total that were polymorphic (i.e., informative), and number and percentage of informative tumors that had LOH for each probe.
†DNA segment detected.

Frequent LOH was also found on chromosome 18q in this panel of breast tumor DNAs. LOH was detected in 41%, 69%, and 25% of the informative tumor DNAs for probes pMS1-3, OS-4, and pERT25, respectively (Table 2 and Fig. 2). In tumor DNA 301, LOH was detected with probes pMS1-3, OS-4, and pERT25. Similarly, pMS1-3 and OS-4 also detected LOH in tumor DNA 292. Further localization of a target region for LOH is provided in tumor DNAs 303 and 332. In tumor DNA 303, heterozygosity was maintained at the locus defined by pMS1-3 but not at those defined by OS-4 or pERT25, whereas in DNA 332 heterozygosity was maintained at pERT25 but not at OS-4. This result suggests that another potential tumor-suppressor gene is closely linked to the region of chromosome 18q defined by the OS-4 probe.

Ten mutations are known to occur frequently at different chromosomal sites in our panel of primary human breast

Table 2. Chromosome 18 allelic deletions

DNA probes		Tumors,* no. (% of total)		
Name	Locus†	Total	Informative	Deletions
L2.7	*D18S6*	34	12 (35)	2 (17)
pB16	*BCL2*	32	14 (43)	1 (7)
pMS1-3	*D18S19*	47	22 (46)	9 (41)
OS-4	*D18S5*	47	16 (34)	11 (69)
pERT25	*D18S11*	82	67 (82)	17 (25)

Summary of the data for the chromosome 18 probes.
*Total number of tumors screened, the number and percentage of the total that were polymorphic (i.e., informative), and the number and percentage of informative tumors that had LOH for each probe.
†DNA segment detected; *BCL2* is the gene for B-cell chronic lymphocyte leukemia/lymphoma 2.

carcinomas (6). The significance of the observed deletions is further emphasized by their nonrandom distribution. Analysis of chromosomes 2p13 (α transforming growth factor gene, *TGFA*), 3p28 (somatostatin gene, *SST*), 6q22–q23 (*MYB*), and 22q12.3–q13.1 (β platelet-derived growth factor gene, *PDGFB*) showed a low (<7%) frequency of LOH (ref. 37 and unpublished data). Since several of the mutations have significant associations with clinical parameters associated with the more aggressive tumors, the associations among the mutations were considered. For the 10 mutations, there are 45 pairwise tests of independence. The *P* values of the exact tests using the hypergeometric distribution are reported in Table 3. If all of the mutations occurred independently of one another during tumor development, one would have expected that 2.25 of them ($45 \times 0.05 = 2.25$) would frequently occur together at $P < 0.05$ in the same tumors. In fact, there are eight pairs of mutations, with *P* values < 0.05 that occur together in our tumor panel. Each of these pairs has a positive ϕ coefficient consistent with mutations that tend to occur together. Furthermore, particular subsets of mutations are suggested by the associations shown in Table 3. Thus LOH on chromosomes 11p, 17p, and 18q frequently occurs in the same tumor. Similarly, a different subset of tumors contain frequent LOH on chromosomes 17q and 1p or 3p. In addition, tumors containing LOH on chromosome 11p also contain LOH on chromosome 3p and amplification of either *MYC* or *INT2* DNA sequences.

Table 3. Tests for independence of mutations in primary breast tumors

Chromosomal region or gene*	Informative tumors, no.	ϕ coefficient†	*P* value‡
17p–18q	40	0.371	0.027
11p–17p	33	0.424	0.025
11p–18q	49	0.315	0.041
11p-*MYC*	76	0.327	0.007
11p-*INT2*	89	0.280	0.020
3p–11p	62	0.334	0.017
3p–17q	40	0.483	0.004
1p–17q	40	0.370	0.029

**MYC* is the human homolog of the avian myelocytomatosis virus oncogene, and *INT2* is the human homolog of the murine mammary tumor virus integration site oncogene.
†The phi coefficient (unlike the *P* value) estimates a measure of association that does not depend on the sample size. It is the square root of T/N, where T is the χ^2-square statistic for the 2×2 table, N is the number of informative tumors, and the square root preserves the sign of the association; it ranges between -1 and 1.
‡The *P* value is for the exact, two-sided hypergeometric test for pairwise independence.

DISCUSSION

A likely target for LOH on chromosome 17p is the gene encoding tumor protein p53, *TP53* (17p13), which is fre-

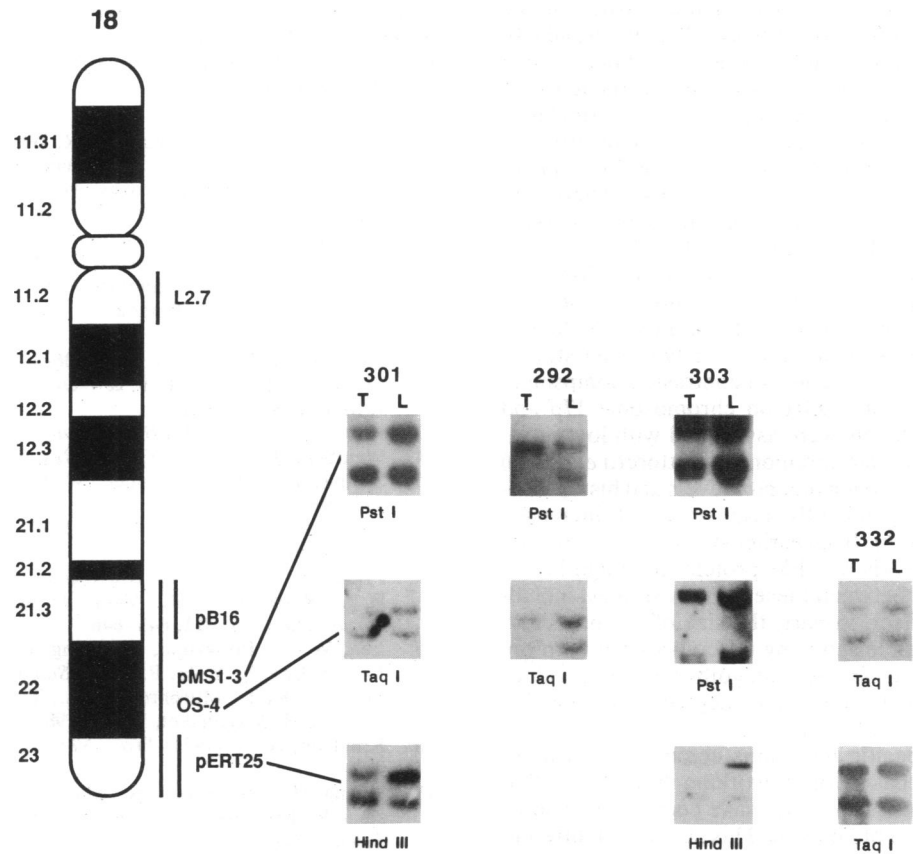

FIG. 2. Representative normal and tumor genotypes showing LOH on chromosome 18.

quently affected by coincident LOH and point mutations in lung (38) and colon (31) carcinomas and in two of three breast tumor cell lines (39). Although homozygous deletions of the *TP53* gene have been detected in lung carcinoma cell lines (38), none was detected in 60 primary breast tumor DNAs (data not shown) when using as probes the TP53 cDNA (php53B) (25) or the closely linked DNA sequences encoding the large subunit of RNA polymerase II (pHRp5.5) (26). The murine *TP53* gene, when altered by certain point mutations, is capable of transforming tissue culture cells to the tumorigenic phenotype (40–42). The point mutations in the *TP53* gene that have been detected in human tumors frequently occur in the same region of the gene (31, 38–39). At the present time, however, it is not known whether the remaining *TP53* allele in primary breast tumors containing chromosome 17p LOH also contains a point mutation.

There are several potential candidate genes that may represent the targets of the observed LOH on chromosomes 17q and 18q23–qter in the breast tumor DNAs. Our results with tumor 106 (Fig. 1) suggest that on chromosome 17q, such a gene is located between the centromere and the sequences defined by pRMU3. Within this large region there are several potential candidate targets (43), including the von Recklinghausen neurofibromatosis (*NF1*) gene (44) and acute promyelocytic leukemia translocation break point (45). The gene(s) affected by LOH on chromosome 18 appear to be located between 18q21.3 and 18q23, near the sequences defined by the anonymous DNA probe OS-4. A likely candidate is the recently described *DCC* (deleted in colorectal carcinomas) gene, which is frequently altered by LOH, homozygous deletions, and insertional mutations in colorectal tumors (46). Other potential candidate target genes mapping to this region include those for gastrin-releasing peptide and plasminogen-activator inhibitor type II and the *YES* protooncogene (47).

Previous studies have reported significant associations between specific tumor mutations and particular clinical parameters of the patient's history, characteristics of the tumor, or the patient's prognosis (reviewed in refs. 6 and 48). LOH on chromosome 17q for at least one marker occurred in 64% (38 of 59) of the informative cases and correlated with estrogen receptor-negative cancers (χ^2, $P < 0.02$). Similarly, LOH for at least one marker on chromosome 18q occurred in 34% (23 of 67) of the cases and was associated with histopathological grade III cancers (χ^2, $P < 0.04$). Therefore, LOH on chromosomes 17q and 18q appears to be associated with the more aggressive breast tumors, although neither was predictive of tumor stage nor patient prognosis. However, despite the high frequency of LOH on chromosome 17p, there was no association with any of the clinicopathological parameters of the tumors in our panel or in two other studies of primary breast cancers (35, 36). In contrast, Thompson *et al.* (37) reported that both LOH on chromosome 17p and increased TP53 RNA levels were associated with low estrogen receptor levels in breast carcinomas. Cattoretti *et al.* (49) also claimed that low estrogen receptor levels and histopathologic grade III were significantly associated with increased levels of the TP53 protein. In an earlier study, Crawford *et al.* (50) found elevated levels of TP53 protein in benign breast lesions. The disparity between these various studies, including our own, may reflect, in part, the size of the particular panel studied, or possible sampling differences between the panels. A more likely possibility is that different cutoff values were used to define estrogen receptor-negative and -positive tumors in the various studies (13).

Our finding that specific mutations commonly occur together provides the basis for a working hypothesis that different subsets of mutations may make comparable contributions to the malignant phenotype. This concept of different subsets of mutations possibly acting in a complementary fashion is consistent with the heterogeneous nature of the etiological factors that provide the selective pressure for mutations during breast carcinogenesis (51, 52). Moreover, it suggests that it may be possible to determine how the different sets of mutations might collaborate in effecting cancer development. Although an overall test of independence using the Bonferroni adjustment (in which each pairwise P value is multiplied by the number of tests done—i.e., 45) gives no evidence of significant associations, other reports are consistent with this working hypothesis. For instance, the association between LOH on chromosomes 11p and 17p in primary breast cancers has also been reported by MacKay *et al.* (35). They found that 10 of 14 tumors (71%) with LOH on chromosome 11p also had LOH on chromosome 17p. The grouping of concomitant mutations is also similar to the findings in other solid malignancies. Thus, in lung carcinomas, mutations of *MYC*, 3p, 11p, and 17p are commonly observed (53–58). Likewise, in colorectal cancer, mutations of 17p and 18q frequently occur in the same tumor and are associated with its development and/or progression (59–63).

The commonality of both dominantly acting gene amplifications and regions of the genome subject to frequent LOH among various carcinomas raises the possibility that certain target genes may play a common role in oncogenic progression irrespective of the tumor site (1–6). LOH on chromosomes 17p and 18q in breast and colorectal carcinomas is especially interesting in view of the well known clinical observation that patients with breast cancer have a higher risk of developing colorectal cancer and vice versa (64–66). Studies of transgenic mouse strains containing either the *ERBB2* (also called *HER2*, *NGL*, and *neu*) or *TP53* oncogenes as transgenes demonstrate, however, that the relative contribution of a particular mutation to neoplasia can depend on the tissue in which it is expressed (67, 68). In the case of LOH at specific chromosomal regions, it is also conceivable that there are closely linked genes that are selectively mutated in a tissue-specific manner. In this regard it will be important to determine whether the *TP53* and *DCC* genes are the targets for LOH on chromosomes 17p and 18q in primary breast carcinomas.

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