Am. J. Hum. Genet. 55:666–677, 1994

A Common Region of Deletion on Chromosome 17q in Both Sporadic and Familial Epithelial Ovarian Tumors Distal to BRCA1

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Summary

Linkage analysis in familial breast and ovarian cancer and studies of allelic deletion in sporadic ovarian tumors have identified a region on chromosome 17q containing a candidate tumor-suppressor gene (referred to as *BRCA1*) of likely importance in ovarian carcinogenesis. We have examined normal and tumor DNA samples from 32 patients with sporadic and 8 patients with familial forms of the disease, for loss of heterozygosity (LOH) at 21 loci on chromosome 17 (7 on 17p and 14 on 17q). LOH on 17p was 55% (22/40) for informative 17p13.1 and 17p13.3 markers. When six polymorphic markers flanking the familial breast/ovarian cancer susceptibility locus on 17q12-q21 were used, LOH was 58% (23/40), with one tumor showing telomeric retention. Evaluation of a set of markers positioned telomeric to BRCA1 resulted in the highest degree of LOH, 73% (29/40), indicating that a candidate locus involved in ovarian cancer may reside distal to BRCA1. Five of the tumors demonstrating allelic loss for 17q markers were from individuals with a strong family history of breast and ovarian cancer. More important, two of these tumors (unique patient number [UPN] 57 and UPN 79) retained heterozygosity for all informative markers spanning the BRCA1 locus but showed LOH at loci distal to but not including the anonymous markers CMM86 (D17S74) and 42D6 (D17S588), respectively. Deletion mapping of seven cases (two familial and five sporadic) showing limited LOH on 17q revealed a common region of deletion, distal to GH and proximal to D17S4, that spans ~25 cM. These results suggest that a potential tumor-suppressor gene involved in both sporadic and familial ovarian cancer may reside on the distal portion of chromosome 17q and is distinct from the BRCA1 gene.

Received December 13, 1993; accepted for publication June 9, 1994.

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0002-9297/94/5504-0009$02.00

Introduction

It is well recognized that cancer, especially adult cancer, is a multistep process and that the accumulation of genetic changes is important for cancer development and/or progression. Loss of heterozygosity (LOH), shown by RFLP and PCR-based simple tandem repeat polymorphism (STRP) analyses of paired normal/tumor DNA, has been used extensively to identify regions of chromosomes that contain candidate tumor-suppressor genes. In some cases, when these data are combined with linkage information from families in whom susceptibility to the tumor in question appears to be inherited, positional cloning methods have been used to isolate the genes (e.g., WT1, APC, and DCC) responsible for a number of disease phenotypes (for review, see Godwin et al. 1992; Knudson 1993).

In the case of ovarian cancer, frequent genetic alterations have been reported. Allelic losses for polymorphic DNA markers in ovarian carcinomas have been observed on nearly every chromosome arm. Lee et al. (1990) have reported high frequencies of allelic losses (>30% of informative tumors studied) on chromosomes 6q, 11, and 17 in ovarian carcinomas, and L. Dubeau and colleagues have also observed, by means of RFLP analysis, a high degree of LOH on chromosomes 3p, 6, and 11p (Ehlen and Dubeau 1990; Zheng et al. 1991). Sato et al. (1991*b*) have reported LOH for various polymorphic DNA markers on chromosomes 4p, 6p, 7p, 8q, 12, 16, and 19p in the 37 ovarian tumors analyzed. In addition to these changes, frequent allelic losses on chromosomes 6q, 13q, and 19q were observed uniquely in serous and serous papillary cystadenocarcinomas; LOH was detected only rarely on these chromosomal arms in the other subtypes of common-epithelial tumors of the ovary. The allelotyping of a large number of ovarian cancers by Yang-Feng et al. (1993) revealed that >40% of the tumors studied showed LOH on chromosomes 13q, 17, and Xp. In separate studies investigators have reported that the tumor-suppressor gene, TP53, on 17p13.1 is frequently mutated and that its altered protein is overexpressed in ovarian carcinomas (Marks et al. 1991; Okamoto et al. 1991; Kohler et al. 1993). On the basis of LOH studies, several groups have suggested that, in addi-

tion to TP53 at 17p13.1, there may be genes at 17p13.3, 17q21, and 17q25 that are acting as tumor suppressors or regulators in ovarian cancer (Foulkes 1993; Phillips et al. 1993; Saito et al. 1993; Tavassoli et al. 1993; Yang-Feng et al. 1993). In addition, at least four other genes that are important in neoplasia are present in the proximal portion of 17q (i.e., NF1, ERBB2, RARA, and NME1), further indicating that a large number of genes might be involved in ovarian cancer development and progression (Bevilaqua et al. 1989; Collins et al. 1989; Salmon et al. 1989; Berchuck et al. 1990; Kakizuka et al. 1991; Varesco et al. 1992).

Ovarian cancer is known to have a familial component, and there is evidence that this is at least in part the result of autosomal dominant inheritance of a recessive gene(s) with high penetrance. Moreover, the risk of breast cancer is significantly increased in individuals with ovarian cancer and in their relatives, and vice versa. In a landmark article published in 1990, Hall et al. used genetic linkage to identify chromosome 17q21 as the location of the early-onset breast cancer–predisposing gene, now termed *BRCA1*. Shortly thereafter, this observation was confirmed and extended to a proportion of hereditary ovarian cancers (Narod et al. 1991). Families in which ovarian and breast cancer are thought to result from inherited BRCA1 mutations have been identified by many groups, and in some instances investigators have begun to provide genetic counseling for high-risk members of hereditary breast cancer and hereditary breast/ovarian cancer syndrome families. In >70% of breast-ovarian families and in an estimated 45% of "breast cancer only" families, inheritance of the disease is consistent with linkage to this as-yet-uncloned gene (for review, see Easton et al. 1993). Furthermore, recent studies have shown that this predisposing gene has the characteristics of a tumor-suppressor gene (Smith et al. 1992). Following the first reports by Hall et al. (1990) and Narod et al. (1991), there have been great strides in the refinement of the location of the BRCA1 gene. A recent set of collaborative studies by the International Breast and Ovarian Cancer Linkage Consortium have indicated that the BRCA1 gene lies between the gene encoding the thyroid-hormone receptor A (THRA1) and an anonymous marker, B43 (D17S183) (Easton et al. 1993). This region includes a number of known genes, including the EDH17B genes (B1 and B2) encoding the enzyme 17β-estradiol dehydrogenase, which catalyzes the conversion of estrone to 17β-estradiol. However, at present, germ-line or somatic mutations in the EDH17B gene, in affected members of families thought to be linked to BRCA1 and in patients with sporadic ovarian cancer, have not been identified (Foulkes et al. 1993; Kelsell et al. 1993; Simard et al. 1993). Very recent genetic linkage studies have now narrowed the region in which the early-onset breast and ovarian cancer gene resides, to less than a few million nucleotides (Bowcock et al. 1993; Kelsell et al. 1993; Tonin et al. 1993).

A number of groups have found a high degree (~80%) of LOH in sporadic unselected ovarian cancers by using markers localized to the distal portion of 17q (Eccles et al. 1990, 1992; Russell et al. 1990). Interestingly, using a telomeric minisatellite marker THH59 (D17S4), Eccles et al. (1992) have reported LOH on 17q in benign and borderline tumors, indicating that there may be a gene(s) residing distally to BRCA1 that is important in early developmental stages of sporadic ovarian cancer. A recent study by Jacobs et al. (1993) has uncovered a 16-cM region located on chromosome 17q22-q23 that shows preferential LOH in sporadic forms of the disease. Here we report the identification of a common region of deletion on the long arm of chromosome 17 in both sporadic and familial cases of ovarian cancer, distal to BRCA1 and distinct from other reported loci. The ramifications of a second candidate tumor-suppressor gene in both sporadic and familial forms of ovarian cancer are discussed.

Material and Methods

Tumor Samples and Histology

Tumors were collected from consenting patients undergoing surgery for ovarian cancer. A portion of the tumor was used immediately for DNA isolation, while additional tumor sample was either frozen in liquid nitrogen and stored at −70°C or frozen in isopentane for histology. The 40 tumors studied include 35 malignant common-epithelial tumors (15 papillary serous, 8 undifferentiated, 4 endometrioid, 4 mixed Müllerian, 3 mucinous, and 1 clear cell), 1 teratoma, and 4 benign or borderline tumors (1 serous cystadenofibroma, 1 mucinous cystadenoma, 1 Brenner tumor, and 1 thecoma). The histopathological classification was based on the typing criteria of the World Health Organization.

Ascertainment of Family History

Ovarian tumor samples were obtained from breast/ovarian families followed by the Family Risk Assessment Program at the Fox Chase Cancer Center, Philadelphia, and by the Department of Preventive Medicine at Creighton University School of Medicine, Omaha. Tumor specimens from individuals with a family history of breast and/or ovarian cancer who were followed at Fox Chase (i.e., unique patient number [UPN] 17, 57, 58, 79, and 98) came to medical attention as the result of the diagnosis of a new case within the family. For each of these families, a pedigree was prepared on the basis of a detailed family history from an informed family member, and, for those with the suggestion of a diagnosis of cancer, confirmation was sought either by pathology report, hospital records, or death certificate when such records were available. A disease was considered potentially familial in its inheritance pattern if the family contained at least three women

who were first- or second-degree relatives who had been diagnosed with invasive cancer of the breast and/or ovary. Pedigrees collected by the group at Creighton University have been described elsewhere (Lynch et al. 1993).

Isolation of DNA from Tumors and Matched Blood Samples

To extract high-molecular-weight DNA, the fresh tumor specimens were finely minced, suspended in 10 ml of TNE (0.5 M Tris pH 8.9, 10 mM NaCl, 15 mM EDTA) with 500 μg proteinase K/ml and 1% SDS, and incubated at 48°C for 24 h with constant gentle rocking. After two extractions with phenol (equilibrated with 0.1 M Tris pH 8.0), the DNA was spooled from two volumes of 100% ethanol, air-dried, and resuspended in 10 mM Tris, 20 mM EDTA. The DNA was then treated sequentially with RNase A (100 μg/ml) for 1 h at 37°C and with proteinase K (100 μg/ml, 1% SDS, at 48°C for 3 h), followed by two extractions with saturated phenol. The DNA was retrieved from the aqueous phase by ethanol precipitation, was washed extensively in 70% ethanol, and, after air-drying, was suspended in TE (10 mM Tris [pH 8.0], 1 mM EDTA).

Lymphocytes were extracted from blood taken at the time or within a few days of the surgery. White blood cells isolated from the buffy coat of 15–20 ml of blood from each patient by centrifugation at 800 *g* for 20 min were mixed with two volumes of buffer containing 1.6 M NH_4Cl, 0.1 M $KHCO_3$, 1 mM EDTA to lyse the residual red blood cells and were centrifuged at 500 *g* for 5 min at 4°C. DNA was extracted from the resulting leukocyte pellet, as described above.

RFLP Typing

DNA samples (5 μg) were digested to completion with restriction enzymes, according to the manufacturer's recommendations (Bethesda Research Laboratories) and were size-fractionated by electrophoresis in 1% agarose gels. Transfer to nylon membranes (GeneScreen Plus; NEN) was achieved under alkaline conditions (0.4 M NaOH, 1.5 M NaCl) by capillary blotting. DNA probes were labeled to a specific activity of $\sim 5 \times 10^8$ cpm/μg with [^{32}P]dATP by the random priming method (Stratagene Prime-It II kit). The filters were hybridized at 1.5×10^6 cpm/ml of hybridization solution (0.5 M $NaPO_4$, 2 mM EDTA, 7% SDS, 0.1% sodium pyrophosphate [NaPpi] [pH 7.1]) at 65°C for 20 h. The filters were sequentially washed at 65°C for 1 h each in the following: 2 × SSC, 0.5 × SET, 0.1% sodium pyrophosphate; and 0.1 × SSC, 0.5 × SET, 0.1% NaPpi (1 × SET = 10 mM Tris, 1% SDS, 5 mM EDTA [pH 7.5]) and were exposed to Kodak XAR-5 film with a Lightning Plus intensifying screen (DuPont and NEN) at −70°C. DNA probes containing repetitive sequences were preannealed using human placental (Sigma) and Cot-1 (BRL/Gibco) DNA prior to hybridization.

Allelic Loss

Allelic loss was scored when constitutional DNA was heterozygous and one of the expected bands in tumor DNA was completely absent or markedly reduced in intensity. To control for possible DNA degradation in ovarian tumors showing loss of the higher-molecular-weight bands by RFLP analysis, the same blots used to assess allelic loss were analyzed with additional DNA gene probes that detect large fragements. The autoradiographic band densities were quantitated using an UltraScan XL laser densitometry (Pharmacia LKB Biotechnology) within the linear range of the film.

DNA Probes

The DNA probes used in this study, together with the restriction endonucleases used to reveal the fragment length polymorphisms, are listed in table 1.

PCR Analysis of STRPs

Primers used for the analysis of STRPs are detailed in table 1 and were used in assays at a concentration of 1.0 μM. Polymorphisms were typed by PCR using 30 ng of genomic DNA or DNA extracted from archival pathology blocks in 20-μl volumes. PCR was performed with [α-^{33}P]-dATP (~1,600 Ci/mmol; NEN and DuPont), at 1.0 μCi/reaction, dATP, dCTP, dGTP, and TTP at 5 μM, using 0.5 units of *Taq* DNA polymerase (Perkin Elmer)/reaction. The alleles were amplified through 27 cycles of 1 min at 94°C, 1 min at the appropriate annealing temperature, and 1 min at 72°C, with a final extension at 72°C for 5 min. Reaction products were diluted 1:1 in loading buffer (90% formamide, 20 mM EDTA, 0.3% bromophenol blue, 0.3% xylene cyanol), heated at 90°C for 5 min, and loaded (3 μl) onto a 6% denaturing polyacrylamide gel. After electrophoresis, gels were dried at 70°C under vacuum and were exposed to X-ray film for 24 h at −70°C.

Results

DNA isolated from 40 ovarian tumors (35 malignant epithelial tumors, 1 teratoma, 1 serous cystadenofibroma, 1 mucinous cystadenoma, 1 Brenner tumor, and 1 thecoma) was analyzed with polymorphic DNA markers for allelic losses on chromosome 17 in order to identify genetic alterations that may contribute to the genesis of ovarian cancer. By use of eight RFLP DNA probes and 13 STRP PCR-primer pairs (table 1), LOH at one or more loci was observed in 73% (29/40) of tumors. The distal region of 17q showed the highest frequency of deletion with losses at GH, detected by a PCR-based polymorphic analysis, peaking at 81% (29/36). Representative results for this polymorphism are shown in figure 1. In 11 tumors both alleles were retained at all informative loci.

LOH on chromosome 17 was analyzed by histopatho-

Table 1

RFLP DNA Probes and STRP Primer Pairs Used to Identify LOH in Ovarian Cancers

Probe[a]	Locus	Chromosome Map Position	Restriction Enzyme or Primer Sequence
144D6	D17S34	17p13.3	*Rsa*I
YNH37.3	D17S28	17p13.3	*Taq*I
YNZ22.2	D17S5	17p13.3	*Msp*I
LL132	D17S379	17p13.3	1. (CA) GACCACATCTGTCCTCACCTGT 2. (TG) CGAGTCCTCACTGTAAACAAGG
pR4-2	TP53	17p13.1	*Ban*II or *Bgl*II
TP53	TP53	17p13.1	1. (GT) AGGGATACTATTCAGCCCGAGGTG 2. (AC) ACTGCCACTCCTTGCCCCATTC
12G6	D17S513	17p13	1. (CA) TTCACTTGTGGGCTGCTGTC 2. (TG) TAAGAAAGGCTCCCACAAGCA
Mfd15	D17S250	17q11-q12	1. (CA) GGAAGAATCAAATAGACAAT 2. (TG) GCTGGCCATATATATATTTAAACC
THRA1	THRA1	17q11-q12	1. (CA) CTGCGCTTTGCACTATTGGG 2. (TG) CGGGCAGCATAGCATTGCCT
UM8	D17S846	17q12-q21	1. (GGAA) TGCATACCTGTACTACTTCAG 2. (TTCC) TCCTTTGTTGCAGATTTCTTC
OF2	D17S856	17q12-q21	1. (AAAG) AAGGCAAGACTTCGTCGAGA 2. (CTTT) CATTCCCTGGTCCTGTGC
248YG9	D17S855	17q12-q21	1. (CA) GGATGGCCTTTTAGAAAGTGG 2. (TG) ACACAGACTTGTCCTACTGCC
B43	D17S183	17q21	1. (CA) ACAAACTGATGTGGGCTCTAG 2. (TG) GTACATAGCATGGGTGCAGCT
Mfd188	D17S579	17q21	1. (CA) AGTCCTGTAGACAAAACCTG 2. (TG) CAGTTTCATACCAAGTTCCT
42D6	D17S588	17q21	1. (CA) CCTGGTCTAGGAAGAGTGTCA 2. (TG) GTGTAAAGCATCTGTGTATACTAC
NM23	NME1	17q21-q22	1. (CA) TCTCAGTACTTCCCGTGACC 2. (TG) TTGACCGGGGTAGAGAACTC
CMM86	D17S74	17q22	*Hin*fI
GH	GH	17q22-q23	1. (AAAG) TCCAGCCTCGGAGACAGAAT 2. (CTTT) AGTCCTTTCTCCAGAGCAGGT
EW101	D17S40	17q22-q23	*Msp*I
THH59	D17S4	17q23-qter	*Pvu*II or *Taq*I
KKA35	D17S75	17q25	*Msp*I or *Taq*I

[a] Original references for description of the following DNA probes or sequences of the PCR primer pairs can be found in the work of Hall et al. (1992), Jones and Nakamura (1992), Anderson et al. (1993), Carrozzo and Ledbetter (1993), Easton et al. (1993), Flejter et al. (1993), and Oliphant et al. (1993).

logical subtype, and the results are shown in table 2. It can be seen that LOH for loci on 17q was nearly 100% in undifferentiated and serous adenocarcinomas, as compared with mucinous adenocarcinomas, where no cases of LOH were seen at any of the informative polymorphisms examined. All three mucinous tumors were either benign or stage I; therefore the absence of LOH in these cases may be related to the stage rather than to the histopathological subtype. However, allelic loss on 17p and 17q was observed in two of four mixed Müllerian tumors that were stage I disease. Table 3 demonstrates that both the number of tumors showing LOH and the extent of the region of chromosome 17 involved increase with tumor grade. In particular, LOH was more common in high-grade, late-stage carcinomas. Total chromosome loss, defined as reduction to homozygosity for all informative markers on chromosome 17, was most frequent in poorly differentiated tumors and was absent in all benign and borderline tumors and in the majority of well-differentiated ovarian tumors. Only the benign Brenner tumor (UPN 91) showed LOH for 17q markers and retention of heterozygosity for all informative 17p polymorphisms (tables 2 and 3 and data not shown).

Of the cases showing LOH, most (21/29) lost heterozygosity for all informative markers on chromosome 17 (table 2). However, the ovarian tumors from patients 53, 57,

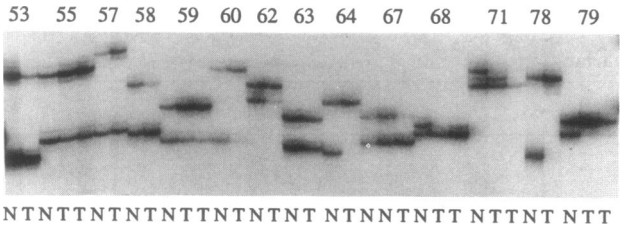

Figure 1 Representative autoradiograph showing allele loss on 17q at the GH locus. DNA pairs from normal (N) and tumor (T) tissue were assayed as described in Material and Methods. Allele losses were scored as a reduction in intensity of one allele relative to the other, in tumor vs. normal samples. LOH was observed in all tumors shown except for UPN 57 and UPN 63. In UPN 55, UPN 59, UPN 68, UPN 71, and UPN 79, allelic loss was detected in both the primary and metastatic lesion.

59, 63, 79, and 84 revealed LOH only for markers on the long arm of chromosome 17, whereas heterozygosity was maintained for loci on the short arm, including TP53 and the candidate loci at 17p13.3. More important, none of these tumors showed allelic losses when markers flanking the BRCA1 locus were used. The pattern of LOH for the long arm of chromosome 17 in these tumors is illustrated in figure 2, and selected examples of the affected loci obtained from RFLP and STRP analyses are shown in figure 3. Analysis of tumors 53, 59, and 79 revealed LOH for markers distal to D17S588 but retention of both alleles at all informative proximal loci on 17q, including the loci flanking BRCA1 and TP53 on 17p. Similar results were observed for tumors 57 and 63, in which heterozygosity was retained at loci proximal to D17S74 (more commonly known as *CMM86*) and GH, respectively, but allelic deletion was observed at distal loci, suggesting that the loss was due to somatic recombination events. In tumor UPN 84 (a stage II endometrioid adenocarcinoma), there was allelic loss spanning the region between the NME1 and the D17S4 loci, indicating the presence of a large interstitial deletion. The pattern of allelic loss in these tumors defines a common region of loss that spans ~25 cM and is flanked by but does not include GH and D17S4. However, if tumor UPN 78, which shows LOH at markers proximal to D17S40 but retains the more telomeric loci, is considered, this region would narrow to ~8 cM, and its boundaries would be defined by GH and D17S40. However, since the BRCA1 locus and the TP53 gene, as well as other potentially important genes proximal to GH, may be altered, this smaller common region of deletion cannot yet be excepted.

Of the 40 total tumors analyzed, 8 were from individuals with family histories of ovarian and breast cancer. Six of these ovarian tumors showed allelic losses on chromosome 17. Interestingly, two of these familial tumors (UPN 57 and UPN 79) showed LOH at loci distal to the BRCA1 region (figs. 1 and 2). Table 4 shows the relationship between family history of breast and ovarian cancer and LOH on chromosome 17. The frequency of LOH for the markers defining the 17q22-q23 locus was higher than that

Table 2

Relationship between LOH on Chromosome 17 and Histopathological Subtype

Histopathological Subtype	LOH of 17p[a]	LOH of 17q[a]	LOH of All Informative 17p13.1-p13.3 Markers[b]	LOH of All Informative BRCA1-defining Markers (17q12-q21)[c]	LOH of All Informative 17q22-q23 Markers[c]
Serous papillary adenocarcinoma[d]	12/15	14/15	12/12	12/14	14/14
Undifferentiated adenocarcinoma	5/8	8/8	5/5	5/8	8/8
Endometrioid adenocarcinoma	2/4	3/4	2/2	2/3	3/3
Mixed Müllerian tumor	2/4	2/4	2/2	2/2	2/2
Mucinous adenocarcinoma	0/3	0/3	. . .	. . .	. . .
Brenner tumor	0/1	1/1	. . .	1/1	1/1
Serous cystadenofibroma	0/1	0/1	. . .	. . .	. . .
Mucinous cystadenoma	0/1	0/1	. . .	. . .	. . .
Clear cell	0/1	0/1	. . .	. . .	. . .
Teratoma	1/1	1/1	1/1	1/1	1/1
Thecoma	0/1	0/1	. . .	. . .	. . .
Total (%)	22/40 (55%)	29/40 (73%)	22/22 (100%)	23/29 (79%)	29/29 (100%)

[a] LOH with any informative marker on the chromosomal arm, of the total number of tumors in that histopathological category.

[b] Number of cases of LOH affecting informative 17p13.1-p13.3 markers, of those tumors with any LOH on the short arm of chromosome 17.

[c] Number of cases of LOH affecting informative 17q22-q23 markers or markers flanking BRCA1 (17q12-q21), of those tumors with any LOH on the long arm of chromosome 17.

[d] Includes serous papillary cystadenocarcinomas, serous carcinomas, and papillary carcinomas.

Table 3

Relationship between LOH on Chromosome 17 and Tumor Stage and Differentiation Grade

	LOH on 17p (%)	LOH on 17q (%)	LOH of the Markers Defining the BRCA1 Locus[a] (%)	LOH of the Markers Defining the 17q22-q23 Locus[a] (%)
Tumor stage:				
Benign	0/3 (0%)	1/3 (33%)	1/1 (100%)	1/1 (100%)
Stage I	2/5 (40%)	2/5(40%)	2/2 (100%)	2/2 (100%)
Stage II	0/4 (0%)	1/4 (25%)	0/1 (0%)	1/1 (100%)
Stage III	13/18 (72%)	15/18 (83%)	13/15 (87%)	15/15 (100%)
Stage IV	7/10 (70%)	10/10 (100%)	7/10 (70%)	10/10 (100%)
Tumor differentiation:				
Benign	0/3 (0%)	1/3 (33%)	1/1 (100%)	1/1 (100%)
Borderline	0/1 (0%)	0/1 (0%)	. . .	. . .
Well differentiated	1/4 (25%)	1/4 (25%)	1/1 (100%)	1/1 (100%)
Moderately differentiated	6/12 (50%)	9/12 (75%)	6/9 (76%)	9/9 (100%)
Poorly differentiated	15/20 (75%)	18/20 (90%)	15/18 (83%)	18/18 (100%)

[a] The number of cases of LOH affecting informative 17q22-q23 markers or markers flanking BRCA1 (17q12-q21), of those tumors with any LOH on the long arm of chromosome 17.

for the BRCA1 locus (75% vs. 50%), as a result of these two interesting cases; no differences in the pattern of LOH were observed in tumor samples from individuals previously determined to carry the disease-bearing chromosome (table 4 and data not shown). The GH locus has been mapped ~30 cM distal to D17S183, indicating that the putative ovarian cancer gene at 17q22-q23 is quite distant from BRCA1, as well as from D17S74 (the original marker used to demonstrate linkage in breast cancer families). Our results suggest that a potential tumor-suppressor gene at 17q22-q23 may play a role in both sporadic and hereditary forms of the disease. Genetic-linkage studies are currently in progress on the family members of UPN 57 and UPN 79 to determine whether disease occurrence cosegregates with markers defining the BRCA1 locus or possibly the 17q22-q23 locus.

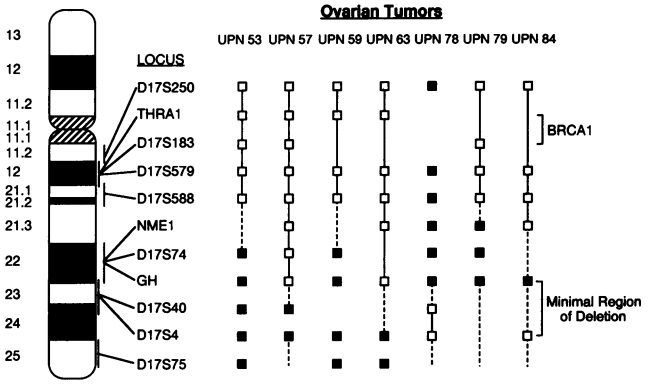

Figure 2 Allelic deletion patterns of ovarian tumors for the long arm of chromosome 17. DNA samples from normal blood and ovarian tumor tissue were typed with RFLP DNA markers, dinucleotide, and tetranucleotide repeats on chromosome 17q. For each tumor, all informative loci are shown: blackened squares represent constitutional heterozygosity with LOH; unblackened squares represent constitutional heterozygosity with no LOH; blank spaces represent homozygosity. Under the assumption that alleles in all regions between loci showing allelic loss are lost, solid lines indicate retained regions of chromosome 17q, and open areas show regions of allelic loss. Dashed lines represent regions that are uncertain in tumors with LOH for some loci. (The physical locations of the polymorphisms are based on data presented in papers by Anderson et al. [1993] and O'Connell et al. [1993].)

Discussion

It is becoming more and more apparent that multiple genetic alterations are involved in the genesis of ovarian cancer. Much of the attention has been drawn to chromosome 17, as a result of convincing studies that have established that in families with early-onset breast cancer and breast and ovarian cancer, disease occurrence is often linked to a small region on chromosome 17q21, referred to as *BRCA1* (Hall et al. 1992; Bowcock et al. 1993; Tonin et al. 1993). We have shown in this study that ~73% (29/40) of tumors for ovarian cancer patients display deletions on the long arm of chromosome 17; however, only 79% (23/29) of these tumors showed LOH for the markers proximal to and flanking the BRCA1 locus (fig. 2 and table 2). Interestingly, two of the six tumors showing LOH only at loci distal to BRCA1 were from individuals with a family history of breast and ovarian cancer (table 4). Deletion mapping of a total of seven cases showing limited LOH on 17q revealed a common region of loss distal to the GH locus and proximal to the anonymous marker THH59

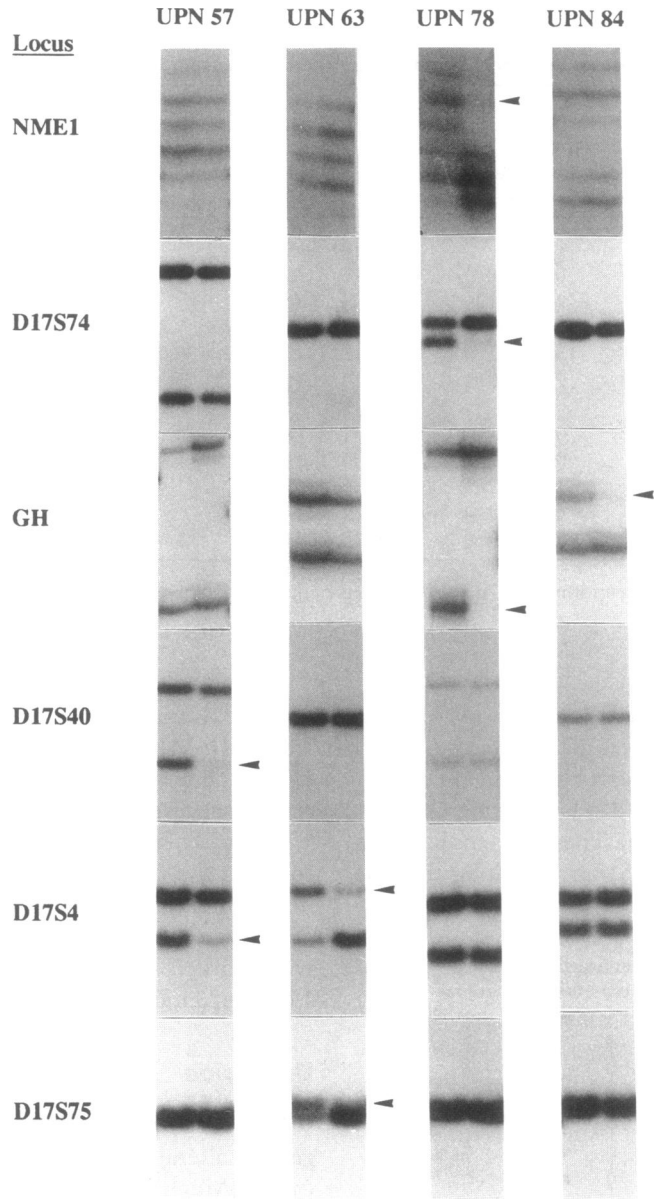

Figure 3 Selected examples from fig. 2 that define the minimal region of deletion. The locus names are shown next to the bands. Tumor numbers are indicated above the panels. In each pair, the normal DNA is on the left, and the tumor DNA on the right. The arrowheads indicate the alleles that are lost in the tumor DNAs.

(D17S4), suggesting the presence of a second candidate locus, distal to BRCA1, that is altered or lost in a high percentage of both sporadic and familial ovarian cancers.

LOH on the distal portion of chromosome 17q has been shown to be frequent in most studies of ovarian cancer. Eccles et al. (1990, 1992) and Russell et al. (1990) observed LOH for D17S4 at 17q23-qter in nearly 80% of informative cases. Foulkes et al. (1993) reported frequent allelic losses at the NME1, D17S74, D17S4, and D17S308 loci in 82%, 70%, 63%, and 82%, respectively, in the ovarian tumors they examined. Milner et al. (1993) reported 68% (15/22) loss in sporadic ovarian tumors and 100% loss in familial tumors (one breast and one ovarian), for the D17S4 locus. Taken together with our results, these studies are consistent with the presence of a second gene that is relevant to ovarian cancer and that lies distal to BRCA1.

LOH on chromosome 17q has also been noted in breast cancer; however, allelic loss is generally less frequent and varies widely (10%–60%) (Sato et al. 1991*a*; Futreal et al. 1992; Lindblom et al. 1993; Saito et al. 1993). Interest-

Table 4

Relationship between LOH on Chromosome 17 and Family History of Breast and Ovarian Cancer

Individual	No. of Breast Cancers	Median Age at Breast Cancer Diagnosis (years)	No. of Ovarian Cancers	Median Age at Ovarian Cancer Diagnosis (years)	LOH of the Markers Defining the BRCA1 Locus[a]?	LOH of the Markers Defining the 17q22-q23 Locus[b]?
UPN 17	2	63	2	51	Yes	Yes
UPN 57[c]	3	47	1	43	No	Yes
UPN 58	2	42	1	41	Yes	Yes
UPN 79[d]	1	NA[e]	3	NA	No	Yes
UPN 91	5	35	2	44	Yes	Yes
UPN 96[f]	14	39.5	10	49	No	No
UPN 97[f]	5	38	8	54.5	Yes	Yes
UPN 98[g]	2	34	4	44	No	No
Overall (%) ...					4/8 (50%)	6/8 (75%)

[a] Markers used to evaluate LOH at the BRCA1 locus: D17S250, THRA1, GAS, D17S856, D17S855, D17S183, and D17S579. Yes = one or more loci show LOH for this set of markers.

[b] Markers used to evaluate LOH at the 17q22-q23 locus: D17S74, GH, D17S40, and D17S4. Yes = one or more loci show LOH for this set of markers.

[c] Diagnosed with ovarian cancer at age 43 years, with stage I intraductal breast carcinoma at age 49 years and with comedo carcinoma of the breast at age 50 years. The tumor sample used in this study was a recurrent poorly differentiated ovarian adenocarcinoma obtained at age 49 years.

[d] Family has a high incidence of cancer in addition to breast and ovarian; i.e., brother, prostate and thyroid; brother, lung and skin; and maternal grandparents, colon.

[e] NA = information not available at this time.

[f] Breast-ovarian cancer families were previously tested for linkage to four markers that flank the BRCA1 locus, and the individual listed was determined to carry the disease-bearing chromosome (Feunteun et al. 1993).

[g] DNA used for LOH studies was extracted from archival paraffin-embedded normal and tumor tissue.

ingly, LOH was generally greater with telomeric markers, as compared with more proximal 17q loci, which is in agreement with the ovarian cancer studies cited above. Furthermore, preliminary deletion mapping of a panel of breast tumors revealed two common regions of deletion; the first was defined by the polymorphic markers D17S250 and GH and included the region believed to house the BRCA1 gene. The second was distal to this region, and its boundaries were defined by GH and D17S4 (A. K. Godwin, unpublished data). These results imply that there is no single common region of loss on chromosome 17q and identify two distinct regions of loss that may contain tumor-suppressor sequences. Although frequent LOH affecting similar 17q loci has been observed in sporadic breast and ovarian cancers, it is still not known whether the same or different 17q genes may be involved in the genesis of these different diseases.

The high rate of allele loss on 17 may be related to the existence of one or more tumor-suppressor genes on this chromosome, the loss or inactivation of which is an important step in the development of ovarian cancer. It has been noted that chromosome 17 contains a number of genes important in human neoplasia (e.g., TP53, NF1, ERBB2, NME-1 and -2, RARA, and prohibitin). Mutations in TP53 are probably the most common genetic abnormality detected in human cancers (Nigro et al. 1989; Vile 1993), and LOH of chromosome 17p is present in nearly half of all ovarian tumors studied (Okamoto et al. 1991; Foulkes et al. 1993; Phillips et al. 1993). Evaluation of seven loci on chromosome 17p showed LOH in 55% of the informative cases. While it has been postulated that in the telomeric region of 17p there may be another gene important in ovarian cancer, we did not observe a difference in the frequency of LOH for the TP53 and the 17p13.3 loci (data not shown).

The observation of allelic losses distal to the BRCA1 locus in ovarian tumors from individuals with a family history of breast and ovarian cancer raises the possibility of the existence of a second predisposing gene for familial breast and ovarian cancer. There is considerable evidence of genetic heterogeneity among breast-cancer-only families, with only an estimated 45% being linked to markers flanking the BRCA1 locus (Easton et al. 1993). In addition, as few as 10% and as many as 40% of breast-ovarian cancer–prone families have been reported unlinked, suggesting that another gene(s) predisposing to breast and ovarian cancer must exist (Easton et al. 1993; Feunteun et al. 1993; Goldgar et al. 1993; Smith et al. 1993). The breast-ovarian cancer–susceptibility locus was originally linked to D17S74 on chromosome 17q21. More recent evidence indicates that BRCA1 is located near D17S183, ~16–17 cM proximal to D17S74 (Bowcock et al. 1993). It is therefore

of interest that Narod and colleagues (Feunteun et al. 1993) have observed that a large breast-ovarian family (15 breast cancers [mean age at onset 44 years] and 2 ovarian cancers [mean age at onset 46 years]), which showed linkage to D17S74 (a positive lod score), was negative when typed using a marker closer to the BRCA1 locus. This suggests that by typing more telomeric polymorphic markers in families displaying linkage heterogeneity with respect to BRCA1, one could identify a small subset of kindreds that show linkage to more distal loci (e.g., the 17q22-q23 locus). In this regard, the families of the two individuals identified through our study (UPN 57 and UPN 79) are potential candidates for these types of studies. In contrast, there is considerable evidence against this scenario. Easton et al. (1993) have reported that of the breast cancer families and breast-ovarian cancer families typed by the Breast Cancer Linkage Consortium for a series of 17q markers (i.e., GH, D17S74, NME1, D17S588, D17S579, and D17S250), evidence of linkage was observed for all polymorphisms except the most distal, GH. There is, however, some evidence for linkage to alternative loci in BRCA1-unlinked breast cancer and breast-ovarian cancer families. Mutations of TP53 have been shown to be linked to the occurrence of tumors in Li-Fraumeni syndrome, in which breast cancer is common (Malkin et al. 1990). An estimated 1% of women diagnosed with breast cancer before age 40 years have germ-line mutations in TP53 (Malkin et al. 1990; King 1992; King et al. 1993). Interestingly, Jolly et al. (1994) recently identified a germ-line mutation in TP53 in a family with early-onset breast and ovarian carcinomas. Furthermore, evidence has been reported for linkage to the estrogen receptor on chromosome 6 in a late-onset breast cancer family (Zuppan et al. 1991).

It might also be argued that individuals UPN 57 and UPN 79, who show limited LOH affecting the 17q22-q23 locus, are sporadic cases and that the cancers arising in their respective families are merely chance aggregations. Even though the number of cancers in these two families is relatively small (table 4), epidemiological criteria support inherited susceptibility, rather than acquired mutations, as the factor responsible for the observed frequency of breast and ovarian cancers. Furthermore, it has recently been reported that male relatives of women with breast cancer have an increased risk of prostate cancer. In addition, there is an increased frequency of other cancers (e.g., colon, uterus, thyroid, and male breast) in association with familial breast and ovarian cancer (King 1992; Lobaccaro et al. 1993). This phenomenon is particularly evident in the family of UPN 79, where cases of prostate, thyroid, skin, lung, and colon cancers are observed (table 4), further supporting a potential role for a second tumor-suppressor gene on chromosome 17q in hereditary ovarian cancers, as well as other familial neoplasia.

The study of ovarian cancer bears intrinsic difficulties because the majority of patients with ovarian cancer are diagnosed in the advanced stage of their disease; therefore it is difficult to identify the early events that influence ovarian cancer development. LOH analysis, for example, has effectively been used to identify many of the genetic changes occurring in the multistep lineage of colorectal cancer (Fearon and Vogelstein 1990). It is not clear whether ovarian cancer develops through a similar progression, i.e., from benign to malignant (Zheng et al. 1993). In our small series of benign, borderline, and grade I tumors, 2 of 8 cases displayed LOH for chromosome 17q, compared with 27 of 32 grade II and III tumors (table 3). Furthermore, the observation that limited regions of deletion were detected in both sporadic and familial forms of the disease, including the benign Brenner tumor discovered during prophylactic surgery (H. Salazar, A. K. Godwin, H. T. Lynch, T. C. Hamilton, unpublished information), raises the possibility that alterations affecting the 17q22-q23 locus are relatively early events in ovarian carcinogenesis. In support of this observation, Eccles et al. (1992) have demonstrated that LOH on chromosome 17q occurs in benign and borderline tumors, but at a much lower frequency than in malignant carcinomas.

More specific support for the existence of a distal locus on 17q that is important in the genesis of ovarian cancer comes from recent reports. During the course of our studies, Jacobs et al. (1993) reported the identification of a common region of deletion in sporadic epithelial ovarian tumors that maps distal to BRCA1. This region spans 16 cM and was defined by the NME1 and GH loci. In addition, Yang-Feng et al. (1993) identified a group of ovarian tumors that showed LOH at loci distal to D17S41/D17S74 and proximal to D17S24. In these tumors heterozygosity was retained at all informative loci proximal to D17S74. Our results position this candidate ovarian cancer locus at a more distal location with respect to Jacobs et al.'s work and within the large region defined by Yang-Feng et al.'s studies. The discrepancy between the region identified by Jacobs et al. and that by our group may be the result of the relatively small number of informative samples and useful polymorphic markers available to define a common region of deletion within this distal portion of 17q. For example, if tumors UPN 57 and UPN 63 were excluded from our study, the common region of loss would extend proximally to NME1 and would thereby overlap the region identified by Jacobs et al. (see fig. 2). Of 40 tumors studied, only 8 showed LOH while retaining heterozygosity at other loci. Of these, only one tumor (UPN 84) possessed an interstitial deletion, while six of the remaining seven cases showed telomeric deletions, most likely the result of mitotic recombination. Foulkes et al. (1993) have suggested that LOH at any one site on chromosome 17 is most commonly explained by LOH over the whole of the chromosome. In agreement, we observe that

for the majority (21/29) of our cases showing LOH, all informative markers on chromosome 17 were reduced to either homozygosity or hemizygosity, indicating loss (probably due to nondisjunction) of the whole chromosome, with or without reduplication. It is apparent that unless either linkage to disease occurrence is detected (which is unlikely) or a very large number of tumors are thoroughly studied, it will be difficult to accurately define the boundaries of this putative ovarian cancer locus. Therefore, further studies are needed to provide a more precise map of the chromosome 17q22-q23 locus before identification, by positional cloning, of the candidate ovarian cancer gene can be initiated. We are presently evaluating an additional set of breast and ovarian normal/tumor pairs for LOH by using a panel of highly informative markers flanking the 17q22-q23 locus.

Acknowledgments

The authors would like to thank Jim Elliott, Fred Cooper, and Dr. Hernando Salazar for their invaluable support, without whose help the collection of the samples used in this study would not have been possible. We are also grateful to Susan Slominski and Theresa Conway for their assistance in obtaining family history information and tumor specimens. This study was supported by grants from the National Cancer Institute (CA60643) and the Mary Smith Charitable Lead Trust to A.K.G.

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