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Linkage Studies with 17q and 18q Markers in a Breast/Ovarian Cancer Family

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Summary

Genes on chromosomes 17q and 18q have been shown to code for putative tumor suppressors. By a combination of allele-loss studies on sporadic ovarian carcinomas and linkage analysis on a breast/ovarian cancer family, we have investigated the involvement of such genes in these diseases. Allele loss occurred in sporadic tumors from both chromosome 17p, in 18/26 (69%) cases, and chromosome 17q, in 15/22 (68%) cases. In the three familial tumors studied, allele loss also occurred on chromosome 17 (in 2/3 cases for 17p markers and in 2/2 cases for a 17q allele). Allele loss on chromosome 18q, at the DCC (deleted in colorectal carcinomas) locus, was not as common (6/16 cases [38%]) in sporadic ovarian tumors but had occurred in all three familial tumors. The results of linkage analysis on the breast/ovarian cancer family suggested linkage between the disease locus and 17q markers, with a maximum lod score of 1.507 obtained with Mfd188 (D17S579) polymorphism at 5% recombination. The maximum lod score for DCC was 0.323 at 0.1% recombination. In this family our results are consistent with a predisposing gene for breast/ovarian cancer being located at chromosome 17q21.

Introduction

In 1981, a family was described by Matheson et al. (1981) in which predisposition to ovarian cancer appeared to be segregating in an autosomal dominant manner. More recently, individuals in the family have presented with early-onset (i.e., at <40 years of age) breast cancer, and thus this family now has the characteristics of a breast/ovarian cancer family (Cruickshank et al. 1992). Linkage analysis has not previously been carried out on this family.

Early-onset familial breast cancer has been linked to chromosome 17q21, using a highly informative VNTR marker called "CMM86" (Hall et al. 1990). Similarly, results in three of five families with breast/ovarian cancer have suggested that linkage to the same locus exists (Lynch et al. 1991). These studies suggest that a mutation, possibly in a tumor suppressor gene on 17q, may be responsible for cancer predisposition in some breast/ovarian cancer families. Mutations of the p53 gene, a tumor suppressor which maps to 17p13.1, 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; Srivastava et al. 1990). In one family with late-onset breast cancer, linkage to the estrogen receptor locus on chromosome 6q has been suggested, by a lod score of 1.85 (Zuppan et al. 1991).

Allele loss at tumor suppressor loci has been found to be accompanied by mutation in the remaining allele in familial and sporadic cancer. Thus, identifying regions of significant allele loss is a useful method for identifying loci containing putative tumor suppressor genes which may be involved in familial predisposition (Goodrich and Lee 1990).

To date, allele loss in breast cancer has been well documented, notably from 17p (Mackay et al. 1988), 11p (Ali et al. 1987), 17q, and 18q (Cropp et al. 1990). Similarly, in ovarian cancer, allele loss has been reported for 11p, 6q, 17p, and 17q (Eccles et al. 1990,

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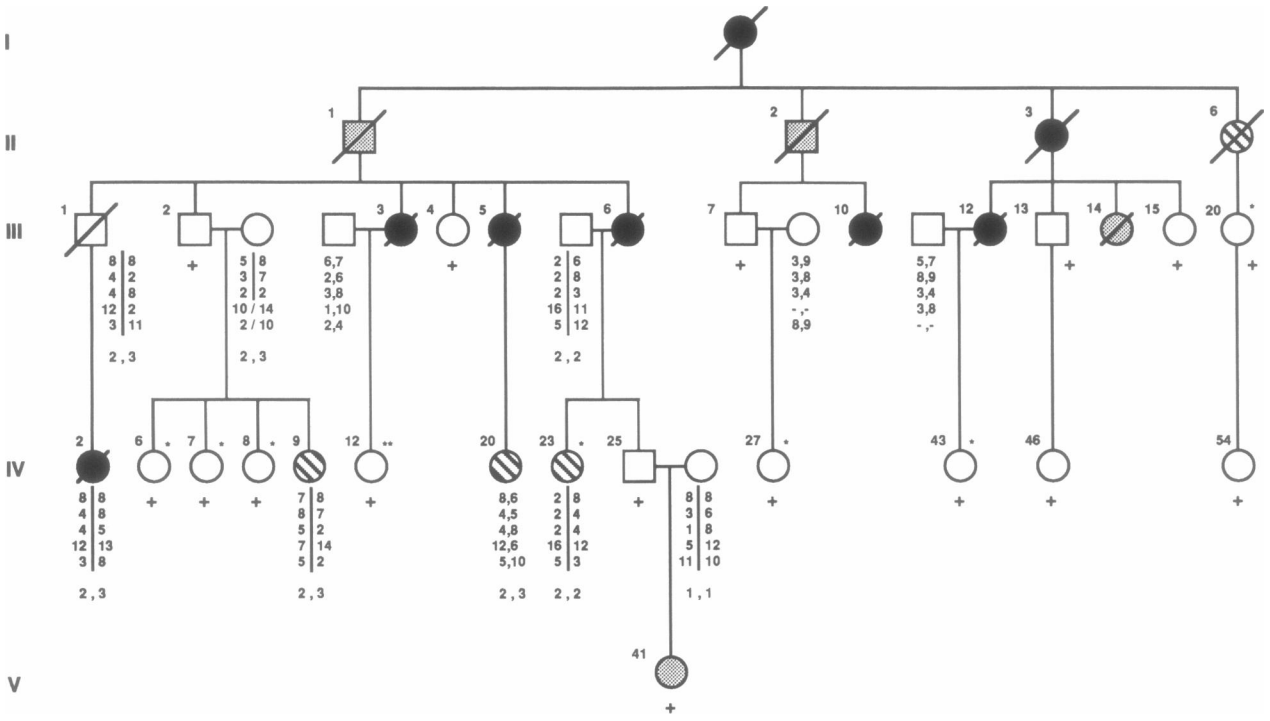

Figure 1 Allele segregation in the breast/ovarian cancer family. The polymorphic markers are, from top to bottom, Mfd15, Mfd188, 42D6, CMM86, and GH, all from chromosome 17, and p15–65 from chromosome 18. Blackened symbols represent ovarian cancers; hatched symbols represent breast cancers; and stippled symbols represent other cancers. Unaffected living family members marked with a plus sign (+) were typed for all markers; however, because of ethical reasons, the results are not shown. A single asterisk (*) at the upper right of a symbol denotes that the individual underwent prophylactic pelvic clearance; and a double asterisk (**) at the upper right of a symbol denotes that the individual underwent oophorectomy only.

1992; Lee et al. 1990; Russell et al. 1990) and at other loci (Sato et al. 1991).

The characterization of the DCC (deleted in colorectal carcinomas) gene on chromosome 18q (Fearon et al. 1990) is particularly interesting, given that ovarian and breast carcinomas are sometimes found in familial association with cancers of the gastrointestinal tract (Schildkraut and Thompson 1988). The gene itself has tumor suppressor qualities and is therefore a candidate for possible familial cancer predisposition. By a combination of allele-loss studies on sporadic ovarian carcinomas and linkage analysis on our breast/ovarian cancer family, we have investigated the involvement of loci on chromosomes 17 and 18 in both familial and sporadic forms of the disease.

Material and Methods

We have been studying the breast/ovarian cancer family reported by Matheson et al. (1981) and, more recently, by Cruickshank et al. (1992). A simplified family pedigree is shown in figure 1, with a total of eight women affected with ovarian cancer and four affected with breast cancer.

Table 1 shows the early age at diagnosis (6/8 of the ovarian cancer patients are <50 years of age, and 3/4 of the breast cancer patients are <40 years of age). There have been four additional individuals with cancer in the family: one with cancer of the kidney (II.1), two with cancers of the gastrointestinal tract (II.2 and III.14), and one with acute lymphocytic leukemia (V.41) which we have considered to be sporadic.

Fresh tumor material and blood were available from one ovarian cancer (IV.2) and two breast cancers (IV.9 and IV.20). In addition, a large number of sporadically occurring epithelial ovarian tumor/blood pairs were studied: 28 invasive carcinomas, 13 with borderline malignant potential, and 11 benign.

Both blood and tumor samples were collected fresh

Table 1

Clinical Details of Family Members

Tumor Type and Pedigree Number	Age at Diagnosis	Survival Time	Histology
Ovarian:			
I.1	Not known	Died at age 60 years	Not known
II.3	66 years	6 mo	Papillary adenocarcinoma
III.3	45 years	2 years	No biopsy taken at laparotomy
III.5	47 years	1 year	Poorly differentiated adenocarcinoma
III.6	44 years	1 year	Cytology-confirmed ovarian carcinoma
III.10	44 years	2 years	Poorly differentiated adenocarcinoma
III.12	49 years	6 mo	Poorly differentiated adenocarcinoma
IV.2	41 years	17 mo	Poorly differentiated papillary serous
Breast:			
II.6	Not known	Died at age 50 years	Not known
IV.9	35 years	33 mo treatment	Poorly differentiated ductular carcinoma
IV.20	30 years	20 mo treatment	Medullary and ductular carcinomas
IV.23	38 years	3 years	Ductular carcinoma

from the surgery theater and were stored frozen at −20°C. DNA extraction and Southern blot analysis were carried out by conventional methods (Sambrook et al. 1989). Allele-loss analysis was carried out by comparing tumor/blood pairs by using the following genomic DNA probes in conjunction with appropriate restriction endonucleases: pYNZ22 (D17S30 at 17p13.3) with *Bam*HI, pYNH37.3 (D17S28 at 17p13.3) with *Msp*I, pBHP53 (tp53 at 17p13.1) with *Bam*HI, pMCT35.1 (D17S31 at 17p13.1) with *Msp*I, pTHH59 (D17S4 at 17q23-qter) with *Pvu*II or *Taq*I (all reported by Nakamura et al. 1988*a*, except for pBHP53, which was reported by Hoyheim et al. 1989), and p15–65 (18q21-qter) with *Msp*I (Fearon et al. 1990).

For linkage studies on chromosome 17q, peripheral blood DNA samples were analyzed either by using PCR polymorphisms as described by Easton et al. (1993) or, in the case of genomic DNA probe CMM86, by detecting a *Hin*fI polymorphism (D17S74 at 17q22) (Nakamura et al. 1988*b*), by Southern blot analysis. The PCR polymorphisms were Mfd15 (D17S250 at 17q11.2), Mfd188 (D17S579 at 17q12q21), 42D6 (D17S588 at

Table 2

Allele-Loss Results

	Frequency [N[a]] of		
Tumor Type	Total 17p Loss	17q23-25.3 pTHH59	18q21.3-qter p15–65
Sporadic ovarian:			
Malignant	18/26 (69%) [28]	15/22 (68%) [28]	6/16 (38%) [38]
Borderline	0/9 [14]	0/10 [13]	0/4 [6]
Benign	1/12 (8%) [12]	0/6 [11]	0/1 [3]
Familial:			
Ovarian (IV.2)	Loss	Loss	Loss
Breast (IV.9)	No Loss	Loss	Loss
Breast (IV.20)	Loss	Noninformative	Loss

[a] No. of tumors studied.

Table 3

Lod Scores Calculated Using the LINKAGE Computer Program

	Lod Score at Recombination Fraction =					
DNA Marker	.001	.01	.05	.1	.2	.3
Chromosome 17:						
Mfd15	−.306	−.263	−.127	−.030	.054	.074
Mfd188	1.458	1.478	1.507	1.464	1.248	.930
42D6	.274	.295	.356	.382	.355	.283
CMM86	−.169	−.135	−.013	.088	.187	.196
GH	−1.445	−1.433	−1.343	−1.187	−.893	−.651
Chromosome 18:						
DCC	.323	.316	.285	.244	.162	.080

17q21), and GH (growth hormone at 17q23). Linkage analysis to the DCC locus (18q21.3-qter) was carried out by Southern blot analysis using the *Msp*I polymorphism detected by probe p15–65. The linkage analysis was performed using the LINKAGE program and criteria, previously described by Easton et al. (1993), in which all non–breast/ovarian cancers are classed as unaffecteds.

Results

Of a total of 28 sporadic malignant ovarian tumors studied, 26 were informative, and 18 (69%) of these 26 demonstrated allele loss for one or more of the chromosome 17p loci (table 2). Similarly, for chromosome 17q loci, 15/22 (68%) tumors showed the loss of one allele. Analysis of the three familial tumors demonstrated a similar degree of allele loss for both 17p and 17q, with both informative tumors showing loss for the 17q marker pTHH59.

The frequency of allele loss in sporadic ovarian tumors for the chromosome 18q DCC locus was 6/16 (38%), which is significantly less than the frequency of 17p loss ($.02 > P > .01$). In contrast, loss of a DCC allele was found in all three of the familial solid tumors studied.

Figure 1 shows the results of allele segregation in a portion of the extended family, for the five 17q markers and one 18q marker. Table 3 shows the lod scores obtained from linkage analysis.

Discussion

A mutation in a tumor suppressor gene can be inherited recessively, having no phenotypic effect, until, as a consequence of one of a number of genetic mechanisms, the functioning copy of the normal gene is lost, thereby allowing the dominant expression of the aberrant allele. The very fact that the mutated genes can be recessive, often lying latent until after childbearing age, accounts for their being maintained in the genetic pool.

By a combination of allele-loss studies in sporadic cancer and linkage analysis in suitable families, it is possible to identify potential tumor suppressor genes whose loss may lead to cancer development. Their involvement in an inherited predisposition can then be examined.

Allele loss on chromosome 17p—and, indeed, mutations of the p53 gene—are common in many cancers (Harris 1990). From the studies of Eccles et al. (1990) and Russell et al. (1990), supported by our own studies (Eccles et al. 1992), it seems that a gene on chromosome 17q is also involved in ovarian cancer. For this reason and because of the results of previous studies on breast/ovarian cancer families (Lynch et al. 1991), we have studied linkage between 17q loci and cancer occurrence in the present family. A maximum positive lod score of 1.507 was obtained using Mfd188 (D17S579) at 5% recombination. All Mfd188 alleles were given an equal frequency of .1 for the purpose of linkage analysis (see Easton et al. 1993). Increasing the allele frequency of the apparently linked allele 4 to .2 had the effect of decreasing the maximum lod score to 1.296 at 5% recombination. While, on the basis of the available allele frequency data, there is no reason to suspect that allele 4 has a higher frequency than that used in the initial analysis, a lod score of 1.296 was still considered high enough to support linkage of this allele to disease occur-

rence. Individual IV.9, affected with breast cancer at 35 years of age, has inherited from her father a chromosome 17q which, at all five loci, differs from that apparently segregating with cancer predisposition in this family.

The results of analysis of the DCC polymorphism (table 2) showed that there was less allele loss than on both arms of chromosome 17 in the sporadic ovarian carcinomas, suggesting that perhaps mutations of the DCC gene are less frequently involved in sporadic disease development. In contrast, all three of the familial solid tumors tested—i.e., one ovarian cancer and two breast cancers—showed allele loss at the DCC locus. When the RFLP marker p15-65, which is within the DCC gene, was used for linkage analysis, a lod score of 0.323 at 0.1% recombination was obtained. No crossovers were detected. This low positive lod score reflects the lack of informativity for the DCC polymorphism. The likelihood of linkage to DCC was reduced, though, as two of the three familial tumors tested had lost the apparent at-risk allele (data not shown).

Although no crossovers were detected between the DCC locus and predisposition to breast/ovarian cancer, the information content for this locus is low compared with that for the Mfd188 locus. The linkage results contained in this study favor the existence of a mutant gene, at chromosome 17q21, as a candidate for cancer predisposition in this family.

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