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Linkage Analysis of 19 French Breast Cancer Families, with Five Chromosome 17q Markers

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Summary

Nineteen French breast and breast-ovarian cancer families were tested for linkage with five chromosome 17q markers. The five breast-ovarian cancer families as a group give positive evidence for linkage, whereas the 14 breast cancer–only families do not. Heterogeneity of linkage of breast and breast-ovarian cancers is significant in France and supports the existence of more than one susceptibility gene.

Introduction

Family history of breast cancer is a significant risk factor in women (Easton and Peto 1990). For other tumor types, the same genetic alterations may be responsible for both inherited and sporadic disease; therefore, identifying susceptibility genes may be relevant for the screening, prevention, and treatment of breast cancer, on a population-wide basis.

Familial early-onset breast cancer has been linked to chromosome 17q12-q21 (Hall et al. (1990). In that study, there was no evidence for linkage heterogeneity among the families with early-onset disease (mean age at detection <46 years), but older-onset families did not appear to be linked to chromosome 17. A reanalysis of these data, allowing for a higher proportion of sporadic cases, suggested that linkage may not be restricted to early-onset disease (Margaritte et al. 1992). Mérette et al. (1992) also analyzed the same data set. They included age at onset as a covariate and concluded that linkage heterogeneity by age was, in fact, present. Genetic heterogeneity of early-onset familial breast cancer has since been reported (Sobol et al. 1992). In a further study, the chromosome 17 locus was assigned for a proportion of breast and ovarian cancer syndrome families (three of five), but no trend between positive linkage and median age at onset was observed (Narod et al. 1991).

We have tested 14 French families with breast cancer and 5 families with breast and ovarian cancers, with five polymorphic DNA markers from chromosome 17q. With these markers, we observed positive lod scores for the breast-ovarian cancer families but negative lod scores for the breast cancer families.

Subjects and Methods

Families

The genetic analysis is based on 93 affected women from 19 families residing in France. All persons are Caucasian. These families were identified from the records of the French Cooperative Network and through interested physicians. The network is a multidisciplinary group formed to investigate inherited tumors and to develop protocols for their screening and treatment.

Eligible families contain at least three women who are first- or second-degree relatives who have been diagnosed with invasive cancer of the breast and/or ovary. The pedigrees are compatible with the segregation of a dominant gene. An affected family member is

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Table 1

Breast and Breast-Ovarian Cancer Families

Family	No. of Individuals with Breast Cancer	Mean Age at Onset (years)	Age Range (years)	No. of Individuals with Ovarian Cancer	Mean Age at Onset (years)	Age Range (years)	Other Cancer(s)[a]
1	11	45	36–54				Pr
2	3	38	29–46				
3	4	44	33–58				
6	6	45	33–55				Lx
7	6	42	30–55				Co, Pr, Ut, and Lu
8	6	52	37–72				
9	5	48	33–60				
10	3	54	42–75				
11	4	57	54–62				
12	3	59	44–79				
13	7	45	30–60				Lu, Co, and Test
14	3	59	54–65				Blad
15	4	38	30–44				
19	3	45	40–54				Co, Me, and E
4	2	36	29–43	3	54	32–75	Lu
5	3	38	35–42	3	49		Lu
16	3	54	41–71	1	45		
17	3	36	29–40	2	55	45–66	
18	3	54	42–60	2	64	55–74	Le, Lu, Cx, and CSU

[a] Co = colon; Le = leukemia; Lu = lung; Cx = cervix; E = esophagus; Me = melanoma; Lx = larynx; Pr = prostate; Ut = uterus; Test = testis; Blad = bladder; and CSU = cancer site unknown.

defined as one with a documented pathology report or a medical record. For a few cases diagnosed before 1960, a history of breast or ovarian cancer, reported by the family, was considered sufficient.

Fourteen of the 19 families contain three or more women with breast cancer (mean = 4.8 cases) but without ovarian cancer (table 1). Mean age at diagnosis of these families was 48 years.

One of the early-onset breast families (family 1) has already been reported to be unlinked to an interval defined by four of the markers tested in this study (Sobol et al. 1992). Other cancers (and total number of affected individuals) in these families include colon (3), lung (2), prostate (2), larynx (1), uterus (1), testis (1), melanoma (1), esophagus (1), and bladder (1).

Five additional families contain two or more women with breast cancer (mean = 2.8 cases) and one or more women with ovarian cancer (mean = 2.2 cases). One woman was affected with both tumors. Mean age at breast cancer diagnosis for these families was 44 years. Other cancers (and total number of affected individuals) include lung (3), cervix (1), and leukemia (1).

Typing of DNA Polymorphisms

Genomic DNA from 69 cases and from 123 informative relatives was prepared from either whole blood or Epstein-Barr virus–immortalized lymphoblastoid cells. Five polymorphic loci on chromosome 17q were tested (from centromere to telomere): D17S250 (Weber et al. 1990), D17S579 (Hall et al. 1992), 42D6 (provided by M. Skolnick, through the Breast Cancer Linkage Consortium), NM23-H1 (Hall et al. 1992), and D17S74 (Nakamura et al. 1988). For typing the minisatellite locus D17S74, 5 μg of genomic DNA were digested with *Hin*fI, electrophoresed on a 0.8% agarose gel, transferred to a nylon membrane, and then hybridized with the CMM86 probe labeled by random primer extension. For typing the microsatellite loci D17S250, D17S579, 42D6, and NM23-H1, 80–200 ng of genomic DNA were amplified in 50-μl reaction volumes containing 20 pmol of each primer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 50 mM KCl, 10 mM Tris pH 8.3, and 1 unit *Taq* polymerase (Perkin Elmer Cetus), according to a method described elsewhere (Weber et al. 1990; Hall et al. 1992; Sobol et al. 1992). One-half

Table 2

Lod Scores for Chromosome 17 Probes and Breast versus Breast/Ovarian Cancer

Locus and Cancer Type	Lod Score at θ = .001	.01	.05	.10	.20	.30	LOD2	θ
D17S250:								
Breast alone	−5.16	−4.53	−3.54	−2.44	−1.17	−.46	.00	.50
Breast-ovarian	−1.97	−1.17	−.03	.12	.24	.17	.24	.20
Both	−7.13	−5.70	−3.57	−2.32	−.93	−.29	.00	.50
D17S579:								
Breast alone	−4.30	−3.50	−1.76	−.83	−.14	.01	.05	.01
Breast-ovarian	2.29	2.24	2.01	1.71	1.12	.59	2.29	.00
Both	−2.01	−1.26	.25	.88	.98	.60	1.05	.00
42D6:								
Breast alone	−3.55	−2.90	−1.44	−.65	−.06	.06	.07	.30
Breast-ovarian	1.01	.99	.86	.71	.44	.22	1.01	.00
Both	−2.54	−1.91	−.58	.06	.38	.28	.36	.10
NM23:								
Breast alone	−5.13	−4.48	−2.82	−1.73	−.67	−.22	.00	.50
Breast-ovarian	1.30	1.27	1.15	.98	.65	.34	1.30	.00
Both	−3.83	−3.21	−1.67	−.75	−.02	.12	.27	.00
D17S74:								
Breast alone	−9.96	−8.23	−4.99	−3.17	−1.34	−.33	.00	.50
Breast-ovarian	.45	.08	.74	.90	.78	.28	.91	.12
Both	−10.41	−8.15	−4.25	−2.27	−.56	−.05	.36	.00

Note.—Lod scores at particular θ values are calculated assuming homogeneity. The maximum lod scores (LOD2) and θ are calculated assuming genetic heterogeneity.

microliter of Perfect Match (Stratagene) was added to the reaction mixture for typing the NM23-H1 locus. Amplified DNA was then electrophoresed on nondenaturing acrylamide gels and was visualized either with ethidium bromide or by silver staining. 42D6 alleles were typed by labeling one oligonucleotide with T4 polynucleotide kinase, according to the method described by Weber and May (1989).

Linkage

Hereditary breast cancer was modeled as a dominant disease with a gene frequency of .003 (Claus et al. 1991). The penetrance of the gene was assumed to be .10, .20, .30, .40, .60, and .80 for the age groups 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, and 70 years and older, respectively. The risk values for noncarriers were set at those of the general population, using age-specific cumulative incidence rates (Young et al. 1981). Lod scores were calculated with the LINKAGE program, and the proportion of linked families was estimated using the admixture test with the HOMOG program (Ott 1985). Lod scores were calculated assuming homogeneity (LOD1) and assuming heterogeneity (LOD2), according to the method of Risch (1989). Heterogeneity of lod scores for the groups of families with and without ovarian cancer was assessed with Morton's likelihood-ratio test by using the MTEST program.

Results

When all 19 families are combined and homogeneity is assumed, there is modest evidence for linkage to D17S579 (lod score = 0.98; recombination fraction [θ] = .20), which is the marker the most closely linked to breast cancer (Hall et al. 1992). Because of previous reports of genetic heterogeneity in breast cancer syndromes (Hall et al. 1990; Narod et al. 1991; Sobol et al. 1992), we examined all two-point linkages, under the assumption of heterogeneity, by allowing the proportion of linked families to vary (Risch 1989). LOD2 scores for all loci, except D17S250, were positive but

Table 3

Individual Lod Scores for D17S579 and 19 Breast Cancer Families

Cancer Type and Family	Lod Score at θ = .001	.01	.05	.10	.20
Breast:					
1	.26	.25	.22	.18	.10
2	−1.35	−1.12	−.68	−.44	−.20
3	−.92	−.82	−.54	−.36	−.16
6	−1.11	−.91	−.51	−.31	−.12
7	.58	.46	.40	.33	.20
8	−.39	−.36	−.25	−.17	−.07
9	−.92	−.79	−.47	−.28	−.11
10	.19	.19	.16	.13	.08
11	−.39	−.36	−.25	−.17	−.07
12	−.05	−.05	−.04	−.03	−.01
13	.64	.63	.54	.44	.25
14	−.13	−.12	−.10	−.08	−.04
15	.40	.39	.35	.29	.19
19	−.96	−.82	−.50	−.31	−.13
Breast-ovarian:					
4	−.11	−.11	−.09	−.07	−.04
5	.40	.39	.35	.30	.19
16	.72	.71	.64	.56	.38
17	.56	.45	.49	.42	.28
18	.66	.64	.56	.46	.28

NOTE.—Lod scores are calculated assuming homogeneity.

did not reach significance. For each of these probes, the five breast-ovarian cancer families as a group gave evidence of linkage (table 2). There was no evidence of genetic heterogeneity among this subgroup, although one family (family 4) gave slightly negative lod scores. The estimated θ for the breast-ovarian cancer families was .00 for the three interior loci (D17S579, 42D6, and NM23), .20 for D17S250, and .12 for D17S74. In contrast, there was little or no evidence of linkage for the group of 14 families with only breast cancer. Five of 14 families gave positive lod scores for $\theta < .20$ (table 3), but none of these families independently gave a lod score sufficiently high for evidence of linkage. A common θ between the disease susceptibility locus and D17S579, for the two subgroups of families, i.e., those with and those without ovarian cancers, was rejected ($\chi^2 = 5.89$; 1 df; $P = .015$).

Discussion

The 5 French breast-ovarian cancer families as a group give evidence of linkage to chromosome 17q, whereas the group of 14 French breast cancer families as a whole do not. However, it is likely, on the basis of the results of the collaborative study group, that some of our breast cancer families with positive lod scores are, in fact, linked.

While it is most probable that the differences observed are due to true genetic heterogeneity, there are several other possibilities. The observed heterogeneity of linkage, depending on the syndrome (breast-only vs. breast-ovarian cancer), among these families may be due to chance, but this is unlikely ($P = .015$).

It has been suggested that linkage to chromosome 17q is restricted to early-onset (mean age at diagnosis <46 years) breast cancer families (Hall et al. 1990). The majority of the French breast cancer families studied (8 of 14) satisfy this early-onset criterion, and we observed no trend between positive lod score and mean age at onset, in agreement with the finding by Narod et al. (1991).

Ovarian cancer is infrequent and occurs at the rate of 10.6/100,000 per year, as compared with the breast cancer rate of 75.8/100,000 per year in French women

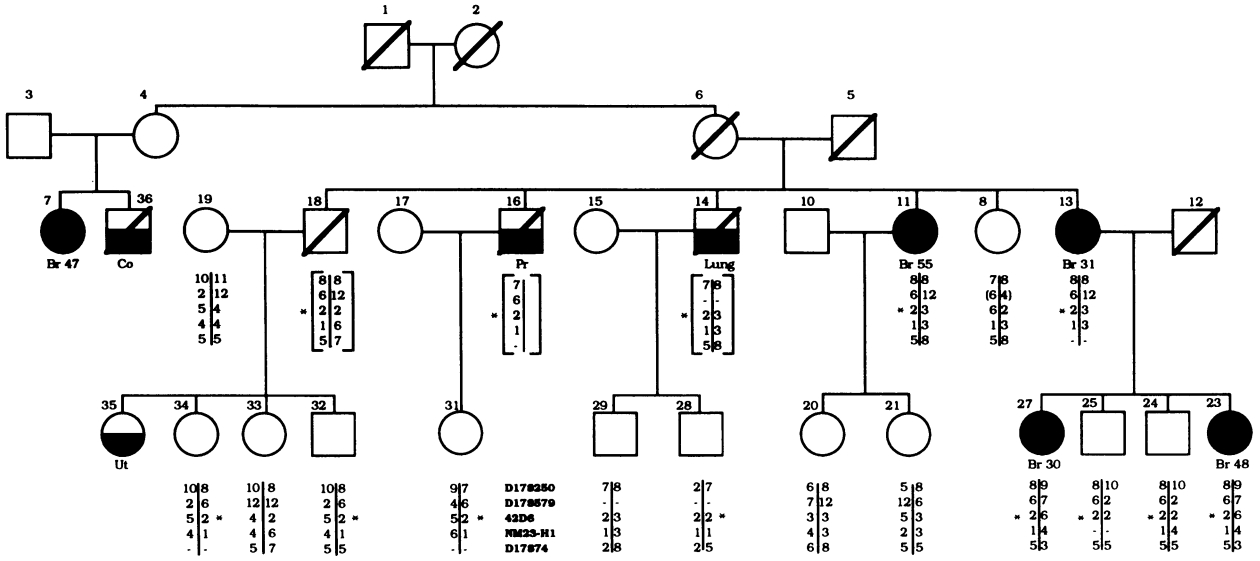

Figure 1 Linkage of breast cancer to chromosome 17q in family 7. Blackened circles represent women with breast (Br) cancer; and unblackened circles and squares represent nonaffected women and men, respectively. A diagonal line indicates that the individual is deceased. The age at first breast cancer diagnosis is given. A dash indicates an untested marker. Half-blackened symbols indicate other cancers (Co = colon; Pr = prostate; and Ut = uterus). For clarity, one woman with breast cancer (the daughter of woman 6 and man 5) has been omitted. Reconstructed genotypes are in brackets and have been verified with two more telomeric markers (data not shown). An asterisk indicates that the genotype is putatively linked to susceptibility.

(Muir et al. 1987). As a result, the breast-ovarian cancer syndrome may be better defined than site-specific familial breast cancer. Our definition of a breast cancer family (three or more cases) may not be sufficiently stringent, and we may have included some families in which the observed clustering is due to chance or to polygenic inheritance. The presence of sporadic cases will, on average, lead to negative lod scores. Chance aggregation is more likely for families with few cases. Eight of 14 of our site-specific breast cancer families contain fewer than five cases. However, there is no trend between the number of cancer cases and lod score. In family 1, no more than three of eight affected woman share a common haplotype (Sobol et al. 1992). Although the lod score with the D17S579 marker is slightly positive for this family (table 3), a multipoint analysis of the family effectively ruled out the region between D17S250 and D17S74 (data not shown). Family 6 contains six cases of breast cancer, with an average age at onset of 45 years. Four women have a common chromosome 17 haplotype, but this is not shared by the other two affected women, diagnosed at ages 39 and 44 years. Because of these early ages at onset, these two women are unlikely to be sporadic cases.

Four of the 19 breast cancer families contain three or more cancers of other sites (table 1). While these cancers were not used to define a breast cancer family, it is possible these cancers are part of the familial syndrome. Interestingly, all four families with multiple additional cases of cancer give positive lod scores with D17S579. Family 7 (fig. 1), which contains four additional cancers (colon, prostate, uterus, and lung), gives a lod score of 0.58 with D17S579. The two brothers who developed lung and prostate cancer and the father of the woman with uterus cancer carry the haplotype putatively linked to susceptibility between D17S579 and NM23-H1.

Four members of breast-ovarian cancer family 18 (fig. 2) (lod score = 0.66) developed other cancers, including leukemia, cervix, lung, and a primary cancer of an unknown site. All four carry the haplotype that appears to be linked to susceptibility.

Four endometrial and two thyroid cancers were reported among 5 of the 23 families studied by Hall et al. (1990). Three of these families appear to be linked, and two were unlinked. When two endometrial cancer patients belonging to linked families were considered to be affected, lod scores increased (Hall et al. 1992). It is

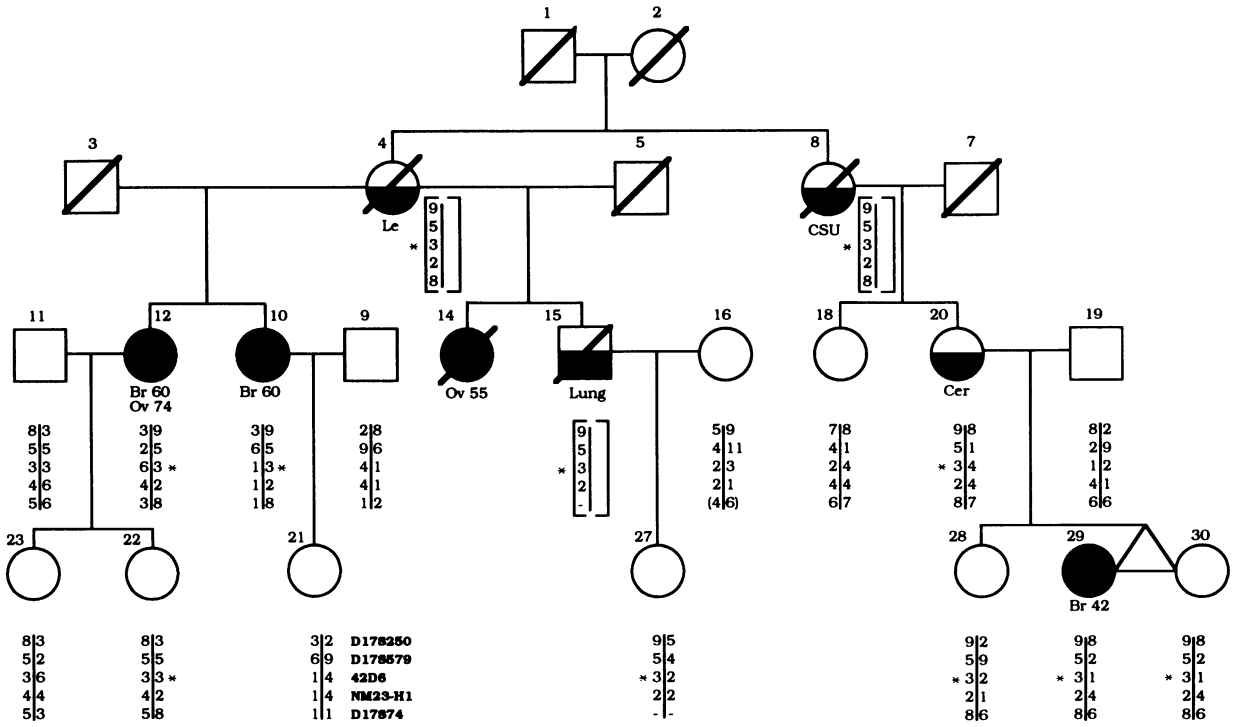

Figure 2 Linkage of breast-ovarian cancer to chromosome 17q in family 18. Blackened circles represent women with breast (Br) or ovarian (Ov) cancer. Half-blackened symbols indicate other cancers (Le = leukemia; Cer = cervix; and CSU = cancer site unknown). All other symbols are as in fig. 1.

possible that the gene for predisposition to breast and ovarian cancer is also related to the development of other cancers. Further collaborative studies would be useful to investigate this interesting hypothesis.

It is likely that observed heterogeneity of linkage findings is at least partially due to the existence of more than one gene for inherited breast cancer. The distribution of susceptibility genes may be different in France and in North America. To date, all linked families reported are from the United States (Hall et al. 1990; Narod et al. 1991).

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