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A Breast-Ovarian Cancer Susceptibility Gene Maps to Chromosome 17q21

Jean Feunteun,* Steven A. Narod,† Henry T. Lynch,‡ Patrice Watson,‡ Theresa Conway,‡ Jane Lynch,‡ Jillian Parboosingh,† Peter O'Connell,§ Ray White,§ and Gilbert M. Lenoir‖

*Laboratoire d'Oncologie Moléculaire, URA 1158 CNRS, Institut Gustave Roussy, Villejuif, France; †Centre for Human Genetics and Departments of Medicine and Oncology, McGill University, Montreal; ‡Department of Preventive Medicine, Creighton University School of Medicine, Omaha; §Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City; and ‖International Agency for Research on Cancer, Lyon

Summary

Nineteen North American Caucasian families that contain a minimum of four confirmed cases of breast or ovarian cancer have been studied. Four polymorphisms (cLB17.1, D17S579, D17S588, and D17S74), which span a region of approximately 15 cM on chromosome 17q12, were typed. Our data confirm the location of a dominant gene conferring susceptibility to breast and ovarian cancer (maximum lod = 9.78) and suggest that the breast-ovarian cancer syndrome is genetically heterogeneous. Two recombinants in one large family suggest that the breast-ovarian cancer locus lies between D17S588 and D17S579.

Introduction

The breast-ovarian cancer syndrome is a dominant predisposition to cancer of the breast and of the ovaries (Lynch 1981; Go et al. 1983). Although breast cancer is relatively common in middle age and in the elderly (Young et al. 1981), tumors appearing early in life, bilateral tumors, or tumors affecting additional organs, particularly the ovary, suggest an underlying genetic susceptibility.

A locus associated with inherited early-onset breast cancer has been identified on the long arm of chromosome 17 by linkage analysis using the marker D17S74 (Hall et al. 1990). In this study, genetic heterogeneity was suggested, as linkage was restricted to early-onset breast cancer families (mean age at diagnosis <45 years). However, heterogeneity was no longer significant when liability classes that assigned a higher probability of a late-onset cancer patient being sporadic (Margaritte et al. 1992) were used. We subsequently confirmed the presence of a breast cancer susceptibility locus on chromosome 17q and showed that predisposition to ovarian cancer (within the context of hereditary breast cancer) is determined at the same locus or at a tightly linked locus (Narod et al. 1991). Genetic heterogeneity of linkage to D17S74 was present in the five breast-ovarian cancer families tested. The present study is part of the hereditary breast cancer linkage consortium and extends our linkage data to 15 breast-ovarian cancer families and 4 breast-specific cancer families, by using four markers from the region of chromosome 17q21.

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Address for correspondence and reprints: Gilbert M. Lenoir, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon, Cedex 08, France.

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Subjects and Methods

Origin of the Families

The 19 North American Caucasian families included in this study are followed by the Department of Preventive Medicine at the Creighton University School of Medicine, Omaha. They were selected because they contain a minimum of four confirmed cases of breast or ovarian cancer, at least two of which were breast cancers. Ten families had two or more ovarian cancers; five had only one ovarian cancer; and four had only breast cancer. Twenty-three (16%) of the 133 breast cancers were bilateral, and 12 (37%) of the 42 ovarian cancers were diagnosed in women with a history of breast cancer. In all cases the breast cancer preceded

Table 1

Recombination Fractions and Pairwise Two-Point Lod Scores for Chromosome 17 Probes Used in the Analysis

	cLB17.1	D17S579	D17S588	D17S74
cLB17.1	...	.03 (.01–.06)	.06 (.03–.10)	.15 (.11–.20)
D17S579	52.03	...	.05 (.02–.09)	.14 (.10–.19)
D17S588	44.53	38.61	...	.10 (.06–.14)
D17S74	26.27	20.52	39.94	...

NOTE.—Recombination fractions and 1-lod support intervals (in parentheses) are presented above the diagonal; maximum lod scores are presented below the diagonal.

the ovarian cancer. No male breast cancer was detected in these families.

Genotyping

A total of 370 individuals have been genotyped. The number of sampled individuals was between 5 (family 1882) and 105 (family 1816). All typings have been performed on DNA extracted from lymphoblastoid cell lines immortalized by Epstein-Barr virus. Two VNTR markers, cLB17.1 and D17S74, were typed by conventional Southern blot analysis in the presence of human placental DNA. cLB17.1 revealed at least 10 different alleles and more than 90% heterozygosity. Sample DNAs were digested by *Hae*III for cLB17.1 and by *Hin*fI for D17S74. Two markers, D17S579 and D17S588, are defined by (CA) in repeats. Their characteristics are described in the accompanying consortium paper (Easton et al. 1993). They were typed by PCR amplification according to standard procedures. PCR amplification products were run on 8% nondenaturing gel electrophoresis.

Linkage Analysis

Hereditary breast-ovarian cancer was modeled as a dominant disease with incomplete penetrance and a gene frequency of .003, on the basis of segregation analysis (Claus et al. 1991; Iselius et al. 1991). The penetrance of the gene was modeled with six liability classes: .10, .20, .30, .40, .60, and .80 for 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 in the Connecticut Tumor Registry (Young et al. 1981). Individuals with multiple primary tumors were considered more likely to be genetic cases than were those with a single tumor, and the liability class for these individuals was reduced by one. This model differs slightly from that used in the accompanying consortium paper (Easton et al. 1993). Our families differ because they were selected for the presence of ovarian cancer. Although there is little data about age-specific cancer rates in breast-ovarian cancer families, we do not see a decline in incidence rate with age in our families. We chose to estimate liability classes by using cumulative incidence rates, rather than incidence density rates, but the data set analyzed under both models gave nearly identical results. This similarity reflects the lack of elderly cases in our families.

Table 2

Linkage of Breast-Ovarian Cancer and Chromosome 17 Probes

	LOD SCORE AT θ =							
LOCUS	.001	.01	.05	.10	.20	LOD1 (θ)[a]	LOD2 (θ)[b]	PROPORTION OF LINKED FAMILIES
cLB17.1	6.96	7.37	8.45	8.14	6.36	8.45 (.05)	8.50 (.02)	.81
D17S579	−.55	.63	2.47	3.04	2.72	3.08 (.12)	4.38 (.001)	.55
D17S588	2.20	3.14	4.48	5.30	4.60	5.30 (.10)	5.49 (.06)	.73
D17S74	−11.92	−7.45	−.89	2.09	3.86	3.88 (.22)	4.07 (.15)	.68

[a] Calculated assuming genetic homogeneity.
[b] Calculated assuming heterogeneity.

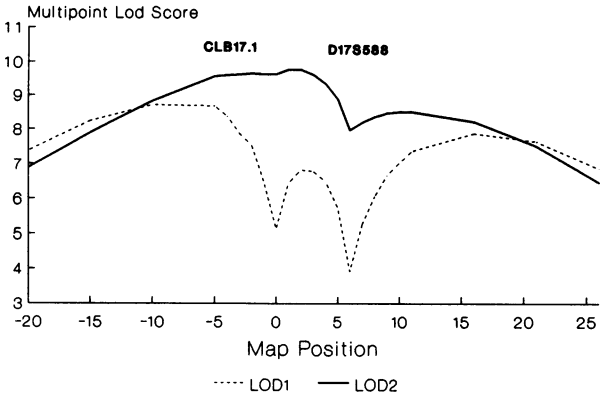

Figure 1 Three-point analysis of linkage between the breast-ovarian cancer locus and loci cLB17.1 and D17S588, performed on 19 families. The map origin represents the cLB17.1 locus. The LOD2 curve was constructed by assuming that 65% of the families are linked.

Lod scores were calculated with the LINKAGE program, and support intervals were based on the 1-lod method (Conneally et al. 1985). Lod scores were calculated assuming homogeneity (LOD1) and assuming heterogeneity (LOD2), according to the method of Risch (1989). The LOD2 score is calculated by allowing the recombination fraction (θ) and the proportion of linked families to vary simultaneously. The proportion of linked families was estimated by using the admixture test with the HOMOG program (Ott 1985). Equal θ values in males and females were assumed for the region.

Results

Four polymorphisms, which span a region of approximately 15 cM on chromosome 17q12 were typed on the 19 families. The θ values and pairwise lod scores for these four markers calculated from our family data are presented in table 1. The genetic map order, from centromere to telomere, of these markers, from two-point linkage and when the Kosambi mapping function is assumed, are cLB17.1---*3*---D17S579----*5*----D17S588-------*10*--------D17S74. The distance from cLB17.1 to D17S74 is 15 cM, from cLB17.1 to D17S588 is 6 cM, and from D17S579 to D17S74 is 14 cM. No recombinant between cLB17.1 and D17S579 has been observed in the CEPH families (D. Black, personal communication), and the relative position of these markers is still uncertain.

The two-point linkage results for each of these four markers versus the disease locus, summed from the 19 families, are presented in table 2. The lod scores were calculated alternately under LOD1 and LOD2. When heterogeneity was assumed, the proportion of linked families was estimated simultaneously. The estimated θ of .15 between the disease locus and D17S74 is similar to our previous result based on only five breast-ovarian cancer families (Narod et al. 1991). cLB17.1, D17S579, and D17S588 show maximum lod scores at much lower θ values (θ = .02, θ = .001, and θ = .06, respectively), indicating that these three markers are closer to the disease locus. When the proportion of linked families was held fixed (e.g., at 70%), the estimated θ values differed very little from those presented in table 2 (data not shown).

We performed a three-point analysis of linkage between the breast-ovarian cancer locus and the loci cLB17.1 and D17S588, on the 19 families (fig. 1). A maximum LOD2 score of 9.78 was seen at a position between the two probes, 2 cM from D17S588. However, this position was only marginally favored over the second most likely position, 1 cM centromeric to cLB17.1 (LOD2 = 9.66). These orders were both favored over a position of the breast-ovarian cancer gene telomeric to D17S588 (relative odds 18:1). Under homogeneity, the maximum LOD1 of 8.72 was observed at a position 10 cM centromeric to cLB17.1. Because this value is significantly less than the maximum LOD2 assuming heterogeneity (9.78), genetic homogeneity could be rejected (χ^2 = 4.82; P = .015). Among the 15 breast-ovarian cancer families, genetic heterogeneity was borderline significant (P = .08). With the multipoint lod scores the proportion of linked families is estimated to be 65%. This estimate incorporates the most linkage information and is probably the most accurate.

Individual multipoint lod scores were estimated for each family, at the maximum point on the curve in figure 1, in the interval between D17S588 and cLB17.1 (table 3). When it is assumed that 65% of families are linked overall, these lod scores can be converted into posterior probabilities of linkage. When the families are ordered by decreasing probability of linkage, there appear to be no obvious factors that distinguish those with a high probability of linkage from those that are likely to be unlinked. The 11 families with probabilities of linkage of 70% or greater differ only slightly from the 6 families with probability of linkage of less than 50%, in terms of average number of breast cancers (6.9 vs. 7.8, respectively), average median age at onset of breast cancer (39.6 vs. 41.5 years), average number of ovarian cancers (2.6 vs. 1.8), average median age at on-

Table 3

Families Used in the Analysis, with Corresponding Lod Scores and Probabilities of Being of the Linked Type

Family	No. of Breast Cancers	Median Age at Diagnosis (years)	No. of Ovarian Cancers	Median Age at Diagnosis (years)	Lod Score[a]	Probability of Linkage[b]
1816	14	39.5	10	49	3.03	.99
2775	10	43	0	...	2.15	.99
2770	9	31	3	44	2.04	.99
1086	8	40.5	1	66	1.71	.99
2090	5	32	2	42	1.06	.96
32	7	38	2	59.5	.97	.95
1234	5	38	8	54.5	.90	.94
2979	2	39	2	48	.77	.92
2749	4	47	0	...	.57	.87
1813	5	34	1	51	.28	.78
2887	7	54	0	...	.10	.70
1882	6	53	1	72	.00	.65
1812	4	53	1	17	.00	.65
2619	2	56	3	62	−.29	.49
2944	3	27	2	49	−.75	.24
2850	15	44	2	46	−1.21	.10
1252	9	36	1	84	−1.34	.08
2651	5	42	3	49	−1.45	.06
2932	13	44	0	...	−1.73	.03
Overall	133	42	42	50.5		

[a] Represents the maximum value estimated from the three-point analysis in the interval between cLB.17.1 and D17S588.

[b] Estimates based on a prior probability of 65%.

set of ovarian cancer (51.8 vs. 58.0 years), or the proportion of families with one or more cases of ovarian cancer (72.7% vs. 83.3%). None of these differences are significant.

The identification of recombination events in affected individuals from informative families is critical to the precise mapping of the susceptibility gene. In this respect, family 1816, with 28 affected individuals and for which linkage is highly probable (lod score = 3.03), is a valuable resource (fig. 2). The comparison of individual 193, who carries haplotype [2,1,3,3] for the markers cLB17.1, D17S579, D17S588, and D17S74, respectively, and individual 126 [haplotype 2,1,6,2] indicates that a recombination has occurred between cLB17.1/D17S579 and D17S588 and places the disease locus centromeric to D17S588. The linked haplotype for individual 25 is [4,3,3,3], in contrast to that for individual 59 [2,1,3,3], indicating that a recombination event has occurred between D17S579 and D17S588. Breast cancer was diagnosed in individual 25 at age 57 years, an age significantly higher than the mean age at onset of breast cancer in her family (41.5 years). If individual 25 is in fact a gene carrier, then this recombination places the disease locus below D17S579. Together, these two meioses suggest that the breast cancer locus is located within the 6-cM interval between D17S579 and D17S588, in keeping with the multipoint lod scores (fig. 1).

Discussion

In an attempt to minimize the anticipated effect of genetic heterogeneity on linkage power, 19 large families were selected for study. Most (15/19) of them display a predisposition to both breast and ovarian cancer. It was originally postulated that the hereditary breast-ovarian cancer syndrome was a genetically homogenous condition within the hereditary breast cancer spectrum. Our data confirm the location of a dominant gene conferring susceptibility to breast and ovarian cancer and suggest that the breast-ovarian cancer syndrome is genetically heterogeneous. The confidence

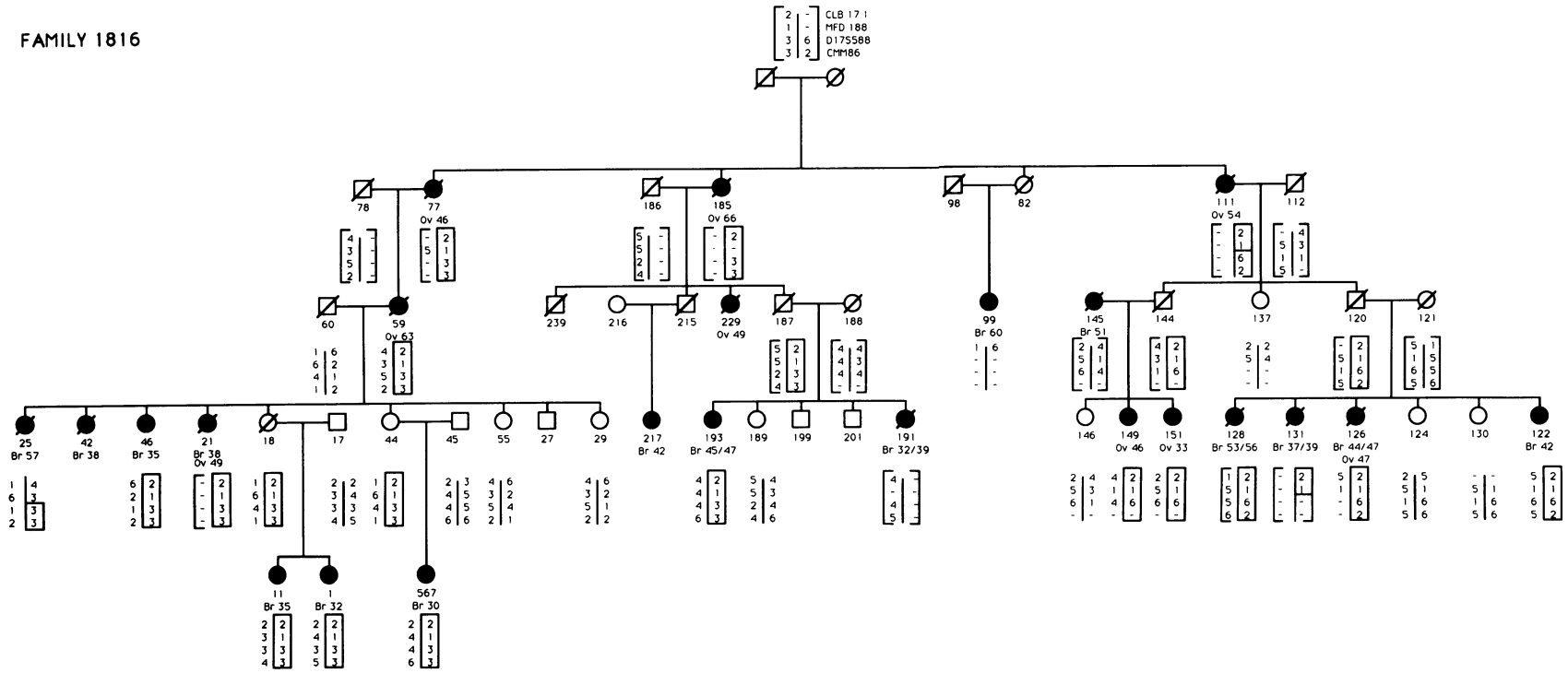

Figure 2 Pedigree of family 1816. A dash indicates marker alleles for which phase is unknown or that are not typed. Ov = ovarian cancer; and Br = breast cancer. Haplotypes in brackets are inferred from offspring. Haplotypes in boxes are intended to represent mutation-bearing chromosomes. CMM86 = D17S74; and 42D6 = D17S588. Several members and haplotype data have been omitted for clarity and for confidentiality. Age at diagnosis is indicated.

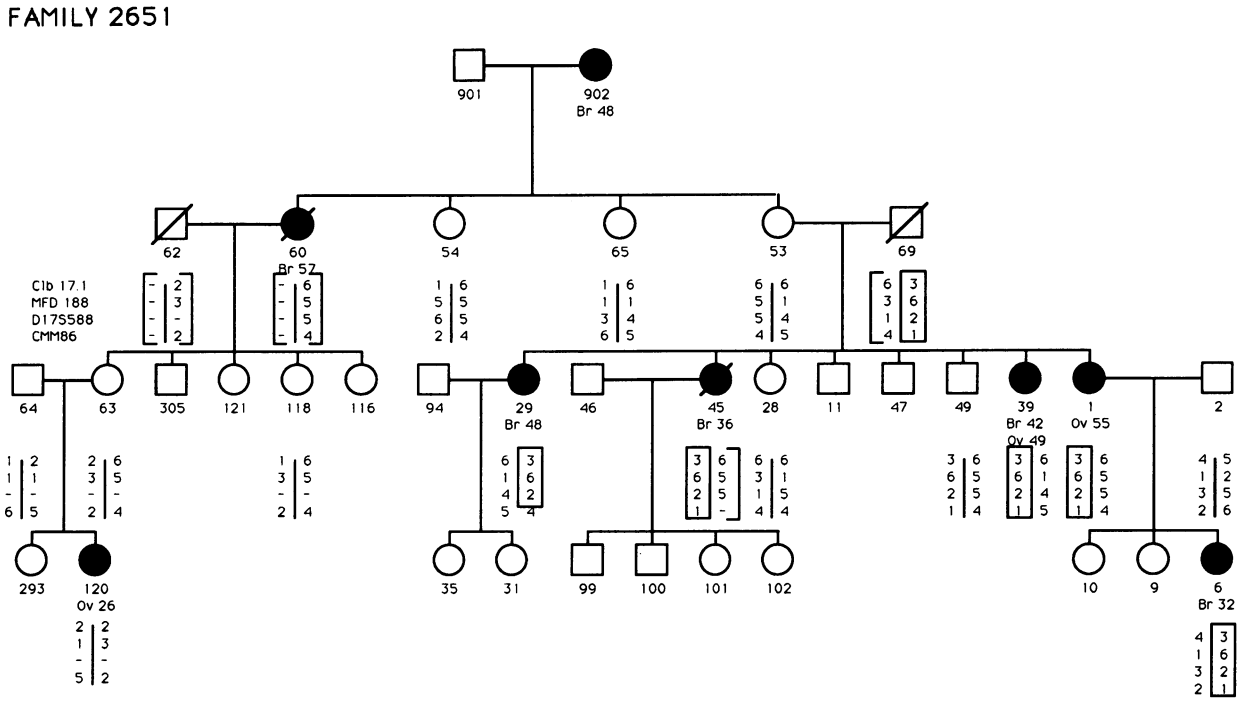

Figure 3 Pedigree of family 2651. Ov = ovarian cancer; and Br = breast cancer. Haplotypes in brackets are inferred from offspring. Haplotypes in boxes are intended to represent mutation-bearing chromosomes. Several members have been omitted for clarity and for confidentiality. Age at diagnosis is indicated.

with which genetic homogeneity of all breast cancer families is now rejected ($P = .015$) is higher than in our previous study ($P = .025$). This is as expected, because the power to detect heterogeneity increases (*a*) with the number of families tested, (*b*) with more closely linked markers (Risch 1989; Narod 1991), and (*c*) when flanking markers are employed (Martinez and Goldin 1991). Among the five breast-ovarian cancer families initially reported (Narod et al. 1991), two (2770 and 2651) were believed to be unlinked to D17S74. Three additional families (32, 1812, and 1813) give negative lod scores with this marker. In contrast, typing with the closer marker cLB17.1 suggests that four of these families (2770, 32, 1812, and 1813) are, in fact, linked. Conversely, family 2850, which was positive with D17S74, is negative with cLB17.1. The status of families 1816 and 2090 (positive lod scores) and family 2651 (negative lod scores) is identical with both markers.

Among the six families showing negative multipoint lod scores (table 3), four (2619, 2944, 2850, and 2651) have two or more cases of ovarian cancer, one (1252) has nine cases of breast cancer and a single case of ovarian cancer at age 84 years (possibly a chance finding), and one (2932) is a site-specific breast cancer family. The median age at onset—44 years—in family 2932 qualifies the latter as an unlinked, early-onset, site-specific breast cancer family, according to the criteria proposed by Hall et al. (1990).

Family 2651 illustrates some of the difficulties encountered in the selection of informative individuals (fig. 3). Five women (1, 29, 39, 45, and 6) in the right-hand branch are affected. In these individuals the disease cosegregates with a common paternal chromosome [3,6,2,1], suggesting linkage. However, the pedigree can be extended to include three additional women (902, 60, and 120) who were affected at a young age, one of whom (120) developed ovarian cancer at age 26 years. None of these three individuals shares the [3,6,2,1] haplotype with the affected people from the right-hand branch. Consequently, linkage to the 17q21 locus is unlikely (lod score = −1.45). It is also possible that the two branches of this family carry distinct mutations.

The two recombinants observed in family 1816 and discussed above suggest that the breast-ovarian cancer locus lies between D17S588 and D17S579. The cross-

over that places the cancer gene centromeric to D17S588 is strongly supported by its identification in several affected individuals. In contrast, the recombination that places the cancer gene below D17S579 is evident only in woman 25. She developed breast cancer at age 57 years, an age significantly higher than the mean age at onset (41.5 years) of breast cancer in the family. None of her five daughters (ages between 20 and 37 years) is affected. If this case of breast cancer is sporadic, the recombinant has no mapping value.

Further linkage studies are needed to provide a more precise map of the chromosome 17q21 locus, before identification of the susceptibility gene by positional cloning can be initiated. The possibility that chromosome 17 marker data may be used to evaluate individual risks for women from high-risk families will certainly now be raised. It is therefore essential to accurately estimate both the location of the breast-ovarian cancer susceptibility gene with respect to a panel of chromosome 17 markers and the proportion of linked families in the population. The probability of a family being of the linked type is likely to vary with the number of affected individuals in the family, the proportion of women with ovarian cancer, their ages at cancer onset, their country of origin, and the number and types of additional cancer cases. Because of the linkage heterogeneity, it is problematic to attempt marker-based counseling at present. It is therefore important to accumulate and synthesize data from as many centers as possible. Nevertheless, for a few very large families where linkage is not in doubt (e.g., family 1816; lod score = 3.03), we feel that marker information may now be introduced, with caution, into the interpretation of individual risk.

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References

Claus EB, Risch N, Thompson WD (1991) Genetic analysis of breast cancer in the Cancer and Steroid Hormone Study. Am J Hum Genet 48:232–242

Conneally PM, Edwards JH, Kidd KD, Lalouel JM, Morton NE, Ott J, White R (1985) Report of the Committee on Methods of Linkage Analysis and Reporting. Cytogenet Cell Genet 40:356–359

Easton DF, Bishop DT, Ford D, Crockford GP, the Breast Cancer Linkage Consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. Am J Hum Genet 52:678–701

Go RCP, King MC, Bailey-Wilson J, Elston RC, Lynch HT (1983) Genetic epidemiology of breast cancer and associated cancers in high risk families. I. Segregation analysis. J Natl Cancer Inst 71:455–461

Hall JM, Lee MK, Morrow J, Anderson L, King MC (1990) Linkage of early onset familial breast cancer to chromosome 17q21. Science 250:1684–1689

Iselius L, Slack J, Litter M, Morton NE (1991) Genetic epidemiology of breast cancer in Britain. Ann Hum Genet 55:151–159

Lynch HT (1981) Genetics and breast cancer. Van Nostrand Reinhold, New York

Margaritte P, Bonaiti-Pellie C, King M-C, Clerget-Darpoux F (1992) Linkage of familial breast cancer to chromosome 17q21 may not be restricted to early-onset disease. Am J Hum Genet 50:1231–1234

Martinez M, Goldin LR (1991) Detection of linkage for heterogeneous disorders by using multipoint linkage analysis. Am J Hum Genet 49:1300–1305

Narod SA (1991) Power of admixture test to detect genetic heterogeneity. Genet Epidemiol 8:209–216

Narod SA, Feunteun J, Lynch HT, Watson P, Conway T, Lynch J, Lenoir G (1991) Familial breast-ovarian cancer locus on chromosome 17q12-23. Lancet 338:82–83

Ott J (1985) Analysis of human genetic linkage. Johns Hopkins University Press, Baltimore

Risch N (1989) Linkage detection test under heterogeneity. Genet Epidemiol 6:473–480

Young JL, Percy CL, Asire AJ (1981) Surveillance, epidemiology and end results: incidence and mortality data, 1973–1977. Natl Cancer Inst Monogr 57:1–1082