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Closing in on a Breast Cancer Gene on Chromosome 17q

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Summary

Linkage of early-onset familial breast and ovarian cancer to 11 markers on chromosome 17q12-q21 defines an 8-cM region which is very likely to include the disease gene BRCA 1. The most closely linked marker is D17S579, a highly informative CA repeat polymorphism. D17S579 has no recombinants with inherited breast or ovarian cancer in 79 informative meioses in the seven families with early-onset disease (lod score 9.12 at zero recombination). There is no evidence for linkage heterogeneity in the families with early-onset disease. The proportion of older-onset breast cancer attributable to BRCA 1 is not yet determinable, because both inherited and sporadic cases occur in older-onset families.

Introduction

We recently reported linkage of early-onset familial breast and ovarian cancer to a gene, BRCA 1 (designated 113705 in *Mendelian Inheritance in Man*), on chromosome 17q12-q21 (Hall et al. 1990). This linkage was subsequently confirmed in at least six independent series of families (Narod et al. 1991; Breast Cancer Consortium, unpublished data). However, the region of chromosome 17q linked to breast cancer was nearly 50 cM long. We report here linkage results with 11 markers linked to breast cancer genotyped on the 23 families from our earlier study. These markers define a region of 8 cM which is very likely to include BRCA 1. One of these markers (Mfd188) is highly informative, with no recombinants with breast cancer in the seven families with early-onset disease.

Subjects and Methods

The 23 families in our series include 7 families in which average age at female breast cancer diagnosis is ≤45 years and 16 families in which average age at female breast cancer diagnosis is >45 years (Hall et al. 1990). Two of the early-onset families also include women with ovarian cancer. Since our previous report, one additional case of breast cancer and one case of ovarian cancer have appeared in family 5, the only early-onset family in which breast cancer did not previously seem linked to a gene on chromosome 17q. In all families, breast and ovarian cancer patients were considered affected; two women with endometrial cancer (in families 1 and 4) were not included in the analyses at all. (If endometrial cancer patients were considered affected, lod scores would be higher.)

The model for multipoint analysis using LINKAGE (Lathrop et al. 1985) postulated a rare autosomal dominant breast cancer gene with age-at-onset distribution and frequency of sporadic disease as described elsewhere (Newman et al. 1988). Lod scores were calculated separately for early-onset families (mean age at breast cancer diagnosis is ≤45 years) and for older-onset families (mean age at breast cancer diagnosis is >45 years). Because the region between D17S250 and D17S40 includes 11 markers with a total of 63 alleles in the families, a single multipoint analysis was not feasible. Hence, multipoint analyses were carried out for breast cancer versus (*a*) D17S250, HER2, and D17S579; (*b*) D17S579, D17S78, GIP, D17S293, and NM23; (*c*) NM23, D17S41, and D17S74; and (*d*)

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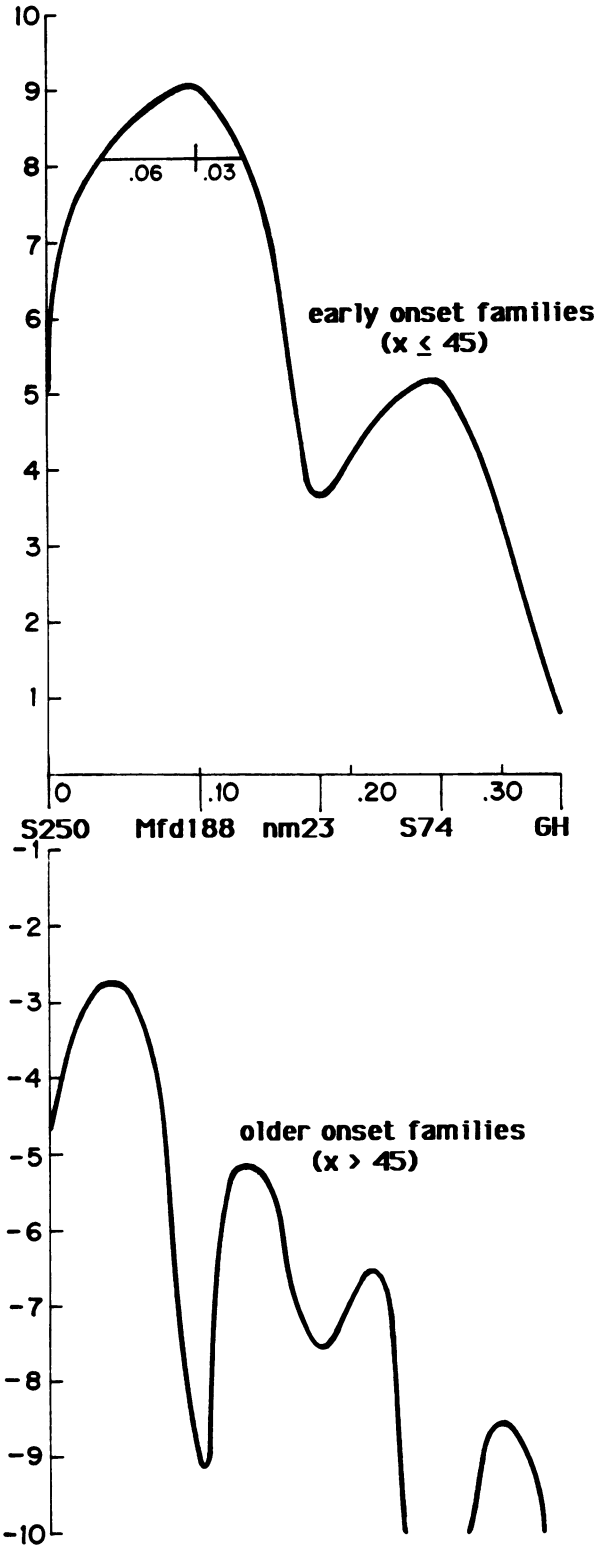

Figure 1 Multipoint analysis of linkage between breast and ovarian cancer and the region of chromosome 17q12-q23 between D17S250 and GH. The top curve represents lod scores for the seven families in which average age at breast cancer diagnosis is ≤45 years; the lower curve represents lod scores for the 16 families in which average age at breast cancer diagnosis is >45 years.

D17S74 and GH. Lod scores between the endpoints of each interval are plotted in figure 1. Lod scores at common endpoints of adjacent intervals are the average of lod scores from the component analyses. Distances between markers are based on data in table 1.

We also applied a model with liability classes representing (1) cumulative incidence of breast cancer, by age, among unaffected women, as we had done in an earlier study (Hall et al. 1990), and (2) age-specific incidence risks of breast cancer among affected women (Margaritte et al. 1992) The effect of this model is to give older-onset cases a much higher probability of being sporadic, rather than of being due to inheritance of susceptibility. Since sporadic and inherited cases are not distinguishable except by age, this renders families with primarily older-onset breast cancer considerably less informative. The models yield very similar results for families with early-onset disease. For two-point linkage analysis, we continued to use LIPED with log normal age-at-onset distributions based on cumulative incidence (Ott 1991), postulating different mean ages for onset of inherited versus sporadic cases (Hall et al. 1990).

Genomic DNA was prepared from lymphoblastoid cell lines of 375 informative relatives by methods described elsewhere (Hall et al. 1989). The following seven polymorphic loci on chromosome 17q were added to those already screened in these families: D17S250 (Weber et al. 1990); HER2 (Hall and King 1991); D17S579; GIP (Johnson et al. 1990); NM23-H1; D17S293; and GH (Polymeropoulos et al. 1991). For typing the microsatellite loci D17S579, NM23-H1, D17S293, D17S250, and GH, 50-μl reaction volumes containing 100–200 ng genomic DNA, 50 pmol each primer, 1.5 mM $MgCl_2$, 100 μM each of dATP, dGTP, and dTTP, 0.5 μCi ^{32}P-dCTP, 50 mM KCl, 10 mM Tris pH 8.3, and 2.5 units *Taq* polymerase were amplified for 35 cycles, each comprising 1 min at 94°C, 1 min at the annealing temperatures specified in table 1, and 1 min at 72°C, followed by electrophoresis in acrylamide sequencing gels. HER2 was screened as previously described (Hall and King 1991). Primers and map positions of these 11 markers are shown in table 1. Order and distances between adjacent markers on chromosome 17q were determined by typing CEPH and breast cancer families and by calculating lod scores and distances with the LINKAGE program. Odds in favor of the locus order shown

Table 1

Polymorphisms on Chromosome 17q Linked to Breast and Ovarian Cancer

Locus	Position	θ[a]	Primers or Probe/Enzyme	Annealing Temperature[b] (°C)
D17S250	q12		GGA AGA ATC AAA TAG ACA AT GCT GGC CAT ATA TAT ATT TAA ACC	55
		.02		
Her2	q12-q21		CTG GAA TGG GAA GCA GCC AGC AAA GAA ATC TTA GAC GT	56
		.04		
D17S579 (Mfd188)	q21		AGT CCT GTA GAC AAA ACC TG CAG TTT CAT ACC AAG TTC CT	55
		.01		
D17S78	q21		131A8/*Msp*I	
		.01		
GIP	q21		CAC AAT GGG CTC GAC TTA GCA TAA CTT GCT GGA TCA GAC AAA CCT CTG	62
		.02		
D17S293 (6C1)	q21		ACA GTG CCA GAG ATA TAC CG GCT ATG AGC CTG GCA GAC C	55
		.04		
NM23	q21.3-q22		TTG ACC GGG GTA GAG AAC TC TCT CAG TAC TTC CCG TGA CC	56
		.02		
D17S41	q22		ew102/*Pst*I	
		.07		
D17S74	q22		CMM86/*Hin*fI	
		.07		
GH	q23		TCC AGC CTC GGA GAC AGA AT AGT CCT TTC TCC AGA GCA GGT	56
		.08		
D17S40	q23-q24		ew101/*Msp*I	

[a] Distance between adjacent markers.
[b] For microsatellite repeat polymorphisms.

in table 1 are >1,000:1 for all pairs of adjacent markers except D17S579:D17S78 and D17S78:GIP, for which odds in favor of the specified order are 270:1 and 150:1, respectively.

Three polymorphisms in this analysis, all defined by $(CA)_n$ repeats, have not been previously described. D17S579 (Mfd188) includes 12 alleles of size 111–133 bp. Allele frequencies are .01 (A = 133 bp), .01 (B = 131 bp), .01 (C = 129 bp), .09 (D = 127 bp), .29 (E = 125 bp), .23 (F = 123 bp), .05 (G = 121 bp), .04 (H = 119 bp), .13 (I = 117 bp), .01 (J = 115 bp), .05 (K = 113 bp), and .08 (L = 111 bp). Heterozygosity of D17S579 is 87% in the CEPH grandparents. NM23-H1 has five alleles of size 94–104 bp. Allele frequencies are .26 (A = 104 bp), .04 (B = 102 bp), .33 (C = 100 bp), .17 (D = 98 bp), and .20 (E = 94 bp). D17S293 includes seven alleles of size 115–130 bp, with heterozygosity of approximately 70%. No null alleles (due to polymorphism in the primer sequences) have appeared in our analyses for any of these markers.

Results

Linkage of breast and ovarian cancer to loci in this region for the seven families in which mean age at diagnosis is ⩽45 years and for the 16 families in which mean age at diagnosis is >45 years are shown in figure 1. The maximum lod score for the families with early-onset breast cancer is 9.12 at D17S579, with a 95% confidence interval extending 6 recombination units proximal and 3 recombination units distal to D17S579. For the older-onset families as a group, lod scores are less than −2, throughout the region, on the basis of the cumulative-incidence model. With the age-specific incidence model, no conclusions can be

drawn about the older-onset families as a group. In three older-onset families (14, 17, and 21 of Hall et al. 1990), breast cancer cosegregated with alleles of D17S250, D17S579, NM23, with all intervening markers.

The location of BRCA 1 vis-à-vis other markers can be evaluated by identifying critical recombination events in affected relatives from informative families. Seven linked, recombinant cases from families 1, 3, 5, and 7 suggest that the breast cancer gene lies below D17S250 and above GIP (figs. 2–5). Family 4 is not shown; a recombination event in an affected woman

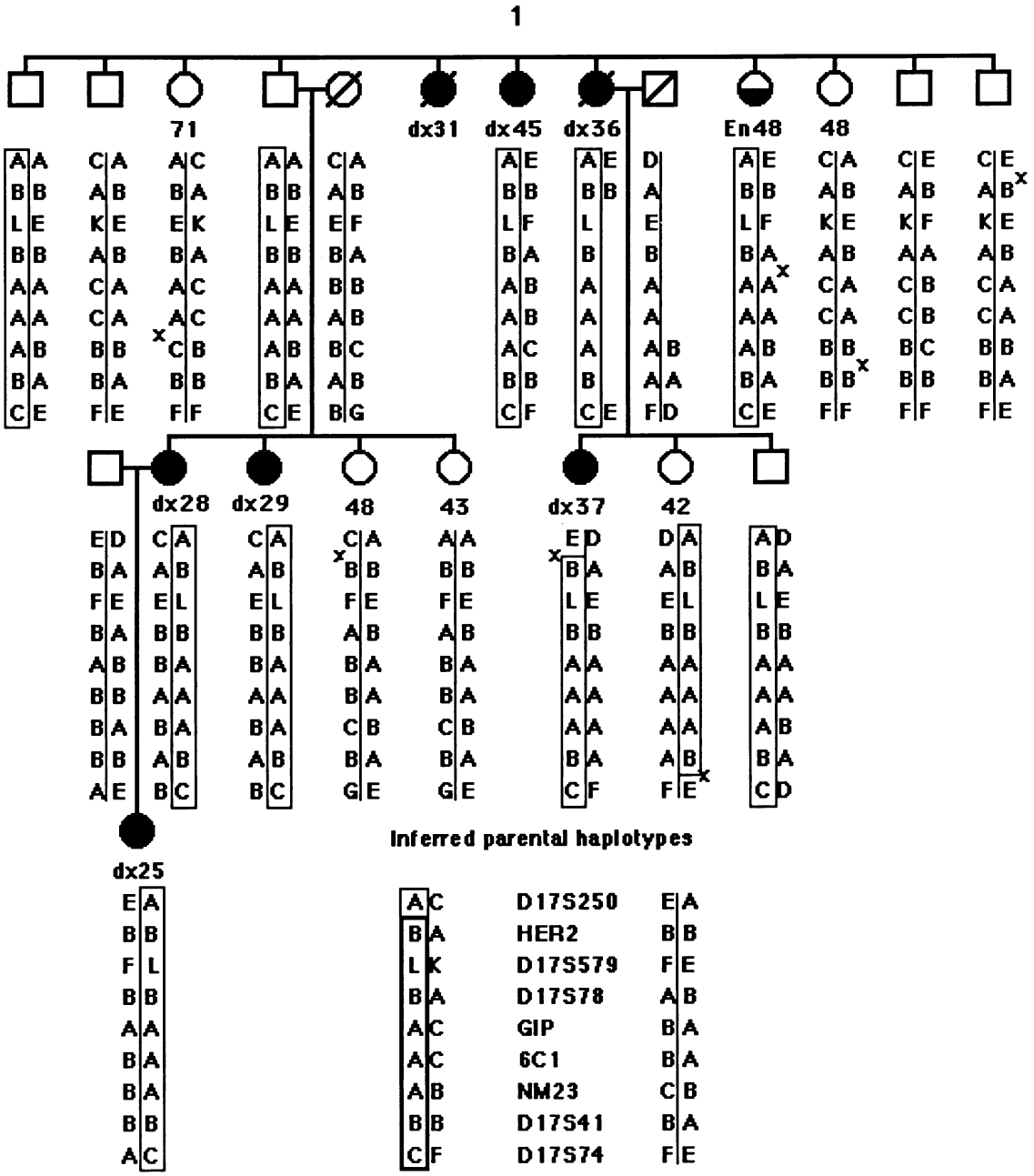

Figure 2 Linkage of breast cancer to chromosome 17q in family 1. Darkened circles represent women with breast cancer. Symbols with diagonal lines indicate deceased individuals whose genotypes were reconstructed from their relatives. Endometrial cancer (En) appears linked to the same region, but the endometrial cancer patient was not included in the calculation of lod scores. Recombination in the patient diagnosed at age 37 years suggests that BRCA 1 lies below D17S250.

in family 4 excludes only D17S40, the most distal marker in the set of 11; family 4 has no recombinants among any of the more proximal markers. No recombination within the 11-marker region appears among either the four affected women from family 2 or the six affected women from family 6 (data not shown).

D17S579 was informative for 79 adult women in families 1–7. Lod scores between breast or ovarian cancer and D17S579 were highest at zero recombination: 1.72, 0.71, 0.64, 1.06, 1.75, 1.16, and 2.08 for families 1–7, respectively. Thus, there is no evidence for linkage heterogeneity among the early-onset families. Of the 79 women informative for D17S579 in the seven families with early-onset breast cancer, 51 carried the allele linked to breast cancer in their families. The risk of breast or ovarian cancer among these women was .50 by age 41 years and .90 by age 50 years.

Of the 28 women in families 1–7 who carried D17S579 alleles *not* linked to breast or ovarian cancer, two women have developed breast cancer. These two patients with apparently sporadic disease are both from family 5 (fig. 5). One patient was diagnosed at age 59 years; her mother died at age 86 years, with no history of cancer. The other patient was diagnosed at age 36 years; her paternal grandmother is still alive and cancer free at age 92 years, and the two paternal aunts with whom she shares her grandmother's 17q genotypes are still cancer free at ages 73 and 68 years. This is reflected in the positive lod score (1.75 at zero recombination) between BRCA 1 and D17S579 in family 5. This lod score was calculated both by assuming that the background lifetime risk of breast cancer is .08 and without any prior assignment of specific sporadic cases. The observed cumulative risk of sporadic breast cancer in all early-onset families was approximately .10 by age 60 years.

Discussion

This analysis of 11 markers linked to breast cancer defines more precisely than was previously possible the region of chromosome 17q which contains BRCA 1. D17S579 is now the most informative marker closely linked to early-onset breast cancer. However, the region of close linkage is still 8 cM long. Hence, additional highly polymorphic markers from chromosome 17q21, particularly microsatellites near D17S579, will be necessary to further delimit the region that resolves all recombinant inherited cases. The critical distance can be refined further by combining

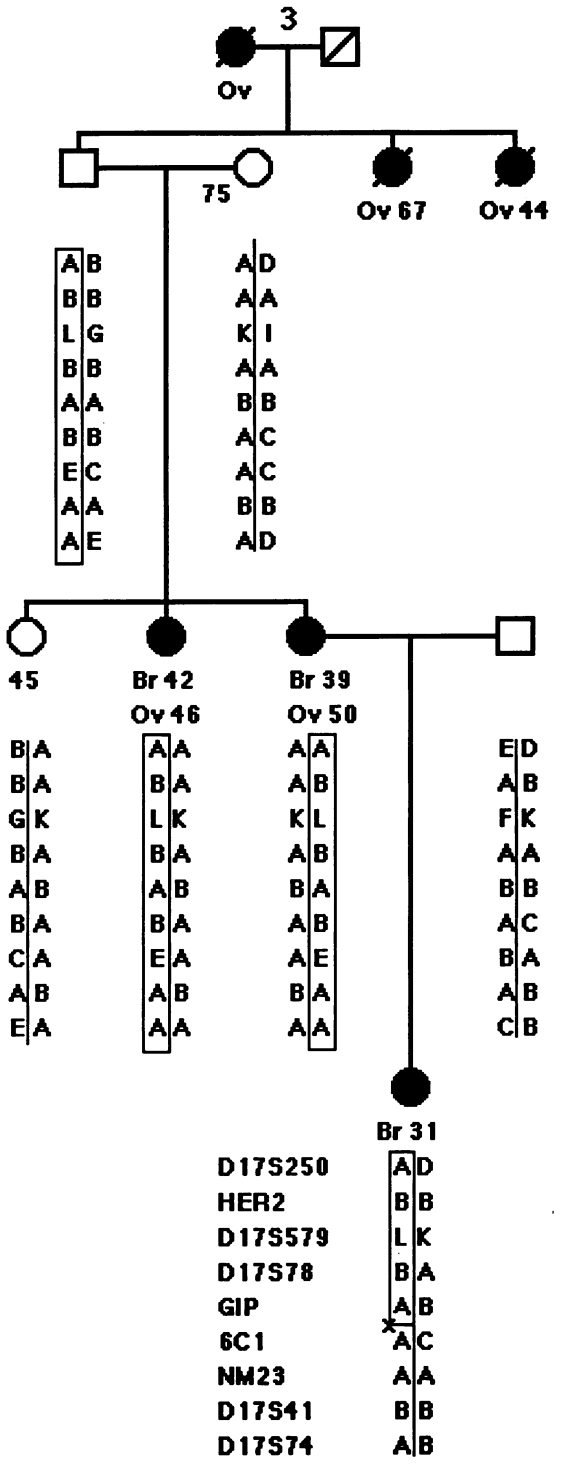

Figure 3 Linkage of breast (Br) and ovarian (Ov) cancer to chromosome 17q in family 3. Recombination in the youngest patient suggests that BRCA 1 lies above D17S293 (which is here termed "6C1").

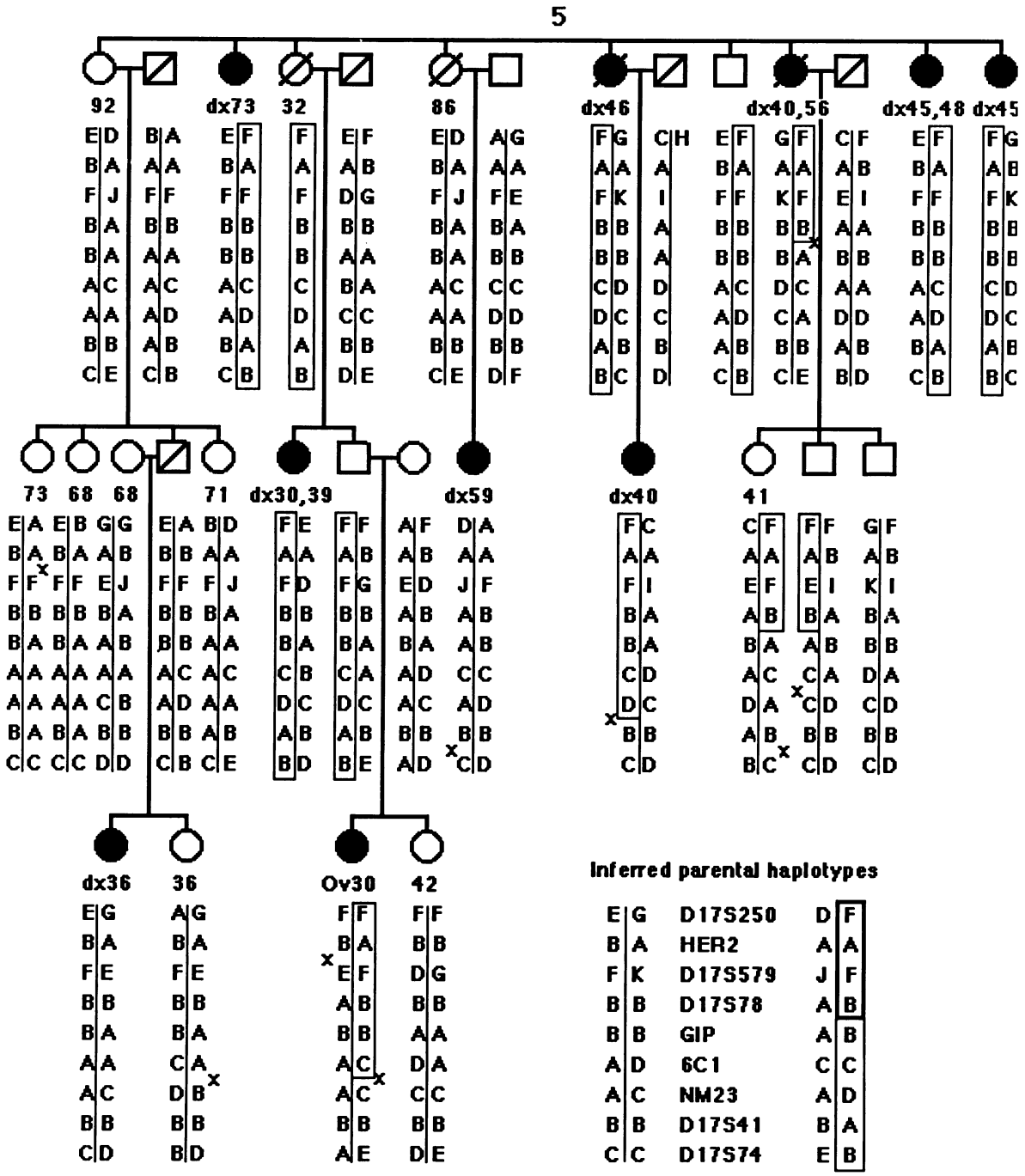

Figure 4 Linkage of breast and ovarian cancer to chromosome 17q in family 5. Two breast cancers, both in women with unaffected elderly female ancestors, appear to be sporadic. Recombination in the ovarian cancer patient (Ov30) suggests that BRCA 1 lies above NM23; recombination in a breast cancer patient in the original sibship suggests that BRCA 1 lies above GIP.

data from these families and data from other families in which breast cancer appears linked to 17q.

Biologically plausible candidate genes at chromosome 17q12-q21 above NM23-H1 and below D17S250 include HER2, RARA, EDBH17. NGFR, HOX2, and WNT3 (see references in Hall et al. 1990). The chromosomal region containing BRCA 1 may now be sufficiently well-defined to encourage screening for polymorphic markers within or very near these candidates. NM23-H1 was a biologically plausible

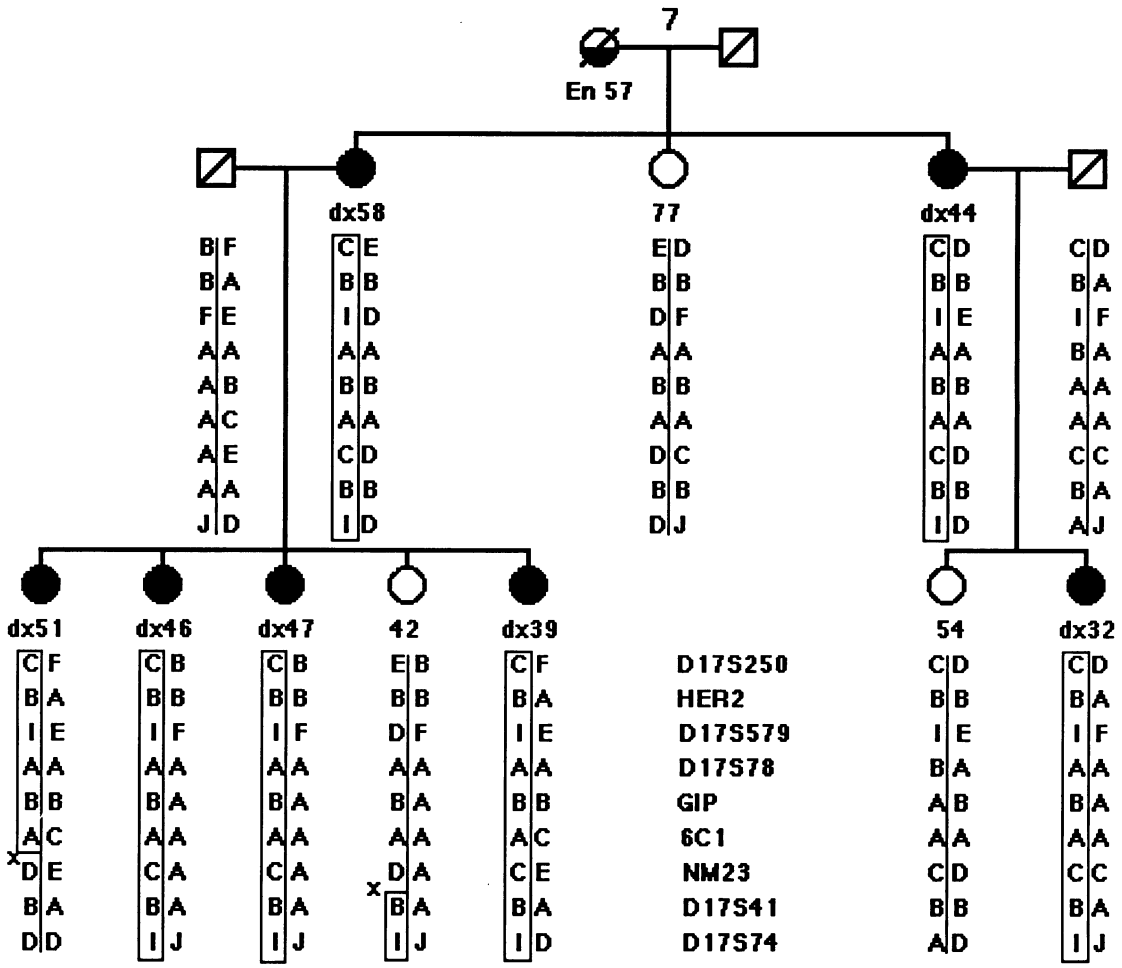

Figure 5 Linkage of breast cancer to chromosome 17q in family 7. Recombination events suggest that BRCA 1 lies above NM23-H1.

candidate (Leone et al. 1991) but appears to be excluded by recombinant, affected individuals in families 3, 5, and 7.

The possible involvement of BRCA 1 in sporadic breast and ovarian cancer has been approached by comparing malignant breast tissue to normal cells of the same patients, in an attempt to determine whether there is loss of alleles in this region. In a large series of primarily postmenopausal breast cancer patients, 31% of patients heterozygous at D17S74 had tumors hemizygous at this locus, a rate significantly above background for this series of patients but somewhat lower than the frequency of loss of heterozygosity at either p53 (37%) or D17S30 (50%) (Sato et al. 1991). In an independent series of 25 breast cancer patients heterozygous for NM23, 16 (64%) of the tumors were hemizygous (Leone et al. 1991). Furthermore, among sporadic ovarian cancers, 65% lost an allele at D17S74 and 80% lost an allele at a more distal marker on chromosome 17q (Foulkes et al. 1991). Loss of heterozygosity at chromosome 17 loci in primary breast tumors was very strongly correlated with HER2 amplification in the same tumors: HER2 was amplified in 20% of tumors hemizygous for loci on chromosome 17, but in only 1 of 67 tumors with no apparent loss of heterozygosity on chromosome 17 (Sato et al. 1991). HER2 amplification was not associated with loss of heterozygosity on other chromosomes. This may suggest that loss of function of BRCA 1 is required for subsequent amplification of HER2. The very high frequencies of allele loss on chromosome 17q in ovarian tumors suggest that loss of function at BRCA 1 may be involved in ovarian cancer. The more variable rates of allele loss on chromosome 17q in breast tumors may reflect simply the lack of information for markers in the right place. It would be useful to have informa-

tion on allelic imbalance in the small region around D17S579 in breast tumors from the general population of patients.

The observed risk of breast cancer among women in the early-onset families carrying alleles linked to susceptibility is consistent with the prediction, based on population data (Newman et al. 1988; Claus et al. 1991), of high penetrance of the breast cancer phenotype among women with inherited susceptibility. However, it is not possible to generalize the risk of breast cancer among gene carriers from these early-onset families to the population as a whole, since these families were ascertained specifically for early-onset disease. In addition, two apparently sporadic cases of breast cancer are to be expected, given that the predicted rate for sporadic disease in families with inherited susceptibility is 8% by age 75 years (Newman et al. 1988; Claus et al. 1991).

Heterogeneity of linkage of breast cancer to chromosome 17q could be due either to the existence of more than one gene for inherited breast cancer or to a high frequency of sporadic cases in older-onset families, or to both. The possibility that there may be additional genes for inherited breast cancer comes from three sources: (1) the negative lod scores for most older-onset families, although this could reflect confounding by sporadic cases; (2) the demonstration of linkage heterogeneity at D17S74 among families with early-onset breast and ovarian cancer (Narod et al. 1991), although some of these recombinants may be resolved by the more proximal markers reported here; and (3) the possible linkage of some postmenopausal breast cancer to the estrogen-receptor gene (Zuppan et al. 1991). Nonetheless, BRCA 1 appears to be responsible for inherited breast cancer in most early-onset families and in some older-onset families. Since sporadic and inherited breast cancer cannot be distinguished on the basis of clinical criteria, the role of BRCA 1, both in families and in sporadic breast and ovarian cancer, will be defined by the identification of the BRCA 1 gene and the mutations leading to disease.

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