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Linkage Analysis of Chromosome 17q Markers and Breast-Ovarian Cancer in Icelandic Families, and Possible Relationship to Prostatic Cancer

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Summary

Seven families, selected for breast cancer segregation, have been analyzed for chromosome 17q12-q23 linkage to breast and ovarian cancer. In two of them, linkage is seen with most markers tested, increasing toward the most proximal region, but without informative recombinations above NM23. In the remaining families, no linkage is observed. Families with 17q linkage are not easily distinguished by clinical characteristics such as early onset (mean age at diagnosis $\leq$45 years) or organs involved. In fact, the family with the highest lod scores ($\geq$2.3) belongs to the "later onset" (>45 years) category of families. Interestingly, prostatic cancer is the most frequent malignancy, after breast cancer, in the families that we studied (13 cases total, all metastasizing) and is especially prevalent in males presumed to carry the trait. Of 16 paternal carriers, 7 (44%) had developed prostatic cancer. Haplotype analysis in families with 17q linkage reveals two further prostatic cases as potential carriers. We propose that breast cancer genes may predispose to prostatic cancer in male carriers.

Introduction

Recently, linkage of breast-ovarian cancer to a gene on chromosome 17q has been reported for a subset of families segregating these traits (Hall et al. 1990; Narod et al. 1991). The gene has been assigned the name "BRCA 1" (Solomon and Ledbetter 1991). To test this linkage and to narrow the chromosomal region in question, we have genotyped members of seven families for six markers mapping to 17q12-q23. As the degree and nature of linkage heterogeneity is of interest, as well as the chromosomal location of BRCA 1, we introduce all the families here with their clinical history (age at diagnosis and organs involved), along with the results of the linkage analysis. This work is a part of the joint analysis of the Breast Cancer Linkage Consortium (Easton et al. 1993).

An incidental observation of prevalent prostatic cancer in the families led us to analyze the relationship of this cancer to breast cancer gene carrier status. Surprisingly, we found that no prostatic cancers were observed in fathers marrying into the family, whereas 9 of 13 cases of prostatic cancer occurred in males considered to carry the trait, either by pedigree analysis (paternal nonfounders) or by haplotype analysis in "linked" families. The four remaining prostatic cases were without progeny informative of carrier status and did not belong to families with 17q linkage. Therefore, nothing can be concluded from their haplotypes in this respect.

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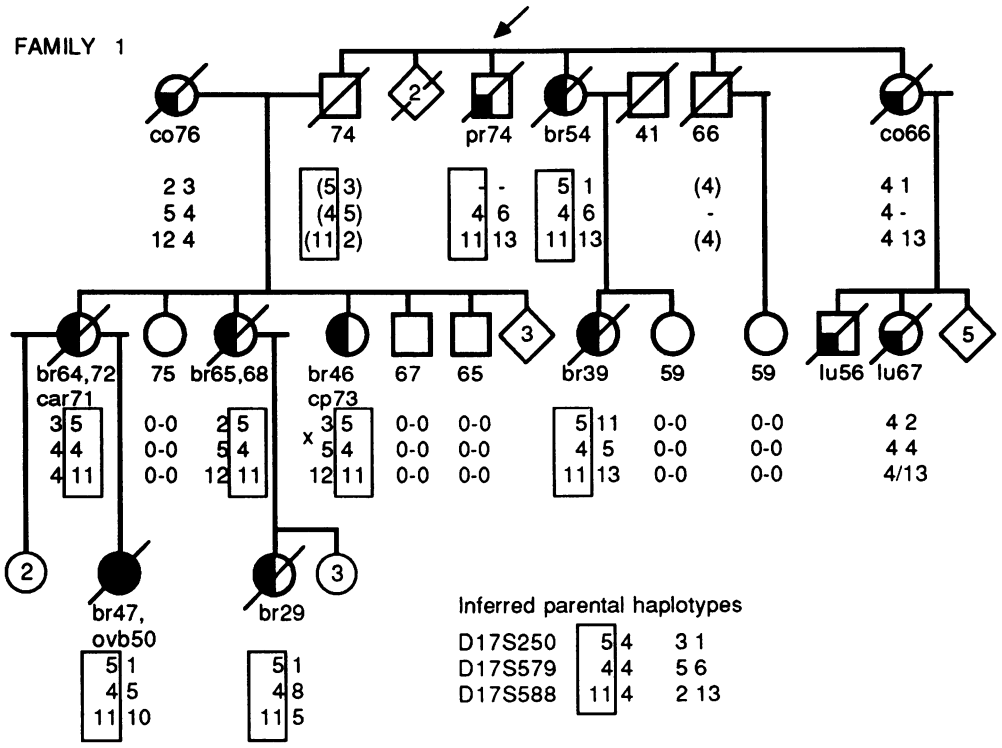
Subjects and Methods

Families

Our analysis is based on seven unrelated Icelandic families, traced from breast cancer–affected probands. In four of these families, ovarian cancer also occurred. Various other types of cancer were observed in all families (figs. 1 and 2) but were disregarded in the linkage analysis. Of 158 members typed, 40 were affected: 1 male and 35 females with breast cancer (7 cases were bilateral), 1 with ovarian cancer, and 3 with both breast and ovarian cancer. Of the last three, two developed bilateral ovarian cancer. The genotypes of an additional five affected females (four with breast cancer and one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74
2
pr74
br54
41
66
co66
2 3
5 4
12 4
(5 3)
(4 5)
(11 2)
- -
4 6
11 13
5 1
4 6
11 13
(4)
-
(4)
4 1
4 -
4 13
br64,72
car71
3 5
4 4
4 11
75
0-0
0-0
0-0
br65,68
2 5
5 4
12 11
br46
cp73
x
3 5
5 4
12 11
67
65
3
0-0
0-0
0-0
0-0
0-0
0-0
br39
5 11
4 5
11 13
59
0-0
0-0
0-0
59
0-0
0-0
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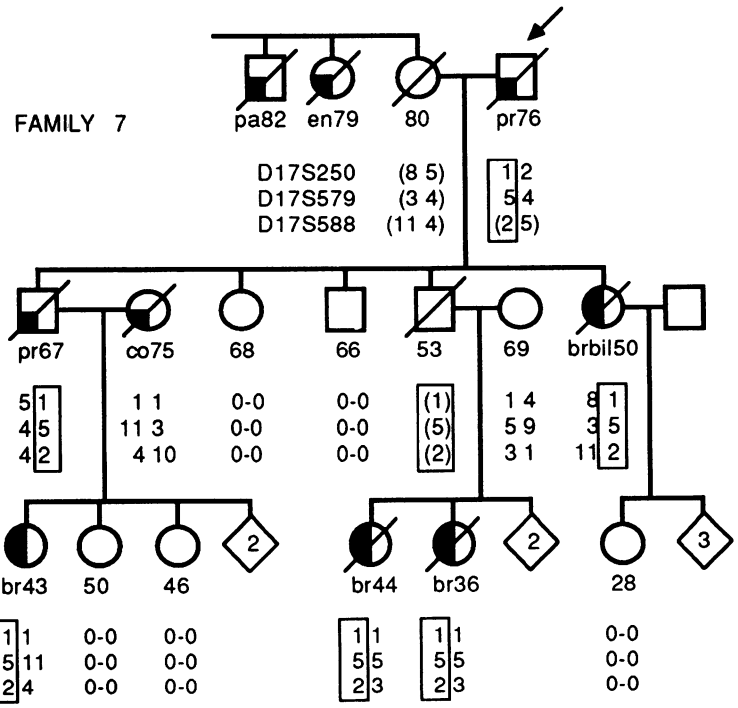
lu56
lu67
5
4 2
4 4
4/13
2
br47,
ovb50
5 1
4 5
11 10
br29
3
5 1
4 8
11 5
Inferred parental haplotypes
D17S250 5 4 3 1
D17S579 4 4 5 6
D17S588 11 4 2 13


FAMILY 7
pa82
en79
80
pr76
D17S250 (8 5)
D17S579 (3 4)
D17S588 (11 4)
1 2
5 4
(2 5)
pr67
co75
68
66
53
69
brbil50
5 1
4 5
4 2
1 1
11 3
4 10
0-0
0-0
0-0
0-0
0-0
0-0
(1)
(5)
(2)
1 4
5 9
3 1
8 1
3 5
11 2
br43
50
46
2
br44
br36
2
28
3
1 1
5 11
2 4
0-0
0-0
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1 1
5 5
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with ovarian cancer) could be at least partially reconstructed from those of typed offspring. The number of affected, average age at diagnosis, and other characteristics for each family are summarized in table 1.

Three of the families (families 4–6) were included in a previous study (Barkardóttir et al. 1989) and, together with families 1–3, were selected for the present study, without emphasis on features reported to be associated with 17q-linkage—namely, early-onset history (Hall et al. 1990) or family history of ovarian cancer (Narod et al. 1991). An additional family (family 7) was identified after we had come across an allele loss at the D17S74 locus in breast tumors from both members of a sister pair (R. B. Barkardóttir, unpublished results) and was therefore traced and selected on a different basis. The retained allele was identical in both these tumors, and this was taken as a possible sign of a linked germ-line mutation in a suppressor gene.

The malignancies of all cases with breast or ovarian cancer were histologically confirmed, as were other cancer types indicated in figures 1 and 2—with three exceptions (belonging to the oldest generations of families 2, 4, and 5), where only death certificates based on clinical examination existed.

DNA Analysis

Genomic DNA was isolated from whole blood by conventional methods (Barkardóttir et al. 1989) and from paraffin-embedded tissue according to the method of Jackson et al. (1990). Families were genotyped for the following five markers on chromosome 17q: D17S250 (Weber et al. 1990), D17S579 (Hall et al. 1992), D17S588 (Appendix in Easton et al. 1993), NM23 clone H1 (Hall et al. 1992), and D17S74 (Nakamura et al. 1988). In addition, the marker GH (Polymeropoulos et al. 1991) was screened in family 1 only. For typing the marker D17S74, *Hin*fI-digested genomic DNA was hybridized by methods described elsewhere (Barkardóttir et al. 1989), except that whole plasmid (pCMM86; ICRF Human Genetic Resources) was labeled using a multiprime DNA labeling kit (Amersham International). For typing all other markers, PCR amplification was carried out in 25- or 50-μl reaction volumes and was scored according to the method of D. Kelsell (personal communication) by EtBr staining of nondenaturing 12% acrylamide gels. A 25-μl reaction volume typically contained 75–150 ng genomic DNA, 75 ng each primer, 1.5 mM $MgCl_2$, 50 μM each dNTP, 50 mM KCl, 10 mM Tris pH 8.3, and 0.6 units *Taq* polymerase (Amersham International). The samples were amplified in 30–40 cycles for time intervals of 0.5, 0.5, and 1 minute at 94°C, 55°C, and 72°C, respectively.

Linkage Analysis

Two point lod scores were calculated by the method of Easton et al. (1993).

Results

Two-point lod scores for BRCA 1 and each of the chromosome 17q markers are presented in table 2. Pedigrees with clinical information are shown in figures 1 and 2, along with haplotype information in the former. For two families (families 1 and 7) compatible with linkage, the two-point lod scores increased toward the most proximal region but failed to identify the closest marker, because of the lack of informative recombinants for the four most proximal markers (fig. 1; and results not shown). Family 6, a very large one, shows interesting features. Although the family gives negative lod scores, close examination of haplotypes (not shown) shows some evidence of linkage in a central, large family branch (fig. 2) with one male breast cancer and seven female breast cancers. It remains unclear

Figure 1 Pedigrees of families 1 and 7, and chromosome 17q haplotypes for the three most proximal markers studied. Genotypes 0–0 denote typed unaffected members (who, for ethical reasons, are not shown). Boxes outlining either haplotypes or alleles within recombined haplotypes denote chromosomes compatible with transmission of the trait. Reconstructed genotypes are shown within parentheses. A minus sign (−) denotes that allele type is unknown. × denotes the position of a recombination event. Squares denote males; circles denote females; and diamonds denote that sex was not specified. The number within a symbol denotes the number of siblings. A diagonal slash through a symbol denotes the individual is deceased. The number immediately below a symbol denotes the age (in years) at diagnosis, death, or last observation. Arrows point to prostatic cases, whose carrier status is not predictable on the basis of pedigree structure. ● = breast and ovarian cancer; ◐ and ◧ = breast cancer only; ◑ = ovarian cancer only; and ◒ and ◨ = other malignancies. ad = adrenocortical adenocarcinoma; bl = urinary bladder cancer; br = breast cancer; brbil = bilateral breast cancer; car = carcinoid malignant colon cancer; cx = cervical invasive cancer; cxs = cervical cancer in situ; co = colon cancer; cp = choledocho-pancreatic duct cancer; en = endometrial cancer; es = esophageal cancer; ga = gall bladder cancer; gen = female genital cancer (unknown); ho = Hodgkins disease; le = leukemia; lu = lung cancer; me = mesothelioma; ov = ovarian cancer; ovb = bilateral ovarian cancer; pa = pancreatic cancer; pr = prostatic cancer; re = renal cancer; st = stomach cancer; te = testis teratocarcinoma; th = thyroid cancer; and un = carcinoma of unknown origin.

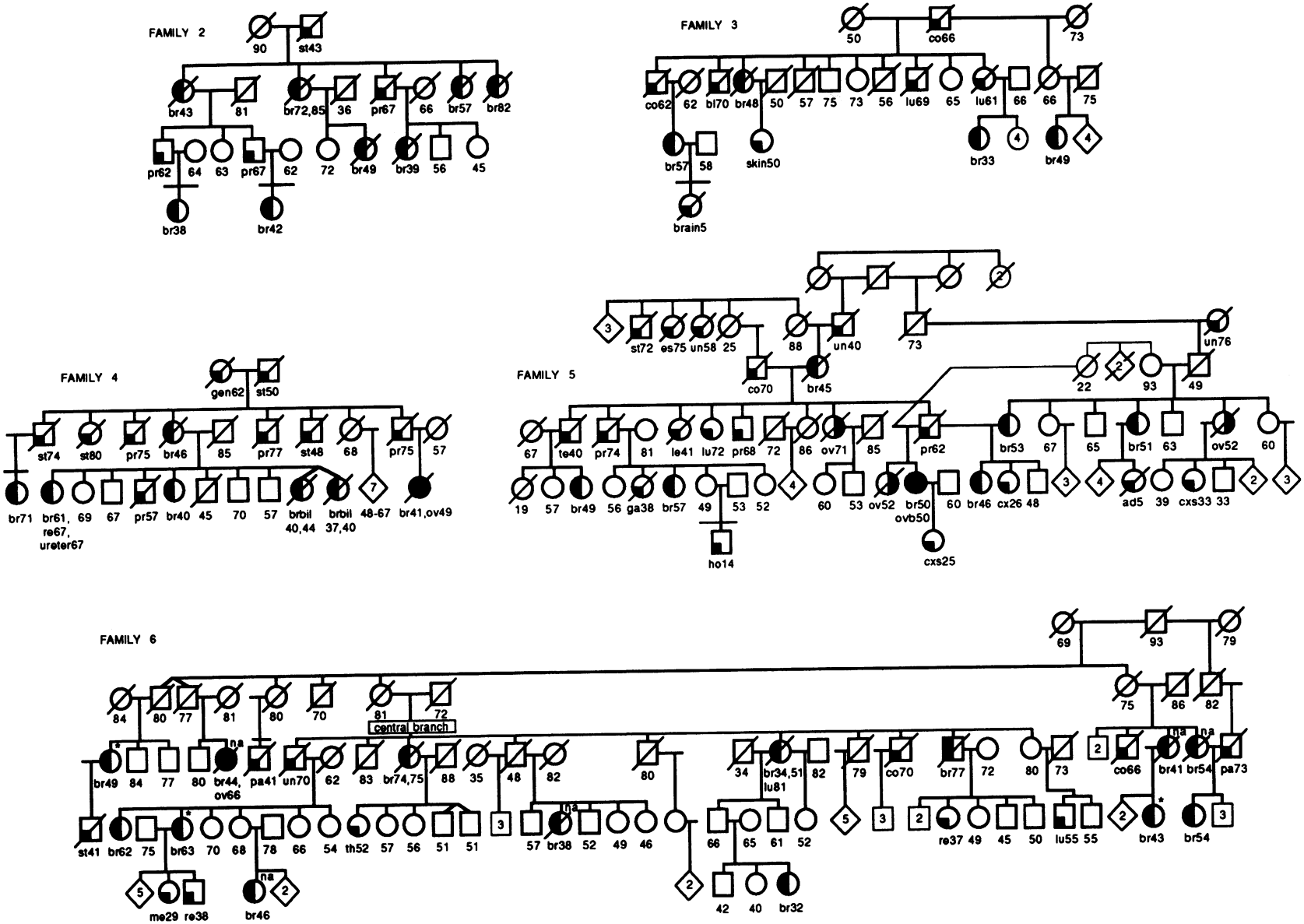

Figure 2 Pedigrees of families 2–6. Asterisks denote cases in family 6 who are discussed, in the text, as being possibly sporadic. na = case not typed. Other abbreviations and symbols are as in fig. 1.

Table 1

Summary of the Main Clinical Characteristics of the Families

Family	No. of Affected Individuals	Tumor Type(s)	Average Age at Diagnosis (range) (years)
1	7	Breast cancer, ovarian cancer	49 (29–65)
2	8	Breast cancer	53 (38–82)
3	4	Breast cancer	47 (33–57)
4	6	Breast cancer, ovarian cancer	48 (37–71)
5	10	Breast cancer, ovarian cancer	53 (45–71)
6	14	Male breast cancer and breast cancer, ovarian cancer	63 (38–74)
7	4	Breast cancer	43 (36–50)

whether this family is linked; if linked, two relatively "early" sporadic cases (diagnosed at ages 43 and 49 years) occur in distant branches. Linkage is also unclear in family 4, which appears positive at D17S588 and D17S250 but negative at D17S579, the marker between (table 2). Both the positive markers were homozygous in an affected mother of four cases and therefore were not informative; on the other hand, D17S579 provides full information, and haplotyping based on this marker and D17S74 (not shown) suggests sporadic cases/recombinations among her children if linkage is present. We feel that more information is needed on this family, for any final conclusion regarding linkage. The remaining families (families 2, 3, and 5) provide no evidence for linkage.

We also made the following observation: in our families, prostatic cancer (13 cases total) was the most frequent cancer type after breast cancer and always behaved as a metastasizing disease. It occurred in five of our families but, notably, not in the largest family (family 6, with over 100 members). Where observed, it occurred only in oldest-generation male founders or their descendants—never in males married into the family (figs. 1 and 2). In order to evaluate the relationship of breast cancer segregation to this finding, we identified as a subset of males those presumed by pedigree structure to carry a breast cancer gene (i.e., fathers of affected daughters, excluding founders). Of a total of 16 males in this subset, 7 (44%) had developed prostatic cancer. Six remaining prostatic cases had unknown carrier status by the above definition. However, two of those belonged to families (families 1 and 7) of the 17q-linked type and were shown by our genetic analysis to cosegregate the haplotype in question (fig. 1). Therefore, altogether 9 of the 13 prostatic cases were believed to carry a breast cancer–predisposing gene, and the 4 remaining prostatic cases had an unpredictable carrier status.

Discussion

In agreement with previous reports on chromosome 17q linkage to familial breast or breast-ovarian cancer (Hall et al. 1990; Narod et al. 1991), we observe linkage heterogeneity in the families in our study. Families 1 and 7 are compatible with linkage to the D17S250–D17S579–D17S588 region, recently indicated as the region containing BRCA 1 (Hall et al. 1992; Easton et al. 1993). In family 1, a perfect match is obtained between alleles of all markers proximal to D17S74 and the affected state of seven women, whereas five women unaffected at ages 59–75 years did not segregate these alleles (fig. 1). This is reflected in noticeably high lod scores for BRCA 1 and the markers D17S250 and D17S588 (table 2). The marker (D17S579) between these two is less informative for two-point analyses in this family, because of identical parental genotypes in the oldest generation, and therefore contributes lower values.

Family 6 (fig. 2) is complicated by its size and branching. Sporadic cases are more likely to be found in such a large family, and, because the branches descend from unaffected family members, it is quite possible that different predisposing genes enter the family via married-in spouses. In the central branch, a haplotype was found in four of five typed female cases (two additional cases were untyped) and, noticeably, also in the male breast cancer case. The exception was a female diagnosed at age 63 years and therefore not unlikely to be a sporadic case. Among the four female cases with the haplotype were a grandmother-granddaughter pair. The evidence for linkage to this haplotype is further strengthened by its occurrence in a 54-year-old case

Table 2

Two-Point Lod Scores for BRCA 1 and Five Chromosome 17 Markers

Markers and Family	Lod Score at Female Recombination Fraction of					
	.001	.01	.05	.10	.20	.30
D17S250:						
1	2.34	2.31	2.18	2.00	1.62	1.18
2	−.65	−.57	−.31	−.09	.13	.23
3	−.13	−.12	−.10	−.07	−.03	−.00
4	.16	.16	.15	.13	.10	.06
5	−.42	−.39	−.32	−.24	−.12	−.05
6	−.90	−.73	−.34	−.12	.06	.11
7	.57	.56	.53	.49	.40	.28
Total	.97	1.23[a]	1.80[a]	2.10	2.15[a]	1.80[a]
D17S579:						
1	.43	.42	.38	.34	.24	.15
2	−.96	−.87	−.56	−.30	−.01	.14
3	−.19	−.18	−.17	−.15	−.10	−.06
4	−.35	−.30	−.14	−.04	.05	.06
5	−.48	−.45	−.35	−.25	−.12	−.05
6	−.27	−.25	−.19	−.15	−.12	−.11
7	.26	.26	.24	.22	.16	.11
Total	−.156	−1.37	−.79	−.33	.10	.23[a]
D17S588:						
1	2.05	2.02	1.91	1.75	1.42	1.04
2	−.40	−.37	−.26	−.17	−.07	−.02
3	.15	.15	.13	.12	.08	.05
4	.38	.37	.32	.27	.17	.09
5	−.74	−.60	−.30	−.14	−.02	.01
6	−.49	−.47	−.37	−.26	−.07	.05
7	.57	.56	.53	.49	.40	.28
Total	1.51[a]	1.66	1.96	2.06	1.91	1.51[a]
NM23[b]	.68	.76	.96	1.09	1.13	.99
D17S74[c]	−3.21	−2.86	−1.79	−.96	−.00	.42

NOTE.—For the sake of brevity we show lod scores only according to recombination fractions for the female map, assuming female distance to be twice the male distance. For the three most proximal markers, results are shown by family; for the other two markers, results are shown only as total lod scores. GH is not included, because only one family was typed for this marker.

[a] Because of rounding errors, entries do not exactly sum to the total.

[b] Not typed in families 5 and 6.

[c] Not typed in family 7.

remote to the central branch, but its absence in two additional "remote" cases (females with breast cancer at ages 43 and 49 years) obviously argues against linkage. Taken together, these results do not provide a final answer to the question of linkage in this family.

No clinical characteristics are identified which parallel the 17q linkage results. Only one of the two families with positive linkage exhibits the reported characteristic of early average age at onset (≤45 years; Hall et al. 1990) (see table 1). Ovarian cancer is seen in the families independently of linkage results.

In our analysis, prostatic cancer is frequent in males presumed, on the basis of pedigree structure, to carry a breast cancer gene (i.e., nonfounders with affected daughters), and this is irrespective of 17q linkage. As the breast cancer phenotype very rarely penetrates in males, those in the parental generation and those without affected daughters cannot be assigned a carrier status on the basis of pedigree structure alone. It is therefore difficult to measure the association between breast cancer genes and prostatic cancer in these males, unless they are shown by genetic analysis to cosegregate breast cancer–linked haplotypes. As regards 17q linkage, this is seen to have happened in fact in two instances in our study—namely, one male in each of families 1 and 7 (fig. 1). These two, as well as one already defined as a carrier on the basis of pedigree structure in family 7, are the only prostatic cases in these two families. We thus propose that genes predisposing to breast or breast-ovarian cancers also carry an increased risk of prostatic cancer in males. This is considered not to be a feature of BRCA 1 alone, as prostatic cancer is prevalent in at least two families without 17q linkage (families 2 and 5; see fig. 2). Recently, it has been found that in Iceland the risk of prostatic cancer in relatives of breast cancer patients is significantly increased (Tulinius et al. 1992). Similar indications were reported by Thiessen (1974). A more detailed discussion on this matter would exceed the scope of the present paper, but we suggest that this be examined further in future studies of familial breast and breast-ovarian cancer.

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