

## ORIGINAL INVESTIGATION

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## Linkage analysis of 26 Canadian breast and breast-ovarian cancer families

Received: 15 September 1993 / Revised: 15 September 1994

**Abstract** We have examined 26 Canadian families with hereditary breast or ovarian cancer for linkage to markers flanking the BRCA1 gene on chromosome 17q12–q21. Of the 15 families that contain cases of ovarian cancer, 94% were estimated to be linked to BRCA1. In contrast, there was no overall evidence of linkage in the group of 10 families with breast cancer without ovarian cancer. A

genetic recombinant in a breast-ovarian cancer family indicates a placement of BRCA1 telomeric to D17S776, and helps to define the region of assignment of the cancer susceptibility gene. Other cancers of interest that appeared in the BRCA1-linked families included primary peritoneal cancer, cancer of the fallopian tube, and malignant melanoma.

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### Introduction

The majority of families with hereditary breast and ovarian cancer are linked to a locus on chromosome 17, BRCA1 (Hall et al. 1990; Narod et al. 1991, 1995; Easton et al. 1993). BRCA1 has been assigned to a 1-cM interval on chromosome 17q12–q21, flanked by the markers EDH17B2 and D17S78 (Tonin et al. 1994; Simard et al. 1993). In the report of the Breast Cancer Linkage Consortium, it was estimated that 45% of breast cancer families without ovarian cancer were also linked to BRCA1 (Easton et al. 1993). We report linkage results for 26 Canadian families, which were not included in the Consortium analysis. These families have been studied to improve the localization of BRCA1 and to better estimate the proportion of linked families in the two subgroups.

### Materials and methods

The 26 Caucasian breast cancer families were ascertained through the genetics clinics of McGill University, the University of British Columbia, and through other participating Canadian physicians. A total of 133 affected women have been identified in the families, including 109 cases of breast cancer (mean age of diagnosis 45.7 years) and 33 cases of ovarian cancer (mean age of diagnosis 50.0 years). All families contained at least three cases of breast or ovarian cancer. Ten families contained only breast cancer, one family contained only ovarian cancer, and 15 families contained cancers of both types. Two families (family 101 and family 102) were included in the report of Simard et al. (1993) and 10 of the breast-ovary families have been submitted to the second Consortium linkage analysis (Narod et al. 1995). The other 16 families have not been reported elsewhere. Families were typed with five highly

polymorphic CA-repeat probes. From centromere to telomere these include: D17S250, THRA1, D17S855, D17S579, D17S588 (described in Easton et al. 1993). BRCA1 is located between THRA1 and D17S579, and is near the D17S855 locus (Easton et al. 1993; Simard et al. 1993; Tonin et al. 1994). The relative position of BRCA1 and D17S855 is not yet known. Family 101 was also typed with D17S800 and D17S776 to better characterize the informative genetic recombinant. Lod scores were calculated with the LINKAGE program (Lathrop and Lalouel 1984) using the FASTLINK modifications (Cottingham et al. 1993). Genetic homogeneity of linkage was tested with the HOMOG program (Ott 1985). The genetic model used for hereditary breast and ovarian cancer is described in Feunteun et al. (1993). Cancers at sites other than breast or ovary were not used to define the phenotype. All CA-repeat loci were analyzed as systems of eight equally frequent alleles.

## Results

Descriptions of the families, and multipoint lod scores calculated midway between the two flanking markers THRA1 and D17S579 appear in Table 1. It is estimated that 94% (95% CI 41%–100%) of the 16 breast-ovarian and ovarian cancer families were linked to BRCA1 (HOMOG test). An example of a linked breast-ovarian cancer family is presented in Fig. 1. In contrast, it was estimated that 0% (95% CI 0%–51%) of the 10 breast cancer specific families were estimated to be linked to BRCA1. These two linked proportions were significantly different ( $P < 0.01$ ).

Family 101 showed recombination to D17S250 and THRA1, but not to D17S855, D17S579, or D17S588 (Fig. 2). To further characterize this recombinant, D17S800 and D17S776, which map between THRA1 and D17S855 (Weissenbach et al. 1992), were also tested on this family. The haplotype analysis in this family places the crossover event below D17S800, but there is no evidence for recombination with D17S776. The lod score for the family to D17S579 was 0.75. Assuming that 92% of all breast-ovarian families are linked to BRCA1 (Narod et al. 1995), the probability that this family is linked to BRCA1 is 98%. Under our model, the probability that 37-year-old women with breast cancer in this family is a gene carrier is greater than 99%.

Three breast-ovarian families gave negative lod scores to the region. In family 133 (lod = -0.33) three sisters, who of whom are affected, share a common chromosome 17q haplotype (Fig. 3). The fourth sister, with breast cancer diagnosed at age 63, has a discordant genotype and may represent a sporadic case or the family may be unlinked to BRCA1. Family 254 (lod = -1.02) has two women with breast cancer and two with ovarian cancer. The two ovarian cancer cases (ages 46 and 47) share no chromosome 17 haplotype. Family 121 (lod = -0.02) was essentially uninformative with these markers.

Although there was no overall evidence for linkage in the families with breast cancer without ovarian cancer, a few families of this type gave positive multipoint lod scores and were consistent with linkage to the BRCA1 markers (Table 1). Several of the breast cancer families that showed segregation of markers consistent with link-

**Table 1** Multipoint lod scores between the chromosome 17q markers and the breast and breast-ovarian cancer traits

Breast-ovary families ( $n = 15$ )			
Family	No. of cases of breast cancer	No. of cases of ovarian cancer	Lod scores <sup>a</sup>
71	6	1	2.13
101	2	2	0.12
102	5	1	0.20
121	2	1	-0.02
133	11	2	-0.33
178	4	2	0.31
183	4	1	0.45
185	1	4	0.82
211	2	1	0.25
213	3	1	0.01
218	3	1	0.44
235	4	2	0.12
254	2	2	-1.02
255	6	6	1.45
270	4	3	0.36
Total	59	30	5.29
Breast cancer families ( $n = 10$ )			
107	7	0	-1.54
130	3	0	-0.97
151	5	0	-1.22
174	8	0	-0.48
176	5	0	-1.83
182	6	0	0.25
186	3	0	0.06
219	3	0	-0.96
279	4	0	0.52
372	6	0	-0.68
Total	50	0	-6.85
Ovarian cancer families ( $n = 1$ )			
240	0	3	0.19

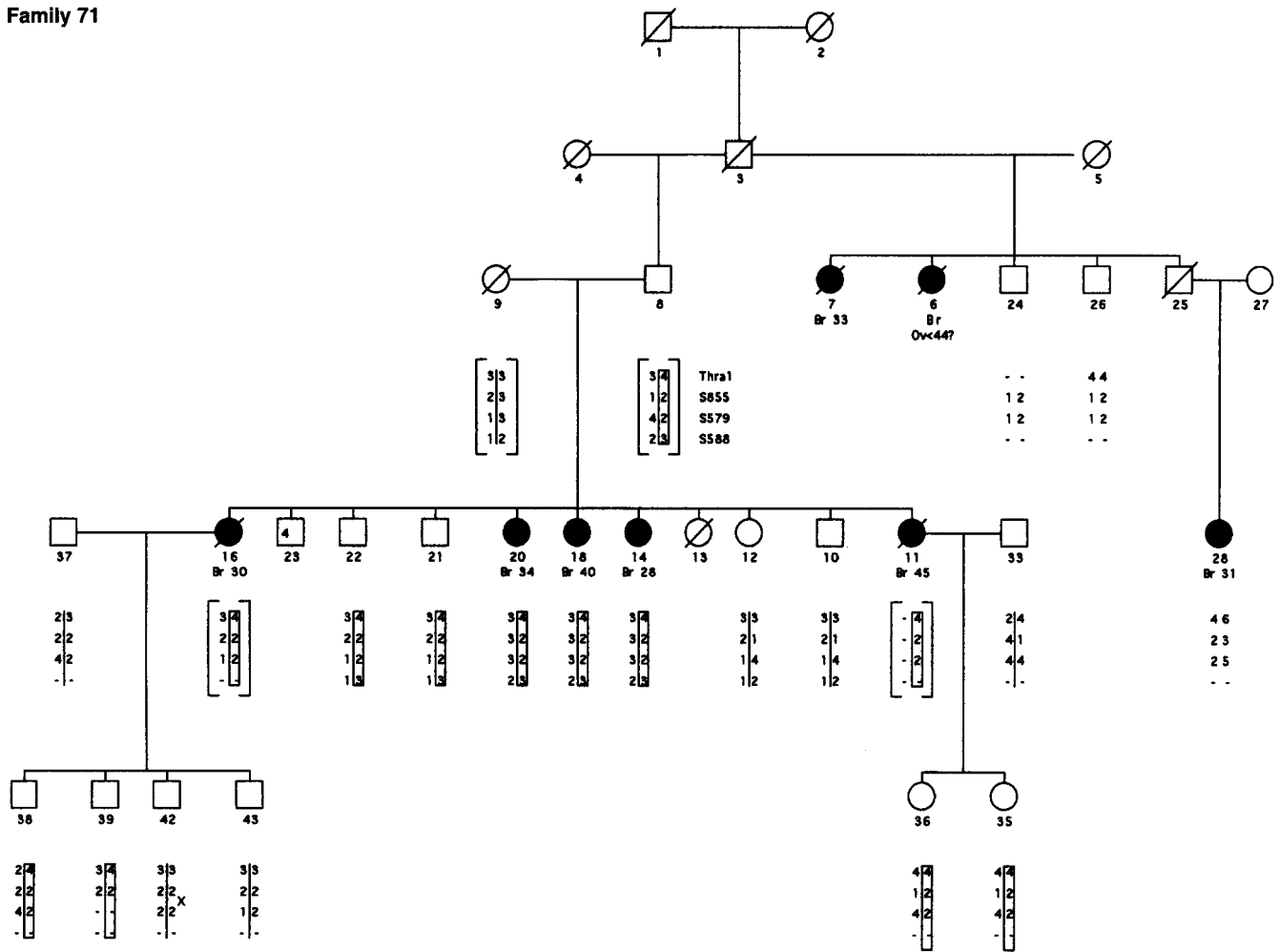
<sup>a</sup>Lod scores are computed at a point midway between the interval THRA1–D17S579, assuming a genetic distance (males) of 2 cM and a 2:1 female:male recombination distance

age to BRCA1 included cancers of other types, including one case of primary cancer of the peritoneum, and one case of fallopian tube cancer (Fig. 4). Family 240 contained three cases of ovarian cancer and two early onset melanomas (Fig. 5).

## Discussion

The estimated proportion of linked families with breast cancer, but without ovarian cancer, in our study (0%) is lower than that reported by the consortium study of 157 breast cancer families (45%), but the confidence limits of both estimates are wide. Our sample size is small, and the observed difference may be due to chance or may reflect

Family 71



**Fig. 1** Pedigree of family 71. *Ov* ovarian cancer, *Br* breast cancer. The numbers following these abbreviations indicate age at diagnosis. *Diagonal slash* indicates deceased. The numbers arranged vertically below the individual symbols indicate the marker alleles arranged into haplotypes. Haplotypes in brackets are inferred. The haplotype enclosed in the vertical rectangle is that believed to be segregating with the BRCA1 mutation. Marker alleles separated by a comma cannot be phased. A dash in the place of a marker typing indicates missing information. *x* indicates position of genetic recombination

different distribution of cancer susceptibility genes in Canada and elsewhere. Some of our families are relatively small, and the breast cancers may be due to susceptibility genes with lower penetrance than BRCA1. Our experience of the absence of linkage in families with breast cancer alone is similar to the findings of Lindblom et al. (1993) in Sweden, Mazoyer et al. (1993) in France, and Smith et al. (1993) in Great Britain. These studies, together with our results, suggest that the proportion of linked families with only breast cancer is less than 45%. Family 183 (lod = 0.45) was initially thought to be a linked site-specific breast cancer family (four cases of breast cancer). However, in 1993 one of the women who had been previously diagnosed with breast cancer was diagnosed with ovarian cancer as well. This case illus-

trates that the risk of ovarian cancer may be increased in all 17q-linked breast cancer families. Among the linked breast-ovary families, family 102 (lod = 0.68 with D17S855) is unusual because the mean age of diagnosis of the five breast cancer cases was 64 years (range 44–80 years). This is much older than the average of the other 104 hereditary breast cancer cases in our series (44.7 years).

Our estimated proportion of linked breast-ovarian families (94%) is lower than the first consortium estimate of 100%, but is consistent with more recent Consortium data (Narod et al. 1995). In the second Consortium analysis it was estimated that 88% of 132 breast-ovary families (without cases of male breast cancer) were linked to BRCA1. Two of our 15 breast-ovary cancer families appear not to be linked to BRCA1. It is important to improve the precision of the linked estimate of breast-ovarian families if genetic counseling with DNA markers is to be offered.

Because of genetic heterogeneity and because of the possibility of sporadic cases of breast or ovarian cancer, no single genetic recombinant is definitive for the mapping of BRCA1. Tonin et al. (1994) report a recombinant in a 45-year-old woman that places BRCA1 distal to EDG17B2; Goldgar et al. (1994) report a recombinant

Family 101

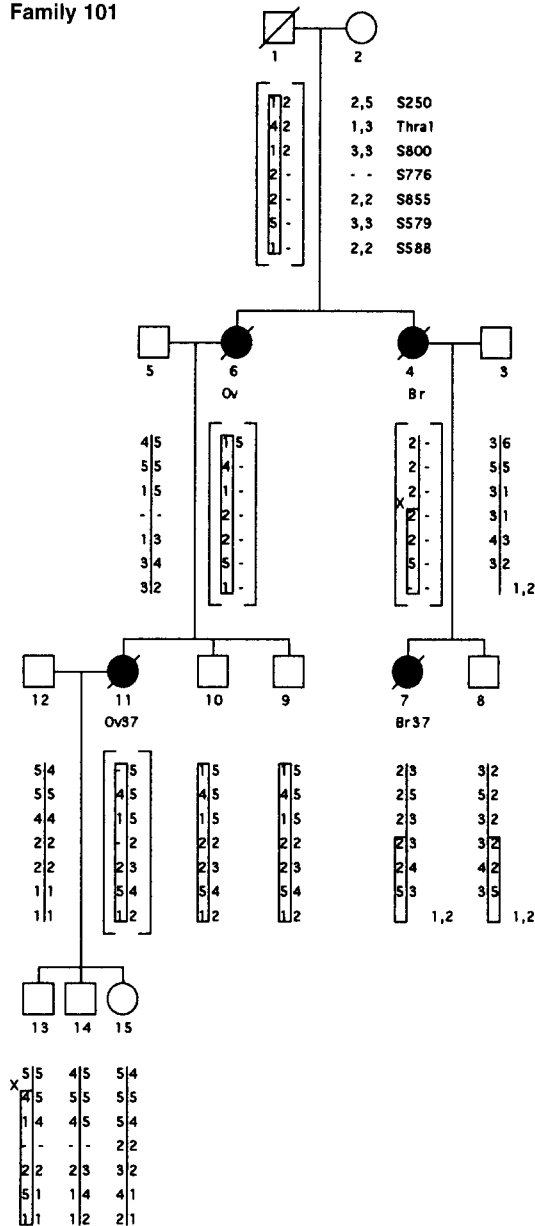
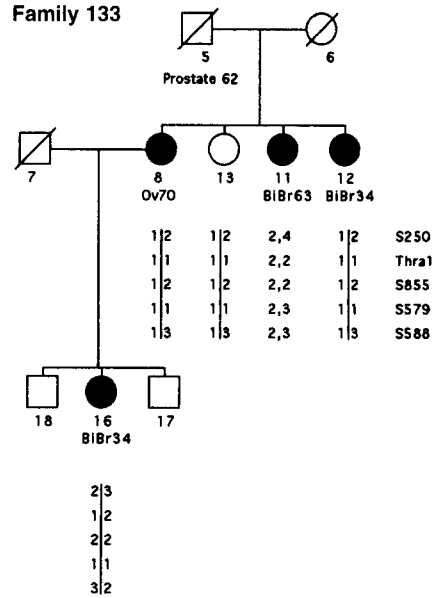


Fig.2 Pedigree of family 101. Symbols as in Fig. 1

that places BRCA1 distal to D17S776. Our recombinant in family 101 strenghtens the assignment of BRCA1 to a position distal to D17S800.

Other cancers observed in these families, and which may also be due to BRCA1 mutations, included cancers of the peritoneum, fallopian tube, and melanoma. Primary cancer of the peritoneum has also been observed in women in high risk families following prophylactic removal of the ovaries (Tobacman et al. 1982). The fallopian tube is a rare site of cancer, and the histology of these tumors is similar to that of serous ovarian tumours (the most common hereditary form; Hamilton 1992). It is important to establish if these rare tumour types are to be considered as phenotypic variants of ovarian cancers, because of the implications for genetic counseling using

Family 133



Family 254

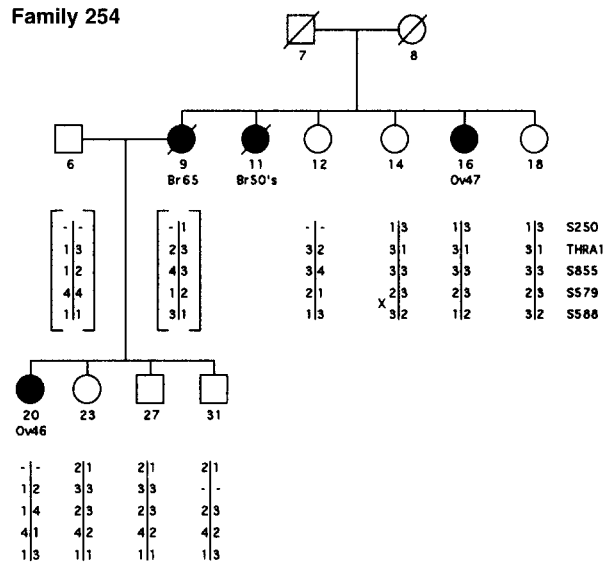


Fig.3 Examples of unlinked breast-ovary families (families 133 and 254). Symbols as in Fig. 1. BiBr bilateral breast cancer

BRCA1 markers. For example, if family 279 were to be considered a site-specific breast family, then the probability of linkage to BRCA1 would be estimated to be only 77% (assuming a prior probability of linkage of 45%). If it were to be considered a breast-ovary family, then the probability of linkage would be 93%, and predictive testing could be considered.

It has not been established if the incidence of melanoma is increased in BRCA1 carriers (Ford et al. 1994), but family 240 represents an interesting example of familial aggregation of ovarian cancer and malignant melanoma in young women. Cancer susceptibility in this small family is consistent with a single BRCA1 mutation.

In conclusion, it appears that the proportion of site-specific breast cancer families that show linkage to BRCA1

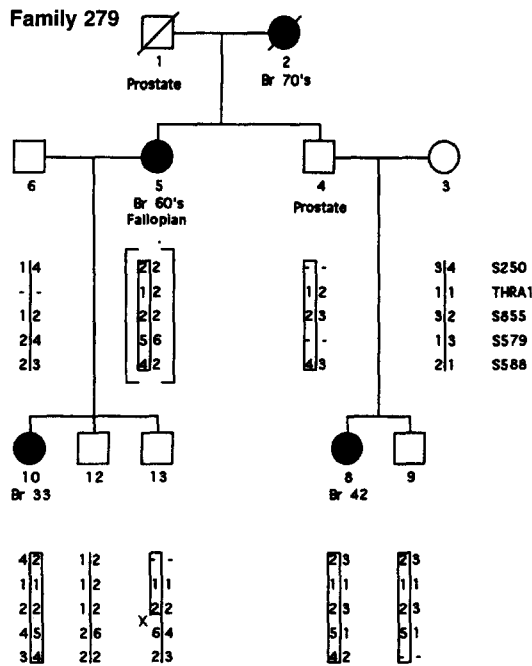


Fig. 4 Pedigree of family 279. Symbols as in Fig. 1. Falloplan indicates fallopian tube carcinoma

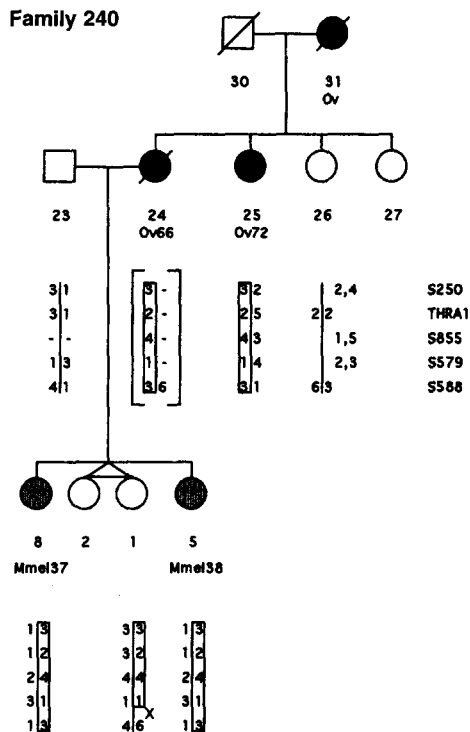


Fig. 5 Pedigree of family 240. Symbols as in Fig. 1. Grey circles indicate malignant melanoma

in Canada is small, and it is unlikely that these markers will be useful for presymptomatic testing in these families. In contrast, we have begun DNA-based genetic counseling for several of the breast-ovarian cancer families described here.

Table 2 Lod scores for five chromosome-17 probes

Marker families	Breast-ovary families (n = 16) <sup>b</sup>		Breast-cancer (n = 10)	
	Ø <sup>a</sup>	Lod	Ø	Lod
D17S250	0.06	1.06	0.50	0.00
THRA1	0.07	1.45	0.50	0.00
D17S855	0.01	5.14	0.41	0.02
D17S579	0.01	5.42	0.37	0.03
D17S588	0.00	4.00	0.35	0.03

<sup>a</sup> Theta values (Ø) are recombination fractions estimated at the maximum lod score. A 2 : 1 female : male recombination ratio is assumed

<sup>b</sup> The single site-specific ovarian cancer family (family 240) is included with the breast-ovarian families

**Acknowledgements** This work was supported by grants from the National Cancer Institute of Canada Breast Cancer Initiative, the Canadian Genetic Diseases Network and Endorecherche. P.T. is a fellow of the Cedars Cancer Institute of the Royal Victoria Hospital. J.T. is a scholar of the Medical Research Council of Canada. S.A.N. is supported by the Fonds de La Recherche en Sante due Quebec. We thank Ophira Ginsburg, Shari Miller, and Linda Bradley for help with the family studies, and France Dion, Anne Vivier, Marius Vienozinskis, and Thierry Normand for technical expertise.

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