

THRA1 and D17S183 Flank an Interval of <4 cM for the Breast-Ovarian Cancer Gene (BRCA1) on Chromosome 17q21

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Summary

In order to pinpoint the locale of the gene for early-onset familial breast and ovarian cancer (BRCA1), polymorphisms were developed within the locus for thyroid hormone receptor alpha (THRA1) and for several anonymous sequences at chromosome 17q12-q21. The THRA1 polymorphism is a dinucleotide repeat with 10 alleles and heterozygosity .79. Gene mapping in extended families with inherited, early-onset breast and ovarian cancer indicates that BRCA1 is distal to THRA1 and proximal to D17S183 (SCG43), an interval of <4 cM. This locale excludes HER2, THRA1, WNT3, HOX2, NGFR, PHB, COLIA1, NME1, and NME2 as candidates for BRCA1 but does not exclude RARA or EDH17B. Resolving the remaining recombination events in these families by new polymorphisms in the THRA1-D17S183 interval will facilitate positional cloning of the breast-ovarian cancer gene on chromosome 17q12-q21.

Introduction

Early-onset familial breast and ovarian cancer is linked to a gene, BRCA1 (MIM 113705), on chromosome 17q12-q21 (Hall et al. 1990; Narod et al. 1991). Polymorphisms closely linked to BRCA1 in early-onset families have facilitated more precise localization of the disease gene (Hall et al. 1992; Easton et al. 1993). In this report, we identify polymorphisms at the thyroid hormone receptor alpha locus (THRA1) and in several anonymous sequences (D17S183, D17S190, and D17S181). Recombination events in families with inherited disease localize BRCA1 to the interval between THRA1 and D17S183, a distance of <4 cM.

Material and Methods

In order to localize BRCA1, polymorphic markers from chromosome 17q12-q21 were screened on fami-

lies in our series with early-onset breast and ovarian cancer. Since our most recent report (Hall et al. 1992), three more cases of breast cancer have appeared in family 1, in individuals II-3, III-5, and IV-2 (fig. 1). Family 81, which has not been previously published, includes 11 women who have developed breast cancer, one of whom (II-1) developed ovarian cancer as well (fig. 2). The average age at breast cancer diagnosis in family 81 is 39.6 years.

Table 1 indicates the polymorphic markers included in this analysis. Genomic DNA was prepared according to a method described elsewhere for lymphocyte cell lines (Hall et al. 1992). Paraffin-embedded tissue from pathology specimens was used as a source of both tumor and normal DNA, which were separately obtained by dissecting tumor cells from adjacent normal cells in paraffin blocks. DNA from each cell type from paraffin-embedded tissues was prepared according to a method described elsewhere (Wright and Manos 1990).

Polymorphisms D17S250, HER2, D17S78, D17S579, GIP, and D17S293 were tested according to a method described elsewhere. Five other polymorphisms are new to this analysis: D17S518, THRA1, D17S183, D17S190, and D17S181. A polymorphic CA dinucleotide repeat within THRA1 was developed from a

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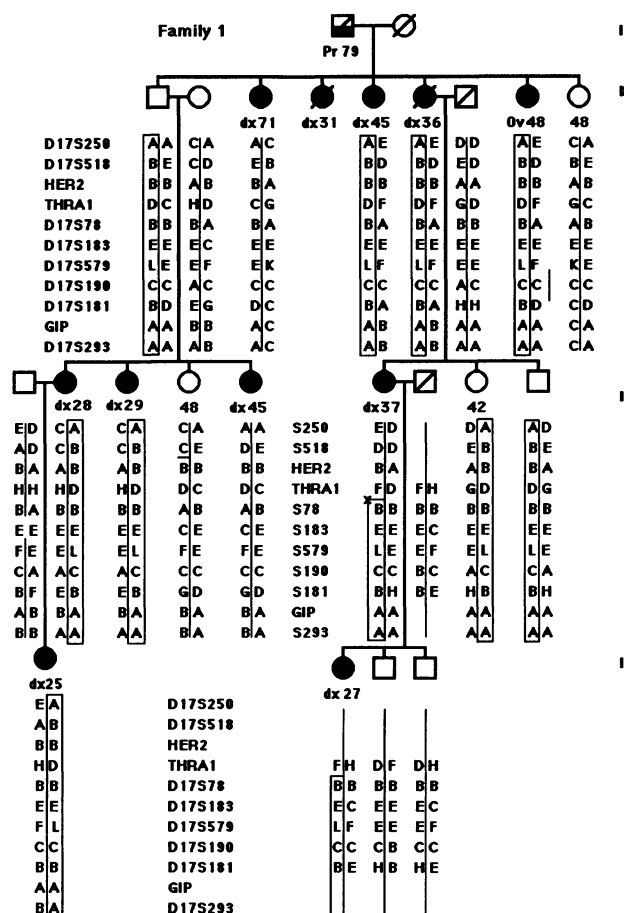


Figure 1 Linkage between breast cancer (blackened circles; with age at diagnosis [dx] indicated) and markers on chromosome 17q21 in family 1. Boxed haplotypes carry the BRCA1 disease allele. Breast cancers in II-3, III-5, and IV-2 have appeared since our last report of this family (Hall et al. 1992). Genotypes of II-6, II-7, and III-7 were reconstructed completely or in part from their surviving relatives. I-1 developed prostatic cancer at age 79 years; II-8 developed ovarian cancer at age 48 years. A recombination event between THRA1 and D17S78 in patient III-6, which was inherited by patient IV-2, suggests that the disease gene is distal to THRA1.

[CA]18 sequence beginning at bp 2004 in intron 9 of the human thyroid hormone receptor alpha locus (THRA1, or c-erbA-1) (Laudet et al. 1991). This sequence was screened with the program PRIMER (version 0.5; E. Lander, M. Daly, and S. Lincoln, Whitehead Institute, Boston), and oligonucleotides were selected to yield a PCR product of 143 bp. These were THRA-L, GGG CAA AAA TGT CTT AAG C; and THRA-R, CAG CAT AGC ATT GCC TTC.

For analysis of THRA1 genotypes, two methods were used. For the development of the polymorphism

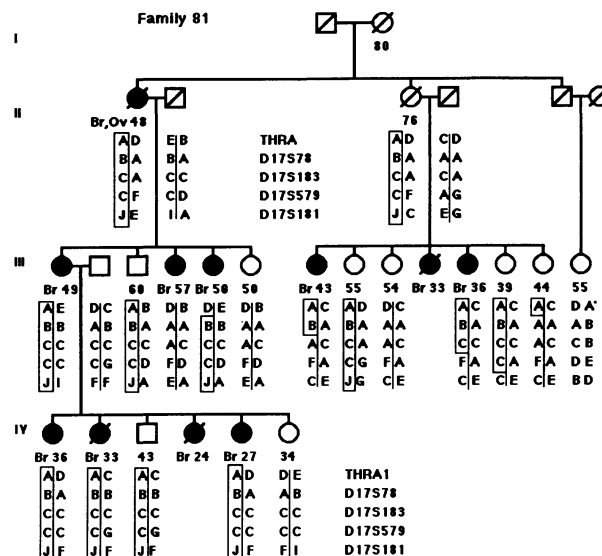


Figure 2 Linkage between breast cancer and chromosome 17q21 markers in family 81. Notation is the same as in fig. 1. Genotypes of individuals in generation II were reconstructed from their surviving relatives. Chromosome phase for individual III-14 is not determinable with certainty. Recombination between THRA1 and D17S78 in individual III-6 suggests that the disease gene is distal to THRA1. Recombination between D17S183 and D17S579 in individual III-11 suggests that BRCA1 is proximal to D17S579. Recombination between D17S78 and D17S183 in individual III-7 further suggests that BRCA1 is proximal to D17S183.

and for typing on CEPH families, 33 ng total human genomic DNA was amplified by PCR. Reactions were performed in a 20-ml volume with 0.25 mM of each oligonucleotide primer, 0.5 units *TaqI* polymerase

Table 1

Ordered Polymorphic Markers on Chromosome 17q12-q21 Linked to BRCA1

Gene/Locus	Name	Reference
D17S250	Mfd15	Weber et al. 1990
D17S518		Couch et al. 1991
HER2		Hall and King 1991; Papewalis et al. 1991
THRA1		Present report
D17S78	p131	Hall et al. 1990
D17S183	B43, SCG43	Black et al. 1993
D17S579	Mfd188	Hall et al. 1992
D17S190	C54, SCG41	Black et al. 1993
D17S181	SCG40	Black et al. 1993
GIP		Johnson et al. 1990
D17S293	6C1	Hall et al. 1992

(Promega), 0.25 mM spermidine, and 200 mM each of dATP, dGTP, dTTP, and dCTP was added to the standard PCR buffer. One 5'-labeled oligonucleotide (10^4 cpm) was included in the PCR reaction. Samples were overlaid with mineral oil (DNase and RNase-free; Sigma) and amplified for 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, followed by 7 min at 72°C. Products were electrophoresed in 4.5% denaturing polyacrylamide gels containing 35% formamide. Sizes of alleles were determined by comparing the most intense band, for each allele, with an M13 sequencing ladder. Allele frequencies were estimated from 210 unrelated CEPH parents and grandparents. Alternatively, and for typing the breast cancer families, we followed the procedure described elsewhere (Hall et al. 1992), with the addition of 2 mg BSA/ml to the amplification reaction.

The smallest interval including BRCA1 was defined by identifying critical recombinants among breast and ovarian cancer patients in families with early-onset disease. Only genotypes obtained directly or reconstructed unambiguously were considered.

Results

Allele sizes and frequencies for THRA1 are as follows: A = 157 bp (frequency .01); B = 155 bp (frequency .01); C = 153 bp (frequency .08); D = 151 bp (frequency .13); E = 149 bp (frequency .04); F = 147 bp (frequency .31); G = 145 bp (frequency .08); H = 143 bp (frequency .29); I = 141 bp (frequency .02); and J = 135 bp (frequency .04).

Figures 1, 2, and 3 indicate linkage of breast and ovarian cancer to this interval of chromosome 17q12-q21, for families 1, 81, and 4, respectively. In family 1, recombination between THRA1 and D17S78 in breast cancer patient III-6 suggests that BRCA1 is distal to THRA1 (fig. 1). This suggestion was strengthened when patient IV-2, who inherited the recombinant haplotype from her mother, was diagnosed with breast cancer at age 27 years this year (as the consequence of going to her physician to have blood drawn for this study). However, breast cancer diagnosed at age 71 years in individual II-3 and at age 45 years in individual III-5 does not appear linked to 17q, although the possibility of a double recombination or gene conversion cannot be excluded.

In family 4, breast cancer patient II-9, whose genotype was obtained from normal cells from paraffin-embedded tissue, carries the alleles linked to susceptibility in the family (fig. 3). Also in family 4, recombination

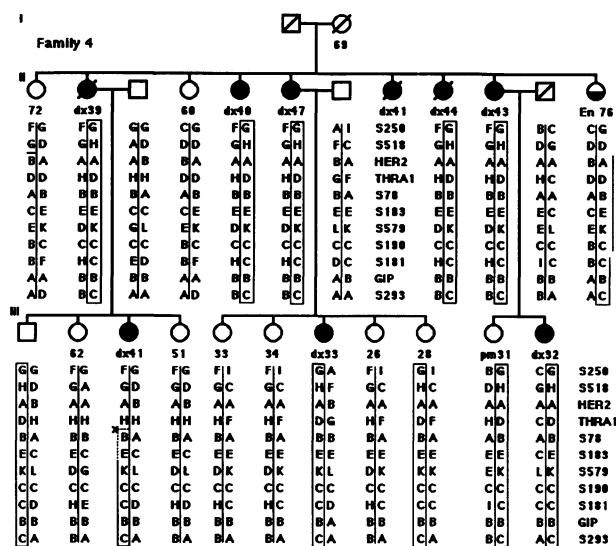


Figure 3 Linkage between breast cancer and chromosome 17q21 markers in family 4. Notation is the same as in figs. 1 and 2. III-10 had a prophylactic mastectomy (pm) at age 31 years, prior to the beginning of this study. Since our previous report on this family (Hall et al. 1990), DNA extraction from paraffin-embedded tissue has enabled us to type PCR-based markers for deceased individual II-9. The genotype of II-2 was reconstructed from her spouse and children. Recombination between THRA1 and D17S579 in patient III-3 suggests that the disease gene is distal to THRA1.

between THRA1 and D17S579 in patient III-3 places BRCA1 distal to THRA1. D17S78 and D17S183 are uninformative in this family.

In family 81, eight of the nine genotyped breast cancer patients carry the same alleles for the critical region of chromosome 17q12-q21 (fig. 2). Patient IV-2 was typed from paraffin-embedded tissues; she carries the alleles linked to susceptibility in the family. Breast cancer in patient III-4 does not appear linked to BRCA1. This patient's breast cancer occurred at age 57 years, the oldest age at diagnosis in the family. Three patients in family 81 have informative recombination events in the BRCA1 region. In patient III-11, recombination between D17S183 and D17S579 suggests that BRCA1 is proximal to D17S579. In patient III-7, recombination between D17S78 and D17S183 suggests that BRCA1 is proximal to D17S183 as well. In patient III-5, recombination between THRA1 and D17S78 strengthens the conclusion, based on families 1 and 4, that BRCA1 is distal to THRA1. D17S190 is uninformative in family 81.

Several other families in our series carry recombination events placing BRCA1 between HER2 and

D17S293. However, the recombination events described above represent all those we have detected between THRA1 and D17S579.

Discussion

Multiple recombination events reported by the Breast Cancer Linkage Consortium place BRCA1 in the interval between D17S250 and D17S588 (Easton et al. 1993). However, as far as we know, the recombinants in families 1, 4, and 81 are the first to place BRCA1 distal to THRA1 and proximal to D17S183. There are at least two other reports of convincing recombinants placing BRCA1 proximal to D17S579 (Chamberlain et al. 1993; Devilee et al. 1993).

The recombination events placing BRCA1 distal to THRA1 in families 1 and 4, as well as those placing it proximal to D17S183 and D17S579 in family 81, appeared in patients with young ages at diagnosis who are members of extended, early-onset, 17q-linked families. These patients are not likely to be sporadic cases. Breast cancer in patient III-5 in family 81 was diagnosed at age 50 years, so it might be sporadic disease. However, patient III-5 represents the third THRA1 recombinant in this series and is therefore less critical.

The distance between THRA1 and D17S183 is <4 cM. This rough estimate is based on the 4.1-cM genetic distance between THRA1 and D17S509 (data from the Chromosome 17 Workshop, March 11–12, 1992), the absence of recombination between D17S509 and D17S579 in more than 30 informative CEPH families (CEPH, version 5; Anderson et al., in press), and recombination between D17S183 and D17S579 in both CEPH and breast cancer families, placing D17S183 ≥ 0.3 cM proximal to D17S579 (authors' unpublished data).

This locale for BRCA1 excludes HER2, THRA1, WNT3, HOX2, NGFR, PHB, COLIA1, NME1, and NME2 as candidates for BRCA1 (Lemons et al. 1990; Anderson et al., in press). On the other hand, RARA and EDH17B remain candidates in the linked interval. Clearly, the gene-mapping approach to identifying BRCA1 has not yet been exhausted. Within the THRA1-D17S183 interval, four informative recombination events remain to be resolved in these three families alone. Additional polymorphisms in this interval will enable us to pinpoint the BRCA1 locale more precisely by genetic mapping (Anderson et al., in press). Finally, as a means to test whether breast and/or ovarian cancer is/are attributable to inherited alterations of BRCA1 in newly ascertained, multiply affected families, the three

markers THRA1, D17S579 (Mfd 188), and D17S181 (SCG40) provide an informative and easily screened set of polymorphisms.

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