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A Radiation Hybrid Map of the BRCA1 Region

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Summary

A locus on chromosome 17q, designated "BRCA1," has been identified as a predisposition gene for breast cancer. A panel of chromosome 17–specific radiation-reduced somatic cell hybrid clones has been assembled for high-resolution mapping of chromosome 17. A series of 35 markers, known to span the BRCA1 locus, were tested against this hybrid panel by PCR assays. Statistical analysis of these data yields a BRCA1 radiation hybrid map at a density sufficient to initiate YAC cloning and pulsed-field gel electrophoretic mapping of the candidate region. In addition, many of the markers reveal genetic polymorphisms and may be tested in breast cancer families and in loss-of-heterozygosity studies of sporadic breast cancers to better define the BRCA1 gene candidate region.

Introduction

Breast cancer is an often fatal neoplastic disease of mammary tissue, most often arising in the terminal ductal structures of the adult female breast. Among women, it is second only to lung cancer in numbers of cancer-related fatalities (Silverburg and Lubera 1989). The majority of the cases arise in individuals without any known family history of this disorder. However, a hereditary form of this disease is observed in 5%–10% of cases. One such predisposition gene, designated "BRCA1," is characterized by multiple cases of breast and/or ovarian cancers in families. Identification of this predisposition gene is a critical step in gaining insights into the pathogenesis of this devastating condition, in both familial and sporadic breast cancer patients.

Mapping of the BRCA1 locus to chromosome 17 (Hall et al. 1990, 1992; Narod et al. 1991) has generated intense interest in the 17q12-q22 region of this chromosome. These studies have stimulated several mapping efforts (e.g., Hall et al. 1992; Easton et al. 1993; Feunteun et al. 1993), all directed at better defining a candidate region for the breast cancer gene. These studies have shown that the markers D17S509 and D17S579 are very tightly linked to the BRCA1 gene, and they have identified a 20-cM region, bounded by D17S250 and D17S588, containing the BRCA1 gene (Easton et al. 1993); an additional report suggests that the BRCA1 gene resides between THRA1 and D17S183 (Bowcock et al. 1993).

Detailed meiotic maps based on genotyping of normal reference families exist for this region (see, e.g., O'Connell et al. 1993), for a combination of normal and breast cancer families (see, e.g., Anderson et al. 1993; Easton et al. 1993). However, as these maps become increasingly dense, the limitations of the resolution of the linkage approach complicate determination of an unambiguous order for all the markers known to map to the BRCA1 region.

Radiation-reduced somatic cell hybrid technology (Goss and Harris 1975; Cox et al. 1990) offers several advantages for high-resolution mapping. This technique utilizes ionizing radiation to fragment chromosomes in a donor cell line, with subsequent recovery of portions of the fragmented donor genome in a recipient cell line. The panel of radiation hybrid cell lines can be assayed for retention of specific loci, and the distance between loci can be calculated by the concordance or

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discordance of marker retention across the panel. The order of multiple loci can be deduced from statistical analysis of these patterns of locus retention. Because the assay used to analyze these hybrids is for presence or absence of a marker in the cell lines that make up the panel, markers do not need to reveal genetic polymorphisms. The resolution of the radiation hybrid mapping resource is proportional to the number of fragment-containing cell lines under study. Recent applications of this concept have permitted investigators to isolate cell lines containing a panel of fragments from particular human chromosomes (Cox et al. 1990; Glaser et al. 1990; Burmeister et al. 1991; Warrington et al. 1992) and to produce recently a high-resolution radiation hybrid map of the BRCA1 region (Abel et al. 1993). We have utilized such a radiation-reduced hybrid panel specific to chromosome 17 to study additional markers in the BRCA1 region.

Genetic markers based on PCR amplification of microsatellite loci are a powerful tool for human gene mapping (Weber and May 1989; Economou et al. 1990). To better characterize the BRCA1 region, we mapped a series of chromosome 17–specific genetic markers, based on microsatellite repeats (Melis et al. 1993; accompanying article [Albertsen et al. 1994]) and chromosome 17 genes, to an interval spanning this region, with respect to a framework somatic cell hybrid panel (van Tuinen et al. 1987). Our study includes markers in the D17Z1–MPO region and centered on the BRCA1-linked D17S579 described by Hall et al. (1992). A PCR-based format was used exclusively for these studies (Saiki et al. 1988). We then tested these markers on the chromosome 17 radiation-reduced hybrid panel to assess the order and spacing of these markers, with respect to markers previously tested in BRCA1 families. The resulting map indicates that a series of closely spaced PCR-based markers span the candidate region, making possible further genetic and physical characterization of this region.

Material and Methods

Primers

Primer sequences for NF1, D17S509, D17S608, D17S620, D17S648, D17S652, D17S672, D17S677, D17S702, D17S733, D17S742, D17S747, D17S750, D17S754, D17S760, D17S964, D17S902, D17S907, and D17S965 were determined from dideoxynucleotide sequencing of M13 clones (Sanger and Coulson 1975) on an ABI model 373 sequencer. Primers were selected using the computer program OLIGO (J.-M. Lalouel, personal communication). The D17S183 primers was from Black et al. (1993). This group of primers was synthesized on an ABI model 394 DNA synthesizer. Primers for GIP (Johnson et al. 1991), ALDOC, CRYB1, PENT, GAS, EPB3, PPY, GP2B, and MPO were designed on the basis of DNA sequence data from their 3′ UTRs, found in the GenBank DNA sequence database, by using the methods of Theune et al. (1991). Primers for D17Z1 were designed on the basis of DNA sequence data obtained from GenBank. The HOX2B primer sequences were from Deinard et al. (1992), and primer sequences for D17S250, D17S579, D17S293, ERBB2, and NME1 were from Hall et al. (1992). These primers were synthesized commercially by Genosys (Houston).

Cell Lines and Culture Conditions

A human × rat hepatoma microcell hybrid, designated "7AE-4" (Leach et al. 1989), containing a *neo*-marked human chromosome 17 as its only human component was cultured in 1:1 Ham's F12:Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum and 250 μg G418/ml. This line served as the donor for the radiation-reduced hybrid fusion. The recipient line was a hypoxanthine phosphoribosyl transferase–deficient Chinese hamster cell line, GM459, which was cultured in DMEM with 10% fetal bovine serum, 500 U penicillin/ml, and 0.5 mg streptomycin/ml. Prior to fusion, GM459 cells were cultured in 10 μg 6-thioguanine/ml for 10 d to reduce the possibility of revertants in the GM459 cell population.

Hybrid Construction

The 7AE-4 donor cells were exposed to 3- or 6-krad doses of ^{137}cesium irradiation and were fused to the recipient cells, GM459, by the method of Cox et al. (1990) with the following alterations: The fused population was plated at approximately 1×10^6 cells/75-cm^2 flask and placed in complete DMEM-selective-medium containing hypoxanthine aminopterin thymidine (HAT). After 3 wk, HAT-resistant hybrids were isolated using cloning cylinders. No more than three clones were picked from any one flask, and all clones were isolated from a flask at the same time. A total of 61 hybrids were isolated from the two fusions, over a period of 6 d, 17 from the 3-krad dose and 44 from the 6-krad dose. No clones were observed in flasks containing only GM459 placed in complete HAT medium or in flasks containing 7AE-4-irradiated cells placed in complete nonselective medium.

Table 1

BRCA1-Region Sequence-tagged-Sites Markers

Locus (name)	Cytogenetic Localization	Allele	Primer Sequences	Annealing Temperature (°C)/[Mg^{++}]	Amplicon (bp)
ALDOC (aldolase C)	17q11.2[a]	1	TCCCTGCTTTGAGCCTTGCACAAA GGTAGTACAGAAGCCCTGACTCAT	68/1.5	403
CRYB1 (crystallin, beta polypeptide 1)	17q11.2[a]	1	CTGGGTTTGCTACCATTATCTTGG GTGGCGAACAAGATAGCGCCATTA	60/1.5	424
EPB3 (erythrocyte surface protein 3)	17q12-q21	1	AGGGGCACCTGTGTTTAAGCAG TCCACTGCCTGCAGGTACTGT	60/1.5	158
ERBB2 (erb-B2 oncogene, Her 2)	17q11.2-q12	1	CTGGAATGGGAAGCA GCCAGCAAAGAAATCTTAGACGT	55/2.0	1,100
GAS (gastrin)	17q12-q23[a]	1	ATGCTAGTCGGTGTAGAGCCATG TTGTACCTCATAGGGCTGCGTGA	60/1.5	297
GIP (gastric inhibitory polypeptide)	17q21.3-q22	3	CACAATGGGCTCGACTTAGCATAA CTTGCTGGATCAGACAAACCTCTG	60/1.5	1,960
GP2B (platelet glycoprotein IIb integrin, alpha 2b, ITGA2B)	17q21-q32	1	TCTCCGTCTTCTGTTACACCTACC CCTACACTATTCTAGCAGGAGGGT	60/1.5	361
HOX2B (homeobox 2B)	17q21-q22	1	TCATCTCCAATATGATGTGGCATTCC GCGCCCAAATTAACACACCTGTGAT	50/2.0	135
MPO (myeloperoxidase)	17q21.3-q2.3	1	CACTTCCTGCATTGAACCTGGCTT CTCAAGGTCACATAGCTAGCAAGC	60/1.5	417
NF1 (neurofibromatosis 1; GXAL)	17q11.2	3	CAAGAAAAGCTAATATCGGC GGAACCATTAAGTTCACTTAG	52/1.5	395
NME1 (nonmetastatic cells 1, nm23)	17q22	1	TTGACCGGGGTAGAGAACTC TCTCAGTACTTCCCGTGACC	55/1.5	100
PENT (phenylethanolamine N-methyl transferase)	17q11.2-q12[a]	1	CTAGCCTCAAGGAAGCTTCTGGAA ACCTTCAGACAGGCGTAGATGATG	60/1.5	269
D17S183 (c5/B43)	17	4	ACAAACTGATGTGGGCTCTAG GTACATAGCATGGGTGCAGCT	52/2.0	510
D17S250 (Mfd15)	17q11.2-q12	10	GGAAGAATCAAATAGAGAAT GCTGGCCATATATATATTTAAACC	60/2.0	153
D17S293 (LCN6C1)	17q21-q23[a]	7	ACAGTGCCAGAGATATACCG GCTATGAGCCTGGCAGACC	55/1.5	123
D17S509 (LB17.1)	17	4	GAGAGTGCAGAGTTTTACA CTTTCTCTCTCTCTCCCTG	57/1.5	146
D17S579 (Mfd188)	17q12-q23[a]	10	AGTCCTGTAGACAAAACCTG CAGTTTCATACCAAGTTCCT	60/2.0	123

D17S608 (UT148)	17q12-q23[a]	1	TAGGTTCACCTCTCATTTTCTTCAG GTCTGGGTCTTTATGGNGCTTGTG	57/1.0	136
D17S620 (UT159)	17p11-q11[a]	3	GGGAAGGTGTCTGAAACCCAAGG CCCACCACTACCTATTGTTCTATAC	60/1.0	143
D17S648 (UT185)	17q12-q23[a]	2	TGGGCGACAAGGCAAGACTTCGT CCTGAGTGAAGGTGGCTTAAAAAAG	57/1.5	351
D17S652 (UT189)	17q12-q23[a]	5	TTTCTTATCACCTTCCCTCCCACTG CTGCAATCTATCAGTGTCCAAGATG	57/1.5	159
D17S672 (UT22)	17q12-q23.1[a]	1	CGGGCGTGGTGGCGGACCC ACGGGCATCTTTTCAGGTCGGT	60/1.0	223
D17S677 (UT224)	17q12-q23[a]	6	CTCCATCATAGGTGACAAATTG TTTGAGCTTTGCCACTTGCCAGC	57/1.5	255
D17S702 (UT394)	17q12-q23[a]	3	GACATAGCGAGACTCCCTGTCCA GGCACATAGTAGGAGTGCTACATG	55/1.5	100
D17S733 (UT50)	17q12-q23[a]	6	TGGGCAACAGAGCAAAACTCTGTT GACAGAGGGGAGGGGAGACGG	60/1.0	201
D17S742 (UT59)	17q12-q23.1[a]	2	ATCTTTAGGGAAAACACTCCACACT TCTATGTGTGTATACCAATGACCCA	58/1.5	221
D17S747 (UT63)	17q12-q23.1[a]	3	GTTGGCTACTCTTGCTTCCTTCC GCCTATCACCATGTGTGTGACTG	58/1.5	116
D17S750 (UT67)	17q12-q23[a]	5	AGCGAAGAGCACTCTGACTAGAATC ATGGGGTAAGGTCTACAGAATGCCT	55/1.0	175
D17S754 (UT71)	17	2	TGGATTCACTGACTCAGCCTGC GCGTGTCTGTCTCCATGTGTGC	55/1.0	145
D17S760 (UT8)	17	4	ACACGTTGGACACCAACACACA AACCGGAGCTTCTGTGTAGGC	60/1.0	152
D17S902 (UT573)	17	5	GAGGTTGCAGTGAGTTGAGA GGAACATCCTCCTTCACTCTT	62/1.5	155
D17S907 (UT752)	17	4	ACTCCAACGTGGGCGACAG CCTTCGTTTTATGTCCCGAG	60/1.0	338
D17S964 (UT401)	17	2	GTTCTTTCCTCTTGTGGGG AGTCAGCTGAGATTGTGCC	54/1.25	224
D17S965 (UT956)	17	5	GAAGTGCCAAAGGCCAGGAACC CTGCACTTCATTCAGCCTGGGTG	62/1.0	185
D17Z1	17cen	1	CATCCTCAGAAGCTTCTCTG AGATGATTCCGTTTCCAACGA	55/1.5	149

[a] Regional localization refined in this study.

The framework hybrids GM10661 (17q11.2-qter), GM10659 (17q11.2-pter), and GM10502 (17q23-qter; O'Connell et al. 1989) were tested to identify markers near the BRCA1 region and used as controls for the PCR assays. Human genomic and 7AE-4 (intact human chromosome 17) DNAs were included as positive controls; GM459 (hamster) and PCTA-7A (rat) DNAs were included as negative controls. All GM-series cell lines were obtained through the National Institute of General Medical Science's Camden Mutant Cell Line Repository.

DNA Marker Analysis

Hybrid cell DNA was extracted according to the method of Bell et al. (1981). All data were collected by PCR analysis of the radiation-reduced hybrid panel. For each set of primers, Mg^{++} concentration and annealing temperatures were determined empirically using either a DNA Thermal Cycler (Perkin-Elmer) or a 96-well PHC-3 thermocycler (Techne). All of the primers produced a species-specific product under the described conditions (see table 1). PCR reactions consisted of 500 ng of template DNA, 10 ng of each primer, 0.1 mM each of dNTPs, and 1.2 U of *Taq* DNA polymerase (Perkin-Elmer or Promega) in a 0.025 ml volume. PCR reactions were denatured for 3 min at 94°C, followed by 30 cycles (each comprising 94°C, 1 min; annealing temperature, 1 min; and 72°C, 1 min). The final cycle was followed by a 5′ extension at 72°C. PCR products were electrophoresed on a 2.0% LE agarose (FMC) and were visualized with ethidium bromide staining. PCR assays were routinely done at least twice, and the data sets for the assays were merged. Data were entered as 1 (present), 0 (not present), or 8 (ambiguous). An average of 10 cR were seen in Radiation Hybrid TWOPOINT analysis of uncorrected data for duplicate tests of markers.

Radiation Hybrid Data Analysis

The distances and order between the markers were estimated using the Statistical Package for Radiation Hybrid Mapping (RHMAP) (Cox et al. 1990; Boehnke 1992). The TWOPOINT option of Cox's RHMAP program was used to estimate recombination fractions and retention frequencies. An anchor map was generated for a subset of robustly mapped markers spanning the region. Additional markers were added to this map as the result of TWOPOINT analysis. Trial map orders were then validated and tested for support, with the FOURPOINT option of Cox's RHMAP program.

Results

Radiation Hybrid Panel

Sixty-one radiation-reduced hybrids were tested, but the 44 6-krad lines carried the majority of the breakpoints in the BRCA1 region (37 lines with a fragment or multiple fragments of the BRCA1 region). To better define dose-specific centiray distances (cR_{6000}), subsequent analysis was confined to data from the 6-krad radiation hybrids. Retention frequency for loci tested against the 6-krad chromosome 17 radiation hybrid panel was .60–.31 in a gradient from proximal to distal across the BRCA1 region.

Analysis of PCR Primers

Table 1 documents the characteristics of the 35 BRCA1-region PCR-primer pairs used in this study. PCR annealing temperatures and $MgCl_2$ optima were redetermined and may differ from those in other reports. Nineteen of the PCR markers reveal genetic polymorphisms, including 14 new microsatellite markers for the BRCA1 region.

Radiation Hybrid Data Analysis

Two-point analysis was carried out on the data, and these results are summarized in table 2. Two markers, D17Z1 and D17S620, near the centromere demonstrated extremely high retention frequencies. As a result, these markers failed to demonstrate significant linkage to the rest of the radiation hybrid map. A number of markers formed clusters of fully linked loci: D17S964–D17S677, D17S760–D17S702, D17S183–EPB3, D17S509–D17S602–D17S579, D17S652–D17S750–D17S965, and D17S733–HOX2B. This resulted in 25 loci that were subsequently ordered by multipoint analysis.

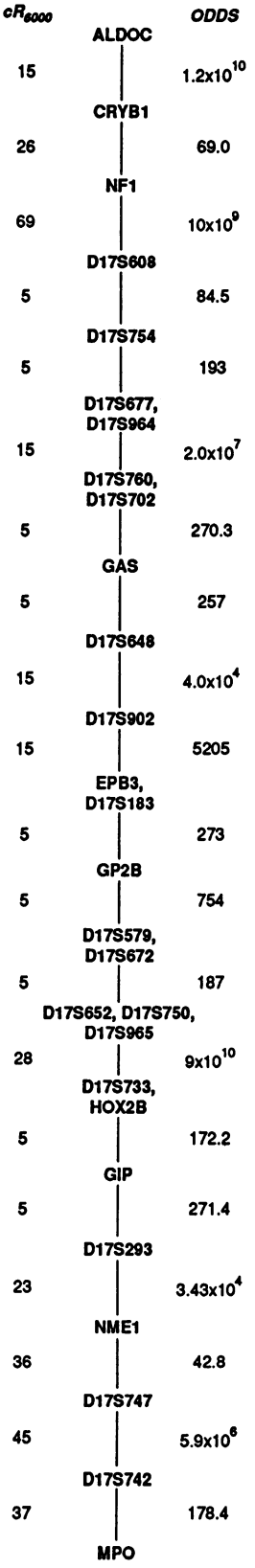
To simplify error checking and to reduce the number of potential orders to consider, trial marker orders were determined with Boehnke's RHMINBRK program. A small number of potential orders were favored, differing from each other by the inversion of adjacent pairs of closely linked loci. Data were then reexamined to detect inconsistences. Marker data were arranged in the order indicated by RHMINBRK, and "exceptional" hybrids, i.e., those testing negative or positive for an isolated marker contrary to data obtained with adjacent markers, were retested. In some cases, duplicate typing of markers yielded inconsistant results. In these cases, the marker data for that hybrid were entered as unknown. Data for four markers—PENT, D17S907, D17S250, and ERBB2—although linked to each other

Table 2

Two-Point Lod Score Analysis of BRCA1 Region

	D17Z1	S620	ALDOC	CRYB	NF1	S907	PENT	S250	ERBB2	S608	S754	S677 S964	S702 S760	GAS	S648	S902	EPB3 S183	GP2B	S579 S672 S509	S652 S750 S965	S733 HOX2B	GIP	S293	NME1	S747	S742	MPO
D17Z1		145	U	270	135	400	U	253	627	U	400	U	U	U	U	U	U	U	U	U	627	282	336	241	U	U	377
S620	.5		84	73	106	213	118	135	151	104	119	136	208	251	204	236	326	U	326	244	204	244	200	170	199	U	266
ALDOC	0	1.8		15	46	127	187	187	151	110	127	148	158	177	151	159	200	177	151	168	151	168	144	213	297	U	473
CRYB	.04	2.2	8.1		26	113	126	163	136	98	113	130	140	157	136	144	177	157	16	151	177	201	170	278	U	U	U
NF1	.7	1.1	4.2	6.4		60	89	114	99	69	79	89	100	88	78	83	99	113	99	88	99	112	126	189	225	U	326
S907	0	.1	.8	1.0	3.0		70	53	61	38	32	26	32	26	32	57	61	70	80	71	80	91	104	118	98	201	489
PENT	0	.9	.3	.8	1.7	2.5		46	39	32	38	32	39	46	39	68	71	81	92	82	119	137	159	139	114	259	U
S250	.1	.7	.3	.4	1.0	3.5	4.1		14	25	30	20	26	32	39	50	71	81	71	63	92	82	94	107	151	363	U
ERBB2	0	.5	.5	.7	1.4	3.0	4.7	8.3		15	20	26	21	27	21	30	50	56	48	41	65	57	67	79	106	194	321
S608	0	1.3	1.1	1.4	2.5	4.8	5.6	7.4	8.3		5	10	26	32	26	35	54	62	54	47	71	82	94	107	114	209	U
S754	0	.9	.8	1.0	2.1	5.6	4.8	8.5	7.4	11.1		5	20	26	32	41	61	70	61	53	80	91	104	118	128	246	U
S964																											
S677	0	.6	.5	.7	1.7	6.5	5.7	7.4	6.6	9.7	11.2		15	20	26	48	53	61	69	61	89	101	115	130	110	235	U
S760																											
S702	0	.2	.4	.6	1.3	5.6	4.8	6.4	7.2	6.4	7.4	8.5		5	10	29	33	39	47	40	63	73	84	97	78	155	U
GAS	0	.1	.3	.4	1.7	6.4	4.1	5.5	6.1	5.5	6.4	7.5	11.0		5	23	27	33	40	34	56	65	76	88	70	137	499
S648	0	.2	.5	.7	2.2	5.6	4.7	4.7	7.0	6.3	5.6	6.6	9.5	10.8		17	21	27	34	28	48	57	67	79	60	122	321
S902	0	.1	.5	.6	1.9	3.1	2.4	3.6	5.3	5.0	4.4	3.8	5.6	6.3	7.2		17	23	17	11	45	54	65	67	78	91	215
S183																											
EPB3	0	.02	.2	.3	1.4	3.0	2.4	2.4	3.7	3.4	3.0	3.6	5.4	6.1	7.0	7.2		5	10	10	34	41	50	79	60	93	321
GP2B	0	0	.3	.5	1.0	2.5	2.0	2.0	3.3	2.9	2.5	3.0	4.7	5.4	6.1	6.3	10.8		5	10	40	48	57	88	69	79	224
S509																											
S672																											
S579	0	.02	.5	.7	1.4	2.0	1.5	2.4	3.8	3.4	3.0	2.5	4.0	4.6	5.2	7.2	9.2	10.8		5	34	41	50	79	81	81	196
S652																											
S750																											
S965	0	.1	.4	.5	1.8	2.5	1.9	2.9	4.4	4.0	3.5	3.1	4.6	5.3	6.0	8.2	9.3	9.3	10.6		28	35	43	70	71	94	259
S733																											
HOX2B	0	.2	.5	.3	1.4	2.0	.9	1.5	2.7	2.4	2.0	1.7	2.8	3.3	3.8	3.8	5.2	4.6	5.2	6.0		5	10	28	29	60	107
GIP	.04	.1	.4	.2	1.1	1.6	.6	1.9	3.1	1.9	1.6	1.3	2.3	2.7	3.1	3.0	4.4	3.8	4.4	5.0	10.6		5	23	36	71	96
S293	.02	.2	.5	.4	.8	1.3	.4	1.5	2.5	1.5	1.3	1.0	1.8	2.3	2.5	2.3	3.7	3.2	3.7	4.2	9.1	10.4		17	30	84	114
NME1	.1	.3	.2	.04	.3	1.0	.6	1.2	2.0	1.2	1.0	.8	1.4	1.7	5.0	2.3	2.0	1.7	2.0	2.4	5.8	6.6	7.5		23	54	76
S747	0	.2	.04	0	.1	1.4	.1	.5	1.1	.1	.8	1.1	2.0	2.5	2.9	1.8	2.9	2.4	1.9	2.3	5.6	4.7	5.5	6.3		45	98
S742	0	0	0	0	0	.2	.1	.02	.2	.2	.1	.1	.4	.6	.8	1.4	1.4	1.9	1.9	1.4	2.9	2.3	1.8	3.2	3.8		37
MPO	.01	.1	0	0	.02	0	0	0	.02	0	0	0	0	0	.02	.1	.02	.1	.2	.2	1.1	1.4	1.0	2.0	1.3	4.6	

NOTE.—U = undefined.



and to other elements of this map, could not be ordered on the radiation hybrid map (for two-point analysis placement of these loci, see table 2 and Discussion).

These corrected data were analyzed by the FOUR-POINT option of RHMAP to assess the relative order and distance between these markers. The resulting radiation hybrid map spans 369 cR_{6000} and is shown in figure 1. The meiotic recombination rate across the NF1–MPO (the most distal elements of our map) map interval is estimated to be 35 cM. We estimate a 328-cR_{6000} radiation hybrid map distance for this interval. Thus, 1 cR_{6000} = ~0.1 cM. Chromosome 17 covers 168 cM according to genetic mapping (O'Connell et al. 1992, 1993), covers 196 cM according to chiasma counts, and has an estimated 92 Mb of DNA (Morton 1991). Taken together, these data suggest that 1 sex-averaged cM of chromosome 17 equals ~500 kb of DNA or that 1 cR_{6000} of the BRCA1 region averages 50 kb.

Discussion

A panel of somatic cell hybrids containing chromosome 17 fragments generated by radiation/fusion technology has been characterized with 35 PCR-formatted markers spanning the BRCA1 locus. A gradient of retention across the panel almost certainly results from selective retention of the chromosome 17 centromere. This results in dramatic map expansion across the centromere, although significant linkage can be detected if the 3-krad hybrids are included in the analysis (R. Leach and P. O'Connell, unpublished data).

Multipoint analysis can position 31 of these markers, defining 21 loci, on a radiation hybrid map spanning 369 cR_{6000}. Two centromeric markers (D17Z1 and D17S620) did not show significant linkage to the map, and an additional cluster of four markers (D17S907, PENT, D17S250, and ERBB2) could not be uniquely ordered relative to the rest of the map, because of certain influential hybrids that introduced data inconsistent with adjacent markers (i.e., either the absence of the marker within a line positive for the adjacent markers or the presence of the marker in a line negative for the adjacent markers). We detect a higher-than-

Figure 1 Radiation hybrid map for 28 markers in the BRCA1 region. Distances are shown in dose-specific centirays. Odds are given against the inversion of adjacent loci, i.e., the likelihood of order ABCD vs. the likelihood of ACBD, except for the extreme left (ABCD vs. BACD) and right (ABCD vs. ABDC) ends of the map. Fully linked markers cannot be ordered relative to one another.

average correlation of the latter form of aberrant data with faint PCR products and suspect that both these phenomena result from clonal variation in the hybrid population; that is, only a subset of the population retain a given human chromosome fragment. The resulting variation in the presence or absence of loci across that hybrid is a function of the sensitivity of the PCR markers tested. However, in the instance of the PENT–D17S907–D17S250–ERBB2 marker cluster, strong PCR amplification was observed. We suspect that key hybrids in this region may have rearrangements, such as interstitial deletions, relative to the rest of the chromosome, resulting in difficulty in merging this region's data with the rest of the map. Empirical comparison of the radiation hybrid map with the genetic linkage map shows very good correlation between the two (accompanying paper [Albertsen et al. 1994]). A different radiation hybrid panel of chromosome 17, prepared from the identical 7AE-4 donor, has recently been reported by Abel et al. (1993). This separate chromosome 17 radiation hybrid panel was prepared at an 8-krad dose and has retention frequencies in the range .667–.440, across the BRCA1 region. Both panels yield remarkably similar estimates of recombination frequency, suggesting that it may be possible to combine these panels to increase resolving power for this chromosome.

The 50-kb cR_{6000} estimate is based on genetic and physical analysis of the entire chromosome 17 and may not be completely accurate for this specific region. However, this estimate is consistent with those suggested by other radiation hybrid mapping results (Cox et al. 1990; Warrington et al. 1992). The BRCA1 region is conservatively mapped between markers D17S250 and D17S579 (Devilee et al. 1993; Goldgar et al. 1993). It has been further refined to the region between markers THRA1 and D17S183 (Bowcock et al. 1993). This region spans ~134 cR_{6000} and includes 15 mapped loci between NF1 and D17S183. The markers in this ~6.7-Mb region should serve as good starting points to complete YAC coverage of the BRCA1 region and will aid in the pulsed-field gel electrophoresis analysis of this region. In addition, the PCR-primer pairs for D17S648 and D17S902 encompass polymorphic microsatellite repeats and can be of use in the genetic analysis of BRCA1 families and in loss-of-heterozygosity analysis of sporadic breast cancers. Finally, if the BRCA1 gene transcript is expressed in the radiation hybrid cell lines, the small fragments of human chromosome 17 present within this panel can be tested for human-specific gene expression, potentially aiding in the isolation of this important gene.

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