GENOMICS 17, 632–641 (1993)

A Radiation Hybrid Map of the *BRCA1* Region of Chromosome 17q12–q21

KENNETH J. ABEL,*·⊥·[1] MICHAEL BOEHNKE,†·‖ MURALI PRAHALAD,‡·§·‖ PEGGY HO,* WENDY L. FLEJTER,‡·§·‖ MELANIE WATKINS,§·‖ JILL VANDERSTOEP,† SETTARA C. CHANDRASEKHARAPPA,‡·‖ FRANCIS S. COLLINS,*·‡·‖·⊥ THOMAS W. GLOVER,‡·§·‖ AND BARBARA L. WEBER*

*University of Michigan, Departments of *Internal Medicine, †Biostatistics, ‡Human Genetics, and §Pediatrics; ‖Michigan Human Genome Center; and the ⊥Howard Hughes Medical Institute, Ann Arbor, Michigan*

Received December 28, 1992

The chromosomal region 17q12–q21 contains a gene (*BRCA1*) conferring susceptibility to early-onset familial breast and ovarian cancer. An 8000-rad radiation-reduced hybrid (RH) panel was constructed to provide a resource for long-range mapping of this region. A large fraction of the hybrids (~90%) retained detectable human chromosome 17 sequences. The complete panel of 76 hybrids was scored for the presence or absence of 22 markers from this chromosomal region, including 14 cloned genes, seven microsatellite repeats, and one anonymous DNA segment. Statistical analysis of the marker retention data employing multipoint methods provided both comprehensive and framework maps of this chromosomal region, including distance estimates between adjacent markers. The comprehensive RH map includes 17 loci and spans 179 cRays$_{(8000)}$. Likelihood ratios of at least 1000:1 support the 10-locus framework order: cen–D17S250–*ERBB2*–(*THRA1*, *TOP2A*)–D17S855–*PPY*–D17S190–*MTBT1*–*GP3A*–BTR–D17S588–tel. The order obtained from RH mapping, when used in conjunction with other methods, will be useful in linkage analysis of breast cancer families and will facilitate the development of a physical map of this region. © 1993 Academic Press, Inc.

INTRODUCTION

Breast cancer is the second most common cause of cancer death among American women. Family history of breast cancer has long been recognized as a significant risk factor, increased risk being associated with two or more affected first-degree relatives and with decreasing age of the index case (Sattin *et al.*, 1985; Claus *et al.*, 1990, 1991). Several reports suggest that increased risk in these families is best explained by autosomal dominant inheritance of a highly penetrant susceptibility allele (Newman *et al.*, 1988; Claus *et al.*, 1991). Recently, chromosome 17q12–q21 was identified as the location of a gene conferring susceptibility to familial breast and ovarian cancer (Hall *et al.*, 1990; Narod *et al.*, 1991), now designated *BRCA1* (Solomon and Ledbetter, 1991). Multipoint linkage analysis and identification of critical recombinants in families demonstrating linkage between breast cancer and genetic markers flanking *BRCA1* has narrowed the most likely region for the gene to a <4-cM interval flanked by the markers *THRA1* and D17S183 (Chamberlain *et al.*, 1993; Easton *et al.*, 1993; Hall *et al.*, 1992; Bowcock *et al.*, 1993). Further delineation of the critical region by genetic analysis, however, is limited by the need for informative recombinants in breast cancer families and by the availability of well-ordered and highly polymorphic markers. Efforts to isolate this gene will be aided by additional mapping strategies that provide order and distance estimates for markers in this region.

Radiation-reduced hybrids (RHs) represent a valuable somatic cell resource for the long-range analysis of chromosomal regions independent of genetic recombination. This technique has been used to construct maps for a number of chromosomal regions (Cox *et al.*, 1990; Burmeister *et al.*, 1991; Ceccherini *et al.*, 1992; Gorski *et al.*, 1992; Warrington *et al.*, 1992) and to generate cloning resources enriched for regions of interest (Pritchard *et al.*, 1989; Glaser *et al.*, 1990; Brook *et al.*, 1992). One of the strengths of RH mapping is the ability to order markers at a level of resolution intermediate between that of other methods, such as linkage analysis on the one hand and pulsed-field gel electrophoresis on the other (Cox *et al.*, 1990). Also, since RH mapping involves the analysis of a single chromosome copy, all markers are informative in each typed hybrid and nonpolymorphic markers can be included in the analysis. Statistically derived estimates for distance between adjacent markers, and for relative likelihoods of different marker orders, also are possible (Cox *et al.*, 1990; Boehnke *et al.*, 1991).

We constructed a radiation hybrid panel for chromosome 17 to order markers within the *BRCA1* region.

[1] To whom correspondence should be addressed at University of Michigan, Department of Internal Medicine, 5514 MSRB I, Box 0680, Ann Arbor, MI 48109.

0888-7543/93 $5.00

These markers included polymorphic microsatellite repeats and cloned genes previously mapped to this region. A number of these genes represented particularly interesting candidates for *BRCA1*, including *EDH17B* (estradiol 17-β dehydrogenase, loci 1 and 2), *ERBB2* (*HER/2, NEU*), *RARA* (retinoic acid receptor α), *THRA1* (thyroid hormone receptor α1), *TOP2A* (topoisomerase IIα), and *NME* (nonmetastatic proteins, loci 1 and 2). Although radiation hybrids alone cannot be used to map disease loci, superposition of the RH and genetic linkage maps will help to refine the locations of key recombinants, decreasing the size of the *BRCA1* candidate interval.

In addition to providing a new resource for mapping chromosome 17 markers in general, this RH panel will complement other mapping approaches aimed toward isolation of the *BRCA1* gene. The eventual identification of this gene will permit improved risk assessment for family members and will help determine whether mutations at the same locus are responsible for sporadic tumors.

MATERIALS AND METHODS

Cell lines, culture conditions. 7AE-4 (donor), a rat hepatoma microcell hybrid cell line carrying a *neo* gene-tagged 17 as its only human chromosome (Leach *et al.*, 1989), was maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 1000 units/ml penicillin, 1 mg/ml streptomycin, and 4 m*M* glutamine (complete DMEM), supplemented with 200 m*M* proline and containing 250 μg/ml active G418 (GIBCO). GM459 (recipient), a near diploid Chinese hamster line deficient for hypoxanthine-guanine phosphoribosyltransferase activity ($HPRT^-$; Cox *et al.*, 1990), was maintained in complete DMEM. For 10 days prior to fusion, GM459 cells were grown in the presence of 10 μg/ml 6-thioguanine to select against possible $HPRT^+$ revertants.

Radiation hybrid panel construction. Donor cells were irradiated and fused to recipients as previously described (Cox *et al.*, 1990), with the following modifications: On the day of fusion, 4×10^7 7AE-4 cells were trypsinized and resuspended in serum-free DMEM medium at a concentration of 2×10^6 cells/ml. Inoculated flasks were placed on ice and γ-irradiated with 8000 rads using a ^{137}Cs source at a rate of approximately 260 rads/min. During irradiation, approximately 3×10^7 GM459 cells were trypsinized and resuspended in serum-free DMEM as above. Recipient and irradiated donor cells were gently mixed and fused in the presence of PEG 1500. Eighty 100-mm dishes containing 10 ml of complete DMEM each were inoculated with 0.5 ml of the cell fusion mixture and incubated at 37°C. The medium was replaced the next day with HAS medium (complete DMEM plus 200 m*M* proline, 100 m*M* hypoxanthine, 10 m*M* azaserine) to initiate selection for $HPRT^+$ hybrids. Plates were incubated 2–4 weeks at 37°C with replacement of fresh HAS medium every 5–7 days. No colonies were observed in control plates containing only GM459 or irradiated 7AE-4 cells in HAS medium (3 plates each; ~10^6 cells/plate). Seventy-six HAS-resistant colonies were isolated using cloning cylinders and expanded. DNA was extracted from nearly confluent cultures and additional cells were harvested for storage in liquid nitrogen.

Polymerase chain reactions (PCR). Initial characterization of hybrids and generation of probe DNA for fluorescence *in situ* hybridization (FISH) relied on PCR-amplification of inter-*Alu* DNA segments using individual hybrid DNAs as template. Fifty-microliter reactions included 250 ng DNA, 1× *Taq* polymerase buffer (Boehringer-Mannheim), 200 μ*M* each dNTP, 1.0 m*M* *Alu* primer TC-65 (Nelson *et al.*, 1989) or *Alu-5′* and *Alu-3′* (Tagle and Collins, 1992) and 1.25 units *Taq* DNA polymerase (Boehringer-Mannheim). Following a 4-min 94°C denaturation, amplification proceeded for 30 cycles of: 94° (1 min), 55° (1 min), 72° (4 min), followed by a 7-min 72° extension. Amplification products (10 μl) were separated by electrophoresis on 1.2% agarose gels in 0.5× TBE.

Primer sequences for locus-specific sequence-tagged sites (STSs) were obtained from the following sources: D17S250 (Mfd15), *ERBB2* (*HER2/NEU/NGL*; avian erythroblastic leukemia viral oncogene homolog), *TOP2A* (topoisomerase II, α subunit), GAS (gastrin), *PPY* (pancreatic polypeptide), *EPB3* (erythrocyte surface protein band 3), *GP2B* (platelet glycoprotein IIb), *GIP* (gastric inhibitory peptide), and *NGFR* (nerve growth factor receptor) were all obtained from the proceedings of the Second International Workshop on Human Chromosome 17 Mapping (Fain *et al.*, 1991); D17S579 (Mfd188, Hall *et al.*, 1992); D17S588 (42D6, Easton *et al.*, 1993); *GP3A* (platelet glycoprotein IIIa, Stoffel and Bell, 1992); *NME1* (nm23-H1, nonmetastatic protein, Chandrasekharappa *et al.*, 1993); D17S190 (SCG41, Black *et al.*, 1993; Anderson *et al.*, 1993); BTR, an anonymous DNA segment isolated from near the end of a chromosomal rearrangement (A. Futreal, Research Triangle Park, NC, 1992, pers. communication); D17S855 (JW248yg9) and D17S856 (OF2) (Anderson *et al.*, 1993). The following STSs were designed from sequences deposited in the GenBank database or from published sequences: *THRA1* (thyroid hormone receptor α, Nakai *et al.*, 1988); *RNU2* (U2 small nuclear RNA, Westin *et al.*, 1984); *MTBT1* (microtubule β associated protein tau1, Goedert *et al.*, 1988); D17S409 (LL154, J. Chamberlain, Ann Arbor, MI, 1992, pers. communication); and *EDH17B* (estradiol 17-β dehydrogenase, Luu-The *et al.*, 1990). Twenty-five-microliter PCR reactions included 50–100 ng genomic DNA, 1× reaction buffer (Boehringer-Mannheim), 200 μ*M* each dNTP, 2–10 ng/μl each oligonucleotide primer, and 0.75–1.0 units *Taq* polymerase. The primer sequences, concentrations, and annealing temperatures for each STS are shown in Table 1. After denaturation, amplifications proceeded for 35 cycles of: 94° (1 min), annealing temperature (1 min), 72° (1 min), followed by a 5 min 72° extension. PCR reactions were performed using either a DNA Thermal Cycler (Perkin–Elmer–Cetus) or a PTC-100 Programmable Thermal Controller (MJ Research). Samples were analyzed on 1.5% NuSieve/1% agarose gels in 0.5× TBE.

Multipoint mapping of the radiation hybrid data. We analyzed the chromosome 17 RH mapping data using the multipoint maximum likelihood approach of Boehnke *et al.* (1991). For this purpose, we used the FORTRAN programs RH2PT and RHMAXLIK from the package RHMAP version 1.1 (Boehnke *et al.*, 1991). In our analysis, we assumed that γ-ray breakage occurred at random along the chromosome, and that the resulting fragments were independently retained in a RH. In the *N*-locus case, the likelihood of the RH data is a function of the *N*-1 breakage probabilities between adjacent loci and one or more fragment retention probabilities. Under the assumption of no interference, breakage probabilities θ can be converted to additive distances d according to the formula $d = -\ln(1 - \theta)$ (Cox *et al.*, 1990). Distances d are expressed in centirays (cR), where 100 $cR_{(8000)}$ corresponds to one expected break per hybrid after exposure to 8000 rads.

To describe the different fragment retention probability models, let r_{ij} be the probability of retaining a fragment on which only loci $i < i + 1 < \cdots < j$ are present. We considered two such models. In the first, all fragment retention probabilities were assumed to be equal ($r_{ij} = r$ for all $i \leq j$). In the second, we allowed for a centromeric effect by setting $r_{1j} = r_1$ and $r_{ij} = r_2$ $(1 < i)$. Use of this second model was suggested by the decrease in observed retention probabilities with increasing distance from the centromere (see Results). For each model, and for a given locus order, we estimated breakage and retention probabilities by those values that maximized the likelihood for the RH mapping data. Orders were compared by their maximum likelihoods, the order with the largest maximum likelihood being best supported by the data. For a given order, models were compared by likelihood ratio tests.

Since it was not practical to consider explicitly all 17!/2 (equal retention) or 17! (centromeric retention) > 10^{14} orders for the 17 distinguishable loci separated by at least one obligate chromosome break, we used a stepwise locus ordering algorithm to identify the best locus order (Boehnke *et al.*, 1991). This algorithm builds locus orders by adding one locus at a time. At each stage, it keeps under consideration those partial locus orders with maximum likelihood no more than K times smaller than the maximum likelihood for the best partial locus

order made up of the same loci. We carried out stepwise locus ordering for both the equal and centromeric models for the 17 distinguishable loci with $K = 10^9$. For each model, the locus order for all 17 distinguishable loci that had the largest maximum likelihood was the comprehensive map. To build a framework map of loci ordered at 1000:1 maximum likelihood ratio, we carried out stepwise locus ordering, adding loci until no additional locus could be added with maximum likelihood ratio of at least 1000:1 for its best position. Possible positions of the remaining loci within the framework were then determined by adding each such locus to the map and noting those locus orders with maximum likelihood ratio within 1000:1 of that for the locus' best position.

FISH analysis of radiation hybrids. Metaphase chromosomes were prepared from phytohemaglutinin (PHA)-stimulated peripheral blood lymphocytes of a healthy female according to standard procedures. *Alu*-PCR products from individual hybrids were labeled by nick-translation with biotin-14-dATP using a commercially available kit (GIBCO/BRL). Biotinylated probe DNAs (500 ng) were separated from unincorporated nucleotides using Sephadex G-50 spin columns, ethanol precipitated along with 10 μg human Cot-1 DNA (GIBCO/BRL), and resuspended in 10 μl of a hybridization mix containing 50% formamide, 10% dextran sulfate, 2× SSC (pH 7.0). Following denaturation and preannealing for 3 h at 37°C, probe hybridization to metaphase chromosomes and detection was performed as described (Lichter *et al.*, 1988). Chromosomes were counterstained in an antifade solution containing 200 ng/ml propidium iodide. Slides were viewed on a Zeiss Axioskop microscope equipped with FITC epifluorescence optics and were photographed with Kodak Ektachrome 400 color film.

RESULTS

RH Panel Construction and Initial Characterization

As noted previously (Cox *et al.*, 1990), the appropriate combination of monochromosomal hybrid (donor) and rodent recipient cell lines is crucial for successful RH panel construction. In our earlier attempts at generating radiation hybrids for chromosome 17, the donor/recipient pairs HDD3B/RJK88 (hamster) and MH22.6/A9 (mouse) produced few hybrids. The RH panel described here was generated using the cell lines 7AE-4 and GM459 (hamster), as only this combination yielded a large number of hybrid clones.

DNAs from the complete panel of 76 hybrids were examined by *Alu*-PCR, using the *Alu* primer TC-65 (Nelson *et al.*, 1989), to estimate the fraction of hybrid clones that retained human chromosome 17 sequences. Although there was no selection for chromosome 17 markers, 69 of the hybrids (91%) retained detectable human sequences by this assay (data not shown). The specificity of the primer for human sequences was confirmed by the absence of products in reactions containing either rat or hamster DNA, both of which are present in the hybrids. The hybrids displayed a range in the number of products generated, most likely reflecting different relative amounts of chromosome 17 retained per hybrid.

Alu-PCR products from several radiation hybrids were used as FISH probes to verify chromosomal fragmentation and determine whether the hybrids retained multiple fragments. PCR products, generated using primers *Alu-5'* and *Alu-3'* (Tagle and Collins, 1992), were biotin-labeled and hybridized to normal human metaphase chromosomes. As shown in Fig. 1a, *Alu*-PCR probes generated from the parental line 7AE-4 painted the entire lengths of both chromosome 17 homologs. Hybridization of *Alu*-PCR products from three RH clones was similarly limited to chromosome 17; however, these probes detected from two to six noncontiguous chromosomal regions (Figs. 1b–1d). These results provided support for breakage of chromosome 17 and retention of multiple chromosomal fragments in at least a portion of the hybrids.

PCR Analysis Using Chromosome 17q Markers

The complete RH panel (76 hybrids) was scored using PCR for the presence or absence of 22 markers flanking *BRCA1,* including 14 cloned genes, 7 microsatellite repeats, and 1 anonymous DNA segment. Table 1 identifies the locus and reaction conditions for each STS. The marker retention scores are shown in Table 2. All scores were performed in duplicate to help identify typing errors, and any discrepant scores were repeated at least once more. A total of 20 discrepancies that could not be resolved were left as untyped. Thirteen hybrids shared identical marker retention scores with other hybrids cloned from the same original culture dish and were considered likely to be daughter clones. Only data from the 63 clearly independent hybrids are shown in Table 2 and were included in the analysis which provided marker order and distance estimates. Partially typed hybrids can be included in this analysis, allowing for those instances in which a particular hybrid/marker combination could not be scored confidently.

Multipoint Analysis of the Radiation Hybrid Data

Marker retention data were analyzed using multipoint methods described in Boehnke *et al.* (1991). In the 63 clearly independent hybrids, no breaks were detected between loci *THRA1* and *TOP2A,* between loci *GIP* and *NGFR,* and between loci D17S579, *EPB3,* and *GP2B.* To simplify the computations, we excluded *THRA1, NGFR, EPB3,* and *GP2B* from the multipoint analysis. In this analysis, we carried out stepwise locus ordering for both the equal and the centromeric retention models. Both models gave the same best order for the 17 distinguishable loci. The centromeric model fit the data significantly better than the equal retention probability model ($\chi^2 = 6.69$, $df = 1$, $P < .01$), consistent with our observation of decreasing retention as a function of distance from the centromere. The best comprehensive map under the centromeric retention probability model is presented in Fig. 2, together with distance estimates between adjacent loci and observed locus retention frequencies. The map spans a distance of 179 $cR_{(8000)}$. Distance estimates under the equal retention model were very similar. The most likely order agrees well with orders determined using other methods (Fain, 1992; Hall *et al.*, 1992; Easton *et al.*, 1993) and with orders determined by FISH and genetic analysis of this region using many of these same STSs (Flejter *et al.*, 1993; Anderson *et al.*, 1993).

The framework map for these loci under the centro-

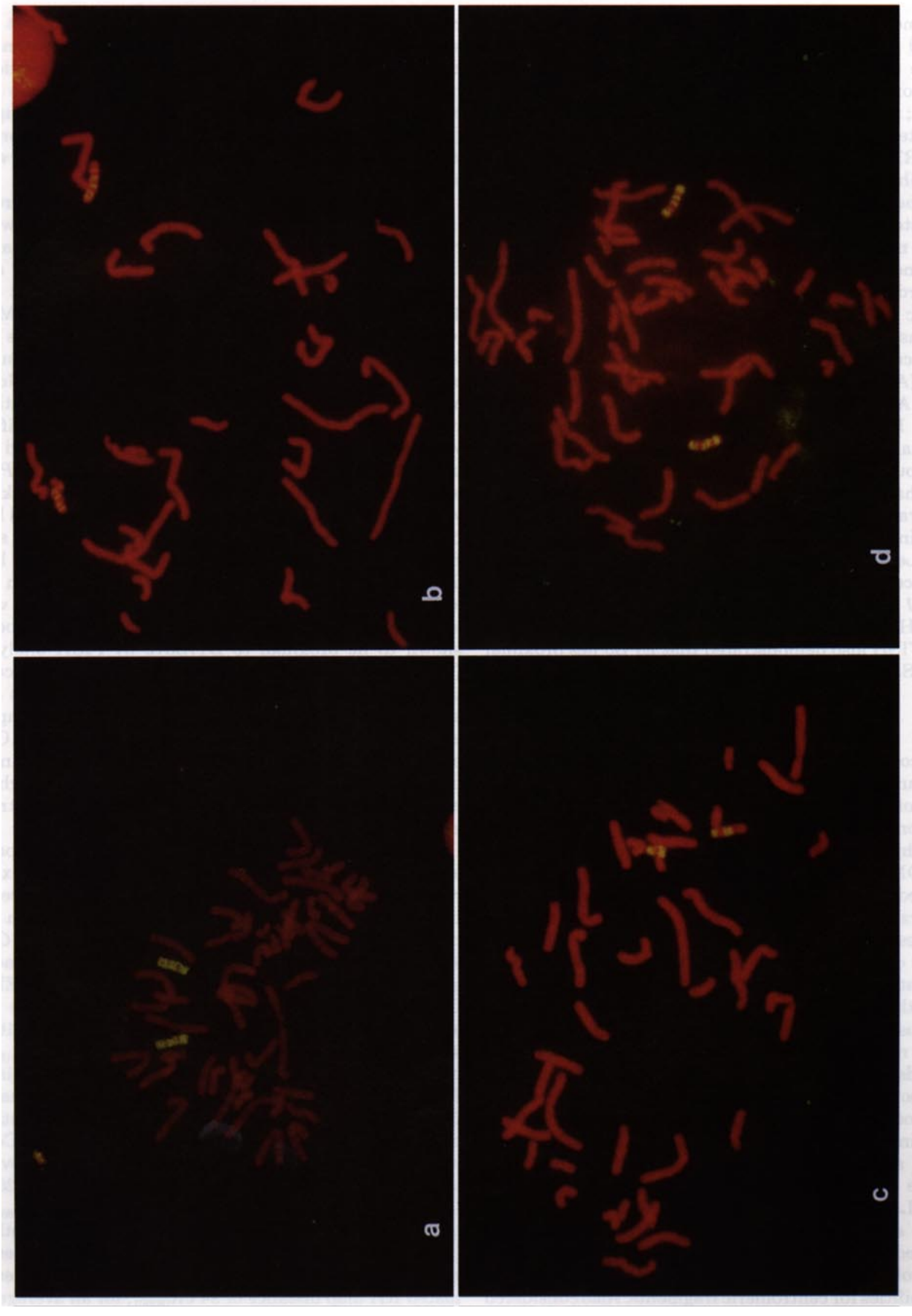

FIG. 1. FISH analysis of radiation hybrid (RH) derived *Alu*-PCR products. Genomic DNAs from the parental chromosome 17 donor cell line 7AE-4 and from several RHs were templates for *Alu*-PCR using the primers *Alu-5'* and *Alu-3'* (Tagle and Collins, 1992). Pooled reaction products were biotin-labeled and used as hybridization probes to normal human metaphase chromosomes. Hybridization of probe amplified from: **(a)** 7AE-4; **(b–d)** RH clones 11, 27a, and 29, respectively.

meric model is shown in Fig. 3. In this map, 10 loci are ordered at 1000:1 maximum likelihood ratio. Positions for the nonframework loci that are consistent with 1000:1 ordering within this framework map are also indicated; most of them can be placed in one of two adjacent intervals. The shorter length of the framework map (139 cR) relative to the corresponding portion of the comprehensive map (D17S250–D17S588, 165 cR) suggests the presence of undetected typing errors, despite duplicate typing and scoring.

Two marker pairs that could not be resolved by the RH panel (*THRA1/TOP2A* and *EPB3*/D17S579) have been ordered using different mapping approaches. The somatic cell hybrid, P12.3B (vanTuinen *et al.*, 1987) contains the derivative chromosome 17 of a t(15;17) translocation associated with acute promyelocytic leukemia (APL). *THRA1* was previously localized proximal to the APL breakpoint (Sheer *et al.*, 1985). More recently, PCR analysis of P12.3B DNA confirmed the proximal location for *THRA1* relative to the APL breakpoint, but indicated that *TOP2A* mapped distal (Chandrasekharrappa *et al.*, in preparation). Given that the APL translocation breakpoints have been localized to the retinoic acid receptor α(*RARA*) locus (Borrow *et al.*, 1990; Lemmons *et al.*, 1990), these results suggest the order: cen–*THRA1*–*RARA*–*TOP2A*–tel. The order for *THRA1* and *TOP2A* is consistent with that determined by FISH analysis (Flejter *et al.*, this issue). The analysis using FISH also demonstrated that *EPB3* lies proximal to D17S579.

DISCUSSION

We constructed an 8000-rad radiation-reduced hybrid panel and used it to order markers within the *BRCA1* region of human chromosome 17. Despite the absence of selection for chromosome 17 sequences, a large fraction of the hybrids (91%) were shown to have retained human DNA. The complete panel of 76 hybrids was screened using PCR for the presence or absence of 22 markers in the 17q12–q21 region. Marker retention frequencies ranged from 44 to 67%, with an average frequency of 55%. Although it is not known whether these observations are representative for the entire chromosome, they suggest that, on average, roughly half the chromosome may have been retained per hybrid.

The retention data were analyzed using multipoint methods which rely upon maximum likelihood. The analysis also determined order under different marker retention models and when different assumptions were made regarding hybrid independence. Data from several groups suggest that fragments nearer the centromere may be retained at higher frequencies (Benham *et al.*, 1989; Lawrence *et al.*, 1991; Ceccherini *et al.*, 1992; Gorski *et al.*, 1992). Essentially identical results were obtained under models allowing for either equal retention probabilities for all markers, or for higher retention probabilities for centromeric fragments. Also considered was the possibility that hybrids cloned from the same culture dish originally may not be independent, but instead may be daughter clones. The order and distances presented were based upon the 63 clearly independent hybrids. When the data from all 76 hybrids were included, the most likely orders were largely unchanged (although their relative rankings were altered somewhat), and the same framework order for 10 loci was supported at 1000:1 maximum likelihood ratio.

Statistical analyses also provided a most likely order including 17 loci. This order was in good agreement with recently proposed orders including many of these same markers and which derive from other mapping approaches (Easton *et al.*, 1993; Fain, 1992; Hall *et al.*, 1992; Flejter *et al.*, 1993; Anderson *et al.*, 1993). Map locations were determined for a number of markers for which little information was previously available, such as BTR, D17S190, *MTBT1*, D17S409, and *GP3A*. However, several markers could not be ordered by radiation hybrid analysis alone. Three regions were identified which included two or three markers not resolved by obligate breaks. Of these, the regions *THRA1/TOP2A* and *EPB3/GP2B*/D17S579 potentially contain markers within the *BRCA1* candidate interval. A somatic cell hybrid containing an APL translocation breakpoint and FISH analysis have been useful for ordering the loci *THRA1* and *TOP2A*, and for localizing *RARA* with respect to the RH maps. Although no rearrangement was available to subdivide the other group, *EPB3* has been shown to lie proximal to D17S579 by FISH analysis (Flejter *et al.*, 1993), and analysis of YAC clones containing *EPB3* and *GP2B* indicate that these genes are separated by no more than 130 kb (Chandrasekharappa *et al.*, in preparation). Thus, as observed previously (Cox *et al.*, 1990; Gorski *et al.*, 1992), radiation hybrids can be useful in the identification of those loci close enough to examine using higher resolution physical mapping strategies.

The complete RH map from D17S250 to *NME1* spans an estimated distance of 179 $cR_{(8000)}$. Although the exact relationship between $cR_{(8000)}$ and physical distance in this region is not clear, estimates can be made from genetic mapping data. The interval from D17S250 to *GIP* spans an estimated RH distance of 160 $cR_{(8000)}$ and a genetic distance of 8.3 cM (Hall *et al.*, 1992; NIH/CEPH Collaborative Mapping Group, 1992). Making the assumption that 1 cM is approximately equivalent to 1000 kb, we estimate that within the *BRCA1* region, 1 $cR_{(8000)}$ corresponds to about 50 kb. This estimate, determined for a relatively large region, is consistent with estimates for several other regions based upon similar radiation dosages (Cox *et al.*, 1990; Burmeister *et al.*, 1991). Consideration of smaller subregions, however, can provide very different estimates. The interval D17S250–D17S579 has estimated distances of 6 cM and 66 $cR_{(8000)}$, giving an average of about 90 kb/$cR_{(8000)}$. On the other hand, the adjacent interval D17S579–*GIP* has an estimated genetic distance of only 2 cM, yet has an estimated RH map distance of 94 $cR_{(8000)}$, for an average of about 20 kb/$cR_{(8000)}$. Although data exist suggesting that

TABLE 1

PCR Conditions for 17q12–q21 STSs

Locus	Primer sequences	PCR product (bp)	Primer conc. (ng/μl)	Anneal temp. (degrees C)
BTR	TGCAAGTGCACAGTGTCAGC GGATGTGTGGACCCTTTCTC	~150	2	63
D17S190	ACCTGACCCATCTCTCTGTC GGTGACCTGTCATATGCACG	~110	2	65
D17S250	GGAAGAATCAATAGACAAT GCTGGCCATATATATATTTAAAACC	~165	10	55
D17S409	TTTCTAGAACCCGATCCACG TGCCCTTCACTCTCGACTG	~170	2	55
D17S579	AGTCCTGTAGACAAAACCTG CAGTTTCATACCAAGTTCCT	~120	10	60
D17S588	CCTGGTCTAGGAAGAGTGTCA GTGTAAGCATCTGTGTATACTAC	156	2	60
D17S855	GGATGGCCTTTTAGAAAGTGG ACACAGACTTGTCCTACTGCC	145	2	55
D17S856	AAGGCAAGACTTCGTCGAGA CATTCCCTGGTCCTGTGC	260	2	63
EDH17B	AAAGAAGCCAGCCCAGGATCAC GGTCTCATGGCTAATGGGGTCC	150	2	65
EPB3	AGGGGCACCTGTGTTTAAGCAG TCCACTGCCTGCAGGTACTGT	158	10	66
ERBB2	CTGGAATGGGAAGCA GCCAGCAAAGAAATCTTAGACGT	~1100	10	60
GAS	ATGCTAGTCGGTGTAGAGCCATG TTGTACCTCATAGGGCTGCGTGA	297	10	67
GIP	CTTGCTGGATCAGACAAACCT CACAATGGGCTCGACTTAGCATAA	~1960	2	65
GP2B	TCTCCGTCTTCTGTTACACCT CCTACACTATTCTAGCAGGAGGGT	361	10	58
GP3A	GCTAAATCATCCTTAGCCTTC AGCCATTGCTCCTAGTGGAG	~165	2	58
MTBT1	CAGCAAGCCCAGTGCAATAC TGATCTCTTCTTTGGCCAGC	155	10	65
NGFR	TTGTGTGTAAGTTTCAGGAGGGCC GAAGGGACTAGGAGCACTGTAGTA	348	10	64
NME1	TTAATCAGATGGTCGGGGAT GATCTATGAATGACAGGAGG	186	2	60
PPY	CCTTTCTCTCCCATGCATTTGGCA TACACTGCCATGTTCTGCCCTGT	258	10	64
RNU2	GAAGAAGCACGGGTGTAAGA TCCCACTCTCATCCACATTC	160	10	60
THRA1	TGCTGATGAAGGTGACTGAC CACACTGTCCTTTCCATAGC	359	10	67
TOP2A	GGGAGAGTGATGACTTCCATATGGA AACACCTTCCCCAAACTAAATTCAG	229	10	60

Note. Locus identification, sources of STSs, and thermal cycling programs are detailed under Materials and Methods.

radiation breaks are uniform on the physical map (Cox *et al.*, 1990; Burmeister *et al.*, 1991), it is possible that nonrandom distribution of breaks will be observed when small intervals are considered. Alternatively, since these estimates have a basis in genetic distances, it is also possible that these differences reflect variation in the fre-

TABLE 2

Retention Scores for Markers Flanking *BRCA1*

	Loci[b]																					
RH[a]	250	ERB	THR	TOP	GAS	856	EDH	855	RNU	PPY	EPB	GP2	579	190	MTB	409	GP3	BTR	GIP	NGF	588	NME
1	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
5	+	−	−	0	−	0	+	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
8a	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	~	−	−	−	−
8b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	~	−	−	−	−
12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+
16a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	0	−	~	−	−	−	~
16b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
17	−	−	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
18a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	~	−	−	+	+
18c	+	−	0	−	−	0	−	−	+	+	+	+	+	+	+	0	+	0	+	0	−	+
19	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
20a	+	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
20b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
21a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	~	−	−	−	~
22a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	+	+	+	+
22b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	~	−	−	−	~
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	~
24	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
25a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	~	−	−	−	~
26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27a	+	−	0	+	−	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	~
27c	−	−	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	+	+	+	+
29	−	−	0	−	−	−	−	−	−	+	+	+	+	+	+	+	−	~	−	−	−	~
31	+	−	0	−	−	−	−	−	0	−	+	+	+	+	+	−	+	+	+	0	+	+
32b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
42a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
43	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
44a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~	−	−	−	~
49b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
49d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
54	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~
55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	~	−	−	−	0
57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
61	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
64a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	~
66	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	~
67	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
68	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	−	+	+
70	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
71	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
72b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+
72c	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	−	−	+	+	+	+
73	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
74b	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
75	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
76a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+

Note. Symbols indicate that the locus was typed and either present (+), absent (−), or could not be typed (0).

[a] RH, radiation hybrids. Scores for only the 63 clearly independent hybrids are shown.

[b] All numbered loci have the prefix D17S. ERB, *ERBB2*; THR, *THRA1*; TOP, *TOP2A*; EDH, *EDH17B*; RNU, *RNU2*; EPB, *EPB3*; GP2, *GP2B*; MTB, *MTBT1*; GP3, *GP3A*; NGF, *NGFR*.

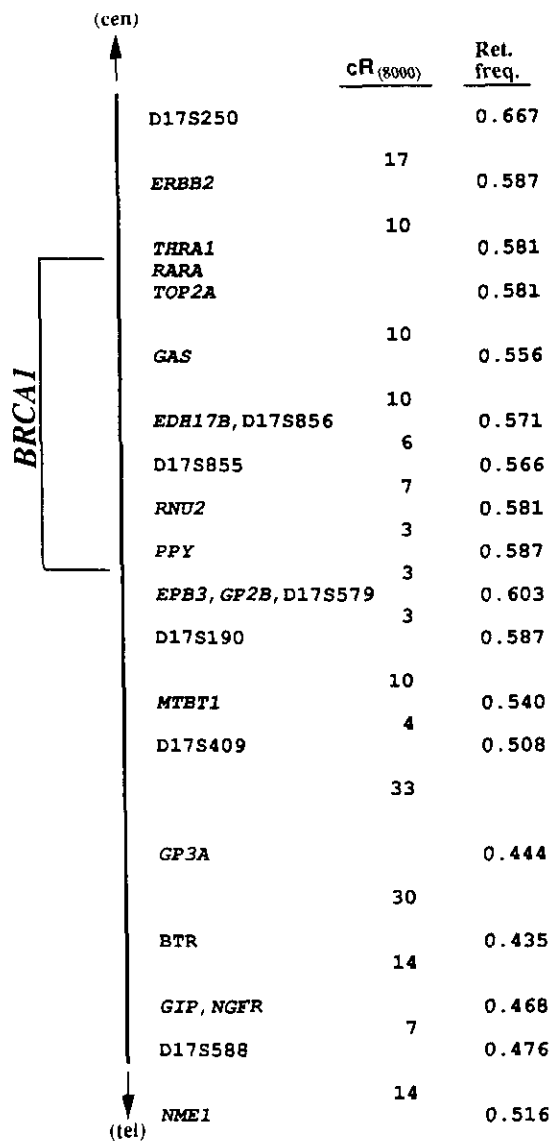

FIG. 2. Comprehensive RH map of the *BRCA1* region of chromosome 17q12–q21. The most likely order for all tested loci determined by multipoint maximum likelihood analysis of the marker retention data is shown. Estimated distances in $cR_{(8000)}$ between adjacent loci and marker retention frequencies (Ret. freq.) are also shown. The region considered most likely to contain *BRCA1* by genetic linkage analysis (Bowcock *et al.*, 1993; Chamberlain *et al.*, 1993) is shown to the left. The loci *THRA1* and *TOP2A* could not be ordered by our panel of radiation hybrids; the order shown is based upon mapping these markers using FISH (Flejter *et al.*, 1993) and relative to a translocation breakpoint at the *RARA* locus (Chandrasekharappa *et al.*, in preparation). Otherwise, *RARA* was not included in the RH analysis and is thus not regarded as a component of the comprehensive RH map.

quency of genetic recombination in the two subregions, or simply reflect random experimental variability in any of the distance estimates. The length of the interval most likely to contain *BRCA1* (*THRA1*–D17S579) is estimated to be only 39 $cR_{(8000)}$. Using the relationship of 50 kb/$cR_{(8000)}$, the size of this critical interval is estimated to be roughly 2000 kb, although allowance for potential variation in this relationship suggests that the estimated size could range between 800 and 3500 kb.

RH mapping, particularly when combined with other mapping techniques, thus represents a powerful tool for analyzing large chromosomal regions. The overall consistency between our RH maps and other reports suggests that this panel will be a valuable resource for the rapid mapping of new markers which derive from chromosome 17q12–q21. Screening the panel with a new marker and obtaining likelihood ratios for candidate intervals on the framework map are possible within several days. We have found that the use of a small subset of carefully chosen hybrids can now be extremely useful for excluding from further analysis those markers which do not derive from the well-characterized region. The order of genes and polymorphic markers obtained from RH mapping will assist in efforts both to narrow the critical region containing *BRCA1* and to exclude candidate disease loci. Also, knowledge of marker order and distance estimates will facilitate the development of a physical map of this region, including the construction of contigs of cloned genomic DNA segments. It is anticipated that with further characterization, this panel should also provide a useful resource for mapping other regions of human chromosome 17.

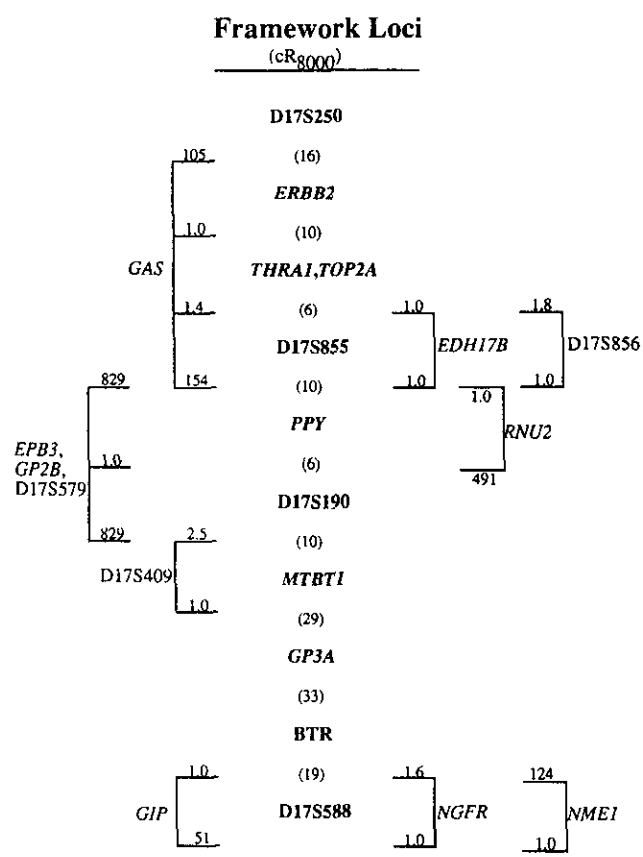

FIG. 3. Framework RH map of the region 17q12–q21. Likelihood ratios of at least 1000:1 support the order shown for the 10 loci in bold type. Numbers in parentheses indicate the estimated distances in $cR_{(8000)}$ between adjacent framework loci. The likelihood estimates for placement of the remaining markers within the framework map are also shown. A value of 1 identifies the most likely interval to contain a particular marker; values for other candidate intervals are the likelihood ratios against localization of that marker in each respective interval.

ACKNOWLEDGMENTS

We thank J. Chamberlain, A. Futreal, D. Black, M.-C. King, and J. Weissenbach for contributing STS primer sequences prior to publication., R. Leach and D. Cox for providing cell lines, and J. Gorski for helpful discussions. This work was supported by grants from the NIH (CA57601 to B.L.W., HG00209 to F.S.C.) and from the Rackham School of Graduate Studies (B.L.W.). F.S.C. is an investigator, and K.J.A. was a postdoctoral associate, of the Howard Hughes Medical Institute.

REFERENCES

Anderson, L. A., Friedman, L., Osborne-Lawrence, S., Lynch, E., Weissenbach, J., Bowcock, A., and King, M.-C. (1993). High-density genetic map of the BRCA1 region of chromosome 17q12–q21. *Genomics* **17:** 618–631.

Benham, F., Hart, K., Crolla, J., Bobrow, M., Francavilla, M., and Goodfellow, P. N. (1989). A method for generating hybrids containing nonselected fragments of human chromosomes. *Genomics* **4:** 509–517.

Black, D. M., Nicolai, H., Borrow, J., and Solomon, E. (1993). A somatic cell hybrid map of the long arm of human chromosome 17 containing the familial breast cancer locus (BRCA1). *Am. J. Hum. Genet.* **52:** 702–710.

Boehnke, M., Lange, K., and Cox, D. R. (1991). Statistical methods for multipoint radiation hybrid mapping. *Am. J. Hum. Genet.* **49:** 1174–1188.

Borrow, J., Goddard, A. D., Sheer, D., and Solomon, E. (1990). Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. *Science* **249:** 1577–1580.

Bowcock, A. M., Anderson, L. A., Friedman, L. S., Black, D. M., Osborne-Lawrence, S., Rowell, S. E., Hall, J. M., Solomon, E., and King, M.-C. (1993). *THRA1* and D17S183 flank an interval of <4 cM for the breast-ovarian cancer gene (BRCA1) on chromosome 17q21. *Am. J. Hum. Genet.* **52:** 718–722.

Brook, J. D., Zemelman, B. V., Hadingham, K., Siciliano, M. J., Crow, S., Harley, H. G., Rundle, S. A., Buxton, J., Johnson, K., Almond, J. W., Housman, D. E., and Shaw, D. J. (1992). Radiation-reduced hybrids for the myotonic dystrophy locus. *Genomics* **13:** 243–250.

Burmeister, M., Kim, S., Price, E. R., deLange, T., Tantravahi, U., Myers, R. M., and Cox, D. R. (1991). A map of the distal region of the long arm of human chromosome 21 constructed by radiation hybrid mapping and pulsed-field gel electrophoresis. *Genomics* **9:** 19–30.

Ceccherini, I., Romeo, G., Lawrence, S., Breuning, M. H., Harris, P. C., Himmelbauer, H., Frischauf, A. M., Sutherland, G. R., Germino, G. G., Reeders, S. T., and Morton, N. E. (1992). Construction of a map of chromosome 16 by using radiation hybrids. *Proc. Natl. Acad. Sci. USA* **89:** 104–108.

Chamberlain, J. S., Boehnke, M., Frank, T. S., Kiousis, S., Xu, J., Guo, S.-W., Hauser, E. R., Norum, R. A., Helmbold, E. A., Markel, D. S., Keshavarzi, S. M., Jackson, C. E., Calzone, K., Garber, J., Collins, F. S., and Weber, B. L. (1993). BRCA1 maps proximol to D17S579 on chromosome 17q21 by genetic analysis. *Am. J. Hum. Genet.* **52:** 792–798.

Chandrasekharappa, S. C., Gross, L. A., King, S. E., and Collins, F. S. (1993). The human *NME2* gene lies within 18 kb of *NME1* on chromosome 17. *Genes, Chrom., Cancer* **6:** 245–248.

Claus, E. B., Risch, N. J., and Thompson, W. D. (1990). Age at onset as an indicator of familial risk of breast cancer. *Am. J. Epidemiol.* **131:** 961–972.

Claus, E. B., Risch, N., and Thompson, W. D. (1991). Genetic analysis of breast cancer in the Cancer and Steroid Hormone study. *Am. J. Hum. Genet.* **48:** 232–241.

Cox, D. R., Burmeister, M., Price, E. R., Kim, S., and Myers, R. M. (1990). Radiation hybrid mapping: A somatic cell genetic method for constructing high-resolution maps of mammalian chromosomes. *Science* **250:** 245–250.

Easton, D. F., Bishop, D. T., Ford, D., and Crockford, G. P., and the Breast Cancer Linkage Consortium (1993). Genetic linkage analysis in familial breast and ovarian cancer—Results from 214 families. *Am. J. Hum. Genet.* **52:** 678–701.

Fain, P. R., Ledbetter, D. H., and Solomon, E. (1991). Second international workshop on human chromosome 17 mapping. *Cytogenet. Cell Genet.* **57:** 65–77.

Fain, P. (1992). Third international workshop on chromosome 17 mapping. *Cytogenet. Cell Genet.* **60:** 177–186.

Flejter, W. L., Bancroft, C. L., Guo, S-W, Lynch, E. D., Boehnke, M., Chandrasekharappa, S., Hayes S., Collins, F. S., Weber, B. L., and Glover, T. W. (1993). Multicolor FISH mapping with *Alu*-PCR-amplified YAC clone DNA determines the order of markers in the *BRCA1* region on chromosome 17q12–q21. *Genomics* **17:** 624–631.

Glaser, T., Rose, E., Morse, H., Housman, D., and Jones, C. (1990). A panel of irradiation-reduced hybrids selectively retaining human chromosome 11p13: Their structure and use to purify the *WAGR* gene complex. *Genomics* **6:** 48–64.

Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., and Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: Identification as the microtubule-associated protein tau. *Proc. Natl. Acad. Sci. USA* **85:** 4051–4055.

Gorski, J. L., Boehnke, M., Reyner, E. L., and Burright, E. N. (1992). A radiation hybrid map of the proximal short arm of the human X chromosome spanning incontinentia pigmenti 1 (IP1) translocation breakpoints. *Genomics* **14:** 657–665.

Hall, J. M., Lee, M. K., Morrow, J., Newman, B., Anderson, L., Huey, B., and King, M.-C. (1990). Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* **250:** 1684–1689.

Hall, J. M., Friedman, L., Guenther, C., Lee, M. K., Weber, J. L., Black, D. M., and King, M.-C. (1992). Closing in on a breast cancer gene on chromosome 17q. *Am. J. Hum. Genet.* **50:** 1235–1242.

Lawrence, S., Morton, N. E., and Cox, D. R. (1991). Radiation hybrid mapping. *Proc. Natl. Acad. Sci. USA* **88:** 7477–7480.

Leach, R. J., Thayer, M. J., Schafer, A. J., and Fournier, R. E. K. (1989). Physical mapping of human chromosome 17 using fragment-containing microcell hybrids. *Genomics* **5:** 167–176.

Lemmons, R. S., Bilender, D., Waldmann, R. A., Rebentisch, M., Frej, A. K., Ledbetter, D. H., Willman, C., McConnell, T., and O'Connell, P. (1990). Cloning and characterization of the t(15;17) translocation breakpoint region in acute promyelocytic leukemia. *Genes, Chrom., Cancer* **2:** 79–87.

Lichter, P., Cremer, T., Borden, J., Manuelidis, L., and Ward, D. C. (1988). Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. *Hum. Genet.* **80:** 224–234.

Luu-The, V., Labrie, C., Simard, J., Lachance, Y., Zhao, H.-F., Couet, J., Leblanc, G., and Labrie, F. (1990). Structure of two in tandem human 17 β-hydroxysteroid dehydrogenase genes. *Mol. Endocrinol.* **4:** 268–275.

Nakai, A., Sakurai, A., Bell, G. I., and DeGroot, L. J. (1988). Characterization of a third human thyroid hormone receptor coexpressed with other thyroid hormone receptors in several tissues. *Mol. Endocrinol.* **2:** 1087–1092.

Narod, S. A., Feunteun, J., Lynch, H. T., Watson, P., Conway, T., Lynch, J., and Lenoir, G. M. (1991). Familial breast-ovarian cancer locus on chromosome 17q12–q23. *Lancet* **338:** 82–83.

Nelson, D. L., Ledbetter, S. A., Corbo, L., Victoria, M. F., Ramirez-Solez, R., Webster, T. D., Ledbetter, D. H., and Caskey, C. T. (1989). Alu polymerase chain reaction: A method for rapid isolation of hu-

man-specific sequences from complex DNA sources. *Proc. Natl. Acad. Sci. USA* **86:** 6686–6690.

Newman, B., Austin, M. A., Lee, M., and King, M.-C. (1988). Inheritance of human breast cancer: evidence for autosomal dominant transmission in high risk families. *Proc. Natl. Acad. Sci. USA* **85:** 1–5.

NIH/CEPH Collaborative Mapping Group (1992). A comprehensive genetic linkage map of the human genome. *Science* **258:** 67–86.

Pritchard, C. A., Casher, D., Uglum, E., Cox, D. R., and Myers, R. M. (1989). Isolation and field-inversion gel electrophoresis analysis of DNA markers located close to the Huntington disease gene. *Genomics* **4:** 408–418.

Sattin, R. W., Rubin, G. L., Webster, L. A., Huezo, C. M., Wingo, P. A., Ory, H. W., Layde, P. M., and the Cancer and Steroid Hormone Study (1985). Family history and the risk of breast cancer. *JAMA* **253:** 1908–1913.

Sheer, D., Sheppard, D. M., LeBeau, M., Rowley, J. D., San Roman, C., and Solomon, E. (1985). Localization of the oncogene c-erbA1 immediately proximal to the acute promyelocytic leukemia breakpoint on chromosome 17. *Ann. Hum. Genet.* **49:** 167–171.

Solomon, E., and Ledbetter, D. H. (1991). Report of the committee on the genetic constitution of chromosome 17. *Cytogenet. Cell Genet.* **58:** 686–738.

Stoffel, M., and Bell, G. I. (1992). Microsatellite polymorphism in the human platelet glycoprotein IIIa gene (GP3A) on chromosome 17. *Nucleic Acids Res.* **20:** 1172.

Tagle, D. A., and Collins, F. S. (1992). An optimized *Alu*-PCR primer pair for human-specific amplification of YACs and somatic cell hybrids. *Hum. Mol. Genet.* **1:** 121–122.

vanTuinen, P., Rich, D. C., Summers, K. M., and Ledbetter, D. H. (1987). Regional mapping panel for human chromosome 17: Application to neurofibromatosis type 1. *Genomics* **1:** 374–381.

Warrington, J. A., Bailey, S. K., Armstrong, E., Aprelikova, O., Alitalo, K., Dolganov, G. M., Wilcox, A. S., Sikela, J. M., Wolfe, S. F., Lovett, M., and Wasmuth, J. J. (1992). A radiation hybrid map of 18 growth factor, growth factor receptor, hormone receptor, or neurotransmitter receptor genes on the distal region of the long arm of chromosome 5. *Genomics* **13:** 803–808.

Westin, G., Zabielski, J., Hammarstroem, K., Monstein, H.-J., Bark, C., and Pettersson, U. (1984). Clustered genes for human U2 RNA. *Proc. Natl. Acad. Sci. USA* **81:** 3811–3815.