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Characterization of 10 New Polymorphic Dinucleotide Repeats and Generation of a High-Density Microsatellite-Based Physical Map of the BRCA1 Region of Chromosome 17q21

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A familial early onset breast cancer gene (BRCA1) has been localized to chromosome 17q21. To aid in the identification of this gene a number of new microsatellite markers from the D17S857 to D17S78 region were isolated and characterized. These markers, along with previously published markers from the region, were localized on a physical map by STS content mapping of cosmids from the BRCA1 interval. This high-density STS map of the BRCA1 region will be useful for linkage studies of families with apparent inherited breast cancer and for loss of heterozygosity analysis of breast tumor DNAs.

INTRODUCTION

Breast cancer is a leading cause of death among women, afflicting over 170,000 individuals per year in the United States (Silverberg and Boring, 1990). Family history of breast cancer has been identified as a major risk factor in the development of early onset breast cancer. Several reports have suggested that the increased risk of early onset breast and ovarian cancer in some families is due to an autosomal dominant susceptibility allele (Newman *et al.*, 1988; Claus *et al.*, 1991). Chromosome 17q12–q21 has recently been identified as the site of a familial early onset breast and ovarian cancer susceptibility gene (BRCA1) (Hall *et al.*, 1990; Narod *et al.*, 1991). A collaborative study utilizing 214 breast cancer families determined that breast/ovarian cancer was linked to markers in this region in greater than 90% of families with at least one case of ovarian cancer along with breast cancer. However, linkage between breast cancer and 17q12–q21 markers was observed in just 45% of families with breast cancer only (Easton *et al.*, 1993).

Examination of recombination events in families demonstrating linkage between breast cancer and genetic markers in the 17q12–q21 region has suggested that the BRCA1 gene is located between D17S857 and D17S78, a region estimated to be 1–1.5 Mb in length (Kelsell *et al.*, 1993; Simard *et al.*, 1993). Currently, the published genetic map of the BRCA1 region, located between D17S857 and D17S78, contains 10 polymorphic markers (Anderson *et al.*, 1993). Two distinct pairs of these markers can be regarded as single loci because of their close association: 17β-estradiol dehydrogenase genes (EDH17B1 and EDH17B2) have been localized within a 13-kb genomic DNA fragment (Friedman *et al.*, 1993), and two other markers, PPY and D17S78, have been localized within a 45-kb genomic DNA fragment (Chandrasekharappa *et al.*, 1994). Several of these markers also display low levels of heterozygosity. Two of these (PPY and D17S78) (Human Gene Mapping 11, 1991) are non-PCR-based restriction fragment length polymorphisms (RFLPs).

Loss of heterozygosity (LOH) of a marker in tumor DNAs is thought to reflect the presence of a tumor suppressor gene in the region of the marker. The loss of the wildtype allele of a marker in a tumor sample compared to allelic heterozygosity in the normal DNA may be detected by Southern blotting or PCR amplification. This method has recently been successfully used to identify the p16 tumor suppressor gene (Kamb *et al.*, 1994). LOH has also been observed across the BRCA1 region in breast tumor DNAs using markers flanking the candidate interval (Smith *et al.*, 1992). Further reduction of the size of the BRCA1 region by LOH will require the identification of small regions of allelic loss within the BRCA1 interval. Identification of informa-

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TABLE 1

Characteristics of 10 New Polymorphic Markers from the BRCA1 Region

Locus	Name	Primers	Type	Size (bp)	% heterozygosity	Annealing temp. (°C)
D17S1138	UM39	ACTTGAACAAGTCATTAGCCTCCC GTGCTTAGCCAGGCACTGTG	CA	175	75	62
D17S1139	UM40	CTGGTAACAATGTTCGCATTCTG CCTGGAAAATGCTCAAACTGC	CA	151	54	62
D17S1140	UM64	ATTATAGGTGTGTGCCACCACG CTGGGGAACAGAGCAAGACC	CA	182	65	60
D17S1141	UM44	GCACTCTTTGAGATTTCACCT GATCCCCCCCAAAAGTAAATG	CA	108	15	60
D17S1142	UM45	GTTGTCTGGCTTAACACCGCG CTGTCTCAGCCCTCCCAAAGTG	CA	107	44	62
D17S1143	UM41	ACACCATAACGTGCAAACACG GTTTCTGTGCATGGTGGCAC	CA	97	37	58
D17S1144	UM46	GCCCGCCTCGACCTCCCCAAAG CCTTGGGGTGGAGGTGCTGAAT	CA	196	68	60
D17S1145	UM42	CCTCCAGCCTGGCAATAGAC TCACTCAAAGAGTGCAATTCAATG	CA	205	34 or 88	55
D17S1146	UM47	GATCCAAAACTAAAGCTATTATAC GTAACAGCGTGAGACCTTGTC	CA	125	85	52
D17S1147	UM43	AGGTGCTGACTCTGGCTCTTC GCCACGTCTTTTCTGTGTTCC	CA	146	71	62

tive recombinants within the BRCA1 region in linked families may also reduce the size of the BRCA1 interval. However, both linkage and LOH studies in the BRCA1 interval have been limited by the lack of a dense set of well-ordered, highly polymorphic markers.

To define the BRCA1 region more precisely by either LOH or linkage studies, we developed an additional 10 PCR-based polymorphic markers from the BRCA1 region. Furthermore, we have localized these markers and several other recently published markers (Albertsen *et al.,* 1994a; Weissenbach *et al.,* 1993) on the physical map, resulting in the integration of the sequence tagged site (STS)-based physical map and the microsatellite-based genetic map of the BRCA1 region.

MATERIALS AND METHODS

Construction of the physical map. The known STSs D17S856, EDH17B1, EDH17B2, D17S855, RNU-2, D17S859, D17S858, and D17S78 were used to isolate clones from the CEPH and Washington University yeast artificial chromosome (YAC) libraries by PCR-based analysis (Couch *et al.,* submitted). All sequences are available through GenBank and GDB. Additional clones were identified using information from Albertsen *et al.* (1994b). The YAC 22HE5 was obtained from the ICI library, and the YACs 167B7, 173B7, and 300C2 were obtained from the CEPH library. All YACs were assembled into a contig by PCR-based STS content mapping as shown in Fig. 1. Newly isolated polymorphic STSs were further utilized to identify and to isolate YAC clones by PCR screening to generate a greater density of YACs and to bridge YAC gaps.

Identification of dinucleotide repeats. Yeast artificial chromosomes isolated by PCR with STSs from the BRCA1 region were used to isolate cosmids from the Los Alamos chromosome 17-specific flow-sorted cosmid library provided by L. Deaven. The markers D17S1138–D17S1147 were identified by screening 400 cosmids from the BRCA1 region with an oligonucleotide probe containing the repeat sequence $(CA)_{22}$. Cosmids were digested with *Eco*RI, the fragments were separated on 1% agarose gels, and the DNA was Southern blotted. Membranes were hybridized with 100 ng of the $(CA)_{22}$ oligonucleotide end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Hybridization was performed for 12 h at 65°C in 6× SSC, 0.5% SDS, 2× Denhardt's, 25% formamide, and 100 μg/ml of denatured salmon sperm DNA. Membranes were washed twice for 30 min at 65°C in 2× SSC, 0.1% SDS. Cosmids containing fragments that hybridized strongly with $(CA)_{22}$ were digested with *Sau*3AI and subcloned into pBluescript II KS(+) (Stratagene). Replica plated membranes of the *Sau*3AI transformants were prepared and hybridized with the $(CA)_{22}$ probe. Clones that hybridized with ^{32}P-labeled $(CA)_{22}$ were sequenced using a Sequenase kit (US Biochemicals) for the development of PCR primers. Primers were selected from nonrepetitive regions using the program PRIMER, version 0.5 (1991, Whitehead Institute).

Characterization of dinucleotide repeats. PCR conditions were developed for each of the 10 polymorphic markers. Annealing temperatures are shown in Table 1. The PCR-based markers were then genotyped on a panel of 160 unrelated CEPH parent DNAs. Amplification of all microsatellite repeats was carried out in 10-μl volumes of 1× Cetus buffer containing 4 ng forward primer, 4 ng ^{32}P-end-labeled reverse primer, 1.5 m*M* $MgCl_2$, 200 μ*M* each dNTP, 10–50 ng of human genomic DNA template, and 0.1 units of *Taq* polymerase on a programmable thermal controller (MJ Research). Reactions were denatured at 94°C for 3 min followed by 35 cycles of 94°C for 30 s, the specific annealing temperature for 30 s, and 72°C for 30 s. Alleles were separated on 6% denaturing polyacrylamide sequencing gels and visualized by autoradiography. Allele sizes and frequencies for the microsatellites were estimated from genotypes of the panel of CEPH parents.

Mapping the dinucleotide repeats. YACs and cosmids from the BRCA1 region that had been assembled into contigs were used as template for PCR amplification with microsatellite oligonucleotide primers. Amplification was performed in 25-μl reaction volumes containing buffer as stated above, 100 ng of each cosmid or YAC DNA as reaction template, 1 unit of *Taq* polymerase, and primers at 150 ng per reaction. PCR reactions for the markers D17S1138–D17S1147 were carried out under the temperature conditions shown in Table 1. Conditions for the markers D17S648, D17S750, D17S652, D17S902, and D17S965 were as previously published (Albertsen *et*

TABLE 2

Allele Size and Frequency of 10 New Polymorphic Markers

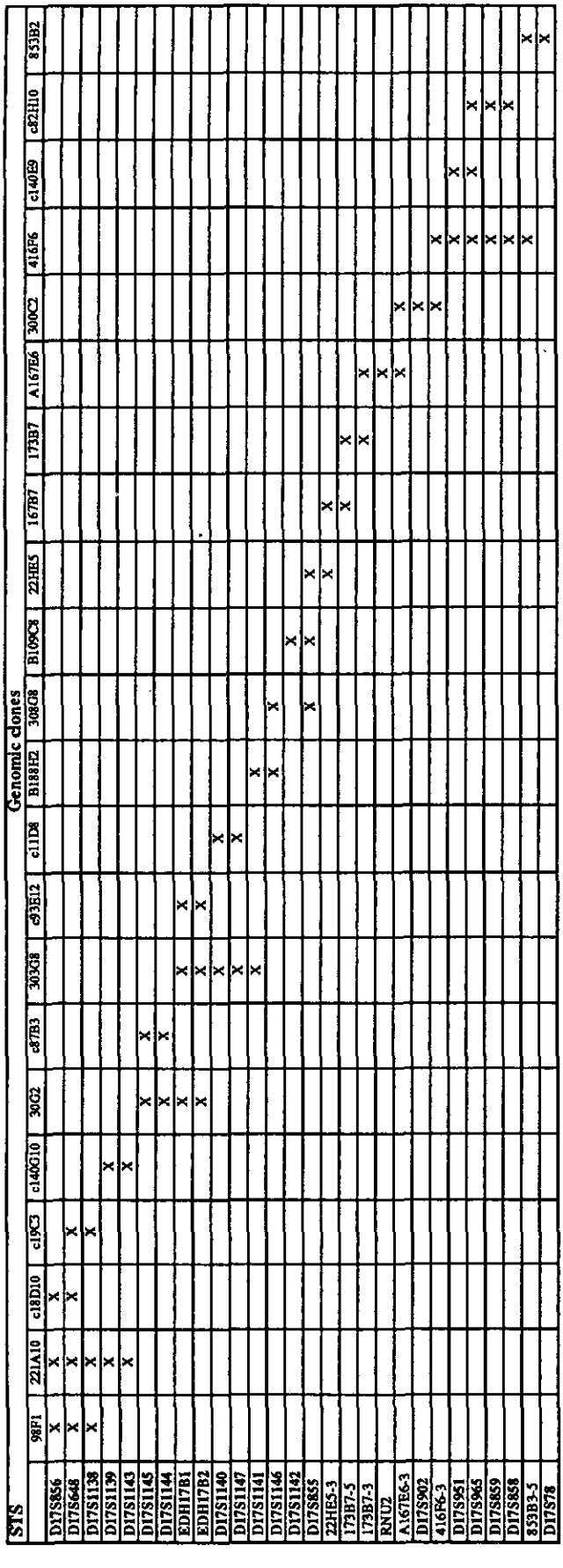
Locus	Alleles	Basepairs	Frequency	Basepairs	Frequency
D17S1138	8	183	0.0072	175	0.3913
		181	0.0217	173	0.1014
		179	0.0724	171	0.0072
		177	0.3550	169	0.0434
D17S1139	6	155	0.0562	149	0.0250
		153	0.1188	147	0.0438
		151	0.6625	145	0.0938
D17S1140	5	190	0.0062	184	0.4625
		188	0.0250	182	0.0313
		186	0.4750		
D17S1141	4	110	0.0015	106	0.0385
		108	0.8590	104	0.0641
D17S1142	10	127	0.0064	117	0.0383
		125	0.0766	115	0.0064
		123	0.1075	109	0.0511
		121	0.0319	107	0.6519
		119	0.0192	105	0.0064
D17S1143	6	101	0.0062	95	0.0188
		99	0.1062	93	0.0750
		97	0.7875	91	0.0062
D17S1144	10	210	0.0067	200	0.1067
		208	0.0600	198	0.0400
		206	0.2200	196	0.2533
		204	0.0667	194	0.1333
		202	0.0933	192	0.0200
D17S1145	CEPH	207	0.0857	203	0.0571
	4	205	0.7857	201	0.0714
	Random	221	0.0200	211	0.0400
	10	219	0.0400	209	0.1200
		217	0.0200	207	0.0400
		215	0.2000	205	0.3000
		213	0.0600	203	0.1600
D17S1146	17	149	0.0072	130	0.0072
		143	0.0072	129	0.0869
		141	0.0072	128	0.0507
		137	0.0289	127	0.1087
		135	0.0797	125	0.1667
		134	0.0072	124	0.0579
		133	0.2174	123	0.0362
		132	0.0145	119	0.0072
		131	0.1087		
D17S1147	9	150	0.0063	140	0.0570
		148	0.0253	138	0.2975
		146	0.0570	136	0.0380
		144	0.0696	118	0.0063
		142	0.4430		

al., 1994a) and similarly for the markers D17S857 (Anderson *et al.*, 1993), GAS (Human Gene Mapping 11, 1991), D17S856, D17S855, D17S858, D17S859 (Anderson *et al.*, 1993), D17S951 (Weissenbach *et al.*, 1993), and EDH17B1 and 2 (Friedman *et al.*, 1993). PCR products were separated on 2% agarose gels, stained with ethidium bromide, and visually scored for the presence or absence of individual microsatellites in each cosmid and YAC.

RESULTS

Ten polymorphic microsatellites were identified and developed from cosmids in the 17q21 BRCA1 region, which is flanked by the markers D17S857 and D17S78 (Anderson *et al.*, 1993). After determination of PCR conditions, each anonymous polymorphism was typed on CEPH parent DNAs to determine the number, size, and frequency of the alleles and to calculate the heterozygosity of the markers. The results of these studies are presented in Tables 1 and 2.

To improve the usefulness of all the markers in the BRCA1 region, we attempted to place the 10 new markers described above, along with several recently identified markers (Albertsen *et al.*, 1994a; Weissenbach *et al.*, 1993), on the physical map of the BRCA1 region. The current genetic map of the D17S857–D17S78 interval includes 10 previously known markers ordered by analysis of recombination events in CEPH and



breast cancer families and by STS mapping on a YAC contig. The order of markers is as follows: D17S857–GAS – D17S856 – EDH17B1 – EDH17B2 – D17S855 – D17S859–D17S858–PPY–D17S78 (Anderson *et al.*, 1993). The order of markers in this genetic map is consistent with that in the physical map generated by both fluorescence *in situ* hybridization (Flejter *et al.*, 1993) and radiation hybrid mapping (Abel *et al.*, 1993). Therefore, localization of microsatellite markers on the physical map by inference also places the markers on the genetic map of the BRCA1 region.

The markers were mapped by PCR as STSs onto a cosmid and YAC contig of the BRCA1 region. The contig was based on the published physical map of Albertsen *et al.* (1994b), which utilized many novel STSs to generate a contig of YAC and cosmid clones. Three previously unpublished YACs B188H2, 308G8, and B109C8 in association with many of the YACs from Albertsen *et al.*, (1994b) are shown in Fig. 1. All clones were typed with the known STSs (D17S856, EDH17B1, EDH17B2, D17S855, RNU2, D17S859, D17S858, and D17S78) from the published physical (Flejter *et al.*, 1993; Abel *et al.*, 1993) and genetic (Anderson *et al.*, 1993) maps of the region.

All 10 newly derived markers were observed to map between D17S856 and D17S855 in the order shown in Fig. 2, while other recently developed markers (Albertsen *et al.*, 1994a; Weissenbach *et al.*, 1993) were scattered throughout the D17S857–D17S78 interval. Mapping of the markers resulted in the identification of several marker clusters. The markers D17S856, D17S648, and D17S1138 were placed within a 100-kb region by colocalization to the two overlapping cosmids 18D10 and 19C3. The markers D17S1139 and D17S1143 mapped to the 140G10 cosmid, or a 50-kb region, which overlapped with the 19C3 cosmid containing the D17S1138 markers. Similarly, the D17S1144/D17S1145 and the D17S1140/D17S1147 intervals were also shown to be less than 50 kb by colocalization of the markers to single cosmids 87B3 and 11D8, respectively. The within-pair orders from centromere to telomere of (D17S1139/D17S1143), (D17S1145/

FIG. 1. A YAC- and cosmid-based physical map of the BRCA1 region between the markers D17S856 and D17S78. The map indicates which clones, placed across the top of the figure, are positive for the STS markers and polymorphisms indicated in the address column. The polymorphisms used to construct Fig. 2 are indicated in boldface type. Cosmid clones are identified by the letter c. The map is composed of a minimal set of reagents, 14 YACs and 7 cosmid clones, which cover this interval. The contig is based on the published physical map of Albertsen *et al.* (1994b), in which many STSs were used to generate an overlapping contig of YAC and P1 clones. Only those STSs from Albertsen *et al.* (1994b) required to demonstrate minimal overlap are shown above. The sizes of YACs B188H2, 308G8, and B109C8 (Couch *et al.*, submitted) that were not reported in Albertsen *et al.* (1994b) are 375, 360, and 100 kb, respectively. A deletion in the YAC 308G8 was identified by the absence of the marker D17S1142.

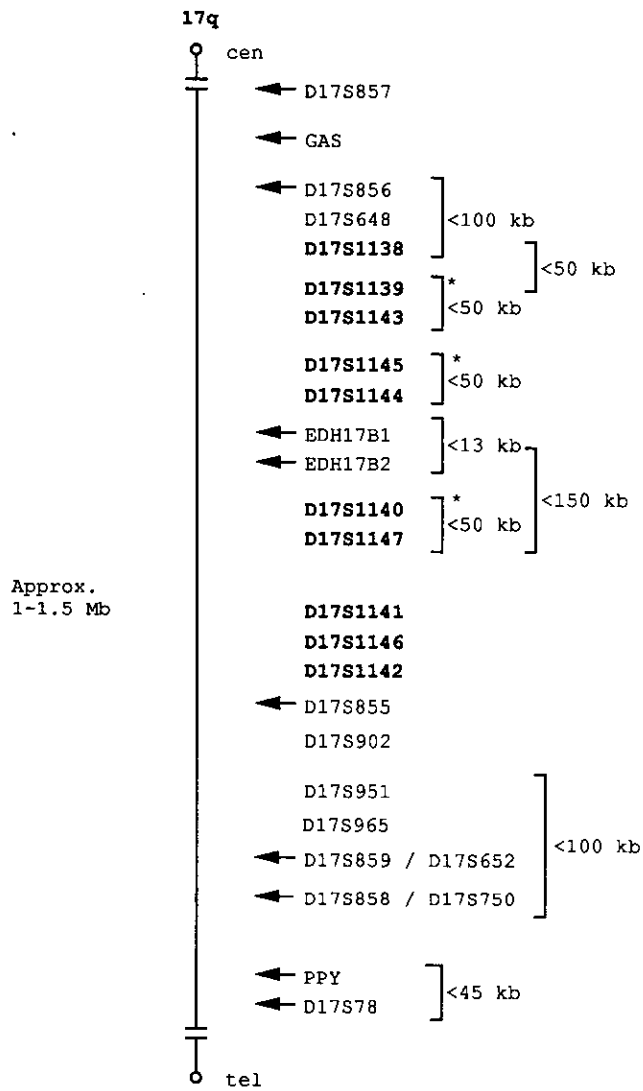


FIG. 2. A map of the BRCA1 region from centromere to telomere indicating the position of genetic markers as determined by STS mapping on a cosmid and YAC contig of the region. New anonymous markers described in this paper are in boldface lettering. Arrows show the position of previously known markers on a genetic map of the BRCA1 region (Friedman *et al.,* 1993). The remaining markers were previously reported (Albertsen *et al.,* 1994a; Weissenbach et al., 1993). The asterisk indicates marker pairs with undefined order. Maximum physical distances between markers are indicated by brackets.

D17S1144), and (D17S1140/D17S1147) are currently unknown. The markers D17S1140 and D17S1147 mapped a maximum of 150 kb, or three cosmid lengths, distal to the two EDH17B genes. The cosmids 93E12, 53B12, and 11D8 were shown to overlap by restriction digestion and whole cosmid hybridization (Couch *et al.,* submitted). Another cluster of markers was detected surrounding the marker D17S858 (OF3), which had previously been placed on the genetic map of the region (Anderson *et al.,* 1993). The marker D17S858 was determined to be the same microsatellite as the marker D17S750 (UT67) by comparison of DNA sequences present in GDB and GenBank. Similarly, D17S859 (OF4) is identical to the marker D17S652 (UT189). The markers D17S858/D17S750 and D17S859/D17S652, the marker D17S965 (UT956), and the marker D17S951 (afm298wg5) were all localized to the 140E9 and 82H10 cosmids. The remaining markers mapped sufficiently distant to known markers that estimates of the intervening distances could not be made.

Several of the new markers were typed both on the CEPH parent panel and also on a panel of unrelated individuals from the general population (described under Materials and Methods). The levels of heterozygosity in both populations were consistent in most cases. However, the heterozygosity values generated for the marker D17S1145 were very different. Initially, 35 of the CEPH parents were typed, which resulted in the identification of four alleles yielding a heterozygosity value of 34%. The marker was subsequently typed on 25 unrelated individuals from the general population. Ten alleles were identified with a heterozygosity value of 88%.

One other dinucleotide repeat was isolated from the BRCA1 cosmids. Sequence analysis of a *Sau*3AI clone known to contain a repeat identified two dinucleotide repeats within a 155-bp region. A $(CA)_{17}$ repeat was shown to be nonpolymorphic when typed on the CEPH parent panel, while the other $(CA)_{18}$ repeat generated a heterozygosity value of 15%. The monomorphic repeat was not assigned a D segment number, while the polymorphic repeat is represented in this paper as D17S1141.

DISCUSSION

We have isolated 10 new polymorphic dinucleotide repeat markers from the BRCA1 region of chromosome 17q12–q21. The new markers were isolated in an attempt to reduce the BRCA1 candidate interval by identification of recombination events in linked families and by LOH analysis of breast tumor DNAs. These and other closely spaced markers have assisted in defining the position of recombination events in linked families, thereby reducing the size of the BRCA1 critical region. Furthermore, detailed LOH analysis within the D17S857–D17S78 1 to 1.5-Mb region using these markers has allowed accurate determination of the boundaries of allelic loss in those rare tumors that display interstitial LOH specific to this small region (data not shown). The prevalence of informative markers in the BRCA1 region, flanking the BRCA1 gene, will allow accurate linkage analysis of breast cancer families to be performed for diagnostic purposes. As shown in Fig. 2, several of the markers are separated by distances of less than 50 kb. These marker pairs can usually be treated as single loci for linkage analysis of BRCA1 families and LOH studies of BRCA1 tumors.

The markers were mapped in relation to markers on the previously published genetic and physical maps of

the region by localization on a cosmid and YAC contig. All 10 new markers mapped into the D17S857–D17S78 BRCA1 region between the genetic map markers D17S856 and D17S855, while other markers were distributed between D17S857 and D17S78. It was not possible to order all of the markers by radiation hybrid analysis. Analysis of known breakpoints within the hybrids suggested that only two breaks existed between the markers D17S856 and D17S855 and three breaks between D17S855 and D17S78. Therefore, the markers could be separated into only six segments. However, the placement of the markers on the STS-based physical map allows incorporation of the new markers into the genetic map. No genetic distances are currently available for these intramarker intervals, but the known location on the physical map should allow the markers to be integrated and used in linkage studies. The markers can also serve as STSs in the BRCA1 region. Screening of the markers on YACs and cosmids from the region has identified overlaps between genomic clones and has subsequently served as a framework for completion of both YAC and cosmid contigs in the BRCA1 region (Couch *et al.*, submitted).

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REFERENCES

Abel, K. J., Boehnke, M., Prahalad, M., Ho, P., Flejter, W. L., Watkins, M., Vanderstoep, J., Chandrasekharappa, S. C., Collins, F. S., Glover, T. W., and Weber, B. L. (1993). A radiation hybrid map of the BRCA1 region of chromosome 17q12–q21. *Genomics* **17:** 632–641.

Albertsen, H., Plaetke, R., Ballard, L., Fujimoto, E., Connolly, J., Lawrence, E., Rodriguez, P., Robertson, M., Bradley, P., Milner, B., Fuhrman, D., Marks, A., Sargent, R., Cartwright, P., Matsunami, N., and White, R. (1994a). Genetic mapping of the BRCA1 region on chromosome 17q21. *Am. J. Hum. Genet.* **54:** 516–525.

Albertsen, H. M., Smith, S. A., Mazoyer, S., Fujimoto, E., Stevens, J., Williams, B., Rodriguez, P., Cropp, C. S., Slijepcevic, P., Carlson, M., Robertson, M., Bradley, P., Lawrence, E., Harrington, T., Mei Sheng, Z., Hoopes, R., Sternberg, N., Brothman, A., Callahan, R., Ponder, B. A. J., and White, R. (1994b). A physical map and candidate genes in the BRCA1 region on chromosome 17q12–21. *Nature Genet.* **7:** 472–479.

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:** 618–623.

Chandrasekharappa, S. C., Friedman, L., King, S. E., Lee, Y.-H., Welsch, P., Bowcock, A. M., Weber, B. L., King, M.-C., and Collins, F. S. (1994). The gene for pancreatic polypeptide (PPY) and the anonymous marker D17S78 are within 45 kb of each other on chromosome 17q21. *Genomics* **21:** 458–460.

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.

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.

Flejter, W. L., Barcroft, 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.

Friedman, L. S., Lynch, E. D., and King, M.-C. (1993). Two independent polymorphisms at the 17-beta-hydroxysteroid dehydrogenase (EDH17B) gene (17q21). *Hum. Mol. Genet.* **2:** 821.

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

Human Gene Mapping 11 (1991). *Cytogenet. Cell Genet.* **58:** 1–2200.

Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. H. (1994). A cell cycle regulator potentially involved in genesis of many tumor types. *Science* **264:** 436–440.

Kelsell, D. P., Black, D. M., Bishop, D. T., and Spurr, N. K. (1993). Genetic analysis of the BRCA1 region in a large breast/ovarian family: Refinement of the minimal region containing BRCA1. *Hum. Mol. Genet.* **2:** 1823–1828.

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.

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

Silverberg, E., and Boring, C. (1990). Cancer statistics—1990. *CA Cancer J. Clin.* **40:** 9–26.

Simard, J., Feunteun, J., Lenoir, G., Tonin, P., Normand, T., Luu The, V., Vivier, A., Lasko, D., Morgan, K., Rouleau, G. A., Lynch, H., Labrie, F., and Narod, S. A. (1993). Genetic mapping of the breast–ovarian cancer syndrome to a small interval on chromosome 17q12–21: Exclusion of candidate genes EDH17B2 and RARA. *Hum. Mol. Genet.* **2:** 1193–1199.

Smith, S. A., Easton, D. F., Evans, D. G. R., and Ponder, B. A. J. (1992). Allele losses in the region 17q12–21 in familial breast and ovarian cancer involve the wild type chromosome. *Nature Genet.* **2:** 128–131.

Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, Vaysseix, G., and Lathrop, M. (1993). A second generation linkage map of the human genome based on highly informative microsatellite loci. *Nature* **359:** 794–801.