

## Evidence for Involvement of BRCA1 in Sporadic Breast Carcinomas

Craig S. Cropp, Heli A. Nevanlinna, Seppo Pyrhönen, Ulf-Håkan Stenman, Paul Salmikangas, Hans Albertsen, Ray White, and Robert Callahan

Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, MD 20892 [C. C., R. C.]; Departments I and II of Obstetrics and Gynecology [H. A. N., U-H. S., P. S.] and Radiotherapy and Oncology [S. P.], Helsinki University Central Hospital, Haartmaninkatu 2, 00290 Helsinki, Finland; and Eccles Institute of Human Genetics and Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah 84112 [H. A., R. W.]

### Abstract

The hereditary breast cancer gene *BRCA1* previously has been localized to chromosome 17q21. We looked for evidence of involvement of this region of chromosome 17 in 130 sporadic breast cancers. Seventeen polymorphic sequence tagged site markers were examined in these tumors between the *D17S250* and *D17S579* loci to screen for deletions as measured by loss of heterozygosity. The smallest common region that was deleted occurred in the approximately 120-kilobase interval between the *D17S846* and *D17S746* loci within the *BRCA1* region. Delineation of this commonly deleted area should accelerate attempts to identify the involved gene(s) and its relationship to *BRCA1*.

### Introduction

It is widely believed that there are one or more tumor suppressor genes on chromosome 17q for breast carcinoma (1–3). One candidate gene, *BRCA1*, is genetically linked to the development of some familial breast cancers and is located at 17q21 (4). Ovarian cancer is also known to have a hereditary component (5–7). Furthermore, a woman with ovarian cancer is at increased risk for developing breast cancer as a second primary tumor, and *vice versa* (8–10). Recently, the syndrome for familial breast/ovarian cancer has also been linked to the same region as *BRCA1* (11). The observed LOH<sup>1</sup> affecting the wild type chromosome in tumors from affected breast/ovarian cancer patients (12, 13) is consistent with the hypothesis that *BRCA1* is a tumor suppressor gene. However, whether the target genes for familial breast and breast/ovarian cancers are the same or are different closely linked genes is not known. In a variety of hereditary neoplasias the affected gene (*e.g.*, *RB*, *TP53*, *APC*, etc.) has also been found to be frequently mutated in sporadic forms of the disease (14, 15). Previously, we (1) and others (2, 3, 16) have shown that sporadic breast carcinomas are frequently affected by LOH in the general region of *BRCA1*. The availability of additional polymorphic STS which have been genetically mapped to the region of chromosome 17q21 containing *BRCA1* (17) has provided us the opportunity to further define the region of this portion of the genome which is affected by LOH in sporadic primary human breast tumors. When this study was begun the consensus among published reports on the genetic and physical boundaries of *BRCA1* suggested that the centromeric boundary was *D17S250* (18–20). The telomeric boundary was less clear. In some reports it was *D17S588* while in others it was the more centromeric locus, *D17S579* (19, 21, 22). In our study we chose *D17S579* as our telomeric border. The interval between *D17S250* and *D17S579* is estimated to be roughly 3500 kilobases (23). In the present study we

describe a high density deletion map using 17 PCR-based polymorphic STS markers located between the *D17S250* and *D17S579* loci in 130 primary human breast tumors.

### Materials and Methods

Primary breast carcinomas and matching peripheral lymphocytes were collected at the Helsinki University Central Hospital in Helsinki, Finland, from 130 patients who had received no prior therapy.

Genomic DNA was extracted and diluted to 100–200 ng/μl. PCR was performed with 100–200 ng template DNA, 10 mM Tris-HCl, 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, gelatin 0.1 mg/ml, 250 μM concentration of each nucleoside triphosphate, 0.4 unit Taq polymerase (Boehringer Mannheim), and 30 pmol of each primer in a total volume of 25 μl. The PCR product was identified by end labeling primers with [ $\gamma$ -<sup>32</sup>P]ATP. All PCR reactions were performed on a GeneAmp PCR System 9600 starting with denaturation for 6 min at 94°C followed by 25 cycles of denaturation at 94°C for 10 s, annealing temperature for 10 s, and extension at 72°C for 20 s. The primers used, their annealing temperatures, and references are shown in Table 1.

The PCR products were diluted with loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol), heat denatured and rapidly cooled. Samples were run in pairs (tumor and lymphocyte PCR product from the same patient) on a denaturing gel (7% acrylamide-32% formamide-6 M urea-1× Tris-buffered EDTA) at a constant 65–70 W. After electrophoresis the gel was transferred to Whatman No. 3MM paper and autoradiography was performed with Kodak X-Omat AR film at –70°C.

### Results and Discussion

We have previously defined three regions of chromosome 17q that are frequently affected by LOH in primary human breast tumors (1). In that study a putative target gene(s) was suggested in the interval between *D17S73* and *NME1* on chromosome 17q12–q21. In the present study we have examined an additional 17 polymorphic STS markers between *D17S250* and *D17S579* which is a subregion within the *D17S73* and *NME1* interval. This represents an average of one polymorphism every 210 kilobases since the distance between *D17S250* and *D17S579* is estimated to be 3500 kilobases. The total number of tumors examined, the percentage of the total number of tumors that were informative, and the percentage of the informative tumors which were deleted (*i.e.*, LOH) is shown in Table 1. The overall frequency of LOH varies from 12 to 32% and is the highest between *D17S846* and *D17S776*.

To further define the region containing the putative target gene we have analyzed the genotypes of individual primary breast tumor DNAs for evidence of LOH. Shown in Fig. 1 are autoradiographs of the STS markers of four breast tumors between *D17S702* and *D17S856*. Tumor 20 showed no LOH at *D17S702* and *D17S746* but was deleted for the upper allele of *GAS* and the lower allele of *D17S846*. Markers *D17S776* and *D17S856* were not informative in this tumor. In tumor 26 no LOH was detected at *D17S702*, *D17S846*, and *D17S856*, but the tumor DNA was deleted for the lower allele of

Received 2/24/94; accepted 4/5/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> The abbreviations used are: LOH, loss of heterozygosity; STS, sequence tag sites; PCR, polymerase chain reaction

Table 1 Frequency of LOH at chromosome 17q21 loci

The locus number of the STS marker, the PCR annealing temperature, and a reference is listed for each marker used. The total number of tumors examined for each marker is indicated by *N*. The percentage of the total number of tumors (*N*) that were informative is shown as %I, and the numbers in parentheses are the expected heterozygosity for that STS marker. The percentage of the informative tumors (*I*) that were deleted is indicated by %LOH.

Locus/STS	Annealing temperature (°C)	Ref.	<i>N</i>	% I	% of LOH
<i>D17S250</i>	55	24	101	80 (81)	21
<i>THRA1</i>	55	25	95	39 (81)	27
<i>D17S700</i>	60	17	100	54 (44)	19
<i>D17S857</i>	55	26	102	67 (NP)	25
<i>D17S702</i>	52	17	102	90 (88)	23
<i>GAS</i>	60	27	97	52 (NP)	26
<i>D17S846</i>	55	28	95	78 (84)	32
<i>D17S746</i>	58	17	108	33 (44)	28
<i>D17S776</i>	58	29	105	60 (55)	30
<i>D17S856</i>	55	26	103	51 (NP)	19
<i>D17S648</i>	60	17	107	36 (29)	18
<i>D17S855</i>	55	26	60	50 (NP)	27
<i>D17S902</i>	60	17	50	74 (78)	27
<i>D17S859</i>	55	26	49	35 (NP)	12
<i>D17S750</i>	65	17	103	57 (66)	24
<i>D17S183</i>	55	30, 31	103	35 (40)	22
<i>D17S579</i>	55	32	109	86 (87)	13

*D17S746*. Tumor 63 was informative and unaltered at the *D17S702*, *GAS*, *D17S846*, and *D17S856* loci but was deleted for the upper allele of *D17S776*. In this patient the STS marker *D17S746* was not informative. Tumor 117 was informative and unaltered at the *D17S702*, *D17S776*, and *D17S856* loci but was deleted for the upper allele of *D17S846*. In this patient *D17S746* was not informative.

The genotypes of these four tumors and seven additional tumors for loci lying between *D17S250* and *D17S579* is shown in Fig. 2. Ten of the tumors examined in this study have clearly defined interstitial deletions within the *BRCA1* interval, while tumor 127 appears to have lost a large portion of the long arm of chromosome 17. In this study a total of nine tumors appeared to have lost an entire chromosome 17q arm, since all markers in the *BRCA1* region as well as telomeric markers, such as *D17S4*, were deleted. Six other tumors had LOH at all the markers in the *BRCA1* region but were informative and retained heterozygosity at *D17S4*. These data taken together indicate that the smallest commonly deleted region is located between *D17S846* and *D17S746*. This conclusion is both consistent with and extends previous studies in which fewer loci were examined within the *BRCA1* region (2, 3, 16). *D17S846* and *D17S746* are located on two overlapping recombinant P1 bacteriophage clones. The distance between them is estimated to be 120–150 kilobases based on physical mapping data<sup>2</sup> and could possibly contain several undiscovered genes.

The increased availability of polymorphic STS markers in the region of *BRCA1* has also led to a continued shrinkage of the size of this region through genetic linkage studies in breast and breast/ovarian cancer families. Thus, in successive studies the telomeric border has moved centromerically from *D17S579* (18, 20, 21) to *D17S183* (located between *D17S750* and *D17S579*; Fig. 2) (26) to *D17S78* (located between *D17S750* and *D17S183*; Fig. 2) (33). Similarly the centromeric border has moved in the telomeric direction from *D17S250* (19, 20, 31) to *THRA1* (21, 30) to *D17S857* (13) to *D17S702* (34). The region we have defined as containing the target gene for LOH on chromosome 17q21 in sporadic breast tumors is compatible with these linkage studies defining *BRCA1* in familial breast and breast/ovarian cancer families. However, there is one report (29) which is potentially inconsistent with this conclusion. They show that in one family having the familial breast/ovarian cancer syndrome, one

affected member had a recombinational event telomeric of the locus *D17S776*. This locus is more telomeric than *D17S746* which is the telomeric boundary of the affected region defined in our study (Fig. 2). There are at least three explanations for this apparent paradox: (a) the locus we have defined may be relevant only in sporadic breast cancer and not in hereditary breast cancer; (b) possibly there are two closely linked genes which are independently altered in breast-only versus breast/ovarian families. A comparison of the linkage studies done in breast-only families versus those done in breast/ovarian families show that in the former group the altered gene lies between *THRA1* and *D17S579* (35), whereas in the latter group the location of the altered gene lies distal to *D17S776* and proximal to *D17S78* (29, 32). The region we have defined is consistent with the current linkage analysis of the location of the altered gene in the breast-only families; (c) our results are not incompatible with the linkage data of Goldgar *et al.* (29). However, this would require that the *BRCA1* gene is large (1–2 megabases) and that the recombinational event described by Goldgar *et al.* occurred within the gene. There are precedents for each of these requirements. For instance, the *DCC* gene on chromo-



Fig. 1. Autoradiographs of four tumors for the six STS polymorphic markers between *D17S702* and *D17S856*. Arrows, deleted allele indicating LOH. Tumor 20 is deleted for *GAS* and *D17S846*; tumor 26 is deleted for *D17S746*; tumor 63 is deleted for *D17S776*; and tumor 117 is deleted for *D17S846*. T, tumor; L, lymphocyte.

<sup>2</sup> H. Albertsen *et al.*, manuscript in preparation.

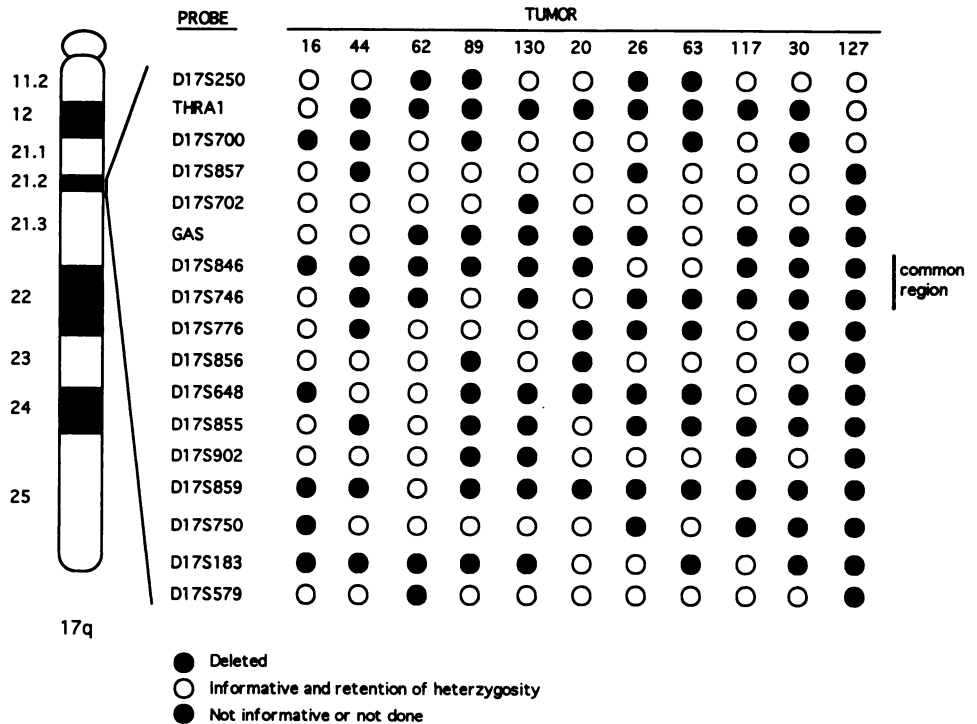


Fig. 2. Genotypes of 11 tumors between STS markers *D17S250* and *D17S579*. ●, markers with LOH (i.e., deleted); ○, STS markers which are informative and normal; ◐, either the tumor was not examined for that marker, or the marker was not informative. The commonly deleted region is indicated at the right side of the figure. The genetic order of the STS marker probes is according to published linkage studies (17, 26).

some 18q is approximately 1.4 megabases (36), and the Duchenne muscular dystrophy (*DMD*) gene on Xp21 is approximately 2.3 megabases (37). In the search for the *DMD* gene, linkage analysis was somewhat confusing because of the frequency of recombination events within such a large gene (38–40). Clearly, identification of the target gene(s) in sporadic breast carcinomas as well as the familial breast and breast/ovarian carcinoma loci will be required to distinguish between these possibilities.

## References

- Cropp, C. S., Champeme, M.-H., Lidereau, R., and Callahan, R. Identification of three regions on chromosome 17q in primary human breast carcinomas which are frequently deleted. *Cancer Res.*, 53: 5617–5619, 1993.
- Cornelis, R. S., Devilee, P., van Vliet, M., Kuipers-Dijkshoorn, N., Kersenmaeker, A., Bardoel, A., Meera Khan, P., and Cornelisse, C. J. Allele loss patterns on chromosome 17q in 109 breast carcinomas indicate at least two distinct target regions. *Oncogene*, 8: 781–785, 1993.
- Saito, H., Inazawa, J., Saito, S., Kasumi, F., Koi, S., Sagae, S., Kudo, R., Saito, J., Noda, K., and Nakamura, Y. Detailed deletion mapping of chromosome 17q in ovarian and breast cancers: 2-cM region on 17q21.3 often and commonly deleted in tumors. *Cancer Res.*, 53: 3382–3385, 1993.
- Hall, J. M., Lee, M. K., Newman, B., Horrow, J. E., Anderson, L. A., Huey, B., and King, M. C. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* (Washington DC), 250: 1684–1689, 1990.
- DiCioccio, R. A., and Piver, M. S. The genetics of ovarian cancer. *Cancer Invest.*, 10: 135–141, 1992.
- Matheson, J. A. B., Matheson, H., and Anderson, S. A. Familial ovarian cancer. How rare is it? *J. R. Coll. Gen. Pract.*, 31: 743–745, 1981.
- Perez, R. P., Godwin, A. K., Hamilton, T. C., and Ozols, R. F. Ovarian cancer biology. *Semin. Oncol.*, 18: 186–204, 1991.
- Curtis, R. E., Hoover, R. N., Kleinerman, R. A., and Harvey, E. B. Second cancer following cancer of the female genital system in Connecticut, 1935–82. *Natl. Cancer Inst. Monogr.*, 68: 113–137, 1985.
- Harvey, E. B., and Brinton, L. A. Second cancer following cancer of the breast in Connecticut, 1935–1982. *Natl. Cancer Inst. Monogr.*, 68: 99–112, 1985.
- Curtis, R. E., Boice, J. D., Jr., Kleinerman, R. A., Flannery, J. T., and Fraumeni, J. F., Jr. Summary: multiple primary cancers in Connecticut, 1935–82. *Natl. Cancer Inst. Monogr.*, 68: 219–242, 1985.
- Narod, S. A., Feunteun, J., Lynch, H. T., Watson, P., Conway, T., Lynch, J., and Lenoir, G. M. Familial breast-ovarian cancer locus on chromosome 17q12–q23. *Lancet*, 338: 82–83, 1991.
- Smith, A. A., Easton, D. F., Evans, D. G. R., and Ponder, B. A. J. Allele losses in the region 17q12–21 in familial breast and ovarian cancer involve the wild-type chromosome. *Nat. Genet.*, 2: 128–131, 1992.
- Kelsell, D. P., Black, D. M., Bishop, D. T., and Spurr, N. K. 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, 1993.
- Ponder, B. A. J. Molecular genetics of cancer. *Br. Med. J.*, 304: 1234–1236, 1992.
- Finlay, G. J. Genetics, molecular biology and colorectal cancer. *Mutat. Res.*, 290: 3–12, 1993.
- Futreal, P. A., Soderkvist, P., Marks, J. R., Iglehart, J. D., Cochran, C., Barrett, J. C., and Wiseman, R. W. Detection of frequent allelic loss on proximal chromosome 17q in sporadic breast carcinoma using microsatellite length polymorphisms. *Cancer Res.*, 52: 2624–2627, 1992.
- 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. Genetic mapping of the BRCA1 region on chromosome 17q21. *Am. J. Hum. Genet.*, in press, 1994.
- Devilee, P., Cornelis, R. S., Bootsma, A., Bardoel, A., van Vliet, M., van Leeuwen, I., Cleton, F. J., de Klein, A., Lindhout, D., Vasen, H. F. A., Cornelisse, C. J., and Khan, P. M. Linkage to markers for the chromosome region 17q12–21 in 13 Dutch breast cancer kindreds. *Am. J. Hum. Genet.*, 52: 730–735, 1993.
- Smith, S. A., Easton, D. F., Peto, J., Anderson, K., Averill, D., Stratton, M., Ponder, M., Pye, C., and Ponder, B. A. J. Genetic heterogeneity and localization of a familial breast-ovarian cancer gene on chromosome 17q12–21. *Am. J. Hum. Genet.*, 52: 767–776, 1993.
- Spurr, N. K., Kelsell, D. P., Black, D. M., Murday, V. A., Turner, G., Crockford, G. P., Solomon, E., Cartwright, R. A., and Bishop, D. T. Linkage analysis of early-onset breast and ovarian cancer families, with markers on the long arm of chromosome 17. *Am. J. Hum. Genet.*, 52: 777–785, 1993.
- Goldgar, D. E., Cannon-Albright, L. A., Oliphant, A., Ward, J. H., Linker, G., Swensen, J. H., Tran, T. D., Fields, P., Uharriet, P., and Skolnick, M. H. Chromosome 17q linkage studies of 18 Utah breast cancer kindreds. *Am. J. Hum. Genet.*, 52: 743–748, 1993.
- Chamberlain, J. S., Boehnke, M., Frank, T. S., Kioussis, 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. BRCA1 maps proximal to D17S579 on chromosome 17q21 by genetic analysis. *Am. J. Hum. Genet.*, 52: 792–798, 1993.
- Abel, K. J., Boehnke, M., Prahald, M., Ho, P., Flejter, W. L., Watkins, M., Vanderstoep, J., Chandrasekharappa, S. C., Collins, F. S., Glover, T. W., and Weber, B. L. A radiation hybrid map of the BRCA1 region of chromosome 17q12–21. *Genomics*, 17: 632–641, 1993.
- Weber, J. L., Kwitek, A. E., May, P. E., Wallace, M. R., Collins, F. S., and Ledbetter, D. H. Dinucleotide repeat polymorphisms at the D17S250 and D17S261 loci. *Nucleic Acids Res.*, 18: 4640, 1990.
- Futreal, P. A., Barrett, J. C., and Wiseman, R. W. Dinucleotide repeat polymorphism in the *THRA1* gene. *Hum. Mol. Genet.*, 1: 66, 1992.
- Anderson, L. A., Friedman, L., Osborne-Lawrence, S., Lynch, E., Weissenbach, J., Bowcock, A., and King, M.-C. High-density genetic map of the BRCA1 region of chromosome 17q12–q21. *Genomics*, 17: 618–623, 1993.
- Epstein, N., Nahor, O., and Silver, J. The 3' ends of alu repeats are highly polymorphic. *Nucleic Acids Res.*, 18: 4634, 1990.
- Flejter, W. L., Kukowska-Latallo, J. F., Kioussis, S., Chandrasekharappa, S. C., King, S. E., and Chamberlain, J. S. Tetranucleotide repeat polymorphism at *D17S846* maps

- within 40 kb of GAS at 17q12–22. *Hum. Mol. Genet.*, 2: 1080, 1993.
29. Goldgar, D. E., Fields, P., Lewis, C. M., Tran, T. D., Cannon-Albright, L. A., Ward, J. H., Swensen, J., and Skolnick, M. H. A large kindred with 17q-linked breast and ovarian cancer: genetic phenotypic, and genealogical analysis. *J. Natl. Cancer Inst.*, 86: 200–209, 1994.
  30. Black, D. M., Nicolai, H., Borrow, J., and Solomon, E. 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, 1993.
  31. Easton, D. F., Bishop, D. T., Ford, D., Crockford, G. P., and Consortium, B. C. L. Genetic linkage analysis in familial breast and ovary cancer—results from 214 families. *Am. J. Hum. Genet.*, 52: 678–701, 1993.
  32. Hall, J. M., Friedman, L., Guenther, C., Lee, M. K., Weber, J. L., Black, D. M., and King, M-C. Closing in on a breast cancer gene on chromosome 17q. *Am. J. Genet.*, 50: 1235–1242, 1992.
  33. Simard J., F., J., Linoir, G., Tonin, P., Normand, T., The, V. L., Vivier, A., Lasko, D., Morgan, K., Rouleau, G. A., Lynch, H., Labrie, F., and Narod, S. A. 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, 1993.
  34. Smith, S. A., DiCioccio, R. A., Struwing, J. P., Easton, D. F., Gallion, H. H., Albertsen, H., Mazoyer, S., Johansson, B., Steichen-Gersdorf, E., Stratton, M., Ford, D., Marshall, G., White, R. L., Piver, M. S., and Ponder, B. A. J. Localization of the breast-ovarian cancer susceptibility gene (*BRCA1*) on 17q12–21 to an interval of  $\leq 1$  cM. *Genes Chromosomes Cancer*, 10: in press, 1994.
  35. 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. *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, 1993.
  36. Cho, K. R., Oliner, J. D., Simons, J. W., Hedrick, L., Fearon, E. R., Preisinger, A. C., Hedge, P., Silverman, G. A., and Vogelstein, B. The *DCC* gene: structural analysis and mutations in colorectal carcinomas. *Genomics*, 19: 525–531, 1994.
  37. Worton, R. G. Duchenne muscular dystrophy: gene and gene product; mechanism of mutation in the gene. *J. Inher. Metab. Dis.* 15: 539–550, 1992.
  38. Koenig, M., Hoffman, E. P., Bertelson, C. J., Monaco, A. P., Feener, C., and Kunkel, L. M. Complete cloning of Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the *DMD* gene in normal and affected individuals. *Cell*, 50: 509–517, 1987.
  39. Fischbeck, K. H., Ritter, A. W., Tirschwell, D. L., Kunkel, L. M., Bertelson, C. J., Monaco, A. P., Hejtmancik, J. F., Boehm, C., Ionasescu, V., Ionasescu, R., Pericak-Vance, M., Kandt, R., and Roses, A. D. Recombination with *PERT87* (*DXS164*) in families with X-linked muscular dystrophy. *Lancet*, 2: 104, 1986.
  40. Kunkel, L. M., *et al.* Analysis of deletions in DNA from patients with Becker and Duchenne muscular dystrophy. *Nature (Lond.)*, 322: 73–77, 1986.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## Evidence for Involvement of BRCA1 in Sporadic Breast Carcinomas

Craig S. Cropp, Heli A. Nevanlinna, Seppo Pyrhönen, et al.

*Cancer Res* 1994;54:2548-2551.

**Updated version** Access the most recent version of this article at:  
<http://cancerres.aacrjournals.org/content/54/10/2548>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cancerres.aacrjournals.org/content/54/10/2548>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.