variants. Furthermore, probe cyclization reactions depend on an intramolecular reaction as opposed to reaction between pairs of independent probe molecules as in amplification by the polymerase chain reaction. Thus, there should be fewer problems with nonspecific reactions resulting from interactions between noncognate pairs of probe segments with cyclizable probes. The present probe design should permit the simultaneous analysis of multiple gene sequences in a DNA sample.

In conclusion, the nucleic acid probe presented here permits highly specific detection of nucleotide sequences and, although the target is not amplified, highly sensitive detection is possible through efficient reduction of nonspecific signal. Circularizable probes should be applicable in a number of other contexts, including the detection of specific RNA molecules expressed in tissue sections as T4 DNA ligase can assist in ligation reactions involving RNA strands (8). Moreover, immobilized padlock probes could be useful for preparative purposes, such as trapping circular target molecules from solution when screening gene libraries.

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5. The upper faint bands observed in lanes 3 and 4 probably represent small amounts of linear dimer molecules, appearing as a consequence of ligation of one end each of two different probe molecules. This material proved susceptible to exonuclease, digestion. The extra lower bands in these lanes were not reproducible between experiments. Small amounts of uncatenated, circular probes appearing in lane 7 most likely were a consequence of endonuclease activity in the exonuclease preparation. With increasing amounts of exonuclease, catenated probes are lost and more free circular probes appear (M. Nilsson *et al.*, unpublished data).
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12. We thank E. Johnsen for technical assistance and T. Hansson for molecular modeling. U. Pettersson offered critical comments on this manuscript. Supported by the Beijer, Procordia, and Borgström foundations; by NUTEK, the Technical and Medical Research Councils of Sweden; and by the Swedish Cancer Fund.

18 July 1994; accepted 1 September 1994

Localization of a Breast Cancer Susceptibility Gene, *BRCA2*, to Chromosome 13q12-13

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A small proportion of breast cancer, in particular those cases arising at a young age, is due to the inheritance of dominant susceptibility genes conferring a high risk of the disease. A genomic linkage search was performed with 15 high-risk breast cancer families that were unlinked to the *BRCA1* locus on chromosome 17q21. This analysis localized a second breast cancer susceptibility locus, *BRCA2*, to a 6-centimorgan interval on chromosome 13q12-13. Preliminary evidence suggests that *BRCA2* confers a high risk of breast cancer but, unlike *BRCA1*, does not confer a substantially elevated risk of ovarian cancer.

In 1990, a breast cancer susceptibility gene, known as *BRCA1*, was localized to chromosome 17q (*1*). Subsequent studies demonstrated that *BRCA1* accounts for most families with multiple cases of both early-onset breast and ovarian cancer and about 45% of families with breast cancer only, but few if any families with both male and female breast cancer (*2*). Several other genes can confer susceptibility to breast cancer. Germline mutations in the p53 gene on chromosome 17p cause a wide range of neoplasms including early-onset breast cancer, sarcomas, brain tumors, leukemias, and adrenocortical cancer (*3*). Certain rare abnormalities of the androgen receptor appear to be associated with breast cancer in men (*4*), and epidemiological studies have suggested that heterozygotes for the ataxia telangiectasia gene, *AT*, on chromosome 11q are at elevated risk of breast cancer (*5*). However, mutations in *p53* and *AT* can only be responsible for a small minority of breast cancer families that are unlinked to *BRCA1* (*6*).

To localize other genes that predispose to breast cancer, we performed a genomic linkage search using 15 families that had multiple cases of early-onset breast cancer and that were not linked to *BRCA1*. These families were classified according to the number of cases of female breast cancer, male breast cancer, and ovarian cancer (Table 1). In addition to a negative lod score (logarithm of the likelihood ratio for linkage) with markers flanking *BRCA1*, all but one of the families used for this study had at least one breast cancer case diagnosed before age 50 that did not share a *BRCA1* haplotype with other breast cancer cases in the family. The exception, CRC 136, had an obligate sporadic case diagnosed at age 53. Families were genotyped with polymorphic microsatellite repeat markers (*7*, *8*). Typing of the markers *D13S260* and *D13S263* provided provisional evidence for the presence of a susceptibility gene on chromosome 13, which was subsequently confirmed by analysis of additional polymorphisms in the region.

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Two-point lod scores were calculated for a set of closely spaced markers on chromosome 13q (Table 2) (9). Ten other markers were typed to confirm the segregation of haplotypes. The order of markers and intervals between them (in centimorgans) is 13cen-*D13S283*-(3)-*D13S221*-(2)-*D13S120*-(2)-*D13S217*-(5)-*D13S289*/*D13S290*-(3)-*D13S260*-(1)-*D13S171*-(2)-*D13S267*-(2)-*D13S220*/*D13S219*-(5)-*D13S218*-(5)-*D13S263*-(8)-*D13S155*-(2)-*D13S153*-13qter (*10*). The maximum total multipoint lod score with markers *D13S260* and *D13S267* was 9.58 at a location 5 cM proximal to *D13S260*. However, the admixture test indicated significant evidence of heterogeneity ($P = 0.001$) with an estimated proportion of 13q-linked families of 74% (95% CI 35 to 97%). Under the assumption of heterogeneity, the maximum total lod score was 11.65 and the most likely location for *BRCA2* was coincident with *D13S260*. Multipoint lod scores at *D13S260* for each family are shown in Table 2. The haplotypes confirmed cosegregation of chromosome 13q markers with the disease (an example from CRC 186 is shown in Fig. 1). Two recombinants place *BRCA2* telomeric to *D13S289*, in breast cancer cases diagnosed at ages 43 and 39 (in families IARC 2932 and CRC 186, respectively). One recombinant in a bilateral breast cancer case diagnosed at ages 38 and 41 in Utah 107 places the gene centromeric to *D13S267*. The distance between these two markers is estimated to be 6 cM (*7*), and these flanking markers place *BRCA2* in a physical region defined by 13q12-13.

The proximal part of chromosome 13 in which *BRCA2* is situated commonly shows loss of heterozygosity (LOH) in sporadic breast and ovarian cancers, suggesting that *BRCA2* is inactivated during oncogenesis (*10*). However, the tumor suppressor gene *RB1* is also located in this region and may account for the LOH observed. Indeed, somatic mutations in *RB1* have been reported in sporadic breast cancers (*11*). However, the presence of numerous recombinants between *RB1* [the marker *D13S153* is within the *RB1* gene (*12*)] and the disease in linked families indicates that *BRCA2* is not *RB1*. Other candidate genes within 13q11-14 include members of a family of tyrosine kinase genes that are related to the *FMS* proto-oncogene (*13*) and the *FTE1* gene, which may act as an effector of the v-*fos* oncogene and is a mammalian homolog of a yeast gene involved in protein import into mitochondria (*14*).

Like *BRCA1*, *BRCA2* appears to confer a high risk of early-onset breast cancer in females; previous segregation analysis of the largest *BRCA2*-linked family (Utah 107) indicated a risk of 87% by age 80 (*15*), which is comparable to the *BRCA1* penetrance. However, other aspects of the *BRCA2* phenotype may differ from the *BRCA1* phenotype. For example, in the two families showing the strongest evidence of

Table 1. Breast cancer families used in the genome search for *BRCA2*. FBC, female breast cancer; MBC, male breast cancer; OvC, ovarian cancer.

Family	Number of FBCs	Number of FBCs under age 50	Number of MBCs	Number of OvCs	Lod score at *BRCA1**	Number of sporadic cases†
CRC 007	7	5	0	0	−1.45	2
CRC 018	5	3	0	0	−0.41	1
CRC 028	3	2	1	0	0.04	1
CRC 135	6	4	0	0	−0.49	1
CRC 136	6	4	0	0	−0.02	1
CRC 186	16	15	1	1	−2.61	7
IARC 2932	15	10	0	0	−2.02	3
Leiden 49	4	4	1	4	−1.11	1
Utah 107	38	25	3	6	−3.57	7
Utah 1001	14	11	0	0	−0.48	2
Utah 1929	4	4	0	0	−0.41	1
Utah 2027	4	4	0	0	−1.14	1
Utah 2043	2	2	1	1	−0.44	1
Utah 2044	9	6	1	4	−1.40	4
Utah 9018	5	5	0	0	−0.53	1

*Multipoint lod score based on *D17S250* and *D17S579*, which flank *BRCA1* in an interval of approximately 6 cM, or closer flanking markers. †Minimum number of cases affected with breast cancer under age 60 or ovarian cancer that do not share a 17q haplotype.

Table 2. Two-point and multipoint lod scores for chromosome 13q markers in breast cancer families showing evidence against linkage to *BRCA1*.

Family	Two-point lod scores at recombination fractions of 0.00 and 0.05										Multipoint lod score*
	D13S289		*D13S260*		*D13S267*		*D13S219*		*D13S263*		*D13S260-D13S267*
	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05	0.00	0.05	
CRC 007	−1.23	−0.81	0.77	0.65	0.22	0.16	0.29	0.24	−0.30	−0.11	0.97
CRC 018	0.73	0.62	0.78	0.67	0.26	0.21	0.10	0.08	−0.48	−0.27	0.84
CRC 028	0.00	0.00	−0.02	0.00	0.26	0.21	0.02	0.01	−0.67	−0.41	0.15
CRC 135	0.43	0.36	0.15	0.12	0.14	0.12	−0.06	−0.04	−0.54	−0.32	0.29
CRC 136	−1.26	−0.84	−1.07	−0.75	−0.57	−0.42	−0.28	−0.10	−1.06	−0.71	−1.24
CRC 186	−0.30	0.03	1.84	1.60	2.35	2.08	1.00	0.84	4.08	3.67	3.70
IARC 2932	−0.03	0.10	1.33	1.22	0.80	0.67	1.62	1.38	−0.72	−0.17	1.93
Leiden 49	−0.65	−0.37	0.08	0.06	−0.21	−0.16	−0.65	−0.25	−0.75	−0.37	−0.44
Utah 107	0.24	1.11	1.84	2.11	−0.86	−0.23	0.24	0.20	1.26	1.66	3.48
Utah 1001	−0.81	−0.41	−3.25	−1.99	−1.63	−0.96	−0.39	−0.17	−1.91	−1.02	−3.40
Utah 1929	−0.46	−0.33	−0.33	−0.26	−0.47	−0.37	−0.16	−0.14	−0.25	−0.19	−0.45
Utah 2027	0.39	0.34	0.14	0.13	−0.06	−0.05	0.19	0.16	0.69	0.59	−0.11
Utah 2043	0.93	0.81	0.85	0.74	−0.39	−0.27	−0.01	−0.02	−1.13	−0.52	0.86
Utah 2044	−0.59	−0.44	1.54	1.37	1.15	0.99	0.88	0.76	−1.29	−0.72	2.11
Utah 9018	0.23	0.19	0.16	0.12	0.00	0.00	−0.11	−0.09	−0.86	−0.47	0.00
Total	−2.39	0.34	4.80	5.78	1.00	1.98	2.67	2.87	−3.95	0.65	

*Multipoint lod scores were calculated at *D13S260*, the most likely position of *BRCA2* in the heterogeneity analysis.

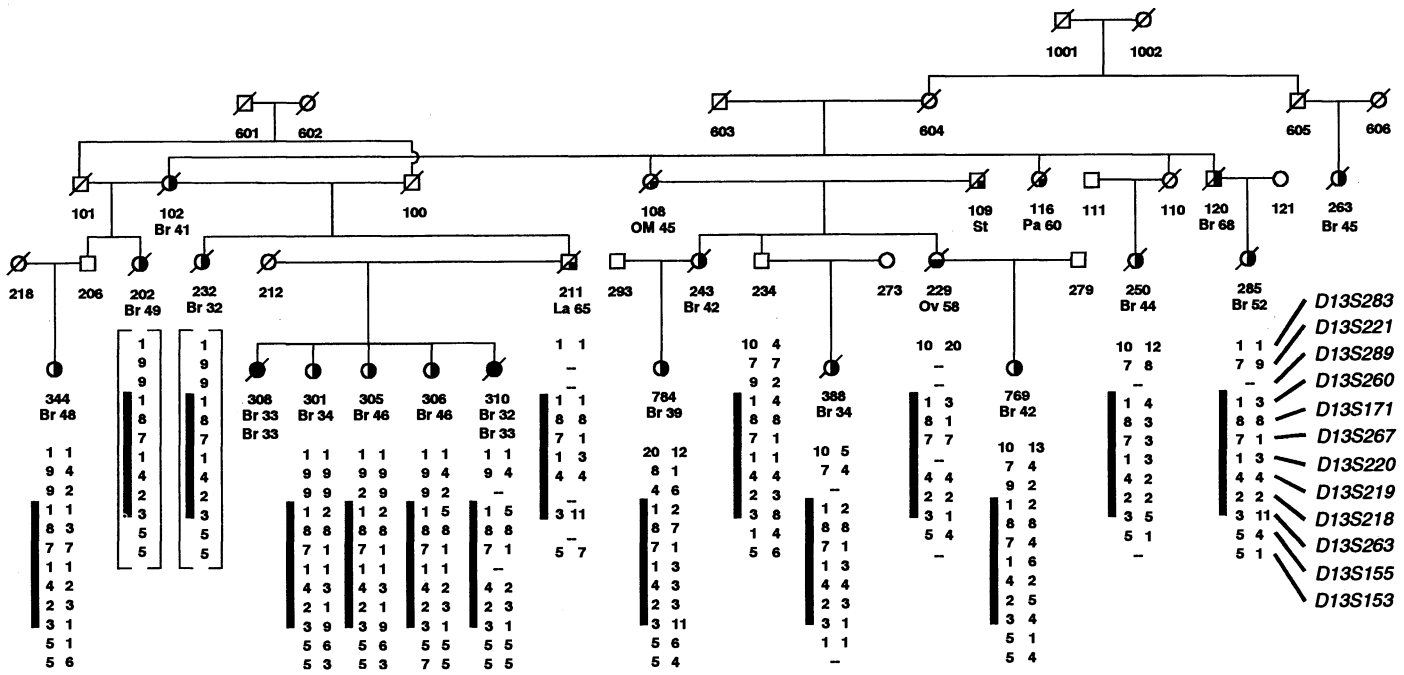

Fig. 1. Pedigree of CRC 186. Half shading (right side), breast cancer; full shading, bilateral breast cancer; half shading (bottom), ovarian cancer; quarter shading, other cancer. Types of cancer: Br, breast; Ov, ovary; Pa, pancreas; La, larynx; St, stomach; OM, ocular melanoma. The number after the cancer type is the age of diagnosis. Unaffected individuals who are potential gene carriers have been omitted. Marker numbers are shown on the right adjacent to the haplotype of individual 285. The black bar indicates the haplotype shared by all affected individuals. Genotypes in square brackets are inferred.

linkage to *BRCA2* (multipoint lod score greater than 3.0), there are 49 reported cases of breast cancer, 39 under age 50, and only 3 ovarian cancers (excluding 5 cases of breast cancer and 4 of ovarian cancer in Utah 107 that do not carry the linked haplotype). This suggests that the risk of ovarian cancer attributable to *BRCA2* may be lower than that for *BRCA1*, which confers an estimated 63% risk by age 70 (*16*). There may also be a difference in the risk of male breast cancer. In the same two families, there were four cases of male breast cancer and three more cases in other families showing some evidence of linkage to *BRCA2*. By contrast, no male breast cancers have been observed in families showing strong evidence of linkage to *BRCA1*. Thus, the risk of breast cancer in men carrying *BRCA2* mutations, though still small, is probably greater than in men carrying *BRCA1* mutations. However, the absolute risk of male breast cancer is still small, and many families where the risk of breast cancer is attributable to *BRCA2* will be characterized by female breast cancer only (for example, IARC 2932).

Although in the majority of families in our data set breast cancer can now be attributed to *BRCA1* or *BRCA2*, it is likely that these genes still do not account for all breast cancer caused by high-risk susceptibility genes (of the order of 5% of all cases). Both the overall evidence for genetic heterogeneity and inspection of haplotypes in individual families indicate that an additional gene (or genes) conferring susceptibility to breast cancer remains to be discovered.

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17. All studies were carried out with the full informed consent of the families. We thank the Cancer Research Campaign, the Medical Research Council, the Committee for Clinical Research of the Royal Marsden Hospital, the National Institutes of Health (grants CA-48711, HG-00571, CN-05222, and RR-00064), grant DAMB17-94-J-4260 from the U.S. Army, Myriad Genetics, the European Community Concerted Action on Genetics and Epidemiology of Hereditary Breast Cancer Linkage (PL920890), the Chetwood Aiken fund, and the Ligue Nationale contre le Cancer for their support. We also thank the families for their encouragement and cooperation, the many clinicians who have helped in the identification of cases, in particular the late Dr. E. Gardner, all the members of the International Breast Cancer Linkage Consortium, F. Lennard, M. Ponder, and T. Harrington.

25 July 1994; accepted 26 August 1994