Special Lecture

The Search for BRCA1

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Abstract

BRCA1, a gene predisposing to breast and ovarian cancer, was mapped to chromosome 17q21 by linkage analysis. Loss of heterozygosity in breast and ovarian tumors from BRCA1-linked patients always involved loss of wild-type alleles from chromosome 17q21, suggesting that BRCA1 acts as a tumor suppressor gene. Meiotic recombination in linked families constrained the BRCA1 region to an estimated physical size of 650 kilobases. Twenty-two candidate genes were isolated by screening complementary DNA libraries with yeast artificial chromosomes and cosmids from the critical region. Of these, 8 were known human genes, 7 were homologues of genes identified in other species, and 7 encoded novel transcripts. Each gene was sequenced and analyzed for variation, revealing 44 variants, including 8 known human genes, 7 were homologues of genes identified in other species, and 7 encoded novel transcripts. Each gene was sequenced and analyzed for variation, revealing 44 variants, including two missense mutations in two genes which segregated with breast cancer and were not found in controls. However, no frameshift, nonsense, or regulatory mutations were found.

Genetic Epidemiology of Breast and Ovarian Cancer

Linkage of early-onset breast cancer to chromosome 17q21 was discovered 4 years ago (1). The existence of a gene that increases susceptibility to breast and ovarian cancer was verified with subsequent work, with odds >1026:1 (2, 3). BRCA1 has recently been cloned (4, 5), and experiments were performed to verify that the announced candidate is BRCA1 (6). In what follows, we present our approach to the search for BRCA1, the 22 genes cloned and sequenced in the BRCA1 region, the mutations identified in these genes, and the implications of the cloning of BRCA1 for breast cancer research.

BRCA1 appears to explain most of inherited breast cancer and inherited ovarian cancer. Of the 104 families in our series, predisposition to breast and/or ovarian cancer are linked to BRCA1 in 57% of families with five or more affected relatives (14). Similarly, in a series of 214 families from Europe and America, 60% of families with at least three breast cancer cases and more than 90% of families with multiple relatives with breast and ovarian cancer trace susceptibility to BRCA1 (3). Furthermore, among families with at least three cases of ovarian cancer and no early-onset breast cancer, the proportion with cancer linked to BRCA1 is 78% if risk is assumed to be restricted to ovarian cancer, and 100% if predisposition to both breast and ovarian cancer is assumed (7).

Risks of breast cancer to women inheriting BRCA1 are extremely high, exceeding 50% before age 50 years and reaching 80% by age 65 years (3, 8—10). However, families who have participated in genetic studies were selected because multiple relatives developed breast cancer and may not be representative of all families with BRCA1 mutations. That is, there may exist other families with breast cancer linked to less severe mutations in BRCA1. Now that the BRCA1 gene is cloned, it will be possible to identify women carrying variant alleles and to begin to evaluate the risk associated with each mutant sequence.

Inherited breast cancer is not restricted to early-onset disease. Inherited predisposition influences a higher proportion of breast cancer at younger ages, accounting for an estimated 30% of breast cancer diagnosed before age 40 years, compared to less than 5% of breast cancer diagnosed after age 60 years (8, 11). However, it is likely that the absolute number of patients with inherited disease is greater among older patients. Reanalysis of the original BRCA1 linkage data with a different liability class structure suggested that inherited breast cancer occurs in all age classes and that linkage of BRCA1 to early-onset disease was easier to identify because far fewer sporadic cases appear (12). This hypothesis can now be tested directly.

Other genes responsible for inherited breast cancer have been identified. BRCA2, on chromosome 13q12—13, is linked to breast cancer in families with both females and males affected, as well as in some families with only female breast cancer (13). In our series, breast cancer is linked to BRCA2 in approximately 10% of families (i.e., ~30% of high-risk families with breast cancer not linked to BRCA1), including families with breast cancer in males and females and families with ovarian cancer (14). The first gene identified for inherited breast cancer was p53, which is responsible for the Li-Fraumeni syndrome (15). In addition to female breast cancer, families with Li-Fraumeni syndrome have extremely high rates of brain tumors and adrenocortical cancers among children with a mutant p53 allele. About 1% of women diagnosed with breast cancer before age 30 years have germline mutations in p53. Additionally, a rare point mutation in the androgen receptor gene on the X chromosome can lead to breast cancer and androgen insufficiency among males (16). Breast cancer in other families with multiple cases is probably due to other, as yet unidentified, predisposing genes. However, given the very high rate of noninherited breast cancer, many remaining families with multiple cases of breast cancer may represent coincidental occurrences of breast cancer, rather than inherited predisposition due to other genes.

The great majority of breast and ovarian cancers are due solely to acquired mutations: only 5 to 10% of breast cancer patients and 0 to 15% of ovarian cancer patients have inherited mutations leading to the disease. However, although inherited breast cancer is a small fraction of the breast cancer burden, it is a common genetic disease (17). Five % of a disease affecting 1 in 10 women over the life span means that roughly 1 in 200 women, or more than 600,000 women in the United States, will develop breast cancer by reason of inherited susceptibility. Therefore, as an inherited trait, breast cancer is one of the most common genetic diseases in the world.
Materials and Methods

DNA Samples. Genomic DNA from lymphoblasts was transformed with Epstein-Barr virus or extracted directly as described elsewhere (18). Breast or ovarian tissue embedded in paraffin blocks was extracted using a 2-mm skin biopsy punch. Paraffin was sliced off using a sterile scalpel, and each tissue punch was treated with 100 μg of proteinase K in 200 μl of 100 mM Tris-4 mM EDTA, pH 8. Proteinase K was inactivated by incubating the sample at 95°C for 10 min, and the sample was centrifuged to pellet cell debris and paraffin. Polymorphic markers were typed on families and tumors as described elsewhere (9).

cDNA Isolation. Two cDNA libraries were constructed in Agt10 with C600 host, using normal ovarian tissue and a nontransformed fibroblast cell line. The cDNA libraries were plated at 4 × 10⁷ plaques/plate (as instructed in Ref. 19) and transferred to Colony/Plaque Screen Hybridization Transfer Membranes (GeneScreen Plus) per NEN Dupont instructions.

YAC probes were prepared by running 1% HGT agarose pulsed field gel electrophoresis, cutting out the unique YAC band, purifying YAC DNA with a Gene Clean II kit (Bio101 Inc., La Jolla, CA), and radioactively labeling the YAC for use as a probe with a Multiprime DNA Labeling System (Amersham) and [α-32P]dCTP and [α-32P]dATP (from NEN Dupont).

Cosmid probes were prepared by digesting the DNA from cosmids with NotI, running 1% HGT agarose gel electrophoresis in 1× TBE, purifying the insert DNA with a Gene Clean II kit (Bio101 Inc., La Jolla, CA). Each cosmid was radioactively labeled with a Multiprime DNA Labeling System (Amersham) and [α-32P]dCTP (from NEN Dupont) and combined into pools of less than 8 cosmids/pool.

Hybridization of YACs and cosmids onto the fibroblast and ovarian cDNA libraries was carried out in a solution of 6X SSC, 1% SDS, 5% Dextran Sulfate, 0.25 mg/ml human placental DNA (Sigma). Wash stringency was 0.1× SDS and 0.1× SSC at 65°C; duplicate filters were aligned, and positive hybridization signals were used to pull which filters were diluted into 1 ml SM (19) plus 20 μl chloroform. Twenty μl of a 1:1000 dilution of the plug in SM were plated for secondary screening. Lifts of secondary filters were used to pull tertiary positive cDNA clones, which were plated and screened in the same manner.

Constructing a Physical Map and Contig of the Region. Pulsed field gel electrophoresis was done on genomic DNA samples digested with NotI, MluI, RsrII, NruI, SacII, or EcoRl and run on 1% HGT gels in 0.5× TBE. Southern blot filters were probed with cDNA clones from the BRCA1 region. Sequence-tagged sites and polymorphic markers on chromosomes 17q21 were used to identify YACs from the Centre d’Etude du Polymorphisme Humain and Washington University libraries. Cosmids were identified by hybridizing Alu-PCR products of YACs to a flow-sorted chromosome 17 cosmid library.

Identification and Amplification of cDNA Clones. PCR amplification of clones isolated from bacteriophage Agt10 fibroblast cDNA library screened with YAC or cosmid probes was done in 50-μl reactions, containing 1 μl phage DNA from bacteriophage plaque in 0.5 ml SM buffer, 200 μM concentrations of each deoxynucleotide triphosphate (Perkin-Elmer), 1× PCR buffer (Perkin-Elmer), 50 pmol each primer, and 1.25 units AmpliTaq DNA polymerase (Perkin-Elmer). Thermocycling was done for 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min. Primer sequences used were: Agt10 Left primer -40: 5’ GCC CTA TAG ACT GCT GGG TAG TC 3’, and Agt10 Right primer -38: 5’ AAG ATT GCG GGT AAA TAA CAG AG 3’. Amplified products of λ clone inserts were run on 1% HGT agarose gel in 1× TBE to ascertain the insert sizes, and the DNA was purified with Gene Clean II kit for use in hybridizations against Southern blot membranes. Northern blot membranes, transcript grid membranes, and cosmid grid membranes. Hybridization of cDNA Clones to Determine Redundancy and Chromosomal Location. Transcript grid membranes were made by growing λ cDNA clones identified in the library screen in an array on LB plates and transferring to GeneScreen Plus hybridization membranes. Cosmid grids were produced by growing cosmids in an array on LB plates and transferring to GeneScreen Plus hybridization membranes. YACs were grown in adenine hemisulfate casein liquid cultures, and total yeast and YAC DNA were isolated (as described in Ref. 20), and digested with EcoRI, run on a 1% HGT agarose gel (20 cm long) in 1× TBE, and Southern blot transferred onto Hybond N (Amersham) as described in Ref. 19.

PCR-amplified products of cDNA were used as probes in hybridizations against YAC Southern blots, cosmid grids, and transcript grids. Primers from cDNA clones were used to amplify DNA from somatic cell hybrids including parts of human chromosome 17 (21), as well as YACs and cosmids from the region.

DNA Sequence Analysis. PCR-amplified products of cDNA clones were either sequenced directly after PCR amplification using a USB PCR Product Sequencing Kit or digested with EcoRI, then subcloned into M13 mp18, and sequenced with USB Sequencing Kit version 2.0. Sequences were resolved on 6% denaturing polyacrylamide gels and exposed to Kodak X-ray film for 24–72 h. Sequencing primers used were the M13 universal primer and sequence-specific internal primers. BLAST was used for nucleotide and amino acid searches of databases, including Genbank and EMBL.

Northern and Southern Blot Hybridizations. PCR-amplified products of cDNA clones were radioactively labeled with a Multiprime DNA Labeling System (Amersham) and [α-32P]dCTP (from NEN Dupont) and used as probes in hybridizations against Southern blots and Northern blots.

Southern blots were made using genomic DNA from lymphoblastoid cell lines of one or more relatives from each of 16 BRCA1-linked families and of unaffected controls. Genomic DNA (7–10 μg) was cut with MspI, BanHI, or EcoRI and run on a 1% agarose gel (20 cm long) in 1× TBE, and Southern blot was carried out according to Ref. 19. Blots were probed with cDNA clones as described, with a wash stringency of 0.2× SSC and 0.1% SDS at 65°C.

Northern blots including multiple tissues were made from polyadenylated RNA or purchased from Clontech, probed, and washed up to a stringency of 1× SSC, 0.1% SDS at 65°C. Lymphoblastoid polyadenylated RNA from 15 BRCA1-linked patients and 8 controls were extracted, blotted, probed, and washed at a stringency of up to 0.1× SSC-0.1% SDS.

Mutation Searching Methods. Chemical cleavage mismatch of cDNA PCR products between 200 and 300 base pairs long was done according to the method of Cotton et al. (22). Analysis of SSCP was carried out using PCR as described above, with the use of 50 ng DNA or cDNA as template, using 10 μM dCTP, and adding 0.5 μCi [α-32P]dCTP (NEN Dupont). DNA template for PCR was lymphoblastoid cDNA or genomic DNA from at least 15 unrelated BRCA1-linked patients. Controls included normal breast, normal ovary, and cell lines MCF7 and HeLa, and untransformed IMR90 fibroblast. PCR-amplified samples were diluted 1:10 in formamide buffer (98% formamide, 10 mM EDTA, pH 8–0.05% bromphenol blue-0.05% xylene cyanol). Diluted samples were then held at 95°C for 5 min, cooled rapidly to 4°C and held for 5 min. For each sample, 5 μl were loaded onto a SSCP gel run at 6 W (constant power) for 14 h in 0.6× TBE at room temperature. An 80 μl gel solution contains 0.5% MDEK (AT Biochem), 0.6× TBE, 160 μl 25% ammonium persulfate, and 38 μl N,N,N’,N’’,N’’-tetramethylethylenediamine. Gels were dried on a vacuum gel dryer and exposed to film for 24–48 h. Variant bands were cut out of the gel, rehydrated in 100 μl water overnight at 4°C, amplified with appropriate SSCP PCR primers, and directly sequenced with the USB PCR product sequencing kit.

Results and Discussion

Indirect Evidence That BRCA1 Is a Tumor Suppressor Gene

Analysis of tumors from families with BRCA1-linked breast and ovarian cancer suggests that BRCA1 may act as a tumor suppressor gene. Tumors from BRCA1-linked cases in families 3 and 82 were evaluated for loss of heterozygosity at markers near BRCA1. Of the seven tumors with LOH in the linked region, loss was invariably from the chromosome 17 carrying the normal allele at BRCA1. Fig. 1 illustrates pedigrees for BRCA1-linked families 3 and 82 and results from PCR amplification of informative markers from tumor and adjacent normal cells from the same paraffin-embedded biopsy.
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Family 3

A

Fig. 1. A, LOH in tumors revealed by polymorphic markers from chromosome 17q21 on paired normal and tumor DNA samples from family 3. Filled symbols, breast or ovarian cancer; speckled symbols, tumors. Below each individual is their age at cancer diagnosis, or current age or age at death if unaffected. Persons with reconstructed genotypes are denoted with brackets. For each tumor with LOH, wild-type alleles are lost and mutant BRCA1 alleles were retained. B, LOH in tumors from Family 82. The chromosome 17 carrying the wild-type BRCA1 allele is lost in the ovarian tumor of individual II-2 and the small intestinal tumor of individual II-7.

Family 82

specimen. In family 3, LOH was observed throughout the BRCA1 region in two breast tumors and three ovarian tumors from three patients with cancer linked to BRCA1. In all five tumors, the chromosome carrying the mutant BRCA1 was retained. In family 82, the normal BRCA1 allele was lost in the ovarian tumor of one patient and in the tumor of the small intestine of a male relative, both of whom carry BRCA1 germline mutations. Loss of heterozygosity was not seen in one tumor sample from family 3, which may reflect no deletion, or amplification of contaminating normal tissue, or the presence of a deletion too small to be detectable by genotyping markers several megabases apart. The observation from these families that LOH is always of the chromosome carrying the normal BRCA1 allele confirms data previously reported for other BRCA1-linked families (23, 24).

BRCA1 may also act as a tumor suppressor in the far more common cases of sporadic, rather than inherited, breast or ovarian cancer. Breast and ovarian tumors from patients not selected for family history frequently lack the BRCA1 region of 17q21. The frequency of LOH in the BRCA1 region ranges from 40 to 80% among sporadic
breast carcinomas (25–27) and from 30 to 60% among sporadic ovarian carcinomas (28–30).

Patients with no evident family history of breast or ovarian cancer may nevertheless have germline BRCA1 mutations (5). Breast and ovarian carcinomas from 25 patients with primary tumors at both sites were evaluated for LOH using 21 dinucleotide repeat markers in the BRCA1 region (31). Of the ovarian tumors, 20 (80%) had lost part or all of chromosome 17q. The loss always included at least the entire BRCA1 region. Of the breast tumors from the same patients, 40% had lost the BRCA1 region. Of the 10 patients whose breast and ovarian tumors both revealed LOH, losses were always of the same chromosome 17q. These patients may have inherited a mutant BRCA1 allele on the retained chromosome, even in the apparent absence of a family history of breast or ovarian cancer.

An alternative to the hypothesis that BRCA1 is a tumor suppressor is the possibility that BRCA1 is a dominant predisposing gene located very near a still-unknown tumor suppressor. If this were true, tumor development would require that acquired alterations in the tumor suppressor be associated with selection for retention of the mutant BRCA1 in the tumor. However, the observation that most BRCA1 mutations observed thus far appear to lead to loss of function (4–6) suggests that this alternative hypothesis is unlikely.

Hopeful Digressions

In our experience, positional cloning involves two types of activities in parallel. One pathway is physical mapping, contig construction, library screening, and characterization of candidate genes. The other pathways are “hopeful digressions,” searches for critical patients or tumors that would provide shortcuts to the gene if they can be found. Although none of these shortcuts revealed BRCA1, the same approaches may reveal other cancer genes.

Identification and Characterization of Triplet Repeats. More recent generations of women in breast cancer families develop breast cancer at younger ages than did their ancestors. This observation could represent ascertainment bias, a cohort effect due to changes in prevalence of noninherited risk factors, and/or biological anticipation. The discovery that unstable triplet repeats might play an important role in inherited disease (32, 33) made a search for triplet repeats in the BRCA1 region irresistible. CAG, CCG, GAG, AAG, and AAT triplets were identified from cosmid maps in the region, sequenced, and screened for polymorphism. A few repeats detected by this method were polymorphic, but none underwent large expansions.

Instability of Short Repeats. Genomic instability characterizes tumors from families with inherited colon and endometrial cancer (34, 35). Tumor DNA samples from patients in families with cancer linked to BRCA1 were screened for 20 dinucleotide repeats on various chromosomes. No instability was found, although occasional new alleles did appear in markers with high heterozygosities.

Search for Chromosome 17q Alterations in Patients. A patient with severe developmental disabilities and early-onset breast cancer might have a chromosomal rearrangement involving both BRCA1 and developmentally important genes. Three patients were identified with severe developmental disabilities and breast cancer at ages 28, 32, and 33 years, respectively. The three patients had chromosomal aberrations on three different chromosomes, none of which involved chromosome 17. We will return to these alterations to search for other breast cancer genes.

A balanced translocation involving BRCA1 might be revealed as both breast cancer and a history of miscarriage. Six patients were identified who were diagnosed with breast cancer before age 30 years and who had, or whose mothers had, frequent miscarriages. No translocations were detected in the three patients karyotyped thus far. We tried the same idea in reverse, screening records of amniocenteses for alterations in chromosome 17q and then interviewing parents of pregnancies with 17q alterations to determine family history of breast cancer. Two families were identified who had both balanced translocations involving chromosome 17q21 and family history of breast cancer. The informative relatives in both families were sampled and karyotyped, but in neither family did the balanced translocation segregate with breast cancer.

A Possible Contiguous Gene Syndrome. An extended kindred in which breast and ovarian cancer were coincident with palmoplantar keratoderma was reported 6 years ago (36). Last year, palmoplantar keratoderma was mapped to chromosome 17q12–q21 (37). Hence, the family with both conditions might carry an inversion or deletion involving BRCA1. Pulsed field filters and Southern blots with DNA from both affected and unaffected members of the family were probed with keratin genes, cosmids, and other genes in the BRCA1 region. No rearrangements were detected on chromosome 17q by Southern or by further karyotyping or FISH analysis. Subsequently, a point mutation in keratin 9 was identified as the cause of palmoplantar keratoderma in this family (38). This family probably carries independent mutations on chromosome 17q, one leading to breast and ovarian cancer and the other to palmoplantar keratoderma, certainly not impossible given the estimated frequency of 1 in 200 for carriers of BRCA1 mutations.

FISH Analysis of Possible Chromosomal Rearrangements in Tumors. Two ovarian cancer patients whose tumors had possible rearrangements of chromosome 17q were identified.5 The patients were diagnosed at ages 68 and 79 years, with no family history of breast or ovarian cancer. Both tumors were aneuploid, with 3 and 4 copies of chromosome 17, respectively. In one tumor, a translocated 17 was observed in one preparation only. In the other tumor, one of the four copies of chromosome 17 was consistently observed to carry a translocation, which by FISH analysis was proximal to the region of linkage.

Narrowing the Linked Region by Meiotic Recombination

The original linkage data mapped BRCA1 to a 50-cM region. In order to close in on the gene, new polymorphisms were developed at multiple loci (39–41), and a high density genetic map was constructed for chromosome 17q12–q21 (21). Transformed lymphocyte lines were established for 104 families with multiple cases of breast or ovarian cancer, and all informative relatives were genotyped. The region of 17q21 linked to breast and ovarian cancer was first refined to 8 cM (9), then to 4 cM (40), then to 1 megabase bounded by the markers D17S856 and D17S78 (42, 43).

Positional cloning of candidate genes began when the linked region was 1 megabase long. However, the linked region was further refined by an informative recombination event in Family 1 (Fig. 2). Recombination between breast cancer and D17S7141 (44) constrains the BRCA1 interval to an estimated physical region of about 650 kilobases (Fig. 3). In principle, either or both breast cancer patients whose recombination events define the 650-kilobase interval could be sporadic cases. However, the critical proximal recombinant occurred in a patient diagnosed with breast cancer at age 37 years with a daughter (also recombinant, of course) diagnosed at age 27 years and with 15 other relatives with breast and/or ovarian cancer in the family. This recombinant excluded the gene for 17β-hydroxysteroid dehydrogenase as a BRCA1 candidate.

Isolating and Mapping Candidate Genes in the BRCA1 Region

Candidate genes in the BRCA1 region were isolated by screening fibroblast and ovarian cDNA libraries directly with eight YACs and four
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Family 1

Fig. 2. Alleles defining the chromosome 17q21 haplotype linked to breast and ovarian cancer in Family 1 (outlined). Individual III-12 is recombinant at D17S5141. Her daughter IV-2, diagnosed at age 27 years, also inherited the recombinant chromosome 17.

Definition of 22 Genes in the BRCA1 Region by Sequence Analysis

cDNA clones representing each gene that were sequenced and the databases searched for nucleotide and amino acid homologies (45). The locations of these genes are shown in Fig. 3. Eight genes identified by positional cloning were known human genes: Ras-related protein Rab5c (46); 17β-estradiol dehydrogenase; α-N-acetylglucosaminidase (47); γ-tubulin; Ki nuclear autoantigen; a B-box protein IA13B (48); vaccinia virus VH1-related dual-specificity tyrosine/serine phosphatase (VHR); and adenovirus E1A enhancer binding protein (E1A-F). Additional sequence and analysis of E1A-F is described elsewhere.5

Seven genes are newly cloned human homologues of genes known in other species; Genbank accession numbers for the human genes are shown in Fig. 3. cDNA clones B66 and B60 encode a protein homologous to proton pump cloned from rat (M58758) and Caenorhabditis elegans (Z11115), as well as to bovine vacuolar H+ ATPase (L31770) and a mouse immunosuppressor factor (M31226). cDNA clone LF26 encodes a protein homologous to an acyl-CoA desaturase cloned from mouse (M21285), rat (J02585), and yeast (J05676). cDNA clones B52 and LF96 encode a protein homologous to the Drosophila melanogaster transcription factor Enhancer of zeste (U00180).7 cDNA clone B7F appears to encode a pseudogene of the high mobility group protein HMG17 (M12623), which maps to chromosome 1p and may have more than 50 pseudogenes (49). cDNA clone LF13 encodes the human homologue of Pacific electric ray vesicle amine transport protein VAT1 (P19333) and is described elsewhere.5 cDNA clone EL107 encodes a homologue of human endogenous retrovirus-related pol and protease polyproteins (sp P10266, M14123) and mouse gag polyprotein (pir S31034). cDNA B154 and cross-hybridizing clones encode the human homologue of Saccharomyces cerevisiae PRP22 (X58681), a helicase in-

pools of cosmids from a partial contig of the 1-megabase BRCA1 region. More than 400 independent cDNA clones were isolated from the libraries after the first round of screening. The 17q21 chromosomal locations of the cDNA clones were confirmed by hybridization to multiple chromosome 17 YACs, to chromosome 17 cosmids, and to hybrid somatic cell lines carrying critical portions of chromosome 17q (21). About 40% of cDNAs identified with YACs did not map back to chromosome 17q21. cDNA clones that mapped back to 17q21 were cross-hybridized with each other and fell into clusters that defined 22 genes (Fig. 3). The order of genes on the chromosome was determined by their position in the contig. The BRCA1 gene appears to map to a gap; i.e., a region not represented in the YACs that made up the contig. Physical distances were estimated by analysis of Southern blots from pulsed field gels of genomic DNA samples of BRCA1-linked patients and controls.

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7 K. Abel et al., unpublished data.
volved in pre-mRNA splicing. Human PRP22 is described in more detail elsewhere.8

Seven apparently novel genes were identified. B102, LF98, and other cross-hybridizing clones encode a 6.4-kilobase message with patches of sequence similarity to *Xenopus laevis* Xotch, *D. melanogaster* Notch, human TAN-1, a human trithorax-homologous protein, and rat neurexin. EL76 encodes a 1.0-kilobase message with no significant similarities to known genes. B32 and several cross-hybridizing clones encode a protein with coiled coils and similarity to tropomyosin, myosin, nuclear mitotic apparatus protein, ezrin, and merlin. B169 and B213 encode a novel gene with two transcript sizes (3.0 and 3.8 kilobases) in lymphoblasts and no similarity to known

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8 E. Ostermeyer et al., unpublished data.

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![Fig. 3. The BRCA1 region of chromosome 17q21, defined by meiotic recombinants at D17S856 and D17S78. The proximal boundary was subsequently defined by D17S1141, narrowing the linked region to ~650 kilobases (shown between the horizontal lines), and excluding 10 candidate genes. cDNA clones representing genes isolated in our library screens were mapped to the partial YAC contig (vertical lines on the left) and are listed in order from centromere to telomere. Also shown is MOX1, which we did not find (57). Transcript sizes, homology, and Genbank accession numbers are listed to the right of each clone.](image)
Analysis of Genes for Mutations in BRCA1-linked Families

The genes identified by positional cloning were analyzed for mutations, with thorough analysis of genes between D17S1141 and D17S758. All variants found by Southern blot, by analysis of single-strand conformation polymorphism (50, 51), and by chemical mismatch cleavage (22) are summarized in Table 1. No aberrant transcripts appeared on Northern blots of RNA from lymphoblast lines of BRCA1-linked patients. RFLPs were found in nine of ten genes screened by Southern blot. Fourteen genes contained point mutations, which were identified and sequenced.

Three mutations, one in the PRP22 homologue and two in E1A-F, changed the predicted amino acid sequence. In the PRP22 homologue, SSCP analysis detected a variant that cosegregated with breast cancer in family 94 (Fig. 4). Sequence analysis of this variant revealed a missense mutation (asparagine to serine) in the PRP22 homologue that was not seen in 100 control chromosomes. A missense mutation at nucleotide 457 of the published E1A-F sequence (D12765), whereas the wild-type sequence (right) has only a C. In the images illustrated, lanes from left to right are C, T, A, and G. The sequence on the left is that of individual III-1 and has both C and A nucleotides at base pair 457 of E1A-F (Genbank No. D12765), whereas the wild-type sequence (right) has only a C. The C→A variant appeared in all relatives in family 74 with BRCA1-linked breast cancer chromosomes.

Relevance of BRCA1 to the Control of Breast Cancer

Incidence rates of breast cancer have increased 25% in the past 20 years and are now 10% by age 80 years (52). Mortality rates have not increased, because survival of breast cancer patients has improved. The increase in breast cancer risk almost certainly has nothing to do with changes in frequencies of susceptibility alleles. Rather, breast cancer risk has probably increased as the result of gradual changes in the prevalence of established risk factors for breast cancer, particularly the length of the interval between menarche and first full-term pregnancy (53). At the beginning of this century, the average age at menarche was 14.5 years and the average maternal age at first birth was 21 years (54). Among young women in the United States now, the average age at menarche is 11 years, and child-bearing frequently begins in the late 20s or 30s. Therefore, the interval of exposure of dividing breast ductal cells to hormonal stimulation is twice as long now as a century ago. Epidemiological studies of women exposed to...
radiation as teenagers indicate that the first mutations leading to breast cancer probably occur in this period (55, 56). An association between increased breast cancer risk and an extended interval of menstruation before pregnancy is biologically plausible, because all breast ductal cells, whether normal or mutant, are dividing rapidly during this period therefore abnormal cells would have every opportunity for clonal growth.

The link between inherited predisposition and increasing risk of breast cancer lies in the application of genetics to diagnosis and prevention. Creating molecular tools for earlier diagnosis and developing ways to reverse the first steps of tumorigenesis may be the most effective means of breast cancer control. Now that \( BRCA1 \) has been cloned, additional critical questions can be addressed. What are the normal and mutant products of the gene? What are the biological consequences of these early changes? What are the different \( BRCA1 \) mutations and how does the risk associated with each differ? What proportion of breast and ovarian cancers in the general population involve somatic mutations at \( BRCA1 \)? How do inherited alterations interact with somatic alterations of other genes? Clinically, which compounds are most effective against each tumor genotype? How can a gene altered early in breast cancer be used as a target for detection at the molecular level? Even more fundamentally, can a missing gene product be replaced or the effects of an aberrant one be prevented?

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References


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