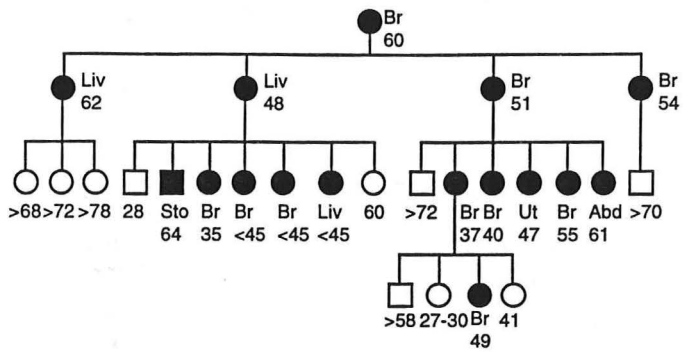
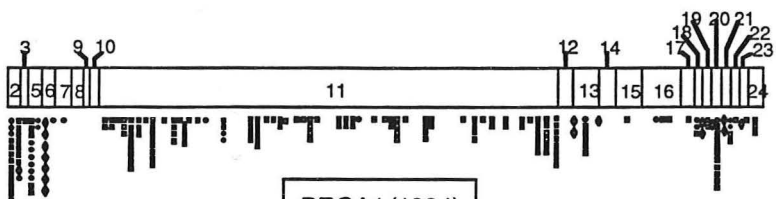
BRCA1 and *BRCA2:* Genes with Frequent Mutations that Predispose to Breast and Ovarian Cancer

Joel D. Taurog, M.D.

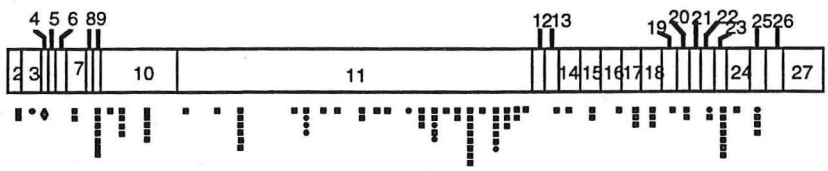
Internal Medicine Grand Rounds
UT Southwestern Medical Center
July 3, 1997



BROCA, P. (1866)



BRCA1 (1994)



BRCA2 (1995)

Joel D. Taurog, M.D.
Professor of Internal Medicine, Division of Rheumatic Diseases
Investigator, Harold C. Simmons Arthritis Research Center

Research Interests: Association of HLA-B27 with rheumatic disease in particular; genetic basis of common diseases in general

I wish to thank Sherry Fariss for secretarial assistance and Drs. Richard Baer, Anne Bowcock, Baruch Modan, and David Goldgar for sharing unpublished data.

This Grand Rounds is dedicated in memory of Susan Perlman, Chaviva Isersky, and Karen Gross.

Cover: The pedigree diagram is taken from a family described in the monograph *Traité des Tumeurs* (1866) by the French surgeon Paul Broca, in which 10 cases of breast cancer, as well as other cancers (liver, stomach, uterus, and an unknown intraabdominal site), were documented in four generations. The age at death in years is indicated for each individual.

MAPPING AND CLONING OF *BRCA1* AND *BRCA2*

The Evidence for Familial Breast and Ovarian Cancer

Breast cancer accounts for 32% of newly diagnosed cancers in women in the United States, making it the most most common cancer in women, with an estimated 182,000 new cases and 46,000 deaths annually (Kelsey *et al.*, 1996). Women in the U.S. are estimated to have a 10-12% cumulative lifetime risk of developing this disorder. The overall mortality rate from breast cancer has remained relatively constant for several decades at ~100 per 100,000 women per year, although the rate has increased for women older than 54 and decreased for women below that age (Bailar *et al.*, 1997). A number of risk factors have been established for breast cancer in women. These are summarized in Table 1. Among the more prominent risk factors is a family history of breast cancer. The observation that breast cancer tends to cluster in certain families, with a tendency to earlier age of onset, bilaterality, multiple primary tumors, and association with other cancers, led to the search for predisposing genes that has resulted in the isolation of the two genes, *BRCA1* and *BRCA2*, that are the topics of this Grand Rounds.

Table 1. Epidemiologic risk factors for breast cancer in women

Relative risk > 4.0
North American or Northern European residence
Mother *and* sister with breast cancer at young age
Relative risk 2.1-4.0
Mother *or* sister with breast cancer
Radiation to chest, moderate to high dose
Relative risk 1.1-2.0
High socioeconomic status
Never married
Urban residence
Residence in Northern US
White
Jewish
Maintaining ovaries ≥ age 40
≥30 yr old at first pregnancy
≤11 yr old at menarche
≥55 yr old at menopause
History of primary cancer in endometrium or ovary
Obesity

From Kelsey *et al.*, 1996

Invasive ovarian cancer is the fourth leading cause of cancer death in women in the US, with the highest mortality rate of all gynecologic cancers. There are an estimated 26,600 new cases of epithelial ovarian cancer annually in the U.S, and 14,500 deaths from the disease, with an overall 5 year survival rate of only 37% (Westhoff, 1996; Boyd & Rubin, 1997; Ozols *et al.*, 1997). As shown in Table 2, a family history of ovarian cancer is a strong epidemiologic risk factor for ovarian cancer, suggesting, as for breast cancer, the possibility of a genetic predisposition.

Reports dating back at least to the mid-19th century have documented aggregation of breast cancer in certain families (Broca, 1866) (see cover) and ovarian cancer in other. More recently, families were reported with clustering of both breast and ovarian cancer. Lynch *et al.* (1978) reported that of 86 families with breast cancer aggregation, twelve families showed a remarkable prevalence of co-existing ovarian cancer, with evidence for a highly penetrant dominant gene predisposing to

either type of cancer. Many of these families also showed cancers at other sites and individuals with two or more primary cancers.

Table 2. Epidemiologic risk factors for ovarian cancer
Age > 60
Residence in Europe, North America, Israel, Australia, New Zealand
Nulliparous
Never used of contraceptives
≥ 2 relatives affected with ovarian cancer
From Westhoff, 1996

Large epidemiologic studies in the 1980's provided strong support for the concept of a dominant gene predisposing to early onset breast cancer. Newman *et al.* (1988) examined 1,579 nuclear families of probands with breast cancer diagnosed before age 55. Their data fit a maximum-likelihood (single locus) Mendelian model, with a highly penetrant susceptibility allele with a frequency of 0.0006 in the general population, conferring a lifetime risk of breast cancer of 0.82 on those carrying the allele, compared with a 0.08 risk in those without it. This inherited susceptibility was predicted to affect only 4% of the families. Very similar predictions arose from the data of Claus *et al.* (1990, 1991), who studied 4,730 patients with histologically confirmed breast cancer diagnosed between ages 20 to 54 years, and a control group of 4,688 women. They also inferred the existence of an autosomal dominant allele with a frequency of 0.0033 and a cumulative lifetime risk of breast cancer of 92% in female carriers and 10% in noncarriers. Similar although less definitive conclusions were drawn from studies of familial ovarian cancer (Schildkraut *et al.*, 1988). The stage was thus set for the application of modern genetic mapping techniques.

The advent of DNA-based methods of linkage analysis, and especially the development of a dense human genetic map of highly polymorphic microsatellite markers detected by the polymerase chain reaction (PCR), made it feasible to carry out linkage analysis in families with multiple cases of breast cancer, and subsequently to undertaken positional cloning of the implicated genes.

Mapping and Identification of *BRCA1*

The linkage study that got the ball rolling was that of Hall *et al.* (1990). This group screened the genome in 23 extended Caucasian families with 146 cases of breast cancer and 329 unaffected relatives, looking for linkage to polymorphic DNA markers. Many of these families showed the epidemiologic features characteristic of familial breast cancer, namely, a younger age at diagnosis, and unusually high frequency of bilateral disease and male breast cancer. In the early-onset families, there was strong evidence for linkage of breast cancer susceptibility to a marker *D17S74* in band 17q21 on chromosome 17, with a lod score of 5.98. Evidence against linkage (negative lod scores) was found in families with late-onset disease. The designation *BRCA1* (BReast CAncer 1) for the locus was subsequently adopted by the 2nd International Workshop on Chromosome 17 (Solomon *et al.*, 1991).

Linkage to the *D17S74* marker on 17q was rapidly confirmed (Narod, 1991) in three of five large families with multiple cases of both breast and ovarian cancer (HBOC, i.e., hereditary breast-ovarian cancer syndrome), and for one large family the lod score was 2.72 at a recombination fraction of 0.07. Many groups subsequently added to the linkage data and mapping. Moreover,

linkage to the *BRCA1* locus was also shown for seven of nine families with multiple cases of ovarian cancer but without cases of breast cancer ("site-specific" ovarian cancer families), suggesting that familial ovarian cancer is not a separate genetic entity (Steichen-Gersdorf *et al.*, 1994). Interational cooperation, in the form of the Breast Cancer Linkage Consortium, led to an analysis by Easton *et al.* (1993) of the data from 214 families (157 with breast CA, 57 with breast and ovarian CA) collected by 13 groups in the U.S. and Europe. The data best fit a model of genetic homogeneity for families with both breast and ovarian cancer, with strongest linkage to the marker *D17S588*, and genetic heterogeneity for families with breast cancer only, with ~45% of these showing linkage to *D17S588* (Table 3). These and other linkage data (Smith *et al.*, 1992; Kelsell *et al.*, 1993) mapped the *BRCA1* gene to a region of ~1-1.5 Mb.

Table 3. Multipoint heterogeneity analysis in 214 HBOC families

Group (no. of families)	LOD Score	Proportion Linked[a]
Breast-ovary (57)	20.79	1.00
Breast-only (153):[b]	6.01	.45
Average age (years) at diagnosis:		
<45 (54)	6.56	.67
45–54 (63)	.40	.19
≥55 (36)	.08	.38
No. of cases diagnosed:		
Before age 45 years:		
Two or fewer (110)	1.46	.44
Three or four (36)	1.89	.39
Five or more (7)	2.88	.72
Before age 60 years:		
Three or fewer (98)	.36	.26
Four or five (38)	3.29	.60
Six or more (17)	2.72	.45

[a] All estimates are at the maximum-likelihood position 9 cM proximal to D17S588 on the female map.
[b] Four families with male breast cancer cases were excluded.

From (Easton *et al.*, 1993)

A number of groups continued to refine the genetic and physical map of the *BRCA1* locus through 1994 (Bowcock *et al.*, 1993; Albertsen *et al.*, 1994a, 1994b; O'Connell *et al.*, 1994), eventually narrowing the search to a region of ~600 kb. A number of candidate genes in this region were investigated and found not to be mutated in the involved families, for example, the genes for estradiol 17β-hydroxysteroid dehydrogenase I and II (Kelsell *et al.*, 1993). The process during this period was described in an excellent review by Anne Bowcock, of the Department of Pediatrics here at UT Southwestern, from the standpoints both of the general problem of positional cloning strategies and of cloning *BRCA1* itself (Bowcock, 1993).

The *BRCA1* gene was eventually identified by a collaborative effort centered at the University of Utah and the company Myriad Genetics, Inc., in Salt Lake City (Miki *et al.*, 1994). The genetic data had mapped the gene within a 600 kb region between two markers, *D17S1321* and *D17S1325*. A total of 65 expressed sequences within this region were characterized by DNA sequence, database comparison, transcript size, expression pattern, genomic structure, and particularly, DNA sequence analysis in individuals from families showing linkage of HBOC to 17q. A single transcription unit was constructed from the data that, in composite, yielded a full length cDNA of a candidate *BRCA1* gene. Probable predisposing mutations were found in 5 of 8

kindreds that were thought to carry *BRCA1* susceptibility alleles, based on strong linkage of cancer to 17q21. Even from the outset, it was evident that a wide variety of mutations would be found in *BRCA1*, since the first 5 mutations included an 11-bp deletion, a 1-bp insertion, a stop codon, a missense substitution, and an inferred regulatory mutation. The polymorphic marker *D17S855* was found to map within the *BRCA1* gene.

Characteristics of the *BRCA1* gene

The *BRCA1* gene consists of 22 coding exons (24 exons total) encoded by 5,592 bp extending over 81 kb of genomic DNA (Miki *et al.*, 1994; Smith *et al.*, 1996). It was subsequently discovered that in the original report by Miki *et al.*, one of the cDNA clones used to reconstruct the open reading frame was aberrant, and what was called exon 4 in this report is in fact an *Alu* element that is not present in the normal mRNA transcript. Nonetheless, the exon numbering was retained, so that all subsequent maps of the coding gene skip from exon 3 to exon 5. There are two alternative transcription start sites in exon 1. The coding sequence is unusually AT-rich and contains an unusually high proportion of charged residues. Protein coding starts with exon 2, and hence most coding maps omit exon 1. The 7.8 kb transcript is expressed in numerous tissues, including breast and ovary, but most abundantly in thymus and testis. It encodes a predicted protein of 1,863 amino acids and predicted molecular mass of 220 kD. The protein shows little homology with known proteins, except for two regions that appear to be of major significance for its function, as discussed below. The gene has an unusually high density of *Alu* repetitive DNA (41.5%), but a relatively low density (4.8%) of other repetitive sequences. *BRCA1* intron lengths range from 403 bp to 9.2 kb, and introns make up 91% of the overall sequence. Two continguous genes have also been sequenced. These are *RHO7*, a member of the *RHO* family of GTP binding proteins, and *VAT1*, an abundant membrane protein of cholinergic synaptic vesicles. The gene order on 17q is centromere-*VAT1-RHO7-BRCA1*-telomere (Smith *et al.*, 1996).

Mutations in *BRCA1* associated with breast and ovarian cancer

As might be expected, identification and sequencing of the *BRCA1* gene led to a flurry of investigation of patients with familial and sporadic cancers for mutations in the gene. So far, no functional assays are available for detecting mutations in *BRCA1*, and therefore all mutation detection has to be carried out at the DNA level. This detection is not a trivial matter technically, since the gene is quite large and it was apparent very early that mutations were distributed throughout its length. The size of the gene largely precludes simple brute-force sequencing of the whole gene, or even all of the exons, as a screening procedure. Two major types of methods of detection have been used to screen for mutations, both using DNA fragments amplified by PCR. The first is the single strand conformation polymorphism (SSCP) assay, based on the principle that the conformation of a double-stranded stretch of DNA in which the two strands differ at one or more bases is different than that of a perfectly matched duplex. Using this strategy, primer pairs can be used that will amplify each of the exons of the *BRCA1* gene, optimally fragments $\leq$ 200 bp (see, for example, Friedman *et al.*, 1994; Langston *et al.*, 1996). The amplified product is denatured and then renatured. If an individual is heterozygous for a mutation, heteroduplexes will form between the wild-type and mutant genes, and these will run aberrantly on gel electrophoresis. The aberrant band can then be isolated from the gel and eventually sequenced to find the mutation. There are variations of this procedure, such as clamped denaturing gel electrophoresis and heteroduplex analysis. One disadvantage of these procedures is that they may fail to detect large deletions, duplications, or insertions, as well as regulatory defects (Shattuck-Eidens *et al.*, 1995). Overall, the sensitivity of these procedures for detecting mutations is ~80%.

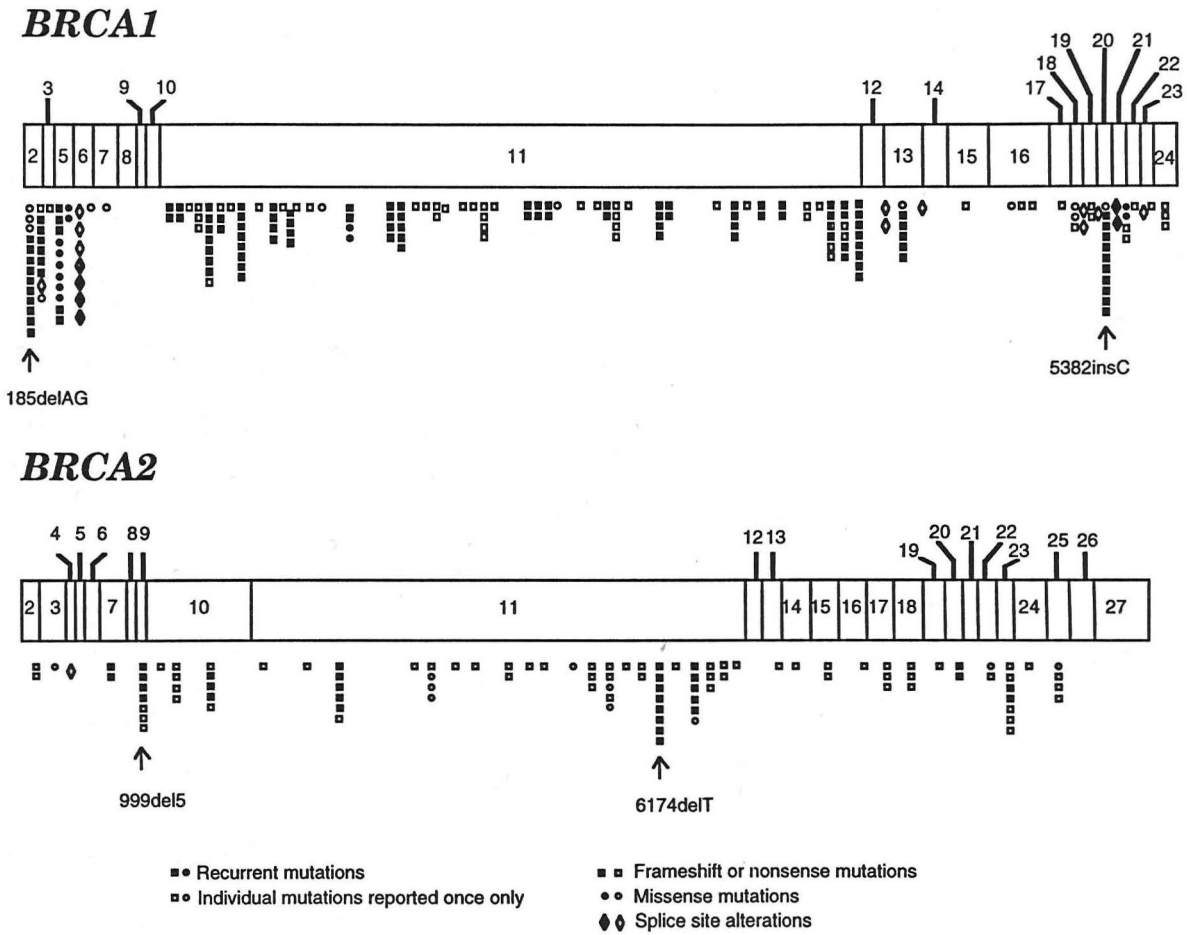


Figure 1. Sites of mutations in *BRCA1* and *BRCA2* genes. Scales are different for the two genes (*BRCA1* ~ 5.6 kb, *BRCA2* ~ 11.4 kb) and are only approximate. Based on BIC database (1997).

The other major form of detection is the protein truncation test (Hogervorst *et al.*, 1995; FitzGerald *et al.*, 1996). Since the vast majority of mutant *BRCA1* genes encode truncated proteins, PCR products can be generated either from genomic DNA, as described above, or from cDNA fragments, and then studied in a transcription/translation system in which the protein products are generated and analyzed for size by gel electrophoresis.

As discussed below, in certain populations with a high frequency of a particular mutation, screening can be done much more simply by PCR amplification of the target region, followed by hybridization with mutation-specific oligonucleotide probes (allele specific oligonucleotide hybridization).

Approximately 182 separate mutations have been reported in *BRCA1* associated with breast or ovarian cancer (Couch *et al.*, 1996c; BIC, 1997; Szabo & King, 1997). These are illustrated in Figure 1. About 90% are mutations that result in a truncated BRCA1 protein. These include ~70% that are interstitial deletions or insertions of one or a few bases that generate frameshifts; ~20% that involve base substitutions that generate a termination codon (nonsense mutations); ~5% that are mutations at splice junctions that result in a frameshift and premature termination or in deletion of an exon; and ~5% that are missense mutations resulting in a single amino acid substitution. As discussed below, some of the latter involve substitution of cysteine residues at positions 61 or 64 in the N-terminal RING finger domain, and others have been found in the C-terminal domain. A small number of mutations result in the absence of a mRNA transcript, with the site of the mutation unknown. A small number of families have been studied that show strong linkage of HB/OC (hereditary breast and/or ovarian cancer) to 17q21, but in which no abnormalities of the *BRCA1* gene have been found.

Polymorphisms have also been found in the *BRCA1* gene, and in some cases it can be difficult to distinguish between a functional, normal polymorphism and a disease-associated missense mutation. If the variant allele has a nonconservative substitution, particularly in a residue that is shared between the human and mouse genes, and if it cosegregates with cancer in families, then it seems reasonable to consider it at least provisionally as a disease-associated mutation (Stoppa-Lyonnet *et al.*, 1997). This difficulty will presumably be obviated once a functional assay for the BRCA1 protein is established.

Mutations are scattered throughout the length of the *BRCA1* gene. The large exon 11 encodes 61% of the protein, and contains a comparable share of the mutations detected to date. The most 5' mutation truncates all but the N-terminal 22 amino acids, whereas the most 3' mutation truncates only the C-terminal 10 amino acids.

Attempts have been made to correlate the site of mutation with the phenotype of the associated tumors. Initial analysis of the mutations led to the suggestion that the nearer the mutation to the 5' end of the gene, the more highly the gene is associated with ovarian cancer (Friedman *et al.*, 1994), with a relatively sharp demarcation near the boundary of exons 12 and 13 (Gayther *et al.*, 1995). However, there are many exceptions to this generalization, and it now does not seem entirely justified by the accumulated data. A similar claim has been made for *BRCA2*, that ovarian cancer seems to be associated with mutations in the middle of exon 11 (Gayther *et al.*, 1997).

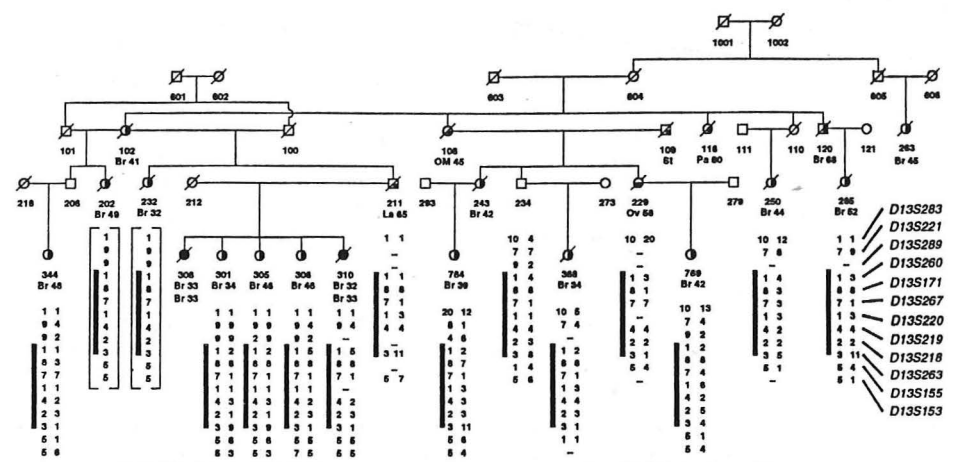
Mapping and Identification of *BRCA2*

Even before the *BRCA1* gene was cloned, several lines of evidence pointed to at least one other dominant susceptibility gene segregating in high-risk breast cancer families. The main evidence

was a lack of linkage to 17q21 in 45% of the high risk breast cancer families, particularly those without ovarian cancer (Table 3). Another clue was the finding that of several families with breast cancer in males as well as females, none showed linkage to the *BRCA1* locus on 17q.

Linkage of HBC to a second locus, *BRCA2*, was reported in the fall of 1994, just a few weeks before the report identifying the gene for *BRCA1* (Wooster *et al.*, 1994). The work was the result of a large international colllaboration, centered at the Institute of Cancer Research in the U.K. The investigators performed a genomic-wide linkage search in 15 high-risk breast cancer families that failed to show linkage to the *BRCA1* locus on 17q21. The *BRCA2* locus was mapped to a 6 cM interval on chromosome 13q12-13. This region was known to harbor the tumor suppressor gene *RB1*, but several recombinant chromosomes within the analyzed families showed that this gene was not *BRCA2*. One of the pedigrees showing linkage to 13q12-13 is shown in Figure 2. Linkage to the 13q12-13 region was subsequently shown for a family with four males with breast cancer (Thorlacius *et al.*, 1995). Other members of this family had other forms of cancer, a phenomenon also seen in the original report by Wooster *et al.* (1994). All the affected men shared the same haplotype.

Figure 2. Linkage of HBOC to chromosome 13q12-13: mapping the *BRCA2* locus



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.

From (Wooster *et al.*, 1994)

Fifteen months later, the *BRCA2* gene was described by Wooster *et al.* (1995), the same group that originally mapped it. The orignal 6 cM region was further narrowed to a 600 kb interval centered around the microsatellite marker *D13S171* by analysis of additional families and microsatellite markers. Meanwhile, other investigators, using a methodology termed representational difference analysis, had mapped a 300 kb homozygous deletion in a pancreatic carcinoma between the markers *D13S260* and *D13S171*, which are ~1 cM apart, exactly in the genetically mapped region of *BRCA2* (Schutte *et al.*, 1995). Wooster *et al.* focused on this region,

centered around *D13S171*, with strategies to identify transcribed sequences on P1 artificial chromosome contigs. In one of the screened exons, they detected a splice site deletion that created a stop codon. The mutation was confirmed in several individuals with breast cancer in the particular family from which it was isolated, who shared only this 13q haplotype. At this stage, 900 kb of sequence data from chromosome 13q became available from the two centers participating in the Human Genome Project, and the both the isolated exon and the rest of the *BRCA2* gene, were found to overlap with the sequenced region. Distinct deletional mutations were identified in a total of six families.

The open reading frame identified by Wooster *et al.* was 7 kb, but northern analysis showed a transcript of 10-12 kb, and it was subsequently shown that their sequence was incomplete. Tavtigian *et al.* (1996) determined the complete coding sequence and intron-exon structure of *BRCA2*, examined its pattern of expression, and developed sequences for a set of PCR primers sufficient to screen the entire coding sequence of *BRCA2* using genomic DNA. They also identified mutations in *BRCA2* in 10 out of 18 families selected on the basis of linkage analysis and/or the presence of one or more cases of male breast cancer. The composite *BRCA2* cDNA sequence consists of 11,385 bp, not including the polyadenylation signal or poly(A) tail. The gene is composed of 27 exons (26 coding exons) distributed over roughly 70 kb of genomic DNA, and contains an unusually high AT content. The predicted open reading frame begins at nucleotide 229 in exon 2 and encodes a protein of 3,418 amino acids. No similarity was detected with other proteins. There was no signal sequence at the N- terminus, and no obvious membrane-spanning regions. Despite the lack of any sequence homology, remarkable similarities between *BRCA2* and *BRCA1* were immediately evident. The highest levels of *BRCA2* expression were observed in breast, thymus, and testis, with slightly lower levels in lung, ovary, and spleen, a pattern very similar to that seen for *BRCA1*. Both genes are very large, with over 20 exons, both have a large exon 11, translational start sites in exon 2, and coding sequences that are AT-rich, both encode proteins that are highly charged with about one fourth of the residues acidic or basic, and both span >70 kb of genomic DNA. A comparison of *BRCA1* and *BRCA2* is shown in Table 4.

Couch *et al.* (1996b) mapped other transcribed genes within the 500 kb segment of chromosome 13q12-13 containing *BRCA2*. Evidence for seven genes, two putative pseudogenes, and nine additional putative transcription units was obtained. All of the identified genes were novel and of unknown function.

Mutations in *BRCA2* associated with breast and ovarian cancer

Analysis of the *BRCA2* gene in affected families by numerous groups showed that mutations in *BRCA2*, like those in *BRCA1*, are scattered throughout the coding region (Wooster *et al.*, 1995; Berman *et al.*, 1996a; Couch *et al.*, 1996a; Lancaster *et al.*, 1996; Phelan *et al.*, 1996; Tavtigian *et al.*, 1996; Thorlacius *et al.*, 1996; BIC, 1997; Schubert *et al.*, 1997; Serova-Sinilnikova *et al.*, 1997). Unlike *BRCA1*, no mutations have been reported in the two C-terminal exons (Figure 1). The most recent analysis (D. Goldgar, personal communication) showed 111 distinct deleterious mutations identified, of which 84 (76%) have been reported in only one individual or family. These include 68% frameshift (microdeletion or microinsertion), 12% nonsense, 13% missense, and 7% splice mutations.

Table 4. Comparison of *BRCA1* and *BRCA2*		
	BRCA1	*BRCA2*
Genomic location	17q21	13q12
Coding exons	22	26
Coding bases	5,592	11,385
Transcript size	7.8 kb	11-12 kb
Genomic size	81 kb	>70 kb
Repetitive elements	46%	?
Amino acids	1,863	3,418
Molecular mass	220 kD	400kD ?
Homology with mouse protein	58%	59%
Tissue distribution	testis, ovary, breast, proliferating tissues	testis, ovary, breast, proliferating tissues
Developmental expression	proliferating tissues	proliferating tissues
Germline mutations	>180; frequent in N,C-termini	≥111; rare in N-terminus, none in C-terminus
LOH in tumors	frequent	frequent
Somatic mutations in tumors	very rare	very rare
Associated tumors	breast, ovary, prostate, others	breast, ovary, pancreas, prostate, many others
Domains identified	RING-finger; BCRT	c-jun-like; BRC repeats
Protein interactions identified	BARD1, Rad51	Rad51

INVESTIGATION OF THE FUNCTION OF THE BRCA1 AND BRCA2 PROTEINS

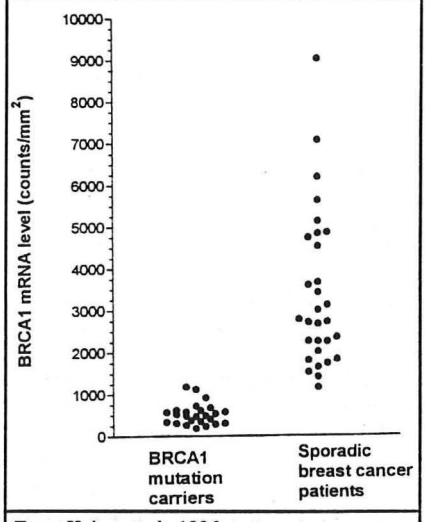
Evidence for tumor suppressor function.

The autosomal dominant nature of the BRCA genes in their association with cancer led most investigators to assume from the beginning that they are tumor suppressor genes. Thus, in accordance with Knudson's "two-hit" hypothesis, which has guided the field of cancer biology for the past two decades (Knudson, 1983), tumorigenesis requires inactivation or deletion of both copies of a tumor suppressor gene. The inactivation of either gene copy can either be inherited or arise through a somatic event. In cases in which one inherited copy of the gene is defective, then progression to neoplasia would require somatic loss of only of the one normal gene. Since this could be expected at a frequency several orders of magnitude greater than the frequency of two somatic inactivating events in the same cell, the development of tumors in individuals with an inherited defect occurs both more frequently and, generally, at an earlier age in life.

Consistent with this hypothesis, even before the *BRCA1* gene was cloned, somatic loss of the 17q21 chromosomal region in breast tumors from families with strong linkage of HB/OC to this chromosomal region showed a strong tendency to involve the normal (i.e., unlinked) chromosome. Subsequently, loss of heterozygosity of the normal allele has been demonstrated in numerous breast and ovarian tumors arising with inherited mutations of *BRCA1* or *BRCA2* (Smith *et al.*, 1992; Collins *et al.*, 1995; Stratton, 1996). However, it has been similarly well established that sporadic breast cancers and ovarian cancers rarely if ever carry somatically acquired mutations of the BRCA genes (Futreal *et al.*, 1994; Hosking *et al.*, 1995; Matsushima *et al.*, 1995; Merajver *et al.*, 1995; Lancaster *et al.*, 1996; Miki *et al.*, 1996; Teng *et al.*, 1996). This would suggest that alterations of BRCA1 or BRCA2 function in tumorigenesis occur predominantly or exclusively in the cancers arising in individuals with inherited BRCA mutations. On the other hand, loss of heterozygosity of the respective chromosomal regions for *BRCA1* or *BRCA2* has been found in 30-60% of sporadic breast or ovarian tumors (Cropp *et al.*, 1994; Feunteun *et al.*, 1996; van den

Berg *et al.*, 1996). Moreover, it was reported that the overall level of *BRCA1* mRNA expression is frequently reduced in sporadic tumors unassociated with BRCA mutations (Thompson *et al.*, 1995). However, assessment of *BRCA1* mRNA by *in situ* hybridization in tumors showed a marked difference between tumors from mutation carriers and sporadic tumors (Kainu *et al.*, 1996) (Figure 3), supporting the concept that the biology of *BRCA1* is fundamentally different in the two different classes of tumor. No alterations in *Brca1* mRNA and only a very low incidence of loss of heterozygosity were found in experimentally induced mammary carcinomas in rats (Chen *et al.*, 1996a), nor were alterations common in a number of murine tumor cell lines (Lane *et al.*, 1995). It remains possible that functional alterations in one or both of the BRCA proteins may play a role in sporadic tumorigenesis in humans, but by mechanisms other than somatic mutation in the genes themselves. However, recent data on the cellular function of these proteins argues against alteration of their function as an essential to neoplasia in most sporadic tumors.

Figure 3. *BRCA1* mRNA expression in sporadic breast cancers and cancers from patients with germline *BRCA1* mutations



From Kainu *et al.*, 1996

BRCA1 protein structure: the RING finger domain

Initially, there were few clues regarding the function of the BRCA1 protein, since it showed little homology to any known protein. One feature that was noticed from the outset was a domain near the N-terminus (amino acids 20-68) called a RING finger domain, a cysteine rich structure found in a number of diverse regulatory proteins. The RING domain has the basic structure Cys3HisCys4, binds two Zn^{++} ions, and has been proposed to serve as an interface for recognition of DNA or for protein-protein interactions (Saurin *et al.*, 1996; Wu *et al.*, 1996). The sequence in BRCA1 is Cys-X2-Cys-X11-Cys-X-His-X2-Cys-X2-Cys-X13-Cys-X2-Cys, where X*n* represents *n* residues other than Cys or His (Miki *et al.*, 1994). Several lines of evidence suggest that this region of the protein is critical in the function of BRCA1. (i) Several missense mutations (i.e., amino acid replacements) in either of the two cysteine residues at positions 61 and 64 within the RING domain are among the cancer-associated inherited *BRCA1* mutations, despite the fact that most of the cancer-associated mutations result in truncations of the protein (Shattuck-

Eidens *et al.*, 1995; Couch *et al.*, 1996c). (ii) The mouse homolog, Brca1, shows only 58% homology with human BRCA1 (Abel *et al.*, 1995; Lane *et al.*, 1995), which is unusually low for tumor suppressor gene, since others such as p53, APC, WT1, and NF1 show amino acid identities of 78-98% between human and mouse (Table 5). However, the 49 amino acid N-terminal RING finger domain is completely conserved between human, mouse, and rat, suggesting that it has a critical, conserved role in the protein's function. (iii) breast tumors from individuals with mutations that disrupt the RING-finger domain tend to be associated with a higher grade mitotic index than tumors from individuals with *BRCA1* mutations in the middle of the gene (Sobol *et al.*, 1996).

Table 5. Conservation of tumor suppressor proteins

Protein	Human/mouse identity (%)
Neurofibromatosis-1 (NF1)	98
Neurofibromatosis-2 (NF2)	98
Wilm's tumor (WT1)	95
Retinoblastoma (RB1)	91
Adenomatous polyposis coli (APC)	90
von Hippel-Landau (VHL)	90
Ataxia telangiectasia (ATM)	84
p53 (TP53)	78
BRCA1	58
BRCA2	59

From (Connor *et al.*, 1997)

Another RING finger protein, BARD1, that binds to BRCA1

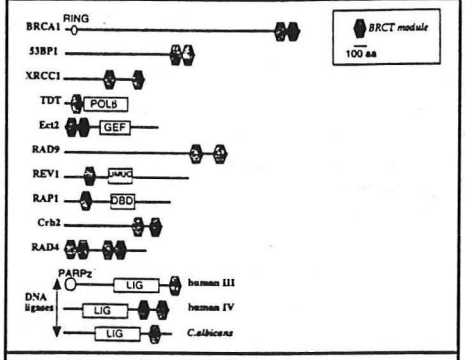
The function of the RING domain is not known. However, recently Drs. Richard Baer (Department of Microbiology) and Anne Bowcock and their colleagues here at UT Southwestern have identified a novel protein, termed BARD1(BRCA1-associated RING domain), that interacts *in vitro* and *in vivo* with the RING domain of BRCA1 (Wu *et al.*, 1996). The protein was discovered using the two-hybrid system in yeast, a powerful *in vitro* tool for isolating novel genes based upon the protein-protein interactions of their products. Interestingly, BARD1 itself contains an N-terminal RING domain, and it also shares a conserved C-terminal domain with BRCA1 (see below). It also has a domain not shared with BRCA1 that contain three ankyrin repeats. The gene for BARD1 maps to the distal end of chromosome 2q (2q33-34, not known to be common site of alteration in cancer) and encodes a protein of ~750 amino acids. BARD1 fails to interact with BRCA1 proteins that have point mutations at cysteine residues 61 or 64, strongly suggesting that these residues are critical for the interaction, and that the interaction is therefore critical in tumor suppression. The interaction with BARD1 requires the N-terminal 101 amino acids of BRCA1, a segment that includes, but is somewhat larger than, the RING domain itself. The biological function of BARD1 remains to be discovered, but it evidently involves binding to BRCA1 as a heterodimer or as two components of a larger complex, disruption of which predisposes to tumor formation. This is discussed further below.

A BRCA1 C-terminal domain, BRCT, also found in DNA repair genes, that activates transcription

Application of sophisticated methods for analyzing protein sequence data for patterns and partial homologies to BRCA1 led to the identification of two tandemly repeated homologous domains of ~95 amino acids near the C-terminus with a predicted globular structure that was termed BRCT (BRCA1 C Terminus) domains (Koonin *et al.*, 1996). These were found to share sequence

homology and spatial organization with 53BP1, a protein known to bind the universal tumor suppressor and cell cycle regulator p53, and also with Rad9, a yeast cell cycle checkpoint protein. Subsequent analysis by these investigators led to the identification of a superfamily of ~40 proteins carrying BRCT domains, many of them associated with the response to DNA damage and with cell cycle checkpoints (Bork *et al.*, 1997). All of the members of this superfamily are large, multidomain proteins (Figure 4. Some of the identifiable domains include enzymatic domains, other common binding domains, and ankyrin repeats. As noted above, BARD1 also falls into this superfamily. The folding of the BRCT domain is predicted to consist of two α-helices and four β-strands, the latter forming a core sheet structure. The widespread distribution of BRCT domains in proteins known to be involved in DNA repair was confirmed by a study using another search method, hydrophobic cluster analysis (Callebaut *et al.*, 1997).

Figure 4. Some proteins with BRCT domains



From Callebaut *et al.*, 1997

Although the function of these BRCT domains is not known, two groups have reported that the BRCA1 C-terminus has transcriptional activation function when fused to the GAL4 DNA binding domain (Chapman *et al.*, 1996; Monteiro *et al.*, 1996). Optimal transcriptional activator activity was contained within residues 1528-1863, which includes both BRCT domains, and the minimal region for transcriptional activator activity was localized to amino acids 1760-1863 (exons 16-24), the lefthand border of which corresponds precisely with the first predicted β-strand of the second BRCT domain. The transcriptional activity was abrogated by germline mutations in this region associated with cancer, including two missense (i.e., nontruncating) mutations. Whether transcriptional activation is a function of the the cell cycle-related activity of BRCA1 (see below) remains to be determined. However, it should be noted that the absolute level of transcriptional activation was quite low in these experiments, and these data cannot yet be taken as establishing transcriptional activation as a major BRCA1 function. No transcriptional activity has been detected for BARD1 (R. Baer, personal communication).

Transcriptional activation by BRCA2 sequences

Transcriptional activation by BRCA2 sequences has recently been reported that is somewhat more convincing than that reported for BRCA1. Milner *et al.* (1997) discovered that a region in BRCA2 exon 3, covering residues 60-105, has significant homology to a portion of the transcription factor c-Jun. They tested a variety of constructs in which this, and/or neighboring, sequences were linked to reporter genes in yeast or mammalian cells. The neighboring sequences, residues 18-60,

showed the strongest activating capacity. Moreover, inhibitory activity was demonstrated for the two flanking regions regions, residues 1-18 and 105-125. Substitution of Cys for Tyr at residue 42, and missense mutation found in HBC families, reduced transcription 5-fold. This domain of BRCA2 shows high conservation between human and mouse, with 97% identity at residues 22-52 (Connor *et al.*, 1997). Five other conserved regions showed no increased transcriptional activitation, providing further evidence for the significance of this finding.

Internal repeats in the BRCA2 sequence

Bork *et al.* (1996) described discovery of eight repeated domains within the large exon 11 of the BRCA2 protein. These were termed BRC repeats. Although the function of these BRC repeats has yet to be discerned, comparison of exon 11 sequences from six mammalian species showed a conserved 26 amino acid core within each of the eight BRC repeats found in all six species (Bignell *et al.*, 1997), suggesting a conserved function for this region.

Granin motifs in BRCA1 and BRCA2?

In a search for functional domains within BRCA1, Jensen *et al.* (1996) reported a granin consensus sequence at residues 1214-1223. Granins are secreted proteins, some of which are located in secretory granules, particularly associated with neuroendocrine tissue. These authors presented evidence that BRCA1 is a secreted protein, but this has not been found by others (see below). They also reported a similar granin consensus sequence in BRCA2 (residues 3335-3344). These motifs are not well conserved in other species (Connor *et al.*, 1997), and the claim that they have any sequence specificity has been criticized (Koonin *et al.*, 1996).

Tissue and developmental expression of *Brca1* and *Brca2* mRNA in mice

To gain insight into the functional role of BRCA1, two groups conducting extensive surveys in mice of the expression of mRNA for the *Brca1* homolog, using northern blot analysis and *in situ* hybridization (Lane *et al.*, 1995; Marquis *et al.*, 1995). Expression in adult tissues was similar to that found in humans (Miki *et al.*, 1994), with the highest expression in spleen, thymus, testis, ovary, and mamary gland. *Brca1* mRNA levels in the mammary gland were increased ~10 fold during mid-pregnancy, falling somewhat during lactation.

The studies in mouse embryos were quite instructive. The expression in embryo whole mounts was most prominent in tissues undergoing rapid proliferation and differentiation, including neuroepithelium during neural tube development and tubular epithelial cells in the developing kidney. Studies of *Brca2* expression have shown similar results (Sharan & Bradley, 1997; Sharan *et al.*, 1997), consistent with the findings, discussed below, that the two genes are coordinately regulated.

Conner *et al.* (1997) carried out a large tissue survey comparing *Brca1* and *Brca2* expression by semi-quantitative PCR of cDNA. The result confirmed the similar tissue localization of expression shown by the two genes in a variety of tissues, including cerebellum, eye, gall bladder, ileum, appendix, mammary gland during pregnancy, testis, epididymis, ovary, placenta, and mid-term fetus.

Cellular function of BRCA1 and BRCA2: likely participation in cell cycle-dependent DNA repair

The first experiments to identify the size and subcellular localization of the BRCA1 protein obtained conflicting results in different laboratories. Some groups found BRCA1 to be a 220 kD nuclear protein, a size consistent with its *in vitro* translation product (Chen *et al.*, 1995; Scully *et*

al., 1996), while others, using similar antibodies, found evidence suggesting it to be a 190 kD secreted protein (Gudas *et al.*, 1995; Jensen *et al.*, 1996). More recently, proteins of 190, 220, and 240 kD have been reported (Coene *et al.*, 1997). These latter investigators also correlated apparently different sites of intracellular localization with different methods of fixation. By far the most productive line of investigation to date is consistent with the data showing a nuclear localization and function for the 220 kD BRCA1 protein, and it is this work that will be the focus of this discussion.

Because the level of *BRCA1* mRNA expression in mice was highest in tissues undergoing rapid proliferation combined with differentiation, and since cell cycle control is a common function for tumor suppressor genes, several groups investigated the levels of BRCA1 during the stages of the cell cycle. Chen *et al.* (1996b) showed that BRCA1 protein levels are undetectable in synchronized T24 bladder carcinoma cells during early G1, increasing sharply to a maximum at the G1/S interface and during S phase, and decreasing somewhat during M phase. Immunostaining by these investigators showed that BRCA1 staining within the nucleus is homogeneous in late G1, but becomes punctate during S phase and remains associated with the chromosomes as they align on the metaphase plate during M phase. Moreover, BRCA1 was shown to become phosphorylated coincident with its level of expression, and to be phosphorylated by known cell cycle-dependent kinases cdk2, cyclinA and cyclinD. These results suggest that BRCA1 participates in cell cycle-regulated events.

Similar results correlating maximal BRCA1 expression with S phase were obtained by Vaughn *et al.* (1996b). These same investigators (Vaughn *et al.*, 1996a) showed that *BRCA2* mRNA is also maximally expressed in late G1 and S phase, consistent with the findings of others that *BRCA1* and *BRCA2* are coordinately regulated in human and mouse breast tissue (Rajan *et al.*, 1996; Spillman *et al.*, 1996; Connor *et al.*, 1997).

Scully *et al.* (1996) found a "nuclear dot" pattern of BRCA1 immunostaining in diploid human fibroblasts and several breast and ovarian cell lines, similar to that found by Chen et al, described above. This pattern of nuclear dots appearing during S phase is also seen for a protein, human Rad51 (Figure 5), and pursuit of this correlation led to a major recent observation by Scully *et al.* (1997). Rad51 is a homolog of the bacterial protein RecA, which functions in DNA repair. These investigators, using two color confocal immunostaining, showed substantial colocalization of BRCA1 and Rad51 in nuclear dots during S phase in synchronized cell lines. Immunoprecipiation of BRCA1 showed partial coprecipitation of Rad51 and vice versa. The region of BRCA1 mediating the binding was localized to amino acids 758-1064, in exon 11, a region that contains a cancer-associated missense mutation. The significance of this observation is further supported by recent findings by Richard Baer and Anne Bowcock and their colleagues, that BARD1, which they had previously shown binds to BRCA1, also co-localizes with BRCA1 to nuclear dots in S phase (Jin *et al.*, 1997).

These observations led Scully *et al.* (1997) to look for an association between BRCA1 and Rad51 in meiotic sperm. It had previously been shown that Rad51 localizes to synaptonemal complexes in various organisms. These are unique DNA-and protein-bearing structures that accompany the pairing of homologous chromosomes during meiosis. Moreover, it had recently been shown that *BRCA1* mRNA is highly expressed in spermatocytes during meiotic prophase (Lane *et al.*, 1995; Zabludoff *et al.*, 1996). Immunostaining of human spermatocytes during meiotic prophase showed localization of BRCA1 on the axial (unsynapsed) elements of developing synaptonemal complexes, which are essential for homologous recombination. From these findings linking

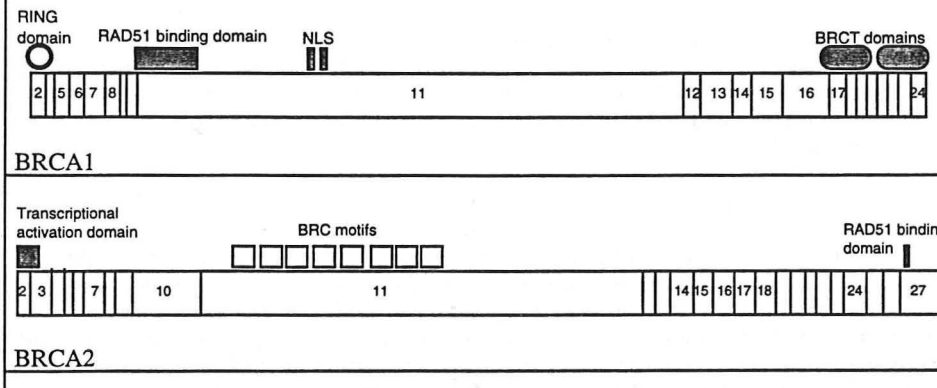
BRCA1 with both mitosis and meiosis, and from other findings showing that Rad51 is involved in resolving double-stranded break repair, it seems likely that BRCA1 is involved in DNA maintenance or repair in some essential way.

Figure 5. BRCA1 localizes to nuclear dots. Immunohistochemical image from the ovarian cancer cell line SKOV-3. Similar results were seen in 20 different cell lines and primary human cells.

From Scully *et al.*, 1996

Subsequently, it was shown by Sharan *et al.* (1997) that Brca2 in the mouse binds to MmRad51, the mouse homolog of of Rad51. The binding domain in Brca2 was localized to a 36 amino acid region in the C-terminus (residues 3,196 - 3,232) that shows 95% conservation between human and mouse (vs. 59% overall). This provided further evidence that both BRCA proteins are somehow involved in processes related to cell cycle-dependent DNA repair. Further support for this hypothesis has come from investigation of mice with induced germline deletions of the *Brca* genes, findings from which are discussed below. A schematic diagram of the identified domains in BRCA1 and BRCA2 is shown in Figure 6.

Figure 6. Identified domains of the BRCA1 and BRCA2 proteins



Domains identified on the basis of structure or function. NLS, conserved nuclear localization signals identified in the *BRCA1* and mouse *Brca1* sequences (Miki et al., 1994; Lane *et al.*, 1995). Other domains are described in the text. The RAD51 binding site in BRCA2 is inferred from functional studies with mouse Brca2 and MmRad51 (Sharan *et al.*, 1997).

Mice with a germline mutation in *Brca1*

The mouse homolog of BRCA1 encodes a 1,812 amino acid protein (Abel, *et al.*, 1995; Lane, *et al.*, 1995). The homology of this protein to human BRCA1 is 58%, but, as noted above, several regions show much higher levels of homology, including the N-terminal ring finger domain. Three groups have reported mice with a germline disruption of the *Brca1* gene by standard gene

knockout technology. To date, there are no reports of increased tumor development in mice heterozygous for this defective gene, so it seems unlikely that these mice will prove useful as a model of cancer. Ironically, a greater clue to the function of Brca1 has come from mice homozygous for the defect. These mice die in embryonic life. Hakem *et al.* (1996) produced mice with deletion of exons 5 and 6. The homozygous *Brca1*$^{-/-}$ embryos failed to survive past day 7.5 of embryogenesis. The major defect appeared in conjunction with the rapid epiblast cell proliferation that accompanies gastrulation and generates mesoderm between days 5.5 and 6.5 of embryogenesis. Mesoderm formation could not be detected. This and other lines of evidence suggested that the *Brca1*$^{-/-}$ embryos had a slowing or block in progression through the cell cycle, and suggested that Brca1 is involved in cell cycle regulation. *In vitro* outgrowth of day 3.5 blastocysts was also defective, particularly of the inner cell mass.

A somewhat different result was obtained by Gowen *et al.* (1996), whose targeting vector deleted a portion of exon 11 of the *Brca1* gene. These embryos typically survived to days E9.5-10.5, with evidence of developmental delay or arrest and defects in neural tube development. Splice variants of BRCA1 that lack exon 11 have been described (Miki *et al.*, 1994; Lu *et al.*, 1996), and this may explain the slightly milder phenotype of the exon 11-disrupted *Brca1*$^{-/-}$ mice, compared with the exon 5/6 disrupted mice (Gowen *et al.*, 1996; Hakem *et al.*, 1996). However, another group, Liu *et al.* (1996), also produced mice with a deletion in *Brca1* exon 11, and these mice had a phenotype similar to those of Hakem *et al.*, which had the exon 5/6 deletion.

Both Hakem *et al.* and Liu *et al.* attempted unsuccessfully to make embryonic stem cells homozygous for defective *Brca1* genes by sequential transfection of genes disrupted by different selection markers, with screening for homologous recombination. These results suggested that Brca1 is essential for the survival and/or proliferation of these cells.

Mice with a germline mutation in *Brca2*

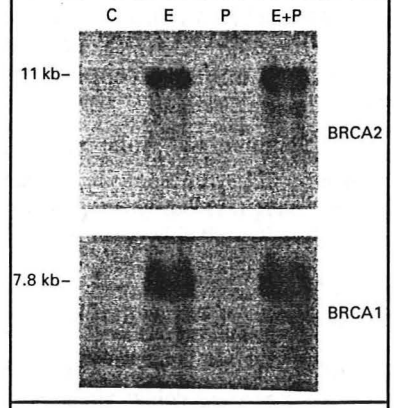
The encoded mouse Brca2 protein was found to contain 3,328 amino acids, with an overall identity of 59% with human BRCA2, although, analogous to the *BRCA1*/*Brca1* comparison, as noted above, some regions are more highly conserved (Sharan & Bradley, 1997). Tissue and developmental expression shows a pattern very similar to *Brca1*, with a marked upregulation at day 7.5 concurrent with a rapid rise in cellular proliferation, consistent with the finding that the two genes are coordinately regulated (Rajan *et al.*, 1996; Sharan & Bradley, 1997; Sharan *et al.*, 1997). Sharan *et al.* (1997) produced *Brca2*$^{-/-}$ mice by standard homologous recombination in embryonic stem cells, using a construct encoding a deleted exon 11. The phenotype of these mice proved to be similar but not identical to that of *Brca1*$^{-/-}$ mice described by Hakem *et al.* and Liu *et al.*, being lethal early in embryogenesis. The embryos were normal up through implantation, but showed developmental arrest after day 6.5. This was thought to be due to a defect in proliferation rather than differentiation, since the embryos were shown to initiate mesoderm formation (unlike the *Brca1*$^{-/-}$ embryos). This was further investigated by attempting to generate homozgygous deletion of the *Brca2* gene in embryonic stem cells. The strategy for this, and the results, were the same as those described for *Brca1*, above, i.e., no homozygously deleted cells could be obtained, again suggesting an essential role for Brca2 in cell proliferation.

As noted above, *BRCA1* was demonstrated to interact with Rad51, implicating possible roles in mitosis, meiosis, and DNA repair, and Sharan *et al.* (1997) showed a similar interaction between Brca2 and MmRad51. It was therefore of great interest that mice with homozygous germline deletion of MmRad51 show a lethal arrest in embryonic development at the stage of extensive cellular proliferation, ~E7.5, similar to that of *Brca1*$^{-/-}$ and *Brca2*$^{-/-}$ embryos (Lim *et al.*, 1996).

Sharan *et al.* (1997) went on to show that Brca2 and MmRad51 mRNA are co-expressed in the developing embryonic nervous system between days E8.5 and 11.5. Finally, it had previously been shown that MmRad51-/- embryos are hypersensitive to ionizing radiation (Lim *et al.*, 1996). Sharan *et al.* tested whether the same is true for *Brca2*$^{-/-}$ embryos by culturing day E3.5 embryos *in vitro* for seven days. Outgrowth of the inner cell mass and the number of trophoblast giant cells were the same for control and *Brca2*$^{-/-}$ embryos (again unlike *Brca1*$^{-/-}$ embryos), but 400 rad of γ-irradiation of the *Brca2*$^{-/-}$ embryos totally ablated the inner cell mass and reduced the number of trophoblast cells 57%, compared with little effect of irradiation on either outcome in the control embryos. Taken together, these data strongly support the concept that Brca2 and MmRad51 interact functionally.

It should be pointed out that two significant differences have been found between the human *BRCA1* and *BRCA2* genes and their mouse homologs. First, as noted above, mice heterozygous for the mutant *Brca1* gene have shown no propensity for tumor formation, and the same has been found for *Brca2*$^{+/-}$ mice (Sharan *et al.*, 1997). Second, a woman with familial breast cancer at age 32 was reported who was found to be homozygous for a *BRCA1* 2800delAA mutation in exon 11 (Boyd *et al.*, 1995), This mutant gene would be expected to encode a truncated, 900 amino acid protein. The finding suggests that in humans, unlike mice, an intact *BRCA1* gene is not essential for normal development.

Figure 7. Induction of *BRCA1* and *BRCA2* mRNA by estrogen in a breast cancer cell line



From Spillman & Bowcock, 1996

Estrogen responsiveness

Several groups have examined the responsiveness of the *BRCA1* and *BRCA2* genes to estrogen. As shown in Figure 7, breast cancer cell lines that express estrogen receptors show a substantial increase in *BRCA1* and *BRCA2* mRNA expression when cultured in the presence of 17β-estradiol (Spillman *et al.*, 1996). Progesterone, either alone or together with estradiol, had no significant effect. This effect could be blocked by the protein synthesis inhibitor cycloheximide, suggesting that the action of estrogen on BRCA transcription is indirect, i.e., mediated by (an)other protein(s) induced by estrogen, consistent with the concept that *BRCA1* and *BRCA2* transcription is coordinately regulated. Since, as noted above, *BRCA1* and *BRCA2* transcription are regulated with the cell cycle, with maximal levels just before the onset of DNA synthesis in S phase, the

mitogenic effect of estrogen on these cell lines may account for the responsiveness of *BRCA1* and *BRCA2* mRNA seen (Marks *et al.*, 1997).

Current understanding and concepts of BRCA1 and BRCA2 protein function
BRCA1 and BRCA2 thus both appear to be coordinately regulated, expressed in a cell cycle dependent manner in a variety of proliferating tissues, including breast epithelium under the influence of estrogen. They both bind RAD51, and thus are probably part of a multi-protein complex that is involved in DNA maintenance and/or repair during mitosis and meiosis. This is even more interesting in view of the evidence that RAD51 interacts directly with p53 (Stürzbecher *et al.*, 1996). BRCA1 associates with BARD1, which shows similar cell cycle-dependent nuclear localization. Neither *BRCA1* and *BRCA2* is somatically mutated in the vast majority of sporadic breast or ovarian cancers, and neither gene shows human/mouse similiarity seen in classic tumor suppressor genes. How does one put this information together?

In recent commentaries, Kinzler and Vogelstein (1996; 1997), who have elegantly and extensively characterized the genes involved in familial colon cancer, have made a distinction between *gatekeeper genes* and *caretaker genes* in the determination of cancer. They define gatekeepers as the genes that directly regulate the growth of tumors by inhibiting growth or promoting cell death. Each cell type has only one or a few specific gatekeepers, and inactivation of the gatekeeper for a given cell leads to cancer with a specific tissue distribution. Thus, germline mutations of the retinoblastoma (*RB1*), von Hippel-Lindau (*VHL*), neurofibromatosis type I (*NF1*), and adenomatous polyposis coli (*APC*) genes predispose specifically to tumors of the retina, kidney, Schwann cells, and colon, respectively, once both copies of the gene are altered (one by germline mutation, the other somatically). They classify *BRCA1* and *BRCA2* as caretaker genes, rather than as gatekeepers. Neoplasia occurs indirectly with mutation of this class of genes, through genetic instability that potentially affects all genes, including gatekeepers. A tumor that arises through this indirect inactivation of a gatekeeper gene may progress rapidly because of an accelerated rate of mutation in other genes that directly control cell proliferation or death. Previously described caretaker genes asssociated with human cancer include the nucleotide-excision-repair genes that are responsible for xeroderma pigmentosum, the mismatch-repair genes that cause hereditary nonpolyposis colorectal cancer, and probably the *ATM* gene, which is responsible for ataxia-telangiectasia, the product of which is involved in the cell cycle checkpoint pathway. It is interesting to note that ovarian cancer is part of the familial cancer syndrome associated with hereditary nonpolyposis colorectal cancer (Boyd & Rubin 1997), and that breast cancer is increased in women heterozygous for the *ATM* gene (Swift *et al.*, 1991; Walberg, 1996).

There is an interesting, although preliminary, parallel between BRCA-associated tumors and hereditary nonpolyposis colorectal cancer, which arises as a result of genetic instability due to DNA mismatch repair genes. Patients with this entity evidently have a significantly better prognosis than those with sporadic colorectal cancer (Sankila *et al.*, 1996). A recent study of *BRCA1*-associated ovarian cancer also suggests a better prognosis, compared with age-matched patients with sporadic disease (Rubin *et al.*, 1996). Marcus *et al.* (1996) made an extensive comparison of the pathology and prognosis of breast cancer in *BRCA1*-linked HBC, in "other HBC" (some of which was *BRCA2*-linked), and in sporadic breast cancer. They found that, despite a higher degree of aneuploidy and strikingly higher proliferation rates, which usually are associated with a poorer prognosis, there was a lower recurrence rate in the *BRCA1*-associated group. Prospective studies would be needed to clarify this issue.

There are grounds for some optimism that rational therapy for cancer related to *BRCA* mutations may arise from an understanding of the function of the BRCA proteins. As pointed out by Kinzler and Vogelstein (1997), tumors arising through defective caretaker genes present an additional intrinsic therapeutic target, since they would be expected to respond favorably to therapeutic agents that induce the type of genomic damage that is normally detected or repaired by the particular caretaker gene involved. The finding by Sharan *et al.* (1997) that *Brca2*$^{-/-}$ mouse embryos are sensitive to γ-irradiation suggests that tumors associated with inherited BRCA mutations may be more sensitive to such radiation than other cancers.

POPULATION GENETICS OF *BRCA1* AND *BRCA2* MUTATIONS

International, national, and local mutations

To date, inherited mutations of *BRCA1* and/or *BRCA2* have been examined in HBC/HBOC families from the U.S., Austria, Belgium, Canada, Denmark, Finland, France, Germany, Holland, Israel, Italy, Japan, Norway, Russia, Sweden, and the U.K. In some of these studies, unselected patients with breast or ovarian cancer have also been examined. The results from these studies have been summarized in a recent review (Szabo & King, 1997), which examined the frequencies of particular mutations in high risk families and populations of unselected patients. A number of interesting conclusions were drawn from these integrated data.

The proportion of high risk HB/OC families with *BRCA1* mutations varies widely, ranging from a high of 74% in Russia to ~10% in Japan and even less in Iceland. In European countries apart from Iceland, the proportion ranges from 14 to 29%. In the U.S., Canada, and Israel, the proportions are 39, 40, and 47%, respectively. As discussed in more detail below, there is a very high prevalence of mutant *BRCA1* genes in Jewish populations, and the higher frequencies found in North American families may in part reflect inclusion of a number of Jewish families in these studies. Some of the variation may be due to ascertainment. For example, the Russian families all had at least two cases of ovarian cancer.

In almost all countries where mutations in both genes have been examined, the proportion of high risk HB/OC families with *BRCA1* mutations is higher than that for *BRCA2*, by a factor of ~1.5-2.0 The most dramatic exception is Iceland, where most families have been found to have a single *BRCA2* mutation, and only one family has been reported with a *BRCA1* mutation. In Israel, 24% of the HB/OC families were found to have a *BRCA2* mutation. In U.S. and Canadian families, the percentages are 25 and 16, respectively.

In at least 30% of high-risk families, no mutations in either *BRCA1* or *BRCA2* have been detected. This group includes some very striking families, including three Hungarian families with ≥ 6 cases of breast or ovarian cancer (Ramus *et al.*, 1997); 4 of 25 Swedish families with breast cancer and ≥2 cases of ovarian cancer (Håkansson *et al.*, 1997); and 15 of 23 U.S. families with ≥ 3 cases of female breast cancer and ≥ 1 case at age < 45 yr (Serova *et al.*, 1997). These observations support the possibility of at least one additional *BRCA* gene. In this regard, it is of particular interest that Drs. Bowcock and Baer have recently identified a germline missense mutation in the *BARD1* gene in a patient who has developed primary cancers of the breast, ovary, and endometrium (A. Bowcock, personal communication and manuscript in preparation).

The nature of the mutations within different individual populations reveals some striking contrasts that reflect the widely differing histories of those populations. Perhaps the most dramatic example of a founder mutation that has become widespread within a population is the finding of a single

mutation, *BRCA2* 999delTCAAA (999del5), in 16 of 21 high-risk families in Iceland (Johannesdottir *et al.*, 1996; Tavtigian *et al.*, 1996; Thorlacius *et al.*, 1996; Thorlacius *et al.*, 1997). Outside of Iceland, this mutation has only been found in two families in Finland (Vehmanen *et al.*, 1997). Further studies of the *BRCA2* 999del5 mutation in Iceland are discussed below.

In Russia, nine of 14 families with predominantly ovarian cancer shared the *BRCA1* mutation, 5382insC, which is also widely distributed throughout Europe, whereas other mutant alleles, 4153delA (three families) and 2073delA (one family), have so far not been observed outside of Russia.

In a more extreme case of private alleles, most of the *BRCA1* mutations found in Italian high-risk families have been uniquely found only in one family (Caligo *et al.*, 1996; De Benedetti *et al.*, 1996; Montagna *et al.*, 1996). For example, in an analysis of 20 high-risk families from the Tuscany region, five separate mutations were found in 7 families (Caligo *et al.*, 1996). Three of the families shared one of the mutations, 1499insA. Although these families were thought to be unrelated, in two of them that could be tested, there was evidence for sharing of a common ancestral haplotype. Altogether, 19 or 25 Italian *BRCA1* mutations are unique to individual (Szabo & King, 1997).

The mutations can thus be classified as recurring or unique in distribution, although obviously mutations initially appearing as unique can subsequently be found to be recurring. Szabo and King (1997) have applied the term "ancient" to recurring mutations, which are either national or international in distribution. Both types of recurrent mutations have been found in most European countries. For example, of 8 recurrent *BRCA1* and 6 recurrent *BRCA2* mutations found in the U.K., 6 and 3, respectively, have been found in other European countries, whereas the remainder have been found only within the U.K alone or in the U.K. and North America alone (Szabo & King, 1997). In an amazing study from Holland (Peelen *et al.*, 1997), *BRCA1* mutations were identified in 79 out of a set of 666 high-risk HBOC families (643 from 7 centers in Holland, 23 from one center in Belgium). Of 28 distinct mutations found, 17 have not been reported outside of the Low Countries. This included one, 2804delAA, which was found in 19 different families from 6 different centers in Holland, but has so far not been identified anywhere outside of Holland. Five other mutations, found in 3 to 7 families, have not been found in any other European country, and only two of these have been found outside of Holland, in each case in one family in the U.S. There was evidence for a common 17q21 haplotype for each of 9 different recurring mutations, suggesting a common ancestor in each case. Different statistical methods were applied to the haplotype data to estimate the age of the 2804delAA mutation in generations, with results ranging from 15 to 49.

Of the recurrent mutations (27 *BRCA1* and 14 *BRCA2*) described in Europe and Israel, approximately half (14 *BRCA1* and 7 *BRCA2*) have been reported in the U.S. and/or Canada. In addition, 31 and 22 novel mutations in *BRCA1* and *BRCA2*, respectively, have been reported in the U.S. and Canada.

A recurrent *BRCA2* mutation in Iceland

As noted above, studies of familial aggregation of breast and ovarian cancer led to the prediction of a highly penetrant dominant allele predisposing to the HBOC syndrome, with a predicted population frequency of 0.0006 to 0.0033 and cumulative lifetime risk for breast cancer of ~80-90% within high-risk families (Newman *et al.*, 1988; Claus *et al.*, 1990, 1991). The subsequent

identification of *BRCA1* and *BRCA2*, and the mutations in these genes associated with HBOC, strikingly confirmed most of these predictions. However, the frequency of these mutations in general populations has been more difficult to determine because of the great technical burden required to screen for large numbers of mutations in large numbers of individuals. An estimate based on assumptions on the family *BRCA1* data placed the *BRCA1* gene frequency at 0.0006 (Ford *et al.*, 1995).

Populations with a limited number of mutations are more amenable to this type of analysis, since they can be screened with allele-specific oligonucleotide hybridization of individual exons. As noted above, the *BRCA2* 999del5 mutation was found in a high proportion of familial breast cancer in Iceland. Iceland was settled in the 9th century by people from western Norway and from Ireland (Thorlacius, *et al.*, 1997; Szabo & King, 1997). The population is thought to have remained at ~50,000 - 60,000 for several centuries until about 100 years ago, and the current population is 265,000. The *BRCA2* 999del5 mutation appears to be a classic founder mutation, with at a common haplotype and at least six families traced back to a common ancestor in the 16th century (11-12 generations) (Thorlacius *et al.*, 1996).

Table 6. Frequency of the *BRCA2* 999del5 mutation in Iceland in patients with breast cancer and in the general population

Sample	n	% positive
BC patients		
Women		
age < 40 yr	50	24.0
age 41 -50 yr	143	14.0
age >50 yr	439	3.9
Total	632	7.7
Men	30	40.0
General population	520	0.6

From Thorlacius *et al.*, 1997

To examine the frequency of this mutation in the general population and in patients with breast cancer, Thorlacius, *et al.* (1997) screened a random population of 520 Icelanders (~0.2% of the entire population), and tissue samples from 632 unselected women with breast cancer (23% of female breast cancer during the past 40 yr) and 30 men with breast cancer (100% of all cases during the past 40 yr) for the *BRCA2* 999del5 mutation. The results are summarized in Table 6.

The mutant allele was found in 3 individuals of the sample population, i.e., 0.6%. This is 10-fold higher than the estimated frequency of *all* BRCA1 mutations (Ford *et al.*, 1995). The mutation was found in 16.6% of all women with breast cancer diagnosed at age ≤50 yr, and 7.7% of all women with breast cancer. This is similar to the 8.5% frequency of the mutation found in another sample of 459 Icelandic women with breast cancer (Johannesdottir, *et al.*, 1996), although it is not clear how much overlap there was between the two populations. The median age of onset of breast cancer in the women with the mutation was 45 yr, vs. 60 yr for the women without the mutation. The oldest age of diagnosis for a mutation carrier was 77 yr. Of the cancer patients with the mutation, there were 61 whose family histories could be analyzed. Only nine had no first-, second-, or third-degree relatives with breast cancer. As will be seen, these data are largely consistent with those obtained from studies of *BRCA1* and *BRCA2* mutations in a population very different from Icelanders but also with a high population frequency of mutations, namely, Ashkenazi Jews.

BRCA1 and *BRCA2* mutations in Jewish populations

The origins of the Ashkenazi Jewish population. Another population that has been amenable to this type of analysis is the Ashkenazi Jewish population, and some space will now be devoted to a discussion of the BRCA mutations in this population. The term Ashkenazi is somewhat imprecise, but is generally used to refer to Jews originating, at least in recent centuries, from Europe, particularly Central and Eastern Europe. The name Ashkenaz (אשכנז) is mentioned in Genesis as one of the descendants of Noah's son, Japheth (*Genesis* 10:3). Most classical commentators, including the Jerusalem Talmud, interpret this to be a reference to an Asiatic nation (Zlotowitz, 1977). The name Ashkenaz is also mentioned in *Jeremiah* 51:27, as one of the kingdoms destined to conquer Babylon, again likely an Asiatic nation (Freedman, 1949). In later Jewish literature, Ashkenaz came to denote Germany. The first existing mention of the term in this context dates from the 9th century (Zlotowitz, 1977). The term is definitely used with this meaning by the 11th century commentator, Rashi (Rabbi Shlomo ben Yitzhak, 1040-1105), who lived most of his life northern France (commentaries to *Deut.* 3:5 and to Tractate *Sukkah* 17a of the Babylonian Talmud).

Jewish settlement in Western Europe dates back to antiquity, perhaps even before the return from the Babylonian exile ~350 B.C.E. (Wein, 1993). There were undoubtedly Jewish settlements throughout France and Germany during the era of Roman conquest of these areas. It is estimated that these settlements numbered approximately 1 million in population at the beginning of the 4th century C.E., but their numbers diminished considerably following the rise of Christianity and fall of the Roman Empire, and it is estimated that at most a population of 10,000 survived as Jews by the end of the 8th century (Agus, 1969, quoted in Wein, 1993). Modern recorded Jewish history in Western Europe dates from the time of Charlemagne (742-814). Under his reign, the Jewish population in France and Germany increased, with Jews immigrating to these communities from Italy, the Balkans, Babylonia (present day Iraq), and Asia Minor. This nuclear Ashkenazi population grew in succeeding centuries, but the founder population of

> "five to ten thousand souls who survived as Jews in Italy, Germany, and France by the end of the 8th century, were the true ancestors of the Ashkenazic Jews of the past 12 centuries...in the sense that all the Yiddish-speaking Jews of the year 1900, numbering more than 10 million, were the descendants of these five to ten thousand" (Agus, 1969, quoted in Wein, 1993).

Between the 8th and 11th centuries, the Ashkenazi population in France and Germany grew and developed. However, beginning with the first crusade at the end of the 11th century, and continuing in subsequent centuries, frequent persecutions, expulsions, pogroms, and massacres at the hands of the larger population drove the surviving Ashkenazi population eastward. Through the 14th and 15th centuries, Jews from the west emigrated in increasing numbers to Poland, Lithuania, Bohemia, and neighboring areas of Central and Eastern Europe (Wein, 1993; Greenbaum, 1995). As noted above, it is primarily these emigrees who are thought to be the ancestors of the modern Ashkenazi population, members of which have been studied for *BRCA* mutations, as described below.

Much has been written regarding the Mendelian genetic disorders that have an increased prevalence in the Ashkenazi population, including Gaucher, Tay-Sachs, Niemann-Pick, and Canavan diseases, idiopathic torsion dystonia, cystic fibrosis, Bloom syndrome, mucolipidosis IV, familial dysautonomia, factor XI deficiency, pentosuria, and non-classical 21-hydroxylase deficiency (Bonné-Tamir *et al.*, 1992; Motulsky, 1995). There is general agreement that founder effects probably account for much of the data, but the potential contributions of genetic drift and selective

advantage in some of these disorders has been controversial (Jorde, 1992; Motulsky, 1995; Risch *et al.*, 1995; Zoossmann-Diskin, 1995).

The BRCA1 185delAG mutation. Once the *BRCA1* gene was identified, the high-risk families that had been studied to establish linkage and to map *BRCA1* were studied for mutations. It soon became clear that a number of families shared a single mutation, 185delAG, and further investigation indicated that these families all shared Ashkenazi Jewish descent (Simard *et al.*, 1994; Friedman *et al.*, 1995; Shattuck-Eidens *et al.*, 1995; Struewing *et al.*, 1995b; Takahashi *et al.*, 1995; Tonin *et al.*, 1995). Moreover, haplotype analysis indicated that the apparently unrelated families sharing this mutation also shared a common haplotype for the 17q21 chromsomal region carrying the mutation (Simard *et al.*, 1994; Friedman *et al.*, 1995; Struewing *et al.*, 1995b). Struewing *et al.* (1995a) then tested DNA samples from 858 unrelated Ashkenazi individuals from the U.S. and Israel who had previously sought genetic testing for Tay-Sachs disease and/or cystic fibrosis. A total of 8 individuals with the 185delAG mutation were found, compared with none out of a mixed ethnic control group of 815 individuals. This result suggested a frequency of this single mutation of 0.9% in the Ashkenazi population, up to 15-fold higher than the predicted frequency of *all BRCA1* mutations in the general population (Ford *et al.*, 1995).

These findings concerning 185delAG were rapidly confirmed and extended. Analysis by a group in Boston of 39 Jewish women with breast cancer occurring before age 40, 8 (21%) carried the 185delAG mutation .(FitzGerald *et al.*, 1996). None of these women came from a classical high-risk family, although 7 of the 8 had a first or second degree relative with breast cancer.

Similar results were reported by Offit *et al.* (1996), who studied a group of 107 Ashkenazi Jewish women from the New York area for the 185delAG mutation by an allele-specific PCR protocol. Of 80 who were diagnosed with breast cancer before age 42 years, chosen without regard to family history, 16 (20%) carried the 185delAG mutation. Of these 11 of 35 (35%) with, and 5 of 49 (10%) without, an affected first degree relative carried the mutation. (However, each of these five had a second-degree relative). They also studied 27 women with breast cancer diagnosed between ages 42 and 50 and with a family history of early onset breast or ovarian cancer. Eight of these women (30%) carried the 185delAG mutation. There was also a strong correlation of the mutation with ovarian cancer: of 10 of 24 (42%) probands with the mutation had a history of ovarian cancer in themselves or a relative, compared with 8 of 83 (10%) of probands without the mutation.

Berman *et al.* (1996b) examined a group of 163 subjects from HBOC families and 178 individuals with breast and/or ovarian cancer unselected for family history, all from the Philadelphia area. Of these, 64 were Ashkenazi Jewish women, and 13 of these carried the 185delAG mutation (20%), 10 from the HBOC families and 3 from the patients unselected for family history. This study also found another *BRCA1* mutation, 188del11, in 10 individuals, including 4 Jewish individuals with breast and/or ovarian cancer screened on the basis of ethnicity. This mutation has not been found in any other study of Jewish subjects. This study also identified two non-Jewish individuals carrying the 185delAG mutation, but evidently on a haplotype distinct from the one found in the Jewish families, suggesting the likelihood of at least two independent origins for the mutation. Similarly, Neuhausen *et al.* (1996b), found that out of 19 families with the 185delAG mutation, two had no known Jewish ancestry. These two families were from Yorkshire, U.K., and shared a common 17q haplotype quite distinct from the Ashkenazi-associated haplotype carrying 185delAG.

These three studies showed that in Jewish women with early onset breast cancer and at least one affected second degree relative, the prevalence of the 185delAG mutation is at least 20%, and

higher in the group with cases of ovarian cancer in the family. This contrasted with a similar population-based study in a predominantly non-Jewish Caucasian group of women with breast cancer diagnosed before age 35 (Langston *et al.*, 1996), in which only 6 of 80 women had a cancer-associated *BRCA1* mutation, including only 4 of 41 with an affected first or second degree relative.

At least two similar studies examined Jewish women with ovarian cancer. Muto *et al.* (1996) examined 31 Jewish women with ovarian cancer and 23 Jewish controls matched for telephone prefix. Six of the 31 patients (19%), but none of the controls, carried the mutation. Modan *et al.* Modan *et al.* (1996) studied 79 Jewish Israeli women with ovarian cancer, 18 with, and 61 without, a first degree relative affected with breast or ovarian cancer. The 185delAG mutation was detected in 7/18 (39%) of those with, and in 8/61 (13%) of those without an affected first degree relative. The mutation was detected in only 1/182 controls. In both of these studies (Modan *et al.*, 1996; Muto *et al.*, 1996), the mutation carriers had a lower age of cancer diagnosis than the patients without the mutation (48, 50 yr vs. 57, 60 yr, respectively). The study of Modan *et al.* is also significant for the finding that one of the patients with the 185delAG mutation is from an Iraqi Jewish family, suggesting that the mutation may be prevalent in non-Ashkenazi Jews as well. Other studies have made similar findings, and this topic is discussed further, below.

The BRCA2 6174delT mutation. Within months of the cloning of the *BRCA2* gene, mutational analyses of additional HB/OC families were published. Tavtigian *et al.* (Tavtigian *et al.*, 1996) reported 9 mutations in addition to the 6 described in the initial report by Wooster *et al.* (Wooster *et al.*, 1995) . Amongst these was a mutation in exon 11, 6174delT, identified in an individual of Ashkenazi Jewish descent. This prompted an analysis of Ashkenazi Jewish women with early onset breast cancer who had previously been examined for *BRCA1* mutations. Investigators at Sloan Kettering in New York (Neuhausen *et al.*, 1996a) studied 80 Ashkenazi Jewish women and 93 non-Jewish women, both groups selected for having breast cancer before age 42, without regard to family history. Six of the 80 (8%) carried the *BRCA2* 6174delT mutation, which was not found in any of the 93 non-Jewish controls with breast cancer or in 70 Caucasian controls without cancer. Out of a second group of 27 Ashkenazi Jewish women with breast cancer diagnosed at age 42-50 yr and at least one first or second degree relative with breast or ovarian cancer, two were found to have the *BRCA2* 6174delT mutation. These results are summarized in Table 7. Out of the entire study population of 107 women with breast cancer, of the 32 women with either of the two mutations, 12 (38%) had either a personal or family history of ovarian cancer, compared with only 6 of 75 (8%) lacking both mutations.

Table 7. *BRCA1* 185delAG and *BRCA2* 6174delT mutations in Ashkenazi Jewish women with breast cancer

Age of onset (yr)	Family history	n	Mutations					
			185delAG		6174delT		Either	(%)
<42	unselected	80	16	20%	6	8%	22	28
42-50	positive	27	8	30%	2	7%	10	37

From Neuhausen *et al.*, 1996a

Berman *et al.* (1996a) identified 8 individuals with the *BRCA2* 6174delT mutation in their cohort of 176 patients with breast and/or ovarian cancer from the Philadelphia area. Five were from high-risk HB/OC families, and the other three (1 breast, 2 ovarian cancer) patients were unselected for family history. Seven of the 8 individuals had Jewish ancestry. Surprisingly, at least in regard to the findings with *BRCA1* 185delAG, analysis of polymorphic markers on 13q in the *BRCA2* 6174delT carriers showed an absence of haplotype sharing, suggesting that the mutation had arisen on several different haplotypes. This finding has apparently not yet been confirmed.

High frequency of BRCA2 6147delT in the Ashkenazi population. Given these results, it was of interest to know the prevalence of the *BRCA2* 6174delT mutation in the Jewish population, and this was promptly addressed in several studies. Oddoux *et al.* (1996) tested 1255 Ashkenazi Jewish individuals who had participated in other genetic screening programs at NYU or the NIH, and compared them with 519 non-Jewish controls studied at these two sites. Twelve individuals (1%) with the *BRCA2* 6174delT mutation were found among the Jewish subjects, whereas none were found among the non-Jewish subjects. This frequency was comparable to that of the *BRCA1* 185delAG mutation, although as noted above, the studies of cancer patients and high risk families suggested a lower relative risk of breast or ovarian cancer associated with the *BRCA2* mutation, compared with the *BRCA1* mutation.

In a similar study (Roa *et al.*, 1996), a group from Baylor College of Medicine in Houston screened approximately 3,000 Ashkenazi Jewish individuals (85% from the from the U.S., 15% from Israel) for the two common mutations, *BRCA1* 185delAG and *BRCA2* 6174delT, as well as for the European/Russian mutation, *BRCA1* 5382insC, which had previously been found in a few Jewish HBOC families. This study confirmed the ~1% frequency of the *BRCA1* 185delAG mutation, and found a frequency of the *BRCA2* 6174delT mutation of 1.5% (1.38% in the U.S., 2.51% in Israel, p=0.12). The 5382insC mutation was found in 0.15% of the individuals in the U.S, but was not found in Israel.

To determine the frequency of these mutations in cases of familial breast cancer in Jewish families, Tonin *et al.* (1996) studied 220 Ashkenazi Jewish families from 10 centers in the U.S. and Canada. Each family had a minimum of two cases of breast cancer, at least one diagnosed at age ≤50. Of these, 82 families also had cases of ovarian cancer. Four mutations were sought: *BRCA1* 185delAG, 5382insC, 188del11, and *BRCA2* 6175delT. The data are summarized in Table 8. No 188del11 mutations were found. However, 100 families, or 45.5% overall, carried one of the other three mutations. Ninety one of these (41%) carried the *BRCA1* mutations, and only 9 (4%) carried the *BRCA2* mutation, despite the higher frequency of this latter mutation in the Ashkenazi populations previously surveyed. This suggests a low penetrance for this gene, but this will be addressed further later. In contrast, there was a disproportionately high frequency of families with the 5382insC mutation. Even in the families with two or three cases of breast cancer without ovarian cancer, the prevalence of mutations was ~25%. These families would not have been included as classical high risk families, but the data are consistent with the finding of the earlier studies that 20-30% of Jewish women with breast cancer before age 50 carry one of the recurrent *BRCA1* mutations, and that cases of breast cancer in first (and second) degree relatives of these women are very common.

The prevalence of *BRCA1* or *BRCA2* mutations was substantially higher in the 82 families with both breast and ovarian cancer, reaching an astounding 89% in the families with two or more cases of ovarian cancer. Overall, 60% of the families with mutations had at least one case of ovarian cancer, and a family history of ovarian cancer conferred at 6.7-fold increased risk of a *BRCA1* mutation. Four families reported cases of cancer of the fallopian tubes, but the authors did not indicate whether these families also had cases of ovarian cancer. Apart from this higher prevalence of ovarian cancer, there was little else that distinguished the families with mutations from those in which no mutation was found. As shown Table 9, the number of cases of breast cancer per family and the age of onset of breast cancer were similar in the different groups. Rather strikingly, none of the four families in which there were cases of breast cancer at an extremely young age (<25 yr)

showed mutations. Other *BRCA1* or *BRCA2* mutations that were not screened for could explain this finding. These families showed a high prevalence of other cancers.

Table 8. Mutations in *BRCA1* or *BRCA2* in Jewish families with breast with or without ovarian cancer

Families	n	Mutations							
		185 delAG (1.05%)		5382 insC (0.11%)		6174 delT (1.36%)		Any (2.52%)	(%)
2 BC	48	10		2		0		12	25
3 BC	43	7		3		1		11	26
≥4 BC	47	11		2		4		17	36
Total	138	28	(20%)	7	(5%)	5	(4%)	40	29
≥2 BC, 1 OC	54	22		9		4		35	65
≥2 BC, ≥2 OC	28	21		4		0		25	89
Total	82	43	(52%)	13	(16%)	4	(5%)	60	73
Grand total	220	71	(32%)	20	(9%)	9	(4%)	100	46

From Tonin *et al.*, 1996

Table 9. Familial breast cancer in Jewish families with or without *BRCA* mutations

Mutation	n	BC cases/family	BC Age	BC at <25
BRCA1	91	3.58	45.1	0
BRCA2	9	3.89	42.2	0
Neither	120	3.41	47.1	4

From Tonin *et al.*, 1996

A similar study, but conducted at the level of individual patients with breast or ovarian cancer, was recently reported from Israel (Abeliovich *et al.*, 1997). Among Ashkenazi women with cancer, tested for the three recurrent mutations 185delAG, 5382insC, and 6174delT, one of the three mutations was found in 23 of 43 women with ovarian cancer, 5 of 6 with both breast and ovarian cancer, 13 of 43 with breast cancer diagnosed before age 40 yr, and 11 of 97 with breast cancer diagnosed after age 40 yr. Of the mutation carriers from whom a complete family history could be obtained, 25 of 28 had an affected first degree relative. Of the subjects with a negative or unknown family history, 12 of 109 were carriers. Both the 185delAG and 6174delT mutations had a similar prevalence among the carriers; the 5382insC mutation was not found in any of the patients with ovarian cancer. Although most of the patients were not selected for family history, approximately half had an affected first degree relative, and of these families, 65 of 90 lacked any of the three mutations. Cancers at sites other than the breast or ovary were reported in many of the families and some of the patients, both with and without identified mutations.

These studies examining both *BRCA1* and *BRCA2* confirmed that the recurrent BRCA mutations are very common in familial breast and ovarian cancer in the Ashkenazi population, but the penetrance of the *BRCA2* 6174delT mutation seemed to differ among them, and neither addressed the risk of breast or ovarian cancer in healthy carriers. These findings emphasized the need for population-based studies to assess more accurately the true risk of carrying the *BRCA1* and *BRCA2* mutations and to provide further data to assess the feasibility of population screening and genetic counselling. Some of these questions were addressed in several recent papers.

In the first of these, conducted by a group at the NIH, Struewing *et al.* (1997) recruited over 5,000 Jewish men and women over age 20 in the Washington, D. C. area, enrolled at 15 sites over a 9

week period. Over half of these subjects were ≥ 50 yr old and 70% were women. The subjects were given a self-administered questionnaire and were asked to list relatives who might also be participating. The investigators were therefore able to create two groups of family sets, one that took into account the participation of related individuals and one that included those who had no participating relatives. The entire study population was screened for the three common mutations 185delAG, 5382insC, and 6174delT. A random set of 1000 subjects was screened for the 188del11 mutations, with no carriers being found. The data for the three mutations are shown in Table 10.

Table 10. Recurrent mutations in *BRCA1* and *BRCA2* in a large Jewish population

BC or OC	n	Mutations							
		185 delAG		5382 insC		6174 delT		Any	(%)
Personal hx	302	10	3.3%	6	2.0%	11	3.6%	27	8.9
Dx at < 50 yr	143								14.0
Dx at ≥ 50 yr	153								4.6
Family hx	1061	17	1.6%	10	0.9%	17	1.6%	44	4.1
Neither	3949	14	0.4%	4	0.1%	31	0.8%	49	1.2
Total	5318	41	0.8%	20	0.4%	59	1.2%	120	2.3

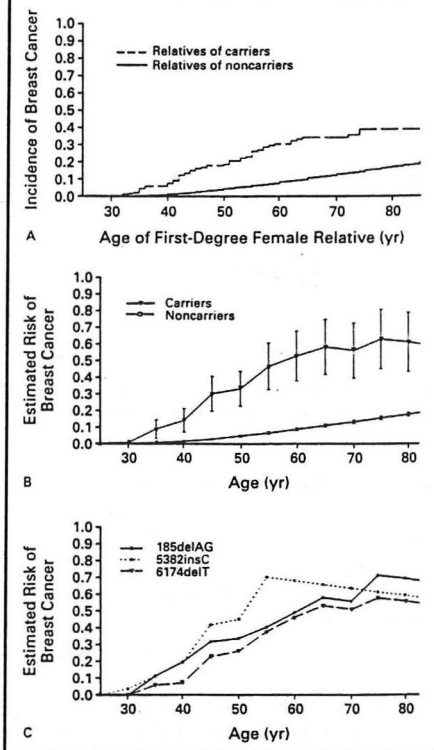
From Struewing *et al.*, 1997

A total of 120 participants were found to carry a mutation (2.26%). The overall frequencies of the *BRCA1* 185delAG and *BRCA2* 6174delT mutations, (0.8 and 1.2%) were similar to those previously reported, whereas the frequency of the *BRCA1* 5382insC mutation (0.4%) was substantially higher than previously found. The data from the study subjects and their reported family histories were then used to generate life-table estimates of the incidence or risk of breast and ovarian cancer. These are shown in Figures 8 and 9.

These data cannot be considered a completely unbiased sample of the Ashkenazi population, since over 5% of the participants were women who had survived breast or ovarian cancer, and 20% of the participants reported at least one first degree relative with breast or ovarian cancer. This probable bias would tend to inflate the estimate of risk associated with the mutations. Nonetheless, these are the most comprehensive population data so far available, and a number of important questions can be addressed from the data. Mutations in both *BRCA1* and *BRCA2* were associated with a substantially increased risk of breast and ovarian cancer. The estimated risk of breast cancer by the age of 50 among carriers was 33%, compared with 4.5% for noncarriers; by age 70, these figures were 56% and 13%. For ovarian cancer, carriers showed a risk of 7% by age 50 and 16% by age 70, compared with risks of 0.4% and 1.2%, respectively, in noncarriers. The breast cancer risks associated with each of the three mutations were not significantly dissimilar, contrary to the suggestion from previous studies, and the same pattern was found for ovarian cancer. Although subjects with a family history of breast or ovarian cancer were more likely to have a mutation, there were 31 carriers identified who did not report any breast or ovarian cancer in either first or second degree relatives. At least 5 of these carriers had three or more first degree female relatives over 40. Thus, especially considering the probable overestimate of true risk attributable to the nonrandom nature of the study population, the risk of cancer seem to be lower than lifetime risk of 85% attributed to *BRCA1* carriers in high risk families (Easton *et al.*, 1993). Various manipulations of the data to reduce the effects of biased sampling led to a predicted carrier-associated risk of breast cancer of 42-54% by age 70. Most likely, this difference from 85% is attributable to modifying

genetic factors (i.e., the difference between risk to those in the general population compared with those in a family with documented high risk), but environmental or statistical factors may also explain part of the difference. Nonetheless, the sobering conclusion remains that at least 2.5% of Jewish women have a ~60% lifetime risk of breast or ovarian cancer. Moreover, as shown in Figure 9 and discussed below, the *BRCA* mutations are associated with cancers at other sites as well.

Figure 8. Incidence or risk of breast cancer in a large Jewish population: Kaplan-Meier estimates

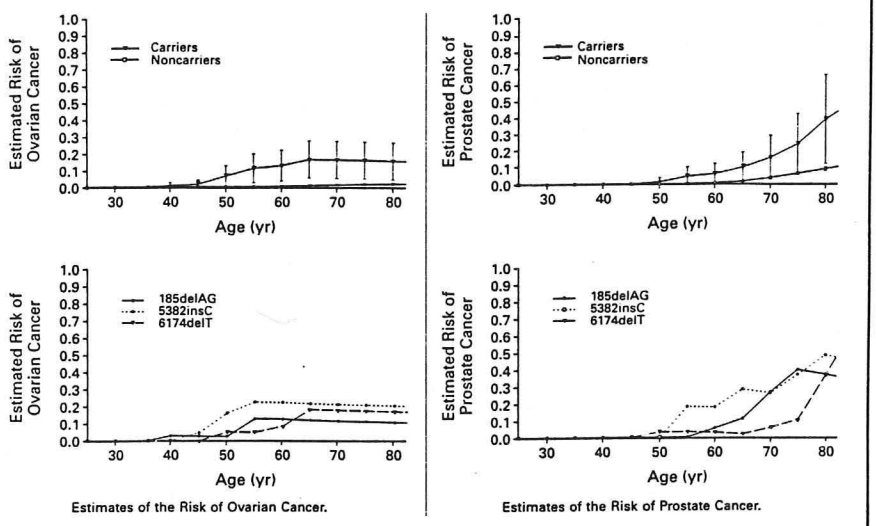


From Struewing *et al.*, 1997

Another question raised by the data in Jewish populations is whether the frequency of *BRCA* mutations is similar in families with breast cancer of different ethnic backgrounds. Couch *et al.* (1997) studied 169 women with breast cancer who were specifically referred because of a familial risk factor for breast cancer (average of 4.0 breast, and 1.5 ovarian, cancer cases per family). The *BRCA1* genes of these individuals were completely screened for mutations thoughout the entire coding region and intron-exon boundaries. Twenty-seven mutations were identified. Twenty-five of the families (15%) reported Ashkenazi Jewish ancestry, and only the 185delAG and 5382insC mutations were found in these families. Almost all of the non-Jewish patients were Caucasian. The overall frequency of *BRCA1* mutations was surprisingly low in the non-Jewish families (16% overall, vs. 26% in the Jewish families), and did not correlate with either number of breast cancers

per family or with number of cases of bilateral breast cancer. The median age of breast cancer in the families with mutations was 41.0 years, vs. 50.7 for families without mutations. From the

Figure 9. Estimates of the risk of ovarian and prostate cancer in a large Jewish population



From Struewing *et al.*, 1997

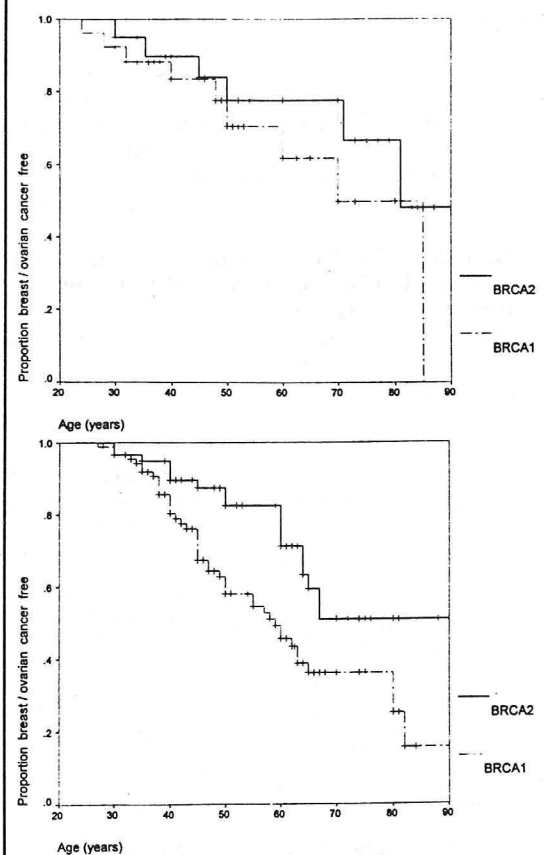
data, the authors developed a model of predicted probability estimates for the likelihood of *BRCA1* mutations in families with breast cancer, with or without ovarian cancer, based on average age of diagnosis of breast cancer in family members. The most striking finding from this study is that, at any age, the likelihood of a *BRCA1* mutation is substantially higher in Jewish families, compared with the probability for the group as a whole. This sugests that *BRCA1* (and probably *BRCA2*) mutations account for a larger proportion of familial breast, and even breast and ovarian, cancer in high risk Jewish families, compared with non-Jewish families.

The role of *BRCA2* mutations in early onset breast cancer was addressed by investigators in Boston in a group of 73 women with breast cancer diagnosed at ≤ 32 years of age mutations (Krainer *et al.*, 1997). using protein truncation assays. Only two *BRCA2* mutations were found, along with 8 *BRCA1* mutations. They also studied a group of 39 Jewish women with breast cancer diagnosed before age 40, in whom they found 1 *BRCA2* 6174delT, 1 *BRCA1* 5382insC, and 8 *BRCA1* 185delAG mutations (26% for all three mutations). The low frequency of *BRCA2* mutations, even in the Jewish cohort, suggests a low penetrance of the *BRCA2* mutation. However, the data of Struewing et al. (1997), shown in Fig. 8C, suggests that the risk of breast cancer at ages under 55 conferred by the *BRCA2* 65174delT mutation lags behind that of the two *BRCA1* mutations, with a rather low risk below age 40. Thus, the data from Krainer *et al.* are actually consistent with the NIH population study.

The issue of penetrance of *BRCA1* and *BRCA2* was specifically addressed in a recent paper from Israel (Levy-Lahad *et al.*, 1997). These authors studied 33 patients with ovarian cancer (22 Ashkenazi, 11 non-Ashkenazi), unselected for family history, as well as 44 families with two or

more individuals affected with breast and/or ovarian cancer (42 Ashkenazi), for the three common *BRCA1* and *BRCA2* mutations previously found in Jewish populations. Ten of the 22 Ashkenazi ovarian cancer patients had mutations (5, 2, and 3, respectively for 185delAG, 5382insC, and 6174delT). One of the non-Ashkenazi women, who was of Iranian-Jewish origin, also carried the 185delAG mutation. Twenty-five of the 42 Ashkenazi families showed one of the three mutations. Data from 301 subjects from these families (208 for *BRCA1*, 93 for *BRCA2*) were used to compute life-table and penetrance estimates, as shown in Figure 10. The penetrance, both for

Figure 10. Risk of breast or ovarian cancer in relatives of patients with ovarian cancer who are carriers of *BRCA1* or *BRCA2* mutations



Top: families ascertained from consecutive OC patients
Bottom: families with known prevalence of OC
From Levy-Lahad *et al.*, 1997

breast cancer alone and for breast and ovarian cancer, of the two *BRCA1* mutations was significantly greater than that of the *BRCA2* mutation, with a hazard ratio of 2.1. This finding is somewhat more consistent with most of the studies examining both genes in Jewish populations, although not with the large NIH population study.

Genetic screening for BRCA1 and BRCA2 mutations. The high penetrance of the *BRCA* mutations, together with the high frequency of a small number of mutant alleles in the Ashkenazi Jewish population, all provide a strong impetus for genetic screening in this population, as well as similar populations (e.g., Icelanders). Many issues — ethical, medical, psychological, and legal — are relevant to such an undertaking. Statements recommending against population-based screening have been issued by several organizations, including the American Society for Human Genetics (1994), the National Advisory Council for Human Genome Research (Collins *et al.*, 1994), and the National Breast Cancer Coalition (1995), and by private individuals such as former NIH Director Bernadine Healy (1997). One way to address the feasibility and advisability of such screening is to conduct pilot screening projects on an investigative basis, and the results of such a project have recently been reported that was carried out in a Jewish population in Houston by a group at Baylor College of Medicine (Richards *et al.*, 1997). Unlike the survey conducted by the NIH, described above, in which the individual participates were not informed of the outcome, this study reported the results to the participants and assessed the outcomes, including the educational processes before and after the screening.

Of 333 individuals who attended an educational session, 309 consented to participate (88% women). Of these 289 requested a DNA test for the *BRCA1* 185delAG mutation (the only test initially offered). Of the 20 individuals who declined the test (19 women), most cited concern about the potential impact on their health insurance status, with excessive worry over a positive result the second most common reason. The results of the screening are shown in Table 11. Of those who were tested, six participants (2.1%) were found to have the *BRCA1* 185delAG mutation and seven (2.4%) the *BRCA2* 6147delT mutation. All but one had either a personal history of breast or ovarian cancer and/or a family history meeting study criteria (one first degree relative or two second degree relatives with onset of breast cancer at ≤ 50 yr of age and/or ovarian cancer at any age), and the one exception had a history of two second degree relatives with breast cancer after age 50. As a result, the authors proposed as a guideline for cost-effective screening for inherited breast or ovarian cancer in the Ashkenazi Jewish population that individuals be

Table 11. Genetic screening in a Jewish population

History of BC and/or OC	n	185delAG	6147delT
Negative	190	1*	0
Positive family	64	4	5
Positive personal	23	1	1
Positive personal & family	12	0	1

*Family history negative by study criteria, but positive for two second degree relatives with postmenopausal breast cancer
From Richards *et al.*, 1997

screened who have one first- or second-degree relative with breast or ovarian cancer, diagnosed at any age. By these criteria, they would have screened 176 individuals instead of 289 and still detected all 13 individuals with mutations. They argue against wholesale testing in the general Ashkenazi population, since no mutations were found in individuals without any family history of breast or ovarian cancer. It is interesting that only 3 of the 35 participants with personal histories of breast or ovarian cancer carried mutations. However, the *BRCA1* 5382insC mutation was not

tested for, and this mutation accounts for an appreciable percentage of the familial breast and ovarian cancers that have been found in the Azhkenazi Jewish population in the U.S., as described above in a number of studies.

The BRCA1 185delAG mutation in non-Ashkenazi Jews. As noted above, several studies have identified the *BRCA1* 185delAG mutation in women with breast or ovarian cancer living in Israel who are of Iraqi or Iranian Jewish descent (Modan *et al.*, 1996; Sher *et al.*, 1996; Abeliovich *et al.*, 1997; Levy-Lahad *et al.*, 1997). There are as yet no published data on the prevalence of any of the mutations in non-Ashkenazi Jewish populations, but Modan *et al.*, have unpublished data showing a 0.5% prevalence of the 185delAG mutation in Iraqi Jewish women in Israel (3 out of 600 in a population survey; with at least one 17q21 haplotype matching that of the Ashkenazi mutation, B. Modan, personal communication). Does this mean that the 185delAG mutation antedates the dispersion of the Jewish people in Roman times or earlier, as proposed by Szabo and King (1997)? At this point, one can only speculate on the basis of inadequate data. The Jewish communities of Iran and Iraq are among the oldest continuous Jewish communities, commencing with the Babylonian exile in the 5th century before the common era and continuing up to our own time (Wein, 1993). Following the destruction by the Romans of the Second Temple and subsequent Roman persecutions of the Jewish population in the Land of Israel in the first and second centuries in the common era, the Jewish community in Babylonia (present day Iraq) became the dominant Jewish community in the world for the next eight centuries. The ascent of Islam after the 7th century coincided with somewhat of a decline in the fortunes of the Babylonian Jewish community, and over the next few centuries there was a substantial emigration from this community, primarily to North Africa and Spain. As noted above, there was evidently also some immigration from Babylonia into the Ashkenazi population in the 8th and/or 9th century, and it is conceivable that the founder gene in the Ashkenazi population was actually introduced during this period of immigration. Alternatively, the mutation may have been transferred from West to East during the period when the Babylonian community was the center of the Jewish world (Abeliovich *et al.*, 1997). However, as speculated by Szabo and King (1997) and by Modan (personal communication), the mutation might have existed in the Jewish population in the time of the Second Temple or even before. Perhaps additional genetic epidemiologic studies will answer the question of the origin of this mutation.

Other mutations in Ashkenazim. One of the problems with screening for specific mutations, e.g., by allele specific oligo hybridization, particularly in cases of familial cancer, is the possibility of not detecting mutations that are either less common and/or not yet described. In a recent paper, Schubert *et al.* (1997) studied 17 Ashkenazi Jewish families with ≥ 4 cases of breast or ovarian cancer. Only eight of them carried one of the three recurrent mutations in *BRCA1* or *BRCA2*. Of the remaining nine, none showed any mutations in *BRCA1*, which was completely screened by SSCP and protein truncation. The *BRCA2* gene was completely examined in two of these families, and partially screened in a third, and one of these three families was found to carry a novel mutation, *BRCA2* 6425delTT. Of the eight unresolved families, three include cases of ovarian cancer, one includes a case of male breast cancer, and one includes six women diagnosed with breast cancer before age 45 yr. Whether these families carry novel mutations in *BRCA2* or have an as yet unidentified gene is unclear, but in any case, it would seem most imprudent to reassure a female member of any of the eight families lacking the three common Ashkenazi mutations regarding her risk of cancer.

Male breast cancer

As noted above, *BRCA2* was originally mapped by stuyding families with cases of male breast cancer, since this entity did not seem to be a part of *BRCA1*-linked HBC. A number of cases of male breast cancer have indeed been found in families with *BRCA2* mutations, as well as two cases in families with *BRCA1* mutations (Hogervorst *et al.*, 1995; Struewing *et al.*, 1995b; Thorlacius *et al.*, 1995; Wooster *et al.*, 1995; Couch *et al.*, 1996a; Tavtigian *et al.*, 1996; Ramus *et al.*, 1997). Since male breast cancer is rare (~1,000 new cases annually in the U.S.), it might be expected that a high percentage of apparently sporadic cases would have a genetic basis, and the role of germline mutations in the *BRCA* genes could be assessed by screening for mutations in patients unselected for family history. As noted in Table 6, 40% of the 30 male breast cancer cases recorded in Iceland during the past 40 years were associated with the *BRCA2* 999del5 mutation. In a study of a more diverse population, Friedman *et al.* (1997) examined 54 male breast cancer cases from southern California for germline mutations in the *BRCA1* and *BRCA2* genes. Sixteen of the 54 had a first or second degree relative affected with breast and/or ovarian cancer. No *BRCA1* mutations were found. Two of the patients (4% of the total) were found to carry novel truncating mutations in the *BRCA2* gene. Only one of these had a family history of cancer. These data suggest that most male breast cancer is not associated with germline mutations in *BRCA1* or *BRCA2*, despite the clearly established increased susceptibility to male carriers of *BRCA2* mutations. This low prevalence of *BRCA* mutations, even among the patients with a positive family history, would seem to provide additional evidence for the association of other genes with familial breast cancer. The basis for the 10-fold discrepancy in the frequency of mutation carriers between the 1997 studies of Friedman *et al.* and Thorlacius *et al.* is not clear, and, in the absence of other identified risk factors, might be difficult to resolve without an accurate assessment of the prevalence of male breast cancer in the two study populations.

Other cancers

A recurring theme as one reads descriptions of HC/OG families with or without known mutations, as well as the family histories of individuals found to have *BRCA1* and *BRCA2* mutations, is the high frequency of cancers at other sites. Cancers of the prostate and pancreas seem particularly frequent, but cancers of the adrenal cortex, brain, cervix, colon, endometrium, follopian tube, gall bladder, lip, kidney, larynx, liver, lung, peritoneum, skin, stomach, testis, thyroid, and urethra, as well as leukemia, lymphoma, melanoma, mesothelioma, and myeloma have been reported in families with either *BRCA1* or *BRCA2* mutations, (Gudmundsson *et al.*, 1996; Phelan *et al.*, 1996; Serova *et al.*, 1996; Tavtigian *et al.*, 1996; Thorlacius *et al.*, 1996, 1997; Abeliovich *et al.*, 1997; Schubert *et al.*, 1997). Cancers of unknown primary site have also been noted. In the NIH study of a Jewish population in the Washington D. C. area (Figure 9), a significantly elevated estimated risk of prostate cancer in *BRCA1* carriers and their relatives was found, comparable to the risk for ovarian cancer. Family histories of some other cancers, including lung cancer, multiple myeloma, and Hodgkin's disease, were significantly elevated in carriers of mutations in *BRCA1* or *BRCA2*, compared with non-carriers.

As noted above, somatic deletion of the chromosomal region carrying the *BRCA2* gene in a pancreatic tumor aided in the original cloning of this gene, and *BRCA2* mutations have recently been directly implicated in pancreatic cancer, an estimated 5% of which is thought to be associated with genetic predisposition (Lynch *et al.*, 1996). Özçelik *et al.* (1997) found two germline *BRCA2* mutations in 41 unselected patients with pancreatic cancer. Of these, 13 were Jewish, and one of these carried the 6174delT mutation. Screening archival pancreatic cancers from 26 Jewish and 55 non-Jewish patients for this mutation identified three cases in the Jewish group, but none in the non-Jewish group. Thus, 4 of 39 (~10%) Jewish patients with pancreatic cancer unselected for

family history had the *BRCA2* 6174delT mutation. These data specifically support the concept that *BRCA2* predisposes to pancreatic cancer, as well as the more general concept that *BRCA2* is a low penetrance cancer gene predisposing to a variety of cancers (Boyd, 1996).

A recent analysis by the Breast Cancer Linkage Consortium has identified an increased risk of other cancers, notably pancreas and prostate, in individuals with germline mutations of *BRCA2*, but with a lower penetrance than for breast or ovarian cancer (D. Goldgar, personal communication). A formal comparison of risk associated with *BRCA1* and *BRCA2* mutations remains to be done.

In an intriguing report, Garcia-Marco *et al.* (1996) used fluorescence *in situ* hybridization to analyze deletions in chromosome 13 in chronic B-cell lymphocytic leukemia (CLL). They found deletion of the 1-Mb 13q12.3 region that encompasses the *BRCA2* gene in 28 of 35 cases of CLL (80%) and homozygous deletion of *BRCA2* in 21 (60%). These findings raise the possibility that *BRCA2* may function as a tumor suppressor gene in B-cell CLL.

Other genes

As noted above, individuals heterozygous for mutations in the *ATM* gene are thought to be at increased risk for breast cancer. Germline mutations of several other genes have also been associated with increased breast cancer susceptibility. These include the gene for the tumor suppressor p53 (Li-Fraumeni syndrome), the androgen receptor on chromosome Xq11.2-12, the gene for Cowden syndrome on 10q22-23, and a locus linked to *HRAS1* on chromosome 11p15.5 (reviewed in Greene, 1997). Also, as noted above, the mutations in DNA mismatch repair genes that cause hereditary nonpolyposis colon cancer are also associated with an increased susceptibility to ovarian cancer (reviewed in Boyd & Rubin, 1997).

The consequences of genetic testing

In this Grand Rounds, I have attempted to review the current understand of the biology of human cancer associated with mutations in *BRCA1* and *BRCA2*. The suitability and consequences of genetic testing for cancer in terms of clinical practice is beyond the scope of this presentation. This large and difficult topic is scheduled to be covered in a subsequent Grand Rounds on August 14, 1997, by Dr. Andrew Zinn. At present, the most drastic course for an asymptomatic woman with an inherited *BRCA1* or *BRCA2* mutation is bilateral prophylactic mastectomy and oophorectomy. There are no data on the long-term outcome of these procedures. A recent paper presents a decision analysis for estimating the benefits and risks of these procedures (Schrag *et al.*, 1997).

Summary and Conclusions

The identification of the *BRCA1* and *BRCA2* genes and the characterization of hundreds of mutations in these genes associated with breast and ovarian cancer constitute a triumph of modern clinical and molecular genetics.

The majority of cancer-associated *BRCA1* and *BRCA2* mutations are small insertions or deletions that induce frameshifts and premature protein truncation. Mutations are located throughout both genes. The mutations range in prevalence from those that are widely dispersed throughout many populations, to those that are recurrent within individual populations, to those found only in single families or individuals. Most of the investigation of mutations has so far been carried out in Europe, North America, Israel, and Japan. Investigation of populations in other parts of the world is needed as well.

Women with mutations in either *BRCA1* or *BRCA2* are at significant lifetime risk for developing breast or ovarian cancer. The best estimates of this risk have come from studies of the Askhenazi Jewish population, which has an unusually high carrier frequency of ~2.5% for three recurrent mutations in *BRCA1* and *BRCA2*. The lifetime risk of breast cancer is probably at least 50%, and for ovarian cancer the risk probably approaches 20%. These risks are only modestly lower than those predicted from studies of families with multiple cases of cancer.

A large majority of mutation carriers with breast and/or ovarian cancer have a history of a similarly affected first or second degree relative. This argues against screening of large populations without regard to family history.

The three common mutations in *BRCA1* and *BRCA2* that have been identified in the Ashkenazi Jewish population do not account for all of the familial breast and/or ovarian cancer in this population, and therefore the absence of all three mutations does not necessarily reduce the risk to someone who has a family history breast or ovarian cancer.

Both *BRCA1* and *BRCA2* mutations are associated with other cancers, most notably of the pancreas and prostate, but probably others as well. The penetrance for any particular cancer appears to be lower than for breast or ovarian cancer. A formal assessment of the risk of other cancers to carriers of *BRCA1* and *BRCA2* mutations has not yet been done.

The *BRCA1* and *BRCA2* genes appear to be coordinately regulated, and to be expressed in a cell cycle-dependent manner in proliferating cells. Both BRCA1 and BRCA2 proteins appear to play a role in a pathway involving DNA mainenance or repair, probably as part of a multi-protein complex involving RAD51 and the recently identified protein BARD1. The best current guess as to the function of this complex is that it is involved in repairing breaks in double-stranded DNA associated with homologous recombination. Cells carrying a germline mutation in one of these genes may thus progress to neoplasia as a result of somatic mutations that accumulate once the corresponding normal allele is lost. Elucidation of the function of BRCA1 and BRCA2 will likely lead to new insights into the processes controlling cell division.

In many but not all studies comparing the two, the penetrance of *BRCA1* mutations exceeds that of *BRCA2* mutations, and in most populations, a greater proportion of hereditary breast and ovarian cancer is associated with *BRCA1* mutations. Mutations in *BRCA1* or *BRCA2* appear to account for most but not all cases of hereditary breast cancer.

BIBLIOGRAPHY

Abel KJ, Xy J, Yin GY, Lyons RH, Meisler MH *et al.* (1995). Mouse Brca1: localization sequence analysis and identification of evolutionary conserved domains. *Hum Mol Genet* 4:2265-2273.

Abeliovich D, Kaduri L, Lerer I, Weinberg N, Amir G *et al.* (1997). The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. *Am J Hum Genet* 60:505-514.

Agus IA (1969). *The Heroic Age of Franco-German Jewry.* NY.

Albertsen H, Plaetke R, Ballard L, Fujimoto E, Connolly J *et al.* (1994a). Genetic mapping of the BRCA1 region on chromosome 17q21. *Am J Hum Genet* 54:516-525.

Albertsen HM, Smith SA, Mazoyer S, Fujimoto E, Stevens J *et al.* (1994b). A physical map and candidate genes in the BRCA1 region on chromosome 17q12-21. *Nat Genet* 7:472-479.

American Society of Clinical Oncology (1996). Statement on genetic testing for cancer susceptibility. *J Clin Oncol* 14:1730-1736.

American Society of Human Genetics. (1994). Statement on genetic testing for breast and ovarian cancer predisposition. *Am J Hum Genet* 55:i-iv.

Bailar JCI, Gornik HL. (1997). Cancer undefeated. *N Engl J Med* 336:1569-1574.

Berman DB, Costalas J, Schultz DC, Grana G, Daly M *et al.* (1996a). A common mutation in BRCA2 that predisposes to a variety of cancers is found in both Jewish Ashkenazi and non-Jewish individuals. *Cancer Res* 56:3409-3414.

Berman DB, Wagner-Costalas J, Schultz DC, Lynch HT, Daly M *et al.* (1996b). Two distinct origins of a common BRCA1 mutation in breast-ovarian cancer families: a genetic study of 15 185delAG-mutation kindreds. *Am J Hum Genet* 58:1166-1176.

BIC (Breast Cancer Information Core). (1997). http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic.

Bignell G, Micklem G, Stratton MR, Ashworth A, Wooster R. (1997). The BRC repeats are conserved in mammalian BRCA2 proteins. *Hum Mol Genet* 6:53-58.

Bonné-Tamir B, Adam A, eds. (1992). *Genetic Diversity Among Jews.* New York, Oxford University Press.

Bork P, Blomberg N, Nilges M. (1996). Internal repeats in the BRCA2 protein sequence. *Nat Genet* 13:22-23.

Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF *et al.* (1997). A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. *FASEB J* 11:68-76.

Bowcock AM. (1993). Molecular cloning of BRCA1: a gene for early onset familial breast and ovarian cancer. *Breast Cancer Res Treat* 28:121-135.

Bowcock AM, Anderson LA, Friedman LS, Black DM, Osborne-Lawrence S *et al.* (1993). THRA1 and D17S183 flank an interval of < 4 cM for the breast-ovarian cancer gene (BRCA1) on chromosome 17q21. *Am J Hum Genet* 52:718-722.

Boyd J. (1996). BRCA2 as a low-penetrance cancer gene. *J Natl Cancer Inst* 88:1408-1409.

Boyd J, Rubin SC. (1997). Hereditary ovarian cancer: molecular genetics and clinical implications. *Gynecol Oncol* 64:196-206.

Boyd M, Harris F, McFarlane R, Davidson HR, Black DM. (1995). A human BRCA1 gene knockout. *Nature* 375:541-542.

Broca P (1866). *Traité des Tumeurs. Tome Une. Tumeurs des Général.* Paris. pp. 149-154.

Caligo MA, Ghimenti C, Cipollini G, Ricci S, Brunetti I *et al.* (1996). BRCA1 germline mutational spectrum in Italian families from Tuscany: a high frequency of novel mutations. *Oncogene* 13:1483-1488.

Callebaut I, Mornon JP. (1997). From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. *FEBS Lett* 400:25-30.

Chapman MS, Verma IM. (1996). Transcriptional activation by BRCA1. *Nature* 382:678-679.

Chen KS, Shepel LA, Haag JD, Heil GM, Gould MN. (1996a). Cloning, genetic mapping and expression studies of the rat Brca1 gene. *Carcinogenesis* 17:1561-1566.

Chen Y, Farmer AA, Chen CF, Jones DC, Chen PL *et al.* (1996b). BRCA1 is a 220-kDa nuclear phosphoprotein that is expressed and phosphorylated in a cell cycle-dependent manner. *Cancer Res* 56:3168-3172.

Chen Y, Chen CF, Riley DJ, Allred DC, Chen PL *et al.* (1995). Aberrant subcellular localization of BRCA1 in breast cancer. *Science* 270:789-791.

Claus EB, Risch N, Thompson WD. (1991). Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am J Hum Genet* 48:232-242.

Claus EB, Risch NJ, Thompson WD. (1990). Age at onset as an indicator of familial risk of breast cancer. *Amer J Epidemiol* 131:961-972.

Coene E, Van Oostveldt P, Willems K, van Emmelo J, De Potter CR. (1997). BRCA1 is localized in cytoplasmic tube-like invaginations in the nucleus. *Nat Genet* 16:122-124.

Collins FS, Benjamin LJ, Botstein D, Cox JR, Davidson N *et al.* (1994). Statement on use of DNA testing for presymptomatic identification of cancer risk. *JAMA* 271:785.

Collins N, McManus R, Wooster R, Mangion J, Seal S *et al.* (1995). Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. *Oncogene* 10:1673-1675.

Connor F, Smith A, Wooster R, Stratton M, Dixon A *et al.* (1997). Cloning, chromosomal mapping and expression pattern of the mouse Brca2 gene. *Hum Mol Genet* 6:291-300.

Couch FJ, Farid LM, DeShano ML, Tavtigian SV, Calzone K *et al.* (1996a). BRCA2 germline mutations in male breast cancer cases and breast cancer families. *Nat Genet* 13:123-125.

Couch FJ, Rommens JM, Neuhausen SL, Belanger C, Dumont M *et al.* (1996b). Generation of an integrated transcription map of the BRCA2 region on chromosome 13q12-q13. *Genomics* 36:86-99.

Couch FJ, Weber BL. (1996c). Mutations and polymorphisms in the familial early-onset breast cancer (BRCA1) gene. Breast Cancer Information Core. *Hum Mutat* 8:8-18.

Couch FJ, DeShano ML, Blackwood A, Calzone K, Stopfer J *et al.* (1997). BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. *N Engl J Med* 336:1409-1415.

Cropp CS, Nevanlinna HA, Pyrhonen S, Stenman UH, Salmikangas P *et al.* (1994). Evidence for involvement of BRCA1 in sporadic breast carcinomas. *Cancer Res* 54:2548-2551.

De Benedetti VM, Radice P, Mondini P, Spatti G, Conti A *et al.* (1996). Screening for mutations in exon 11 of the BRCA1 gene in 70 Italian breast and ovarian cancer patients by protein truncation test. *Oncogene* 13:1353-1357.

Easton DF, Bishop DT, Ford D, Crockford GP, Consortium TBCL. (1993). Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. *Am J Hum Genet* 52:678-701.

Feunteun J, Lenoir GM. (1996). BRCA1, a gene involved in inherited predisposition to breast and ovarian cancer. *Biochim Biophys Acta* 1242:177-180.

FitzGerald MG, MacDonald DJ, Krainer M, Hoover I, O'Neil E *et al.* (1996). Germ-line BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med* 334:143-149.

Ford D, Easton DF, Peto J. (1995). Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. *Am J Hum Genet* 57:1457-1462.

Freedman H (1949). *Jeremiah.* London, Soncino Press.

Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED *et al.* (1994). Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nat Genet* 8:399-404.

Friedman LS, Szabo CI, Ostermeyer EA, Dowd P, Butler L *et al.* (1995). Novel inherited mutations and variable expressivity of BRCA1 alleles, including the founder mutation 185delAG in Ashkenazi Jewish families. *Am J Hum Genet* 57:1284-1297.

Friedman LS, Gayther SA, Kurosaki T, Gordon D, Noble B *et al.* (1997). Mutation analysis of BRCA1 and BRCA2 in a male breast cancer population. *Am J Hum Genet* 60:313-319.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K *et al.* (1994). BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266:120-122.

Garcia-Marco JA, Caldas C, Price CM, Wiedemann LM, Ashworth A *et al.* (1996). Frequent somatic deletion of the 13q12.3 locus encompassing BRCA2 in chronic lymphocytic leukemia. *Blood* 88:1568-1575.

Gayther SA, Warren W, Mazoyer S, Russell PA, Harrington PA *et al.* (1995). Germline mutations of the BRCA1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. *Nat Genet* 11:428-433.

Gayther SA, Mangion J, Russell P, Seal S, Barfoot R *et al.* (1997). Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. *Nat Genet* 15:103-105.

Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH. (1996). Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Genet* 12:191-194.

Greene MH. (1997). Genetics of breast cancer. *Mayo Clin Proc* 72:54-65.

Greenbaum M (1995). *The Jews of Lithuania. A History of a Remarkable Community 1316-1945.* Jerusalem, Gefen Books.

Gudas JM, Nguyen H, Li T, Cowan KH. (1995). Hormone-dependent regulation of BRCA1 in human breast cancer cells. *Cancer Res* 55:4561-4565.

Gudmundsson J, Johannesdottir G, Arason A, Bergthorsson JT, Ingvarsson S *et al.* (1996). Frequent occurrence of BRCA2 linkage in Icelandic breast cancer families and segregation of a common BRCA2 haplotype. *Am J Hum Genet* 58:749-756.

Håkansson S, Johannsson O, Johansson U, Sellberg G, Loman N *et al.* (1997). Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. *Am J Hum Genet* 60:1068-1078.

Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M *et al.* (1996). The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* 85:1009-1023.

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA *et al.* (1990). Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* 250:1684-1689.

Healy B. (1997). BRCA genes--bookmaking, fortunetelling, and medical care. *N Engl J Med* 336:1448-1449.

Hogervorst FB, Cornelis RS, Bout M, van Vliet M, Oosterwijk JC *et al.* (1995). Rapid detection of BRCA1 mutations by the protein truncation test. *Nat Genet* 10:208-212.

Hosking L, Trowsdale J, Nicolai H, Solomon E, Foulkes W *et al.* (1995). A somatic BRCA1 mutation in an ovarian tumour. *Nat Genet* 9:343-344.

Jensen RA, Thompson ME, Jetton TL, Szabo CI, van der Meer R *et al.* (1996). BRCA1 is secreted and exhibits properties of a granin. *Nat Genet* 12:303-308.

Jin Y, Xu XL, Yang M-C, Wei F, Bowcock AM, Baer R. (1997). Cell cycle-dependent accumulation of BARD1 polypeptides into BRCA1 nuclear dots. Submitted.

Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA *et al.* (1996). High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res* 56:3663-3665.

Jorde LB (1992). Genetic diseases in the Ashkenazi population: evolutionary considerations. In *Genetic Diversity Among Jews*, B. Bonné-Tamir and A. Adam, eds. Oxford Unversity Press, NY. pp. 305-318.

Kainu T, Kononen J, Johansson O, Olsson H, Borg A *et al.* (1996). Detection of germline BRCA1 mutations in breast cancer patients by quantitative messenger RNA in situ hybridization. *Cancer Res* 56:2912-2915.

Kelsell DP, Black DM, Bishop DT, Spurr NK. (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.

Kelsey JL, Bernstein L. (1996). Epidemiology and prevention of breast cancer. *Annu Rev Public Health* 17:47-67.

Kinzler KW, Vogelstein B. (1996). Lessons from hereditary colorectal cancer. *Cell* 87:159-170.

Kinzler KW, Vogelstein B. (1997). Gatekeepers and caretakers. *Nature* 386:761-763.

Knudson AG. (1983). Hereditary cancers of man. *Cancer Invest* 1:187-193.

Koonin EV, Altschul SF, Bork P. (1996). BRCA1 protein products. Functional motifs. *Nat Genet* 13:266-268.

Krainer M, Silva-Arrieta S, FitzGerald MG, Shimada A, Ishioka C *et al.* (1997). Differential contributions of BRCA1 and BRCA2 to early-onset breast cancer. *N Engl J Med* 336:1416-1421.

Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C *et al.* (1996). BRCA2 mutations in primary breast and ovarian cancers. *Nat Genet* 13:238-240.

Lane TF, Deng C, Elson A, Lyu MS, Kozak CA *et al.* (1995). Expression of Brca1 is associated with terminal differentiation of ectodermally and mesodermally derived tissues in mice. *Genes Dev* 9:2712-2722.

Langston AA, Malone KE, Thompson JD, Daling JR, Ostrander EA. (1996). BRCA1 mutations in a population-based sample of young women with breast cancer. *N Engl J Med* 334:137-142.

Levy-Lahad E, Catane R, Eisenberg S, Kaufman B, Hornreich G *et al.* (1997). Founder BRCA1 and BRCA2 mutations in Ashkenazi Jews in Israel: frequency and differential penetrance in ovarian cancer and in breast-ovarian cancer families. *Am J Hum Genet* 60:1059-1067.

Lim D-S, Hasty P. (1996). A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol Cell Biol* 16:7133-7143.

Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH. (1996). Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev* 10:1835-1843.

Lu M, Conzen SD, Cole CN, Arrick BA. (1996). Characterization of functional messenger RNA splice variants of BRCA1 expressed in nonmalignant and tumor-derived breast cells. *Cancer Res* 56:4578-4581.

Lynch HT, Harris RE, Guirgis HA, Maloney K, Carmody LL *et al.* (1978). Familial association of breast/ovarian carcinoma. *Cancer* 41:1543-1549.

Lynch HT, Smyrk T, Kern SE, Hruban RH, Lightdale CJ *et al.* (1996). Familial pancreatic cancer: a review. *Semin Oncol* 23:251-275.

Marcus JN, Watson P, Page DL, Narod SA, Lenoir GM *et al.* (1996). Hereditary breast cancer: pathobiology, prognosis, and BRCA1 and BRCA2 gene linkage. *Cancer* 77:697-709.

Marks JR, Huper G, Vaughn JP, Davis PL, Norris J *et al.* (1997). BRCA1 expression is not directly responsive to estrogen. *Oncogene* 14:115-121.

Marquis ST, Rajan JV, Wynshaw-Boris A, Xu J, Yin GY *et al.* (1995). The developmental pattern of Brca1 expression implies a role in differentiation of the breast and other tissues. *Nat Genet* 11:17-26.

Matsushima M, Kobayashi K, Emi M, Saito H, Saito J *et al.* (1995). Mutation analysis of the BRCA1 gene in 76 Japanese ovarian cancer patients: four germline mutations, but no evidence of somatic mutation. *Hum Mol Genet* 4:1953-1956.

Merajver SD, Pham TM, Caduff RF, Chen M, Poy EL *et al.* (1995). Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. *Nat Genet* 9:439-443.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K *et al.* (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266:66-71.

Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y. (1996). Mutation analysis in the BRCA2 gene in primary breast cancers. *Nat Genet* 13:245-247.

Milner J, Ponder B, Hughes-Davies L, Seltmann M, Kouzarides T. (1997). Transcriptional activation functions in BRCA2. *Nature* 386:772-773.

Modan B, Gak E, Sade-Bruchim RB, Hirsh-Yechezkel G, Theodor L *et al.* (1996). High frequency of BRCA1 185delAG mutation in ovarian cancer in Israel. National Israel Study of Ovarian Cancer. *JAMA* 276:1823-1825.

Montagna M, Santacatterina M, Corneo B, Menin C, Serova O *et al.* (1996). Identification of seven new BRCA1 germline mutations in Italian breast and breast/ovarian cancer families. *Cancer Res* 56:5466-5469.

Monteiro AN, August A, Hanafusa H. (1996). Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc Natl Acad Sci USA* 93:13595-13599.

Motulsky AG. (1995). Jewish diseases and origins. *Nat Genet* 9:99-101.

Muto MG, Cramer DW, Tangir J, Berkowitz R, Mok S. (1996). Frequency of the BRCA1 185delAG mutation among Jewish women with ovarian cancer and matched population controls. *Cancer Res* 56:1250-1252.

Narod SA, Feunteun J, Lynch HT, Watson P, Conway T *et al.* (1991). Familial breast-ovarian cancer locus on chromosome 17q12-q23. *Lancet* 338:82-83.

National Breast Cancer Coalition. (1995). Presymptomatic genetic testing for heritable breast cancer risk. Press release, Sept. 28, 1995, Washington, D.C.

Neuhausen S, Gilewski T, Norton L, Tran T, McGuire P *et al.* (1996a). Recurrent BRCA2 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nat Genet* 13:126-128.

Neuhausen SL, Mazoyer S, Friedman L, Stratton M, Offit K *et al.* (1996b). Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. *Am J Hum Genet* 58:271-280.

Newman B, Austin MA, Lee M, King M-C. (1988). Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. *Proc Natl Acad Sci USA* 85:3044-3048.

O'Connell P, Albertsen H, Matsunami N, Taylor T, Hundley JE *et al.* (1994). A radiation hybrid map of the BRCA1 region. *Am J Hum Genet* 54:526-534.

Oddoux C, Struewing JP, Clayton CM, Neuhausen S, Brody LC *et al.* (1996). The carrier frequency of the BRCA2 6174delT mutation among Ashkenazi Jewish individuals is approximately 1%. *Nat Genet* 14:188-190.

Offit K, Gilewski T, McGuire P, Schluger A, Hampel H *et al.* (1996). Germline BRCA1 185delAG mutations in Jewish women with breast cancer. *Lancet* 347:1643-1645.

Özçelik H, Schmocker B, Di Nicola N, Shi X-H, Langer B *et al.* (1997). Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. *Nat Genet* 16:17-18.

Ozols RF, Rubin SC, Thomas G, Robboy S (1997). Epithelial ovarian cancer. In *Principles and Practice of Gynecologic Oncology*. W. J. Hoskins, C. A. Perez and R. C. Young, eds. Lippincott-Raven, Philadelphia. pp. 919-986.

Peelen T, van Vliet M, Petrij-Bosch A, Mieremet R, Szabo C *et al.* (1997). A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. *Am J Hum Genet* 60:1041-1049.

Phelan CM, Lancaster JM, Tonin P, Gumbs C, Cochran C *et al.* (1996). Mutation analysis of the BRCA2 gene in 49 site-specific breast cancer families. *Nat Genet* 13:120-122.

Rajan JV, Wang M, Marquis ST, Chodosh LA. (1996). Brca2 is coordinately regulated with Brca1 during proliferation and differentiation in mammary epithelial cells. *Proc Natl Acad Sci USA* 93:13078-13083.

Ramus SJ, Kote-Jarai Z, Friedman LS, van der Looij M, Gayther SA *et al.* (1997). Analysis of BRCA1 and BRCA2 mutations in Hungarian families with breast or breast-ovarian cancer. *Am J Hum Genet* 60:1242-1246.

Richards CS, Ward PA, Roa BB, Friedman LC, Boyd AA *et al.* (1997). Screening for 185delAG in the Ashkenazim. *Am J Hum Genet* 60:1085-1098.

Risch N, de Leon D, Ozelius L, Kramer P, Almasy L *et al.* (1995). Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. *Nat Genet* 9:152-159.

Roa BB, Boyd AA, Volcik K, Richards CS. (1996). Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nat Genet* 14:185-187.

Rubin SC, Benjamin I, Behbakht K, Takahashi H, Morgan MA *et al.* (1996). Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1. *N Engl J Med* 335:1413-1416.

Sankila R, Aaltonen L, Jarvinen HJ, Mecklin JP. (1996). Better survival rates in patients with MLH1-associated hereditary colon cancer. *Gastroenterology* 110:682-687.

Saurin AJ, Borden KLB, Boddy MN, Freemont PS. (1996). Does this have a familiar RING? *Trends Biochem Sci* 21:208-214.

Schildkraut JM, Thompson WD. (1988). Familial ovarian cancer: a population-based case-control study. *Am J Epidemiol* 128:456-466.

Schrag D, Kuntz KM, Garber JE, Weeks JC. (1997). Decision analysis--effects of prophylactic mastectomy and oophorectomy on life expectancy among women with BRCA1 or BRCA2 mutations. *N Engl J Med* 336:1465-1471.

Schubert EL, Lee MK, Mefford HC, Argonza RH, Morrow JE *et al.* (1997). BRCA2 in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance, and the possibility of families whose cancer is not attributable to BRCA1 or BRCA2. *Am J Hum Genet* 60:1031-1040.

Schutte M, da Costa LT, Hahn SA, Moskaluk C, Hoque AT *et al.* (1995). Identification by representational difference analysis of a homozygous deletion in pancreatic carcinoma that lies within the BRCA2 region. *Proc Natl Acad Sci USA* 92:5950-5954.

Scully R, Chen J, Plug A, Xiao Y, Weaver D *et al.* (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* 88:265-275.

Serova O, Montagna M, Torchard D, Narod SA, Tonin P *et al.* (1996). A high incidence of BRCA1 mutations in 20 breast-ovarian cancer families. *Am J Hum Genet* 58:42-51.

Serova OM, Mazoyer S, Puget N, Dubois V, Tonin P *et al.* (1997). Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast cancer-susceptibility genes? *Am J Hum Genet* 60:486-495.

Serova-Sinilnikova OM, Boutrand L, Stoppa-Lyonnet D, Bressac-de-Paillerets B, Dubois V *et al.* (1997). BRCA2 mutations in hereditary breast and ovarian cancer in France. *Am J Hum Genet* 60:1236-1239.

Sharan SK, Bradley A. (1997a). Murine Brca2: Sequence, map position, and expression pattern. *Genomics* 40:234-241.

Sharan SK, Morimatsu M, Albrecht U, Lim D-S, Regel E *et al.* (1997b). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* 386:804-810.

Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S *et al.* (1995). A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene. Implications for presymptomatic testing and screening. *JAMA* 273:535-541.

Sher C, Sharabini-Gargir L, Shohat M. (1996). Breast cancer and BRCA1 mutations. *N Engl J Med* 334:1199.

Simard J, Tonin P, Durocher F, Morgan K, Rommens J *et al.* (1994). Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. *Nat Genet* 8:392-398.

Smith SA, Easton DF, Evans DGR, Ponder BAJ. (1992). Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. *Nat Genet* 2:128-131.

Smith TM, Lee MK, Szabo CI, Jerome N, McEuen M *et al.* (1996). Complete genomic sequence and analysis of 117 kb of human DNA containing the gene BRCA1. *Genome Res* 6:1029-1049.

Sobol H, Stoppa-Lyonnet D, Bressac-de-Paillerets B, Peyrat JP, Kerangueven F *et al.* (1996). Truncation at conserved terminal regions of BRCA1 protein is associated with highly proliferating hereditary breast cancers. *Cancer Res* 56:3216-3219.

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

Spillman MA, Bowcock AM. (1996). BRCA1 and BRCA2 mRNA levels are coordinately elevated in human breast cancer cells in response to estrogen. *Oncogene* 13:1639-1645.

Steichen-Gersdorf E, Gallion HH, Ford D, Girodet C, Easton DF *et al.* (1994). Familial site-specific ovarian cancer is linked to BRCA1 on 17q12-21. *Am J Hum Genet* 55:870-875.

Stoppa-Lyonnet D, Laurent-Puig P, Essioux L, Pagès S, Ithier G *et al.* (1997). BRCA1 sequence variations in 160 individuals referred to a breast/ovarian family cancer clinic. *Am J Hum Genet* 60:1021-1031.

Stratton MR. (1996). Recent advances in understanding of genetic susceptibility to breast cancer. *Hum Mol Genet* 5:1515-1519.

Struewing JP, Abeliovich D, Peretz T, Avishai N, Kaback MM *et al.* (1995a). The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. *Nat Genet* 11:198-200.

Struewing JP, Brody LC, Erdos MR, Kase RG, Giambarresi TR *et al.* (1995b). Detection of eight BRCA1 mutations in 10 breast/ovarian cancer families, including 1 family with male breast cancer. *Am J Hum Genet* 57:1-7.

Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M *et al.* (1997). The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med* 336:1401-1408.

Stürzbecher H-W, Donzelmann B, Henning W, Knippschild U, Buchhop S. (1996). p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *EMBO J* 15:1992-2002.

Scully R, Ganesan S, Brown M, De Caprio JA, Cannistra SA *et al.* (1996). Location of BRCA1 in human breast and ovarian cancer cells. *Science* 272:123-126.

Scully R, Chen J, Plug A, Xiao Y, Weaver D *et al.* (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* 88:265-275.

Swift M, Morrell D, Massey RB, Chase CL. (1991). Incidence of cancer in 161 families with ataxia-telangiectasia. *N Engl J Med* 325:1831-1836.

Szabo CI, King M-C. (1997). Population genetics of BRCA1 and BRCA2. *Am J Hum Genet* 60:1013-1020.

Takahashi H, Behbakht K, McGovern PE, Chiu HC, Couch FJ *et al.* (1995). Mutation analysis of the BRCA1 gene in ovarian cancers. *Cancer Res* 55:2998-3002.

Tavtigian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D *et al.* (1996). The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 12:333-337.

Teng DH, Bogden R, Mitchell J, Baumgard M, Bell R *et al.* (1996). Low incidence of BRCA2 mutations in breast carcinoma and other cancers. *Nat Genet* 13:241-244.

Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT. (1995). Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat Genet* 9:444-450.

Thorlacius S, Tryggvadottir L, Olafsdottir GH, Jonasson JG, Ogmundsdottir HM *et al.* (1995). Linkage to BRCA2 region in hereditary male breast cancer. *Lancet* 346:544-545.

Thorlacius S, Olafsdottir G, Tryggvadottir L, Neuhausen S, Jonasson JG *et al.* (1996). A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat Genet* 13:117-119.

Thorlacius S, Sigurdsson S, Bjarnadottir H, Olafsdottir G, Jonasson JG *et al.* (1997). Study of a single BRCA2 mutation with high carrier frequency in a small population. *Am J Hum Genet* 60:1079-1084.

Tonin P, Serova O, Lenoir G, Lynch H, Durocher F *et al.* (1995). BRCA1 mutations in Ashkenazi Jewish women. *Am J Hum Genet* 57:189.

Tonin P, Weber B, Offit K, Couch F, Rebbeck TR *et al.* (1996). Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. *Nat Med* 2:1179-1183.

van den Berg J, Johannsson O, Hakansson S, Olsson H, Borg A. (1996). Allelic loss at chromosome 13q12-q13 is associated with poor prognosis in familial and sporadic breast cancer. *Br J Cancer* 74:1615-1619.

Vaughn JP, Cirisano FD, Huper G, Berchuck A, Futreal PA *et al.* (1996a). Cell cycle control of BRCA2. *Cancer Res* 56:4590-4594.

Vaughn JP, Davis PL, Jarboe MD, Huper G, Evans AC *et al.* (1996b). BRCA1 expression is induced before DNA systhesis in both normal and tumor-derived breast cells. *Cell Growth & Diff* 7:711-715.

Vehmanen P, Friedman LS, Eerola H, Sarantaus L, Pyrhönen S *et al.* (1997). A low proportion of BRCA2 mutations in Finnish breast cancer families. *Am J Hum Genet* 60:1050-1058.

Walberg M. (1996). Ataxia telangiectasia: cell cycle checkpoints and cancer, apoptosis and aging. UT Southwestern Internal Medicine Grand Rounds, Nov 14, 1996.

Wein B (1993). *Herald of Destiny. The Story of the Jews in the Medieval Era 750-1650.* Brooklyn, NY, Shaar Press.

Westhoff C. (1996). Ovarian cancer. *Annu Rev Public Health* 17:85-96.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S *et al.* (1995). Identification of the breast cancer susceptibility gene BRCA2. *Nature* 378:789-792.

Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D *et al.* (1994). Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* 265:2088-2090.

Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A *et al.* (1996). Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nat Genet* 14:430-440.

Zabludoff SD, Wright WW, Harshman K, Wold BJ. (1996). BRCA1 mRNA is expressed highly during meiosis and spermiogenesis but not during mitosis of male germ cells. *Oncogene* 13:649-653.

Zlotowitz M, Ed. (1977). *Bereshis*. Brooklyn, NY, Mesorah Publications, Inc.

Zoossmann-Diskin. (1995). ITD in Ashkenazi Jews--genetic drift or selection? *Nat Genet* 11:13-14.