
Subject Review

Genetics of Breast Cancer

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Familial breast cancer is characterized by young age at diagnosis, an increased risk of bilateral breast cancer, an increasing risk in conjunction with increasing numbers of affected family members, and a strong association with ovarian cancer. At least eight candidate breast cancer susceptibility genes have been identified. Mutations in *BRCA1*, *BRCA2*, *p53*, and the Cowden disease gene are relatively uncommon, are highly penetrant, and produce striking familial clusters of breast cancer. *BRCA1* and *BRCA2* are the most important of these, accounting for an estimated 80% of hereditary breast cancer and 5 to 6% of all breast cancers. Specific *BRCA1* and *BRCA2* mutations are of particular importance in population subgroups, such as those identified among Jewish women of central European (Ashkenazi) origin. Mutations in the ataxia-telangiectasia gene and the rare *HRAS1* vari-

able number of tandem repeats polymorphisms are much more common but also much less penetrant. They do not produce dramatic familial aggregations of breast cancer but may prove to be responsible for a substantial proportion of all breast cancers if their epidemiologic association with breast cancer is confirmed. Predictive genetic testing for breast cancer risk is under way. Oncologists and primary-care physicians must become familiar with these genetic disorders and the issues surrounding predictive testing in order to make appropriate management decisions about women thought to have a high genetic risk of breast cancer.

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AT = ataxia-telangiectasia; VNTR = variable number of tandem repeats

Familial clusters of breast cancer date back to at least AD 100. In 1866, French surgeon Paul Broca described his wife's family in which 10 of 24 women from 5 generations died of breast cancer. The modern analytic epidemiologic literature consistently demonstrates a twofold to threefold increase in breast cancer risk among mothers and sisters of patients with breast cancer.¹ The magnitude of this risk varies by age at diagnosis of breast cancer, laterality, and closeness of relationship. An analysis of 328 consecutive women with breast cancer revealed that the disease seemed to be "familial" in 23%; 9% of all pedigrees had a pattern compatible with a hereditary disorder.² Ultimately, formal segregation analysis demonstrated the existence of an autosomal dominant pattern of inheritance for a small percentage of cases of breast cancer.³ The data suggested that one or more rare but highly penetrant genes contributed to the development of breast cancer. The recent cloning of the breast cancer susceptibility genes *BRCA1*⁴ and *BRCA2*^{5,6} mark the first chapter in the

molecular genetics of breast cancer. The related explosion of new information provided the impetus to summarize the status of current knowledge in this rapidly evolving field.

FAMILY HISTORY AS A RISK FACTOR FOR BREAST CANCER

Multiple epidemiologic studies have documented that a reported history of breast cancer among relatives is a reproducible predictor of breast cancer risk.^{1,7-9} In general, a "positive family history" of breast cancer confers a relative risk of 2.0 to 3.0 for breast cancer. The risk conferred by a positive family history varies directly with closeness of the relationship to the proband. In a recent survey, relative risks were 2.4, 1.8, and 1.4 for first-, second-, and third-degree relatives, respectively.⁹ Affected family members in maternal and paternal bloodlines contribute to breast cancer risk in a similar fashion. A population-based analysis of breast cancer risk factors suggested that a positive family history of breast cancer accounted for 9.1% of all breast cancer in the United States.¹⁰

The relationship between risk factors for breast cancer in women with and without a positive family history of this disease has been explored.¹¹ In contrast with the expected relationships, patients with breast cancer and a positive family history did not experience protection from the following

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factors: later age at menarche, multiple births versus multiparity, or early versus later age at birth of first offspring. An adverse effect associated with the first pregnancy persisted among women with a positive family history in that parous women up to age 70 years had a higher risk of breast cancer than did nulliparous women. The relationship between family history and risks associated with estrogen replacement therapy has been assessed in several studies, and results have been conflicting. Among women with a positive family history, some studies suggested a greater risk from exogenous estrogen exposure, whereas others found no evidence of an association.¹² Data from a survey of reproductive risk factors in 333 *BRCA1* mutation carriers were conflicting.¹³ Increasing parity was protective for breast cancer (as has been traditionally observed), whereas it was associated with an *increasing* risk of ovarian cancer (the opposite of what has been described for unselected patients with ovarian cancer). These findings, which raise the possibility that traditional breast cancer risk factors may operate differently in women with familial breast cancer, complicate efforts to formulate a risk reduction strategy for women who have an increased risk of breast cancer because of a positive family history.

Most studies suggest that having a relative with bilateral breast cancer is associated with a greater increase in the risk of the development of breast cancer than is having a relative with unilateral disease.¹⁴ A widely held belief is that the presence of bilateral breast cancer is a clinical clue suggesting the presence of an inherited susceptibility to breast cancer.¹⁴ Bilateral breast cancers are 3 to 4 times more common in high-risk families than in normal families. Although not all studies demonstrate this relationship,⁷ the consensus is that bilaterality is a feature of familial breast cancer.¹⁵

A major epidemiologic characteristic of familial breast cancer is its strong association with ovarian cancer. The occurrence of breast cancer and ovarian cancer as double independent primary neoplasms in the same woman has long suggested the existence of shared risk factors between these two malignant diseases.¹⁶ Multiple families have been described in which clusters of both breast and ovarian cancer were noteworthy.¹⁷ Data from the Cancer and Steroid Hormone (CASH) survey documented a 1.5- to 1.9-fold increase in the reported frequency of ovarian cancer among family members of breast cancer probands,¹⁸ an observation confirmed in the United Kingdom.¹⁹

The most striking epidemiologic feature of familial breast cancer is the powerful inverse relationship between age at diagnosis of breast cancer and risk of breast cancer among close relatives of patients with breast cancer. This association was documented most thoroughly in the CASH study.⁷ For example, for a woman who is the unaffected sister of a proband with breast cancer, the relative risk of breast cancer is 4.3 if the proband was diagnosed at age 30 years, 2.7 if

diagnosed at age 40, and 1.7 if diagnosed at age 50. These risks escalate dramatically if multiple family members are affected with breast cancer. Thus, if the unaffected sister has, in addition to the proband, an affected mother and another affected sister, the corresponding relative risks are 44, 28, and 17 if the proband is diagnosed at ages 30, 40, and 50 years, respectively. Relative risks of this magnitude advocate strongly for the existence of a genetic mechanism as an explanation for at least a proportion of the striking familial aggregations of breast cancer. Conversely, the data also suggest that for the vast majority of women with a positive family history of breast cancer (one or two affected relatives), the risks conferred are much smaller. Such families may be accounted for by chance, nongenetic risk factors, or the effect of a breast cancer susceptibility allele with low penetrance.

SEGREGATION AND LINKAGE ANALYSES OF FAMILIAL BREAST CANCER

Segregation analysis is the formal, quantitative method for assessing whether the distribution of a particular trait within families is compatible with a mendelian mode of inheritance. This technique has consistently supported the existence of an autosomal dominant, inherited predisposition to breast cancer, in which the gene has a low frequency and a high penetrance. The largest of studies that used this technique suggested an autosomal dominant gene with a population frequency of 0.0033.³ This hypothetical gene conferred a cumulative breast cancer risk of 38% by age 50 years and 67% by age 70 among mutation carriers in comparison with 1.5% and 5.0%, respectively, in noncarriers. The estimated proportion of breast cancer in the general population attributable to this gene ranged from 33% among women diagnosed before age 30 years to 2% among women diagnosed between the ages of 70 and 79.²⁰ Current estimates suggest that 5% of cases of breast cancer in the general population are attributable to germline mutations in dominant, highly penetrant susceptibility genes.²¹

A genomic search was undertaken with use of a panel of polymorphic genetic markers distributed at known sites on all human chromosomes in an effort to identify the chromosomal site of this hypothetical gene. By using "linkage analysis," evidence of cosegregation was sought between the breast cancer trait and one of the genetic markers, the chromosomal sites of which were known. Finding "linkage" between the breast cancer trait and a specific genetic marker would imply that a breast cancer gene might be located close to the marker gene, a situation that would provide the beginning for the molecular biologic studies needed to isolate the gene. Hall and associates²² performed such a study with 23 extended families with breast cancer who had a mean of six cases of breast cancer per family. Evidence for linkage

between the breast cancer trait and the anonymous marker D17S74 (located on chromosome 17q21) was obtained. The linkage evidence was strikingly positive among the seven families with "early-onset breast cancer" (mean age at diagnosis, younger than 45 years) and "negative" among families with later-onset breast cancer. Approximately 40% of the families in the study had linkage to the 17q21 locus, which was designated "*BRCA1*."

Confirmatory evidence was provided by Narod and colleagues,²³ who reported that three of five families with both breast and ovarian cancer had linkage to the same marker. This study provided further impetus to isolate *BRCA1* physically from 17q21, confirmed the critical relationship between breast and ovarian cancer in these high-risk families, and provided additional evidence of genetic heterogeneity in the cause of hereditary breast and ovarian cancer—that is, some families in both the Hall and Narod reports had *no* linkage to *BRCA1*. This finding suggested the existence of more than one breast-ovarian cancer susceptibility gene; additional candidate genes have been identified (Table 1).

BRCA1

An international consortium pooled linkage and clinical data from 214 families and has provided much of the descriptive data related to the *BRCA1* syndrome.²⁴ Most of the *BRCA1*

linkage evidence in this data set was obtained from families in which at least four women had early-onset breast or ovarian cancer. Approximately 45% of families with pure, site-specific breast cancer have linkage to this gene, and the cumulative breast cancer risk among gene carriers is 51% at age 50 years and 85% at age 70.²⁵ The estimated gene frequency for *BRCA1* in the consortium database is 0.0006.²¹ The estimated proportion of breast cancers in the general population due to *BRCA1* is 8.2% for those younger than age 40 years, 4.0% for those between 40 and 49, and 1.0% for those between the ages of 50 and 70.^{21,26} The corresponding figures for ovarian cancer are 5.6%, 4.2%, and 1.8%, respectively. In most families with breast cancer who have less than four cases of breast cancer and no ovarian cancer, the cause is *not* related to *BRCA1*. The presence of two or more cases of early-onset breast cancer and two or more cases of ovarian cancer is associated with a 92% probability of being linked to *BRCA1*.²⁷ The consortium database has been used to compute the estimated likelihood that a *BRCA1* mutation will be found in women of various ages, cancer patterns, and relationship combinations (Table 2). These data are useful in counseling women who are deciding whether to undergo *BRCA1* testing.

Among *BRCA1* carriers with a first breast cancer, the risk of contralateral breast cancer is estimated to be 48% by age

Table 1.—Breast Cancer Susceptibility Genes*

Gene	Chromosome site	Gene frequency	Gene penetrance	Defining cancers	Possible associated conditions	Attributable risk of breast cancer (%)
<i>BRCA1</i>	17q21	Rare	High	Breast, ovary	Colon cancer, prostate cancer, <i>not</i> male breast cancer	5
<i>BRCA2</i>	13q12-13	Rare	High	Breast, ovary	Male breast cancer, pancreatic cancer, ? colon and prostate cancer	3
? <i>BRCA3</i>	? 8p12-22	?	?	Breast	...	?
p53 (Li-Fraumeni)	17p13.1	Rare	High	Sarcoma, breast, brain, adrenal, leukemia	? Melanoma	<1
Cowden	10q22-23	Very rare	High	Breast	Thyroid cancer; hamartomas of skin, breast, thyroid, oral mucosa, GI mucosa; cerebellar gangliocytomas	<<1
Androgen receptor	Xq11.2-12	Very rare	?	Male breast	...	?
<i>ATM</i>	11q22-23	Common	Low	Lympho-proliferative	Neurologic degeneration, telangiectasia, immunodeficiency, ionizing radiation sensitivity; ? breast cancer (heterozygotes)	7
<i>HRAS1</i> VNTR	11p15.5	Common	Low	...	? Breast, colon, rectum, and bladder cancer	9

**ATM* = ataxia-telangiectasia mutated; GI = gastrointestinal; VNTR = variable number of tandem repeats.

50 years and 64% by age 70. The risk of ovarian cancer in these same women is 29% by age 50 years and 44% by age 70. Colon cancer is estimated to occur 4 times more frequently among *BRCA1* mutation carriers than in the general population—the absolute risk is 6% by age 70 years (1 to 2% in noncarriers). Prostate cancer may occur 3.3 times more often than expected in male *BRCA1* mutation carriers, with an absolute risk of 8% by age 70 years.²⁹ Male breast cancer does not seem to be part of the *BRCA1* disease spectrum.³⁰

An analysis of 60 high-risk families produced evidence of a significant correlation between the site of the mutations within the *BRCA1* gene and the risk of ovarian cancer within each family.³¹ This correlation between genotype and phenotype suggested that *BRCA1* mutations located in the 3' third of the gene are associated with a significantly lower risk of ovarian cancer. A much larger database is needed to refine this observation, which, if true, would have important implications relative to genetic counseling of women with *BRCA1* mutations.

The *BRCA1* gene was identified by positional cloning methods.⁴ The gene has 5,592 nucleotides that are distributed over 100,000 bases of genomic DNA composed of 22 coding exons and encodes a protein of 1,863 amino acids. About 80% of all *BRCA1* mutations are frameshift or non-sense mutations that shift the codon reading frame, an outcome that causes premature protein termination. Genetic susceptibility to breast cancer is postulated to result from inactivation of one *BRCA1* allele in the germline and subsequent loss of the other allele in somatic breast tissue. The prospect that the cloning of *BRCA1* would quickly lead to a simple genetic test was thwarted because of the large size of the gene and the enormous number of distinct, disease-related mutations that have been identified to date. A recent summary documented 132 distinct mutations among 254 mutation carriers; the most common mutations are 185delAG (12%) and 5382insC (10%).³² This situation is further complicated by the fact that, in several families with extremely strong evidence of linkage to *BRCA1*, no *BRCA1* mutations have yet been identified. Thus, even the most sophisticated tests currently available seem unable to detect important abnormalities in the *BRCA1* gene. Therefore, failure to find a *BRCA1* mutation in a high-risk but currently cancer-free family member is clinically useful *only* if a specific *BRCA1* mutation has been identified in an affected first-degree relative. A negative test result (that is, no *BRCA1* mutation was found) in a patient with breast cancer from a multiplex family in which a *BRCA1* mutation has not been previously proved has multiple possible explanations: (1) a *BRCA1* mutation is present but has been overlooked (that is, a false-negative result); (2) a different, highly penetrant gene (for example, *BRCA2*) is responsible for the family's inherited susceptibility; (3) multiple genes of lower penetrance

Table 2.—Estimated Prior Probability of Carrying a *BRCA1* Mutation, Stratified by Cancer Pattern, Age at Diagnosis, and Family Pattern

Family pattern and age (yr) at diagnosis	Probability of <i>BRCA1</i> mutation (%)
Single affected patient	
Breast cancer, <30	12
Breast cancer, 30-39	6
Breast cancer, 40-49	3
Ovarian cancer, <50	7
Sisters	
2 Breast cancers, <40	37
2 Breast cancers, 40-49	20
Breast cancer <50, ovarian cancer <50	46
2 Ovarian cancers, <50	61
Families	
≥3 Breast cancers, <50	40
≥2 Breast cancers, ≥1 ovarian cancer	82
≥2 Breast cancers, ≥2 ovarian cancers	92

Modified from Shattuck-Eidens and associates.²⁸ By permission.

are the basis for the familial aggregation; (4) the cluster is a chance event—that is, not an inherited problem—despite appearances to the contrary; or (5) this is a genetic family, but the specific patient chosen for initial testing and screening represents a sporadic case. Additional evidence of genetic heterogeneity was obtained from a recent analysis of 145 families with both early-onset breast cancer and at least one case of ovarian cancer. The original consortium analysis had suggested that most, if not all, such families had linkage to *BRCA1*. The new data suggest that up to 25% of such families have no linkage to *BRCA1*—that is, are related to a different gene (see subsequent discussion).²⁷ The negative linkage evidence was from families that had either a case of male breast cancer or only one case of ovarian cancer.

MECHANISM OF ACTION FOR *BRCA1*

The mechanism through which *BRCA1* induces the development of breast cancer is unknown, and the function of the *BRCA1* protein has not been precisely identified. *BRCA1* is expressed in rapidly proliferating cells that are undergoing differentiation in the developing mouse. In the mouse mammary gland, *BRCA1* expression is increased during puberty, during pregnancy, and after treatment of oophorectomized animals with estradiol-17 β and progesterone.³³ Mice that are heterozygous for a deletion of exons 5 and 6 of *BRCA1* appear normal, are fertile, and do not have development of tumors up to 11 months after birth.³⁴ Mice that are homozygous for this deletion die before day 7.5 of embryogenesis, but no cancer develops. The mouse may not be the best model for studying *BRCA1*.

An estrogen-dependent, estrogen receptor-mediated effect on *BRCA1* expression has been observed in breast and ovarian cancer cells.³⁵ The basis for a novel, alternative hypothesis about *BRCA1* function is the identification of an amino acid sequence with strong homology for the granin protein family.³⁶ The chromogranin-secretogranin proteins are acidic secretory proteins that are found in a wide spectrum of endocrine and neuronal cells. If *BRCA1* proves to be a regulated secretory protein, it would be a unique mechanism of action for a tumor suppressor gene product. Finally, investigators have demonstrated that the *BRCA1* protein can function as a regulator of gene expression, although the physiologic target genes have not yet been identified.³⁷

On the basis of previous models of genetic cancer, an assumption is that *BRCA1* would be a tumor suppressor gene. One prediction of this model is the expectation that sporadic (nonhereditary) cancers would be found to carry acquired mutations in the same gene found to underlie the inherited variant of that cancer. Thus far, that has not proved to be true for sporadic breast cancer and ovarian cancer with reference to both *BRCA1*³⁸ and *BRCA2*.³⁹ Somatic point mutations in either *BRCA1* or *BRCA2* are rare in sporadic primary breast and ovarian cancers.

The most compelling evidence to date that *BRCA1* inhibits growth of breast and ovarian cancer at the cellular level is from experiments in which retroviral gene transfer of wild-type and mutant *BRCA1* genes into cultured cells was used.⁴⁰ These studies revealed that wild-type *BRCA1* inhibits the growth of breast and ovarian cancer cells in vitro. MCF7 breast cancer cells lost their ability to form tumors in nude mice when transfected with wild-type *BRCA1*. Intraperitoneal injection of *BRCA1* as a retroviral vector into nude mice with growing intraperitoneal MCF7 tumors resulted in increased *BRCA1* expression by those cancer cells and concomitant reduction in their rate of growth, with increased animal survival. These data support the hypothesis that *BRCA1* is a tumor suppressor gene and open the door to the possibility that *BRCA1*-based gene therapy might be effective in the treatment of human breast and ovarian cancers.

BRCA2

A genomic search performed with 15 families at high risk for breast cancer in which linkage to *BRCA1* had been excluded suggested that a second breast cancer susceptibility gene (*BRCA2*) might be located on chromosome 13q12-13.⁴¹ Male breast cancer seems to be part of the *BRCA2* tumor spectrum, and the risk of ovarian cancer seems to be lower than that in families with *BRCA1*. Familial male breast cancer has been linked to *BRCA2* as well.⁴² *BRCA2* was recently cloned.^{5,6} This large gene has 11,385 nucleotides that are distributed over 70,000 bases of genomic DNA composed of 27 coding exons and encodes a protein of 3,418

amino acids. Like *BRCA1*, multiple distinct mutations in *BRCA2* have been identified, scattered rather evenly throughout this gene. To date, all *BRCA2* mutations have resulted in premature termination of *BRCA2* protein synthesis. It is estimated (but far from certain) that *BRCA1* and *BRCA2* together will account for approximately 80% of inherited breast cancer. As with *BRCA1*, several families have strong linkage evidence relative to *BRCA2* in which specific *BRCA2* mutations have not yet been found.

The vast majority of familial breast and ovarian cancers in Iceland are linked to *BRCA2* rather than to *BRCA1*.⁴³ Pancreatic cancer occurs excessively in some of these families, although the magnitude of this risk (if real) has not been defined. A survey of 49 families with site-specific breast cancer found *BRCA2* mutations in 8 families.⁴⁴ The presence of pancreatic cancer in a family was a significant predictor (relative risk, 7.2) of the likelihood that a *BRCA2* mutation would be found. In this series, all four families with a case of male breast cancer had *BRCA2* mutations.

In the United States, *BRCA2* mutations were found in 7 of 50 men with breast cancer (14%) who were *not* selected on the basis of family history.⁴⁵ All but one of these seven men proved to have a positive family history of male or female breast cancer (or both). Families with *BRCA2* are also likely to have women with very early-onset breast cancer (age at diagnosis, 35 years or younger).⁴⁴ Using unpublished data from the International Breast Cancer Linkage Consortium, the same investigators estimated that, in 12% of families with four or five cases of breast cancer and in 61% with six or more cases, the cancer is due to *BRCA2* mutations. The data further suggest that ovarian cancer and perhaps prostate and colon cancer occur excessively in families with *BRCA2*.^{41-44,46}

FOUNDER EFFECTS INVOLVING BRCA1 AND BRCA2

Genetic disorders can be expected to vary in their prevalence among genetically distinctive subsets of the general population. Furthermore, if a genetically identifiable population can trace its progenitors to a relatively restricted set of ancestors or "founders," specific genetic mutations may be more common or even unique within such groups. This latter concept, referred to as the "founder effect," may be of real importance in understanding the genetics of *BRCA1* and *BRCA2* and may have pronounced implications for genetic testing strategies.

BRCA1 Mutation 185delAG.—One of the more intriguing observations of *BRCA1* has been the recognition that one specific mutation, 185delAG (that is, a two-base deletion of adenine and guanine at position 185 [codon 23 of exon 2]), occurs with very high frequency among persons of Ashkenazi Jewish extraction. This observation began with

the recognition that multiple families carrying the 185delAG mutation of *BRCA1* were of Ashkenazi Jewish origin.^{47,48}

This genetically distinctive Jewish population of central European origin is known to be affected with various specific genetic conditions. Recent data suggest that the Ashkenazi population is descended from a limited group of founders.⁴⁹ A study was performed in which this specific *BRCA1* mutation was sought in a group of 858 healthy Ashkenazi men and women who had participated in a genetic testing program for cystic fibrosis and Tay-Sachs disease. The stored DNA of these persons was tested, and 0.9% of Ashkenazi Jews *without cancer* carried this mutation.⁵⁰ This estimate is 10-fold higher than the estimated prevalence of all *BRCA1* mutations in the general population—1 in 833 (0.1%). This one mutation might account for an estimated 16% of all breast cancer and 39% of all ovarian cancer diagnosed in Ashkenazi women before the age of 50 years. The estimated contribution of all *BRCA1* mutations combined in the non-Ashkenazi population is 4.1% and 12%, respectively.

A study of 418 women in Boston with breast cancer diagnosed when they were 40 years of age or younger included specific 185delAG testing in a Jewish subgroup (N = 39).⁵¹ Of these women, 21% carried the 185delAG mutation. Another study assessed 107 Ashkenazi Jewish women with breast cancer from New York City: 80 were diagnosed before age 42 years; 27 were diagnosed between the ages of 42 and 50 and also had a positive family history of breast or ovarian cancer (or both).⁵² Twenty percent of the former group had the 185delAG mutation, as did 30% of the latter group. In this series, all the *BRCA1* mutation carriers had a positive family history of breast or ovarian cancer (or both). Several subsequent surveys have confirmed the importance of 185delAG in Ashkenazi families with breast or ovarian cancer. A study of 37 high-risk families revealed the 185delAG mutation in 5 Ashkenazi families.⁵³ Haplotype analysis confirmed a common ancestral origin for this mutation in these five apparently unrelated families. The pattern of cancers observed in these five families varied from early-onset bilateral breast cancer, both with and without ovarian cancer, to late-onset breast cancer. These findings have been confirmed,⁵⁴ and non-Jewish families with 185delAG have occasionally been identified.^{54,55} The "non-Jewish" variant of 185delAG seems to have a genetic origin that differs from that in Ashkenazi families. Finally, two additional *BRCA1* mutations (5382insC and 188del11) seem to be overrepresented among Ashkenazi Jews.^{48,54}

On the basis of case-control studies, a well-known fact is that Jewish women have a mildly increased risk of breast cancer in comparison with non-Jewish women. A large population-based case-control study of breast cancer was evaluated to reassess the relationship between Jewish reli-

gion and risk of breast cancer.⁵⁶ Overall, Jewish religion conferred a 10% (not statistically significant) excess risk of breast cancer; however, the relative risk was 3.8 for Jewish women with a first-degree relative affected by breast cancer, and this risk increased to 10.5 among Jewish women 50 years of age or younger. These effects of family history and age were significantly stronger for Jewish women than for Catholic or Protestant women. These data are compatible with those previously cited in regard to 185delAG and suggest that Jewish women are significantly more likely to inherit a breast cancer predisposition than are women from other religious groups.

BRCA2 Mutation 6174delT.—*BRCA2* also has a specific mutation that occurs excessively among Ashkenazi Jews. One of the high-risk families in the original Wooster and associates⁴¹ series of *BRCA2* kindred carried a 6174delT mutation; this family was of Ashkenazi Jewish extraction. In order to analyze this finding further, the New York Jewish women studied previously by Offit and colleagues,⁵² in regard to *BRCA1*, were tested for the presence of 6174delT.⁵⁷ Six of 80 (8%) Ashkenazi Jewish women diagnosed with breast cancer before the age of 42 years carried this mutation, as did 2 of 27 (7%) women with breast cancer diagnosed between the ages of 42 and 50 who also had a positive family history of breast or ovarian cancer. The investigators of this study estimated that *BRCA1* 185delAG and *BRCA2* 6174delT together may account for a quarter of all early-onset breast cancer and two-thirds of early-onset breast cancer in the setting of a family history of breast or ovarian cancer among Ashkenazi Jewish women. A survey of 176 women with breast and ovarian cancer (one-quarter of whom had a positive family history) identified 8 (4.5%) with 6174delT,⁵⁸ 7 of whom were of Ashkenazi Jewish descent.

Several recent surveys confirmed that, like *BRCA1* 185delAG, the *BRCA2* 6174delT mutation occurs in about 1% of Ashkenazi Jews who are free of cancer,^{59,60} an extraordinarily high prevalence rate for mutations in a specific population. Surprisingly, the 185delAG mutation is 4 times more common among Ashkenazi women with breast cancer than is the 6174delT mutation, despite their similar population prevalence. These observations suggest that the penetrance of 185delAG (that is, the likelihood that a person with the mutation will actually have development of cancer) is significantly greater than the penetrance of 6174delT. This supports the possibility that some breast cancer gene mutations are associated with a higher risk than others, a finding that further complicates genetic counseling in this setting.

BRCA2 Mutation 999del5.—In a pattern analogous to that described for Ashkenazi Jews, the *BRCA2* abnormalities in Icelanders (a genetically isolated population) are linked to a single specific mutation, 999del5.⁴³ Despite the presence

of a single mutation in 16 Icelandic families, considerable family-to-family heterogeneity was noted in the pattern of tumors observed. This suggests the existence of important risk modifying factors, either genetic or environmental, that influence the expression of *BRCA2* phenotype.

A survey of the prevalence of the 999del5 mutation in Icelandic residents with sporadic cancers of the breast, ovary, prostate, and miscellaneous sites revealed mutations in 8.5%, 7.9%, 2.7%, and 1.0%, respectively.⁶¹ With reference to breast cancer, the risk of carrying this specific *BRCA2* mutation varied inversely with age, ranging from 27% in women diagnosed between the ages of 30 and 39 years to 3.3% for women diagnosed after age 79. An estimated 40% of all male breast cancer in Iceland is attributable to the *BRCA2* 999del5 mutation.⁴³ These observations indicate that, at least in Iceland, *BRCA2* mutations may account for a significant proportion of nonfamilial breast and ovarian cancers.

Finally, preliminary data suggest that families of French-Canadian descent with breast and ovarian cancer have a limited number of *BRCA1* and *BRCA2* mutations^{44,62} and that Japanese families only rarely carry *BRCA1* mutations.⁶³ All these data combined support the existence of the founder effect in breast cancer susceptibility genes—that is, highly prevalent, specific breast cancer gene mutations exist within identifiable subgroups of the general population. The breast cancer risk (penetrance) associated with mutations identified in the screening of healthy populations in comparison with high-risk cancer-prone families has not yet been quantified. If the risk proves to be high, this phenomenon may allow the development of relatively inexpensive population-specific screening tests that could be used more readily in appropriately selected persons.

ADDITIONAL DOMINANT BREAST CANCER SUSCEPTIBILITY GENES

“BRCA3”...?—Investigators have consistently noted that perhaps 10 to 20% of families at high risk for breast cancer have no linkage to either *BRCA1* or *BRCA2*. This situation implies the existence of additional, yet to be discovered, dominant susceptibility genes. A linkage analysis of eight families with linkage to neither *BRCA1* or *BRCA2* yielded preliminary evidence that a third gene might be on chromosome 8p12-22.⁶⁴ None of these families had women with ovarian cancer. This “syndrome,” if it exists, is not yet well characterized. Additional breast cancer susceptibility genes will likely be identified.

Li-Fraumeni Syndrome.—In 1969, Li and Fraumeni⁶⁵ described four families in each of which a pair of children had soft tissue sarcoma. These families were also noteworthy for the occurrence of onset of breast cancer at a young age and a diversity of additional malignant diseases, includ-

ing leukemia, adrenocortical carcinoma, and brain tumors. A prospective study of these families confirmed large excesses of breast cancer, soft tissue sarcomas, and other cancers.⁶⁶ A formal definition was proposed: a proband with sarcoma diagnosed at an age younger than 45 years; a first-degree relative having sarcoma, breast cancer, primary brain tumor, adrenocortical carcinoma, or leukemia diagnosed at an age younger than 45; and a cancer diagnosed in a second-degree relative before the age of 45 or a sarcoma at any age.⁶⁷ Segregation analysis has suggested that the effect of a rare autosomal dominant gene could explain the distribution of cancer in these families.⁶⁸

In 1990, Malkin and associates⁶⁹ reported germline mutations of the tumor suppressor gene p53 in five families with the Li-Fraumeni syndrome. Multiple studies have confirmed this observation, although p53 mutations are currently detectable in only about half the families that fulfill the aforementioned definition. The risk of breast cancer in carriers of p53 mutations in these families is not known precisely, but an estimate is that at least 50% will have breast cancer by age 50 years.⁷⁰ Relatively few breast cancers occur in postmenopausal women from families with the Li-Fraumeni syndrome. Several surveys have sought evidence of germline p53 mutations in more typical families with site-specific breast cancer, but no evidence for such a role has been found.^{71,72} Prevalence surveys of germline p53 mutations in unselected women with breast cancer have found abnormalities in less than 1%.^{73,74} Thus, p53 seems to be a major contributor to the occurrence of inherited breast cancer in the specific setting of the Li-Fraumeni syndrome.

Cowden Disease.—In 1963, Lloyd and Dennis⁷⁵ described a 20-year-old woman with papillomatosis of the lips and oropharynx, scrotal tongue, a high arched palate, jaw hypoplasia, thyroid adenomas, fibrocystic disease of the breasts, central nervous system anomalies, breast cancer, and thyroid cancer. Similar abnormalities were found in other family members, including two maternal aunts with breast cancer. They named the syndrome after the index patient, Rachel Cowden.⁷⁵ Since then, additional families have been identified, but the disorder seems to be extremely rare. It is considered an autosomal dominant disorder in which an estimated 30% of affected women have breast cancer, often bilateral and typically at a younger than average age.⁷⁶⁻⁷⁸ A genomic search localized the Cowden gene to chromosome 10q22-23,⁷⁹ although the gene itself has not yet been cloned. Although rare (probably less than 200 reported cases), the Cowden disease locus seems to be a legitimate breast cancer susceptibility gene.

Androgen Receptor Mutations.—Male breast cancer occurs approximately 100 times less often than does female breast cancer and, except for families with *BRCA2* as previously described, the occurrence of multiple cases of male

breast cancer in families is indeed uncommon.⁸⁰ An epidemiologic case-control study of men with breast cancer provided supportive evidence of a relationship between male and female breast cancer.⁸¹ Among first-degree relatives of male breast cancer probands, women were 2.3 times and men were 6.1 times more likely to have breast cancer in comparison with control subjects. A meta-analysis of seven case-control studies of male breast cancer confirmed that the presence of breast cancer among first-degree relatives of patients conferred a 2.5-fold risk of breast cancer.⁸² As previously noted, familial male breast cancer has, in a few instances, been linked to *BRCA2*,⁴² with 40% of all male breast cancers in Iceland attributable to *BRCA2* 999del5.⁴³ Two families have been described in which multiple male breast cancers and germline mutations in the androgen receptor (chromosome Xq11.2-12) have been observed.^{83,84} Data cited but not yet published in full indicate that a survey found no androgen receptor mutations in site-specific female breast cancer.²¹

LOW-PENETRANCE BREAST CANCER SUSCEPTIBILITY GENES

The susceptibility genes previously described are relatively rare but highly penetrant. Thus, they would be expected to produce dramatic familial breast cancer clusters that would be uncommon but impressive. Susceptibility genes may also exist that are much more common but less penetrant. In general, such genes would not be expected to produce striking familial breast cancer clusters (because of their low penetrance) but, theoretically, could account for a substantial proportion of all breast cancers (because they are common). Such breast cancers would be of genetic origin but not familial in their clinical manifestation.

Ataxia-Telangiectasia.—Ataxia-telangiectasia (AT) is a rare autosomal recessive disorder in which patients experience progressive neurologic degeneration (for example, ataxia, choreoathetosis, and oculomotor abnormalities), telangiectases of skin and eyes, immunodeficiency, sensitivity to ionizing radiation, and an increased risk of lymphoproliferative malignant disease.⁸⁵ Epidemiologic studies that have evaluated the risk of cancer among relatives of patients with AT have suggested that female relatives who are heterozygous for the AT mutation may experience an increased risk of breast cancer.⁸⁶⁻⁸⁹ Easton⁹⁰ performed a quantitative review of the published data and estimated that the relative risk of breast cancer for AT heterozygotes was 3.9.

A series of families with site-specific breast cancer underwent study for evidence of linkage to the AT locus, but no evidence of linkage was found.⁹¹ The observation that suggests potential importance for the AT gene as a risk factor for breast cancer is its high prevalence—an estimated 1% of the

general population may be AT heterozygous. Consequently, this gene could account for as much as 8% of breast cancer in patients younger than age 40 years and 2% of all cases in patients between the ages of 40 and 60.⁹⁰ If this is true, the proportion of all breast cancer accounted for by the AT gene would be greater than that for *BRCA1* and *BRCA2* combined. Thus, although AT may not be an important contributor to the risk of familial breast cancer, it may be a major genetic risk factor for breast cancer in general.

This issue is of particular importance since the AT gene has been cloned.⁹² The gene, located on chromosome 11q22-23, encodes a 12-kb protein that shares close homology with *TEL1*, a gene responsible for telomere length,⁹³ and *MEC1*, a yeast cell cycle checkpoint gene.⁹⁴ Population-based surveys to determine the actual prevalence of AT heterozygosity are under way. If the actual prevalence proves to be as high as has been estimated, determination of whether the epidemiologic association between AT heterozygosity and breast cancer risk is real will be critical. If it is real, population-based screening for AT gene mutations as a breast cancer control measure may warrant consideration.

HRAS1 Alleles.—One kilobase downstream from the *HRAS1* proto-oncogene on chromosome 11p15.5 is the *HRAS1* variable number of tandem repeats (VNTR) polymorphism site.⁹⁵ VNTR are remarkably hyperallelic; it is not uncommon for such a locus to display dozens of alleles. In regard to *HRAS1*, the consensus repeat unit is 28 base pairs long. Four “common” alleles, varying in size from 1,000 to 2,500 base pairs, account for 90% of the alleles at this locus. Some of the rare alleles have been associated with a twofold increase in the risk of breast cancer.⁹⁶ These alleles are only relatively “rare”; they are found in up to 6% of the general population. As a result, up to an estimated 9% of all breast cancer may be attributable to this genetic polymorphism. Like the AT gene, this genetic locus represents a low-penetrance but relatively common genetic abnormality. The mechanism by which this locus influences cancer susceptibility is unknown. Investigators recently suggested that an interaction between the *HRAS1* VNTR locus and *BRCA1* produces a twofold increase in ovarian cancer risk among *BRCA1* mutation carriers.⁹⁷ In that study, breast cancer risk was unaffected by the *HRAS1* polymorphism, but the data suggest that *HRAS1* alleles may modify the expressivity of a major breast cancer susceptibility gene and provide a possible mechanism through which this genetic locus might influence cancer susceptibility. If these epidemiologic associations are confirmed, this genetic locus could prove to be more important than *BRCA1* or *BRCA2* in terms of its etiologic contribution to the total number of cases of breast cancer. Currently, although the concept of common low-penetrance breast cancer susceptibility genes remains provocative and of potential importance, it is unproved.

PREDICTIVE TESTING FOR *BRCA1* AND *BRCA2*

The issue regarding the appropriate time to offer predictive genetic testing for *BRCA1* and *BRCA2* is proving remarkably contentious. Most investigators generally agree that none of the currently available cancer susceptibility tests are appropriate for the screening of asymptomatic persons in the general population, although the population-specific mutations described among Ashkenazi Jews and Icelanders may achieve that status in the future. In assessing persons from high-risk families with well-defined syndromes, the situation is less clear. Most academic, professional, and government organizations that have addressed this issue have concluded that offering *BRCA1* and *BRCA2* testing as a routine clinical service is premature. Dr. Francis Collins,⁹⁸ director of the National Center for Human Genome Research, recently wrote that "the uncertain risks and benefits lead most observers to conclude that testing...should now be done only in a research setting, with a protocol approved by an institutional review board and full informed consent." He cites numerous scientific, technical, socioeconomic, psychosocial, medical, ethical, and legal issues to support his viewpoint that, currently, such testing should be undertaken only as part of a highly structured research-oriented program at a limited number of highly specialized referral centers. The American Society of Clinical Oncology recently published its position paper on this subject.⁹⁹ This statement and the accompanying comments provide an excellent summary of this issue as of the spring of 1996.

The basic elements of informed consent for germline DNA testing are complex and extensive. The following factors must be discussed with patients: (1) information on the specific test being done, (2) implications of a positive or negative test result, (3) possibility that the test will be uninformative, (4) options for risk examination without genetic testing, (5) risk of "passing" a mutation to their children, (6) technical accuracy of the test, (7) fees involved in testing and counseling, (8) risk of psychologic distress, (9) risk of employment and insurance discrimination, (10) need for confidentiality, and (11) options and limitations of medical surveillance and screening after testing. Current problems and limitations inherent in genetic testing include, in part, the following: (1) major technical issues relative to the laboratory procedures used, including sensitivity, specificity, and quality control; (2) inability to ascertain whether a negative test result is a true negative or false-negative result; (3) difficulties in recognizing false-positive results in which missense variations in genetic sequence are found that are not causative of disease (that is, neutral polymorphisms); (4) inability to apply the techniques to small families; (5) existence of multiple breast cancer susceptibility genes, of which *BRCA1* and *BRCA2* are only two; (6) extremely high costs; (7) absence of data correlating the cancer risks associated

with specific *BRCA1* and *BRCA2* mutations, which may vary considerably; (8) limited management options proved to be beneficial for those found to carry mutations; (9) difficulties in communicating uncertain or uninformative test results to patients; and (10) potential problems with insurability and employability for those found to be mutation carriers.

What are the possible benefits for those who undergo predictive genetic testing? The consequences of a negative test result in a family previously unknown to carry a germline mutation have already been described (see section on *BRCA1*). In testing members of a family known to carry a *BRCA1* or *BRCA2* mutation, most persons tested will achieve knowledge of their gene status, and thus pretest ambiguity is eliminated. For those who do not have a mutation, additional potential benefits are as follows: (1) relief from fear of genetic cancer risk, for both themselves and their children; (2) elimination of the need to consider prophylactic breast or ovarian surgical treatment; (3) ability to make choices about exogenous hormone use without concern for genetic interactions; (4) ability to make choices about marriage and childbearing without genetic constraints; and (5) general improvement in their sense of well-being. For those who have a positive test result, one might anticipate the following factors: (1) more accurate quantification of cancer risks; (2) less uncertainty about the potential benefits of a prophylactic operation; (3) ability to elect more intensive, site-specific cancer surveillance; and (4) enhanced motivation to make prudent lifestyle changes that might reduce cancer risk. The absence of proof that factors 2 through 4 are actually beneficial dampens the enthusiasm of many patients and physicians about the utility of predictive genetic testing. These limitations contribute to the note of caution from patient advocacy groups and various organizations of genetics professionals about widespread introduction of genetic testing in light of our current state of knowledge.

In contrast to this cautious approach to the introduction of predictive testing are the commercial interests that hold the patents to the tests to be marketed and that could profit enormously if the tests are widely used. Several such firms are currently in the process of marketing the availability of *BRCA1* and *BRCA2* testing. Fortunately, they are making an effort to ensure that testing is being done as part of a comprehensive genetic testing and counseling program; however, the ultimate responsibility will rest with busy clinicians who are relatively unfamiliar with the subtleties and complexities of predictive genetic testing yet who will find themselves increasingly pressed by their patients to "order the test." On the basis of the data presented, guidelines for referring a patient for specialty assessment for possible genetic testing might include the following: (1) a woman with breast cancer diagnosed before age 30 years; (2) a woman with breast or

ovarian cancer diagnosed before age 50 who has a sister, mother, or daughter with breast or ovarian cancer diagnosed before age 50; (3) an affected woman from a family with two or more cases of breast cancer and one or more cases of ovarian cancer; (4) an unaffected first-degree relative of someone with a known *BRCA1* or *BRCA2* mutation; and (5) an Ashkenazi Jewish woman with breast cancer diagnosed before age 40 or ovarian cancer diagnosed at any age. As previously noted, the subgroup of families most likely to have linkage to *BRCA1* is that with two or more cases of early-onset breast cancer and two or more cases of ovarian cancer; for *BRCA2*, it is families with six or more cases of breast cancer. Such families are rare. The American Society of Clinical Oncology also recommends that, to the greatest extent possible, genetic testing for cancer susceptibility be performed in the setting of long-term outcome studies. Genetic testing should be made available to selected patients as part of the preventive oncologic care of families and only in conjunction with appropriate patient education, informed consent, support, and counseling. It notes that the medical benefit of *BRCA1* carrier identification is "presumed but not established" and emphasizes that the commercial availability of a new genetic test does not ensure that the test is indicated for clinical use.⁹⁹

CONCLUSION

Hereditary breast cancer is real. At least eight genes have been identified that may contribute to inherited breast cancer susceptibility, and others are likely to be found. *BRCA1* and *BRCA2* are currently the most important of these, and predictive genetic testing to identify mutations at these loci is under way. Families in which *BRCA1* and *BRCA2* are functioning are relatively uncommon and probably account for only 5 to 6% of all cases of breast cancer. Specific *BRCA1* and *BRCA2* mutations may be of special importance in selected populations. The relatively rare but highly penetrant genes—for example, *BRCA1*, *BRCA2*, and p53—produce dramatic familial aggregations of breast cancer. The role of low-penetrance but relatively common genetic abnormalities exemplified by AT heterozygosity and the "rare" *HRAS1* VNTR polymorphisms has been less widely recognized but may prove to be of major importance as more is learned about their prevalence and the actual magnitude of their associated breast cancer risk. These genes do not produce multiplex families with breast cancer but may prove to be responsible for a substantial proportion of all breast cancers. The process of predictive genetic testing is complex and probably best done at specialty referral centers until more information is known. Currently, predictive genetic testing is not routine. This is the beginning of an exciting period in genetic oncology, but much remains to be learned. The ultimate outcome of the work now under way will be the

identification of the molecular mechanisms by which inherited breast cancers develop, thereby allowing actual primary prevention to be accomplished as the pathogenic genetic lesions are being repaired.

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