Chapter 17
Inherited Breast Cancer

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Molecular Basis of Disease

Breast Cancer

Breast cancer is the most common cancer among women in Western countries, with about 180,000 new cases and 40,000 deaths occurring annually in the United States. Epidemiologic factors consistently associated with breast cancer risk include a family history of breast cancer, breast biopsy features, and hormonal risk factors such as age at menarche, parity, and age at first live birth. After female gender and age, family history of breast cancer is the most significant risk factor. In a meta-analysis of family history of breast cancer as a risk factor, the relative risk ranged from 1.5 for a second-degree relative to 3.6 for a mother and sister with breast cancer. Relative risks are significantly influenced by the degree of relationship of affected relatives and their age of breast cancer onset, with closer degrees of relationship and younger age of onset conveying higher risks. An analysis of family history as a risk factor using data from the Swedish Family-Cancer Database showed a population-attributable fraction of about 11%.2

Breast cancer is a complex disease, resulting from an incompletely characterized interplay of genetic and environmental factors. In the majority of cases, the level of genetic analysis currently available cannot be used to stratify risk. However about 5% to 10% of breast cancer is hereditary, that is, due to the transmission of highly penetrant mutations in breast cancer-predisposing genes. Within hereditary breast cancer families, mutation status is the overriding risk factor, and genetic analysis can be used to clarify risk and guide medical management in a highly effective way. Genetic risk assessment consists of evaluating the pattern of cancers in the family, judging which of the known hereditary breast cancer syndromes fits the pattern, and pursuing genetic analysis.3

A specific genetic syndrome can be elucidated in about half of hereditary breast cancer families. Additional genes remain to be described.4 Risk-conferring alleles are conceptualized as high-penetration genes with low prevalence (e.g., BRCA1 and BRCA2 [hereditary breast-ovarian cancer (HBOC) syndrome], TP53 [Li-Fraumeni syndrome], PTEN [Cowden syndrome], LKB1[Peutz-Jeghers syndrome]) or low-penetration genes with high prevalence (possibly CHEK2,6 ATM,6 and the TGFBR1*6A allele7). The latter category may have small effects in individuals, but large aggregate effects in populations because they are common.

Using data from the Anglian Breast Cancer Study, Pharoah et al.8 found that the best-fitting genetic model hypothesized that susceptibility to breast cancer is due to several loci, each conferring a modest independent risk. Assuming that all the susceptibility genes could be identified, they showed that the half of the population at highest risk would account for 88% of all affected individuals. Clinical testing for one or a few low-penetration genes at a time would be unsatisfying in many respects. Mutations are found in many individuals but convey small risks, and gene-gene and gene-environment interactions are unknown, limiting clinical utility. Ultimately, whole genome screening might be used in combination with knowledge about such interactions to achieve higher predictive power and allow for efficient breast cancer risk stratification.

BRCA1 and BRCA2

Newman et al. published the first study providing quantitative evidence for an autosomal dominant breast cancer susceptibility allele, accounting for an estimated 4% of breast cancer families and conveying an 82% lifetime risk of breast cancer.9 Following the report by Hall et al. of linkage to chromosome 17q21 for early-onset hereditary breast cancer,10 BRCA1 was isolated using a positional cloning strategy in 1994.11 Subsequently, BRCA2, a second breast-ovarian cancer susceptibility gene, was localized to chromosome 13q12-q13 and cloned.12,13 GenBank, the National Institutes of Health genetic sequence database, lists entries for BRCA1 and BRCA2 as U14680 and U43746, respectively (http://www.ncbi.nlm.nih.gov/Genbank/).
Elucidation of the functions of BRCA1 and BRCA2 has lagged behind the technical capability of carrier detection, delineation of the clinical syndrome, and demonstration of the efficacy of medical management strategies. The manifold functions of BRCA1 and BRCA2 are incompletely characterized. *BRCA1* and *BRCA2* encode very large proteins with 1863 and 3418 amino acids, respectively; each bears little homology to other known proteins or to each other. BRCA1 appears to play a role in numerous cellular functions including transcriptional regulation and influence of estrogen receptor activity, chromatin remodeling, DNA damage repair (homologous recombination and repair of transcription-coupled oxidation-induced DNA damage), centrosome duplication, cell growth, apoptosis, and cell cycle checkpoint control.14 BRCA1 contains an N-terminal RING domain that interacts with BARD1. Two BRCA1 C-terminal (BRCT) domains are present, which are found in proteins involved in DNA repair and control of the cell cycle. *BRCA2* contains eight highly conserved BRC repeats of 30 to 40 residues in exon 11, which bind to RAD51, a key recombinational repair protein. After exposure of cells to DNA damage, BRCA1 relocates from nuclear foci to sites of DNA synthesis and becomes hyperphosphorylated. BARD1, BRCA2, and RAD51 all relocalize with BRCA1.15

### Available Assays

#### Overview of Mutation Types

The *BRCA1* locus spans 100 kilobases (kb), encompasses 22 coding exons, and encodes an open reading frame of 5.5 kb (of 24 exons, exons 1 and 4 are noncoding). *BRCA2* has 26 coding exons and an open reading frame of 10 kb. While the size of these genes presents an enormous challenge to mutational analysis, the problem is compounded by the distribution of mutations throughout the coding regions and introns.16 Interpretation of results is further complicated by the occurrence of numerous polymorphisms of uncertain significance. All types of mutations have been found, including truncating mutations, frameshift mutations, and missense mutations. Furthermore, loss of gene function also occurs through large duplications, deletions, and rearrangements, including promoter deletions. Because the complete genetic characterization of *BRCA1* and *BRCA2* is an ongoing process, the technique(s) selected for mutation detection must be comprehensive in order to provide an accurate clinical result. In general, both genes must be assayed because the *BRCA1* and *BRCA2* clinical syndromes cannot be readily distinguished in individual families.

*BRCA1* and *BRCA2* mutations and polymorphisms are cataloged in the Breast Cancer Information Core (BIC), an open-access online database hosted by the National Human Genome Research Institute (http://research.nhgri.nih.gov/bic/). At the time of this writing, the BIC database contained 6672 entries for *BRCA1*, with 1236 distinct mutations, polymorphisms, and variants, 709 of which had been reported only once. For *BRCA2*, BIC contains 5624 entries, including 1380 distinct mutations, polymorphisms, and variants, 871 of which had been reported only once. Among 7461 consecutive full-sequence analyses of *BRCA1* and *BRCA2* performed at a commercial reference laboratory, 689 (61%) of the mutations identified occurred in *BRCA1* and 440 (39%) in *BRCA2*. Of the 424 different mutations detected, 256 (60%) were frameshifts, 106 (25%) were nonsense, 9 (2.1%) were missense, and 53 (12.5%) occurred in the analyzed regions of introns.16 Genomic rearrangements are discussed below. The technical and financial limitations imposed by the complexity of genetic analysis impacts the availability of clinical genetic testing and the ability to perform genetic epidemiologic studies.

#### Functional and Phenotypic Screening

Given the multifunctional nature of *BRCA1* and *BRCA2*, the development of functional assays amenable to clinical testing is not readily anticipated. An immunoassay based on diminished anticarboxy vs antimano immunoreactivity to *BRCA1* and *BRCA2* mutations has been applied to buccal cells17 and ovarian cancers.18 This assay would be useful for screening truncating mutations, which comprise the majority of mutations, but has not been further characterized.

There is no simple pathologic correlate of the presence of *BRCA1* or *BRCA2* mutations in breast tumors (akin to microsatellite instability for colorectal cancer and mismatch repair gene deficiency) to serve as an effective screen. An immunohistochemical tumor phenotype (typically negative for estrogen receptor, progesterone receptor, and HER2 and positive for TP53) and distinctive morphology (high mitotic count, lymphocytic infiltrate, smooth noninfiltrative pushing borders, and an excess of medullary and atypical medullary types) have been discerned for breast cancers with *BRCA1* mutations.19 However, these features are neither sufficiently sensitive nor specific to reliably predict germline mutation status.

Initial reports on expression profiling in breast tumors have found expression patterns indicative of *BRCA1* loss of function.20,21 If these results can be replicated and if they are widely applicable to a variety of mutation types, then an expression-profiling-based screen for breast tumors could be developed.

#### Sequence Analysis, Gene Scanning Techniques, and Linkage Analysis

A comparison of analytical methods for *BRCA1* and *BRCA2* is presented in Table 17–1. Direct sequence analysis is considered the gold standard test. The BIC Web site provides extensive information on methodologies includ-