

Breast Cancer Genes: Looking for BRACA's Lost Brother

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ABSTRACT: Breast cancer, specifically mammary carcinoma, is the most common cause of death from cancer in women worldwide, with a lifetime risk of one in nine, and its prevalence is increasing. It represents around 30% of all cancer in females and approximately 40,000 deaths in the United States per year. Important advances have been made in detection and treatment, but a significant number of breast cancers are still detected late. This summary of its epidemiology and history, the molecular aspects of detection and the main implicated genes emphasizes the etiology and heterogeneity of the disease. It is still not clear whether the remaining cases of breast cancer negative to *BRCA* are due to mutations in another high penetrance gene or to unknown factors yet to be discovered.

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Recognized risk factors for breast cancer include birth date after 1930 (population studies of immigrants found that immigrants acquire the level of cancer risk of the host country), hormonal influences associated with estrogen exposure (e.g., age at menarche, contraceptive pill usage, pregnancies), dietary habits (e.g., saturated fat intake), and other environmental factors [1]. Important genetic aspects have been indicated by both the occurrence of breast cancer in families and bilateral involvement. Familial clustering adds a relative risk increase: first-degree relatives of affected individuals have a twofold increased risk, which is also influenced by diagnosis at a younger age or the person's family history.

Analysis of the genetics of breast cancer gained momentum more than 15 years ago with the identification of the two main breast cancer-associated genes, *BRCA1* and *BRCA2*. The risk of breast cancer for carriers of *BRCA1* mutation by age 70 years has been estimated to range from 40 to 87%, and for ovarian cancer from 16 to 68% [2]. The risks for *BRCA2* mutation carriers were estimated to be 40–84% for breast cancer and 11–27% for ovarian cancer. For carriers of *BRCA2* mutations, a lifetime breast cancer risk of 60–85% and a lifetime ovarian

cancer risk of 10–20% were cited. Men with germline mutations in *BRCA2*, unlike those with germline mutations in *BRCA1*, had an estimated 6% lifetime risk of breast cancer, a 100-fold increase over the overall male population risk. The majority of multi-case families with both breast and ovarian cancers are due to inherited *BRCA1* mutations, whereas those in families that include male breast cancer cases are more often due to *BRCA2* mutations [3]. Penetrance estimates as low as 36% have been reported in a series of Jewish breast cancer cases selected without regard to family history [4].

HOW EVERYTHING STARTED

Clustering of breast cancer within families is said to have been recorded by the ancient Romans but was not seriously addressed in the medical literature until the 18th century when Le Dran related the experience of a colleague in Avignon who had diagnosed a 19 year old nun with cancer of the right breast. The patient refused a mastectomy due to the expected pain of surgery as well as the belief that the operation would be futile. Her grandmother and a maternal grand-uncle had died from breast cancer and she was convinced that this malady was hereditary and that “her blood was corrupted by a cancerous ferment natural to her family” [5]. In the 19th century the French physician Paul Broca and his contemporary, the London surgeon Sir James Paget, conducted a specific study of the phenomenon and collected a considerable number of illustrative families. Broca's interest in the subject is believed to have been stimulated by his own wife's family history, denoted in his treatise as the descendants of Madame “Z.” Steel cites Broca in 1866 saying: “It is highly unlikely that any explanation other than a major gene effect could account for that pattern of disease over four generations.” Neither Broca nor Paget could possibly have progressed beyond the collection and description of case material. They lacked any knowledge of the principles of inheritance (although G. Mendel was another contemporary) and certainly could not apply mathematical modeling to tease apart the component elements of multifactorial etiology and the role of genetic factors; this remained an enigma until the final decades of the 20th century.

In 1990, after substantial developments in molecular biology and genetics, Hall and co-authors [6] reported a Lod score

We are still looking for BRCA3

of +5.98 associated with the CMM86 locus on chromosome 17 at q21 in breast cancer family pedigrees of early-onset disease [7]. Thereafter, an international effort was launched, using six 17q markers to improve the accuracy of mapping for the breast cancer locus and to establish the characteristics of 17q-linked cancer families.

In April 1993 Easton et al. [8] reported their findings from 214 families, pointing to a gene named *BRCA1* situated about 20 cM centromeric of CMM86 with a substantial number of breast cancer families linked to it. These findings gave enormous impetus to subsequent attempts to clone *BRCA1*. Calculations were also made for the mutation rate on many familial clusters showing an overall frequency of 0.0007, indicating that about 1 woman in 700 can be catalogued as a heterozygous carrier. In most cases the linkage was stronger for families with early age at onset, and there was some evidence that did not support linkage for male breast cancers. Mark Skolnick's group, the University of Utah, and Myriad Genetics independently identified mutations that segregated the disease in affected members of breast cancer kindreds. Some of them were given substantial positive scores for 17q12 markers [5].

BRCA2 was reported for the first time by Wooster and team [9] and was mapped at 13q12-13, proximal to the retinoblastoma gene. The study included 15 families reporting many cases of breast cancer without evidence of linkage to *BRCA1*, and male and ovarian cancers were included in 5 of the families. *BRCA2* was the main implicated gene in these studies with no evidence of linkage to other genes, including *BRCA1*. Risks for female breast, male breast, and ovarian cancers carried a penetrance of 87% by age 80. The gene was cloned for the first time in December 1995 [10].

THE BRACAS

• *BRCA1*

BRCA1 is located on chromosome 17q. It contains 22 exons spanning some 100 kilobases (kb) of genomic DNA and encoding a protein of 1863 amino acids that also encode a 220 kilodalton (kD) nuclear protein with a zinc-binding RING domain at the amino terminus and a conserved acidic carboxyl terminus. *BRCA1* binds to *BRCA2*, p53, RAD51 and many other proteins involved in cell cycling and DNA damage response [11]. The main downstream targets identified so far are p53-responsive genes, including p21. The involvement of *BRCA1* in response to DNA damage is supported by extensive data, including evidence that *BRCA1* is phosphorylated by the mutated ataxia telangiectasia (*ATM*) and checkpoint kinase 2 (*CHK2*) proteins in response to DNA damage [12]. Cells without functional *BRCA1* do not arrest in G2 after DNA damage and are deficient in transcription-coupled repair of DNA double-stranded breaks, but the specificity of cancer risk, mostly limited to

breast and ovarian cancer, has not been explained. *BRCA1* may regulate the G2-M checkpoint by controlling mitotic spindle assembly and thus chromosome segregation. It is also involved in apoptosis, and the effects of its deficiency are cell cycle dependent [13]. *BRCA1* has a RING-finger domain that is likely to be involved in protein interactions that could target proteins for degradation. Mice with homozygously deleted exon 11 of *BRCA1* demonstrate chromosomal instability with aneuploidy and chromosome rearrangements [14].

• *BRCA2*

BRCA2 lies on chromosome 13q and codes for a protein of 3418 amino acids. *BRCA2* is even larger than *BRCA1*, with a 10.3 kb open-reading frame encoding a 384 kD nuclear protein. *BRCA2* bears no obvious homology to any known gene and the protein contains no well-defined functional domains. *BRCA2* binds to *BRCA1* and to RAD51. Binding to RAD51 allows *BRCA2* to reach sites of DNA breaks, indicating involvement of *BRCA2* as well in recombination-mediated repair of double-stranded breaks and the maintenance of chromosome integrity. *BRCA2* mutations are also associated with an increase in colon, prostate, pancreatic, gallbladder, bile duct and stomach cancer as well as malignant melanoma. *BRCA2* mutants are deficient in spindle assembly checkpoints [15].

Both genes encode proteins involved in double-stranded DNA repair, specifically in homologous recombination, a highly specific method of error-free DNA repair. Both *BRCA1* and *BRCA2* contain BRCT repeats and both interact with RAD51 – this interaction being along most of the *BRCA2* protein, but along only 5% of *BRCA1* [16]. In cells with deficient *BRCA1* and *BRCA2* proteins, DNA repair proceeds by the more error-prone alternative repair pathways of non-homologous end-joining. Transcriptional regulation may also be a function of *BRCA1* or *BRCA2* by interaction with RNA Pol II and RNA helicase A. (Both proteins are normally nuclear and their mRNAs are preferentially expressed during the late G1–early S phase of the cell cycle.) Punctate foci of *BRCA1*, *BRCA2* and RAD51 may be detected in the nucleus in the S phase, and in meiotic cells they associate with unsynapsed regions of synaptonemal complexes. None of the functions of *BRCA1/2* appear to be specific to breast tissue. The reason for the tissue specificity in cancer susceptibility is unclear, although the obvious involvement of estrogen-target organs makes it likely that the secondary effects of gene mutation are enhanced by a responsive tissue environment.

MUTATION ANALYSIS

Deleterious mutations are found throughout both genes. Mutations in *BRCA1* and *BRCA2* have a prevalence of 0.11% in the general UK population and account for approximately 60% of cases of familial breast cancer. To date, the mutations reported are at different positions and include frameshifts, missense,

In view of the heterogeneity of breast cancer, screening should be mandatory for all

nonsense, base substitutions and non-transcription of one allele (presumed to be due to a control region mutation). They may be sequenced directly or tested for the presence of a mutation by an indirect approach. More than two decades ago such testing was made by single-strand conformational polymorphism (SSCP), heteroduplex analysis, or mismatch cleavage. SSCP was widely favored because of its simplicity, but with the handicap of being insensitive to single base substitutions. Nevertheless, it has been substituted by denaturing high performance liquid chromatography (DHPLC) [17], which uses the same principle for detecting heteroduplexes according to the way they migrate in a medium. Methods of determining their pathogenicity included the use of reverse-transcriptase polymerase chain reaction (RT-PCR) and analysis of gene transcripts. Even protein truncation assays proved to be highly appropriate for *BRCA1*. Today, the use of multiplex ligation-dependent probe amplification (MLPA) and exome sequencing is used routinely for genotyping and detection of deletions and duplications. With the availability of the human genome sequence it is possible to approach cancer genes using high throughput analysis of changes in tumors. Also, for the entire cytogenetic age, strategies have been reported that can detect gains and losses of genetic material as well as small intragenic mutations that are essential to cancer gene identification. Currently, the capacity to analyze thousands of single nucleotide polymorphisms (SNPs) and nucleotide sequences through the entire genome is moving faster with new techniques using systems other than gel-based, such as next-generation sequencing and microarray technologies which utilize the high throughput method while maintaining specificity and sensitivity. The Cancer Genome Project at the Sanger Centre in the UK is now identifying cancer-related genes based on homozygous deletions and panels of thousands of cell lines as well as mutation analyses in genes across the human genome. Breast cancer tumors containing germline mutations provide lists of cancer-related genes to be screened through family trees and are used in large case-control studies to define the basis of cancer susceptibility due to mutations in low, moderate and high penetrance genes [Table 1] [18].

Microarray-based comparative genomic hybridization (CGH) to cDNA arrays revealed specific somatic genetic alterations within the BRCAX subgroups. These findings illustrated that when gene expression-based classifications are used, BRCAX families can be grouped into homogeneous subsets, thereby potentially increasing the power of conventional genetic analysis. One such approach being developed is the stratification of breast cancer families based on a molecular profile of the associated tumors. The first example of this approach showed that transcriptional profiling using cDNA arrays can separate sporadic tumors from those found in women with *BRCA1* mutations. Three common founder mutations – 187delAG, 5385insC and 6174delT – are present in *BRCA1* and

Research on the genetic etiology of breast cancer will continue

Table 1. Genes with germline mutations conferring high, intermediate or low risk for breast cancer [2,18]

Gene	Protein	Function
High penetrance <i>BRCA1</i> <i>BRCA2</i> <i>TP53</i>	Breast cancer gene 1 Breast cancer gene 2 Tumor protein 53	DNA repair DNA repair DNA repair
Intermediate penetrance <i>CHEK2-1100delC</i> <i>ATM</i> <i>BRIP1</i> <i>PALB2</i>	Check point kinase 2 Ataxia telangiectasia mutated gene <i>BRCA1</i> -interacting protein Partner and localizer of <i>BRCA2</i>	DNA repair DNA repair DNA repair DNA repair
Low penetrance <i>CASP</i> <i>TGF-β1</i> <i>FGFR2</i> <i>MAP3K1</i> <i>LSP1</i> <i>TNRC9</i> Locus on 2q35	Caspase cysteine protease Transforming growth factor beta type 1 Fibroblast growth factor receptor type 2 Serine/threonine kinase Cytoskeletal protein Chromatin protein Unknown	Apoptosis Cell growth/signaling Cell growth/signaling Cell growth/signaling F-actin bundling Transcription factor? unknown
Other <i>PTEN</i> <i>STK11</i> <i>CDH1</i> <i>NF1</i>	Phosphatase and tensin homolog Serine/threonine kinase 11 Cadherin-1 Neurofibromin 1	Tumor suppressor Tumor suppressor Tumor suppressor Tumor suppressor

Table 2. Mutations in *BRCA1* and *BRCA2* [20]

Mutation type	<i>BRCA1</i>	<i>BRCA2</i>
Missense/nonsense	292	162
Splicing	72	39
Regulatory	0	0
Small deletions	261	246
Small insertions	88	90
Small indels	11	11
Gross deletions	47	10
Gross insertions	9	4
Complex rearrangements	8	2
Repeat variations	0	0
Public total (HGMD Professional 7.3 total)	788 (1004)	564 (735)

BRCA2 in the Ashkenazi Jewish group, which overall occur in up to 2% of this ancestry. The Ashkenazi founder mutation 6174delT seems to be an example of a low penetrance breast cancer mutation, with estimates as low as 28%. Larger genomic deletions/duplications occur often in *BRCA1* compared to *BRCA2*, and some are founder mutations.

Similarly, a common *BRCA1* mutation, 2800delAA, is found mainly in Northern Ireland and on the west coast of Scotland, whereas the *BRCA2* 6503delTT mutation is found on the east coast of Scotland, suggesting that it may have been introduced by Viking raiders or Scandinavian fishermen [Table 2] [19,20]. Preservation of a common haplotype has been reported. It

appeared that common *BRCA1* mutations in different families were often accompanied by a number of polymorphic 17q12 markers and seem to be from widely separated geographic regions and communities. This not only suggests founder effects rather than mutational “hot spots” as the basis for the recurrent mutations, but also points to a high degree of genetic stability at this locus, with a relatively low rate of new mutations. Some of the mutations in *BRCA1* involve putative regulatory sequences and are demonstrated through loss of heterozygosity at the cDNA level. Where the individual is constitutionally heterozygous for one or more polymorphic markers within *BRCA1* exons but homozygous for these markers in lymphocyte-derived cDNA, it can be concluded that one of the alleles has been rendered transcriptionally silent. The identification of four highly polymorphic intragenic microsatellite markers – D17S855, D17S754, D17S975 and D17S1323 – has greatly facilitated *BRCA1* allele imbalance studies.

DISCUSSION

Even when 67% of families with 4 or 5 cases of breast cancer were found to be not linked to *BRCA1*, *BRCA2* or other common susceptibility alleles, only 15% of the excess risk for breast cancer in first-degree relatives was attributable to mutations in *BRCA1* and *BRCA2*. Family history remains a predictive factor for breast cancer risk in women without *BRCA1* or *BRCA2* mutations [21].

So, what is responsible? A high penetrance gene, low penetrance susceptibility genes or alleles? Or perhaps the main perpetrator is not utterly genetic. Cumulative evidence indicates that there are common elements of risk in the population that are shared between women with breast cancer and their relatives. Positional cloning efforts are greatly facilitated by the detailed sequence information now available from the human genome. The set of polymorphic genetic markers is an essential resource for initial gene localization on denser maps, and fine mapping has become available through the Single Nucleotide Polymorphism (SNP) Consortium. Researchers have utilized a variety of other approaches, including sib-pair analysis and a combination of linkage and tumor expression profiling to identify families with the same genetic phenotype [22]. One of the implicit problems in isolating low penetrance genes is that such genes will rarely produce multiple-case families that can be used in traditional linkage analysis, being unlikely to travel with breast cancer in a conventional scheme. Alternatively, large population-based case-control studies are expensive and time consuming. A possible approach might be to evaluate candidate low penetrance susceptibility genes as modifiers of high penetrance genes. Lower penetrance genes can be difficult to identify because they are selected on the basis of biological plausibility; in fact, candidate genes from many cellular pathways have been investigated using this approach.

By 2002, a susceptibility locus in 13q22 gained support for being the hunted *BRCA3*, and consensus was found later by the HUGO Gene Nomenclature Committee (HGNC) [23]. Nevertheless, genetic testing and information concerning the putative sought-after gene is still scarce and limited. So, is this the gene we are really looking for? Or perhaps a gene with similar penetrance as *BRCA1* and *BRCA2* does not exist.

Since TP53 was identified in 1990 as the cause of Li-Fraumeni syndrome [24] (characterized by early-onset breast cancer in association with other childhood cancers, in particular sarcomas and brain tumors), many other genes have been implicated as risk factors for the genetics of breast cancer, as follows. Proto-oncogen HRAS, where individuals with rare alleles (population frequency < 5%) have an increased risk of breast cancer [25]. Androgen receptor genes are suspicious susceptibility alleles for male breast cancer [26]. E-cadherin, mapped on 16q, is a possible candidate for the breast cancer susceptibility gene [27]. PTEN, the cause of Cowden syndrome, is associated with a 20–30% lifetime risk of breast cancer [28]. Mutations in *LKB1* cause Peutz-Jegher’s syndrome, associated with a relative risk for breast cancer of 20.3 compared with non-carriers [29]. Some polymorphisms calculated from large case-control studies confer small relative risks of breast cancer: V1508M in the *COMT* gene, 1462V in *CYP1A1*, *CYP1B13* (different estrogen metabolism genes), and *TGF-β C-509T* [30]. In the human leukocyte antigen (HLA) region, there may be a potential role for the HLA class III sub-region in susceptibility to breast cancer in patients at moderate familial risk [31]. Regarding *BARD1*, cys557-to-ser substitution in this gene is common and a predisposing factor for allele breast cancer [32]. Steroid hormone metabolism genes are associated with a high dominance inherited breast cancer risk, as is the germline R239X mutation in the *CYP17A1* gene [33]. Alterations in regulatory genes of miRNAs are highly prevalent in cancer [34]. *PALB2* is linked to breast cancer susceptibility and the predisposition gene [35]; and plausible genes identified with SNPs – *FGFR2* and *TNRC9* on chromosome 16q – were independently identified in two of the three random genome-wide association studies (GWAS). *MAP3K1* on chromosome 5, *LSP1* on chromosome 11p15.5 and another SNP, rs13281615, on chromosome 18q were not located in any known gene [36]. All these genes have susceptibility alleles that are very common in the UK population which thus shows a small increased disease risk. In combination, the SNPs may become clinically significant; in total, more than 20 genes are involved to date.

The frequency of breast cancer and its partial occurrence, even in positive family trees, plus an increased incidence related to changes in dietary and reproductive patterns have made it necessary to create specific algorithms to allow a closer approach regarding risk assessment. Risk estimation models have been proposed since the early 1990s. Generally, the models consider the strongest risk factors and incorporate them into

an algorithm that combines adjustments for secondary factors, depending on the model applied. Among the more important risks considered are family history, personal information such as age and body mass index, hormonal and reproductive patterns, personal breast diseases, genotype regarding whether or not one or more risk alleles of different penetrance is being carried, and ovarian cancer. Some of the models widely used in the clinic are the Gail model, as well as Claus, BRCAPRO, Cuzick-Tyrer, and Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) [37]. Nevertheless, it is still difficult to differentiate with full accuracy between the family with a mutation and those who will develop breast cancer, regardless of the sum of positive and negative influences. Concerning the search for specific environmental factors such as widespread pollutants (organochlorine pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, etc.), to date none of these factors has been identified as directly responsible. Additionally, the U.S. National Toxicology Program has listed six substances that may cause breast cancer in humans: diethylstilbestrol, steroidal estrogens, X-ray and gamma radiation, alcoholic beverages, tobacco smoking, and ethylene oxide [38]. Still, more research is needed to pinpoint the causes that impact significantly on this specific susceptibility.

After GWAS, more focused research was initiated regarding possible gene variants and SNPs. These were gathered using specific parameters of selection from large consortia like the Breast Cancer Association Consortium (BCAC). One of them, the Large-Scale Collaborative Oncological Gene-Environment Study (COGS), is a massive study that includes more than 200,000 individuals, over 211,000 SNPs and methylation quantitative trait locus (QTL) analyses to characterize the genetic and environmental bases of breast, ovarian and prostate cancers. Generally speaking, the findings include more than 70 new susceptibility loci for those types of cancer, including estrogen receptor negative (ER)-, *BRCA1/2* mutation carriers of European and East Asian ancestry, fine mapping of the TERT region and *CCND1* expression related to risk.

An addendum of environmental risk factors, specifically alcohol consumption and parity, has been confirmed. Interestingly, COGS GWAS data showed single genomic regions comprising different variants and influencing pleiotropically for distinct histological subtypes or sites, especially when sharing hormonal influence. Examples include *MDM4*, *TET2* and *RAD51B* for breast and prostate cancer, and 8q24, 10p12 and 19q13(MERIT40) for cancer of the ovary and breast at 17q12, thus increasing the proportion of familial risk to 28% when sharing the implicated genetic loci. This demonstrates once again the utility of large-scale scientific collaboration and cross-cutting mega-arrays in deciphering what we know about cancer, now recognized as a complex disease, and helping to develop more efficient prediction models [39].

CONCLUSION

Breast cancer results from genetic and environmental factors leading to the accumulation of mutations in essential genes. Germline mutations in *BRCA1* and *BRCA2* and many other rare variants account for 20–28% of breast cancer that clusters in families and less than 8% of breast cancer overall. Genetic predisposition may have a strong effect or it may represent the cumulative effects of multiple low penetrance susceptibility alleles. Segregation analysis in familial breast cancer to find the best-fitting model showed that the majority of familial cases could be accounted for within the BRCA family, with a polygenic cause for the remaining cases. Finally, the question remains: is there evidence for a putative high risk susceptibility gene – “*BRCA3*”?

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