Familial breast cancer: recent advances

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The majority of breast cancer cases are so called sporadic cancers, which do not have a strong genetic component. However, approximately 27% of breast cancer cases are inherited or familial cancers that result from inheriting pathogenic mutations in specific genes. Two of these genes, namely *BRCA1* (BReast CAncer 1) and *BRCA2*, confer a high risk for breast cancer and are known as high penetrance genes. At least 12 more genes associated with breast cancer risk have been identified so far. However, these genes are low penetrance genes that carry a much lower risk for breast cancer than *BRCA1* and *BRCA2*. Four of these low penetrance genes have been identified recently by a large research consortium led by a research group from Cambridge University. It is likely that future research will uncover additional low penetrance breast cancer predisposing genes.

Introduction

The first indication that cancer may be a genetic disease was provided by the influential German scientist, Theodor Boveri, in his celebrated work "Zur Frage der Entstehung Maligner Tumoren" ("The Origin of Malignant Tumours") published in 1914 (1). Although the concept of a gene was unknown at that time, Boveri correctly assumed that chromosomes were carriers of genetic information. His insightful observations of chromosomal abnormalities in tumor cells enabled him to propose that predisposition to cancer may be inherited by inheriting a copy of a chromosome (gene) that is not able to suppress tumor growth efficiently (1).

More recent studies have largely confirmed Boveri's prediction of genetic predisposition to cancer (2). For example, in 1971 Knudson proposed a two-step mutation model of cancer (3). The model is based on

the assumptions that (a) most cancers develop from a single cell and (b) at least two mutational events are required. The model predicts that all cancers can be classified into two categories: 1. sporadic cancers arising as a result of chance (caused by a combination of genetic and environmental factors) and 2. inherited or familial cancers (caused by genetic factors). Both categories involve the same genetic changes or mutations, but the difference between them is in the timing of the mutation's occurrence. In the case of familial cancers individuals are born with the first mutation and all cells in their body carry this mutation. The second mutation is acquired after birth in a relevant somatic cell of these individuals. By contrast, in the case of sporadic cancers both mutations are acquired after birth in somatic cells. Therefore, the chances of two mutations occurring independently in the same somatic cell during the lifetime of an individual (sporadic cancer) are significantly lower than a single mutation occurring in a somatic cell in which another mutation already exists (familial cancer). According to this model, familial cancers should affect younger individuals and can be found at multiple sites in their bodies, whereas sporadic cancers should be confined to older individuals and should appear only at a single site in their bodies. Numerous studies have shown that the Knudson model, which stems from Boveri's early predictions, is applicable to childhood cancers, but also to adult cancers, including breast cancer.

The focus of this article is the familial form of breast cancer. In order to fully appreciate the differences between sporadic and familial forms of breast cancer, basic epidemiological facts will be briefly presented, followed by a more focused description of the molecular mechanisms underlying the familial form of breast cancer. Significant advances in the current understanding of these mechanisms have recently been made (4) and these advances will be briefly described.

Basic facts about breast cancer

According to US estimates, approximately 12% of women will suffer from breast cancer (Fig. 1). Most breast cancers are sporadic and occur with a frequency of approximately 73% (Fig. 1). Sporadic breast cancers are characterized by the lack of family history of disease i.e. no two first-degree relatives in a family are affected. Sporadic breast cancers rarely affect younger women and the cases of the bilateral form of disease (affects both breasts) are rare.

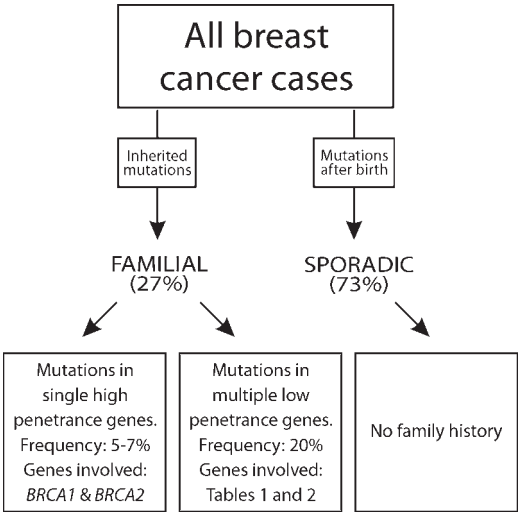

Figure 1. Frequencies of sporadic and familial cancers according to US estimates (for more details see: http://envirocancer.cornell.edu/FactSheet/General/fs48.inheritance.cfm)

The remaining 27% cases of breast cancer are familial cancers (Fig. 1). This type of breast cancer is characterized by a clear family history of disease, which increases a woman's chance of having the disease up to 2-3 times. In addition, patients with familial breast cancer are significantly younger at the time of diagnosis and have a higher frequency of bilateral disease in comparison with their sporadic breast cancer counterparts.

Apart from these epidemiological differences between the two forms of breast

cancer, there are no other obvious differences that affect the clinical and pathological presentation of the disease (5). This is in line with the Knudson's two-step mutation model predicting the same genetic changes or mutations in sporadic and familial cancers. Given that the same genetic events occur in both types of cancer, the underlying molecular mechanism should be the same, thus leading to similar clinical and pathological presentations.

How was the familial form of cancer identified? Researchers realized very early that some breast cancers tend to cluster in families, i.e. multiple first-degree relatives (mother-daughter; sisters) were affected. This suggested a heritable component of the disease. The usual research strategy in such cases is to construct a family tree or a pedigree, collect blood samples from as many family members as possible and subject these samples to genetic investigation. In the early 1990's, genetic analyses of multiple breast cancer families revealed a common gene located on chromosome 17q that was affected (mutated) in some but not all families. This gene was named *BRCA1* (*BReast CAncer gene 1*). After intensive competition between several American and European laboratories, the gene was identified in 1994 (6). Another breast cancer predisposing gene was identified soon after that and named *BRCA2* (7). *BRCA1/2* genes are affected (mutated) in only ~25% of familial breast cancer cases, suggesting involvement of other genes in this form of disease.

BRCA1/2 genes are called "highly penetrant" genes. In medical genetics penetrance is defined as the ability of a gene to express itself, resulting in specific symptoms. Highly penetrant genes will always cause symptoms, irrespective of the environmental effects. By contrast, low penetrance genes produce symptoms only sometimes and can be affected by environmental factors. Therefore, women born with *BRCA1/2* mutations have a high chance of suffering from breast cancer during their lifetime.

Numerous studies have failed to identify another highly penetrant *BRCA* gene, but have instead identified several low penetrance genes involved in familial breast cancer. The full list of low penetrance genes identified by early 2007 (reviewed in reference 8) is shown in Table 1. There are some epidemiological differences between high and low penetrance genes. High penetrance genes, such as *BRCA1/2,* affect many family members and greatly increase breast cancer risk. By contrast, low penetrance genes do not affect as many family members as high penetrance genes and they confer lower cancer risk than high penetrance genes. *BRCA1/2* and the low penetrance genes shown in Table 1 cause approximately 50% of familial breast cancer cases, suggesting that additional low penetrance genes must be involved in this form of disease.

A new study, published in the prestigious journal "Nature" (4), identified several new low penetrance genes using an approach based on recent advances in dissecting the human genome. This approach and the genes identified will be described below. Before that, some basic facts about *BRCA1/2* and low penetrance genes shown in Table 1 will be briefly presented.

Functions of genes involved in familial breast cancer

BRCA1/2 are genes involved in DNA damage response. DNA damage response is the network of cellular mechanisms activated when cells or organisms are exposed to genotoxic stress. These mechanisms typically recognize DNA damage, signal the presence of DNA damage to other relevant molecules, block the cell cycle to allow cells to repair the damage and finally repair the damage via numerous DNA repair pathways. *BRCA1/2* genes are involved in the repair of DNA

Table 1 Familial breast cancer genes as of January/February 2007 according to reference number 8

Gene	Penetrance	Function	Association with other diseases
BRCA1	High	DNA damage response	-
BRCA2	High	DNA damage response	Fanconi anemia
PALB2	Low	DNA damage response	Fanconi anemia
BRIP1	Low	DNA damage response	Fanconi anemia
ATM	Low	DNA damage response	Ataxia telangiectasia
NBS1	Low	DNA damage response	Nijmegen breakage syndrome
RAD50	Low	DNA damage response	-
CHEK2	Low	DNA damage response	-
P53	Low	Tumor suppressor	Li-Fraumeni syndrome
PTEN	Low	Tumor suppressor	Thyroid and colon cancer

double strand breaks through interactions with DNA repair proteins such as Rad51, or interactions with DNA damage signalling molecules such as ATM. The importance of *BRCA1/2* genes in DNA damage response is reflected in the fact that cells defective in these genes show high levels of spontaneous chromosomal abnormalities (9).

It is interesting that *BRCA2* has recently been linked to a human disease – Fanconi anemia (FA). FA is a complex genetic disease, with at least 12 genes known to be involved. The key molecular feature of FA is inability of cells from patients to cope with genotoxic stress, especially the stress caused by the so-called DNA cross-linking agents, such as MMC (mitomycin C) and DEB (diepoxybutane). Sensitivity of FA cells to MMC and DEB suggests inherited failure in DNA damage response in FA patients. All known FA genes are involved in different aspects of cellular DNA damage response, activated as a result of exposure to DNA cross-linking agents. One of the genes, named *FANCD1*, has been identified as *BRCA2* (reviewed in reference 8). Further investigation revealed interaction between the BRCA2 protein and a protein called PALB2 (Partner And Localizer of BRCA 2). Some breast cancer families show mutations in the *PALB2* gene, suggesting that this is a low penetrance breast cancer predisposing gene (8). Another gene involved in FA, called *BRIP1*, was also classified as a low penetrance breast cancer predisposing gene (8). Taken together, these findings demonstrate an interesting link between two human genetic diseases (FA and familial breast cancer) but also reflect the importance of DNA damage response genes in both diseases.

Several more DNA damage response genes have been identified as low penetrance breast cancer predisposing genes. These include: *ATM*, *NBS1*, *CHEK2* and *RAD50* (8). ATM is a DNA damage signalling gene, affected in patients suffering from Ataxia telangiectasia (AT). The major symptom of AT is the sensitivity of patients to ionizing radiation and predisposition to cancer. *NBS1* is a gene affected in a human disease called Nijmegen breakage syndrome (NBS). Symptoms of this disease are very similar to AT. *CHEK2* is a gene encoding a kinase enzyme involved in DNA damage response. *RAD50* is involved in the repair of DNA double strand breaks through homologous recombination. In addition to these 6 low penetrance genes involved in DNA damage response, two more genes have been implicated in familial breast cancer. They are both tumor suppressor genes: *p53*, the function of which is to block cell cycle progression after genotoxic stress and *PTEN*, a gene that increases risk for breast, colon and thyroid cancer (8).

New low penetrance breast cancer predisposing genes

Given that *BRCA1/2* and low penetrance genes shown in Table 1 account for 50% of familial breast cancer cases, it is necessary to identify the remaining 50% genes involved in this disease. Classical genetic analysis, which relies on tracing patterns of inheritance in high-risk families, by using genetic markers inherited together with the disease gene, is limited in the case of low penetrance genes. A potential solution may be large genetic association studies. These studies analyze single genes in a group of people with a particular disease, relative to a group of healthy control people. The power of association studies can be enhanced by applying methods capable of simultaneously analyzing large number of genes/DNA sequences. Such methods have been developed or result directly from the human genome project (HGP).

One of the greatest achievements of the HGP was the identification of genetic variations, or variations in the DNA sequence, between individuals. The DNA sequence is determined by the distribution of four chemical building blocks of DNA, or DNA bases: adenine (A), thymine (T), guanine (G) and cytosine (C). The human genome, defined as the total human DNA sequence, contains three billion of these DNA bases. The DNA sequence in each individual is 99.9% the same. The remaining 0.01% of the human DNA sequence, or 1 in 1200 DNA bases, is different in each individual.

Let us assume a hypothetical DNA sequence on a human chromosome 16 to be: AGGTTCAGATCCT. This sequence is the same in all people, except for the underlined third base G, which can differ in different individuals. One person may have G on that position, whereas others may have A, T, or C bases at the same position. These DNA bases, at specific genomic locations, that differ between individuals, are called single nucleotide polymorphisms (SNPs; pronounced snips). SNPs constitute, by far, the greatest type of genetic variation and it has been estimated that the total number of common SNPs in the human genome is at least 10 million. Geneticists can use SNPs as markers to locate genes in DNA sequences. Let us assume that the SNP above (G in third position) could be associated with a risk for familial breast cancer. In order to test if this assumption is true, we can simply compare a large number of familial breast cancer cases with the same number of healthy individuals, and if we find that G dominates in breast cancer cases and T, or A, or C in healthy individuals, we can say that G, at that particular position, is definitely associated with breast cancer. Using the knowledge generated through HGP, we can then identify the genomic locus carrying the sequence with the above SNP. The identified locus may be within a specific gene, or in the vicinity of a specific gene. That particular gene is now assumed to be associated with breast cancer.

A scenario roughly similar to the above has been recently used to identify several new low penetrance genes, affected in familial breast cancer. A large research consortium, led by a research group from Cambridge University, used the most modern technology to determine or type a large number of SNPs in breast cancer families and healthy individuals, in a three-stage study (4). In the first stage, a panel of 266 722 SNPs were typed in 408 breast cancer cases and 400 healthy individuals. Statistical analysis revealed some SNPs associated with breast cancer cases. In the second stage, approximately 5% of the most significant SNPs (~12 000) were selected and typed in 3990 invasive breast cancers and 3916 control individuals. In the third stage, the 30 most significant SNPs were typed in ~ 22 000 breast cancer cases and a similar number of control individuals. This analysis identified 5 new loci exhibiting a significant association

Table 2 Recently identified (May 2007) low penetrance genes that confer breast cancer risk according to reference 4

Gene	Abbreviation	Function	Chromosome position
FGFR2	Fibroblast growth factor receptor 2	Receptor tyrosine kinase; Tumor suppressor	10q25.3-q26
TNRC9	Trinucleotide repeat containing 9	Transcription factor	16q12.1
MAP3K1	Mitogen activated protein 3 kinase 1	Kinase enzyme	5q11.2
LSP1	Lymphocyte specific protein 1	Regulates leukocyte recruitment to inflamed sites	11p15.5

with breast cancer. Four of these loci contain known genes including: *FGFR2*, *TNRC9*, *MAP3K1* and *LSP1*, which are now assumed to be low penetrance breast cancer genes (Table 2). The gene in the fifth locus has not yet been identified. The only gene that has previously been associated with breast cancer risk is *FGFR2*. This is a receptor tyrosine kinase which is amplified in 5-10% of breast tumors. The remaining three genes, *TNRC9*, *MAP3K1* and *LSP1*, have not been associated with breast cancer risk before.

This study is significant for several reasons. First, the four genes identified are not associated with DNA damage response (see Table 2 for their functions). This is in contrast to *BRCA1/2* and low penetrance genes from Table 1, almost all of which are involved in DNA damage response. This indicates a variety of molecular or genetic pathways that contribute to breast cancer. Second, the study adds further weight to the notion that breast cancer is essentially a polygenic disease in which each inherited gene adds a specific value of risk for breast cancer. Third, the study demonstrates the emerging power of a methodology resulting directly from HGP. It is now possible to apply the same approach to search for genes involved in other types of cancer or any other human disease.

Concluding remarks

Breast cancer is one of the best understood forms of human cancer at molecular and genetic levels. There are at least 14 genes known to be associated with breast cancer risk, two of which are high penetrance genes and the rest are low penetrance genes (Tables 1 and 2). Therefore, familial breast cancer is a polygenic disease (a disease caused by multiple genes). It remains unknown how many more low penetrance breast cancer predisposing genes exist, but it seems likely that future research will uncover new genes. The greatest breast cancer risk is conferred by high penetrance genes *BRCA1/2*. Low penetrance genes confer a much lower risk, which can increase by inheriting more than one of these genes.

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