

# Breast Cancer Susceptibility and the DNA Damage Response

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## Introduction

Breast cancer is a disease caused by a complex combination of genetic and environmental factors. It is one of the most common types of cancer affecting women in the Western world. In 2004 in the United States, it is estimated that more than 200,000 new cases of breast cancer will be diagnosed and over 40,000 will die of this disease. Linkage analysis of families with a high risk of breast cancer has identified two major susceptibility genes: *BRCA1* and *BRCA2*.<sup>1,2</sup> In the context of large, multiple-case families, the *BRCA1* and *BRCA2* genes are numerically the most important, accounting for more than 80% of families with six or more cases of both early-onset breast cancer and ovarian cancer.<sup>3</sup> However, the probability of harboring a mutation is much lower in families with fewer cases of the disease, and population studies have demonstrated that these genes account for only a minority of the overall familial risk of breast cancer. In fact, as many as 60% of families with site-specific female breast cancer cannot be explained by mutations in *BRCA1* and *BRCA2*.<sup>4,5</sup> In addition, mutations in these genes are relatively rare in the general population. Together they account for less than 10% of all breast cancer cases<sup>4,6</sup> (Fig 1). Therefore, the challenge is how to identify individuals at risk for the remaining cases. Conceivably, if we could identify the major genetic factors that contribute to breast cancer risk, we would be able to not only provide comprehensive early identification of individuals at risk but also tailor prevention and treatment regimens to adequately address specific molecular changes in these cancers.

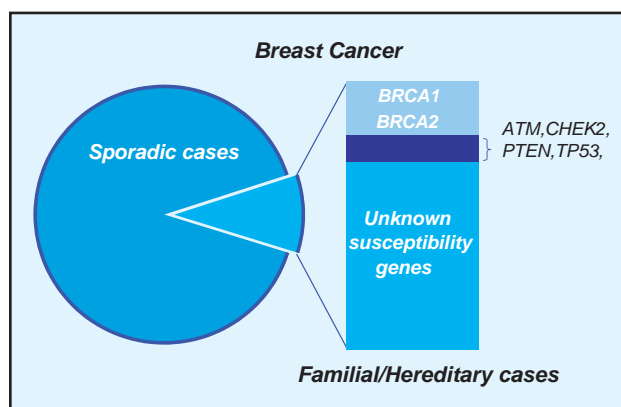


Fig 1. — Breast cancer susceptibility genes. Hereditary breast cancer (right) constitutes only approximately 5% to 10% of all breast cancer cases (left). Germline mutations in the two major susceptibility genes *BRCA1* and *BRCA2* account for less than 5% of all breast cancer cases, while mutations in genes such as *ATM*, *CHEK2*, *PTEN*, and *TP53* account for only about 1% of all breast cancer cases. The genetic factors underlying sporadic breast cancer cases are largely unknown.

*BRCA1* and *BRCA2* were identified and isolated by linkage analysis and positional cloning,<sup>1,2</sup> a strategy that works well for highly penetrant genes. To date, few additional candidate breast cancer susceptibility loci have been identified in families not attributable to any of the known genes. Recently, Kainu et al<sup>7</sup> reported evidence for a novel breast cancer susceptibility locus on chromosome 13q21. However, posterior studies concluded that if a susceptibility gene does exist at this locus, it would account for only a small proportion of non-*BRCA1/2* families with multiple cases of early-onset breast cancer.<sup>8</sup> These findings illustrate the difficulties inherent in efforts to identify additional susceptibility genes for a highly prevalent disease and suggest that the traditional linkage approach may have reached its limit. Indeed, if current models are correct, the remaining predisposition genes are likely to have lower penetrance or be part of a polygenic effect and therefore difficult to isolate by linkage.<sup>9</sup> Some of the candidate low-penetrance genes have been proposed to be proto-oncogenes, genes involved in metabolic, estrogen, and immunomodulatory pathways.<sup>10,11</sup> In particular, genes in hormonal metabolism pathways have received increased attention, but research on the impact of these genes on breast cancer risk is still at an early stage.

In the 10 years since the cloning of the first breast cancer susceptibility gene, *BRCA1*, our knowledge of

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Submitted September 5, 2004; accepted February 8, 2005.

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This work is supported by NIH award CA92309. No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article. Vesna Dapic is a DoD postdoctoral fellow (DAMD17-01-1-0403).

**Abbreviations used in this paper:** AT = ataxia telangiectasia, DSB = double-strand break, HR = homologous recombination.

breast cancer has significantly improved.<sup>1,12</sup> It is possible that understanding the function of the genes identified so far may allow us to make better informed choices of candidate genes to be studied. Besides *BRCA1* and *BRCA2*, several other genes whose inactivation predisposes to breast cancer have been identified such as *ATM*, *TP53*, *CHEK2*, and *PTEN*. Although many of these genes are associated with rare hereditary diseases such as Li-Fraumeni syndrome (*TP53* and *CHEK2*), Cowden disease (*PTEN*), and ataxia telangiectasia (*ATM*) and therefore are unlikely to be major contributors to risk in the general population, they highlight a common characteristic: several play a role in the cellular response to DNA damage (Fig 2). In a simplistic view, one could consider the DNA damage response as composed of processes sensing and signaling the presence of damage and processes involved in the actual repair of the DNA strands. In this view, the known breast cancer predisposition genes seem to be involved in sensing and signaling damage rather than being directly involved in DNA repair.

Damage of genomic DNA occurs spontaneously and constantly throughout the life of an organism and can be further enhanced by exogenous DNA damaging factors. Therefore, an efficient response to DNA damage is essential for cellular life. Spontaneous DNA damage results from errors in fundamental cellular processes such as DNA replication. Exogenous DNA damage factors include environmental pollution, ionizing radiation, ultraviolet rays, and chemotherapeutic drugs. The most detrimental form of DNA damage is chromosomal double-strand break (DSB), which is lethal to the cell if not repaired. DNA DSB can be induced by ionizing radiation, DNA replication errors, or cell oxidative metabolism. Two major pathways

for the repair of DSBs in mammalian cells include homologous recombination (HR) repair, which essentially provides an error-free repair by using a homologous template (the homologous chromosome or the sister chromatid) and the more error-prone nonhomologous end joining.<sup>13,14</sup> Regardless of which mechanism is used, mistakes may introduce mutations that in some cases will promote tumorigenesis. Both pathways consist of a complex network of events that trigger cell cycle checkpoints to prevent cells from progressing through the cycle with damaged DNA and activate a specific DNA repair mechanism (Fig 2). A number of genes involved in the DNA DSB repair pathway have been implicated as breast cancer susceptibility genes. Below we review what is known about the function of these genes in an attempt to understand how it impinges on breast cancer risk and to propose other genes that may be involved in predisposition.

### **BRCA1 (OMIM 113705) and BRCA2 (OMIM 600185)**

One defective copy of *BRCA1* or *BRCA2* in the germline is sufficient for breast cancer predisposition, but the loss of the second allele is required for cancer development. However, little is known about the mechanisms by which the wild-type allele is lost. Surprisingly, despite the association with inherited predisposition, somatic mutations in *BRCA1* and *BRCA2* are rare in sporadic breast cancer.<sup>15,16</sup> *BRCA1* and *BRCA2* encode large nuclear proteins, widely expressed in different tissues, markedly during S and G<sub>2</sub> phases. They bear little resemblance to one another or to other proteins of known function.<sup>17</sup> Orthologs are not found in the yeast or fly, but a *BRCA1* ortholog in the worm *Caenorhabditis elegans* has been recently reported, suggesting a peculiar track in evolution.<sup>18</sup>

Both *BRCA1* and *BRCA2* have been consistently linked to various processes involved in the DNA damage response. These include the repair of DSBs by HR, the repair of oxidative damage by transcription-coupled repair, and a possible role in nonhomologous end joining.<sup>19,21</sup>

*BRCA1* and *BRCA2* are also implicated in the maintenance of chromosome stability, possibly through their function in recombination.<sup>12,17,22</sup> Mouse and human cells null for *BRCA1* and *BRCA2* suffer from chromosome instability and have a heightened sensitivity to DNA lesions that are normally repaired by HR.<sup>23,24</sup> Models have also been proposed to explain the roles of *BRCA1* and *BRCA2* in maintenance of chromosome instability through functions in DNA replication.<sup>25</sup> Stalled replication forks caused by a variety of mechanisms (eg, base lesions, DNA breaks, or strand gaps) are thought to require HR to restart replication. If HR is dysfunctional, then stalled replications forks may lead to persistent DNA breaks and ultimately to gross chromosomal rearrangements, including translocations. These are frequently seen in cells lacking *BRCA1*

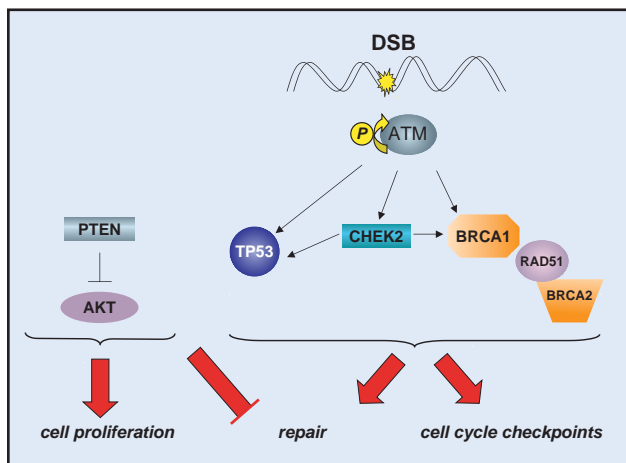


Fig 2. — Breast cancer susceptibility gene products and the DNA damage response pathway. In this simplified view, ATM is activated by the presence of DNA double-strand breaks (DSB) and phosphorylates CHEK2, BRCA1, and TP53. Activated CHEK2 also phosphorylates TP53 and BRCA1. Phosphorylation of these proteins seems to be required for the efficient activation of various cell cycle checkpoints. BRCA2 regulates the function of the RAD51 protein, which bridges the interaction between BRCA1 and BRCA2. Another protein implicated in breast cancer predisposition, PTEN, mediates down-regulation of AKT. Pointed arrowheads indicate activation, and flat arrowheads indicate inhibition.

and *BRCA2*.<sup>24</sup> Such rearrangements may well provide the raw material for the further genetic changes required for tumor progression.<sup>26</sup> Alternatively, chromosomal instability initiated by *BRCA* deficiency may be the result of incorrect routing of DSB processing down an inappropriate pathway rather than the failure of repair per se.<sup>17</sup> In this model, DSBs in *BRCA*-deficient cells are rerouted for repair by mechanisms that are potentially error-prone (nonhomologous end joining or single strand annealing) because the preferred mode of (error-free) processing by HR is unavailable.

The exact molecular functions of BRCA1 in the DNA damage response have remained elusive. Although arbitrary and complicated, the classification of the proteins involved in the DNA damage response as sensors, transducers, or effectors is helpful for a systematic analysis.<sup>27</sup> In this view, BRCA1 is likely to participate as a sensor or transducer rather than directly as a repair factor (effector).<sup>27</sup> Some hints can be gleaned from the protein-protein interaction partners of BRCA1. BRCA1 interacts with Rad51 and the MR11/RAD50/Nbs1 protein complex, which participates in DSB repair.<sup>28-30</sup> BRCA1 may also have local activities at DSB sites through its interaction with enzymes that alter chromatin and DNA structure. BRCA1 interacts with SWI/SNF and other proteins that remodel chromatin, such as regulators of acetylation/deacetylation, and with DNA helicases, including the RecQ homolog encoded by the Bloom's syndrome gene, *BLM*, and the novel helicase BACH1.<sup>31-35</sup> These data suggest a role for BRCA1 as a scaffold or platform to coordinate different activities needed for repair. The molecular role of BRCA2 is somewhat better understood. BRCA2 interacts with and regulates the function of RAD51, the mammalian homolog of *Escherichia coli* RecA that has a catalytic activity central to HR.<sup>36</sup> RAD51 coats single-strand DNA to form a nucleoprotein filament that invades and pairs with a homologous DNA duplex, initiating strand exchange between the paired DNA molecules. The interaction involves a substantial proportion of total cellular pool of each protein, suggesting that BRCA2 works directly to regulate the availability and activity of RAD51 in this key reaction.<sup>37</sup> Taken together, these observations place BRCA1 and BRCA2 firmly in the DNA damage response pathway and suggest a pleiotropic role in this pathway.

## ATM (OMIM 607585)

The ATM (ataxia telangiectasia-mutated) protein was identified as the product of the gene mutated in the rare human autosomal recessive disorder ataxia telangiectasia (AT).<sup>38</sup> ATM is a serine/threonine protein kinase that belongs to the phosphatidylinositol 3-kinase super family. The ATM kinase plays a central role in response to DSB, and loss of ATM abolishes the checkpoints at the G<sub>1</sub>-S transition, in S phase, and at the G<sub>2</sub>-M boundary.<sup>27</sup> AT is characterized by

neurodegeneration, immunodeficiency, genomic instability, hypersensitivity to ionizing radiation, and increased cancer predisposition.<sup>39</sup> It is estimated that approximately 1% to 2% of the general population may be heterozygote carriers of the *ATM* gene but do not show any of the major disease symptoms. However, certain types of ATM mutations in heterozygous carriers seem to increase cancer predisposition, particularly breast cancer.<sup>40-42</sup>

The majority of mutations identified in the gene are truncating mutations resulting in unstable truncated protein products, leaving heterozygous carriers of such mutations with a reduced level of functional ATM protein produced by a wild-type allele.<sup>43</sup> However, *ATM* truncations do not contribute to early-onset breast cancer.<sup>44</sup> The early studies of the relationship between *ATM* heterozygosity and breast cancer risk were inconclusive, and neither linkage analyses nor mutation studies provided supporting evidence for a role of *ATM* in breast cancer predisposition. An explanation that clarified these initial findings came from a missense mutation model.<sup>45</sup> The model defines two groups of *ATM* heterozygous mutations in the general population that cause different degrees of cancer predisposition. One group has a truncated allele and a second group has a missense mutation. These missense mutations allow production of full size, stable, but functionally inactive ATM protein, and they act as dominant negative mutations interfering with the function of the normal allele. Carriers of these mutations have a high predisposition for breast cancer. This explanation is further supported by linkage and penetrance analysis of *ATM* mutations among breast cancer cases.<sup>46</sup> Additional support for this model came from a study of *ATM* "knock-in" heterozygous mice harboring an in-frame deletion corresponding to the human 7636del9 mutation.<sup>47</sup> The *ATM* "knock-in" showed an increased susceptibility to developing tumors. In contrast, no tumors were observed in the *ATM* heterozygous (*Atm*<sup>+/-</sup>) mice.

A mechanistic understanding of the different roles for truncating and missense mutations in breast cancer predisposition came from the elegant work of Bakkenist et al.<sup>48</sup> They found that ATM molecules exist as dimers or higher-order multimers in undamaged cells where the kinase domain of each monomer is bound to an internal domain of another neighboring ATM molecule containing the catalytic site. While in this state, ATM is inactive and unable to phosphorylate its substrates. After DNA damage, the kinase domain of one ATM molecule phosphorylates another ATM molecule in the dimer complex, and the phosphorylated ATM dissociates from the complex to phosphorylate other substrates. Thus, kinase inactive and nonphosphorylatable missense mutants of ATM are locked in the inactive complex. This mechanism of activation provides an explanation for the dominant-negative effect of ATM heterozygous missense mutations. However despite the prevalence of the AT mutations in the population, the risk conferred by AT heterozygosity is still too low to account for a large pro-

portion of familial breast cancers. The degree to which the *ATM* gene contributes to sporadic breast cancer will require further studies and mutation screening. Nevertheless, its central role in the DNA damage response reinforces the notion that this pathway may be intrinsically linked to breast cancer predisposition.

### **TP53 (OMIM 191170)**

Breast cancer is a major component of the rare Li-Fraumeni syndrome, in which germline mutations of the *TP53* gene have been documented.<sup>49,50</sup> Li-Fraumeni syndrome is an autosomal-dominant disease characterized by early occurrence of multiple cancers such as sarcomas, breast cancer, brain tumors, leukemia, and adrenal cortical tumors.<sup>50</sup> It is estimated that 50% of women who survive childhood cancers will develop breast cancer by the age of 50, and lifetime penetrance approaches 100%.<sup>51</sup> Although highly penetrant, the Li-Fraumeni genes account for less than 1% of breast cancer cases.<sup>51</sup>

*TP53* is a tumor suppressor gene encoding a nuclear phosphoprotein that acts as a transcription factor involved in the control of cell cycle progression, repair of DNA damage, genomic stability, and apoptosis.<sup>52</sup> In response to DNA damage, the p53 protein arrests cells in the G<sub>1</sub> phase of the cell cycle, allowing the DNA repair mechanism to proceed prior to DNA synthesis. Loss of p53 function abolishes this growth arrest response to DNA damage. Interestingly, *TP53* mutations are frequently found in *BRCA1*-linked tumors and several studies have suggested that the status of *BRCA1/BRCA2* influences the type and distribution of *TP53* mutations in breast cancer.<sup>53-55</sup>

In conclusion, mutations in p53 are a rare cause of breast cancer except for those associated with Li-Fraumeni syndrome. While *TP53* is one of the most commonly mutated genes in human tumors, among sporadic breast tumors only a small fraction carries a *TP53* mutation.<sup>56</sup> Importantly, p53 is a key regulator of the response to DNA damage and, similar to *BRCA1*, a substrate for damage-induced ATM phosphorylation.

### **CHEK2 (OMIM 604373)**

Germline mutations in the *CHEK2* (*CHK2*) gene have also been implicated in the etiology of Li-Fraumeni syndrome.<sup>57</sup> This gene encodes the human ortholog of yeast checkpoint kinases Cds1 and Rad53 in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, respectively.<sup>58</sup> In mammalian cells, *CHEK2* is phosphorylated by ATM in response to DSB.<sup>59</sup> Activated *CHEK2* phosphorylates a number of target proteins that in turn prevent cellular entry into mitosis and activate DNA repair pathways. In addition, *CHEK2* acts in the G<sub>1</sub>-S checkpoint by phosphorylating p53 and mediating activation and stabilization

of p53 by ATM.<sup>60,61</sup> In another important connection, *CHEK2* phosphorylates Cdc25C and *BRCA1*.<sup>58,62</sup> Mutation screening of the *CHEK2* gene among Li-Fraumeni cases revealed a deletion mutation *CHEK2 1100delC*, which inactivates the kinase activity of the protein.<sup>57</sup> This allele has also been proposed to be a low-penetrance breast cancer susceptibility allele.<sup>63,64</sup> Additional screening of *CHEK2* variants did not identify any other variant that occurs at significantly elevated frequency, indicating that *1100delC* may be the only *CHEK2* allele with a significant contribution to breast cancer susceptibility.<sup>65</sup> Interestingly, *CHEK2 1100delC* is associated with breast cancer only in noncarriers of *BRCA1* and *BRCA2*.<sup>63</sup> A recent search for new breast cancer susceptibility genes among families with no *BRCA1* and *BRCA2* mutation suggested a model in which *CHEK2 1100delC* interacts with an as yet unknown gene to increase breast cancer risk.<sup>66</sup>

Although the *CHEK2 1100delC* allele confers moderate risk, its prevalence suggests that it may be a more important player in breast cancer incidence than genes associated with breast cancer only in the context of rare hereditary syndromes. Again, the cross talk between *CHEK2* and the other breast cancer predisposition gene products in the DNA damage pathway is evident.

### **PTEN/MMAC1 (OMIM 601728)**

*PTEN* (also known as *MMAC1*) was originally identified as a tumor suppressor gene defective in a variety of human cancers.<sup>67,68</sup> Germline mutations in *PTEN* are associated with Cowden disease, a rare autosomal dominant inherited cancer syndrome characterized by a high risk of breast, thyroid, and endometrial carcinomas.<sup>69-71</sup> Most cancer-associated *PTEN* mutations are truncations that cause a 25% to 50% lifetime breast cancer risk among women affected with Cowden disease.<sup>72,73</sup> *PTEN* mutations are rare in sporadic breast cancer and have been found in only 5% of the sporadic cases.<sup>74,75</sup> However, 29% to 48% of sporadic breast cancer cases show loss of heterozygosity at the *PTEN* locus, while no alterations have been found in the remaining allele.<sup>76</sup> In addition, approximately 40% of breast cancers show a decrease or absence of *PTEN* protein levels.

*PTEN* is a phosphatase with dual specificity for proteins and major cellular lipids. Its tumor suppressor function has been linked to its lipid phosphatase activity, which is specific for the position 3 of major cellular lipids phosphatidylinositol 3,4,5-trisphosphate (PIP3) and phosphatidylinositol 3,4-bisphosphate, both byproducts of the lipid kinase activity of the phosphoinositide 3-kinase (PI3K).<sup>77</sup> The PI3K pathway regulates cell growth and survival through signaling to its downstream effectors, the protein kinases AKT and PDK1. Among numerous AKT kinase substrates are members of the FOXO forkhead transcription factors subfamily.<sup>78</sup> Activated AKT kinase pro-

motes phosphorylation and subsequent inactivation of the FOXO family members. Interestingly, in PTEN-deficient cells the FOXO transcription factors are aberrantly localized to the cytoplasm and cannot activate transcription.<sup>79</sup> In addition, PTEN-mediated down-regulation of AKT stimulates transcription of the cyclin-dependent kinase inhibitors p27<sup>Kip1</sup>, p21<sup>Waf1/Cip1</sup>, and p57<sup>Kip2</sup>.<sup>79</sup> Importantly, the FOXO transcription factors modulate expression of several genes that regulate cellular response to DNA damage linking PTEN/PI3K/AKT pathway to DNA damage repair pathway.<sup>80</sup>

## Searching for Additional Genes

It is clear that mutations in *BRCA1* and *BRCA2* genes not only cause defects in DNA repair after DSB but also predispose carriers to breast cancer. In addition, other known breast cancer susceptibility genes such as *ATM*, *CHEK2*, and *TP53* also function in the DNA damage response path-

way. Inactivation of *PTEN*, although less clear than the above-mentioned genes, also seems to impinge on the ability of cells to respond to damage. While it is possible that the apparent clustering of predisposition genes in this pathway may be restricted to the rare hereditary syndromes described above, it is plausible to think that they reveal important common characteristics in the biology of breast cancer susceptibility. The reason why this ubiquitous pathway is specifically tied to breast cancer predisposition remains unknown. Although there is no clear explanation for that, recently several hypotheses, at least in the context of *BRCA1* inactivation, have been formalized and can now be experimentally tested.<sup>12,25,81,82</sup>

If the DNA damage response pathway were a major target of inactivation in breast cancer, we would predict that other known genes would be targets of germline or somatic mutations. In fact, screens to identify mutations in DNA damage response genes as well as association studies using candidate polymorphisms have been undertaken (Table). While no clear major target has emerged,

Mutations in DNA Damage Repair Genes and Breast Cancer Risk

Gene	Polymorphism <sup>a</sup>	Type of Study/Population	Results <sup>b</sup>	Reference
<i>BACH1</i>	Pro919Ser G64A	Kin-cohort study of 2,430 relatives (190 with BC and 2,240 without)	Only Pro919Pro was associated with increased risk.	Sigurdson et al <sup>83</sup>
	Pro919Ser Val193Ile Arg173Cys Glu879Glu 21 other variants	MS 21 families with inherited B/OC not associated with <i>BRCA1/2</i> , 58 early-onset BC patients and 30 controls	No variant could be clearly related to BC risk.	Rutter et al <sup>84</sup>
	Pro919Ser Pro1034Leu G2637A C3411T	MS 214 B/OC patients from 151 families with hereditary B/OC	No variant could be clearly related to BC risk.	Karppinen et al <sup>85</sup>
	Pro47Ala Met299Ile Val193Ile Pro919Ser G2637A C3411T	MS 65 early-onset BC patients not associated with <i>BRCA1/2</i> and 200 controls	Pro47Ala and Met299Ile found only in cases. Functional evidence suggests that it may be pathogenic.	Cantor et al <sup>85</sup>
	Arg173Cys Glu879Glu Pro919Ser Tyr1137Tyr	MS 25 BC and B/OC families and 95 familial BC cases not associated with <i>BRCA1/2</i>	No variant could be clearly related to BC risk.	Luo et al <sup>86</sup>
<i>BARD1</i>	Ser378Arg His506His Val507Met Cys557Ser 3 other variants	MS 126 hereditary BC and B/OC families	Only the Cys557Ser was seen at elevated frequency in cases compared to controls.	Karppinen et al <sup>87</sup>
	None detected in BC	MS 50 breast tumors	Somatic mutation Val695Leu was found but could determine disease association.	Thai et al <sup>88</sup>
	Asn295Ser Lys312Asn Cys557Ser 1144del21bp	MS 40 hereditary BC and B/OC families that were <i>BRCA1/2</i> non-carriers and 20 early-onset sporadic BC cases	Segregation analysis of a family with Cys557Ser had near-borderline significance in linkage to disease.	Ghimenti et al <sup>89</sup>
	Ser241Cys Arg378Ser Asn470Ser His506His Val507Met 1139del21bp	MS 60 familial BC patients not associated with <i>BRCA1/2</i> Followed by a case-control study with 143 BC cases and 155 controls	Asn470Ser was the only one not observed in controls. Case-control study showed its association with increased BC risk in postmenopausal women.	Ishitobi et al <sup>90</sup>

(continues on page 132)

these studies provide enough evidence to keep the issue alive. The reasons for the inability to identify any major additional gene are unknown. Interestingly, although we still have an incomplete understanding of the biochemistry involved in DNA damage response and repair, it could be argued that many of the studies have focused on proteins involved in the DNA repair process, while the known predisposition genes seem to be involved in sens-

ing, signaling, and amplifying the damage signal.<sup>27</sup> Therefore, a candidate gene approach focusing on genes whose products are involved in signaling DNA damage (eg, *CHK1*, *Claspin*, *53BP1*, and *ATRIP*) may prove more fruitful when combined with a better understanding of the biochemistry of the DNA damage response. Another possible reason for the relative failure of the candidate gene approach may be due to the fact that inactivating muta-

Mutations in DNA Damage Repair Genes and Breast Cancer Risk (continued from page 131)

Gene	Polymorphism <sup>a</sup>	Type of Study/Population	Results <sup>b</sup>	Reference
<i>DNA-PKc</i>	C55966T	AS 192 BC cases and 192 controls	No significant association.	Fu et al <sup>91</sup>
<i>GADD45</i>	None detected	MS 59 familial BC	None detected.	Sensi et al <sup>92</sup>
<i>H2AX</i>	None detected	MS 101 hereditary BC not associated with BRCA1/2	None detected.	Monteiro et al <sup>93</sup>
<i>Ku70</i>	Gly593Gly	AS 2205 BC and 1826 controls	No association was found.	Kuschel et al <sup>94</sup>
	Gly593Gly A46922G C61G	AS See Fu et al above	Only C61G had a statistically significant difference between cases and controls, suggesting that it is associated with BC.	Fu et al <sup>91</sup>
	Ku80 G69506A G69632A	AS See Fu et al above	No significant association.	Fu et al <sup>91</sup>
<i>Ligase IV</i>	C299T (5'UTR) Asp501Asp	AS 1,004 BC cases and 1385 controls	No overall association with BC risk.	Han et al <sup>95</sup>
	Asp501Asp	AS See Kuschel et al above	Associated with a decrease in BC risk.	Kuschel et al <sup>94</sup>
	Ile591Val C4062T C4044T	AS See Fu et al above	No significant association.	Fu et al <sup>91</sup>
<i>Mre11</i>	Arg305Trp	MS 151 families with hereditary B/OC	Found in 1/151 patients but not in controls.	Heikkinen et al <sup>96</sup>
<i>NBS1</i>	Leu34Leu Glu185Gln Asp399Asp Pro672Pro	AS See Kuschel et al above	No association was found.	Kuschel et al <sup>94</sup>
	Glu185Gln	AS 223 Finnish BC patients and 172 Polish familial BC cases	Frequency distribution was similar in cases and controls.	Forsti et al <sup>97</sup>
	R215W 657del5	AS 224 BC patients and 1620 controls	657del5 found 3 times more frequently in cases but could not prove the significance of increased BC risk.	Steffen et al <sup>98</sup>
	657del5	AS 150 early onset BC patients, 80 familial BC and 530 controls	Frequency distribution and LOH analysis suggests that it is associated with BC.	Gorski et al <sup>99</sup>
	Leu34Leu Leu150Phe Glu185Gln Asp399Asp Leu574Ile Pro672Pro	MS See Heikkinen et al above	Only Leu150Phe was considered potentially pathogenic.	Heikkinen et al <sup>96</sup>
<i>RAD50</i>	His68His 687delT Ile94Leu Arg224His	MS See Heikkinen et al above	Only 687delT is likely to be disease-associated.	Heikkinen et al <sup>96</sup>

Mutations in DNA Damage Repair Genes and Breast Cancer Risk

Gene	Polymorphism <sup>a</sup>	Type of Study/Population	Results <sup>b</sup>	Reference
RAD51	Gln150Arg	MS 20 hereditary and 25 sporadic BC patients	Present in 2/45 patients with hereditary BC but not in 200 with sporadic BC.	Kato et al <sup>100</sup>
	None detected	MS 120 patients with early onset BC	No sequence variation detected.	Bell et al <sup>101</sup>
	G135C (5'UTR)	AS Ashkenazi Jewish BRCA1/2 carriers: 164 with BC, and 93 without	Elevated risk for BRCA2 but not for BRCA1 carriers.	Levy-Lahad et al <sup>102</sup>
		AS 309 BRCA1/2 carriers; 166 non-carriers BC cases; 155 controls	Elevated risk for BRCA2 but not for BRCA1 carriers or noncarriers.	Kadouri et al <sup>103</sup>
	AS See Kuschel et al above	No increased risk.	Kuschel et al <sup>94</sup>	
AS 83 pairs of female carriers of BRCA1 5382insC mutation	Reduced risk for BRCA1 5382insC mutation carriers.	Jakubowska et al <sup>104</sup>		
MS and AS BRCA1/2 carriers with and without BC	Elevated risk for BRCA2 but not for BRCA1 carriers.	Wang et al <sup>105</sup>		
RAD52	Ser346Ter Tyr415Ter	AS 160 members of B/OC families and 128 healthy controls	No increased risk for BC.	Tong et al <sup>106</sup>
	Ser346Ter Tyr415Ter	MS See Bell et al above	No increased risk for BC.	Bell et al <sup>101</sup>
	Ser346Ter	AS 727 BC cases and 969 controls	No increased risk for BC.	Han et al <sup>107</sup>
	C2259T (3'UTR)	AS See Kuschel et al above	No increased risk.	Kuschel et al <sup>94</sup>
RAD54	Gly325Arg	MS 93 BC cases and 100 controls	Not possible to determine pathogenicity.	Matsuda et al <sup>108</sup>
	Cys657Ser	MS See Bell et al above	Not possible to determine pathogenicity.	Bell et al <sup>101</sup>
XPF	Arg415Gln	AS 253 BC cases and 268 controls	Found at elevated frequency in cases compared to controls.	Smith et al <sup>109</sup>
XPG	Asp1104His	AS 220 BC cases and 308 controls	Marginally significant increased frequency in cancer cases.	Kumar et al <sup>110</sup>
XRCC1	Arg194Trp Arg280His Arg399Gln	AS 254 BC cases and 312 controls	Only Arg280His was associated with increased risk.	Moullan et al <sup>111</sup>
	Arg194Trp Arg399Gln	AS 412 BC cases and 400 controls (Arg194Trp) 639 BC cases and 647 controls (Arg399Gln)	Arg399Gln was associated with risk among blacks but not among whites.	Duell et al <sup>112</sup>
	Arg194Trp Arg399Gln	AS 253 BC cases and 268 controls	Only Arg194Trp was associated with increased risk.	Smith et al <sup>109</sup>
	Arg399Gln	AS 1,088 BC cases and 1,182 controls (Shanghai)	No overall association with BC risk.	Shu et al <sup>113</sup>
	Arg194Trp Arg399Gln	AS 162 BC cases and 302 controls	Weak association of Arg194Trp with risk.	Smith et al <sup>114</sup>
	Arg399Gln	AS 402 cases and 402 controls (Ontario)	No overall association with BC risk.	Figueiredo et al <sup>115</sup>
	Arg194Trp Arg280His Arg399Gln	Kin-cohort study See Sigurdson et al above	Increased risk was noted for homozygous carriers of Arg194Trp and Arg399Gln.	Sigurdson et al <sup>83</sup>
	Arg194Trp Arg399Gln	AS See Forsti et al above	No overall association with BC risk.	Forsti et al <sup>97</sup>

(continues on page 134)

Mutations in DNA Damage Repair Genes and Breast Cancer Risk (continued from page 133)

Gene	Polymorphism <sup>a</sup>	Type of Study/Population	Results <sup>b</sup>	Reference
XRCC2	Arg188His	AS See Han et al above	No overall association with BC risk.	Han et al <sup>95</sup>
	IVS-16bp Leu31Val	MS 105 B/OC families not associated with BRCA1/2 and 200 controls	Leu31Val was detected only once in cases but LOH and segregation analysis indicates this variant is not pathogenic.	Rodriguez-Lopez et al <sup>116</sup>
	Arg188His	AS See Kuschel et al above	The association was marginally significant.	Kuschel et al <sup>94</sup>
	Arg188His	AS 521 BC patients and 895 controls	The association was of borderline statistical significance.	Rafii et al <sup>117</sup>
XRCC3	Thr241Met	AS See Smith et al above	Thr241Met homozygotes may have increased BC risk.	Smith et al <sup>114</sup>
	Thr241Met 2 other variants	AS See Han et al above	No overall association with BC risk.	Han et al <sup>95</sup>
	Thr241Met	AS See Figueiredo et al above	Thr241Met homozygotes were marginally associated with risk.	Figueiredo et al <sup>115</sup>
	Thr241Met	AS See Forsti et al above	Borderline significance in Finnish cohort and not significant in Polish cohort.	Forsti et al <sup>97</sup>
	Thr241Met	AS (Danish prospective cohort) 426 cases and 424 controls	No overall association with BC risk.	Jacobsen et al <sup>118</sup>
	Thr241Met	AS See Kuschel et al above	No overall association with BC risk.	Kuschel et al <sup>94</sup>
XRCC4	Gln82Gln T1394G C1475T	AS See Fu et al above	Only C61G had a statistically significant difference between cases and controls suggesting that it is associated with BC.	Fu et al <sup>91</sup>

MS = mutation screening, AS = association study, BC = breast cancer, B/OC = breast/ovarian cancer, LOH = loss of heterozygosity.  
<sup>a</sup> Missense changes are shown in three-letter code for amino acids. Noncoding changes are indicated by the nucleotide change.  
<sup>b</sup> The results displayed here are a summary of the overall results and are only confined to the findings as pertaining to breast cancer. Results differ for the association of a certain single nucleotide polymorphism with other cancers, or for combinations with other genetic and environmental factors but that is not listed here. Readers are encouraged to consult the original papers for a full analysis and discussion as well as a review by Goode et al<sup>119</sup> discussing polymorphisms in DNA repair genes and cancer in general.

tions in these genes have, in isolation, only small effects on risk. The implication is that significant increases in risk will be apparent only when combined with mutations in additional genes. This scenario would be analogous to synthetic lethality in yeast, where two mutations in separate genes are viable as single mutations but lethal when combined. Several of the association studies mentioned here and presented in the Table suggest that this is the case. For example, while mutations in certain genes had marginal or no association with risk when studied in isolation, they showed significant association when combined with variant alleles in other genes.<sup>91,109</sup> Novel methods to identify synthetic gene interactions in multicellular organisms have only now become possible by exploiting RNA interference.<sup>120</sup>

It is also important not to be limited to the usual suspects. Genome-wide association studies using single nucleotide polymorphisms (SNPs) are unbiased in that there is no preconceived idea about which genes are likely to be involved in the disease process and will be instrumental in identifying other candidate pathways.<sup>121,122</sup> However, large data sets and appropriate SNP genome coverage are needed, putting this approach beyond the reach of smaller laboratories. It is expected that as tech-

nology improves and costs decrease, this approach will have widespread use.

In the near future, we can look forward to the identification of novel breast cancer predisposing genes due to rapid advancement of gene discovery technologies. The identification and functional characterization of such genes will have a significant impact on breast cancer research and early detection. A major challenge for researchers will be to understand the complicated mechanisms and changes that lead to the development and progression of breast cancer and to apply that knowledge to breast cancer detection, prevention, and treatment.

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