REVIEW

Breast cancer susceptibility: current knowledge and implications for genetic counselling

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Breast cancer is the most common malignancy in women in the Western world. Except for the high breast cancer risk in *BRCA1* and *BRCA2* mutation carriers as well as the risk for breast cancer in certain rare syndromes caused by mutations in *TP53*, *STK11*, *PTEN*, *CDH1*, *NF1* or *NBN*, familial clustering of breast cancer remains largely unexplained. Despite significant efforts, *BRCA3* could not be identified, but several reports have recently been published on genes involved in DNA repair and single nucleotide polymorphisms (SNPs) associated with an increased breast cancer risk. Although candidate gene approaches demonstrated moderately increased breast cancer risks for rare mutations in genes involved in DNA repair (*ATM*, *CHEK2*, *BRIP1*, *PALB2* and *RAD50*), genome-wide association studies identified several SNPs as low-penetrance breast cancer susceptibility polymorphisms within genes as well as in chromosomal loci with no known genes (*FGFR2*, *TOX3*, *LSP1*, *MAP3K1*, *TGFB1*, 2q35 and 8q). Some of these low-penetrance breast cancer susceptibility polymorphisms also act as modifier genes in *BRCA1/BRCA2* mutation carriers. This review not only outlines the recent key developments and potential clinical benefit for preventive management and therapy but also discusses the current limitations of genetic testing of variants associated with intermediate and low breast cancer risk.

European Journal of Human Genetics (2009) 17, 722–731; doi:10.1038/ejhg.2008.212; published online 17 December 2008

Keywords: hereditary breast and ovarian cancer; breast cancer susceptibility; low penetrance genes; modifier genes; genetic counselling; single nucleotide polymorphisms (SNPs)

Introduction

With an average lifetime risk of 8–10%, breast cancer is the most common malignancy in women in the Western world. Up to 15% of healthy women have at least one first-degree relative with breast cancer¹ and empirical data show that breast cancer risk doubles in these women. It is assumed that monogenic traits account for 5% of breast cancer overall.² Germline *BRCA1* or *BRCA2* mutations, which account for 20–40% of breast cancer that clusters in

families and less than 5% of breast cancer overall,³ are associated with a high lifetime risk of up to 60–85% for breast cancer as well as an increased risk for ovarian cancer. In addition to this high risk in hereditary breast and ovarian cancer, there are certain heritable syndromes associated with an increased breast cancer risk. However, more than 50% of the genetic predisposition to familial breast cancer remains unexplained.^{4–6}

In this review, an overview of the most recently published breast cancer susceptibility genes and single nucleotide polymorphisms (SNPs) is given. Besides these current findings, many other SNPs linked to breast cancer susceptibility were found in the past, but the objective of this paper is to review the recent findings in breast cancer genetic susceptibility identified in candidate gene approaches of genes involved in DNA repair and huge

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Received 9 January 2008; revised 22 September 2008; accepted 1 October 2008; published online 17 December 2008

genome-wide association studies. One has to distinguish strictly between rare mutations in genes involved in DNA repair that are associated with a moderate breast cancer risk on the one side and common low penetrance polymorphisms causing a slightly increased breast cancer risk on the other.⁷ These common variants consist of SNPs in known genes such as *fibroblast growth factor receptor 2* (*FGFR2*) and in loci in which the gene or genes responsible have not yet been identified. As for most complex diseases, it is expected that the individual risk for breast cancer is determined by the interaction of several or even many genetic variants, that is, a certain pattern of polymorphisms, and environmental factors, for example, hormones or physical exercise, and not by a single genetic variant. So far, neither the cumulative risk associated with patterns of known low-risk variants has been determined nor have risk prediction models integrating the different genetic variants been developed and validated. This clearly limits the clinical use of these susceptibility mutations and polymorphisms in *BRCA1/BRCA2* mutation-negative individuals with an increased risk for familial breast cancer as well as in the general population to estimate the individual breast cancer risk for those women who want to learn more about it.⁸ The situation may change in the near future with the identification of further genetic risk factors, the establishment of valid risk prediction models and the use of new generation high-throughput sequencing technologies. Analogous to the guidelines that have been established to support decision-making on whether or not a family should be offered mutation screening in *BRCA1* and *BRCA2*,^{1,2,9–14} criteria have to be defined for requirements to be fulfilled before genetic testing of intermediate penetrance genes for rare mutations associated with moderate breast cancer risk, and of low penetrance SNPs

for common alleles associated with slightly increased breast cancer risks should be implemented in routine diagnostics.

Breast cancer-associated cancer predisposition syndromes

Hereditary breast and ovarian cancer caused by mutations in *BRCA1* or *BRCA2* is the most frequent autosomal dominant disorder associated with a high breast cancer risk.⁶ In addition, there are several other rare cancer predisposing syndromes associated with an increased breast cancer risk (Table 1). In Li–Fraumeni syndrome (LFS1, MIM no. 151623),^{2,20} caused by germline mutations in *TP53*, breast cancer is one of the multiple neoplasias such as soft tissue sarcomas, leukaemia and brain tumours that recurrently occur at a young age. For organizing the clinical management, please refer, for example, to the National Comprehensive Cancer Network (NCCN) guidelines.²¹

In addition, an increased breast cancer risk is present in individuals with Peutz–Jeghers syndrome (MIM no. 175200) carrying heterozygous germline mutations in *STK11*, formerly known as *LKB1*, causing a polyposis syndrome characterized by melanocytic macules of the lips, buccal mucosa and digits as well as multiple gastrointestinal hamartomatous polyps and an increased risk of various neoplasms, including breast cancer.¹⁹ For age-dependent screening procedures, please see the recent review by Lynch *et al.*²²

Cowden syndrome (MIM no. 158350), characterized by multiple hamartomas occurring in skin, breast, thyroid, gastrointestinal tract, endometrium and brain as well as an increased risk of malignant tumours, is associated with an increased breast cancer risk of up to 30–50% by the age of 70 years. In view of the increased cancer risk, intensified screening procedures are indicated.^{21,22} Disease-causing

Table 1 Breast cancer-associated cancer predisposition syndromes

Syndrome ^a	Gene	Name	Location	Syndrome prevalence ^b	Estimated breast cancer risk
Cowden syndrome	<i>PTEN</i>	Phosphatase and tensin homologue	10q23.3	1–9/1 000 000	30–50% risk by the age of 70 years ¹⁵
Hereditary diffuse gastric cancer/familial lobular breast cancer	<i>CDH1</i>	Cadherin 1, E-cadherin	16q22.1	—	52% risk by the age of 75 years for 2398delC ¹⁶
Li–Fraumeni syndrome 1	<i>TP53</i>	Transformation-related protein 53	17p13.1	1–9/100 000	50–60% risk by the age of 45 years ²
Neurofibromatosis type I	<i>NF1</i>	Neurofibromin	17q11.2	1–5/10 000	SIR: 3.5 ¹⁷
Nijmegen breakage syndrome	<i>NBN</i> ^c	Nibrin	8q21–24	Exceptional	OR: 2.8 for 657del5 ¹⁸
Peutz–Jeghers syndrome	<i>STK11</i> ^d	Serine/threonine protein kinase 11	19p13.3	1–9/100 000	45% risk by the age of 70 years ¹⁹

OR, odds ratio; SIR, standardized incidence ratio.

^aAlphabetical order.

^bAccording to Orphanet (www.orpha.net, 28 June 2008).

^cFormerly known as *NBS1*.

^dFormerly known as *LKB1*.

germline mutations were commonly found in *PTEN*, a crucial tumour suppressor in the phosphatidylinositol 3-kinase pathway.¹⁵ Interestingly, a recent report on the subsequent sequence analysis of *PTEN* in *BRCA1/BRCA2* mutation-negative individuals in 98 French–Canadian breast cancer cases from high-risk breast/ovarian cancer families failed to identify mutations in the coding region of *PTEN*, whereas four variants in intronic sequences were found that do not seem to alter RNA splicing or *PTEN* protein levels.¹⁵ In contrast, Saal *et al*²³ recently reported on *PTEN* expression loss in basal-like breast cancer associated with somatic *PTEN*-coding mutations in *BRCA1* wild-type carriers and ‘gross’ *PTEN* mutations, that is, intragenic chromosome breaks, inversions, deletions and microcopy number aberrations in heterozygous *BRCA1* mutation carriers.

CDH1 mutations are known to cause hereditary diffuse gastric cancer and/or lobular breast cancer.¹⁶ Even in families without diffuse gastric cancer in their medical history, *CDH1* mutations could be found in women with lobular breast cancer.²⁴ In view of the increased cancer risk, intensified surveillance programmes are indicated.^{22,25}

For women with neurofibromatosis type I (NF1) (MIM no. 162200), an autosomal dominant disorder caused by *NF1* mutations, a moderately increased breast cancer risk was reported. Therefore, early screening has to be considered in these women by an interdisciplinary team of experts.¹⁷

Nijmegen breakage syndrome (MIM no. 251260), an autosomal recessive chromosomal instability syndrome characterized by microcephaly, growth retardation, immunodeficiency and predisposition to different malignancies, is caused by mutations in *NBN*, known to be involved in DNA repair.²⁶ Recently, a 5 bp deletion in *NBN* (657del5) was shown to be associated with an almost threefold increase in breast cancer risk for heterozygous female carriers in Central and Eastern Europe.¹⁸

Finally, some studies reported an elevated breast cancer risk in hereditary non-polyposis colorectal cancer (HNPCC/Lynch syndrome, MIM no. 120435) caused by heterozygous mutations in DNA mismatch repair genes, mainly *MLH1*, *MSH2*, *MSH6* and *PMS2*,^{27,28} whereas other investigations showed no or only a slightly increased breast cancer risk.^{29,30} At present, no special breast cancer surveillance is recommended for women with HNPCC,^{30–32} but this is mainly based on the investigation of heterozygous *MLH1* and *MSH2* mutation carriers, and there are indications that cancer risk associated with heterozygous *MSH6* and *PMS2* mutations may differ.³⁰

Rare intermediate-penetrance breast cancer susceptibility genes

As *BRCA1* and *BRCA2* are involved in DNA repair, and heterozygous mutations in DNA repair genes such as *ATM*

and *TP53* have been discussed to be associated with an increased breast cancer risk, candidate gene approaches to identify further breast cancer susceptibility genes in high-risk families concentrated on genes involved in DNA repair such as *CHEK2*, *RAD50*, *BRIP1* and *PALB2*. The link between DNA repair and breast cancer susceptibility became even more intriguing after homozygous mutations in *BRCA2* were found to be responsible for Fanconi anaemia, and *BRCA2* was shown to be identical with *FANCD1*.^{33,34} Notably, mutations in several genes associated with an increased breast cancer risk in monoallelic mutation carriers cause rare subgroups of Fanconi anaemia or other rare autosomal recessive diseases, if both alleles are affected. For example, *BRIP1* and *PALB2*, both associated with a moderately increased risk in heterozygous mutation carriers, turned out to be *FANCF* and *FANCG*, respectively (Figure 1). It is beyond the scope of this review to discuss DNA repair and Fanconi anaemia in detail. Interested readers are referred to the excellent reviews by Wang,³⁴ Hakem,³⁵ and D’Andrea and Grompe.³⁶ Odds ratios for heterozygous mutations in DNA repair genes such as *ATM*, *CHEK2*, *BRIP1*, *PALB2* and *RAD50* lie between 2.0 and 4.3 and are thus associated with a moderately increased breast cancer risk (Table 2).²⁶

Figure 1 Genotype–phenotype correlation of heterozygous and homozygous/compound heterozygous mutation carriers for breast cancer predisposition genes and low-penetrance breast cancer susceptibility SNPs. Besides *BRCA1* and *BRCA2*, genes are grouped by association with cancer predisposition syndromes (Table 1), rare intermediate penetrance breast cancer susceptibility genes (Tables 2 and 3), and common low-penetrance breast cancer susceptibility single nucleotide polymorphisms (Tables 2 and 3). Genes are listed in alphabetical order in each group.

Table 2 Breast cancer susceptibility genes and single nucleotide polymorphisms

Gene/locus ^a	Name	Mutation/ reference SNP	Location	Function	Odds ratio	
					Heterozygous	Homozygous
(A) Rare, moderate-penetrance breast cancer susceptibility genes						
ATM ³⁷	Ataxia telangiectasia mutated	Mutations	11q22–q23	DNA repair	2.4	—
BRIP1 ^{38b}	BRCA1 interacting protein C-terminal helicase 1	Mutations	17q22.2	DNA repair	2.0	—
CHEK2 ³⁹	Checkpoint kinase 2	1100delC	22q12.1	DNA repair	2.0	—
PALB2 ⁴⁰	Partner and localizer of BRCA2	Mutations	16p12.1	DNA repair	2.3	—
RAD50 ⁴¹	RAD50 homologue (Saccharomyces cerevisiae)	687delT	5q23–q31	DNA repair, telomere integrity and meiosis	4.3	—
(B) Common low-penetrance breast cancer susceptibility single nucleotide polymorphisms						
FGFR2 ^{42,43}	Fibroblast growth factor receptor type 2	rs2981582 ⁴³ rs1219648 ⁴²	10q25.3–q26	Receptor for acidic and basic fibroblast growth factors	1.2	1.6
LSP1 ⁴³	Lymphocyte-specific protein 1	rs3817198	11p15.5	Intracellular F-actin binding protein	1.1	1.2
MAP3K1 ⁴³	Mitogen-activated protein kinase kinase kinase 1	rs889312	5q11.2	Integration of cellular responses to mitogenic and metabolic stimuli	1.1	1.3
TGFB1 ⁴⁴	Transforming growth factor, beta 1	L10P	19q13.1	Control of proliferation, differentiation and other functions	1.1	1.2
TOX3 ^{43,45,c}	TOX high mobility group box family member 3	rs3803662	16q12.1	Regulation of transcription, DNA dependent	1.3	1.6 ⁴⁵
2q35 ⁴⁵	—	rs13387042	2q35	—	1.2	1.4 ⁴³
8q ⁴³	—	rs13281615	8q	—	1.1	1.4
					1.1	1.2

^aAlphabetical order.^bFormerly known as *BACH1*.^cFormerly known as *TNRC9*.

ATM mutations are known to cause ataxia telangiectasia (Louis-Bar syndrome), an autosomal recessive disorder characterized by cerebellar ataxia, telangiectases, immune defects and a predisposition to malignancy (MIM no. 208900). Increased breast cancer risk in heterozygous mutation carrier females has been controversial for nearly 20 years.^{37,46} Estimated relative risk varied from 1.3 to 12.7 in different studies.⁴⁷ Most recently, screening of 441 *BRCA1/BRCA2*-negative breast cancer cases from 440 families and 521 controls for *ATM* sequence variants identified 12 mutations in affected individuals and 2 in controls. By segregation analysis incorporating information from the controls and the full pedigrees of the cases, Renwick *et al*³⁷ estimated a relative breast cancer risk of 2.37 in heterozygous mutation carriers. Therefore, *ATM* can currently be classified as a breast cancer susceptibility gene with intermediate penetrance.

Cell cycle checkpoint kinase 2 (*CHEK2*), a conserved signalling component in DNA repair, is activated by *ATM* in response to DNA double-strand breaks and subsequently phosphorylates *BRCA1*. Mutations in *CHEK2* rarely cause LFS2 (MIM no. 609265). No clear relationship with breast

cancer risk was found for several sequence alterations of *CHEK2*.⁴⁸ In contrast, *CHEK2* 1100delC in exon 10, leading to an abolished kinase function, displayed a twofold increased breast cancer risk in female heterozygous mutation carriers.³⁹ Most recently, the results of a meta-analysis for the assessment of breast cancer risk in *CHEK2* 1100delC carriers of more than 50 000 cases and controls reported on a 4.8 relative risk of developing breast cancer for heterozygous *CHEK2* 1100delC mutation carriers with a family history of breast cancer. This leads to an estimated cumulative breast cancer risk of 37% by the age of 70 years.⁴⁹ Carrier frequency in the Netherlands reaches up to 0.8% and in Finland and Sweden up to 0.7 and 0.5%, respectively. Allele frequencies in other European countries, North America and Australia rarely exceed 0.1 or 0.2%, meaning 1 or 2 in 1000 alleles (Table 3).^{39,50} In addition, screening of all 14 coding exons in 516 *BRCA1/BRCA2* mutation-negative hereditary breast cancer families displayed independent segregation of *CHEK2* 1100delC with breast cancer in two of four families analysed,⁵¹ and the initially reported high risk for male breast cancer has not been confirmed.³⁹ However, *CHEK2* 1100delC carriers

Table 3 Frequency of mutations in breast cancer susceptibility genes and common single nucleotide polymorphisms

Gene/locus ^a	Mutation/reference SNP	Allele frequency	%
(A) Rare, moderate-penetrance breast cancer susceptibility genes			
ATM	Mutations	Western population	0.25–0.5 ⁴⁶
BRIP1 ^b	Mutations	UK	0.15 ³⁸
CHEK2	1100delC	The Netherlands	0.65–0.8 ³⁹
		Finland	0.55–0.7 ³⁹
		UK	0.18–0.25 ³⁹
		Germany	0.075–0.125 ³⁹
		Australia	0.07 ³⁹
		Sweden	0.3–0.5 ³⁹
		Poland	0.1–0.125 ³⁹
		Czech Republic	0.15 ³⁹
		Italy	0.055 ³⁹
		USA	0.15–0.2 ³⁹
		Canada	0.1 ³⁹
		Spain	No detection ^{39,50}
PALB2	Mutations	UK	0.25 ⁴⁰
RAD50	687delT	Finland	0.5 ⁴¹
(B) Common low-penetrance breast cancer susceptibility single nucleotide polymorphisms			
FGFR2	rs1219648	USA/European ancestry	39 ⁴²
	rs2981582	UK	38 ⁴³
		Asia	30 ⁴³
LSP1	rs3817198	UK	30 ⁴³
		Asia	14 ⁴³
MAP3K1	rs889312	UK	28 ⁴³
		Asia	54 ⁴³
TGFB1	L10P	BCAC	38 ⁴⁴
TOX3 ^c	rs3803662	Western population	14 ⁴⁵
		UK	25 ⁴³
2q35	rs13387042	Asia	60 ⁴³
8q	rs13281615	Western population	25 ⁴⁵
		UK	40 ⁴³
		Asia	56 ⁴³

BCAC, Breast Cancer Association Consortium, international collaboration group.

^aAlphabetical order.^bFormerly known as *BACH1*.^cFormerly known as *TNRC9*.

seem to have both poorer disease-free and overall survival than non-carriers, and an increased risk of developing a second, mostly contralateral breast cancer has been observed in *CHEK2* 1100delC mutation carriers having received initial radiotherapy.⁵² Weischer *et al*⁴⁹ proposed *CHEK2* 1100delC genotyping of women counselees of Northern or Eastern European descent with a family history of breast cancer. In contrast, Offit and Garber,⁵³ taking into account the very low frequency of *CHEK2* 1100delC mutations in most Western populations, felt it currently does not seem to be justified to routinely test for *CHEK2* 1100delC to guide the clinical management.

Mutations in *BRIP1*, previously known as *BACH1*, were demonstrated to be associated with an increased breast cancer risk. Screening for constitutional *BRIP1* mutations in 1212 individuals with breast cancer from *BRCA1/BRCA2* mutation-negative families and 2081 controls identified nine and two truncating mutations in individuals with breast cancer and controls, respectively. This leads to an estimated relative risk of breast cancer of 2.0 in hetero-

zygous mutation carriers, whereas biallelic mutations cause Fanconi anaemia, complementation group J (MIM no. 609054).³⁸

Investigations of *PALB2*, known to cause Fanconi anaemia, complementation group N (MIM no. 610832), in 923 individuals from different families with hereditary breast cancer and 1084 healthy control individuals led to the identification of 10 truncating *PALB2* mutations in individuals with familial breast cancer, whereas there were no truncating mutations in controls.⁴⁰ Similar to former reports on breast cancer families carrying mutations in *ATM*, *CHEK2* or *BRIP1*,^{37,38,51,54} Rahman *et al*⁴⁰ reported that only two of four first-degree breast cancer-affected relatives of breast cancer probands with *PALB2* mutations also carried the *PALB2* mutations, indicating an incomplete segregation of *PALB2* mutations with breast cancer and thereby mirroring the difficulties of screening these genes in breast cancer families when unaffected women ask for predictive molecular testing to specify their individual breast cancer risk. In addition, *ATM*, *BRIP1*, *CHEK2* and

PALB2 mutations, classified as rare breast cancer susceptibility mutations with intermediate penetrance, collectively account for only approximately 2.3% of the overall familial risk.⁴⁰

RAD50 encodes a protein essential for double-stranded DNA break repair interacting with *BRCA1* and *NBN*. In this gene, Heikkinen *et al*⁴¹ identified the Finnish founder mutation 687delT, which is associated with an increased breast cancer risk (odds ratio 4.3) in the Finnish cohort ($n=317$). But, subsequent analyses in additional cohorts from Sweden, Norway and Iceland ($n=512$) failed to identify the mutation, thus again indicating the high frequency of certain mutations in distinct populations that has to be considered if molecular screening is discussed.

Common low-penetrance breast cancer susceptibility SNPs

New tools such as high-density SNP arrays are now available for genome-wide association studies to decipher genetic variants associated with an increased breast cancer risk in large groups containing hundreds and thousands of patients and controls. The aim of these studies is to identify a number of polymorphisms, each expected to impact only to a minor extent on the individual risk. Nevertheless, as most of these variants occur with high frequency in the investigated populations (Table 3), they have a significant impact on the breast cancer risk but can also frequently be found in control cohorts.⁸ To understand how these genetic variants function and interact, and whether and how they modify environmental risk factors, it will be necessary to characterize their role in tumorigenesis. Currently, there is a growing list of reports on common SNPs in genes or chromosomal loci that have been identified in genome-wide association studies: *FGFR2*,^{42,43} *LSP1*,⁴³ *MAP3K1*,⁴³ *TGFB1*,⁴⁴ *TOX3*,^{43,45} as well as a locus on 2q35⁴⁵ and 8q.⁴³ The odds ratios for heterozygous and homozygous carriers range between 1.1 and 1.3, and 1.2 and 1.6, respectively (Table 2). Notably, although rare mutations in *ATM*, *BRCA1*, *BRCA2*, *CHEK2* and *TP53* are known to be associated with increased breast cancer risk, Baynes *et al*⁵⁵ recently reported that no significant breast cancer association of a common SNP or a certain combination of SNPs could be identified in any of these genes in 4474 investigated breast cancer cases from the British, East Anglian SEARCH study. In addition, Cox *et al*,⁴⁴ screening nine SNPs for which there was some prior evidence of breast cancer association, reported on a common SNP in *CASP8* (rs1045485) that reduces breast cancer risk. For a critical discussion of the epidemiological credibility of these studies that depends on the number of investigations and patients included, replication and protection from bias, and regarding different genome-wide approaches for the identification of susceptibility genes,

please refer to the review by Risch⁵⁶ as well as the most recently published review by Seng and Seng.⁵⁷

For example, two SNPs in intron 2 of *FGFR2* (rs2981582 and rs1219648), a gene which is known to be amplified and overexpressed in sporadic breast cancer cases,⁵⁸ has been observed in two independent multicentre genome-wide association studies of SNPs, leading to an increased breast cancer risk with an odds ratio in a heterozygous situation of 1.23–1.24.^{42,43} However, 47–48% of the healthy control cohorts also carry these SNPs,⁸ mirroring the difficulties to use them in predictive testing in breast cancer families as well as in women without a family history of breast cancer.

Interestingly, a recent report of a three-phase genome-wide association study in Ashkenazi Jews⁵⁹ confirmed the formerly reported^{42,43} association of breast cancer risk with the chromosomal region of *FGFR2* on 10q and observed a new breast cancer risk locus in 6q22.33 (odds ratio 1.41) comparing *BRCA1/BRCA2*-negative high-risk breast cancer cases with unaffected controls. Although functional or biological consequences of most SNPs are as yet unknown,⁷ Meyer *et al*⁶⁰ recently reported on an altered binding affinity for transcription factors OCT1, RUNX2 and C/EBP β as a result of identified breast cancer susceptibility SNPs in intron 2 of *FGFR2* that lead to an increased *FGFR2* expression.

Currently, further genome-wide association studies are underway comparing *BRCA1/BRCA2*-negative high-risk breast cancer and sporadic breast cancer cases with control cohorts. They will certainly identify new susceptibility polymorphisms and will possibly be able to investigate additional or synergistic effects of defined susceptibility polymorphisms.⁶

Modifiers in *BRCA1* and *BRCA2* mutation carriers

There have been several observations supporting the hypothesis that breast cancer risk in *BRCA1* and *BRCA2* mutation carriers is modified by genetic factors.⁶¹ Taking into account that women tested negative for their familial *BRCA1/BRCA2* mutations still face an increased breast cancer risk,⁶² one can speculate that this increased risk might be due to inherited genetic variants that act as modifier genes in *BRCA1/BRCA2* mutation carriers, and also as low penetrance susceptibility polymorphisms in *BRCA1/BRCA2* mutation non-carriers. Currently, vast efforts are being made to identify modifier genes in *BRCA1* or *BRCA2* mutation carriers (see eg, the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA)).⁶³ An SNP 135G>C (*RAD51*: c.-98G>C) in *RAD51* has recently been identified as first modifier in *BRCA2* mutation carriers, whereas no effect of this SNP was found in *BRCA1* mutation carriers and *BRCA1/BRCA2* mutation non-carriers.^{64,65} A multicentre study, investigating 10358 mutation carriers, described modifier roles for SNPs in *FGFR2*, *MAP3K1* and *TOX3* that were initially identified as low-penetrance breast cancer susceptibility

SNPs.⁴³ Importantly, the SNPs rs2981582 and rs889312 in *FGFR2* and *MAP3K1*, respectively, increase the breast cancer risk in *BRCA2* mutation carriers but not in *BRCA1* mutation carriers, whereas rs3803662, an SNP in *TOX3*, is associated with an increased breast cancer risk in both *BRCA1* and *BRCA2* mutation carriers.⁶⁶

Discussion

After the major breast cancer susceptibility genes *BRCA1* and *BRCA2* were cloned in 1994 and 1995, respectively,⁶⁷ considerable efforts have been made to identify another susceptibility gene in high-risk families,²⁶ but the predicted *BRCA3* could not be found.^{4,67} At present, it seems to be widely accepted that no further high-penetrance genes accounting for a relevant number of familial cases exist. Instead, breast cancer is considered a complex disease determined by the combined effect of several or even many genetic variants.⁴³ Generally, two groups of susceptibility factors can be clearly distinguished comparing mean allele frequencies and mean associated risks: rare mutations in genes involved in DNA repair with intermediate penetrance on the one side and low penetrance SNPs on the other (Figure 2).

At present, a vast amount of new data is generated by candidate gene approaches and genome-wide association studies. Although criteria for genetic testing of *BRCA1* and *BRCA2* have been defined,^{9–14} and several models for determining carrier probabilities and cancer risks of individuals with a family history of breast cancer are publicly available,^{68–70} it is still a matter of debate whether, when and how the increasing list of genetic susceptibility factors can be integrated into routine clinical care. So far, no clinical decisions can be based on their presence or absence, and genetic testing may currently only provoke great insecurity,⁸ both for counselees and medical doctors. As discussed by Offit and Garber,⁵³ routine investigation may actually lead to unnecessary interventions in carriers of susceptibility mutations and

Figure 2 Association of mean frequency and relative risk of breast cancer susceptibility genes and loci. On the basis of the information in Tables 2 and 3, the association of mean frequency and mean breast cancer-associated relative risk is displayed illustrating the clear differences between rare mutations in genes involved in DNA repair with intermediate penetrance on the one side and common low penetrance single nucleotide polymorphisms on the other.

non-carriers may feel a false sense of security. Genetic counselling of *BRCA1/BRCA2* mutation carriers regarding modifier polymorphisms such as *FGFR2*, *TOX3*, *MAP3K1* and *RAD51*, may be the easiest task because (i) the additional analysis of certain polymorphisms may specify the breast cancer risk and allow further individualization of the clinical management regarding the surveillance programme, decision-making concerning risk-reducing surgery and therapy, (ii) this is relevant for a small group of about 3% of all breast cancer patients, and most importantly, (iii) clinical programmes to meet this high risk have been set up in most countries.^{14,71} At present, complete sequencing of intermediate penetrance genes such as *ATM*, *BRIP1*, *CHEK2*, *PALB2* or *RAD50* in *BRCA1/BRCA2*-negative high-risk breast cancer families does not appear to be justified. Mutations in these genes are extremely rare in most populations and, even more importantly, the clinical consequences are not yet clear, especially regarding the incomplete segregation of the known mutation with breast cancer in affected families.^{37,38,40,51,72} It has to be considered that mutations, at least in some of these genes, are not specific for *BRCA1/BRCA2* mutation-negative familial cases, but can also occur in sporadic breast cancer cases (Bogdanova *et al*,⁷³ Erkkö *et al*⁷⁴ and A Meindl, unpublished data). Therefore, it is likely that predisposing mutations with moderate penetrance in other genes will be identified in families with multiple cases. Subsequently, this knowledge as well as the potential risk-modifying interaction of these mutations with predisposing polymorphisms may be an attractive tool for risk prediction.

Nevertheless, in the near future, as soon as costs and time of sequencing are reduced to a more reasonable level, it may become cost-effective to screen all genes involved in DNA repair or other breast cancer-associated pathways in high-risk families. Because of the known differences in allele frequency, for example, the rather high frequency of *CHEK2* 1100delC in the Netherlands or Finland, genetic testing of certain mutations and/or polymorphisms associated with a significantly increased breast cancer risk may only be indicated in populations with relatively high allele frequencies, because significant reduction of disease burden can only be achieved in a certain population if an effective preventive management is available, and the polymorphism or mutation is sufficiently frequent.

In addition to the identification of new polymorphisms and the validation of their relative risk in different large cohorts, it will be essential to understand the combined effect of different polymorphisms, and to establish reliable risk prediction models before stepping forward to routine investigations. Given seven established common breast cancer susceptibility SNPs, there are 2187 possible combinations of genotypes.⁷⁵ Although risks conferred by individual loci are rather small, some risk alleles seem to act multiplicatively. Thus, the breast cancer risk among

women carrying 14 risk alleles is estimated to be six times as high as among women carrying none of these risk alleles.⁷⁵ In the future, screening programmes may be adapted to the individual genetic risk, for example, modifying age of initiation and investigation intervals of mammography, integration of magnetic resonance imaging and/or risk-reducing surgery. It is to be hoped that this will lead to an intensified screening in high-risk individuals, but spare unnecessary and even potentially harmful screening in women with very low-risk profiles. However, the clinical utility of such an individualized genome-based approach has to be validated, and principles for the implementation of genetic testing of new susceptibility mutations and/or polymorphisms have to be established from an individual as well as from a population and health-care system point of view.^{76–78} Further studies will probably identify polymorphisms associated with different response rates to certain therapies or histological subtypes and particular SNPs may even serve as therapeutic targets.^{45,79,80} Taking full advantage of these polymorphisms will require functional characterization of the polymorphisms, as for instance for an SNP in intron 2 of *FGFR2* leading to an altered binding affinity for transcriptional factors and thereby, to an increased *FGFR2* expression.⁶⁰

In summary, based on the current knowledge, there is no evidence that justifies screening of common low penetrance genes and loci to specify the individual breast cancer risk. In the near future, with the new generation of low-cost high-throughput sequencing technologies and powerful risk prediction models integrating family history, lifestyle factors and available genetic information, individual risk classification may allow adequate preventive management, intensified in those with higher risks but reduced in those with lower risk levels. With these powerful, validated risk estimation tools in hand, we will hopefully reach a point where statistical significance meets clinical relevance, and screening programmes based on genetic information will lead to reduced breast cancer-associated mortality.

Acknowledgements

We thank Doris Steinemann for her critical review of the paper and Gillian Teicke for her assistance in editing the paper.

References

- 1 Robson M, Offit K: Clinical practice. Management of an inherited predisposition to breast cancer. *N Engl J Med* 2007; **357**: 154–162.
- 2 Bradbury AR, Olopade OI: Genetic susceptibility to breast cancer. *Rev Endocr Metab Disord* 2007; **8**: 255–267.
- 3 Wooster R, Weber BL: Breast and ovarian cancer. *N Engl J Med* 2003; **348**: 2339–2347.
- 4 Nathanson KL, Wooster R, Weber BL: Breast cancer genetics: what we know and what we need. *Nat Med* 2001; **7**: 552–556.
- 5 Meindl A: Comprehensive analysis of 989 patients with breast or ovarian cancer provides BRCA1 and BRCA2 mutation profiles and frequencies for the German population. *Int J Cancer* 2002; **97**: 472–480.
- 6 Turnbull C, Rahman N: Genetic predisposition to breast cancer: past, present, and future. *Annu Rev Genomics Hum Genet* 2008; **9**: 321–345.
- 7 Stratton MR, Rahman N: The emerging landscape of breast cancer susceptibility. *Nat Genet* 2008; **40**: 17–22.
- 8 Willems PJ: Susceptibility genes in breast cancer: more is less? *Clin Genet* 2007; **72**: 493–496.
- 9 American Society of Clinical Oncology: American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility. *J Clin Oncol* 2003; **21**: 2397–2406.
- 10 Bachmann K, Bartram CR, Chang-Claude J, Fonatsch C, Propping P: Richtlinien zur Diagnostik der genetischen Disposition für Krebserkrankungen. *Deutsches Ärzteblatt* 1998; **95**: A1396–A1403.
- 11 Dietel M, Harms E, Honnefelder L *et al*: Bekanntmachungen: Richtlinien zur prädiktiven genetischen Diagnostik. *Deutsches Ärzteblatt* 2003; **100**: A1297–A1305.
- 12 Schmutzler R, Schlegelberger B, Meindl A, Gerber WD, Kiechle M: [Counselling, genetic testing and prevention in women with hereditary breast- and ovarian cancer. Interdisciplinary recommendations of the consortium 'Hereditary Breast- and Ovarian Cancer' of the German Cancer Aid]. *Zentralbl Gynakol* 2003; **125**: 494–506.
- 13 Eisinger F, Alby N, Bremond A *et al*: Recommendations for medical management of hereditary breast and ovarian cancer: the French National *ad hoc* Committee. *Ann Oncol* 1998; **9**: 939–950.
- 14 McIntosh A, Shaw C, Evans G *et al*: *Clinical Guidelines and Evidence Review for the Classification and Care of Women at Risk of Familial Breast Cancer (NICE Guidelines CG014)*. London: National Collaborating Centre for Primary Care, University of Sheffield, 2004.
- 15 Guenard F, Labrie Y, Ouellette G *et al*: Germline mutations in the breast cancer susceptibility gene PTEN are rare in high-risk non-BRCA1/2 French Canadian breast cancer families. *Fam Cancer* 2007; **6**: 483–490.
- 16 Kaurah P, MacMillan A, Boyd N *et al*: Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. *JAMA* 2007; **297**: 2360–2372.
- 17 Sharif S, Moran A, Huson SM *et al*: Women with neurofibromatosis 1 are at a moderately increased risk of developing breast cancer and should be considered for early screening. *J Med Genet* 2007; **44**: 481–484.
- 18 Bogdanova N, Feshchenko S, Schurmann P *et al*: Nijmegen breakage syndrome mutations and risk of breast cancer. *Int J Cancer* 2007; **122**: 802–806.
- 19 Hearle N, Schumacher V, Menko FH *et al*: Frequency and spectrum of cancers in the Peutz–Jeghers syndrome. *Clin Cancer Res* 2006; **12**: 3209–3215.
- 20 Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni Jr JF, Li FP: Follow-up study of twenty-four families with Li–Fraumeni syndrome. *Cancer Res* 1991; **51**: 6094–6097.
- 21 NCCN Clinical Practice Guidelines in Oncology: Genetic/familial high risk assessment: breast and ovarian. Version 1. 2008, www.nccn.org/professionals/physician_gls/PDF/genetics_screening.pdf.
- 22 Lynch HT, Lynch JF, Lynch PM, Attard T: Hereditary colorectal cancer syndromes: molecular genetics, genetic counseling, diagnosis and management. *Fam Cancer* 2008; **7**: 27–39.
- 23 Saal LH, Gruvberger-Saal SK, Persson C *et al*: Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. *Nat Genet* 2008; **40**: 102–107.
- 24 Masciari S, Larsson N, Senz J *et al*: Germline E-cadherin mutations in familial lobular breast cancer. *J Med Genet* 2007; **44**: 726–731.
- 25 Schrader KA, Masciari S, Boyd N *et al*: Hereditary diffuse gastric cancer: association with lobular breast cancer. *Fam Cancer* 2008; **7**: 73–82.
- 26 Walsh T, King MC: Ten genes for inherited breast cancer. *Cancer Cell* 2007; **11**: 103–105.
- 27 Vasen HF, Morreau H, Nortier JW: Is breast cancer part of the tumor spectrum of hereditary nonpolyposis colorectal cancer? *Am J Hum Genet* 2001; **68**: 1533–1535.

- 28 Risinger JI, Barrett JC, Watson P, Lynch HT, Boyd J: Molecular genetic evidence of the occurrence of breast cancer as an integral tumor in patients with the hereditary nonpolyposis colorectal carcinoma syndrome. *Cancer* 1996; **77**: 1836–1843.
- 29 Geary J, Sasieni P, Houlston R *et al*: Gene-related cancer spectrum in families with hereditary non-polyposis colorectal cancer (HNPCC). *Fam Cancer* 2008; **7**: 163–172.
- 30 Watson P, Vasen HF, Mecklin JP *et al*: The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer* 2008; **123**: 444–449.
- 31 Lindor NM, Petersen GM, Hadley DW *et al*: Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. *JAMA* 2006; **296**: 1507–1517.
- 32 Vasen HF, Moslein G, Alonso A *et al*: Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). *J Med Genet* 2007; **44**: 353–362.
- 33 Howlett NG: Fanconi anemia: Fanconi anemia, breast and embryonal cancer risk revisited. *Eur J Hum Genet* 2007; **15**: 715–717.
- 34 Wang W: Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nat Rev Genet* 2007; **8**: 735–748.
- 35 Hakem R: DNA-damage repair; the good, the bad, and the ugly. *EMBO J* 2008; **27**: 589–605.
- 36 D'Andrea AD, Grompe M: The Fanconi anaemia/BRCA pathway. *Nat Rev Cancer* 2003; **3**: 23–34.
- 37 Renwick A, Thompson D, Seal S *et al*: ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet* 2006; **38**: 873–875.
- 38 Seal S, Thompson D, Renwick A *et al*: Truncating mutations in the Fanconi anemia J gene BRP1 are low-penetrance breast cancer susceptibility alleles. *Nat Genet* 2006; **38**: 1239–1241.
- 39 Nevanlinna H, Bartek J: The CHEK2 gene and inherited breast cancer susceptibility. *Oncogene* 2006; **25**: 5912–5919.
- 40 Rahman N, Seal S, Thompson D *et al*: PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* 2007; **39**: 165–167.
- 41 Heikkinen K, Rapakko K, Karpinen SM *et al*: RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. *Carcinogenesis* 2006; **27**: 1593–1599.
- 42 Hunter DJ, Kraft P, Jacobs KB *et al*: A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet* 2007; **39**: 870–874.
- 43 Easton DF, Pooley KA, Dunning AM *et al*: Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* 2007; **447**: 1087–1093.
- 44 Cox A, Dunning AM, Garcia-Closas M *et al*: A common coding variant in CASP8 is associated with breast cancer risk. *Nat Genet* 2007; **39**: 352–358.
- 45 Stacey SN, Manolescu A, Sulem P *et al*: Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet* 2007; **39**: 865–869.
- 46 Ahmed M, Rahman N: ATM and breast cancer susceptibility. *Oncogene* 2006; **25**: 5906–5911.
- 47 Angele S, Hall J: The ATM gene and breast cancer: is it really a risk factor? *Mutat Res* 2000; **462**: 167–178.
- 48 Bell DW, Kim SH, Godwin AK *et al*: Genetic and functional analysis of CHEK2 (CHK2) variants in multiethnic cohorts. *Int J Cancer* 2007; **121**: 2661–2667.
- 49 Weischer M, Bojesen SE, Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG: CHEK2*1100delC genotyping for clinical assessment of breast cancer risk: meta-analyses of 26 000 patient cases and 27 000 controls. *J Clin Oncol* 2008; **26**: 542–548.
- 50 Osorio A, Rodriguez-Lopez R, Diez O *et al*: The breast cancer low-penetrance allele 1100delC in the CHEK2 gene is not present in Spanish familial breast cancer population. *Int J Cancer* 2004; **108**: 54–56.
- 51 Dufault MR, Betz B, Wappenschmidt B *et al*: Limited relevance of the CHEK2 gene in hereditary breast cancer. *Int J Cancer* 2004; **110**: 320–325.
- 52 Schmidt MK, Tollenaar RA, de Kemp SR *et al*: Breast cancer survival and tumor characteristics in premenopausal women carrying the CHEK2*1100delC germline mutation. *J Clin Oncol* 2007; **25**: 64–69.
- 53 Offit K, Garber JE: Time to check CHEK2 in families with breast cancer? *J Clin Oncol* 2008; **26**: 519–520.
- 54 Meijers-Heijboer H, van den OA, Klijn J *et al*: Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in non-carriers of BRCA1 or BRCA2 mutations. *Nat Genet* 2002; **31**: 55–59.
- 55 Baynes C, Healey CS, Pooley KA *et al*: Common variants in the ATM BRCA1, BRCA2, CHEK2 and TP53 cancer susceptibility genes are unlikely to increase breast cancer risk. *Breast Cancer Res* 2007; **9**: R27.
- 56 Risch NJ: Searching for genetic determinants in the new millennium. *Nature* 2000; **405**: 847–856.
- 57 Seng KC, Seng CK: The success of the genome-wide association approach: a brief story of a long struggle. *Eur J Hum Genet* 2008; **16**: 554–564.
- 58 Heiskanen M, Kononen J, Barlund M *et al*: CGH cDNA and tissue microarray analyses implicate FGFR2 amplification in a small subset of breast tumors. *Anal Cell Pathol* 2001; **22**: 229–234.
- 59 Gold B, Kirchhoff T, Stefanov S *et al*: Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. *Proc Natl Acad Sci USA* 2008; **105**: 4340–4345.
- 60 Meyer KB, Maia AT, O'Reilly M *et al*: Allele-specific up-regulation of FGFR2 increases susceptibility to breast cancer. *PLoS Biol* 2008; **6**: e108.
- 61 Antoniou AC, Easton DF: Models of genetic susceptibility to breast cancer. *Oncogene* 2006; **25**: 5898–5905.
- 62 Smith A, Moran A, Boyd MC *et al*: Phenocopies in BRCA1 and BRCA2 families: evidence for modifier genes and implications for screening. *J Med Genet* 2007; **44**: 10–15.
- 63 Chenevix-Trench G, Milne RL, Antoniou AC, Couch FJ, Easton DF, Goldgar DE: An international initiative to identify genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA). *Breast Cancer Res* 2007; **9**: 104.
- 64 Kadouri L, Kote-Jarai Z, Hubert A *et al*: A single-nucleotide polymorphism in the RAD51 gene modifies breast cancer risk in BRCA2 carriers, but not in BRCA1 carriers or noncarriers. *Br J Cancer* 2004; **90**: 2002–2005.
- 65 Antoniou AC, Sinilnikova OM, Simard J *et al*: RAD51 135G→C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. *Am J Hum Genet* 2007; **81**: 1186–1200.
- 66 Antoniou AC, Spurdle AB, Sinilnikova OM *et al*: Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Am J Hum Genet* 2008; **82**: 937–948.
- 67 Narod SA, Foulkes WD: BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer* 2004; **4**: 665–676.
- 68 Marroni F, Aretini P, D'Andrea E *et al*: Evaluation of widely used models for predicting BRCA1 and BRCA2 mutations. *J Med Genet* 2004; **41**: 278–285.
- 69 Barcenas CH, Hosain GM, Arun B *et al*: Assessing BRCA carrier probabilities in extended families. *J Clin Oncol* 2006; **24**: 354–360.
- 70 Antoniou AC, Cunningham AP, Peto J *et al*: The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br J Cancer* 2008; **98**: 1457–1466.
- 71 Silva E, Gatalica Z, Snyder C, Vranic S, Lynch JE, Lynch HT: Hereditary breast cancer: part II. Management of hereditary breast cancer: implications of molecular genetics and pathology. *Breast J* 2008; **14**: 14–24.
- 72 Meijers-Heijboer H, van den OA, Klijn J *et al*: Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in non-carriers of BRCA1 or BRCA2 mutations. *Nat Genet* 2002; **31**: 55–59.
- 73 Bogdanova N, Feshchenko S, Cybulski C, Dork T: CHEK2 mutation and hereditary breast cancer. *J Clin Oncol* 2007; **25**: e26.
- 74 Erkkö H, Xia B, Nikkila J *et al*: A recurrent mutation in PALB2 in Finnish cancer families. *Nature* 2007; **446**: 316–319.

- 75 Pharoah PD, Antoniou AC, Easton DF, Ponder BA: Polygenes, risk prediction, and targeted prevention of breast cancer. *N Engl J Med* 2008; **358**: 2796–2803.
- 76 Cassiman JJ: Research network: EuroGentest – a European Network of Excellence aimed at harmonizing genetic testing services. *Eur J Hum Genet* 2005; **13**: 1103–1105.
- 77 Ginsburg GS: Genomic medicine: ‘grand challenges’ in the translation of genomics to human health. *Eur J Hum Genet* 2008; **16**: 873–874.
- 78 Scheuner MT, Sieverding P, Shekelle PG: Delivery of genomic medicine for common chronic adult diseases: a systematic review. *JAMA* 2008; **299**: 1320–1334.
- 79 Fagerholm R, Hofstetter B, Tommiska J *et al*: NAD(P)H:quinone oxidoreductase 1 NQO1*2 genotype (P187S) is a strong prognostic and predictive factor in breast cancer. *Nat Genet* 2008; **40**: 844–853.
- 80 Garcia-Closas M, Hall P, Nevanlinna H *et al*: Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. *PLoS Genet* 2008; **4**: e1000054.