The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications

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Cancer arising in carriers of mutations in the *BRCA1* and *BRCA2* genes differs from sporadic breast cancer of age-matched controls and from non-*BRCA1/2* familial breast carcinomas in its morphological, immunophenotypic and molecular characteristics. Most *BRCA1* carcinomas have the basal cell phenotype, a subtype of high-grade, highly proliferating, estrogen receptor- and HER2-negative breast carcinomas, characterized by the expression of basal or myoepithelial markers such as basal keratins, P-cadherin, epidermal growth factor receptor, etc. This phenotype is rarely found in *BRCA2* carcinomas, which are of higher grade than sporadic age-matched controls, but tend to be estrogen receptor- and progesterone receptor-positive. The expression of the cell-cycle proteins cyclins A, B1 and E and SKP2 is associated with a *BRCA1* phenotype, whereas cyclin D1 and p27 expression is associated with *BRCA2* carcinomas. Recent studies have shown that hereditary carcinomas that are not attributable to *BRCA1/2* mutations have phenotypic similarities to *BRCA2* tumors, but tend to be of lower grade and proliferation index. Somatic mutations in the *BRCA* genes are rarely found in hereditary tumors; by contrast, *BRCA1* and *BRCA2* loss of heterozygosity (LOH) is found in almost all *BRCA1* and *BRCA2* carcinomas, respectively. Furthermore, all types of hereditary breast carcinomas have a low frequency of HER2 expression. Finally, comparative genomic hybridization studies have revealed differences in chromosomal gains and losses between genotypes. The pathological and molecular features of hereditary breast cancer can drive specific treatments and influence the process of mutation screening. In addition, detecting molecular changes such as *BRCA1/2* LOH in nonatypical cells obtained by random fine-needle aspiration, ductal lavage or nipple aspirate fluid may help to earlier identify carrier women who are at an even higher risk of developing breast carcinoma.

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It is currently estimated that 5–10% of all breast cancers are hereditary and attributable to mutations in high-penetrance susceptibility genes, but only two of these have been identified: *BRCA1* (OMIM 113705)¹ and *BRCA2* (OMIM 600185).² *BRCA1* mutations are associated with families with breast and ovarian cancer, while families with male breast tumors are associated with mutations in the *BRCA2* gene.

While early estimates suggested that *BRCA1* and *BRCA2* mutations were responsible for 75% of multiple-case breast cancer families and for the majority of families with multiple breast and ovarian cancers,^{3,4} recent data have shown that these percentages may have been overestimated. In fact, the percentage of high-risk families associated with mutations in these genes seems to be around 25% in all series investigated, including the Spanish population.⁵ Mutations are found in a high percentage (about 45%) of families with breast and ovarian tumors, while the mutation rate in families with only breast cancer have a low percentage and ranges from 15% (for families with three breast cancers) to 25–35% (for families with more than five breast tumors).

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Currently, it is estimated that the proportion of breast cancer cases in the general population due to *BRCA1* mutations ranges from 5.3% for less than 40 years old, to 1.1% for between 50 and 70 years old.⁶

The penetrance of these mutations is incomplete, and depends on different factors such as the type of mutation, the population and/or exogenous factors. In general, germline *BRCA1* and *BRCA2* mutations confer a breast cancer risk of 80% among mutation carriers at the age of 70 years.⁷ Furthermore, in women with *BRCA1* or *BRCA2* mutations the risk of ovarian cancer is 60 and 27%, respectively, at the age of 70 years.^{8–10} These percentages are lower in the Jewish¹¹ and Italian⁹ populations, among others. The type and location of the mutation is another parameter. For instance, the missense mutations 300 T>G in *BRCA1* and 4486 G>T in *BRCA2*, have been individually found to be associated with high breast cancer risk ($P<0.001$), and mutations in the central region of *BRCA1* may be associated with a lower risk.¹² Lifestyle could influence the lifetime risk in *BRCA1*- and *BRCA2*-mutation carriers. For example, physical exercise and absence of obesity in adolescence are associated with significantly delayed breast cancer onset.¹³

About 85% of all alterations in *BRCA1* and *BRCA2* tumors are frameshift or nonsense mutations, and yield a truncated protein product. The types of mutation differ in distribution by ethnicity and geographic location. For instance, the proportion of families who inherit a mutated *BRCA1* gene seems to be smaller in Japanese families with breast cancer and/or ovarian cancer than in similar Eastern European families. Some mutations appear very frequently in certain populations, and are known as founder mutations. For example, the Jewish population presents a high prevalence of three mutations: 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*.¹⁴ More than 2% of Jewish descendants carry at least one of these three mutations, and 20–30% of Jewish women diagnosed with early breast cancer are carriers of 185delAG or 6174delT. A similar phenomenon is observed in Iceland. The prevalence of the *BRCA2* cancer-predisposing mutation 999del5 in the general population has been estimated to be 0.6%, and affects 7.7% of female and 40% of male patients with breast cancer.¹⁵

BRCA1 and *BRCA2* are considered to be 'gatekeepers': genes whose mutation or altered expression relieves normal controls on cell division, death, or lifespan, promoting the outgrowth of cancer cells. The function of the *BRCA1* and *BRCA2* genes, classified as tumor suppressors, is linked with key metabolic processes such as DNA-damage repair, regulation of gene expression and cell-cycle con-

trol.^{16–18} *BRCA1* and *BRCA2* act to preserve chromosome structure. Recently, several reports have provided insights into the role of *BRCA1* and *BRCA2* in the cellular response to DNA damage.^{19–21} Work by several groups^{22–24} has shown that *BRCA1*- or *BRCA2*-deficient rodent cells or human tumors are specifically deficient in DNA repair by homologous recombination, whereas when measured nonhomologous recombination remains intact after double-strand DNA breaks.

There is a wide range of molecular-genetic tests for *BRCA1* and *BRCA2* cancer-predisposing mutations, but techniques based on sequencing and Denaturing High-Performance Liquid Chromatography or Denaturing Gradient Gel Electrophoresis offer the greatest accuracy. However, these techniques are expensive, complex and time consuming due to the large size of both genes. For this and other reasons, such as the absence of hotspots for mutations in their coding regions and the low percentage of mutated cases, it is necessary to select good candidate families for mutation testing. Until now such selection has been based on clinical criteria, but the function of these genes and the pathology of their tumors is now been established and this information could also be used to select candidate families.

In recent years, it has been demonstrated that breast cancer arising in mutation carriers of *BRCA1* and *BRCA2* genes differs from sporadic breast cancer of age-matched controls and from non-*BRCA1/2* familial breast carcinomas in their morphological, immunophenotypic and molecular characteristics. The recognition of these differences, in addition to improving our understanding of the biology of hereditary breast cancer, could be used to predict *BRCA* mutation status in a given patient. In addition, these features could also be used to select the most appropriate treatment for an affected woman. The aim of this review is to set out our current knowledge of the morphological, immunohistochemical and molecular characteristics of *BRCA1*, *BRCA2* and non-*BRCA1/2* hereditary breast cancer, and to describe how these characteristics may impact on genetic testing and treatment.

Histopathology of *BRCA1* and *BRCA2* Breast Cancer

Invasive Carcinoma

The histopathology of *BRCA*-associated cancer has been studied by different groups. The largest series is that reported by the Breast Cancer Linkage Consortium (BCLC).^{25–27} Their studies have demonstrated that cancer arising in carriers of *BRCA1* and

Figure 1 Typical histological patterns of *BRCA1*, *BRCA2* and non-*BRCA1/2* tumors: (a) Necrosis area, (b) pushing margins, (c) prominent lymphoid infiltration, (d) solid sheets with high pleomorphism and numerous mitosis, (e) *in situ* ductal carcinoma (more frequent in *BRCA2* and non-*BRCA1/2* tumors), and (f)–(h) tumors with well-formed tubules, low pleomorphism and low number of mitosis.

BRCA2 gene mutations differs morphologically from sporadic breast cancer of age-matched controls.^{26–29} Invasive ductal carcinoma-not otherwise specified is the most common histological type in all forms of hereditary breast cancer and seems to be significantly more frequent in *BRCA1*- and *BRCA2*-mutation carriers than in noncarriers.³⁰ In addition, *BRCA1*-mutation carriers have a higher incidence of medullary carcinoma (13%) than *BRCA2*-mutation carriers (3%) and noncarriers (2%).²⁷ When only invasive ductal carcinomas are compared, after excluding medullary carcinomas, *BRCA1* tumors more frequently have a prominent lymphocytic infiltrate, foci of necrosis and pushing margins,²⁶ which are some of the features that define the medullary histotype (Figure 1).

Several studied series exhibited no statistically significant difference in the histological type of *BRCA2* tumors and controls.^{26,27,31} However, Marcus *et al*³² reported a higher incidence of *BRCA2* tumors belonging to a 'tubular lobular group', including invasive lobular, tubular and cribriform carcinomas.^{32,33} Armes *et al*²⁸ found that *BRCA2*-mutation carriers showed an excess of pleomorphic lobular and intraductal carcinomas. It remains to be established whether differences between series are due to the ethnicity of specific mutations.

One of the most constant characteristics of *BRCA1* tumors is their high histological grade. Thus, the reported incidence of grade 3 tumors in *BRCA1*-mutation carriers ranged from 66 to 84% in different studies,^{27,34–36} while the proportion of grade 3 tumors in sporadic age-matched breast cancer controls was between 30 and 40%.^{27,34–36} *BRCA1* tumors show less tubule formation, higher pleomorphism and more mitosis than do sporadic age-matched breast cancer controls.

BRCA2 tumors tend to be of higher grade than sporadic controls, although this association is less strong than for *BRCA1* cases. Most *BRCA2* tumors are grade 2/3, and show less tubule formation but cellular pleomorphism and mitotic counts similar to those of sporadic cases.²⁷ However, in some series more nuclear pleomorphism and higher mitotic

rates have been reported in *BRCA2* tumors than in sporadic tumors³¹ (Table 1).

Many series have demonstrated a high frequency of estrogen receptor-negativity in *BRCA1* tumors.^{25,34,35,37–41} Between 73 and 90% of *BRCA1* carcinomas were estrogen receptor-negative in different series.^{34,35,38} For example, Lakhani *et al*²⁵ reported that 90% of *BRCA1*-related breast cancers were estrogen receptor-negative compared with 35% in controls. Although it has been suggested that this relationship might be explained by the higher grade of tumors and younger age of patients, *BRCA1* tumors are more likely to be estrogen receptor-negative than are sporadic ones, when tumors from patients of the same age are compared. In addition, the likelihood of estrogen receptor-negativity is 4.8 times higher in *BRCA1* grade 3 tumors than in grade 3 sporadic cases.³⁷

Progesterone receptor expression in *BRCA1* tumors is also lower than in sporadic tumors. In the study of Lakhani *et al*²⁵ 79% of *BRCA1* tumors were progesterone receptor-negative, compared with only 41% in sporadic tumors. The same proportion has been observed in other studies^{34,35,38} (Figure 2).

In contrast to *BRCA1* tumors, those arising in *BRCA2*-mutation carriers do not differ from controls with regard to estrogen receptor and progesterone receptor expression. Thus, estrogen receptor expression has been reported in around 65% of *BRCA2* tumors.^{25,38,39} Between 40 and 60% of *BRCA2* carcinomas express progesterone receptor^{25,38,39} (Table 2).

Non-Invasive Lesions

Regarding precursor lesions, the natural history of hereditary breast cancer, from morphologically normal epithelium to invasive disease, is not well understood. The incidence of *in situ* lesions associated with invasive carcinomas is not described in most publications. Overall, an *in situ* component is rarer around invasive ductal carcinomas in *BRCA1*-mutation carriers than in controls.²⁷ In addition, the

Table 1 Histological characterization of familial and sporadic breast tumors

	<i>BRCA1</i> (%)	<i>BRCA2</i> (%)	Non- <i>BRCA1/2</i> (%)	Sporadic tumors (%)	References
Invasive lobular carcinoma	7	13	14	12	30,33,34,36,67,95
Invasive ductal carcinoma	74	71	73	69	30,33,34,36,67,95
Medullary carcinoma	18	3	2	3	28,30,33,36,67,95
Grade 3	71	39	22	36	28,30,31,33,34 35,36,67,95
Mitotic count (score 3)	74	45	17	33	28,31,33,34,95
Tubules (score 3)	81	70	59	65	28,31,33,34,95
Pleomorphism (score 3)	76	46	35	56	28,31,33,34,95
Pushing margins > 75%	49	16	5	27	28,95
Solid sheets > 75%	30	12	8	10	95
Prominent lymphoid infiltration	13	3	6	6	95
Necrosis	71	21	13	23	95,28

Figure 2 Immunohistochemical features of *BRCA1* and *BRCA2* breast carcinomas.

Table 2 Immunohistochemical characterization of familial and sporadic breast tumors

	<i>BRCA1</i> (%)	<i>BRCA2</i> (%)	Non- <i>BRCA1/2</i> (%)	Sporadic tumors (%)	References
Estrogen receptor+	21	65	72	66	30,34,36–39 25,35,67
Progesterone receptor +	20	49	60	56	25,30,34–36,38,67
Ki-67+	56	21	7	22	34,38,67
p53+	45	27	12	27	25,30,34–36,38,67
HER2+	7	6	3	18	25,34–36,67
Cyclin D1+	30	56	—	79	67,38,69
Cyclin E+	47	35	—	27	69
p27+	40	85	—	60	69
Ck5/6+	65	7	—	8	91,92

incidence of *in situ* lesions in the absence of an invasive component in familial breast cancer has not been established.

The study of prophylactic mastectomy specimens from *BRCA1/2*-mutation carriers has been proposed as a means to better understand the different stages of breast cancer development in these patients. However, the few data so far available are not conclusive because of the small number of case studies involved, and the difficulty of selecting appropriate controls and recognizing precursor lesions.

Hoogerbrugge *et al* have reported a study of 67 patients at high risk of breast cancer, 44 of whom had *BRCA* mutations and underwent unilateral or bilateral prophylactic mastectomy. One or more different types of high-risk precursor lesions, such as atypical lobular hyperplasia, atypical ductal hyperplasia, lobular carcinoma *in situ* and ductal carcinoma *in situ*, were present in 57% of the women. High-risk lesions were more frequent in the group aged 40 years than in the older group (73 vs 43%) and less frequent in *BRCA*-mutation carriers (43%) and women with bilateral oophorectomy before prophylactic mastectomy (20%).⁴²

In another study, Kauff *et al*⁴³ compared prophylactic mastectomy specimens from 24 women with *BRCA* mutations and 48 controls extracted from an autopsy registry, and concluded that *BRCA*-mutation carriers have a higher incidence of high-risk lesions (46%) than control cases (6%). Adem *et al*⁴⁴ compared prophylactic mastectomy in patients with a family history of breast cancer including 28 *BRCA1/2*-mutation carriers, 117 women without *BRCA1/2* mutations, 12 unclassified mutation variant carriers and 283 sporadic control cases. They found a similar prevalence of ductal carcinoma *in situ* in all groups (50–60%); however, in *BRCA1/2*-mutation carriers, proliferative fibrocystic changes were less prevalent (7%) than in sporadic cases (25%). By contrast, the proportion of invasive carcinomas was higher in mutation carriers and unclassified mutation variant-carriers than in the control group and the group without mutations, suggesting a faster progression of precursor lesions in mutation carriers.

Molecular alterations in *BRCA1* and *BRCA2* breast cancer

LOH at *BRCA1* and *BRCA2* Loci and Other Chromosome Alterations

The *BRCA* genes act as classical tumor suppressors, since they are nonfunctional in cancer cells as a result of germline mutations following inactivation of the second allele in the tumor (the Knudson two-hit hypothesis). Given that somatic mutations in the *BRCA* genes were rarely found in hereditary tumors,⁴⁵ it was suggested that somatic loss of the wild-type allele is a common mechanism of tumor activation. This hypothesis has been supported by several studies of loss of heterozygosity (LOH) in *BRCA1* and *BRCA2* loci in tumors from mutation carriers. For example, Staff⁴⁶ found *BRCA1* LOH in all informative tumors from *BRCA1*-mutation carriers (15/15) and *BRCA2* LOH in five out of six informative tumors from *BRCA2*-mutation carriers. Similarly, Osorio *et al*⁴⁷ found *BRCA1* LOH in nine out of 10 tumors from *BRCA1*-mutation carriers, and in five out of six tumors from *BRCA2*-mutation carriers. Armes *et al*³⁸ identified *BRCA1* LOH in seven out of eight informative cases of tumors from *BRCA1*-mutation carriers, and in each of seven tumors from *BRCA2*-mutation carriers. Interestingly, these authors studied the *in situ* component in three and five *BRCA1* and *BRCA2* tumors, respectively, and found the same allele to be lost as in the invasive component in all but one *BRCA2* ductal carcinoma *in situ*, which did not show LOH. Finally, Cavalli *et al*⁴⁸ found LOH of the corresponding wild-type allele in six tumors from five *BRCA1*- and one *BRCA2*-mutation carriers. In the latter study, the authors analyzed not only tumor tissue, but also non-neoplastic adjacent tissues, including normal lobules and sclerosing adenoses, and observed *BRCA1/2* LOH in all six cases. In some women, LOH was detected not only in peritumoral areas but also in other quadrants, and even in the contralateral breast. These findings suggested that these nonmalignant tissues already harbor significant amounts of the genetic alterations that may predispose to malignant transformation.

It has been demonstrated that *BRCA1* promoter hypermethylation may be a second inactivation event in *BRCA1* families, but this mode of inactivation is infrequent due to the dominance of genetic deletions as 'second hits'.⁴⁹

Staff *et al*⁴⁶ reported that the somatic loss of the other cancer susceptibility gene locus might be selected in cancer development in tumors from *BRCA1/2*-mutation carriers. They used microsatellite analysis and Fluorescence *in situ* hybridization (FISH) to study somatic loss of *BRCA1* in breast tumors from *BRCA2*-germline mutation carriers and vice versa. They found that eight out of eleven (73%) informative tumors from *BRCA1*-mutation carriers had *BRCA2* LOH, and that five out of six informative tumors from *BRCA2*-mutation carriers had *BRCA1* LOH. Combined LOH in *BRCA1* and *BRCA2* genes was seen in twelve of the 17 (71%) informative hereditary breast tumors, whereas this situation was only seen in 32 and 47% (Silva⁵⁰ and Kelsel,⁵¹ respectively) of sporadic tumors (Table 3).

Comparative genomic hybridization analysis has demonstrated that breast tumors from *BRCA1* and *BRCA2*-germline mutation carriers contain a large number of chromosomal copy number gains and losses. Tirkkonen *et al*⁵² carried out a genome-wide survey by comparative genomic hybridization on breast cancers from 21 *BRCA1*-mutation carriers, 15 *BRCA2*-mutation carriers, and 55 unselected controls. The total number of genetic changes was almost twice as high in tumors from both *BRCA1*- and *BRCA2*-mutation carriers as in the control group. In *BRCA1* tumors, losses of 5q (86%), 4q (81%), 4p (64%), 2q (40%), and 12q (40%) were significantly more common than in the control group (7–13%). *BRCA2* tumors were characterized by a higher frequency of 13q (73%) and 6q (60%) losses and gains of 17q22–q24 (87%) and 20q13 (60%) compared to the prevalence of these changes in the control group (12–18%).

Wessels *et al*⁵³ studied a series of 28 *BRCA1* and 42 control breast carcinomas by comparative genomic hybridization and found that at the chromosome level, *BRCA1* tumors showed more frequent loss of 3p, 4p, 5q, 12q, 16p, and 18q and gain of

chromosome arms 3q, 7p, 8q, 10p, and 12p. Control tumors featured more frequent gain of 16p and 17q. The authors developed a molecular classifier, which assigns a given tumor to either the *BRCA1* or control group based on somatic genetic profiles with an accuracy of 84% (sensitivity of 96% and specificity of 76%). Chromosomal bands used by this classifier include regions on chromosomes 3p, 3q, and 5q.

High-resolution comparative genomic hybridization has been used to build a classifier that allows to differentiate *BRCA1* and *BRCA2* tumors.⁵⁴

Together, these data imply that accumulation of somatic genetic changes during tumor progression may follow a unique pathway in individuals genetically predisposed to cancer, especially in the case of the *BRCA1* gene. Activation or loss of genes in the affected chromosomal regions may be selected for during tumor progression in cells lacking functional *BRCA1* or *BRCA2*.

p53 Expression and Gene Mutations

Around 15–30% of sporadic breast carcinomas have mutations in the tumor-suppressor gene *p53*. Most of them are missense mutations and produce accumulation of stable p53 protein that can be visualized by immunohistochemistry. However, 20% of the mutations do not yield a stable protein and are not detected by immunostaining. For this reason, there is a stronger association between clinicopathological variables and *p53* mutations detected by DNA sequencing than with p53 immunohistochemistry detection in breast cancer.

Considering the proposed function of *BRCA1/2* in DNA double-strand break repair, it was suggested that loss of *BRCA1/2* function activates a p53-dependent checkpoint that prevents genomic instability by promoting cell-cycle arrest or apoptosis. Abrogation of this checkpoint might be essential for malignant transformation in cells with *BRCA1/2*-inactivation and could be brought about by mutation of *p53*.

Several studies have demonstrated a higher incidence of p53 immunostaining in *BRCA1* than

Table 3 Molecular characterization of familial and sporadic breast tumors

	<i>BRCA1</i> (%)	<i>BRCA2</i> (%)	Non- <i>BRCA1/2</i> (%)	Sporadic tumors (%)	References
<i>p53</i> mutation	62	46	—	28	30,55,56
<i>HER2</i> amplification	6	0	5	19	34,61,62
<i>CCND1</i> amplification	18	60	—	20	34
<i>CCNE</i> amplification	0	0	—	0	34
<i>MYC</i> amplification	26	35	12	21	34,62,63
<i>MYB</i> amplification	29	0	—	2	64
<i>BRCA1</i> LOH	92	83	6	47	38,46,47,50
<i>BRCA2</i> LOH	73	89	—	51	38,46,47,50
Chromosome gains	3q, 7p, 8q, 10p	17q22-24, 20q13	1q, 17q, 8q, 16p	8q, 17q, 16p, 10p	52,53,98
Chromosome losses	3p, 5q, 4q, 4p, 2q	6q, 13q	13q, 6q, 11q, 9p, Xp	5q, 4p, 3p	52,53,98

in sporadic cases. *p53* overexpression has been detected in 45–77% of *BRCA1*-associated tumors.^{25,34,35,55,56} The results in *BRCA2* tumors are less conclusive.²⁵ Some studies have found *p53* overexpression in around 50% of *BRCA2* carcinomas, whereas the percentage was lower than 20% in other series.³⁴

The importance of *p53* inactivation in *BRCA*-associated tumors is also demonstrated by genetic studies in which *p53* mutations were present at higher frequencies and in unusual locations in this group compared with sporadic cases.^{55–57} In a review of reported cases by Chappuis *et al*,³⁰ around 40% of *BRCA1* and 30% of *BRCA2* carcinomas had *p53* mutations, whereas only 20% of sporadic controls had them. However, *p53* mutations were not found in *BRCA2* tumors in one series.³⁸

Comparing the spectrum of *p53* mutations in *BRCA1/2* patients with the mutations reported in the IARC *p53* mutation database, Greenblatt *et al*⁵⁸ found that they differed significantly in distribution and base change. Mutations at A:T base pairs were more common in *BRCA1/2*-associated tumors (38%) than in the IARC database (25%). Changes were frequent at *p53* codons that are not mutation hotspots. Thus, in the IARC database, mutations in 73 hotspot codons accounted for 82% of all cases of breast cancer, but in *BRCA1/2*-mutation carriers only 67% occurred at these hotspots. Finally, most nonhotspot mutations in *BRCA1/2*-mutation carriers were located on the opposite side of the *p53* DNA-binding site. Interestingly, Greenblatt *et al*⁵⁸ found no differences in the spectrum and location of *p53* mutations between *BRCA1* (44 mutations) and *BRCA2* (29 mutations) tumors.

Functional characterization of specific mutations of *BRCA1/2* tumors has revealed that they frequently possess properties not commonly associated with those occurring in sporadic cases: they retain apoptosis-inducing, transactivating, and growth-inhibitory activities similar to those of the wild-type protein, yet are compromised for transformation suppression. It has been suggested that the *BRCA*-associated mutants represent the minimal loss of function required to overcome *p53*-dependent tumor suppression. The genetic background in *BRCA* null cells would allow the selection of *p53* mutants whose expression is not tolerated in cells with intact *BRCA1/2* genes.⁵⁹

HER2 Expression and Gene Amplification

Data on HER2 expression in *BRCA*-associated tumors vary from series to series, probably as a consequence of differences in the techniques employed. For example, Armes *et al*³⁸ found no differences in the strong expression of HER2 in *BRCA2* and sporadic breast tumors (44 and 45%, respectively), but did not find HER2 expression in any *BRCA1* tumors. However, subsequent studies

revealed frequencies of 0–3% in HER2 overexpression in both *BRCA1* and *BRCA2* tumors.^{25,34,60}

With regard to *HER2* amplification, few studies have analyzed gene status by FISH in hereditary *BRCA1/2*-associated carcinomas.^{34,61} Grushko *et al*⁶¹ reported that 10 out of 53 (19%) *BRCA1* tumors had a *HER2*:CEP17 ratio ≥ 2 . The mean *HER2*:CEP ratio was 2.4 ± 0.4 , and never exceeded 3.1. The tumors were therefore categorized as having low levels of amplification. The authors also found that the majority of tumors from *BRCA1*-mutation carriers (32 of 53; 61%) were monosomic for chromosome 17, and 19% were polysomic. Palacios *et al*³⁴ and Adem *et al*⁶² did not find any *HER2*-amplified cases in a sample of 43 *BRCA1/2* carcinomas. Apart from amplification, other chromosome aberrations involving chromosome 17 were observed in one of these studies,³⁴ polysomy was observed in two (20%) *BRCA2* carcinomas and monosomy was observed in five (35%) *BRCA1* carcinomas. The different types of chromosomal abnormalities in different genotypes may be relevant, since chromosome 17 monosomy may be the second hit of inactivation in a proportion of *BRCA1*-associated carcinomas. It has been suggested that the low incidence of *HER2* amplification in *BRCA1* carcinomas may be due to a physical codeletion of one *HER2* allele and nearby sequences during the LOH at the *BRCA1* locus.⁶¹ At present, there is no plausible explanation for the low incidence of *HER2* amplification in *BRCA2* carcinomas.

MYC and MYB Amplification

Three studies have reported different frequencies of *MYC* amplification in *BRCA1/2* hereditary breast cancer. However, due to differences in the methodologies used (tissue microarrays vs whole sections) and the genotypes analyzed (*BRCA1* only, *BRCA1* and *BRCA2*, and *BRCA1/2* as a group), it is not clear whether or not *MYC* status differs among *BRCA1*, *BRCA2*, and sporadic breast carcinomas.

Groussko *et al*⁶³ observed that 21 out of 40 (57%) tumors from *BRCA1*-mutation carriers had *MYC* amplification defined as a *MYC*:CEP8 ratio of ≥ 2 ; most of these cases had two to four copies. They also found that most *MYC*-amplified cases had chromosome 8 polysomy. In another study,³⁴ although there were too few cases (10 *BRCA1*, 8 *BRCA2*), differences were observed between genotypes, whereby *MYC* amplification was observed in two (18%) *BRCA1*, in five (62%) *BRCA2*, and in two (12%), non-*BRCA1/2* invasive ductal carcinomas. Two polyploid cases were observed in *BRCA1* tumors. No cases showed amplification of both *HER2* and *MYC*. These results are in contrast to those obtained by Adem *et al*,⁶² who found a high prevalence of *MYC* duplications (57%) but a low frequency of amplification (8%) among invasive carcinomas from *BRCA1/2*-deleterious mutation carriers.

A high regional copy number gain at 6q22–q24 was revealed in *BRCA1* tumors using comparative genomic hybridization.⁶⁴ FISH analysis revealed amplification of *MYB* in five (29%) of 17 *BRCA1* breast tumors, whereas none of eight *BRCA2* tumors and 13 breast cancer cell lines, and only two of 100 sporadic breast tumors exhibited altered *MYB* copy numbers. Gene amplification resulted in mRNA overexpression, as determined by Northern blot and cDNA microarray analysis, and in protein overexpression, as determined by immunohistochemical staining. The authors concluded that *MYB* amplification is infrequent in sporadic breast cancer but common in breast tumors from *BRCA1*-mutation carriers, suggesting a role for this cell-cycle regulator and transcription factor in the progression of some *BRCA1* tumors.

Alterations in Cell-Cycle Regulation

Proliferation rate, gauged through mitotic counts and Ki67 immunostaining, was one of the most important characteristics differentiating familial and sporadic breast cancer,^{26,65} suggesting that the expression of genes involved in cell-cycle control differs between genotypes. Cell-cycle progression is governed by cyclin-dependent kinases (CDKs) that are activated by cyclin binding and inhibited by CDK inhibitors (CDKI).⁶⁶ The passage from G₁ to S phase is regulated by the activities of cyclin D1/CDK4, cyclin E/CDK2, and cyclin A/CDK2 complexes. Cyclin B/CDK1 regulates the G₂–M transition. Two CDKI families regulate the cell-cycle. Members of the CDK4 (INK4) inhibitor family, such as p15^{INK4B} and p16^{INK4A}, inhibit and specifically bind to CDK4 and CDK6. In contrast, members of the kinase inhibitor protein (KIP) family (p21^{CIP1}, p27^{Kip1}, and p57^{Kip2}) have opposite effects on the function of different CDKs. While p27 and p21 have a negative effect on cyclin E/CDK2 and cyclin A/CDK2 activity, they seem to activate cyclin D/CDK complexes.⁶⁶

The expression of these cell-cycle proteins has been extensively studied in sporadic breast cancer and in most cases each marker has been associated with specific morphological (histological grade) or biological (estrogen receptor status) characteristics. However, hereditary breast cancer studies are uncommon and generally limited to the analysis of the expression of individual cell-cycle proteins.^{38,40,41,67} The expression of cyclin D1 has been reported in some series, but there is little information available for most of the other cell-cycle markers.^{38,41,67} Most series have exhibited lower cyclin-D1 expression levels in *BRCA1* than in sporadic tumors.^{38,40,68} Cyclin-D1 expression in *BRCA2* tumors has been found at an intermediate level between those of *BRCA1* and sporadic tumors. For example, Osin *et al*⁴⁰ reported that cyclin D1 was expressed in 27% of *BRCA2* tumors, compared with 5% in *BRCA1* tumors and 35% in sporadic tumors. Likewise,

Armes *et al*³⁸ showed cyclin D1 positivity in 55% of *BRCA2*, 33% of *BRCA1* and 100% of sporadic tumors. In a previous study, our group found that 5, 57, and 56% of *BRCA1*, *BRCA2*, and sporadic age-matched controls, respectively, showed cyclin D1 expression in more than 50% of neoplastic cells.⁶⁹ In support of these results, a lower incidence of cyclin D1 expression in *BRCA1* than in *BRCA2* carcinomas was also revealed by cDNA microarray analysis.^{38,67,70} This is not surprising, considering that cyclin D1 is a protein induced by estrogen, and its association with estrogen receptor positivity has been clearly demonstrated in breast cancer.

CCND1 amplification associated with cyclin D1 overexpression has been reported in around 20% of sporadic breast cancers. Vaziri *et al*⁷¹ used FISH to study *CCND1* amplification in 30 tumors from *BRCA1*-mutation carriers and did not find any amplified cases. In contrast, Palacios *et al*⁶⁹ identified five cases of *CCND1* amplification among 16 *BRCA1/2* cases. The incidence of gene amplification in *BRCA1* tumors (two out of 11; 18%) was similar to that previously reported in sporadic cases. In *BRCA2* tumors, although the frequency of this alteration was very high (three out of five; 60%), the small number of valuable cases precluded any conclusion regarding whether or not this molecular alteration is characteristic of this breast cancer phenotype.

In our previous study, differences between *BRCA1* and *BRCA2* hereditary breast carcinomas in the expression of cell-cycle molecules were observed not only for cyclin D1, but also for its associated CDK (CDK4) and CDKIs (p16, p21, p27), which were downregulated in *BRCA1* with respect to *BRCA2* carcinomas. For example, only eight out of 20 *BRCA1* tumors showed p27 expression in more than 50% of the tumor cells; by contrast, 12 out of 14 (86%) *BRCA2* tumors did. In addition, the expression of p27 was associated with estrogen receptor status, as previously reported in sporadic tumors. Conversely, Chappuis *et al*⁷² found that in 27 *BRCA1/2*-mutation carriers, the frequency of low-level p27 expression (less than 50% of stained cells) was 85% (23 of 27), compared with 59% (87 of 147) in noncarriers of *BRCA1/2* mutations. When *BRCA1*- and *BRCA2*-related breast cancers were analyzed separately, a low p27 expression level was present in all seven (100%) breast cancers in *BRCA2*-mutation carriers and in 16 (80%) of the 20 breast cancers in *BRCA1*-mutation carriers. With regard to estrogen receptor status, they found that all the *BRCA2*-associated breast cancers that were evaluated had low levels of p27 expression, despite the fact that six of these seven tumors were estrogen receptor-positive. The authors suggested that the association between low p27^{Kip1} expression and *BRCA1/2* germline mutations in hereditary breast cancer does not necessarily result from alteration of the estrogen receptor pathway. Differences between studies could be related with ethnicity, since

Figure 3 Differential expression profiles of *BRCA1* vs *BRCA2* tumors. Using the immunohistochemical expression of the markers shown on the right, the majority of the *BRCA1* tumors (1) were grouped in a separate cluster from the *BRCA2* tumors (2). The basal phenotype characterized by CK 5/6- and cyclin E-positivity and estrogen receptor, HER2-, and p27-negativity is observed in *BRCA1* tumors. Red indicates positive expression, green indicates negative expression, and the intensity of the color is a function of the immunohistochemical expression level. White indicates expression not valuable.

Chappuis *et al*⁷² studied only Ashkenazi Jewish women with primary invasive breast cancer due to the three common *BRCA1/2* founder mutations present in this population.

In our cluster analysis of several cell-cycle proteins, markers associated with a *BRCA1* phenotype were cyclins A, B1, and E, and SKP2, among others (Figure 3). Cyclin E has also been related with the *BRCA1* phenotype in an additional study.⁷³ Interestingly, we have not detected any *BRCA1/2* carcinoma showing *CCNE* amplification,³⁴ indicating that in hereditary breast carcinoma *CCNE* amplification is a rare event, as previously reported for sporadic cases.⁷⁴ Based on their cell-cycle regulatory defects, the phenotypes of *BRCA1* and *BRCA2* carcinomas are similar to those proposed by Landberg,⁷⁵ but with fewer markers differentiating at least two subgroups of sporadic breast cancer. One of them, the *BRCA2* carcinomas, was characterized by estrogen receptor-positivity and high cyclin D1 and p27, and seemed to induce cell proliferation through a preserved Rb pathway. The second group, the *BRCA1* carcinomas, was estrogen receptor-negative, and showed defects in p53 and p27. These tumors had a more substantial lack of G1/S control, adopting an Rb-independent mechanism of cell proliferation.⁶⁸

Alterations in Apoptosis Regulation

Dysregulation of apoptosis plays an important role in the pathogenesis and progression of breast cancer,

as well as in responses of tumors to therapeutic intervention. Overexpression of BCL2 is commonly observed in estrogen receptor-positive sporadic breast carcinomas and has been associated with a good prognosis.⁷⁶ Compared with BCL2, far less is known about the expression of other apoptotic markers in breast tumors in general, and in hereditary cases in particular.

Overexpression of BCL2 in *BRCA2* tumors has been reported in several studies, confirming the good correlation between these markers and estrogen receptor status.⁷⁷ By contrast, low levels of BCL2 but high levels of caspase 3 were observed in *BRCA1* tumors.³⁴ Caspase 3 is a cytosolic enzyme that is activated only in cells committed to undergoing apoptosis, and is strongly associated with morphological assessment. Thus, previous studies have shown that the apoptotic index obtained by measuring caspase activation was higher in high-grade, estrogen receptor-negative tumors,⁷⁸ as has been observed in *BRCA1*-associated carcinomas. These data are in accordance with an expression study using cDNA microarrays that showed *BRCA1*-mutation-positive tumors to have increased expression of genes associated with inducing apoptosis (such as *PDCCD5*), and decreased expression of genes involved in suppressing apoptosis (such as *CTGF*).⁷⁰

BRCA1 Carcinomas and the Basal Cell Phenotype

Expression studies using conventional immunohistochemistry on whole sections,^{79–81} tissue microarray immunohistochemistry,^{82–84} and cDNA-array technology^{85,86} have demonstrated that there is a subset of breast carcinomas that express the so-called 'basal cell' or myoepithelial markers, such as high molecular weight cytokeratins (CK) CK5/6, CK14 and CK17, and P-cadherin. These reports have established that breast carcinomas with expression of basal cell markers show specific characteristics in relation to their morphological, proliferative, and prognostic characteristics. Several studies have linked the expression of basal markers to increased proliferation, absence of ER, a characteristic growth pattern, and poor prognosis.^{79,80,87,88} Recently, Sorlie *et al*,⁸⁹ reanalyzing cDNA microarray data from van't Veer *et al*,⁹⁰ which included 18 *BRCA1* and two *BRCA2* carcinomas, observed that 80% of *BRCA1* carcinomas had a basal-type gene expression profile. Subsequently, Foulkes *et al*⁹¹ and Palacios *et al*⁹² found a high prevalence of tumors with a basal phenotype among *BRCA1*-mutation carriers. For example, Palacios *et al*⁹² reported that CK5/6 was expressed in 50% of *BRCA1* tumors, but in fewer than 10% of *BRCA2* tumors and sporadic control cases. In addition, this phenotype is characterized by estrogen receptor- and HER2-negativity and the expression of specific cell-cycle markers, such as overexpression of cyclin E and downregulation of

p27⁷³ (Figure 2). Furthermore, a high proportion (60%) of *BRCA1* carcinomas are known to over-express epidermal growth factor receptor (EGFR), which is considered to be another basal phenotype marker.⁹³

Histopathology and molecular features of familial non-*BRCA1/2* tumors

Very little is known about the genetic basis of non-*BRCA1/2* breast cancer. Genetic linkage analysis of families has been performed and several chromosomal regions potentially harboring breast cancer susceptibility genes have been identified, including 8p12–p22, 13q21, and 2q31–q33. However, these loci have either been shown not to be major predisposing loci, or their status remains to be confirmed, thereby emphasizing genetic heterogeneity and population-specific effects among non-*BRCA1/2* families. Non-*BRCA1/2* hereditary carcinomas represent 67% of familial breast cancers when families with only female breast cancer and four or five affected members are considered.⁸ In the Spanish population, considering families with at least three cases of female breast cancer and one of the affected women being <50 years, 75% of cases were not attributable to *BRCA1/2* mutation.⁹⁴

There are three studies that have defined the histological characteristics of these neoplasias.^{34,36,95} In the three studies, invasive ductal carcinoma was the most frequent histological type: 77% of the cases according to Lakhani *et al*,⁹⁵ 78% in the series of Palacios *et al*,³⁴ and 67% in the study of Eerola *et al*.³⁶ In two of them,^{34,95} an excess of lobular carcinomas was found in familial non-*BRCA1/2* (15%) compared with *BRCA1* (3%), *BRCA2* (9%), and sporadic cases (10%). The difference was only significant with respect to *BRCA1* tumors.⁹⁵

E-cadherin inactivation by gene mutation is characteristic of sporadic lobular carcinomas. In addition, germline mutations of the E-cadherin gene (*CDH1*) have been described in families with early-onset diffuse gastric cancer, some of which are associated with lobular breast carcinomas.⁹⁶ Considering these observations, it is reasonable to speculate that some familial lobular breast cancers that cannot be attributed to *BRCA1* or *BRCA2* mutations could instead be attributed to germline mutations in *CDH1*. However, germline *CDH1* mutation analysis in several non-*BRCA1/2* lobular breast carcinomas has not revealed any genetic alterations.^{69,97}

With respect to tumor grade, breast cancers from familial non-*BRCA1/2* patients were of lesser histological grade than *BRCA1/2*-associated tumors. Thus, grade 1 tumors accounted for 27–50% of the total. In addition, non-*BRCA1/2* tumors showed more tubule formation, a lower mitotic index, and less pleomorphism than *BRCA1/2*-associated carcinomas.^{34,95}

In the immunohistochemical^{34,36,95} and FISH³⁴ analysis in familial non-*BRCA1/2*, these tumors were more frequently estrogen receptor- (73–75%), progesterone receptor- (54–67%), and BCL2-positive (55%), but p53-negative (78–96%); these figures clearly differed from *BRCA1* tumors, but no significant differences were found with respect to *BRCA2* carcinomas. A low incidence of *HER2* expression and amplification (4%) was found in non-*BRCA1/2* carcinomas.³⁴ In addition, some chromosome aberrations involving chromosome 17 were observed: five cases (23%) showed polysomy, and two cases (9.5%) were monosomic. Finally, *c-MYC* amplification was observed in two (12%) non-*BRCA1/BRCA2* tumors.

Kainu *et al*⁹⁸ investigated the molecular alterations of non-*BRCA1/2* tumors by using chromosomal comparative genomic hybridization and linkage analysis to study 61 breast tumors from 37 breast cancer families with no identified *BRCA1* or *BRCA2* mutations. Losses in non-*BRCA1/2* tumors were most often seen on chromosome arms 13q (56%), 6q (41%), 11q (30%), 9p (26%), and Xq (26%). Gains in non-*BRCA1/2* tumors were clustered in regions that are also typical of unselected breast cancers, such as 1q (46%), 17q (39%), 8q (36%), and 16p (34%), whereas losses were more evenly distributed throughout the genome. In non-*BRCA1/2* cancers, the total of copy number alterations (CNAs) was 9.4 ± 5 , which was not significantly different from the sporadic set (7.7 ± 5.1). However, the non-*BRCA1/2*-associated tumors did have fewer CNAs than *BRCA1*-associated (12.2 ± 5.3) and *BRCA2*-associated (12.5 ± 4.9) tumors. Branching and phylogenetic tree models predicted that loss of 13q was one of the earliest genetic events in these non-*BRCA1/2* hereditary cancers.

Hedenfalk *et al*⁹⁹ used the complementary strategy of global gene-expression profiling and comparative genomic hybridization to subclassify non-*BRCA1/2* tumors. The authors found two groups (A and B) of tumors based on the expression of 60 genes. Interestingly, many of the genes with increased expression in group A relative to group B were ribosomal, possibly indicating different capacities for protein biosynthesis for these groups. The two groups were not significantly different from each other with respect to clinical and histopathological factors. However, although not statistically significant, individuals in group A tended to have a later age at onset of disease and the tumors had a low percentage of cells in S-phase. This may indicate a less aggressive appearance of these tumors. Moreover, groups A and B manifested significantly different expression of the *CYP1A1* gene, which encodes a phase I cytochrome P450 enzyme known to play a central role in the metabolism of a wide range of compounds, including steroids. The authors analyzed the genomic content of eight individual tumors by comparative genomic hybridization and identified 262 DNA clones that displayed

significant differences. In particular, a large number of clones were located on 8q24, and this region was significantly amplified in group B compared with group A.

Implications for Genetic Testing

The results presented above on the morphological and immunohistochemical characteristics of hereditary breast cancer illustrate major differences between genotypes. The most important were those between tumors in *BRCA1* carriers and all other categories. These histopathological features, in conjunction with clinical data, can be used to predict *BRCA1* status and, to a lesser extent, *BRCA2* and non-*BRCA1/2* status and this could have implications for the process of mutation screening.

In families with a high incidence of breast and ovarian cancer (six or more cases) there is a high probability of finding a *BRCA1* or *BRCA2* germline mutation. In the absence of other clinical features suggesting the alteration of a specific gene, such as prostate cancer and *BRCA2* mutations, histopathological features can be used to indicate which gene should be screened first. Grade 3, estrogen receptor- and progesterone receptor-negative infiltrating ductal carcinomas are more common among *BRCA1* patients, specially if they show medullary features. These cases should be also tested for CK5/6 and EGFR expression, since the positive staining of any of these makers further increase the probability of being a *BRCA1* mutation carrier. Estrogen receptor- and/or progesterone receptor-positive tumors without evidence of basal differentiation should first be tested for *BRCA2* mutations.

In cases with less familial aggregation, the same rules should be taken into account, however the frequency of non-*BRCA1/2* tumors increases. For example, it would be very unusual to find a *BRCA1* or *BRCA2* mutation in a case with a grade 1 tumor with high tubule formation, that is estrogen receptor- and progesterone receptor-positive and p53-negative and has a very low proliferation index. In such circumstances, it would probably be sufficient to study only the more common mutations in each population in order to reduce the possibility of missing *BRCA1* cases without the typical phenotype.

In the absence of a family history of breast and ovarian cancer, we should suspect a *BRCA1*-hereditary carcinoma in women diagnosed before age 35 years that have a grade 3, estrogen receptor-negative infiltrating ductal carcinoma, since the incidence of *BRCA1* germline mutations in these cases is around 27%.²⁵ These tumors should be analyzed for the expression of CK5/6, HER2 and EGFR, since the identification of a basal phenotype probably increase the likelihood of being a *BRCA1*-mutation carrier, although this hypothesis remains to be tested. In our opinion, in such situations, the pathologist should raise the possibility of a *BRCA1*

mutation in the report. In addition, since a panel with these four antibodies (estrogen receptor, HER2, CK5/6, EGFR) can accurately identify the basal-like phenotype,¹⁰⁰ we recommend testing for these four markers in all grade 3 sporadic tumors, including results in the diagnostic report because of their possible prognostic and therapeutic implications.

Several techniques, such as random fine-needle aspiration, ductal lavage, and nipple aspirate fluid, are being used to detect atypical breast epithelial cells to improve risk stratification for women who already have an elevated risk of developing breast carcinoma. However, taking into account the special characteristics of patients with *BRCA1* or *BRCA2* mutations, there is a need to identify new ways of providing individual women with additional information regarding their risk of breast carcinoma. Detecting molecular changes, such as *BRCA1/2* LOH, in nonatypical cells obtained by these methods may help to earlier identify those women who are at an even higher risk of developing breast carcinoma. In this way, Osorio *et al*⁴⁷ studied LOH at the *BRCA1/2* locus in tumors from women with deleterious mutations, with variants of unknown significance most of which were suspected of being nondeleterious mutations, and with several common polymorphisms. They found that more than 90% of tumors with deleterious mutations had LOH of the wild-type allele, while this was the case in fewer than 5% of tumors with variants of unknown significance and common polymorphisms. The authors concluded that LOH analysis of the tumor is a test of high sensitivity (around 90%) and specificity (around 95%) for determining whether a germline variant in the *BRCA* genes in a case of familial breast cancer is either a deleterious mutation or a common polymorphism. Isaacs *et al*¹⁰¹ took this molecular approach in *BRCA1* patients, but extracting free DNA from the ductal fluid instead of using epithelial cells. The authors studied the presence of LOH in the *BRCA1* and *FHIT* genes, and mitochondrial DNA mutations in the D310 marker. They evaluated 26 ductal lavage and six nipple aspirate fluid samples from 14 women of known *BRCA1* status, who had no clinical evidence of breast tumors: nine mutation carriers and five noncarriers. LOH studies of the *BRCA1* locus were possible in 19 out of 26 ductal lavage samples, and at the *FHIT* locus in 16 out of 26 samples. In four out of nine mutation carriers LOH at the *BRCA1* allele was found, and in two of these LOH at the *FHIT* allele was also found. In one of the mutation carriers with *BRCA1* LOH, invasive breast cancer was subsequently detected, and the tumor showed the same LOH as the ductal lavage.

Therapeutic Implications

From a therapeutic point of view, adjuvant hormone therapy is not indicated in most *BRCA1* tumors,

since they are estrogen receptor- and progesterone receptor-negative, but can be used in most *BRCA2* and non-*BRCA1/2* carcinomas. Antihormonal therapy, including tamoxifen, raloxifene, and other selective estrogen receptor modulators, ovarian ablation (oophorectomy, radiation, or chemical ablation), and aromatase inhibitors, is being used to prevent both primary breast cancer and contralateral cancer after a primary tumor. Of these treatments, only oophorectomy and tamoxifen have been studied in *BRCA1*- and *BRCA2*-mutation carriers. Three studies have reported that prophylactic oophorectomy reduced the risk of developing breast cancer in *BRCA1*-mutation carriers by 50%.¹⁰² However, the benefit of tamoxifen remains controversial. The protective effect of tamoxifen against breast cancer has been demonstrated in women with moderate risk factors, but the data specific for carriers of *BRCA1* mutations remain unclear. A study involving 13 388 women whose risk factors included an age of more than 60 years, a history of preneoplastic breast lesions, or a positive family history of breast cancer, found a 50% reduction in the risk of breast cancer that could be attributed to tamoxifen therapy, although this effect was limited to estrogen receptor-positive tumors. Of 288 women with breast cancer who could be evaluated, only 6.6% had a *BRCA* mutation. A trend towards a protective effect of tamoxifen was found among carriers of *BRCA2* mutations, but not among carriers of *BRCA1* mutations. Breast cancers arising in carriers of *BRCA1* mutations are commonly estrogen receptor-negative, unlike tumors associated with *BRCA2* mutations, suggesting that the effect of tamoxifen might be selective for distinct genotypes.¹⁰³

By contrast with these results, and supporting studies of prophylactic oophorectomy that suggested that hormonal manipulation might be effective in carriers of both *BRCA1* and *BRCA2* mutations, Narod *et al*¹⁰⁴ reported that tamoxifen use reduced the risk of contralateral breast cancer by 50% in women with pathogenic mutations in the *BRCA1* or *BRCA2* gene. However, these results have been questioned based on different methodological issues.¹⁰⁵ Foulkes *et al*¹⁰⁶ also suggested that adjuvant tamoxifen might be an effective treatment for *BRCA1*-related breast cancer, irrespective of the estrogen receptor status of the tumor. The authors hypothesized that tamoxifen might induce cell death via oxidative stress in estrogen receptor-negative cancer cells, and that this effect might be more prominent in *BRCA1*-null cells, which have profoundly defective DNA repair. Thus, tumors that lack both estrogen receptor and *BRCA1* may respond differently to tamoxifen than those that lack only estrogen receptor.

Although these findings suggest that hormonal interventions should help to lower the risk among women who carry mutations, this needs to be confirmed in prevention trials such as the ongoing

study of tamoxifen and raloxifene, and these trials should include young women who carry mutations in *BRCA1* or *BRCA2*.

Finally, given the low incidence of *HER2* amplification and overexpression in most hereditary breast cancers, these tumors are not good candidates for treatment with herceptin. However, the high incidence of EGFR recently reported in *BRCA1* tumors⁹³ offers the possibility that these women could be treated with specific compounds, such as tyrosine kinase inhibitors or anti-EGFR monoclonal antibodies.

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