[CANCER RESEARCH 56, 471–474, February 1, 1996]

Advances in Brief

Germ Line Mutation at BRCA1 Affects the Histoprognostic Grade in Hereditary Breast Cancer[1]

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Abstract

Histoprognostic grade is a determinant parameter to select the initial therapeutic strategy in breast cancer. Our aim was to analyze the grade repartition in *BRCA1*-associated breast cancer (BC) and to explore the possible connections between grade and the *BRCA1* gene function. We first compared 27 *BRCA1*-associated BCs from 14 families with 4461 cases from an administrative district registry and 242 cases from a hospital-based registry, matching for grade and constitutive elements, and then considered their repartition in families.

We observed a prevalence of grade 3 ($P < 0.0001$) in *BRCA1*-associated BC. This was attributed mainly to nuclear polymorphism ($P < 0.0001$), mitotic activity ($P < 0.0001$), and tubular differentiation ($P = 0.0004$), implying that *BRCA1*-associated BCs are highly proliferating tumors. Moreover, it is suggested that grade segregates as a genetic trait within families ($P = 0.0015$), and this was attributed to the mitotic index segregation only ($P = 0.0005$). Therefore grade, through its components, could be interpreted as the morphological translation of the *BRCA1* germ line mutation. Thus, a genotype-phenotype correlation may exist between the type of mutation and the aggressiveness of the disease.

These findings are bound to have an important impact in the care management of hereditary breast cancer at the individual and at the familial level and in the comprehensive approach of breast cancer development.

Introduction

An important breakthrough in the understanding of breast carcinogenesis came with the identification of three major genes predisposing to breast cancer: *p53* associated with the rare Li-Fraumeni syndrome (1), *BRCA1* related to the development of almost 50% of inherited breast cancers (2) and an important proportion of familial ovarian carcinomas (3, 4), and *BRCA2* (5) involved in female as well as in male breast cancer development. The *BRCA1* gene has recently been cloned (6). The function of the protein is presently undetermined and germ line mutations have been found in a high proportion of breast and breast/ovarian cancer families (7–9). Nevertheless, evidence is mounting that germ line mutations of these genes may not account for all familial cases of breast cancer, and at least one other gene may be involved (10, 11).

Until recently, the question of whether HBC[3] and sporadic BC may be considered as equivalent diseases was unresolved. In a previous study, a first difference was observed by analyzing the histoprognostic grade, a crucial parameter in the initial selection of a therapeutic strategy. We showed that *BRCA1*-associated BC was significantly associated with a histoprognostic grade 3 disease (12), implying that the outlook is worse than in sporadic BC.

The aim of the present study was to explore the full biological relevance of this phenomenon through the possible connections between the histoprognostic grade and *BRCA1* gene function. We have compared an enlarged series of *BRCA1*-associated BCs, ascertained by germ line mutation and/or linkage analyses, with cases from a breast cancer registry and with a set of sporadic BCs. Cases were matched according to grade and constitutive scoring criteria, and their distribution was analyzed within families.

Patients and Methods

Family and Patient Selection. We have identified families with a strong history of breast and ovarian cancers from the records of the French Cooperative Network (13) and our cancer genetics clinics. Families were identified who had either three or more female first- or second-degree relatives affected with ovarian and/or BC (two of which had to be 60 years old or less at onset) or two BC cases (one of which was diagnosed by age 40 years).

Families were selected on the basis of both molecular studies of the *BRCA1* locus (to analyze breast carcinomas which have a high probability of being genetic cases) and the availability of paraffin blocks from at least one woman who is a gene carrier with breast cancer. Fourteen families were selected with either a positive linkage with markers at the *BRCA1* locus (lod score above 0.30; of which only two families exhibit a lod score <0.53) and/or a *BRCA1* germ line mutation (7/14 families; Table 1). The selected families were Caucasian, and 9 of them were BC and ovarian cancer families (Table 1). From this series, 51 breast carcinomas and 12 ovarian carcinomas were identified. The blocks from 27 BC cases were collected. All but two were primary tumors, the remaining blocks corresponded to a lymph node and an osteomedullar metastasis.

***BRCA1* Status.** Linkage analyses using chromosome 17q microsatellite markers from the *BRCA1* locus were conducted using a previously reported model (2). Lod scores with *D17S250*, THRA1, and *D17S579* are indicated in Table 1 and have been reported elsewhere (2, 14).

To further ascertain the *BRCA1* status in families with evidence of linkage to the *BRCA1* gene and in families in which linkage could not be performed, detection of sequence variation was conducted to identify the *BRCA1* germ line mutation. Direct DNA automatized fluorescent sequencing analyses were conducted. Both DNA strands were sequenced. In brief, PCR was performed using 40 pairs of primers (19 pairs for exon 11 and 1 pair for the remaining exons), as described previously (7–9), in a 25-μl volume containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 12 μM deoxynucleotide triphosphates, 50 pM each primer, and 25 ng genomic DNA. The reactions were carried out

Received 10/17/95; accepted 12/12/95.

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[1] This work was supported by Paoli-Calmettes Institute, INSERM, and grants from La Ligue Nationale Contre le Cancer (the National Office and the district committees of Alpes de Haute Provence, Bouches du Rhône, Corse du Sud, Haute Corse, Var), Caisse Nationale d'Assurance Maladie, CAMPLP, FEGEFLUC, Association pour la Recherche sur le Cancer, Fédération Nationale des Centres de Lutte Contre le Cancer, and Groupement de Recherche et d'Etude sur les Génomes.

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[3] The abbreviations used are: HBC, hereditary breast cancer; BC, breast cancer; lod, logarithm of the odds of linkage; OR, odds ratio; CI, confidence interval.

Table 1 *BC families and BRCA1* status (lod score using *D17S250*, THRA1, and *D17S579* markers and/or mutation)

Family (n = 14)	Ovarian/breast cancers	BC <40 yr	40 yr ≤ BC <50 y	≥50 yr BC	Lod score/*BRCA1* mutation
F15[a]	1/5	2 (30, 34)[b]	2 (43, 44)	1 (60)	0.53/exon 3 243delA→ter
F16	1/3		1 (41)	2 (52, 71)	0.63
F49[a]	0/4	3 (33, 33, 38)		1 (60)	../exon 20 5382insC→ter
F73[a]	1/4	2 (37, 39)		2 (52, 62)	../exon 11 2565ins5
F75[a]	0/3	3 (27, 32, 36)			../exon 11 1617ins16
F101	0/3	3 (30, 33, 36)			0.31
F153	1/4	3 (32, 35, 39)	1 (41)		0.54
F307	0/4		1 (48)	3 (52, 56, 57)	0.594
F322[a]	2/3	3 (28, 38, 39)			0.56/exon 11 A/C Gln3043Pro
F323[a]	0/2	1 (29)	1 (48)		../exon 11 4184del14→1364
F326	2/4	2 (27, 37)	1 (48)	1 (54)	0.963
F338	2/3		1 (42)	2 (60, 79)	0.66
F417[a]	0/5	1 (33)	2 (42, 48)	2 (50, 51)	../exon 20 5383insC→ter
F519	2/4	1 (36)	1 (42)	2 (51, 53)	0.364
Total	12/51	24	11	16	

[a] Families in which, to date, a *BRCA1* germ line mutation has been found.
[b] Numbers in parentheses, age in years.

using a Perkin Elmer/Cetus thermal cycler model 9600. The PCR products were then purified with the Gene Clean kit (BIO 101, Inc., La Jolla, CA) and resuspended in 25 μl double-distilled H_2O. Ten μl purified fragments were used for sequencing with AmpliTaq Dye Terminator Cycle Sequencing kit, using the PCR primers mentioned above, and the reactions were analyzed on an ABI 373A automated DNA sequencer.

Registries. The Bouches-du-Rhône Administrative District Registry corresponded to a series of 4725 patients with infiltrating breast carcinoma (Bouches-du-Rhône administrative district population = 1,800,000 residents). The registry is almost exhaustive, and the cases had been registered over the past 5 years. In this population (registry BC), the parameters collected were age, histological type, histoprognostic grade, size, and lymph node invasion.

The hospital-based registry corresponded to a series of 320 consecutive cases of breast carcinomas diagnosed and followed up in at the Paoli-Calmettes Institute. Familial history of BC was investigated and was positive for 78 patients (24%). Consequently, 242 cases (76%) could be considered as sporadic breast carcinomas (sporadic BC).

Pathological Analysis. For the *BRCA1*-associated BC and sporadic BC series, additional factors concerning the grading (tubular differentiation, nuclear polymorphism, and mitotic index) were analyzed. Diagnoses were reviewed independently by two pathologists from different institutions according to histological typing of the BC (15). In case of a discrepancy, results were discussed between the two pathologists and a consensus was reached.

The Scarff, Bloom, and Richardson histoprognostic grade was scored according to the Contesso recommendations (16) with three parameters: tubular differentiation (throughout, 1; occasional, 2; not seen, 3), nuclear polymorphism (uniform and regular size, 1; moderate pleomorphism, 2; very pleomorphotic with giant nuclear, 3), and mitotic index (≤1 mitosis, 1; 2, 2; ≥3, 3). The final grade is determined by adding the three scores: grade 1, 3–5; grade 2, 6–7; grade 3, 8–9.

Statistical Analysis. χ^2 and Fisher exact tests were used to compare each parameter. When an expected cell value was <5, the Fisher exact test was used. Statistical analyses were performed using the EPI-INFO version 5.01 package (March 1991).

For the contingency table (R*C) with sparse data, as for the grade repartition in families, the exact P value for the Kruskal-Wallis test (17, 18) was computed using the StatXact package (Cytel Software Corporation).

Results

Age of Onset. Mean age at diagnosis for the 27 *BRCA1*-associated BCs was 42.4 (ranging from 27 to 79) years, with 52% below the age of 40 years. By comparison, the 4725 registry BCs had a mean age of 59 years, with 6% below the age of 40 years (both $P < 0.0001$). The 242 sporadic BCs had a mean age of 59.9 years, with 6% below the age of 40 years (both $P < 0.0001$; Table 2). To avoid an obvious bias of sampling, the mean age at onset was compared between the 27 *BRCA1*-associated BCs and the 24 remaining cases, in the 14 families, for which blocks were not available. We did not observe a significant difference ($P = 0.48$).

Table 2 *Clinicopathological comparison of HBCs with registry BCs and sporadic BC cases*

Type	*BRCA1*-associated BC	*vs.* Registry BC	P	*vs.* Sporadic BC	P
Mean age (yr)	42.4	59	<0.0001	59.9	<0.0001
BC below age 40 yr	14/27[a] (52%)	282/4725 (6%)	<0.0001	15/242 (6%)	<0.0001
Grade 3					
<40 yr	13/14 (93%)	89/271 (33%)	<0.0001	7/15 (47%)	=0.009
40–<50 yr	5/6 (83%)	222/1001 (22%)	=0.003	12/41 (29%)	=0.02
≥50 y	4/7 (57%)	621/3189 (19%)	=0.03	43/186 (23%)	ns[b] (P = 0.06)
All ages	22/27 (81.5%)	932/4461[c] (21%)	<0.0001	62/242 (26%)	<0.0001
Tubular differentiation grade 3					
<40 yr	13/14 (93%)	ND		7/15 (47%)	=0.009
40–<50 yr	6/6 (100%)	ND		20/41 (49%)	=0.02
≥50 yr	5/7 (71%)	ND		102/186 (55%)	ns
All ages	24/27 (89%)	ND		129/242 (53%)	=0.0004
Nuclear polymorphism grade 3					
<40 yr	13/14 (93%)	ND		6/15 (40%)	=0.004
40–<50 yr	5/6 (83%)	ND		9/41 (22%)	=0.006
≥50 yr	5/7 (71%)	ND		47/186 (25%)	=0.02
All ages	23/27 (85%)	ND		62/242 (26%)	<0.0001
Mitotic index grade 3					
<40 yr	11/14 (79%)	ND		7/15 (47%)	ns (P = 0.08)
40–<50 yr	5/6 (83%)	ND		11/41 (27%)	=0.013
≥50 yr	3/7 (43%)	ND		50/186 (27%)	ns
All ages	19/27 (70%)	ND		68/242 (28%)	<0.0001

[a] Only 27 of 51 invasive breast carcinomas were available for review.
[b] ns, not significant; ND, not done.
[c] Grade was obtained for 4461 of 4725 BCs (>94%).

Table 3 *Histoprognostic grade distribution in BC families*

Family (n = 8[a])	Grade 1	Grade 2	Grade 3	BCs
F101	0	0	3 (30, 33, 36)	3
F322	0	0	3 (28, 38, 39)	3
F326	0	0	3 (27, 37, 48)	3
F519	0	0	3 (42, 51, 53)	3
F49	0	0	2 (33, 38)	2
F417	0	0	2 (48, 50)	2
F338	0	3 (42, 60, 79)	0	3
F73	0	2 (37, 62)	0	2
Total	0	5	16	21
				P = 0.0015

[a] At least two paraffin blocks were available in 8 of 14 families.
[b] Numbers in parentheses, age in years.

BRCA1-associated BCs were significantly younger than registry BC cases, and also younger than the sporadic BC cases, as it is usually observed in HBC.

Histological Type. Among the 27 HBCs, 25 infiltrating ductal carcinomas, 1 infiltrating lobular carcinoma, and 1 colloidal carcinoma were diagnosed. When considered as a whole, we observed a predominance of infiltrating ductal carcinoma in *BRCA1*-associated BCs (92.5%) *versus* 66% of the registry BCs and 69% of the sporadic BCs. Both differences were significant (P = 0.003 and P = 0.01, respectively). The differences were not significant in all subclasses of age, with the exception of cases below the age of 40 years in *BRCA1*-associated BCs (93%) compared to the registry BCs (63%; P = 0.002).

Histoprognosis Grade. A grade 3 disease was found in 81.5% of the *BRCA1*-associated BCs *versus* 21% of the registry BCs and 26% of the sporadic BCs (Table 2). Both differences were significant ($P < 0.0001$) with ORs of 16.7 (6.0–50.2; 95% CI) and of 12.8 (4.3–40.4; 95% CI), respectively. The differences were significant in all subclasses of age, with the exception of patients above the age of 50 years in sporadic BCs (Table 2). In the patients below the age of 40 years, 93% of the *BRCA1*-associated BCs were grade 3 *versus* 33% of the registry BCs and 47% of the sporadic BCs ($P < 0.0001$ and P = 0.009, respectively). Of the patients below the age of 50 years at onset, 83% of the *BRCA1*-associated BCs were grade 3 *versus* 22% of the registry BCs and 29% of the sporadic BCs (P = 0.003 and P = 0.02, respectively). And at 50 years of age and above, 57% of the *BRCA1*-associated BCs were grade 3 *versus* 19% of the registry BCs (P = 0.03).

The three criteria of histoprognosis grade were specified on the sporadic BC population and examined for comparison with *BRCA1*-associated BC (Table 2). When considered as a whole, tubular differentiation grade 3 was found in 89% of the *BRCA1*-associated BCs *versus* 53% of the sporadic BCs [P = 0.0004 and OR = 7.0 (1.9–30.0; 95% CI)]. Nuclear polymorphism grade 3 was found in 85% of the *BRCA1*-associated BCs *versus* 26% of the sporadic BCs [$P < 0.0001$ and OR = 16.7 (5.2–59.5; 95% CI)]. Mitotic index grade 3 was found in 70% of the *BRCA1*-associated BCs *versus* 28% of the sporadic BCs [$P < 0.0001$ and OR = 6.1 (2.4–16.0; 95% CI)]. The prevalence of grade 3 was thus attributed mainly to nuclear polymorphism, but also to tubular differentiation and mitotic index.

Segregation of Histoprognostic Grade in Families. We analyzed the distribution of the histoprognostic grade and its constitutive criteria within families. In 8 of 14 *BRCA1*-associated BC families, blocks from at least 2 relatives were available (5 families with tumors from 3 members), corresponding to 21 different individuals (Table 3). In all eight families, patients exhibited the same histoprognostic grade within the family. Six of them were associated with a grade 3 disease and two with a grade 2. Thus, the distribution of the histoprognostic grade was not random and was specific to a given family with P = 0.0015 (0.0–0.0023; 99% CI). This was attributed to the segregation of the mitotic index criteria only, with P = 0.0005 (0.0–0.0018; 99% CI; Table 4). The lowest mitotic index (mitotic index 1) was restricted to only two families (F73 and F338). For the tubular differentiation and the nuclear polymorphism criteria, significance was not reached.

Histoclinical Heterogeneity of *BRCA1*-associated BC Families. To further assess the prominence of the mitotic index, we compared families in which a mitotic index 1 (population 1) was found *versus* the others (population 2). We compared each subgroup on: age of onset, nuclear polymorphism, and tubular differentiation. Mean age at onset in population 1 was 56 years *versus* 39.3 years in population 2 (P = 0.01). The proportion of nuclear polymorphism grade 3 was 40% in population 1 *versus* 95.5% in population 2 (P = 0.013). For tubular differentiation grade 3, differences were not significant (80% in population 1 *versus* 91% in population 2). Thus, the existence of at least one individual with mitotic index 1 could define a subgroup of families (in our sample 22%) associated with a late onset of BC, a lower mitotic index as observed above, and a lower nuclear polymorphism, regardless of tubular differentiation.

Discussion

The care management of familial BC is often based on an unproven hypothesis, *i.e.*, that no major differences exist between the so-called sporadic cases, especially in terms of their prognosis and natural history. This study confirms the prevalence of grade 3 disease in *BRCA1*-associated BC, even after controlling for other factors (*e.g.*, age). A high frequency of grade 3 in young patients below the age of 40 years has been previously reported (19), but this was out of any familial context. The proportion of grade 3 in this series (27%) was not significantly different (P = 0.12) from those observed in registry BC (33%) and sporadic BC (47%).

To further address the issue of a correlation of grade and prognosis in HBC, we compared each of its constitutive elements. The histoprognosis grade is a combination of three criteria: tubular differentiation (degree of differentiation), nuclear polymorphism, and mitotic index. The high frequency of the histoprognostic grade 3 in *BRCA1*-associated BC could be mainly attributed to the prevalence of nuclear polymorphism grade 3, tubular differentiation grade 3, and mitotic index grade 3. The differences were mostly significant when *BRCA1*-associated BC was considered as a whole. These results suggest that *BRCA1*-associated BCs are highly proliferating tumors. The same trend has also been reported in a population of familial breast carcinoma not selected on the basis of a molecular typing (20).

An important and novel result dealt with the striking intrafamilial distribution of the histoprognostic grade. Such an observation favors the hypothesis that grade segregates as a genetic trait within families. To further explore this finding, we have analyzed the intrafamilial repartition of each criteria and observed that the mitotic index, but not tubular differentiation or nuclear polymorphism, was subordinate to a given family.

Table 4 *Histoprognostic grade criteria distribution in BC families*

Family (n = 8)	BCs	Tubular differentiation	Nuclear polymorphism	Mitotic index
F101	3	3 (3)[a]	3 (3)	3 (3)
F322	3	1 (2);2 (3)	3 (3)	3 (3)
F326	3	3 (3)	3 (3)	3 (3)
F519	3	3 (3)	3 (3)	3 (3)
F49	2	2 (3)	2 (3)	1 (2);1 (3)
F417	2	2 (3)	1 (2);1 (3)	1 (2);1 (3)
F338	3	1 (2);2 (3)	1 (2);2 (3)	2 (1);1 (2)
F73	2	2 (3)	2 (2)	2 (1)
	P	ns[b]	ns	P = 0.0005

[a] 3 (3), three tumors with score 3.
[b] ns, not significant.

From our results, we assume the existence of two subgroups of families, the first (22%) is composed of late onset BC cases with a low mitotic index. The remaining 78% are families with earlier age at onset of BC and a high proliferation rate. Such a finding is reminiscent of the work of Easton *et al.* (21), using penetrance data, who first posited the existence of two types of *BRCA1* alleles: one conferring a BC risk of 39% by the age of 60 years (29% of families) and the other a 62% risk.

The mitotic index and nuclear polymorphism are two criteria which reflect, respectively, the proliferation rate and genetic changes in breast tumors. Our study suggests that the former is genetically determined, whereas the latter is apparently not and may result mainly from environmental factors. This is compatible with Knudson's model and what is known about tumor suppressor genes. The first hit is a germ line mutation and involves the *BRCA1* gene. The others are somatic mutations and affect the remaining copy of the *BRCA1* gene (22) as well as additional loci (23). Interestingly, it has recently been reported that *BRCA1* mRNAs are markedly decreased during the transition from *in situ* carcinoma to invasive BC, and that experimental inhibition of *BRCA1* expression accelerates growth in normal and malignant mammary cells (24). Therefore, grade, through its components, could be interpreted as the morphological translation of the loss of tumor suppressor gene function of *BRCA1* and a consequence of the *BRCA1* germ line mutation.

To date, it has not been possible to correlate a given mutation with a particular phenotype (7–9; *i.e.*, breast only or breast ovarian cancer families). Breast or ovarian involvement does not seem to be determined by the type of mutation and could result from epigenetic factors such as the accumulation of somatic mutations in the respective tissues and specific environmental factors, or could be determined by the existence of modifying genes elsewhere in the genome. Alternatively, there may exist a correlation between the type of germ line mutation and the aggressiveness of the disease. These findings will have an important impact in terms of care management of HBC at the individual and at the familial level. Analysis of cases within families, as well as the type of genetic defect, could be helpful in anticipating the design of preventive strategies (mammography, chemoprevention, or prophylactic surgery) and used to select for appropriate therapeutic protocols.

To confirm the link between grade and prognosis in the case of inheritance, one should observe a reduction in overall survival. This requires follow-up of the subjects in a prospective study. The analysis of additional families is also necessary to establish a correlation with the type of mutation. Since alterations of the *BRCA1* gene seem to contribute to only half of the inherited BCs, a similar study involving *BRCA2*-associated carcinomas, as well as familial tumors unlinked to *BRCA1* and *BRCA2*, should be conducted. Differences between the three types may exist. Alternatively, it is possible that the genetically primed breast tumors differ as a whole from the sporadic cases. When the functions of the different proteins encoded by the susceptibility genes are determined, it will be easier to eliminate the remaining uncertainties.

Acknowledgments

We are particularly indebted to patients and their families. We are grateful to Drs. D. Maraninchi, C. Mawas, B. Devictor, R. Sauvan, and T. J. Mayne for helpful discussions and comments, and to S. Pages and G. Ithier for technical assistance.

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Cancer Res 1996;56:471-474.