Immunohistochemical Characteristics Defined by Tissue Microarray of Hereditary Breast Cancer Not Attributable to *BRCA1* or *BRCA2* Mutations: Differences from Breast Carcinomas Arising in *BRCA1* and *BRCA2* Mutation Carriers[1]

José Palacios,[2] Emiliano Honrado, Ana Osorio, Alicia Cazorla, David Sarrió, Alicia Barroso, Sandra Rodríguez, Juan C. Cigudosa, Orland Diez, Carmen Alonso, Enrique Lerma, Lydia Sánchez, Carmen Rivas, and Javier Benítez

Laboratory of Breast and Gynecological Cancer [J. P., D. S.], Human Genetics Department [E. H., A. O., A. B., S. R., J. C. C., J. B.], Cytogenetics Unit [S. R., J. C. C.], Immunohistological Unit, Centro Nacional de Investigaciones Oncológicas, 28029 Madrid [L. S.]; Department of Pathology, Fundación Jiménez Díaz, Universidad Autónoma, Madrid [A. C., C. R.]; and Department of Genetics [O. D.], Oncology [C. A.], and Pathology [E. L.], Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

ABSTRACT

Purpose: **Most familial breast cancers are not associated with *BRCA1* or *BRCA2* germ-line mutations. Therefore, it is of major importance to define the morphological, immunohistochemical, and molecular features of this group of tumors to improve genetic testing and also gain further insight into the biological characteristics of tumors.**

Experimental Design: **We evaluated the morphological characteristics of 37 tumors arising in women without *BRCA1* or *BRCA2* mutations, 20 tumors from *BRCA1* mutation carriers, and 18 from *BRCA2* mutation carriers, all of which were from index patients from breast cancer families. In addition, a tissue microarray was constructed with all tumoral samples to evaluate the immunohistochemical expression of a wide panel of antibodies (11 antibodies) and the amplification of *HER-2* and *c-MYC* genes by fluorescence *in situ* hybridization. An age-matched group with 50 sporadic breast cancers as controls for non-*BRCA1/2* was also included.**

Results: **Non-*BRCA1/2* infiltrating ductal carcinomas (IDCs) showed specific differences from *BRCA1* tumors. They were of lower grade (1 and 2); more frequently estrogen receptor, progesterone receptor, BCL2 positive, and p53 negative; had a low proliferation rate (Ki-67 immunostaining < 5%); and did not express P-cadherin. With respect to *BRCA2* IDCs and control group, non-*BRCA1/2* tumors were of lower grade and had a lower proliferation rate. No cases of *HER-2* amplification and/or overexpression were observed except in the control group (~20%). In contrast, c-*MYC* amplification was present in 18.2, 62.5, and 12.5% of *BRCA1, BRCA2,* and non-*BRCA1/2* IDCs, respectively, and 31% in the control group.**

Conclusions: **This study thus reveals distinct morphological and immunohistochemical features in non-*BRCA1/2* and *BRCA1* tumors, whereas *BRCA2* tumors present characteristics intermediate between the two phenotypes. In addition, the study also demonstrates the usefulness of tissue microarray technology in the evaluation of the immunophenotypic features of hereditary breast cancer.**

INTRODUCTION

It is currently estimated that 5–10% of all breast cancers are hereditary and attributable to mutations in several high penetrance susceptibility genes, of which only two have been identified: (*a*) *BRCA1* (OMIM 113705; Ref. 1); and (*b*) *BRCA2* (OMIM 600185; Ref. 2).

Earlier estimates suggested that *BRCA1* and *BRCA2* mutations were responsible for 75% of site-specific breast cancer families and the majority of breast and ovarian cancer families (3, 4). Recent data show, however, that these percentages may have been overestimated and that the proportion of families classified caused by mutations in *BRCA1* or *BRCA2* is much lower and strongly depends on the population analyzed (5) and the specific characteristics of the selected families (6, 7). In fact, the percentage of high-risk families associated with mutations in these genes is very similar (~25%) in all series, including the one we have found in Spain (8, 9).

Genetic testing for *BRCA1* and *BRCA2* is expensive and time consuming because of the large size of both genes, the absence of hot spots for mutations throughout their entire coding region, and the low percentage of mutated cases. It is therefore important to find clinical or pathological factors that could suggest or exclude the presence of *BRCA1* or *BRCA2* mutations in a given patient.

It has recently been demonstrated that cancer arising in carriers of mutation in the *BRCA1* and *BRCA2* genes differs from sporadic breast cancer of age-matched control (10–13). The differences are more evident and have been more exten-

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[2] To whom requests for reprints should be addressed, at Laboratory of Breast and Gynecological Cancer, Molecular Pathology Programme, Centro Nacional Investigaciones Oncológicas, Melchor Fernández Almagro, 3, 28029 Madrid, Spain. Phone: 34 912246952; Fax: 34 912246923; E-mail: jpalacios@cnio.es.

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sively documented in breast cancer from *BRCA1* mutation carriers. These tumors are poorly differentiated IDCs,[3] with higher mitotic counts and pleomorphism, and less tubule formation than in sporadic tumors. In addition, more cases with the morphological features of typical or atypical medullary carcinoma are observed in these patients. Breast carcinomas from *BRCA2* mutation carriers tend to be of higher grade than sporadic age-matched controls. Some differences also exist in the morphology of non-*BRCA1/2* hereditary breast cancer with respect to cancers attributable to *BRCA1* and *BRCA2*. They are of lower grade, and more lobular carcinomas are diagnosed when compared with *BRCA1* tumors (14).

The immunophenotypic features of breast carcinomas arising in *BRCA1* and *BRCA2* mutation carriers have been evaluated in some series (15–18). *BRCA1* tumors have been found to be more frequently ER and PR negative and p53 positive than age-matched controls, whereas these differences are not usually found in *BRCA2*-associated tumors (17). In addition, *BRCA1* and *BRCA2*-associated breast carcinomas show a low frequency of HER2 expression (16, 18, 19).

To date, there have been no studies evaluating the immunophenotypic characteristics of familial non-*BRCA1/2* breast cancer. Most familial breast cancers are not associated with *BRCA1* or *BRCA2* germ-line mutations, and so it is of major importance to define the immunohistochemical features of this group of tumors to carry out genetic testing more effectively and also to gain insight into the biological characteristics of the tumors. Here we report the morphological, immunohistochemical, and genetic differences, revealed by FISH studies, in a series of familial non-*BRCA1/2* tumors in which *BRCA1*- and *BRCA2*-associated tumor groups are compared by means of a TMA. We have found great differences between *BRCA1* and familial non-*BRCA1/2* tumors, whereas *BRCA2* presented intermediate characteristics.

MATERIALS AND METHODS

Patients. Patients were drawn from three centers in Spain: Centro Nacional Investigaciones Oncológicas and the Fundación Jimenez Díaz in Madrid and the Hospital Sant Pau in Barcelona. They were selected from breast cancer families containing at least three women affected with breast cancer, one of them <50 years of age, or with ovarian cancer, or with male breast cancer (8, 9, 20).

Mean ages were 41.6 and 43 for *BRCA1* and *BRCA2* and 50.8 for non-*BRCA1/2* patients. In addition, we selected an age-matched control group for non-*BRCA1/2* patients that included 50 patients with sporadic breast cancer (mean age 49.9).

Genetic Studies. The index case of each family was screened for mutations in the *BRCA1* and *BRCA2* genes by a combination of single-strand conformational polymorphism, conformation-sensitive gel electrophoresis, and protein truncate technique. Some of these results have been published previously (8, 9, 20). In one patient with a diagnosis of lobular carcinoma and no mutation in *BRCA1/2* genes, the *E-CD* gene was screened for mutations by PCR and sequencing. All 16 exons, including exon-intron boundaries, were PCR amplified individually using the specific primers and conditions reported previously (21, 22).

Table 1 Antibodies used in the immunohistochemical study

Antibody	Clone	Dilution	Supplier
ER	1D5	1:30	Novocastra
PR	1A6	1:30	Novocastra
BCL2	124	1:80	DAKO
Ki-67	MIB1	1:30	DAKO
P53	DO-7	1:50	Novocastra
HER-2 (IHC)	Poly	1:2000	DAKO
E-CD	4A2C7	1:200	Zymed
P-CD	56	1:200	Transduction Labs
β-Catenin	14	1:1000	Transduction Labs
γ-Catenin	15	1:1000	Transduction Labs
$p120^{ctn}$	98	1:500	Transduction Labs

Morphological Evaluation. After the genetic screening, we selected tumor samples from 37 index patients without mutations in *BRCA1* or *BRCA2* and 38 with mutation in *BRCA1* (20 tumors) or *BRCA2* (18 tumors). Two pathologists (J. P. and E. H.), who had no knowledge of the germ-line mutation status or family history, reviewed one representative histological slide from each patient. The Nottingham histological grading system was used to assess the grade of IDCs (23).

TMA Construction. Representative areas of the different lesions were carefully selected on H&E-stained sections and marked on individual paraffin blocks. Two tissue cores (1-mm diameter) were obtained from each specimen. In addition, 10 non-neoplastic breast tissue samples were included as controls. The tissue cores were precisely arrayed into a new paraffin block using a TMA workstation (Beecher Instruments, Silver Spring, MD) as described previously (24). The final TMA consisted of 188 1-mm diameter TMA cores each spaced at 0.8 mm from between core centers. An H&E-stained section was reviewed to confirm the presence of morphologically representative areas of the original lesions.

Immunohistochemistry. Immunohistochemical staining was performed by the Labeled Streptoavidin Biotin method (DAKO, Glostrup, Denmark) with a heat-induced antigen retrieval step. Sections from the tissue array were immersed in boiling 10 mM sodium citrate at pH 6.5 for 2 min in a pressure cooker. Antibodies, dilutions, and suppliers are listed in Table 1.

Three pathologists (J. P., E. H., and C. R.) simultaneously evaluated the immunohistochemical staining. The percentage of stained nuclei, independent of the intensity, was scored for ER, PR, Ki-67, and p53. In the same way, the percentage of cells with cytoplasmic stain was scored for BCL2. To evaluate HER2 and cadherins/catenins, the percentage of cells with membranous staining and intensity were evaluated. For categorical analysis, a case was considered positive when ≥10, 10, 25, and 70% of the cells were stained with ER, PR, p53, and BCL2, respectively (25). Three categories were defined for Ki-67: 0–5, 6–25, and >25% of stained nuclei. HER2 was evaluated ac-

[3] The abbreviations used are: IDC, infiltrating ductal carcinoma; ER, estrogen receptor; P-CD, P-cadherin; E-CD, E-cadherin; TMA, tissue microarray; PR, progesterone receptor; DCIS, ductal carcinomas *in situ*; ILC, infiltrating lobular carcinoma; FISH, fluorescence *in situ* hybridization.

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cording to the four category (0–3+) DAKO system proposed for the evaluation of the HercepTest. A tumor was considered to have preserved expression of E-CD and catenins (β-, γ-catenin, and p120^{ctn}) expression when ≥75% of the cells showed complete membranous staining of similar intensity to normal breast epithelium (26). Other cases were considered to have reduced E-CD or catenin expression. Aberrant P-CD expression was diagnosed when ≥10% of the neoplastic cells expressed this protein in their membrane (27). Cases were evaluated for the possibility of aberrant cytoplasmic or nuclear expression of catenins.

FISH. We used 4-μm sections of the TMAs to carry out FISH analysis, which was performed using two different sets of probes. For the detection of *HER-2* amplification, we used the commercial probe from Vysis (Downer's Grove, IL), which spans the entire *HER-2* gene, and is labeled in SpectrumOrange. This probe also contains a centromeric probe for chromosome 17, which is labeled in SpectrumGreen and hybridizes to the α satellite DNA located at the centromere of chromosome 17 (17p11.1-q11.1). For the detection of *c-MYC* amplification, we used the *IGH/MYC*, CEP 8 Tri-color, Dual Fusion Translocation Probe from Vysis (Downer's Grove, IL). This probe is a mixture of a 1.5-Mb SpectrumGreen-labeled IGH probe, a ~750-kb SpectrumOrange-labeled *MYC* probe, and the SpectrumAqua-labeled CEP 8 probe. The large *c-MYC* probe extends ~400 kb upstream of *c-MYC* and ~350 kb 3′ beyond *c-MYC*. The CEP 8 probe targets the α satellite sequences on chromosome 8. Inclusion of the CEP 8 and 17 probes was used in dual hybridizations with the *c-MYC* and *HER-2* probes, respectively, to differentiate between increases in the number of signals attributable to increases of the number of copies of the genes and those attributable to increases in the number of chromosome homologues. These probes were also used as an internal control for chromosomes 8 and 17 aneusomies.

Hybridization was carried out according to the manufacturer's instructions with slight modifications. The slides were deparaffinized, boiled in a pressure cooker with 1 mM EDTA (pH 8.0) for 10 min, and incubated with pepsin at 37°C for 30 min. The slides were then dehydrated. The probe was denatured at 75°C for 2 min before overnight hybridization at 37°C in a humid chamber. Slides were washed with 0.4× SSC and 0.3% NP40.

The FISH analysis was performed by two investigators (S. R. and J. C. C.) with no previous knowledge of the genetic, clinical, or IHC results. Scoring of fluorescence signals was made accordingly to previous reports (18, 28). Briefly, in each sample, an average of 110 (50–200) well-defined nuclei was analyzed, and the number of single copy gene and centromeric signals was scored. Amplification was defined as the presence (in ≥5% of tumor cells) of either ≥8 gene signals or more than three times as many gene signals as centromere signals of the chromosome (28). The number of whole chromosome copies was scored as monosomy when only signal of the CEP probes was observed or polysomy when three or more signals were observed. The cutoff values for the copy number changes were obtained from the analysis of normal adjacent epithelium in each experiment.

Statistical Analysis. The χ^2 contingency test with Yates correction or Fisher's exact test, for categorical variables, and two-sided Student's *t* test, for continuous variables, were used to determine differences between genotypes. The SPSS for Windows statistical program (SPSS, Inc., Chicago, IL) was used for this analysis.

Table 2 Distribution of morphological characteristics in familial breast cancer

	BRCA1 *n* (%)	*P*	BRCA(−) *n* (%)	*P*	BRCA2 *n* (%)
Histological type					
IDC	19 (95.0)		29 (78.4)		15 (83.3)
DCIS	1 (5.0)		5 (13.5)		2 (11.1)
ILC	0	NS	3 (8.1)	NS	1 (5.6)
Grade[a]					
1	0		14 (50)		1 (7.1)
2	3 (15.8)		8 (28.6)		5 (35.7)
3	16 (84.2)	<0.0001	6 (21.4)	0.014	8 (57.1)
Tubule score[a]					
1	1 (5.3)		9 (32.2)		0
2	3 (15.8)		6 (21.4)		5 (35.7)
3	15 (78.9)	0.049	13 (46.4)	NS (0.055)	9 (64.3)
Mitotic count[a]					
1	2 (10.5)		17 (60.7)		4 (28.6)
2	2 (10.5)		6 (21.4)		2 (14.3)
3	15 (78.9)	<0.0001	5 (17.9)	0.033	8 (57.1)
Pleomorphism score[a]					
1	0		3 (10.7)		0
2	2 (10.5)		16 (57.1)		5 (35.7)
3	17 (89.5)	0.001	9 (32.1)	NS (0.09)	9 (64.3)

[a] Only including the infiltrating ductal tumors. NS, not statistically significant.

RESULTS

Morphological Study. Table 2 summarizes the distribution of morphological features in this series of hereditary breast carcinomas. The most common histological type in all three groups was IDC of no special type. DCISs and ILCs were less frequently seen in *BRCA1*-associated tumors, although the differences were not statistically significant. ILCs in this series included three classic and one pleomorphic variants, this latter occurring in a non-*BRCA1/2* patient.

Non-*BRCA1/2* IDCs were of lower grade than in *BRCA1* ($P < 0.0001$) and *BRCA2* ($P = 0.014$) tumors. Non-*BRCA1/2* IDCs showed more tubule formation ($P = 0.049$), fewer mitotic figures ($P < 0.0001$), and less nuclear pleomorphism ($P = 0.0001$) than did *BRCA1* IDCs. These differences were also found when comparing non-*BRCA1/2* and *BRCA2* tumors, although only the mitotic count was statistically significant ($P = 0.033$; $P = 0.055$ for tubule score, and $P = 0.099$ for nuclear pleomorphism). Representative cases of BRCA1 and non-*BRCA1/2* IDCs are presented in Fig. 1, *A* and *B*. A representative area of the constructed TMA is illustrated in Fig. 1*C*.

Immunohistochemical Study. We analyzed significant differences in the immunohistochemical profile according to genotypes only in the IDC group. DCISs and ILCs were excluded from this analysis because of their scarcity and different biological characteristics. The results are summarized in Table 3.

Immunohistochemical differences for ER, PR, BCL2, Ki-67, and p53 were found after categorization of the variables according to the thresholds defined previously between non-

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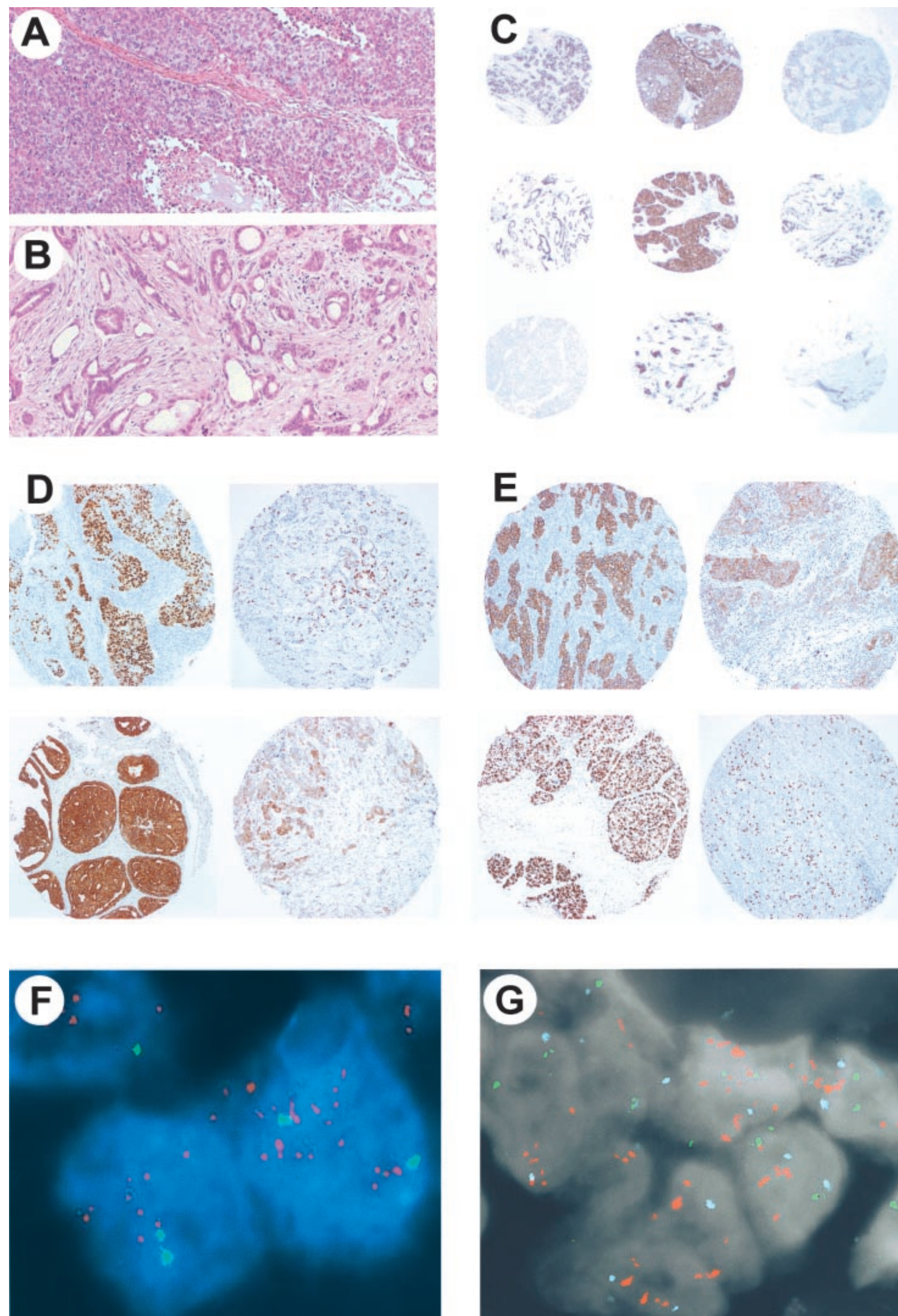

Fig. 1 Morphological, immunohistochemical, and genetic characteristics of familial breast cancer. *A,* poorly differentiated (grade 3) IDC from a *BRCA1* mutation carrier (hematoxylin). *B,* well-differentiated (grade 1) non-*BRCA1/2* IDC (hematoxylin). *C,* overview of part of a TMA section immunostained for ER. *D,* examples of tumors (1-mm diameter single punches) showing different levels of expression of progesterone receptor (*top,* compare *left* with *right*) and BCL2 (*bottom, left,* and *right*). *E,* different levels of HER2 expression in infiltrating ductal tumors (*top,* compare *left* with *right* samples) and Ki-67 (*bottom*). *F, HER-2* amplification in the case overexpressing HER2. The mean copy number was of 16. The picture shows two cells with 10 and 22 copies of *HER-2* (stained in *orange*) in comparison with two centromeric copies for chromosome 17 (*green*). *G, c-MYC* amplification in a BRCA2 tumor with a mean number copies of 8. In the picture, the number of *c-MYC* copies (stained in *red*) ranges from 6 to 10, in comparison with centromeric signals for chromosome 8 (*blue*) and *IG* (chromosome 14; *green*).

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Table 3 Distribution of immunohistochemical characteristics in familial IDCs

	BRCA1 *n* (%)	*P*	BRCA(−) *n* (%)	*P*	BRCA2 *n* (%)
ER					
Negative	14 (73.7)		7 (25.0)		1 (7.1)
Positive	5 (26.3)	0.001	21 (75.0)	NS	13 (92.9)
PR					
Negative	15 (75)		9 (32.1)		3 (21.4)
Positive	4 (21.1)	0.002	19 (67.9)	NS	11 (78.6)
BCL2					
Negative	17 (89.5)		12 (44)		8 (57.1)
Positive	2 (10.5)	0.005	15 (55.6)	NS	6 (42.9)
Ki-67					
0–5%	5 (26.3)		25 (92.6)		8 (57.1)
6–25%	8 (42.1)		2 (7.4)		5 (35.7)
>25%	6 (31.6)	<0.0001	0	0.021	1 (7.1)
P53					
Negative	9 (47.4)		26 (96.3)		11 (84.6)
Positive	10 (52.6)	0.000	1 (3.7)	NS	2 (15.4)
HER-2 (IHC)					
Negative (0/+)	19 (100)		26 (96.3)		9 (64.3)
Positive (++)	0		0		5 (35.7)
Positive (+++)	0	NS	1 (3.7)	0.004	0
E-CD					
Preserved	9 (47.4)		12 (42.9)		12 (80.0)
Reduced	10 (52.6)	NS	16 (57.1)	0.019	3 (20.0)
P-CD					
Absent	15 (78.9)		29 (100)		14 (93.3)
Present	4 (21.1)	0.010	0	NS	1 (6.7)
β-catenin					
Preserved	6 (31.6)		6 (21.4)		7 (46.7)
Reduced	13 (68.4)	NS	22 (78.6)	NS	8 (53.3)
γ-catenin					
Preserved	5 (27.8)		6 (21.4)		7 (46.7)
Reduced	13 (72.2)	NS	22 (78.6)	NS	8 (53.3)
$p120^{ctn}$					
Preserved	4 (22.2)		7 (25.9)		5 (38.5)
Reduced	14 (77.8)	NS	20 (74.1)	NS	8 (61.5)

BRCA1/2 and *BRCA1* tumors (Fig. 1, *C–E*). Non-*BRCA1/2* IDCs were more frequently ER positive, PR positive, BCL2 positive, and p53 negative and had a lower proliferation index than *BRCA1* IDCs. In contrast, we observed that P-CD expression was significantly more frequent in *BRCA1* than in non-*BRCA1/2* IDCs. No statistically significant differences were found in the expression of HER-2, E-CD, β- and γ-catenin, and $p120^{ctn}$. When compared with *BRCA2* IDCs, non-*BRCA1/2* showed a lower proliferation index, a different pattern of expression of HER2, and less frequently preserved expression of E-CD.

A tumor of the lobular histotype from a non-*BRCA1/2* patient was investigated for *E-CD* gene germ-line mutations, but none was found.

FISH Study. FISH analysis of *HER-2* gave valuable results in 45 IDCs (Table 4). *HER-2* amplification was found in 1 non-*BRCA1/2* IDC with high level of HER2 expression (3+; Fig. 1*F*). No cases of amplification were observed in *BRCA1* or *BRCA2* IDCs. In addition to amplification, other chromosome aberrations were observed: 7 polysomy cases (5 non-*BRCA1/2* and 2 *BRCA2* carcinomas) and 7 monosomies (5 *BRCA1* and 2 non-*BRCA1/2* carcinomas).

FISH analysis of *c-MYC* resulted in valuable results in 35

Table 4 Distribution of FISH characteristics in familial IDCs

	BRCA1 *n* (%)	*P*	BRCA(−) *n* (%)	*P*	BRCA2 *n* (%)
HER-2					
Negative	14 (100)		20 (95.2)		10 (100)
Positive	0	NS	1 (4.8)	NS	0
c-MYC					
Negative	9 (81.8)		14 (87.5)		3 (37.5)
Positive	2 (18.2)	NS	2 (12.5)	0.011	5 (62.5)

IDCs. *c-MYC* amplification was significantly more frequent in *BRCA2*-associated cases (Table 4). It was observed in 2 (18.2%), 5 (62.5%), and 2 (12.%) *BRCA1, BRCA2,* and non-*BRCA1/2* IDCs, respectively. A representative case of *c-MYC* amplification in a *BRCA2*-associated IDC is shown in Fig. 1*G*. No cases showed amplification of both *HER-2* and *c-MYC*.

Comparison with the Control Group. Because we observed differences in the morphological and immunohistochemical profile between non-*BRCA1/2* IDCs and those observed in *BRCA1* and *BRCA2* patients, we assessed whether or not differences also existed between non-*BRCA1/2* and an age-matched control group of sporadic breast cancer IDCs (Table 5). We observed that non-*BRCA1/2* IDCs were of lesser grade, had a lower proliferative index, were more frequently p53 negative and HER2 negative, and had more frequently reduced expression of E-CD and β-catenin than sporadic cases. FISH with *HER-2* and c-*MYC* probes were both amplified in ~20 and 30% of cases of control group but without significance.

DISCUSSION

The morphological results from non-*BRCA1/2* breast carcinomas and those arising in *BRCA1* and *BRCA2* mutation carriers are similar to those recently reported by Lakhani *et al.* (14). We observed a low percentage of DCISs and ILCs in *BRCA1*-mutation carriers (only one case). When compared with *BRCA1* and *BRCA2* IDCs, non-*BRCA1/2* IDCs were of lower grade because of the greater tubule formation, lower mitotic count, and less nuclear pleomorphism. These differences were more pronounced between non-*BRCA1/2* and *BRCA1* tumors. Because the present series reflected the morphological differences between distinct genotypes in hereditary breast cancer, we considered that it was an appropriate sample with which to explore immunohistochemical differences further.

We used the recently developed TMA technology for this purpose because it allows the analysis of a large number of samples and markers without producing methodological variations. A major concern surrounding the TMA technique is the extent to which tumor heterogeneity may affect the validity of the results. This issue has been addressed in an earlier series of TMA studies, which demonstrated that all previous findings from large sections could be fully reproduced in TMA studies (24, 28). Thus, our data on ER, PR, and p53, the immunohistochemical markers most commonly studied in *BRCA1/2*-associated breast carcinomas, were similar to those reported previously (16–18), thereby confirming the usefulness of the TMA approach.

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Table 5 Distribution of immunohistochemical characteristics in familial non-*BRCA1/2* tumors and control group

	Non-*BRCA1/2* *n* (%)	*P*	Control group (IDCs) *n* (%)
Grade			
1	14 (50)		10 (20.8)
2	8 (28.6)		15 (31.3)
3	6 (21.4)	0.018	23 (47.9)
ER			
Negative	7 (25.0)		15 (31.3)
Positive	21 (75.0)	NS	33 (68.8)
PR			
Negative	9 (32.1)		22 (44.9)
Positive	19 (67.9)	NS	27 (55.1)
BCL2			
Negative	12 (44)		26 (54.2)
Positive	15 (55.6)	NS	22 (45.8)
Ki-67			
0–5%	25 (92.6)		26 (53.1)
6–25%	2 (7.4)		18 (36.7)
>25%	0	0.002	5 (10.2)
P53			
Negative	26 (96.3)		33 (68.8)
Positive	1 (3.7)	0.005	15 (31.3)
HER-2 (IHC)			
Negative (0/+)	26 (96.3)		36 (73.5)
Positive (++)	0		4 (8.2)
Positive (+++)	1 (3.7)	0.000	9 (18.4)
E-CD			
Preserved	12 (42.9)		32 (68.1)
Reduced	16 (57.1)	0.032	15 (31.9)
P-CD			
Absent	29 (100)		44 (93.6)
Present	0	NS	3 (6.4)
β-catenin			
Preserved	6 (21.4)		30 (65.2)
Reduced	22 (78.6)	0.000	16 (34.8)
γ-catenin			
Preserved	6 (21.4)		13 (28.3)
Reduced	22 (78.6)	NS	33 (71.4)
p120^{ctn}			
Preserved	7 (25.9)		17 (37.0)
Reduced	20 (74.1)	NS	24 (63.0)
HER-2[a]			
Negative	20 (95.2)		37 (80.5)
Positive	1 (4.8)	NS	9 (19.5)
c-MYC[a]			
Negative	14 (87.5)		31 (68.9)
Positive	2 (12.5)	NS	14 (31.1)

[a] By FISH analysis.

In our series, non-*BRCA1/2*- and *BRCA2*-associated IDCs were frequently ER positive: 82 and 92% respectively, compared with only 35% in *BRCA1*-associated tumors. It has been suggested that the immunohistochemical analysis of ER provides a new powerful predictor of *BRCA1* mutation status. It has been estimated that the probability of a woman with familial breast cancer diagnosed before the age of 35 years to be a *BRCA1*-mutation carrier is 25% if the tumor is grade 3 and ER negative. However, the probability is only 5% if the tumor is ER positive (17, 18). In accordance with our results on ER expression, we also observed that non-*BRCA1/2* and *BRCA2* expressed PR and BCL2 more frequently than *BRCA1* tumors. These results imply that hormone receptor characteristics of breast cancer should be taken into account when recommending chemoprevention strategies in these patients. Because tamoxifen does not reduce the incidence of cancer in ER-negative tumors (29), it is not yet clear whether this drug could reduce breast cancer incidence in women carrying *BRCA1* mutations. By contrast, *BRCA2* and non-*BRCA1/2* mutation carriers are probably good candidates for chemoprevention with tamoxifen or other hormonal agents (30).

Our study revealed a very low incidence of p53 immunostaining in non-*BRCA1/2* tumors (3.7%) compared with *BRCA1* (50%) and *BRCA2* (16%) tumors. Most previous studies have demonstrated a higher positivity of p53 in tumors from *BRCA1* mutation carriers, although the results in *BRCA2* tumors are inconclusive (17, 18). Because p53 positivity has been associated with more aggressive tumors, a higher grade, an absence of hormone receptors, and a worse prognosis, our results in non-*BRCA1/2* tumors are consistent with the morphological status that we have observed in this group (~80% grade 1–2 and ER positive). Hence, p53 could represent an important immunohistochemical factor in the evaluation of familial breast cancer.

Our study evaluating Ki-67 expression revealed differences in the proliferation rate between groups. The proliferation rate was high, medium, and very low in *BRCA1, BRCA2,* and non-*BRCA1/2* tumors, respectively. The importance of a high proliferation rate assessed by immunohistochemistry was indicated by a study of familial breast cancers diagnosed before the age of 35 years or in women with a strong history of breast and/or ovarian cancer. In this series, grade 3 tumors that were ER negative and had a high proliferation rate (using Ki-67 immunohistochemistry) showed 53% of mutations in *BRCA1* in contrast with 0–5% in cases that did not fit these criteria (31). At the other end of the spectrum, our study suggests that a tumor that is well differentiated, ER positive, p53 negative, and with a low proliferation rate (<5%) has a high probability of being a non-*BRCA1/2* carcinoma.

Most studies of HER2 expression in *BRCA1*- and *BRCA2*-associated breast carcinomas have revealed a low frequency of HER2 expression when compared with sporadic tumors (18). We have obtained similar results in our series of *BRCA1* and *BRCA2* carcinomas and also observed a low incidence of HER2 expression in non-*BRCA1/2* IDCs. With regard to the *HER-2* amplification, only one previous study has analyzed gene amplification in hereditary *BRCA1*-associated carcinomas (19), but no reports are available concerning *HER-2* gene amplification in *BRCA2*- and non-*BRCA1/2*-associated carcinomas. Our study not only confirms the absence of *HER-2* amplifications in *BRCA1* tumors but also shows for the first time that *HER-2* amplification is infrequent in both *BRCA2* non-*BRCA1/2*-associated IDCs. Results from this and previous studies suggest that women with familial breast cancer are probably not good candidates for Herceptin therapy.

Given that high-grade tumors are usually associated with HER2 overexpression and/or amplification, it has been suggested that the low incidence of *HER-2* amplification in *BRCA1* carcinomas may be caused by a physical codeletion of one *HER-2* allele and nearby sequences during the loss of heterozygosity at the *BRCA1* locus (19). This frequently would occur in these tumors as the second mechanism of inactivation (32). We

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observed 35% of monosomic cases in *BRCA1*-associated tumors but only 9.5 and 0% in non-*BRCA1/2* and *BRCA2* tumors, confirming that monosomy of chromosome 17 is a frequent event. Thus, the presence of both alleles of these genes would be essential for gene amplification of *HER-2,* whereas other pathways would be responsible for the morpho-phenotype and aggressiveness of *BRCA1* tumors (19). In contrast, 23 and 20% of polysomic cases (four to five copies) were found in non-*BRCA1/2* and *BRCA2* tumors, respectively, but none was found in the *BRCA1* tumors. In all these cases, there was low or intermediate HER2 expression (1+/2+), which confirms the usefulness of assessing *HER-2* status by FISH in cases with positive 2+ expression.

This is the first study to analyze *c-MYC* amplification in hereditary breast cancer. *c-MYC* is amplified in ~15–20% of sporadic breast cancers, and it is significantly associated with the absence of ER and PR, high histological grade, and high proliferation rate (33, 34). Here, we have found that 16% of *BRCA1* tumors and 12% of non-*BRCA1/2* tumors had *c-MYC* amplification, which is similar to the observations in sporadic cases. There was, however, a high frequency of *c-MYC* amplification in *BRCA2*-associated IDCs (62%), although the number of analyzed cases was scarce. If these differences in *c-MYC* amplification were to be confirmed in additional series, this marker would be a good predictor of *BRCA2*-associated carcinomas.

In this series, we carried out an analysis of cadherins and catenins, because these molecules participate in tumor initiation, differentiation, and progression in breast cancer. E-CD and P-CD are transmembrane cell–cell adhesion receptors of the cadherin superfamily, which are expressed in epithelial and myoepithelial breast cells, respectively. We observed aberrant P-CD expression only in *BRCA1*-associated IDCs. This is not surprising because aberrant P-CD expression is related to cell proliferation, dedifferentiation, and negative estrogen and PRs in sporadic breast cancer (27), and each of these characteristics is present in *BRCA1*-associated IDCs. We found a similar percentage of E-CD cases with reduced expression in non-*BRCA1/2*-associated carcinomas as has been reported in sporadic tumors (27). Taking into account the morphological characteristics of *BRCA1*- and *BRCA2*-associated IDCs, more cases with reduced E-CD expression would be expected in these groups. In contrast, and despite a high percentage of grade 2 and 3 *BRCA2*-associated carcinomas, most cases in the *BRCA2* group maintained their E-CD expression.

Germ-line mutations of the *CDH1* gene have been described in families with early onset diffuse gastric cancer, some of which are associated with lobular breast carcinomas (35). Considering these previous observations, it is reasonable to speculate that some familial lobular breast cancer that is not attributable to *BRCA1* or *BRCA2* mutations could instead be attributed to germ-line mutations in *CDH1*. We did, however, screen a patient with a non-*BRCA1/2* lobular breast carcinoma for germ-line mutation in the complete coding sequence of *CDH1* and did not find any genetic alterations. Similar results were obtained by Salahshor *et al.* (36) in patients with a positive family history and by Rahman *et al.* (37) in a large sample of lobular carcinoma *in situ*.

Finally, the question of whether or not non-*BRCA1/2* familial IDCs have different morphological and immunohistochemical features than sporadic breast carcinomas has not been investigated previously. In an age-matched control group, we observed that non-*BRCA1/2* familial IDCs had a less aggressive phenotype than sporadic cases, because they had a lower grade and proliferation index and were more frequently p53 and HER2 negative. In contrast, non-*BRCA1/2* familial IDCs showed more frequently reduced cadherin E expression, suggesting that the mechanisms responsible for E-CD-mediated cell adhesion may be different in sporadic and hereditary breast cancer

In summary, although non-*BRCA1/2* tumors can be heterogeneous from a genetic point of view (38), we have found some frequent characteristics in this group. These differences are important when we compare with *BRCA1* tumors and are intermediate *versus BRCA2*. Familial breast carcinomas arising in women who lack germ-line mutations in *BRCA1* and *BRCA2* genes are more frequently low-grade IDCs (grade 1–2) and ER, PR, and BCL2 positive. The proliferation rate assessed by Ki-67 expression is extremely low, and p53 and P-CD expression are very infrequent if not absent in this group of tumors. With regard to *BRCA2* IDCs, the differences are less specific. A higher proliferation rate, frequent normal E-CD expression, and a higher frequency of *c-MYC* amplification were the most striking features distinguishing these tumors from non-*BRCA1/2* carcinomas, although additional studies are necessary to confirm these preliminary results. Our study also shows that non-BRCA1/2 tumors are less aggressive, less proliferative, and have a lower grade than sporadic breast tumors matched by age. Finally, we demonstrate the usefulness of TMA technology in the evaluation of the immunohistochemical characteristics of large samples of familial breast cancer. Delineation of the morphological, immunohistochemical, and molecular features of familial breast cancer will help in the selection of candidate patients for *BRCA* gene studies.

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