Advances in Brief

The Pathology of Familial Breast Cancer: Histological Features of Cancers in Families Not Attributable to Mutations in *BRCA1* or *BRCA2*[1]

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Abstract

Breast cancers arising in carriers of mutations in the breast cancer susceptibility genes, *BRCA1* and *BRCA2*, differ histologically from each other and from breast cancers unselected for a family history. However, a substantial proportion of families with multiple cases of breast cancer is not attributable to these two genes (non-*BRCA1/2* families). We have now characterized the pathology of 82 breast cancers from non-*BRCA1/2* families. Breast cancers in non-*BRCA1/2* families were of lower grade ($P = 0.0018$), showed fewer mitoses ($P < 0.0001$), less nuclear pleomorphism ($P = 0.0014$), less lymphocytic infiltrate ($P < 0.0001$), a lesser extent of the tumor with a continuous pushing margin ($P = 0.004$), a lesser extent of the tumor composed of solid sheets of cells ($P = 0.0047$), less necrosis ($P = 0.002$), and were more likely to be of invasive lobular type ($P = 0.0003$) than breast cancers arising in *BRCA1* mutation carriers. In com-

Received 9/27/99; revised 12/13/99; accepted 12/14/99.
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[1] The pathology review and meetings of the Breast Cancer Linkage Consortium were supported by the European Union Concerted Action. We would also like to acknowledge the support of the Cancer Research Campaign, South Thames Regional Health Authority, Imperial Cancer Research Fund, NIH Grant CA61231 (to B. L. W.), La Ligue Nationale contre le Cancer, FEGEFLUC, Association pour la Recherche sur le Cancer, Groupement de Recherche et d'Etude sur les Genomes, The Dutch Cancer Society, Deutsche Krebshilfe e.V, the Icelandic Cancer Society Science Fund, the Nordic Cancer Union, Schweizer Krebsliga, and Apple Computer United Kingdom Ltd.
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parison with *BRCA2* tumors, non-*BRCA1*/2 tumors were lower grade ($P = 0.017$) and exhibited less pleomorphism ($P = 0.01$) and more tubule formation ($P = 0.05$). In comparison with control breast cancers unselected for a family history of the disease, non-*BRCA1*/2 tumors were of significantly lower grade ($P = 0.001$), showed less pleomorphism ($P = 0.0002$), and had a lower mitotic count ($P = 0.003$). The results indicate that non-*BRCA1/2* breast cancers differ histologically from both *BRCA1* and *BRCA2* breast cancers and are overall of lower grade. They also suggest that non-*BRCA1/2* breast cancers differ from nonfamilial breast cancers, but these differences may be attributable to various types of bias.

Introduction

In a large collaborative study on behalf of the Breast Cancer Linkage Consortium, we characterized previously the histological features of breast cancers arising in carriers of mutations in the *BRCA1* and *BRCA2* genes (1, 2). Breast cancers attributable to mutations in *BRCA1* were of higher grade and were characterized by greater numbers of mitoses, a greater degree of cellular pleomorphism, and less tubule formation than age-matched breast cancers unselected for family history. A number of other histological features were associated with breast cancers in *BRCA1* carriers. These included an excess of medullary and atypical medullary cancers; a greater extent of confluent necrosis; more lymphocytic infiltrate; a greater proportion of cases with a smooth, noninfiltrative edge to the cancer (also known as pushing margins); a greater proportion of cancers that had extensive solid sheets of cells; and the presence of prominent nucleoli. Multifactorial analysis demonstrated that many of these factors were associated with each other. Elevated mitotic count, the presence of a lymphocytic infiltrate, and the presence of a smooth noninfiltrative border to the cancer were independently associated with *BRCA1*, but all other features became nonsignificant.

Breast cancers attributable to *BRCA2* mutations were also of higher overall grade, predominantly as a result of exhibiting less tubule formation, but were not significantly different from controls with respect to mitoses and pleomorphism. Among other features evaluated, a greater proportion of *BRCA2* cancers had a smooth noninfiltrative border than controls. In the multifactorial analysis, both the reduction in tubule formation and the presence of continuous pushing margins were significantly associated with *BRCA2*. The findings with respect to *BRCA1* are broadly in agreement with other smaller series, but only a limited number of breast cancers attributable to *BRCA2* have been evaluated in detail by other groups (3–11).

Recent analyses by the Breast Cancer Linkage Consortium indicate that most families with both breast and ovarian cancer or with six or more cases of early-onset breast cancers are attributable to mutations in *BRCA1* and *BRCA2* (12). However, only one-third of families with four or five cases of breast cancer and no ovarian cancer are attributable to the known genes, providing strong evidence that a substantial proportion of breast cancer susceptibility is not accounted for by *BRCA1* and *BRCA2*. The existence of further susceptibility genes is supported by a recent analysis of a population-based series of early-onset breast cancer cases for mutations in *BRCA1* and *BRCA2*. In this study, <20% of the risk to first-degree relatives conferred by a case of breast cancer diagnosed at age 45 or less was attributable to *BRCA1* and *BRCA2* (13). Both family-based and population-based studies indicate that mutations in the remaining genes are more common and confer, on average, a lower risk of breast cancer than *BRCA1* and *BRCA2* mutations.

The identity of the genes responsible for the remaining predisposition to breast cancer is not known. Mutations in the *PTEN* gene (14), the *TP53* gene (15), and the *ATM* gene (16) are known or suspected to be associated with an increased risk of breast cancer, but none of these genes are likely to explain an important fraction of familial aggregation of the disease. Loci on chromosomes 6q (17) and 8p (18) have been suggested by linkage studies in non-*BRCA1*/2 families, but neither has been established conclusively. In the absence of a set of families attributable to a major known breast cancer susceptibility gene additional to *BRCA1* and *BRCA2*, we now present analyses of the histopathology of breast cancers arising in families that have a high probability of not being attributable to *BRCA1* and *BRCA2*.

Materials and Methods

Breast Cancer Specimens. As described previously (1, 2), we obtained specimens from case subjects with familial breast cancer in the form of unstained or H&E-stained 5-μm-thick sections from the United Kingdom, United States, Ireland, France, Germany, Iceland, Switzerland, and the Netherlands. The vast majority of familial cases were from the last two decades, because it was predominantly from that period that blocks were available. Given the diverse origin of the familial cases, it was logistically impossible to obtain locally matched controls in all cases. However, almost all of the familial cases and controls are Caucasian. A higher proportion of mutation carriers than controls would be Ashkenazi Jewish, but this is still a very small minority of the cases. Moreover, ethnic origin is unlikely to be strongly related to grade or other histopathological features. We therefore chose control specimens from the Departments of Histopathology, Royal Marsden Hospital National Health Services Trust (Sutton, Surrey) and University College Hospital London to give an age distribution similar to that of familial cases. We selected one, or occasionally two, representative H&E-stained sections from each primary breast cancer and coded each section with a random number. We arranged the sets of familial cancer patients and control subjects with sporadic cancers in sequential order, according to their random number for the review. If slides from two or more tumors from the same woman were available, results from the earliest tumor only were included in the analysis, unless the second tumor was clearly recorded as a second primary. The studies were carried out with the consent of the patients and after approval from the local institutional review board.

Conduct of the Histological Review. The results presented in this report derive from three separate histological reviews. The first review was carried out by five pathologists (J. P. S., T. J. A., J. J, M. v. d. V., and B. A. G.), the second review by seven pathologists (J. P. S., T. J. A., J. J, M. v. d. V., B. A. G., L. F., and D. V.), and the third review by two pathol-

ogists (B. A. G. and S. R. L.). In the first study, the pathologists were asked to assess mitotic score, degree of pleomorphism, extent of tubule formation, type of invasive cancer (according to the criteria (in Ref. 19), and the presence and type of *in situ* cancer. In the second review, a number of additional features were registered, including the percentage of tumor present as solid sheets of cells (<25%, 25–75%, and >75%) determined by low-power scrutiny of the section, the total mitotic count per 10 high-power fields using ×40 lens, the presence of continuous pushing margins (*i.e.,* a smooth, noninfiltrative edge to the tumor; subdivided into absent, <25%, 25–75%, and >75% of tumor perimeter) determined by low-power scrutiny of the section, the presence of confluent necrosis, the presence of lymphocytic infiltrate and whether mild or prominent, the presence of discernible cell borders, the presence of vesicular nuclei (defined as nuclei with cleared chromatin, often divided by septae into sac-like compartments), and the presence of prominent, eosinophilic nucleoli. In the third review, all of the indices reviewed in the first and second reviews were evaluated on an additional set of familial cases that had been submitted since the first two reviews. The majority of cases in this set were classified as unlikely to be attributable to mutations in *BRCA1* or *BRCA2*. In most families, this classification depended upon the absence of mutations, detected by screening the full coding sequence and intron-exon junctions of *BRCA1* and *BRCA2*. The third review also included controls from the first and second reviews and a new set of controls. The new controls were an age-stratified random sample from the histopathology archives at University College Hospital London. The numbers of controls in each of the age groups <40, 40–49, 50–59, and 60+ were chosen so that the age distribution of the cases and controls was similar.

In all three reviews, each slide was read independently by two pathologists. The studies were conducted blind, so that pathologists were not aware if the slide being read was from a case subject or control subject. No attempt was made to reconcile differences between pathologists because it was difficult to design such a process that would not introduce other biases. Although there were clearly differences in frequency of diagnoses between pathologists, each pathologist reviewed tumors from individuals carrying *BRCA1* and *BRCA2* mutations, other familial tumors, and control tumors from individuals unselected for a family history. Moreover, all variables examined were adjusted for pathologist.

Classification of Families. As described previously (1, 2), familial cases were attributed to *BRCA1* or *BRCA2* on the basis of either a mutation clearly associated with disease or strong linkage evidence generating a >90% posterior probability of being attributable to one or the other gene. These probabilities incorporate the prior probability of linkage according to the family structure, the linkage evidence at both loci, and the sensitivity of mutation testing undertaken and have been updated since the last report. For those families without mutations, the posterior probability of linkage to *BRCA1* was determined by the following formula:

$$(1-\mu_1)\alpha_1 10^{\mathrm{LOD1}}/[(1-\mu_1)\alpha_1 10^{\mathrm{LOD1}} + (1-\mu_2)\alpha_2\, 10^{\mathrm{LOD2}} + (1-\alpha_1-\alpha_2)]$$

The posterior probability of linkage to *BRCA2* was computed in an analogous manner. α_1 and α_2 are the prior probabilities of linkage to *BRCA1* and *BRCA2*. These were estimated from the numbers of individuals with breast cancer (both female and male) and ovarian cancer in the family, as reported in the recent Breast Cancer Linkage Consortium studies (12). Although α_1 and α_2 theoretically depend on ages of cancer occurrence in a family, precise prior probabilities by exact ages are not known. We have therefore based the prior probabilities on number of cases. μ_1 and μ_2 are the estimated sensitivities of the *BRCA1* and *BRCA2* mutation testing used on the family (12). Methods of mutation testing included DNA sequencing, single-stranded conformational polymorphism analysis, the protein truncation test, and heteroduplex analysis. LOD1 and LOD2 (logarithm of odds ratios) are the LOD scores for linkages to *BRCA1* and *BRCA2*, respectively, using markers close to the gene, as described previously. We calculated posterior probabilities for *BRCA2* in a similar way. We made the assumption that cases in mutation-positive families were mutation carriers, unless information from mutation or linkage analyses indicated that they were noncarriers (these noncarriers were excluded from all analyses). In practice, only one family was classified as being attributable to *BRCA1* and one family attributable to *BRCA2* on the basis of linkage alone.

For this report, we defined a third group of cases with a moderate to strong family history of the disease but a low probability of being attributable to *BRCA1* and *BRCA2*. This group is referred to as "non-*BRCA1/2*." To define the non-*BRCA1/2* group, we first selected families where at least one breast cancer case had been screened for mutations in *BRCA1* or *BRCA2* by a sensitive screen of the coding sequences but where no mutation was found. We excluded cases with a family history of either male breast cancer or of ovarian cancer, because the majority of cases of such families are known to be attributable to *BRCA1* or *BRCA2*. We also excluded families with two cases of the disease, because such families have a high probability of being chance clusters. On the basis of these criteria, the posterior probability of such a case harboring a *BRCA1* or *BRCA2* mutation is <20%. For those families where linkage evidence at *BRCA1* or *BRCA2* was available, these LOD scores were factored into the probability (as described above). Any families not falling into the *BRCA1*, *BRCA2*, or non-*BRCA1*/2 categories were excluded from the analyses.

Statistical Methods. We performed separate analyses comparing non-*BRCA1/2* tumors with tumors in *BRCA1* carriers, *BRCA2* carriers, and control tumors. As in the previous analyses, the effects of each morphological feature on cancer status were summarized in terms of odds ratios, as in standard case control analyses. All analyses were adjusted for age, in groups <30, 30–39, 40–49, 50–59, 60–69, by review (1/2 or 3), and by pathologist within a review. In addition to the overall analyses, we also performed analyses comparing non-*BRCA1/2* tumors and controls separately for reviews 1 and 2 and review 3. The features that were significant in the overall analysis showed similar effects in these two subgroups, and the subgroup analyses are not presented. There were too few *BRCA1* or *BRCA2* tumors in the third review to perform subgroup analyses for these categories.

These adjusted analyses were carried out using multiple

logistic regression analysis, using the program S-Plus (version 3.4; MathSoft, Inc., Seattle, WA). The main complication in the analysis is that the observations by different pathologists on the same slide cannot be considered independent. Using standard logistic regression, therefore, involves maximizing a quasi-likelihood rather than a true likelihood; this leads to unbiased odds ratio estimates but underestimates their standard errors and confidence intervals. To correct for this, we computed confidence limits using Huber's sandwich estimator for the variance-covariance matrix of maximum quasi-likelihood estimates (20), using specially written S-Plus macros. This quasi-likelihood approach allows for the variation in scoring individual samples between the pathologists without explicitly modeling the error distribution. The confidence intervals were also estimated by bootstrapping (21), in which 1000 bootstrap samples were created by resampling (unit of resampling was the case with observations from the two pathologists) the cases (with replacement) within each age group. This method allows confidence limits to be derived without assuming an asymptotic normal distribution and gave very similar results to the sandwich estimator. For simplicity and consistency with previous analyses, the confidence limits using the sandwich estimator are quoted. Significance levels for each factor were derived from the parameter estimates and the covariance matrix (adjusted using the sandwich estimator). All of the factors scored on more than one level, except primary histological class, are naturally considered as ordered categories. We constructed one degree of freedom significance tests based on testing for linear trends in log (odds ratio) with increasing category (22). (Estimated odds ratios were, however, derived separately for each level.) Significance levels <0.10 are quoted in the tables. Heterogeneity χ^2 statistics (based on $k - 1$ degrees of freedom for factors with k levels) have also been presented for those factors with the best-fitting models.

To determine which factors were independently predictive of genetic status, we also performed multiple regression analyses. In these analyses, all factors that were significant at the 5% level for either presence of mutations in *BRCA1* or *BRCA2* genes, together with age of the patient and pathologist who reviewed the slides, were initially included. Factors (other than age and pathologist) were then removed from the model on a stepwise basis until no further factors could be removed at the 5% level.

Results

Classification of Familial Cases. In total, 594 familial breast cancer cases (462 from the first and second reviews, 132 from the third review) and 715 controls (605 from the first and second reviews, 110 from the third review) were reviewed. The analysis was restricted to 149 *BRCA1* cases, 88 *BRCA2* cases, 82 non-*BRCA1/2* cases, and the controls. The remaining 275 familial cases were excluded from the analysis either because they were from families with only two cases of breast cancer, because the family had a probability of >20% of being attributable to *BRCA1* or *BRCA2* but <90% of being attributable to either gene, or because they were noncarriers of the mutation in a *BRCA1* or *BRCA2* family. The age distribution of the control and familial groups was similar. The number of observations was approximately twice the number of cases, because each slide was reviewed by two pathologists for each feature. However, the exact number of observations varies because some cases could not be evaluated by one or other pathologist for individual features.

Analysis of Morphological Features. The distributions of morphological characteristics in *BRCA1*, *BRCA2*, non-*BRCA1/2* and control tumors, obtained from pooling the data from the three reviews, are shown in Table 1. Odds ratios adjusted for age, review, and pathologist are given in Table 2. Non-*BRCA1/2* tumors were of significantly lower grade than *BRCA1* ($P = 0.0018$), lower average mitotic count ($P < 0.0001$), and lower scores for pleomorphism ($P = 0.0014$) but not tubule formation. Non-*BRCA1/2* tumors also showed less lymphocytic infiltrate ($P < 0.0001$), a lesser extent of the tumor with a continuous pushing margin ($P = 0.004$), a lesser extent of the tumor composed of solid sheets of cells ($P = 0.0047$), and less necrosis ($P = 0.002$). There were also significant differences by histological subtype ($P < 0.0001$). Non-*BRCA1/2* tumors were more likely to be of invasive lobular type (odds ratio, 8.23; $P = 0.0003$) and less likely to be of medullary or atypical medullary type (odds ratio, 0.19), although the latter difference did not quite reach statistical significance ($P = 0.07$). There were no clear differences in any other type. There were no significant differences between non-*BRCA1/2* and all other groups in the frequency of *in situ* ductal or lobular carcinoma.

Those factors significant in the above analysis were then included in a multiple logistic regression analysis. After stepwise removal of those factors not significant at the 5% level, the only factors remaining in the model were mitotic count ($P < 0.0001$) and lymphocytic infiltration ($P = 0.0008$; see Table 3).

In comparison with *BRCA2* tumors, non-*BRCA1/2* tumors were also of significantly lower grade ($P = 0.017$) and showed lower scores for pleomorphism ($P = 0.01$) and tubule formation ($P = 0.05$) but not for mitotic count. No other features, including tumor subtype, showed significant differences between *BRCA2* and non-*BRCA1/2* tumors. In the multiple regression analysis, pleomorphism ($P = 0.05$) and tubule formation ($P = 0.04$) remained in the final model (Table 4).

In comparison with the controls, non-*BRCA1/2* tumors were of significantly lower grade ($P = 0.001$) and were of lower score with respect to pleomorphism ($P = 0.0002$) and mitotic count ($P = 0.003$; Table 2). No other features, including histological subtype, were significantly different. In the multiple regression analysis, only pleomorphism remained significantly different.

Discussion

In previous reports (1, 2), we have demonstrated that the pathology of breast cancers arising in *BRCA1* mutation carriers differs from that observed in cancers from *BRCA2* mutation carriers and that both differ from age-matched breast cancers unselected for family history. We have now compared the pathology of breast cancers from families that are highly likely not to be attributable to either *BRCA1* or *BRCA2* mutations with breast cancers unselected for family history and with breast cancers from *BRCA1* and *BRCA2* mutation carriers.

The results of the current study indicate that the pathology

Table 1 Morphological characteristics of breast tumors in *BRCA1* carriers, *BRCA2* carriers, non-*BRCA1/2* carriers, and controls

	All reviews			
	BRCA1	*BRCA2*	Non-*BRCA1/2*	Controls
Cancer type				
Ductal	246 (78%)	133 (77%)	104 (73%)	1120 (77%)
Lobular	10 (3%)	16 (9%)	22 (15%)	142 (10%)
Tubular	5 (2%)	0	3 (2%)	56 (4%)
Mucoid	3 (1%)	3 (2%)	0	4 (0.2%)
Medullary	35 (11%)	4 (2%)	1 (1%)	20 (1%)
Ductal lobular	2 (1%)	5 (3%)	1 (1%)	38 (3%)
Other	13 (4%)	11 (6%)	11 (8%)	83 (6%)
DCIS				
Absent	180 (57%)	80 (47%)	69 (49%)	632 (43%)
Present	134 (43%)	92 (53%)	73 (51%)	831 (57%)
LCIS				
Absent	308 (98%)	168 (98%)	133 (94%)	1382 (94%)
Present	6 (2%)	4 (2%)	9 (6%)	81 (6%)
Grade				
1	22 (8%)	21 (14%)	35 (27%)	280 (20%)
2	66 (23%)	75 (49%)	65 (50%)	571 (41%)
3	197 (69%)	58 (38%)	30 (23%)	531 (38%)
Tubules				
1	10 (3%)	2 (1%)	5 (4%)	93 (7%)
2	35 (12%)	22 (14%)	33 (25%)	315 (23%)
3	249 (85%)	133 (85%)	94 (71%)	986 (71%)
Pleomorphism				
1	12 (4%)	10 (6%)	19 (14%)	116 (8%)
2	70 (24%)	67 (43%)	63 (48%)	532 (38%)
3	213 (72%)	80 (51%)	50 (38%)	747 (54%)
Mitotic count				
0–4	71 (24%)	80 (51%)	84 (65%)	738 (52%)
5–9	38 (13%)	30 (19%)	24 (18%)	219 (16%)
10–19	69 (23%)	28 (18%)	11 (8%)	207 (15%)
20–39	76 (25%)	17 (11%)	6 (5%)	140 (10%)
40+	45 (15%)	2 (1%)	5 (4%)	103 (7%)
Lymph infiltrate				
Absent	101 (34%)	86 (55%)	76 (57%)	806 (57%)
Mild	159 (53%)	66 (42%)	49 (37%)	525 (37%)
Prominent	39 (13%)	5 (3%)	8 (6%)	85 (6%)
Pushing margins				
Absent	154 (52%)	103 (66%)	101 (76%)	1088 (77%)
<25%	35 (12%)	18 (11%)	13 (10%)	135 (10%)
25–75%	57 (19%)	20 (13%)	13 (10%)	123 (9%)
>75%	53 (18%)	16 (10%)	6 (5%)	70 (5%)
Solid sheets				
<25%	139 (46%)	102 (65%)	100 (76%)	1003 (72%)
25–75%	69 (23%)	36 (23%)	21 (16%)	253 (18%)
>75%	91 (30%)	18 (12%)	11 (8%)	145 (10%)
Nucleoli				
Absent	133 (44%)	88 (56%)	81 (61%)	788 (56%)
Present	166 (56%)	68 (44%)	52 (39%)	628 (44%)
Nuclei				
Non vesicular	100 (37%)	66 (43%)	55 (41%)	518 (37%)
Vesicular	173 (63%)	87 (57%)	78 (59%)	898 (63%)
Necrosis				
Absent	131 (48%)	121 (79%)	118 (87%)	1175 (83%)
Present	142 (52%)	32 (21%)	15 (13%)	241 (17%)

of non-*BRCA1/2* familial breast cancers clearly differs on the basis of several measured indices from the pathology of breast cancers attributable to *BRCA1*, with non-*BRCA1/2* tumors being of overall lower grade. Although differences were also observed between cancers arising in *BRCA2* mutation carriers and those in non-*BRCA1/2* families (with the non-*BRCA1/2* tumors again being of lower grade), these differences were much weaker than those observed in the comparison with *BRCA1*.

The number of cases in non-*BRCA1/2* familial breast cancer clusters is smaller than in those because of two known genes (12), and some of these clusters may have occurred by chance. A component of the observed differences may, therefore, be attributable to contamination of the non-*BRCA1/2* set by sporadic breast cancer cases. Overall, however, this seems unlikely to account for all of the differences observed, because the minimum criterion for entry into this study was three cases <60

Table 2 Estimated odds ratios and 95% confidence limits comparing features in non-*BRCA1/2* tumors with other categories

	Odds ratios *vs.* non-*BRCA1/2* (95% CI)		
	Controls	*BRCA1*	*BRCA2*
Cancer type[a]			
Ductal	1.0	1.0	1.0
Lobular	1.74 (0.87–3.48)	8.23 (2.64–25.61)	2.40 (0.91–6.36)
Medullary		0.19 (0.03–1.17)	
	$\chi^2_1 = 2.49$	$\chi^2_2 = 18.74$	$\chi^2_1 = 3.15$
		$P < 0.0001$	
DCIS			
Absent	1.0	1.0	1.0
Present	0.69 (0.45–1.07)	1.22 (0.68–2.20)	0.67 (0.32–1.40)
	$\chi^2_1 = 2.77$	$\chi^2_1 = 0.46$	$\chi^2_1 = 1.12$
	$P = 0.10$		
LCIS			
Absent	1.0	1.0	1.0
Present	1.06 (0.41–2.73)	2.75 (0.47–1.60)	2.24 (0.40–12.7)
	$\chi^2_1 = 0.01$	$\chi^2_1 = 1.26$	$\chi^2_1 = 0.83$
Grade			
1	1.0	1.0	1.0
2	0.63 (0.39–1.02)	0.86 (0.38–1.95)	0.30 (0.12–0.72)
3	0.30 (0.15–0.59)	0.13 (0.05–0.36)	0.33 (0.11–0.98)
	$\chi^2_1 = 10.91$	$\chi^2_1 = 9.79$	$\chi^2_1 = 5.70$
	$P = 0.001$	$P = 0.0018$	$P = 0.017$
Tubules			
1	1.0	1.0	1.0
2	1.80 (0.47–6.92)	1.01 (0.19–5.44)	0.25 (0.03–1.93)
3	1.15 (0.31–4.24)	0.45 (0.09–2.28)	0.09 (0.01–0.78)
	$\chi^2_1 = 0.41$	$\chi^2_1 = 3.81$	$\chi^2_1 = 0.19$
		$P = 0.05$	
Pleomorphism			
1	1.0	1.0	1.0
2	0.51 (0.31–0.85)	0.48 (0.12–1.90)	0.88 (0.13–0.79)
3	0.26 (0.14–0.52)	0.14 (0.04–0.54)	0.25 (0.08–0.77)
	$\chi^2_1 = 13.97$	$\chi^2_1 = 10.27$	$\chi^2_1 = 6.88$
	$P = 0.00018$	$P = 0.0014$	$P = 0.01$
Mitotic count			
0–4	1.0	1.0	1.0
5–9	0.73 (0.41–1.29)	0.46 (0.22–0.97)	0.90 (0.38–2.13)
10–19	0.39 (0.17–0.91)	0.10 (0.03–0.30)	0.46 (0.18–1.15)
20–39	0.34 (0.11–1.03)	0.11 (0.03–0.41)	0.75 (0.17–3.30)
40+	0.32 (0.11–0.94)	0.06 (0.02–0.24)	1.38 (0.23–8.11)
	$\chi^2_1 = 8.70$	$\chi^2_1 = 26.22$	$\chi^2_1 = 0.05$
	$P = 0.003$	$P < 0.0001$	
Lymphocytic infiltrate			
Absent	1.0	1.0	1.0
Mild	0.72 (0.45–1.15)	0.32 (0.17–0.61)	0.62 (0.28–1.33)
Prominent	0.47 (0.18–1.22)	0.13 (0.04–0.38)	0.52 (0.14–1.98)
	$\chi^2_1 = 2.97$	$\chi^2_1 = 18.25$	$\chi^2_1 = 1.34$
	$P = 0.08$	$P = 0.0001$	
Pushing margins			
Absent	1.0	1.0	1.0
<25%	0.80 (0.38–1.69)	0.56 (0.22–1.39)	0.61 (0.21–1.77)
25–75%	1.06 (0.52–2.16)	0.42 (0.19–0.72)	0.75 (0.27–2.07)
>75%	1.08 (0.44–2.65)	0.29 (0.11–0.76)	0.37 (0.10–1.39)
	$\chi^2_1 = 0.003$	$\chi^2_1 = 8.11$	$\chi^2_1 = 2.83$
		$P = 0.004$	
Solid sheets			
<25%	1.0	1.0	1.0
25–75%	0.47 (0.21–1.05)	0.79 (0.33–1.93)	0.89 (0.48–1.64)
>75%	0.30 (0.12–0.75)	1.17 (0.35–3.93)	0.94 (0.40–2.20)
	$\chi^2_1 = 0.06$	$\chi^2_1 = 8.04$	$\chi^2_1 = 0.003$
		$P = 0.0047$	
Nucleoli			
Absent	1.0	1.0	1.0
Present	0.66 (0.41–1.09)	0.56 (0.26–1.20)	1.12 (0.54–2.31)
	$\chi^2_1 = 2.67$	$\chi^2_1 = 3.23$	$\chi^2_1 = 0.09$
	$P = 0.10$	$P = 0.07$	
Nuclei			
Non vesicular	1.0	1.0	1.0
Vesicular	0.72 (0.43–1.21)	0.69 (0.36–1.35)	1.14 (0.50–2.63)
	$\chi^2_1 = 1.55$	$\chi^2_1 = 1.15$	$\chi^2_1 = 0.10$
Necrosis			
Absent	1.0	1.0	1.0
Present	0.70 (0.33–1.48)	0.24 (0.10–0.59)	1.22 (0.44–3.29)
	$\chi^2_1 = 0.88$	$\chi^2_1 = 9.57$	$\chi^2_1 = 0.12$
		$P = 0.002$	

[a] There were too few occurrences of other tumor types for formal analysis.

Table 3 Multiple regression analysis of non-*BRCA1/2* tumors *versus* BRCA1 tumors: final model after stepwise regression

	Odds ratio (95% CI)[a]
Mitotic count	
0–4	1.0
5–9	0.63 (0.28–1.39)
10–19	0.13 (0.04–0.41)
20–39	0.06 (0.03–0.54)
40+	0.06 (0.01–0.33)
	$\chi^2_1 = 15.90$ $P < 0.0001$
Lymphocytic infiltration	
None	1.0
Mild	0.57 (0.28–1.16)
Prominent	0.15 (0.05–0.44)
	$\chi^2_1 = 11.31$ $P = 0.0008$

[a] CI, confidence interval.

Table 4 Multiple regression analysis of non-*BRCA1/2* tumors *versus* BRCA2 tumors: final model after stepwise regression

	Odds ratio (95% CI)[a]
Tubule formation	
1	1.0
2	0.28 (0.04–1.76)
3	0.11 (0.02–0.77)
	$\chi^2_1 = 4.03$ $P = 0.04$
Pleomorphism	
1	1.0
2	0.38 (0.015–0.93)
3	0.36 (0.11–1.14)
	$\chi^2_1 = 3.86$ $P = 0.05$

[a] CI, confidence interval.

years in first- or second-degree relatives. Epidemiological studies suggest that <10% of these families occur by chance alone (23), and mutation testing studies suggest that the majority are attributable to genes other than *BRCA1* or *BRCA2*.[3] In fact, the strength of the observed differences is probably underestimated because the non-*BRCA1/2* set is likely to be contaminated by families in which mutations in *BRCA1* or *BRCA2* have been missed and also because all of the histological indices that have been evaluated show incomplete agreement between pathologists (as illustrated previously in the kappa scores).

The results also suggest that cancers from non-*BRCA1/2* families are of lower grade, even than breast cancers unselected for family history. However, this observation needs to be interpreted with caution and may be partially or completely attributable to two major biases:

(*a*) It is usually easier to locate and obtain on a named basis archival biopsy material relating to living than to deceased persons. Therefore, in the family studies, it is possible that ascertainment of pathological samples is skewed toward surviving cases and hence possibly toward individuals with more indolent and lower grade cancers. By contrast, control breast cancers unselected for family history were ascertained directly from histopathology archives and are not associated with survivor bias.

(*b*) Breast cancers arising within families with multiple cases may have been detected earlier in their natural history as a result of mammographic screening of unaffected family members and hence may present appearances indicative of lower grade. The extent of either bias is difficult to judge and may well be small, particularly because the majority of familial cases were diagnosed at <50 years, and in this age group, mammographic screening would only have been performed on a minority of cases. In conclusion, there is evidence that non-*BRCA1/2* breast cancers differ from control breast cancers, but the results are not definitive. These biases do not, however, apply to comparisons between the *BRCA1*, *BRCA2*, and non-*BRCA1/2* cases, because the *BRCA1* and *BRCA2* cases will be subject to the same effects.

[3] M. R. Stratton, unpublished data.

Previous epidemiological studies have suggested that lobular breast cancers are associated with somewhat higher familial risks than other subtypes (24–26). There is also some suggestion that lobular carcinoma *in situ* is associated with a higher familial risk of breast cancer than other subtypes of *in situ* or invasive breast cancer (27). However, our previous analyses demonstrate clearly that neither *BRCA1* nor *BRCA2* are associated with an increased frequency of either of these phenotypes. Taken together, these observations suggest that there may exist genes that predispose specifically to lobular carcinoma and lobular carcinoma *in situ*. Consistent with this hypothesis, we found some evidence that non-*BRCA1/2* tumors are more likely than any other group to be of invasive lobular subtype, although the difference was only significant when compared with *BRCA1* tumors.

This study, taken together with our previous analyses, has demonstrated major differences between tumors in *BRCA1* carriers and all other categories. There are fewer differences between *BRCA2* tumors, other familial breast cancers, and control breast cancers. We have suggested previously, on the basis of comparisons between mutation carriers and controls, that histopathological features can be used to predict *BRCA1* (and, to a lesser extent, *BRCA2*) mutation status. The present analysis confirms directly that these features may also help to predict carrier status in women with a strong family history of the disease.

Acknowledgments

We thank all of the families, clinicians, and pathologists who have contributed to this study. Thanks are also due to the University of Liverpool, Federation Nationale des Centers de Lutte contre le Cancer, and University College London for providing microscopes and accommodation for the study and to Elaine Peacock for secretarial assistance.

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Clin Cancer Res 2000;6:782-789.