Pathology of Ovarian Cancers in *BRCA1* and *BRCA2* Carriers

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ABSTRACT

Purpose: **Germline mutations in the *BRCA1* and *BRCA2* genes confer increased susceptibility to ovarian cancer. There is evidence that tumors in carriers may exhibit a distinct distribution of pathological features, but previous studies on the pathology of such tumors have been small. Our aim was to evaluate the morphologies and immunophenotypes in a large cohort of patients with familial ovarian cancer.**

Experimental Design: **We performed a systematic review of ovarian tumors from 178 *BRCA1* mutation carriers, 29 *BRCA2* mutation carriers, and 235 controls with a similar age distribution. Tumors were evaluated by four pathologists blinded to mutation status. Both morphological features and immunochemical staining for p53 and HER2 were evaluated.**

Results: **Tumors in *BRCA1* mutation carriers were more likely than tumors in age-matched controls to be invasive serous adenocarcinomas (odds ratio, 1.84; 95% confidence interval, 1.21–2.79) and unlikely to be borderline or mucinous tumors. Tumors in *BRCA1* carriers were of higher grade ($P < 0.0001$), had a higher percentage solid component ($P = 0.001$), and were more likely to stain strongly for p53 ($P = 0.018$). The distribution of pathological features in *BRCA2* carriers was similar to that in *BRCA1* carriers.**

Conclusions: **Use of pathological features can substantially improve the targeting of predictive genetic testing. Results also suggest that *BRCA1* and *BRCA2* tumors are relatively aggressive and may be expected to have poor prognosis, although this may be treatment dependent.**

INTRODUCTION

The *BRCA1* and *BRCA2* genes are the most important known predisposition genes for ovarian cancer. Mutations in these genes cause a high lifetime risk of both breast and ovarian cancer; the risk of ovarian cancer in *BRCA1* mutation carriers is ~40% by age 70, with the corresponding risk in *BRCA2* carriers being ~10% (1). Mutations in these genes account for 5–13% of ovarian cancer cases in Western countries (2, 3) and for the majority of the familial aggregation of this disease (4).

Ovarian neoplasms can be subdivided into three main groups: epithelial/stromal, germ cell, or sex cord/stromal. The

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vast majority (>90%) in the general population are epithelial in origin, and a large proportion of these are benign. All studies performed to date indicate that carcinoma (invasive epithelial malignancy) is the usual histological diagnosis in *BRCA1*- and *BRCA2*-associated ovarian cancer. The detailed pattern of histological characteristics in mutation carriers compared with ovarian cancer in noncarriers is less clear because most studies have been based on relatively small numbers of cases. Most of the available information relates to *BRCA1*-linked disease because *BRCA1* germline mutations are approximately four times more common in ovarian cancer patients than *BRCA2* mutations (4). Most studies have reported that papillary serous adenocarcinoma is the predominant type to occur in *BRCA1* or *BRCA2* carriers. Rubin *et al.* (5) reported that 43 of 53 women with ovarian neoplasms who carried *BRCA1* germline mutations had papillary serous adenocarcinoma. They also found that the tumors were of high grade. Stratton *et al.* (2) and Berchuck *et al.* (6) obtained similar results in 12 of 13 and 15 of 15 individuals studied, respectively. These data are further supported by results reported by Risch *et al.* (3) and more recently by Shaw *et al.* (7). However, three larger investigations have reported that papillary serous carcinomas occurred with similar frequency in *BRCA* mutation carriers compared with control groups (8).

Most studies have shown that malignant mucinous carcinoma is underrepresented in *BRCA1* mutation carriers (2), suggesting that mutations in this gene do not generally play a role in the development of this subtype of epithelial neoplasm. However, occasional invasive (5) and borderline (2) mucinous neoplasms have been described in *BRCA1* mutation carriers.

In a large collaborative study carried out on behalf of the Breast Cancer Linkage Consortium (BCLC), we characterized the histopathological features of breast cancers arising in patients harboring germline mutations in the *BRCA1* and *BRCA2* genes (9–11). The present study extends this approach to ovarian cancer, using cases ascertained through the BCLC resource together with cases identified from the United Kingdom Coordinating Committee on Cancer Research Familial Ovarian Cancer Study Group. It is a systematic blinded detailed review of >200 *BRCA*-associated ovarian cancers compared with population-based controls carried out by specialist gynecological pathologists. To our knowledge, this is the largest study on the morphology and immunophenotype of these tumors.

MATERIALS AND METHODS

Ovarian Cancer Cases and Controls. We reviewed 223 "familial" tumors and 235 tumors unselected for family history. Seventy-five of the familial cases were drawn from the UKCCR study of familial ovarian cancer. All of these cases had at least one first- or second-degree relative diagnosed with ovarian cancer. The remaining familial cases were identified through collaborating centers in the BCLC in the United Kingdom, United States, the Netherlands, Ireland, Finland, Italy, France, Germany, Austria, Portugal, Spain, Iceland, Switzerland, and Hungary. These cases were identified on the basis of a family history of breast and/or ovarian cancer. Of the 223 familial cases, 178 were in women with germline *BRCA1* mutations, 29 in women with *BRCA2* mutations, and 16 had no definite mutation in either gene. This latter group was not considered further in the analysis because of its small size and the fact that it was probably a heterogeneous mixture of mutation carriers and noncarriers. For the purpose of these analyses we included only those mutations that are classified as deleterious according to the Breast Cancer Information Core (protein truncating frameshift or nonsense mutations, large-scale rearrangements, and splice-site and missense alterations classified as deleterious by Breast Cancer Information Core). Nine of the 16 individuals without definite mutations had possible disease-causing missense variants or splice-site alterations in either *BRCA1* or *BRCA2*. Details on age at diagnosis and mutation type (but no other identifying information) were collected.

Controls were drawn from a population-based study covering West and North Yorkshire and Humberside over the calendar year 1993 (190 tumors) and from a consecutive series of patients from University College Hospital, London, over the period 1980–1995 (45 tumors). Stratified random sampling by age group (in decades) was used; a higher fraction of younger cases was selected to minimize the difference in the overall age distribution between the familial and unselected tumors.

We obtained specimens from case and control subjects in the form of blocks or unstained 3-μm-thick sections. All familial case and control samples were allocated randomly generated study numbers.

Morphological Analysis. Samples were analyzed for morphological features by use of an agreed proforma similar to that used in previous BCLC studies (9–11). The forms recorded details of tumor subtype, histological grade (using the Silverberg system), presence or absence of psammoma bodies, percentage of solid component, presence of vascular invasion, presence of necrosis, and total mitotic count. Each slide was read independently by two pathologists (two of S. M., A. M. F., F. P-L., and L. A.). Because the slides were arranged and labeled only by their study number, the pathologists were not aware if the slide being read was from a case subject or a control subject. The numbers in Tables 2 and 4 refer to the number of observations of each histological category (counting the observations by each pathologists separately) rather than the numbers of tumors. No attempt was made to reconcile differences between pathologists because it was difficult to design such a process that would not introduce other biases.

The samples were analyzed for two immunohistochemical markers, p53 and HER2, using the antibodies DO7 (DAKO) and CB11 (Novocastra), respectively, and protocols as described previously (11). Proformas based on those used for the BCLC breast cancer analysis (11) were used to score the slides. For p53, the intensity of staining was recorded as negative, low, moderate, or strong. The pathologists were provided with identical color charts to aid consistency in scoring the intensity of the staining [ranging from white (negative) to dark brown (strong)]. The proportion of positive cells was divided into six categories: 0 to <1%, 1–5%, 6–25%, 26–50%, 51–75%, and >75%. For HER2, tumors in which the majority (>75%) of cells showed a strong complete membrane staining (equivalent to a score of 3 on the DAKO scoring system) were classed as positive. All other cases were recorded as negative. The slides were evaluated independently by two pathologists (S. M., F. P-L.).

Statistical Analysis. Statistical analyses were performed in a manner similar to our previous analyses of breast tumors.

We performed separate analyses comparing tumors in *BRCA1* carriers and *BRCA2* carriers with control tumors. The effects of each morphological feature on cancer status were summarized in terms of odds ratios (ORs). All analyses were adjusted for age in groups of <30, 30–39, 40–49, 50–59, and 60–69 years and by reviewing pathologist. These adjusted analyses were carried out with multiple logistic regression analysis, using the program Stata (version 7.0).

The main complication in the analysis is that the observations by different pathologists on the same slide cannot be considered independent. Use of standard logistic regression therefore leads to unbiased OR estimates but underestimates the SE and confidence intervals (CIs). To correct for this, we computed confidence limits, using the robust sandwich estimator for the variance-covariance matrix (12), with the "robust" option in Stata. This approach allows for variation in scoring individual samples between the pathologists without explicitly modeling the error distribution. Significance levels for each factor were derived from the parameter estimates and the covariance matrix (adjusted using the sandwich estimator). For those factors measured on an ordinal scale (*e.g.,* grade) one-degree of freedom tests based on testing for linear trends in log (OR) with increasing category were derived. Heterogeneity χ^2 statistics (based on $k - 1$ degrees of freedom for factors with k levels) are also presented.

To determine which factors were independently predictive of *BRCA1* status, we also performed multiple regression analyses. In these analyses, all factors that were significant at the 5% level, together with pathologist and age of the patient, 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. (The corresponding analysis was not conducted for *BRCA2* because the number of tumors was too small and none of the risk factor distributions were clearly different from controls.)

Concordance between pathologists was assessed using κ statistics. For characteristics on an ordinal scale, weighted κs were used. Confidence limits were constructed by bootstrapping using 1000 bootstrap replicates.

The predicted prevalence of *BRCA1* mutations in ovarian cancer cases with given pathological characteristics were calculated as in previous BCLC analyses of breast cancer (11). If there are n risk categories with frequencies $p_0, p_1, \ldots p_{n-1}$ and the OR for category j *versus* category 0 according the best model is ψ_j, then the mutation prevalence for cases in category j is given by:

$$q_j = q_0\psi_j \qquad \text{(A)}$$

where $q_0 = K/\Sigma pj\psi_j$; and K is the overall prevalence. For the purpose of this analysis we present age-specific prevalences for the age-groups 30–39, 40–49, and 50–69 years (the last of these based on the average of the prevalences in the 50–59 and 60–69 years age groups). The overall prevalence of *BRCA1* mutations in ovarian cancer cases in these age groups were derived from the studies by Stratton *et al.* (2) and Antoniou *et al.* (1). Stratton *et al.* (2) found an overall prevalence of mutations in ovarian cancer cases without a previous breast cancer (and assuming 70% mutation sensitivity in that study) of 4.2%. On the basis of the penetrance estimates for breast and ovarian cancer from the meta-analysis of Antoniou *et al.* (1), the probability of a *BRCA1* carrier being affected with ovarian cancer before breast cancer in each age group was as follows: <30 years, 0.015%; 30–39 years, 2.2%; 40–49 years, 8.3%; 50–59 years, 4.9%; 60–69 years, 6.9%. On the basis of these figures and the corresponding population risks for England and Wales, an overall prevalence of 4.2% corresponds to a *BRCA1* carrier frequency of 0.3%, and the predicted age-specific prevalence of *BRCA1* mutations in ovarian cancers in the age groups 30–39, 40–49, and 50–69 years are 6.6, 9.3 and 3.7%, respectively. Some studies, notably Risch *et al.* (3) have reported a higher overall prevalence of ovarian cancer. However, because the pathology-specific prevalence estimates are simply proportional to the assumed overall prevalence, these estimates can be scaled as required.

RESULTS

The age distributions of the *BRCA1* and *BRCA2* carriers and controls are shown in Table 1. Thirteen of the controls were under 30 years of age, whereas none of the tumors in carriers were diagnosed in this age-group. These controls were therefore excluded from all of the analyses. After this exclusion, women with *BRCA1* tumors were, on average, younger than the controls, whereas women with *BRCA2* tumors were, on average, older than the controls. The University College Hospital, London controls were (by deliberate selection), on average, younger than the Yorkshire controls.

In the review, three *BRCA1* tumors and nine controls were scored benign by one of the pathologists but borderline/invasive by the other. Six of the control tumors were scored as benign by both pathologists. The three *BRCA1* tumors scored as benign were all scored "not-assessable" by the other pathologist. Of the controls scored benign by one pathologist, the other scored seven borderline, one invasive, and one not-assessable. For consistency, we removed all of these 18 tumors from further analyses.

Consistency between pathologists was assessed on the remaining 403 tumors. As expected, the κ statistic was highest for borderline/invasive (0.72) and lowest for vascular invasion (0.14). κ values for the remaining morphological features varied from 0.39 to 0.57. Agreement was good for p53 staining (κ = 0.89) but weaker for HER2 (κ = 0.14).

The distribution of morphological characteristics is shown

Table 1 Numbers of tumors in the review, by mutation status and age group

Age group years	*BRCA1*, *n* (%)	*BRCA2*, *n* (%)	Controls (*n*)		
			Total	Yorkshire	UCL[a]
<30			13	7	6
30–39	20 (11%)		16	10	6
40–49	65 (37%)	3 (10%)	49	30	19
50–59	64 (36%)	11 (38%)	67	55	12
60–69	22 (12%)	10 (35%)	56	54	2
70–79	7 (4%)	4 (14%)	27	27	0
80+	0	1 (3%)	7	7	0

[a] UCL, University College Hospital, London.

Table 2 Distribution of morphological features in tumors from *BRCA1* and *BRCA2* carriers and controls

		BRCA1		*BRCA2*		Controls	
Factor	Level	*n*	%	*n*	%	*n*	%
Invasion	Invasive	325		51		334	
	Borderline	4	1	5	10	69	20
	NA[a]	15		2		1	
	Total	344		58		404	
Histological type	Serous	145	44	24	48	108	31
	Mucinous	8	3	3	5	42	38
	Endometroid	118	36	20	38	136	40
	TCC	8	2	1	2	2	1
	Sarcoma	2	1	1	2	2	1
	Clear cells	51	16	6	16	71	23
	Giant cells	21	6	3	6	9	3
	Papillary	2	0.6	2	4	0	0
	Squamous	3	1	0	0	9	3
Histological type (one type only)	Serous	131	40	20	39	97	29
	Mucinous	7	2	1	2	36	11
	Endometroid	106	33	15	29	109	33
	TCC	8	2	0	0	1	0.3
	Sarcoma	2	0.6	1	2	2	0.6
	Clear cell	32	10	2	4	44	13
	Other/multiple	39	12	12	24	45	13
Psammoma bodies	Present	60	18	9	19	60	22
	Absent	265		42		274	
Grade	Well	5	1	2	3	36	10
	Moderately	87	28	7	16	115	35
	Poorly	181	57	26	52	153	46
	Undifferentiated	52	15	16	29	30	9
Solid component	Absent	32	9	4	8	62	19
	<25%	75	24	7	14	98	30
	25–75%	127	40	19	41	115	34
	>75%	91	27	21	37	59	18
Vascular invasion	None	229		42		242	
	Present	96	29	9	23	92	26
Necrosis	None	105	32	13	26	102	31
	Focal	116	36	17	31	114	35
	Moderate	58	19	10	24	73	22
	Marked	46	14	11	19	45	13
Mitotic count	<10	58	18	4	8	73	22
	10–19	67	22	1	5	70	21
	20–29	66	21	9	19	51	16
	30–39	35	11	10	17	46	14

[a] NA, not assessible, TCC, transitional cell carcinoma.

in Table 2, and the corresponding ORs and significance levels, adjusted for age and pathologist, are listed in Table 3. The frequency of borderline tumors among *BRCA1* carriers (1%) was markedly lower than in the control group (10%; OR, 0.044; $P < 0.0001$). The frequency of borderline tumors was also lower in the *BRCA2* carriers than in the controls (OR, 0.57; 95% CI, 0.15–2.21), but the difference was less marked and not statistically significant.

The remaining analyses were restricted to tumors scored as invasive. As anticipated from previous reports, the distribution of histological type was markedly different among *BRCA1* tumors than control tumors. Specifically, the frequency of serous tumors was higher among *BRCA1* tumors (OR, 1.84; 95% CI, 1.21–2.79; $P = 0.004$), whereas the frequency of mucinous tumors was much lower (OR, 0.13; 95% CI, 0.05–0.34; $P < 0.0001$). However, the frequency of other histological types in *BRCA1* tumors was higher than in previous reports. Even if attention was restricted to tumors in which only one histological type was recorded, only 45% of tumors were reported to be serous. There was also some evidence of an increased frequency of giant cell type in *BRCA1* tumors (OR, 2.61; 95% CI, 1.17–5.82). Endometrioid and clear cell tumors were less frequent in *BRCA1* carriers but not significantly so. The distribution of histological types in *BRCA2* tumors was very similar to that in *BRCA1* tumors, but (as a result of the small sample size) did not differ significantly from the control distribution.

Both *BRCA1* and *BRCA2* tumors were of higher grade on average than control tumors ($P < 0.0001$ and $P = 0.028$ respectively) and had a higher percentage solid component ($P = 0.0004$ and $P = 0.056$ respectively). The relationship with mitotic count was less clear. There was some evidence of a difference in the distribution of mitotic count between *BRCA1* carriers and controls (heterogeneity $P = 0.049$), but this was mainly due to a higher frequency of the 20–29 mitoses/10hpf

Table 3 Estimated odds ratios for individual morphological features in *BRCA1* and *BRCA2* tumor *versus* control

		BRCA1		BRCA2	
Factor	Level	OR[a]	95% CI	OR	95% CI
Borderline	(*vs.* invasive)	0.044	0.015–013	0.57	0.15–2.21
		$\chi^2_1 = 33.61$	$P < 0.0001$	$\chi^2_1 = 0.67$	
Histological type (*vs.* all other types)	Serous	1.84	1.21–2.79	1.72	0.85–3.51
	Mucinous	0.13	0.05–0.34	0.56	0.06–1.90
	Endometrioid	0.78	0.52–1.16	0.87	0.42–1.81
	Clear cell	0.77	0.45–1.30	0.51	0.20–1.27
	Giant cells	2.61	1.17–5.82	2.24	0.06–1.93
Histological type (one type only)	Serous	1.0		1.0	
	Mucinous	0.10	0.03–0.31	0.18	0.02–1.43
	Endometrioid	0.65	0.39–1.06	0.69	0.28–1.73
	Clear cell	0.59	0.28–1.22	0.24	0.0.05–1.13
		$\chi^2_3 = 16.95$	$P = 0.0007$	$\chi^2_3 = 4.28$	
Psammoma bodies	Present	1.15	0.68–1.94	0.95	0.38–2.35
		$\chi^2_1 = 0.27$		$\chi^2_3 = 0.03$	
Grade	Well	1.0		1.0	
	Moderately	5.98	2.12–16.88	1.04	0.21–5.06
	Poorly	10.30	3.59–29.6	3.20	0.73–14.01
	Undifferentiated	15.44	4.74–50.4	11.92	2.41–59.0
		$\chi^2_1 = 20.34$	$P < 0.0001$	$\chi^2_1 = 4.80$	$P = 0.028$
		$\chi^2_3 = 23.83$	$P < 0.0001$	$\chi^2_3 = 18.22$	$P = 0.0001$
Solid component	Absent	1.0		1.0	
	<25%	1.71	0.98–2.97	1.07	0.26–4.36
	25–75%	2.62	1.43–4.80	2.52	0.61–10.43
	>75%	3.64	1.78–7.43	6.78	1.65–27.86
		$\chi^2_1 = 12.55$	$P = 0.004$	$\chi^2_1 = 3.64$	$P = 0.056$
		$\chi^2_3 = 13.77$	$P = 0.001$	$\chi^2_3 = 13.06$	$P = 0.0014$
Vascular invasion	Present	1.02	0.67–1.56	0.47	0.20–1.10
		$\chi^2_1 = 0.02$		$\chi^2_1 = 3.00$	$P = 0.08$
Necrosis	None	1.0		1.0	
	Focal	0.94	0.61–1.45	1.26	0.61–2.61
	Moderate	0.80	0.46–1.39	1.27	0.48–3.33
	Marked	1.16	0.59–2.26	2.11	0.68–6.51
		$\chi^2_1 = 0.00$	$\chi^2_3 = 1.53$	$\chi^2_1 = 1.32$	$\chi^2_3 = 1.70$
Mitotic count	<10	1.0		1.0	
	10–19	1.20	0.66–2.16	0.27	0.02–2.96
	20–29	1.86	0.96–3.59	2.78	0.54–14.2
	30–39	0.82	0.42–1.60	3.64	0.68–19.6
	40+	1.37	0.74–2.51	5.06	1.05–24.3
		$\chi^2_1 = 0.59$	$P = 0.049$	$\chi^2_1 = 2.01$	$P = 0.16$
		$\chi^2_4 = 7.84$		$\chi^2_4 = 10.91$	$P = 0.012$

[a] OR, odds ratio; CI, confidence interval.

category in the *BRCA1* tumors, with no evidence of an elevated frequency of tumors with 30 or more mitoses/10hpf. Mitotic count was higher on average in *BRCA2* carriers than controls; again however the test for trend with increasing mitotic count was not significant and the effect was only significant when mitotic count was considered as an (unordered) categorical variable ($P = 0.012$). None of the other features considered (presence of psammoma bodies, vascular invasion and necrosis) differed significantly in frequency between *BRCA1* or *BRCA2* tumors and controls.

The results obtained by immunohistochemical examination of the tumors and controls with the antibodies to HER2 and p53 are shown in Table 4; the corresponding ORs and significance levels are shown in Table 5. No differences in HER2 expression were identified. There was some evidence for an increased frequency of p53 staining among *BRCA1* tumors compared with control tumors. There was no apparent effect for mild staining, but the estimated OR for strong staining compared with no staining was 2.96 (95% CI, 1.18–7.44). A similar pattern was observed for the proportion of cells stained positive for p53. The estimated ORs for *BRCA2* were consistent with an effect similar to *BRCA1*, but the numbers were too small to show a significant difference from controls.

To evaluate the independent predictive value of these morphological and immunohistochemical features on *BRCA1* positivity, we next performed a multiple logistic regression analysis. Tumor grade, histological type, and p53 staining remained independently significant, whereas percentage of solid component did not (Table 6). To test the adequacy of this model, we also fitted models that included interactions between histological type, grade, and p53 status and between any of these factors and age at diagnosis. We found no significant evidence of interaction (data not shown).

We used these results to compute the predicted *BRCA1*

Table 4 Distribution of immunohistochemical features in tumors from *BRCA1* and *BRCA2* carriers and controls

		BRCA1		*BRCA2*		Control	
Antibody	Level	*n*	%	*n*	%	*n*	%
HER2	Positive	24	17	4	22	45	14
	Negative	120		14		273	
p53	Negative	13	9	2	11	55	18
	Low	17	12	1	6	68	22
	Medium	37	26	7	39	79	25
	Strong	73	52	8	44	111	35
	<1%	17	12	2	11	67	21
	1–25%	17	12	2	11	62	20
	26–100%	106	76	14	78	185	59

carrier probabilities among ovarian cancer patients with given histological characteristics. Because the distributions of histological types other than serous and mucinous were similar in *BRCA1* tumors and controls, we classified tumors for this purpose as serous, mucinous, or other. Among tumors with more than one histological type reported, serous was more likely to be reported in *BRCA1* tumors (OR, 2.45; 95% CI, 0.49–12.12), and mucinous type was less likely to be reported (OR, 0.30; 95% CI, 0.032–2.81), similar to the pattern in tumors with a unique type. We therefore classified tumors with serous and another type as serous and tumors with mucinous and another type as mucinous. Three tumors reported as both serous and mucinous were excluded from this analysis.

Predicted *BRCA1* carrier probabilities for different subgroups based on age and pathology are given in Table 7. On the basis of age, histological type, and grade, predicted carrier probabilities exceeded 10% for serous tumors that were undifferentiated or poorly differentiated in women 30–49 years of age at diagnosis and moderately differentiated in women 40–49 years of age at diagnosis. They also exceeded 10% for tumors diagnosed as "other histology" for undifferentiated tumors in women diagnosed at age 30–49 or poorly differentiated tumors in women 40–49. In contrast, carrier probabilities were <3% for all categories of mucinous or well-differentiated tumors. Table 7 also illustrates the additional predictive power of p53 staining. Thus, for poorly or undifferentiated serous tumors diagnosed below age 50 with strong p53 staining, the predicted carrier probability exceeded 20%.

DISCUSSION

The present study is the largest histopathological review of its kind and clarifies and extends the morphological and immunological profiles of familial ovarian cancers due to *BRCA1* and *BRCA2*.

Histopathological typing of tumors is commonly subject to significant interobserver variation. Ovarian carcinoma is no exception, and categorization is particularly difficult when a lesion is high grade (13). Furthermore, consensus criteria for grading ovarian carcinomas have not been agreed on and consequently differ among individuals (14, 15), although a recently proposed system by Shimizu *et al.* (16) and validated by Shaw *et al.* (7) has helped provide uniformity among pathologists in this area. The difficulty in subtyping ovarian carcinomas is clearly shown in the publication by Pharoah *et al.* (8) in which 59% (61 of 133 cases) of the *BRCA1*-associated neoplasms and 36% (8 of 26 cases) of the *BRCA2*-associated cancers were classified as unspecified carcinomas. The subjectivity of typing and grading is likely to account, at least in part, for the different results generated from studies undertaken to date. Systematic reviewing of the slides included in familial cancer studies by a group of histopathologists with a specialist interest in gynecological pathology has the benefit of reducing the interobserver diagnostic variation, but this has been performed only in studies by Zweener *et al.* (17), Shaw *et al.* (7) and Werness *et al.* (18). We attempted to minimize the effects of interobserver variability in the present study by blinding the pathologists with respect to mutation status, by arranging for each slide to be scored by two different pathologists, and by adjusting for pathologist as a covariate in the analysis. The concordance as measured by κ values was reasonably high for most features.

Accepting the limitations of morphological analysis, the present results emphasize the greater frequency of serous carcinomas in *BRCA1*-associated tumors, consistent with previous studies by Rubin *et al.* (5), Berchuck *et al.* (6), and more

Table 5 Estimated odds ratios for immunohistochemical features in *BRCA1* and *BRCA2* tumors *versus* controls

		BRCA1 tumors		*BRCA2* tumors	
Antibody	Staining	Odds ratio	95% CI[a]	Odds ratio	95% CI
C-erb-b2	Positive	1.24	0.63–2.44	1.95	0.44–8.61
		$\chi^2_1 = 0.40$		$\chi^2_1 = 0.79$	
p53	Negative	1.0		1.0	
	Low	0.85	0.29–2.47	0.42	0.023–7.46
	Moderate	1.71	0.64–4.57	2.59	0.27–24.5
	Strong	2.96	1.18–7.44	1.97	0.21–18.5
		$\chi^2_1 = 2.54$	$P = 0.11$	$\chi^2_1 = 0.22$	
		$\chi^2_3 = 10.01$	$P = 0.018$	$\chi^2_3 = 3.59$	
	<1%[b]	1.0			
	1–25%	0.82	0.34–1.97		
	26–100%	2.35	1.08–5.10		
		$\chi^2_1 = 1.61$			
		$\chi^2_2 = 9.06$	$P = 0.01$		

[b] There were too few *BRCA2* carriers to provide estimates by percentage of cells stained.
[a] CI, confidence interval.

Table 6 Multiple logistic regression analysis of histological factors in *BRCA1* carriers *vs.* controls[a]

Factor	OR[a]	95% CI
Histological type		
Serous	1.0	
Mucinous	0.14	0.038–0.49
Endometrioid	0.82	0.45–1.49
Clear cell	0.25	0.082–0.79
	$\chi^2_3 = 12.38$	$P = 0.006$
Grade		
Well differentiated	1.0	
Moderately differentiated	1.83	0.61–5.50
Poorly differentiated	3.45	1.07–11.16
Undifferentiated[b]	5.12	1.23–21.25
	$\chi^2_1 = 4.84$	$P = 0.028$
	$\chi^2_3 = 7.15$	$P = 0.067$
p53 staining		
Negative/Low	1.0	
Medium	2.03	0.86–4.82
Strong	3.67	1.56–8.61
	$\chi^2_1 = 7.80$	$P = 0.005$
	$\chi^2_2 = 8.97$	$P = 0.011$

[a] OR, odds ratio; CI, confidence interval.
[b] No controls with undifferentiated mucinous tumours were observed in this study

recently by Shaw *et al.* (7). Conversely, the frequency of mucinous tumors is much lower than among ovarian cancer patients in general. The frequencies of endometrioid and clear cell carcinomas were similar to, or slightly lower than, their frequencies in controls, in accordance with other reports (5). These types therefore represent a significant fraction of tumors in *BRCA1* carriers (36 and 18%, respectively). Although other tumor types, including transitional cell carcinomas, papillary and squamous carcinomas, and sarcomas, were observed, they were rare, accounting for <10% of all tumors. A dysgerminoma arising in a woman with a *BRCA1* germline mutation has recently been reported (19), but we found no examples of malignant germ cell tumors.

We found that borderline tumors are much rarer (as a proportion of all ovarian tumors) in *BRCA1* carriers, in accordance with previous observations (20). The age-adjusted frequency of borderline tumors was ~1/20th of the frequency in unselected cases, whereas the incidence rates for ovarian cancer in women older than age 30 are ~50-fold greater than in noncarriers (1). Given the wide confidence limits on these estimates, it is thus possible that *BRCA1* mutations confer little or no increased risk of borderline ovarian cancer.

Our data demonstrate that *BRCA1*-associated tumors are of higher grade, on average, than control tumors. This difference has been found in several other studies (5, 7, 8, 21). In contrast, Berchuck *et al.* (6) found that although the *BRCA1* cases were all of advanced stage (III/IV), they were less likely to be poorly differentiated compared with cases without mutations, and Johannsson *et al.* (22) did not identify a difference in grade between the ovarian cancers in their *BRCA* mutation carriers and the control population-based cancer registry group. We have also found a greater proportion of solid tumor in *BRCA1* tumors, indicating poor differentiation, an effect also seen by Shaw *et al.* (7). The other morphological features, such as vascular invasion, necrosis, and mitotic count, were not significantly associated with *BRCA1* positivity in this study.

The requirement that the mutation-positive cases be tested implies that cases with very poor survival may not have been included in our study. Because such cases are likely to be high grade, the effect of grade may, if anything, have been underestimated in this study.

Consistent with the association with grade, we found a higher frequency of strong p53 staining in *BRCA1* and *BRCA2* tumors. These results are consistent with those of Ramus *et al.* (23), who analyzed both p53 immunohistochemistry and p53 mutations in 30 *BRCA1*, 18 *BRCA2*, and 33 sporadic ovarian cancers. The frequencies of p53 overexpression in the three groups were 70% for *BRCA1*, 67% for *BRCA2*, and 39% for sporadic ovarian carcinomas, whereas the corresponding mutation frequencies were 60, 50, and 30% respectively. In contrast, our study did not reveal any difference in HER2 expression between *BRCA1* and *BRCA2* ovarian cancers or controls. This

Table 7 Predicted percentage of *BRCA1* carriers among ovarian cancer patients with a given histological type

Type	Age (years)	p53 staining	Well Differentiated	Moderately differentiated	Poorly differentiated	Undifferentiated
Serous	30–39		1.9	7.8	13.6	20.5
	40–49		2.6	10.7	18.8	28.2
	50–59		0.9	3.9	6.8	10.1
Mucinous	30–39		0.2	0.9	1.6	–[a]
	40–49		0.03	1.3	2.3	–[a]
	50–59		0.1	0.5	0.8	–[a]
Other	30–39		1.1	4.6	8.1	12.1
	40–49		1.5	6.3	11.1	16.6
	50–59		0.6	2.2	4.0	6.0
Serous	30–39	None/Low	2.2	3.7	5.9	9.1
		Medium	4.4	7.3	11.7	18.3
		Strong	7.7	12.9	20.5	32.1
Serous	40–49	None/Low	3.2	5.3	8.4	13.1
		Medium	6.3	10.6	16.8	26.2
		Strong	11.1	18.5	29.5	46.0
Serous	50–59	None/Low	1.0	1.6	2.5	4.0
		Medium	1.9	3.2	5.1	7.9
		Strong	3.4	5.6	8.9	13.9

[a] No controls with undifferentiated mucinous tumors were observed in this study.

contrasts with the pattern in breast cancer, in which HER2 overexpression is less frequent in *BRCA*-associated cancers than in controls (11).

This study is the largest formal evaluation of ovarian cancers in *BRCA2* carriers, although the number of tumors is still small. We found that the distribution of histology features in *BRCA2* carriers was very similar to those in *BRCA1* carriers, with a very low frequency of borderline and mucinous tumors, a higher than average frequency of serous tumors, and smaller but significant frequencies of endometrioid and giant-cell tumors. This pattern has been reflected in other, smaller studies (24). We also found that *BRCA2* tumors were of higher than average grade and solid component. This similarity in ovarian cancer pathology between *BRCA1* and *BRCA2* carriers contrasts with the breast cancer pathology, where there is a very marked contrast between *BRCA1*- and *BRCA2*-associated disease. The only notable differences between *BRCA1*- and *BRCA2*-related ovarian cancer are the much lower risk in *BRCA2* carriers and the different age distributions, with *BRCA2*-associated disease occurring later in life (1).

Although there is some disagreement regarding grade as a prognostic factor, the increased frequency of high grade and strong p53 staining, both of which have been shown in some studies to be adverse prognostic factors in tumors in *BRCA1* carriers, raises the possibility that the disease may have a poor prognosis in these women. The direct evidence on the survival in carriers is conflicting. Rubin *et al.* (5) found better survival in carriers, but this effect may, at least in part, have been an artifact because carriers needed to be alive to be tested. Aida *et al.* (25) found it was twice as likely that women with *BRCA*-associated cancers had a negative second-look operation compared with their matched controls, whereas Boyd *et al.* (21) also found that post-chemotherapy disease-free survival was extended compared with that of the nonhereditary group. In contrast, Johannsson *et al.* (22) found an initial survival advantage in their *BRCA1*-associated ovarian cancer group that was lost over time. Pharoah *et al.*(8) found essentially no difference in survival between patients with *BRCA1* or *BRCA2* germline mutations and noncarriers. The apparent absence of a survival disadvantage in carriers might reflect an increased sensitivity to chemotherapy in carriers. The hypothesis might be particularly pertinent in *BRCA2* carriers, given the role of *BRCA2* in DNA cross-link repair (26).

Morphological and immunohistochemical analysis provides a powerful predictor of *BRCA1* mutation status that could aid genetic testing programs. Even in the absence of information on family history, the mutation prevalence in women with poorly differentiated and undifferentiated serous tumors exceeds 10% in most age groups, whereas the prevalence of mucinous and well-differentiated tumors is low. The addition of p53 staining further improves prediction. Because carrier distributions of tumor types are similar in *BRCA1* and *BRCA2* tumors, similar predictions can be made for *BRCA2* tumors.

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REFERENCES

1. Antoniou A, Pharoah PDP, Narod S, et al. Average risks of breast and ovarian cancer associated with mutations in BRCA1 or BRCA2 detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117–30.

2. Stratton JF, Gayther SA, Russell P, et al. Contribution of BRCA1 mutations to ovarian cancer. N Engl J Med 1997;336:1125–30.

3. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001;68: 700–10.

4. Gayther SA, Russell P, Harrington P, Antoniou AC, Easton DF, Ponder BA. The contribution of germline BRCA1 and BRCA2 mutations to familial ovarian cancer: no evidence for other ovarian cancer-susceptibility genes. Am J Hum Genet 1999;65:1021–9.

5. Rubin SC, Benjamin I, Behbakht K, et al. Clinical and pathological features of ovarian cancer in women with germ-line mutations of BRCA1. N Engl J Med 1996;335:1413–6.

6. Berchuck A, Heron KA, Carney ME, et al. Frequency of germline and somatic BRCA1 mutations in ovarian cancer. Clin Cancer Res 1998;4:2433–7.

7. Shaw PA, McLaughlin JR, Zweemer RP, et al. Histopathologic features of genetically determined ovarian cancer. Int J Gynecol Pathol 2002;21:407–11.

8. Pharoah PD, Easton DF, Stockton DL, Gayther S, Ponder BA. Survival in familial, BRCA1-associated, and BRCA2-associated epithelial ovarian cancer. United Kingdom Coordinating Committee for Cancer Research (UKCCCR) Familial Ovarian Cancer Study Group. Cancer Res 1999;59:868–71.

9. Lakhani SR, Jacquemier J, Sloane JP, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. J Natl Cancer Inst (Bethesda) 1998;90: 1138–45.

10. Lakhani S, Easton D, Stratton MR. Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. Breast Cancer Linkage Consortium. Lancet 1997;349:1505–10.

11. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002;20: 2310–8.

12. Huber PJ. The behaviour of maximum likelihood estimates under nonstandard conditions. Proc Berkeley Symp Math Stat Probab 1967; 1:221–33.

13. Sakamoto A, Sasaki H, Furusato M, et al. Observer disagreement in histological classification of ovarian tumors in Japan. Gynecol Oncol 1994;54:54–8.

14. Cramer SF, Roth LM, Ulbright TM, et al. Evaluation of the reproducibility of the World Health Organization classification of common ovarian cancers. With emphasis on methodology. Arch Pathol Lab Med 1987;111:819–29.

15. Lund B, Thomsen HK, Olsen J. Reproducibility of histopathological evaluation in epithelial ovarian carcinoma. Clinical implications. Apmis 1991;99:353–8.

16. Shimizu Y, Kamoi S, Amada S, Akiyama F, Silverberg SG. Toward the development of a universal grading system for ovarian epithelial carcinoma: testing of a proposed system in a series of 461 patients with uniform treatment and follow-up. Cancer (Phila) 1998; 82:893–901.

17. Zweemer RP, Shaw PA, Verheijen RM, et al. Accumulation of p53 protein is frequent in ovarian cancers associated with BRCA1 and BRCA2 germline mutations. J Clin Pathol 1999;52:372–5.

18. Werness BA, Ramus SJ, Whittemore AS, et al. Histopathology of familial ovarian tumors in women from families with and without germline BRCA1 mutations. Hum Pathol 2000;31:1420–4.

19. Werness BA, Ramus SJ, Whittemore AS, et al. Primary ovarian dysgerminoma in a patient with a germline BRCA1 mutation. Int J Gynecol Pathol 2000;19:390–4.

20. Gotlieb WH, Friedman E, Bar-Sade RB, et al. Rates of Jewish ancestral mutations in BRCA1 and BRCA2 in borderline ovarian tumors. J Natl Cancer Inst (Bethesda) 1998;90:995–1000.

21. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. JAMA 2000;283: 2260–5.

22. Johannsson OT, Ranstam J, Borg A, Olsson H. Survival of BRCA1 breast and ovarian cancer patients: a population-based study from southern Sweden. J Clin Oncol 1998;16:397–404.

23. Ramus SJ, Bobrow LG, Pharoah PD, et al. Increased frequency of TP53 mutations in BRCA1 and BRCA2 ovarian tumours. Genes Chromosomes Cancer 1999;25:91–6.

24. Zweemer RP, Verheijen RH, Gille JJ, van Diest PJ, Pals G, Menko FH. Clinical and genetic evaluation of thirty ovarian cancer families. Am J Obstet Gynecol 1998;178:85–90.

25. Aida H, Takakuwa K, Nagata H, et al. Clinical features of ovarian cancer in Japanese women with germ-line mutations of BRCA1. Clin Cancer Res 1998;4:235–40.

26. Tutt A, Ashworth A. The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol Med 2002;8:571–6.