Influence of BRCA1 Mutations on Nuclear Grade and Estrogen Receptor Status of Breast Carcinoma in Ashkenazi Jewish Women

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BACKGROUND. In the Ashkenazim, three recurrent germline mutations have been identified in the breast carcinoma susceptibility genes BRCA1 and BRCA2: 185delAG, 5382insC (BRCA1), and 6174delT (BRCA2). The frequency of these mutations in the general Ashkenazi population approaches 2%. There is little available controlled data comparing the characteristics of breast carcinoma arising in BRCA1 mutation carriers or BRCA2 mutation carriers with that arising in noncarriers, although such data would be relevant to the urgent clinical need to develop risk-reduction strategies for individuals at increased risk due to genetic factors.

METHODS. The authors screened 149 unselected tumors arising in Ashkenazi Jewish women for the 185delAG, 5382insC, and 6174delT mutations and compared tumors arising in mutation carriers with tumors arising in noncarriers with respect to nuclear grade, steroid hormone receptor status, and axillary lymph node status.

RESULTS. In the 149 cases, the authors found 17 BRCA1 mutations (11.4%; 95% confidence interval [ci], 6.8–17.6%), and 4 6174delT BRCA2 mutations (2.7%; 95% CI, 0.8–6.7%). Tumors from women with BRCA1 mutations were significantly less likely to be estrogen receptor positive (age-adjusted odds ratio [or]: 0.091; $P <$ 0.001) and more likely to have a high nuclear grade (OR: 5.55; P 0.001) than tumors in which no mutation was identified. All four BRCA2 positive breast carcinoma specimens were estrogen receptor positive.

CONCLUSIONS. Breast carcinoma arising in Ashkenazim BRCA1 mutation carriers has adverse prognostic features relative to those arising in noncarriers in the same population. This may be relevant to the development of prevention and treatment strategies for these women. For example, if tamoxifen reduces the risk of breast carcinoma via its antiestrogenic effects, it is possible that this effect will be diminished in the largely estrogen receptor negative BRCA1-related hereditary breast carcinoma.

KEYWORDS: BRCA1, BRCA2, estrogen receptor, Ashkenazim, breast carcinoma.

Four mutations in BRCA1 and BRCA2 have been reported in Ashkenazi Jewish women with a family history of breast or ovarian carci-

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noma.[1–3] The most common mutation is 185delAG (in BRCA1), which has a population frequency of approximately 1%.[4,5] The BRCA1 5382insC mutation has also been found predominately in Ashkenazi Jewish women with breast carcinoma,[3] with a population frequency of 0.13%.[5] The BRCA1 mutation 188del11 has been reported by one center,[6] but appears to be infrequent among Ashkenazim with breast carcinoma.[3] A second breast carcinoma susceptibility gene, BRCA2, accounts for approximately 50% of all autosomal dominant hereditary breast carcinoma.[7,8] The common BRCA2 mutation, 6174delT, has a frequency of approximately 1% in the Ashkenazi population,[5,9] and was found to account for 8% of breast carcinomas in Ashkenazi women age $<$ 42 years.[10]

A few studies have determined the frequency of these mutations in small numbers of generally young breast carcinoma patients,[10–12] but there has been no systematic comparison of the pathobiologic characteristics of BRCA1 and BRCA2 positive and mutation negative tumors in an unselected series of women with breast carcinoma. Therefore, the authors determined the frequency of common BRCA1 and BRCA2 mutations in a sample of Ashkenazi Jewish women with breast carcinoma diagnosed at age $<$ 65 years and determined the nuclear grade, steroid hormone receptor status, and lymph node status in tumors arising in mutation carriers and noncarriers.

METHODS

Patient Selection

Patients were identified from the tumor registry data base of the Sir Mortimer B. Davis-Jewish General Hospital. The cases were derived from a population diagnosed with invasive breast carcinoma before age 65 years and who were of self-reported Jewish descent (the authors' data base contains a field for religion). The protocol was approved by the institutional review board of the Sir Mortimer B. Davis-Jewish General Hospital. Using this criteria, 201 Ashkenazi patients were identified. Fifty-two of these patients were excluded because pathologic material was not available or review of the pathology failed to support a diagnosis of invasive breast carcinoma. This left 149 specimens for study. The majority of the samples were from 1990–1995. At this time, the study data base was fixed and a paraffin wax embedded tissue block for each case was identified. Sections were cut from the block and the specimen was assigned a new study number. DNA and pathologic analyses were delinked and performed blinded to ensure patient anonymity.

Histopathologic Type, Nuclear Grade, and Axillary Lymph Node Status Determination

All cases were reexamined by a single pathologist experienced in the histology of breast carcinoma (L.B). All pathologic examinations occurred before DNA was extracted, and therefore researchers were blinded to mutation status. Histopathologic typing was based on standard criteria.[13] Grading of carcinoma, as an estimate of differentiation, was limited to the invasive portion of the tumor. The score was based on a nuclear grading system with cytologic evaluation of the structural features of tumor nuclei, by comparison with the nuclei of normal mammary epithelium. Nuclear assessment was based on criteria introduced by Black et al.,[14] corresponding to the nuclear score of Elston and the nuclear grading scheme of Fisher et al., exclusive of mitotic activity.[15,16] Briefly, in Grade 1 tumors, nuclei were small, had regular outlines and uniform nuclear chromatin, and showed little variation in size. In Grade 2 tumors, the nuclei had an open vesicular pattern, visible nucleoli, and moderate variability in size and shape. Grade 3 tumors had nuclei that were vesicular, often with prominent nucleoli, and exhibited marked variation in size and shape. Axillary lymph node status was determined by conventional hematoxylin and eosin staining of surgical specimens. Nine women did not undergo axillary lymph node dissection.

Steroid Hormone Assays

In 105 cases, conventional biochemical assays were used to determine estrogen receptor (ER) and progesterone receptor (PR) nuclear protein status. A positive ER or PR score was $>$10 fmol/mg protein. These assays were performed in the Department of Laboratory Medicine at the time of initial breast carcinoma surgery using established techniques.[17] In the remaining cases, these assays had not been performed and therefore the ER and PR status was determined by immunohistochemistry using the streptavidin-biotin-peroxidase complex methodology. For ER and PR nuclear protein analyses, antibodies 6F11 and 1A6, respectively (Ventana, Tucson, AZ), were used prediluted. A positive score was based on $>$10% cells showing clear, intense nuclear staining, with no background cytoplasmic staining. Neither biochemical nor immunohistochemical assays were performed in five cases for PR and in two cases for ER.

DNA Extraction

Because leukocytes samples were unavailable, tumor blocks were used to assay for germline mutations of BRCA1 and BRCA2. No microdissections were performed. Two to 5-μm sections were cut from the paraffin blocks using a microtome and DNA was extracted in a standard fashion. Briefly, sections were deparaffinized in xylene followed by absolute alcohol, then digested at 55 °C overnight in 50 mM TRIS (pH 8.3),

1 mM ethylenediamine tetraacetic acid (EDTA), 0.5% Tween 20, and 200 g/mL proteinase K. After heat inactivation and centrifugation, aliquots of the supernatant fluid were used directly in the polymerase chain reaction (PCR) reactions.

BRCA1 Mutation Detection

PCR was performed under standard conditions in 12.5 μL with 2 μL of sample, and 0.5 Ci ^{35}S-dATP. Thirty cycles of PCR were performed with a ''hot start.'' Conditions were: denaturation at 94 °C for 30 seconds, annealing at the optimal temperature for each primer pair for 30 seconds, and extension at 72 °C for 60 seconds. The primers employed and their respective annealing temperatures were: for detection of 185delAG and 188del11: A- 5′ CGTTGAAGAAGTACAAAATGTCA-3′, B- 5′-TCAATTCTGTTCATTTGCATAGG-3′, 55 °C[12]; and for detection of 5382insC: A- 5′-AAGGTCCAAAGC-GAGCAAGA-3′, B- AATAAGTCTTACAAAATGAAGCGG-3′, 55 °C (S. Neuhausen, personal communication). The authors also searched for 185delAG mutations using mutation specific primers: A- 5′-GAAGTTGTCATT-TTATAAACCTTT-3′, B- 5′-TGACTTACCAGATGGGAC-ACTA-3′, and C- 5 -GTATGTAAGGTCAATTCTGTTC-3, at 60 °C (modified from FitzGerald et al.[11]).

Four μL of the PCR reactions were electrophoresed in a 6% polyacrylamide gel at 45 watts for 2.5 hours, dried, and autoradiographed. Using the mutation specific primers, a 185delAG mutation is present if a 170 nucleotide band is observed. Twenty-nine of the 149 specimens were not informative for the 185delAG specific primers; however, all of the specimens were informative using an assay that detects mutations based on size by determining the presence or absence of the 2 base pair deletion. Mutations were detected by the size change of the amplified products, comparing the pattern observed in the tumor specimens with the positive and negative controls. Positive controls had been previously documented by sequencing. Mutations found were confirmed by automated or manual sequencing. The DNA samples that showed a size change for the exon 20 assay (5382insC) were directly sequenced.

Single Strand Conformation Analysis Detection of BRCA2 6174delt Mutation

The 6174delT mutation was identified by PCR amplification of genomic DNA using the primer set A- 5′-CACCTTGTGATGTTAGTTG-3′, B -5′-TATGAAGCT-TCCCTATACT-3′, at an annealing temperature of 55 °C. The reaction products were diluted 1:2 with stop buffer (90% formamide, 10 mM EDTA), heated for 10 minutes at 95 °C, and electrophoresed in a 6% nondenaturing polyacrylamide gel containing 10% glycerol. Gels were transferred to filter paper, dried, and autoradiographed. Single strand conformation analyses of positive control cases that had been sequenced and were shown to have the 6174delT mutation were compared with the electrophoretic pattern observed in the 136 tumors examined. Samples that had an identical pattern to the positive controls were scored as BRCA2 mutation positive.

We were able to use tumor tissue to search for germline mutations because numerous breast carcinomas have been screened for BRCA1 and BRCA2 mutations and very few somatic mutations have been reported, implying that these mutations are not selected for if they occur in tumors. Specifically, there have been no reports of somatic mutations of BRCA1 or BRCA2 in breast carcinoma at the sites analyzed in this study.[18–21] Therefore, the authors did not microdissect the tumor blocks and an assumption of this study was that all the mutations observed in these tumor specimens reflected the occurrence of germline mutations.

Statistical Analysis

The differences between the population incidence of breast carcinoma and the current series was assessed by comparison of proportions using Fisher's exact test. The Student's *t* test was used to compare the age of diagnosis and tumor size in the two groups (mutation positive vs. mutation negative). The confidence intervals (CI) for the frequency of the mutations were derived using exact binomial calculation. The stratum specific *P* values were calculated using Fisher's exact test. Where the odds ratio (OR) was infinity, a logit estimator was used for the OR and CI for that stratum, and was also used to calculate the overall age-adjusted OR in that table. Otherwise, the Mantel-Haenszel adjustment was used to adjust for age in the stratified analysis of pathobiologic variables. CIs were calculated using the Mantel-Haenszel method.

RESULTS

Frequency of Mutations

The average age at diagnosis of the 149 women studied was 51.3 years and did not significantly differ from that observed in the Quebec population as a whole.[22] The authors identified 12 cases of a total of 149 that were positive for the 185delAG mutation (8.1%; 95% CI, 4.2–13.6%). Mutations were confirmed by sequencing of exon 2. The 5382insC mutation was detected in 5 tumors (3.4%; 95% CI, 1.1–7.7) and 4 of 141 tumors analyzed had the 6174delT mutation in BRCA2 (2.8%; 95% CI, 0.8–7.1%). Therefore, the authors found in total 21 mutations in 149 unselected Ashkenazi women with invasive breast carcinoma age

TABLE 1
Age at Diagnosis in All Study Tumors and in Those Showing BRCA1 or BRCA2 Mutations

Age (yrs)	Study population (%)	185delAG BRCA1 mutation (%)	5382insC BRCA1 mutation (%)	6174delT BRCA2 mutation (%)	BRCA1 or BRCA2 mutation positive tumors[a] (%)
25–34	4 (2.67)	0/4	0/4	0/4	0/4
35–44	29 (19.5)	6/29 (20.7)	2/29 (6.9)	0/27	8/29 (27.6)
45–54	52 (34.9)	3/52 (5.8)	2/52 (3.8)	2/50 (4.0)	7/52 (13.5)
55–64	64 (43.0)	3/64 (4.7)	1/64 (1.6)	2/60 (3.3)	6/64 (9.4)
All ages	149	12/149 (8.1)	5/149 (3.4)	4/141 (2.8)	21/149 (14.1)[b]

[a] No 188del11 mutations were observed (0/149).
[b] Eight tumors were not analyzed for BRCA2 mutations.

$<$ 65 years (Table 1). The mean age at diagnosis was 47.8 $\pm$ 8.8 years for patients in whom a BRCA1 mutation was detected compared with 52.3 $\pm$ 8.5 years for affected women without a mutation ($P = 0.027$).

Relationship of Mutations to Histologic Subtype

Examination of histologic subtypes revealed that 18 of 21 tumors containing a mutation were typical infiltrating ductal carcinoma; one lobular and two medullary carcinomas were observed. Ductal adenocarcinomas represented the largest proportion of carcinomas observed with mutation negative, BRCA1 positive, and BRCA2 positive tumors (74%, 82%, and 100%, respectively). However, BRCA1 positive tumors were more likely than mutation negative tumors to be of medullary type (2 of 17 vs. 2 of 128; OR: 8.4; $P = 0.14$).

Relationship of Mutations to Nuclear Grade

High nuclear grade was associated with the presence of BRCA1 mutations. Thirteen of 17 of BRCA1 mutation positive tumors (76.5%) were Grade 3 compared with 35 of 128 mutation negative tumors (27.3%) (weighted OR: 5.55, 95% CI, 2.10–14.7). The difference between tumors arising in BRCA1 mutation carriers versus noncarriers with respect to nuclear grade was more marked in the older age groups (Table 2), but was significant for the entire population ($P < 0.001$). Only one of the 17 tumors in the BRCA1 mutation positive group (5.9%) was classified as Grade 1 compared with 38 of 128 in the mutation negative group (29.7%). It is well known that there is a trend for tumors arising in younger women to have higher nuclear grade than those arising in older women and for tumors in those at increased genetic risk to arise at a younger age than sporadic tumors. However, the relationship between mutation status and nuclear grade cannot simply be attributed to a relationship between age and nuclear grade because mutation of BRCA1 was strongly associated with nuclear grade, even when the analysis was confined to women age $>$ 45 years (Table 2).

Relationship of Mutations to Axillary Lymph Node Status

Seven of 15 BRCA1 mutation positive tumors (46.7%) had axillary lymph node involvement versus 41 of 121 mutation negative tumors (33.9%) (OR: 1.56; 95% CI, 0.51–4.79). There appear to be age differences in the probability of a woman with a BRCA1 mutation and breast carcinoma having positive lymph node involvement, but none of the stratum specific P values reached significance (Table 3). Seven of eight lymph node negative tumors in women with BRCA1 mutations were high nuclear grade.

Relationship of Mutations to Steroid Hormone Receptor Status

Steroid hormone receptors were strikingly less frequently present in the tumors with BRCA1 mutations. Only 18.8% of tumors with BRCA1 mutations were ER positive compared with 73.4% of tumors without a mutation (weighted OR: 0.091; 95% CI, 0.029–0.29) (Table 4). Similarly, PR was positive in 25% of women carrying mutations and 71.1% in those women not carrying mutations (weighted OR: 0.15; 95% CI, 0.05–0.46) (Table 4). These differences were not attributable to the younger age of patients with mutations.

DISCUSSION

It has now believed that 1% of the Jewish population may carry the 185delAG mutation in BRCA1 and an additional 1% carry the 6174delT mutation in BRCA2.[4–6] The second most common BRCA1 mutation in Ashkenazi Jewish breast carcinoma families is 5382insC,[3] which has a population frequency among the Ashkenazim of 0.13%.[5] Approximately 20% of Ashkenazi Jewish women with breast carcinoma diagnosed before age 42 years carry the 185delAG mutation.[11,12] Although these findings are of considerable interest, a

TABLE 2
Relative Risk of High Nuclear Grade According to BRCA1 Mutation Status

Age (yrs)	BRCA1 status	High nuclear grade (3)	Low or intermediate grade (1 and 2)[a]	Odds ratio (95% confidence interval)	*P* value
< 45	Mutation present	5	3		
	Mutation absent	12	13	1.81 (0.35–9.37)	0.69
45–54	Mutation present	4	1		
	Mutation absent	14	31	8.86 (0.91–86.6)	0.05
55–64	Mutation present	4	0		
	Mutation absent	9	49	46.9 (2.33–)	0.001
All ages	Mutation present	13	4		
	Mutation absent	35	93	5.55 (2.1–14.7)	0.001

[a] Grades 1 and 2 were combined because only one mutation positive tumor was classified as being low grade.

TABLE 3
Relative Risk of Positive Axillary Lymph Node Status According to BRCA1 Mutation Status

Age (yrs)	BRCA1 status	Lymph node positive	Lymph node negative	Odds ratio (95% confidence interval)	*P* value
< 45	Mutation present	2	5		
	Mutation absent	10	15	0.6 (0.095–3.76)	0.683
45–54	Mutation present	4	1		
	Mutation absent	18	25	5.56 (0.68–45.2)	0.165
55–64	Mutation present	1	2		
	Mutation absent	13	40	1.54 (0.13–18.5)	0.999
All ages	Mutation present[a]	7	8		
	Mutation absent[b]	41	80	1.56 (0.51–4.79)	0.44

[a] In two cases, lymph node status could not be ascertained because axillary lymph node dissection was not performed.
[b] In seven cases, lymph node status could not be ascertained because axillary lymph node dissection was not performed.

substantial majority of women with breast carcinoma are diagnosed after the age of 42 years. In one study, 30% of slightly older women (age range, 42–50 years) with a positive family history had a 185delAG mutation.[12] The same data set of Ashkenazi Jewish women with breast carcinoma was separately analyzed for the 6174delT BRCA2 mutation; a mutation was observed in 8% of women diagnosed with breast carcinoma at age < 42 years, and in 7% of breast carcinomas arising in women age 42–50 years with a positive family history.[10] In these studies, the criteria for entry and the mutations analyzed were not the same for all affected women, which makes interpretation difficult. In the current study, 17 of 149 Ashkenazi Jewish women diagnosed at age < 65 years (11.4%) had a mutation in BRCA1 and 4 of 141 (2.8%) had a mutation in BRCA2. We restricted our analyses to reported BRCA1 and BRCA2 mutations in the Ashkenazim, so no other mutations were detected that have been reported in BRCA1 and BRCA2. However, among the Ashkenazim, it is unlikely that any such mutations will have a higher frequency than 185delAG, 5382ins C (BRCA1), and 6174delT (BRCA2).

We have confirmed that the ductal histopathologic type is common in BRCA1 or BRCA2 mutation positive tumors and that there is a higher than expected frequency of tumors with medullary features.[23] BRCA1-associated breast carcinoma is more likely than sporadic breast carcinoma to be high grade.[23,24] However, in previous studies, lymph node status and steroid hormone receptor data were not presented. In one study, germline mutations at the conserved 5′ and 3′ ends of BRCA1 were significantly more likely to be associated with high grade breast carcinomas than mutations elsewhere in the gene.[25] It is of particular relevance to the current study that one of the three mutations at the 5′ end was 185delAG and six of the eight mutations at the 3′ end were 5382insC. These data fit well with those reported in the current study and when combined with the current ER data indicate that Ashkenazi Jewish women with breast carcinoma who have germline BRCA1 mutations at these sites

TABLE 4
Relative Risk of Steroid Hormone Receptor Positive Status According to BRCA1 Mutation Status

Estrogen receptor

Age (yrs)	BRCA1 status	ER+	ER−	Odds ratio (95% confidence interval)	*P* value
< 45	Mutation present	1	6		
	Mutation absent	17	8	0.078 (0.011–0.58)	0.027
45–54	Mutation present	1	4		
	Mutation absent	29	16	0.138 (0.018–1.06)	0.143
55–64[a]	Mutation present	1	3		
	Mutation absent	48	9	0.063 (0.006–0.67)	0.022
All ages	Mutation present	3	13		
	Mutation absent	94	33	0.091 (0.029–0.29)	< 0.001

Progesterone receptor

Age (yrs)	BRCA1 status	PR+	PR−	Odds ratio (95% confidence interval)	*P* value
< 45	Mutation present	2	5		
	Mutation absent	20	5	0.01 (0.02–0.59)	0.019
45–54	Mutation present	1	4		
	Mutation absent[b]	29	15	0.129 (0.013–1.26)	0.143
55–64[c]	Mutation present	2	2		
	Mutation absent	42	13	0.31 (0.04–2.25)	0.265
All ages	Mutation present	5	11		
	Mutation absent	91	33	0.15 (0.05–0.46)	0.001

ER: estrogen receptor; +: positive; −: negative; PR: progesterone receptor.

[a] In two cases, (one mutation positive case and one mutation negative case), estrogen receptor status was not evaluable.

[b] In one case, progesterone receptor status was not evaluable.

[c] In four cases (three mutation negative cases and one mutation positive case), the progesterone status was not evaluable.

form a group of women with poor prognosis breast carcinoma. Approximately 75% of the tumors in the current series that were BRCA1 mutation positive were ER negative and had high nuclear grade.

It is clear that a significant proportion of Ashkenazi Jewish women with breast carcinoma will have mutations in BRCA1 or BRCA2. The four tumors in which the BRCA2 mutation 6174delT was detected were all ER positive and occurred in older women (age 48, 50, 59, and 61 years, respectively). The significance of this finding is uncertain given the small number of cases with the 6174delT mutation. However, comparing BRCA1 and BRCA2 mutation positive tumors, only 3 of 16 evaluable BRCA1 positive tumors were ER positive, but all 4 BRCA2 positive tumors were ER positive (OR: 0.29; $P = 0.007$). The older age of onset of the four breast carcinomas observed in BRCA2 mutation carriers in this series implies a different age-dependent penetrance of BRCA2 compared with BRCA1 mutations. The finding that BRCA1 and BRCA2 mutations may be associated with different steroid hormone receptor profiles of the breast carcinomas that arise in mutation carriers could have important implications for the prevention and treatment of hereditary breast carcinoma.

Although highly suggestive, the findings of the current study should be interpreted cautiously because the study sample size is rather small. In addition, the proportion of ER positive women in the age group < 45 years who did not carry BRCA1 mutations is surprisingly high, but cannot be due to systematic bias because these assays were performed before mutation analyses. We also appreciate that the missing data for the older age group (range, 55–64 years) could have had an important influence on the findings in this study. It should be noted that the PR data are entirely consistent with the ER data.

Tamoxifen use has been considered to be a preventive strategy for women with inherited risks for breast carcinoma. The current data show that this strategy may be appropriate for BRCA2 carriers, but raise concern that in BRCA1 carriers this intervention may be effective only if tamoxifen mainly prevents breast carcinoma through its antiproliferative effect because most BRCA1-related tumors would appear to be either de novo ER negative or to have a propensity

to undergo rapid neoplastic progression to an ER negative phenotype and thus may not be susceptible to antiestrogenic measures.

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