

Germ-Line *BRCA1* Mutation Is an Adverse Prognostic Factor in Ashkenazi Jewish Women with Breast Cancer¹

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ABSTRACT

Germ-line mutations in *BRCA1* confer an increased risk of developing breast and ovarian cancer, but little is known about the clinical course of breast cancer in *BRCA1* mutation carriers compared with noncarriers. Two recurrent *BRCA1* mutations (185delAG and 5382insC) are common (~1.3%) in Ashkenazi Jews and account for about 20% of breast cancers diagnosed before age 40 in this group. We assayed paraffin-embedded tumor blocks from 117 unselected Ashkenazi Jewish women with primary breast cancer, diagnosed before age 65 at a single institution, for the presence of either of the two *BRCA1* mutations. We reviewed the medical records and constructed survival curves for *BRCA1*-positive and -negative subgroups. Twelve of the women (10.3%) were found to carry *BRCA1* mutations (eight mutations were 185delAG, and four were 5382insC). The probability of death from breast cancer in the first 5 years was 35.7% in the *BRCA1* mutation-positive group and 4.3% in the 100 women without a mutation ($P = 0.0023$).

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The 5-year distant disease-free survival was 68.2% in *BRCA1* mutation carriers and 88.7% in noncarriers ($P = 0.019$). These data suggest that breast cancer occurring in an Ashkenazi Jewish woman carrying a germ-line *BRCA1* mutation has an adverse prognosis. This information is available before the diagnosis of breast cancer, and therefore, this finding may have important implications for prevention of breast cancer in *BRCA1* mutation carriers.

INTRODUCTION

Two mutations in *BRCA1*, 185delAG and 5382insC, are common in Ashkenazi Jews, with a combined frequency above 1.0% (1, 2). About 20% of Ashkenazi Jewish women with breast cancer diagnosed at less than 42 years of age carry the 185delAG mutation (3, 4). More than 25% of Ashkenazi Jewish families with two cases of breast cancer, one of which was diagnosed before age 50, carry a mutation in *BRCA1* or *BRCA2* (5). The risk for breast cancer in female *BRCA1* mutation carriers is estimated to be 87% before age 70 (6), but it is not yet known whether a germ-line *BRCA1* mutation has any effect on breast cancer prognosis. To answer this question, we have used a hospital-based historical cohort approach using stored pathology specimens.

PATIENTS AND METHODS

Study Population. Study subjects were identified in the tumor registry of the SMBD-JGH⁴ and included all women who self-reported as being Ashkenazi Jewish by birth and who were diagnosed with invasive breast cancer before age 65 years, during the period January 1, 1990, to November 1, 1995. The study was approved by the Research Ethics Committee of the SMBD-JGH. Two women presenting with distant metastatic disease were excluded. Of the 139 eligible women, we obtained a paraffin-embedded primary breast cancer block from the SMBD-JGH Department of Pathology archives from each of 119 (86%). The consent form offered the participants the opportunity to have their samples analyzed anonymously or to receive their results. Two of the women contacted did not wish to take part in the study. All pathological and molecular analyses of the 117 samples were carried out blinded. All specimens were reviewed by one pathologist (L. R. B). Histological tumor type, grade (1–3), and axillary lymph node status (positive or negative for breast cancer metastases, with the number of positive lymph nodes recorded) were determined by specimen and chart review. The specimens were then coded, and DNA was extracted from the paraffin wax-embedded blocks using standard techniques.

⁴ The abbreviations used are: SMBD-JGH, Sir Mortimer B. Davis-Jewish General Hospital; RR, relative risk.

Table 1 Lymph node status in study subjects

Axillary lymph node status	BRCA1 ⁺ (%)	BRCA1 ⁻ (%)	Totals
Negative	6 (54.5)	65 (68.4)	71 (67.0)
1-3 positive	4 (36.4)	22 (23.2)	26 (24.5)
4-9 positive	1 (9.1)	6 (6.3)	7 (6.6)
10+ positive	0	2 (2.1)	2 (19.0)
Totals	11 (100)	95 (100)	106 (100)
No dissection performed	1	5	6

Mutation Analysis. The extracted DNA was amplified by PCR with the use of oligonucleotide primers specific to *BRCA1* exons 2 (185delAG; Ref. 4) and 20 (5382insC; Ref. 7). The products, with suitable positive controls, were electrophoresed overnight in denaturing polyacrylamide gels. After autoradiography, the patterns were compared with the positive controls. DNA from all samples with a band pattern suggestive of a mutation, as revealed by the presence of two alleles (bands) reflecting the presence of a deletion of two nucleotides (185delAG) or the insertion of one nucleotide (5382insC), was sequenced using appropriate primers. A PCR-RFLP endonuclease digestion assay, specific for the 185delAG mutation (8), was also used to detect this mutation. We used breast cancer tissue as our source of DNA, because no somatic mutations have been reported in *BRCA1* (9), and therefore, the mutations observed were assumed to be germ line in origin. As an additional safeguard, all DNA samples were typed using microsatellite markers to confirm the presence of the characteristic haplotypes seen in association with the two *BRCA1* mutations (Ref. 7; data not shown). During the course of the study, we also looked for the common Ashkenazi *BRCA2* mutation (6174delT) by single-strand conformation analysis (10, 11) and by a mutation-specific PCR-RFLP endonuclease digestion analysis (8), and five individuals with mutations (4.3%) were identified. Because of the small numbers, analysis of the effect of *BRCA2* mutations on survival was not attempted, and therefore these 5 cases were excluded, leaving 112 women for the final statistical analysis.

Ascertainment of Cases and Data Abstraction. The clinicopathological details were abstracted without knowledge of mutation status. These data included dates of birth, first diagnosis of breast cancer, date and site of recurrences, and incidence of new primary cancers. Date of last follow-up and death were also obtained. Only pathologically or radiologically verified recurrences were included. Deaths were verified in the general hospital records and were attributed to breast cancer, another cancer, or other noncancer diseases.

Statistical Analysis. We generated Kaplan-Meier survival curves for the group of 12 *BRCA1* mutation-positive patients and 100 *BRCA1* mutation-negative patients with regard to relapse-free survival and distant disease-free survival. We also estimated the cumulative probability of breast cancer-specific death for each group. We stratified *BRCA1* status by two risk factors, tumor size (<2 cm and ≥2 cm) and lymph node status and repeated the procedure. All *P*s are two-sided.

RESULTS

We observed 12 (10.3%) *BRCA1* mutations. Eight mutations were 185delAG (6.9%), and four were 5382insC (3.4%).

Table 2 Classification of tumors using TNM system

TNM stage	BRCA1 ⁺ (%)	BRCA1 ⁻ (%)	Totals
T ₁ N ₀			
T _{1a}	0	3	3
T _{1b}	0	21 (22.1)	21
T _{1c}	3 (27.3)	32 (33.7)	35
T ₂ N ₁ ^a			
T _{2a}	0	0	0
T _{2b}	0	3 (3.1)	3
T _{2c}	3 (27.3)	13 (13.6)	16
T ₃ N ₀	3 (27.3)	9 (9.5)	12
T ₂ N ₁ ^a	2 (18.1)	12 (12.6)	14
T ₃ N ₀	0	0	0
T ₃ N ₁ ^a	0	2	2
Totals	11 (100)	95 (100)	106 (100)
No dissection performed	1	5	6

^a We do not have full details of the condition of the lymph nodes at dissection, but fixed nodes are not generally dissected at this institution. However, some T₂ individuals may be misclassified as T₁.

Women with *BRCA1* mutations were more likely to have high-grade tumors (RR for grade 3 versus grade 1 or 2 tumor, 19.8; *P* = 0.00004) and to have larger tumors (2.48 versus 1.71 cm; *P* = 0.034). The lymph node status and TNM classification of all subjects are shown in Tables 1 and 2. Axillary lymph node involvement did not differ significantly between the two groups (RR = 1.69; *P* = 0.50). The breast cancers were diagnosed at a significantly younger age in the mutation carriers than in the noncarriers (mean, 45.2 versus 52.4 years; *P* = 0.007), and one-quarter of *BRCA1* mutation carriers were diagnosed with breast cancer at less than 40 years of age, compared with 7% of noncarriers. The median number of years of follow-up did not differ significantly between mutation carriers (3.07 years) and noncarriers (3.53). The median follow-up for the whole data set was 3.29 years. Six noncarriers and one mutation carrier were lost to follow-up within 1 month of diagnosis.

The cumulative probability of breast cancer-related death at 5 years was 35.7% in *BRCA1* mutation carriers compared with 4.3% in noncarriers (*P* = 0.002; Fig. 1). There was also a significantly poorer 5-year distant disease-free survival in mutation carriers (68.2%) than in noncarriers (88.7%; *P* = 0.019; Fig. 2). Eleven *BRCA1* mutation carriers and 94 noncarriers underwent breast-conserving surgery. Two carriers and four noncarriers had ipsilateral breast tumor recurrence. The difference in ipsilateral breast tumor recurrence-free survival between *BRCA1* mutation carriers and noncarriers receiving breast-conserving surgery was of borderline significance (80 versus 95%; *P* = 0.051).

Overall, tumor size was predictive of relapse at 5 years (*P* = 0.0093). We stratified the data to establish whether *BRCA1* mutation status was also a prognostic factor in women with small tumors and in those with lymph node-negative cancers. Strikingly, the presence of a *BRCA1* mutation resulted in decreased distant disease-free survival for both of these groups. Only 1 of 65 (1.5%) women without mutations who were lymph node negative at initial surgery relapsed at a distant site, whereas 3 of 6 (50%) lymph node-negative mutation carriers relapsed at a distant site (*P* = 0.0001, log-rank test); thus, all three *BRCA1*

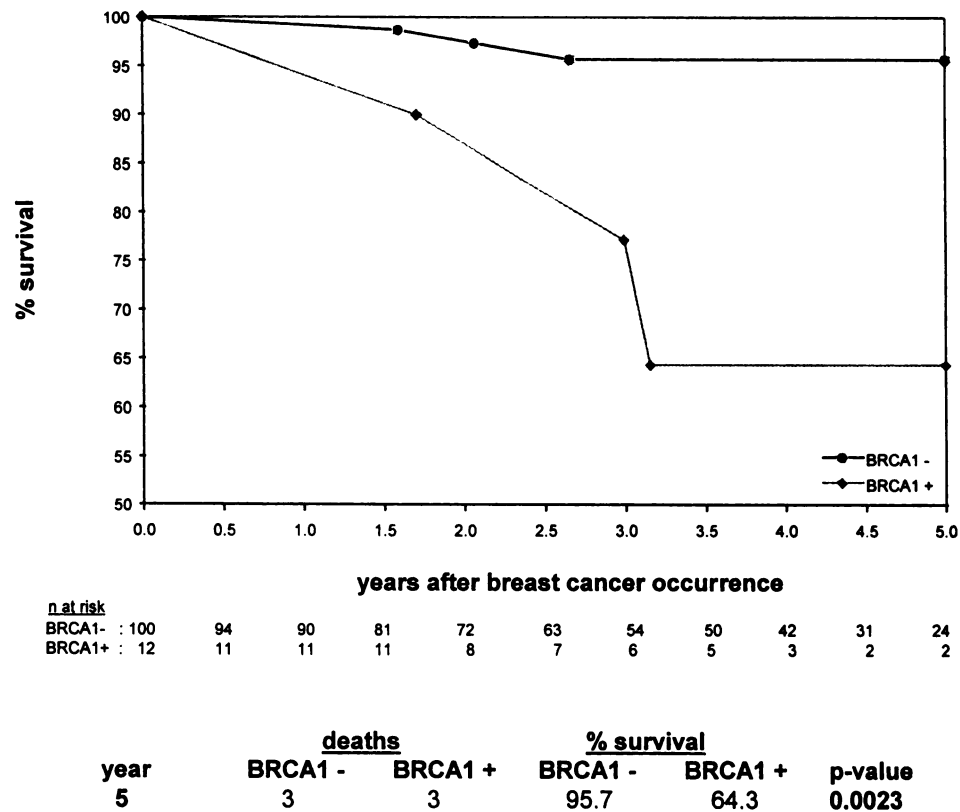


Fig. 1 Kaplan-Meier survival curves with regard to survival until death from breast cancer are shown for *BRCA1* carriers (◆) and *BRCA1* noncarriers (●). The number of people remaining at risk at the end of each half-year is shown beneath the figure. The number of events (deaths) and the survival (% survival) for each of the two subgroups are shown.

carriers who relapsed were lymph node negative. Similarly, 1 of 65 (1.5%) women without mutations who had tumors less than 2 cm in size relapsed at a distant site, whereas 2 out of 6 (33.3%) *BRCA1* mutation carriers with tumors this size relapsed distantly ($P = 0.0001$, log-rank test). When analyzed by TNM classification, 2 of 3 T_1N_0 *BRCA1* mutation carriers relapsed at a distant site, whereas only 1 of 56 noncarriers did so ($P = 0.0001$, log-rank test).

Eleven of the 12 *BRCA1*-positive breast cancers were grade 3, and therefore, we did not stratify mutation status by grade. Of the 11 *BRCA1* carriers with grade 3 tumors, 3 died of breast cancer. Similarly, all 3 deaths in the noncarriers occurred among the 29 women who had grade 3 tumors ($P = 0.4$, log-rank test). We recognize that *BRCA1* mutation positivity may not be acting as a predictive factor independent of grade, but our data suggest that breast cancer developing in a *BRCA1* carrier is likely to have a high grade and a poor prognosis.

DISCUSSION

Tumor size and axillary involvement at primary surgery are prognostic factors in breast cancer (12, 13). However, host genetic factors have not been studied in any great detail in this context. Here, we have shown that germ-line mutations in *BRCA1* in Ashkenazi Jewish women are associated with an increased probability of both breast cancer-related death in the

first 5 years after diagnosis and with a significantly reduced distant disease-free 5-year survival (Figs. 1 and 2).

The poor prognosis for *BRCA1*-positive breast cancer that we observed in women with small or lymph node-negative tumors is of potential importance, because this suggests that *BRCA1* mutation carriers with breast cancer may be especially prone to develop distant metastases without evidence of axillary lymph node involvement. This implies that the natural histories of *BRCA1*-related and sporadic breast cancer may be distinct.

The 5-year survival for the entire patient group was 91.9%. This excellent survival is explained in part by the small size of tumors in this series (mean, 1.79 cm; 63.4% ≤ 2 cm in diameter). This may reflect increased breast cancer awareness in the public served by our institution. In a Surveillance, Epidemiology and End Results-based study, only 33.6% of 24,740 breast cancers diagnosed between 1977 and 1982 were less than 2 cm in diameter, and the 5-year survival in this subgroup was in excess of 90%. Seventy % of these small tumors were lymph node negative, and the 5-year survival in this group was 96.3% (13). In our series, 58.9% of patients who did not have *BRCA1* mutations had T_1N_0 tumors (Table 2), and there were no breast cancer deaths in this group. Only two deaths were observed in 28 $T_{1,2}N_1$ *BRCA1* mutation-negative individuals, partly explaining the excellent survival rate observed in the noncarriers (Fig.

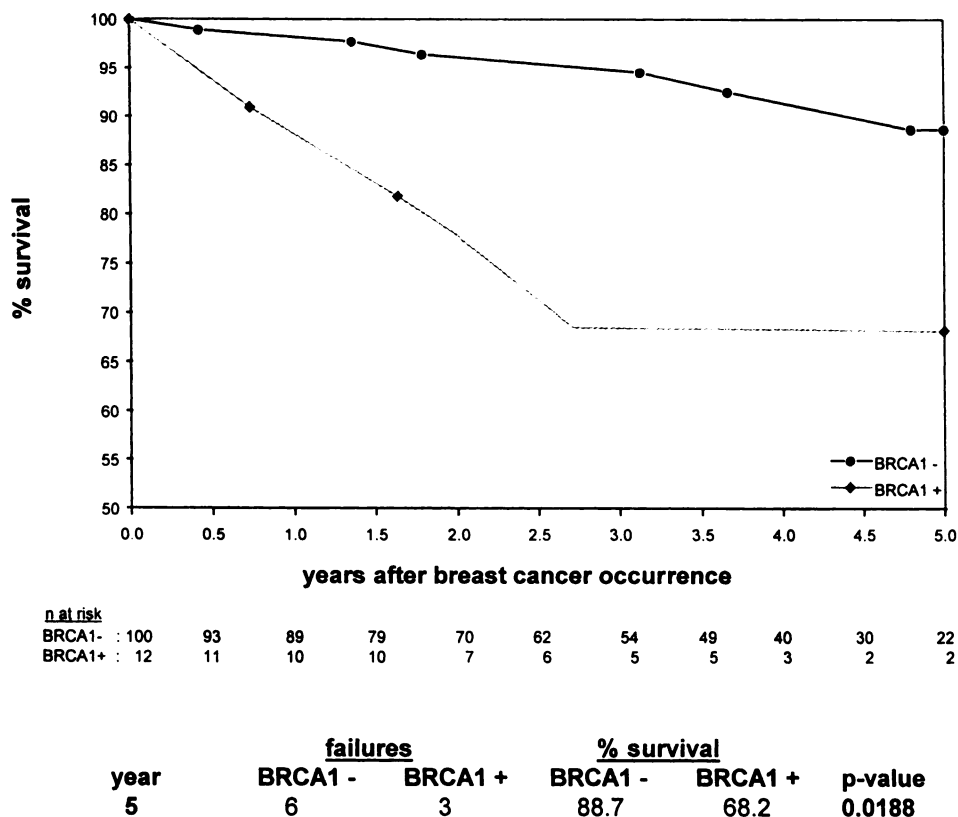


Fig. 2 Kaplan-Meier survival curves with regard to survival until distant disease occurrence are shown for BRCA1 carriers (◆) and BRCA1 noncarriers (●). The number of people remaining at risk at the end of each half-year is shown beneath the figure. The number of events (failures) and the event-free survival (% survival) for each of the two subgroups are shown.

1). The magnitude of the survival ratios produced by the presence of a BRCA1 mutation may differ for other populations.

Women diagnosed with breast cancer at between 20 and 39 years of age are recognized to have a poorer prognosis than women diagnosed at age 50 or more (14–16). In this study, we compared distant recurrence in BRCA1 mutation carriers and noncarriers in two age groups, dichotomized at age 40. BRCA1 mutation carriers in both age groups were more likely than noncarriers to relapse at a distant site (diagnosis at <40 years of age, RR = 4.7 and P = 0.18; diagnosis at ≥40 years of age, RR = 2.0 and P = 0.43). These findings suggest that the survival difference associated with a BRCA1 mutation may be independent of age, but due to the small number of events, this remains uncertain.

Previous studies of breast cancer in BRCA1-linked pedigrees suggested the possibility of a better prognosis for women with BRCA1 mutations compared to those with sporadic breast cancer. Porter *et al.* (17) studied eight breast cancer families and showed an 83% 5-year survival for women with breast cancer and who had a high likelihood of carrying the at-risk BRCA1 haplotype. The 5-year survival for an age-matched control population of sporadic breast cancers diagnosed in Scotland during a similar time period was 61%. In the Surveillance, Epidemiology and End Results registry study (13), the survival for the subgroup with tumors >5 cm was 62.7%, suggesting that the

members of the control population in the Scottish study were diagnosed relatively late. Also, this study was based on large families, among whom knowledge of their elevated breast cancer risk may initiate screening, with a subsequent potential for lead-time bias. Marcus *et al.* (18) reported that the breast cancers in the BRCA1-linked group were more likely to be diagnosed at a young age and to be high grade and aneuploid (Ps all <0.0001), but paradoxically, breast cancers in this group had a lower crude death rate hazard ratio (RR = 0.63; P = 0.051). In fact, after adjusting for age and stage, Marcus *et al.* (18) found that the BRCA1 mutation carriers had a higher crude death rate hazard ratio than did the controls (RR = 1.65; P = 0.12). As in the Scottish study (17), the 5-year survival rate in the comparison group (59%) was much worse than in our study (Fig. 1), and it is not entirely clear that the comparison group in that study (18) was appropriate. The advantage of the present study is that the subjects and controls were derived from an identical population. Another strength is that we ascertained all breast cancer cases and we determined mutation status independently of vital status, eliminating the potential for survivor bias. Also, the collection of clinicopathological information on all of the cases was blinded to the mutation status.

All of our cases were of Ashkenazi Jewish descent. Ashkenazi Jewish women with BRCA1 mutations and breast cancer may have an adverse prognosis compared with women

from other ethnic groups with breast cancer who carry *BRCA1* mutations. Follow-up studies using larger, ethnically heterogeneous populations are therefore indicated. From this study, it appears that *BRCA1* carrier status is an important prognostic marker of particular relevance for women with small or lymph node-negative tumors. Those who carry *BRCA1* mutations may develop tumors that, despite their small size, have a poor prognosis. Because this information is available prior to diagnosis, the findings also have important implications for the evaluation of preventive interventions, such as prophylactic mastectomy or the use of endocrine measures.

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