Primary node negative breast cancer in *BRCA1* mutation carriers has a poor outcome

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Summary

Background: The association between *BRCA1* germ-line mutations and breast cancer prognosis is controversial. A historical cohort study was designed to determine the prognosis for women with axillary lymph node negative hereditary breast cancer.

Patients and methods: We tested pathology blocks from 118 Ashkenazi Jewish women with axillary lymph node negative breast cancer for the presence of the two common *BRCA1* founder mutations, 185delAG and 5382insC. Patients were followed up for a median of 76 months. Somatic *TP53* mutations were screened for by immunohistochemistry, and direct sequencing was performed in the *BRCA1*-positive tumours.

Results: Sixteen breast cancer blocks (13.6%) carried a *BRCA1* mutation. Young age of onset, high nuclear grade, negative estrogen receptor status and over-expression of p53 were highly associated with *BRCA1*-positive status (*P*-values all < 0.01). *BRCA1* mutation carriers had a higher mortality than non-carriers (five-year overall survival, 50% and 89.6%, respectively, $P = 0.0001$). Young age of onset, estrogen receptor negative status, nuclear grade 3, and over-expression of p53 also predicted a poor outcome. Cox multivariate analyses showed that only germ-line *BRCA1* mutation status was an independent prognostic factor for overall survival ($P = 0.01$). Among nuclear grade 3 tumours, the *BRCA1* mutation carrier status was a significant prognostic factor of death (risk ratio 5.8, 95% confidence interval: 1.5–22, $P = 0.009$). Sequencing of *BRCA1*-related breast cancers revealed one *TP53* missense mutation not previously reported in breast cancer.

Conclusions: Using a historical cohort approach, we have identified *BRCA1* mutation status as an independent prognostic factor for node negative breast cancer among the Ashkenazi Jewish women. Those managing women carrying a *BRCA1* mutation may need take these findings into consideration. Additionally, our preliminary results, taken together with the work of others suggest a different carcinogenic pathway in *BRCA1*-related breast cancer, compared to non-hereditary cases.

Key words: *BRCA1*, breast cancer, p53, survival

Introduction

Most women who present with primary breast cancer have axillary lymph nodes that are free from cancer. There have been numerous studies of factors that influence prognosis in lymph node negative breast cancer [1]. The most important factor is tumour size, followed by grade, histological type, proliferation status and to a lesser extent, estrogen receptor (ER) status. More recently, other possibly independent prognostic variables such as p53, $p27^{Kipl}$, ERBB2, microvascular density, and cathepsin D have been identified [2]. Age is a predictor of adverse disease-free survival (DFS) and overall survival (OS), but is probably accounted for by the presence of poor prognostic factor profiles, rather than age itself [3].

Conflicting data exist as to whether the prognosis of familial or hereditary breast cancer differs from that of sporadic cases [4, 5]. Families with multiple cases of early-onset breast and ovarian cancer often carry mutations in tumour suppressor genes, *BRCA1* or *BRCA2* [6]. Nine studies comparing DFS or OS in *BRCA1*-positive and *BRCA1*-negative women with breast cancer have been carried out [7–15] with inconsistent results. In our preliminary study, we noted a striking survival disadvantage for *BRCA1* mutation carriers with small, lymph node negative breast cancers [9].

The carcinogenic pathway which links a constitutional *BRCA1* mutation in histologically normal mammary cells to invasive breast adenocarcinoma harboring particular anatomopathological characteristics is still unknown. Some indirect data support the hypothesis of a distinct molecular pathway in hereditary breast cancer cases compared to their sporadic counterparts. For example, the accumulation of somatic genetic alterations in *BRCA1*-associated breast cancer differs from that of sporadic breast cancer [16]. Recently, it has been observed that breast cancers arising in *BRCA1* mutation carriers are more likely to over-express p53 than their

sporadic counterparts [17]. *In vitro* and *in vivo* experiments have demonstrated the biochemical and functional interactions between the *BRCA1* and *TP53* genes products [18]. For example, in a heterozygous *BRCA1*-deficient mouse model, an increased incidence of mammary tumours was only observed in the context of a *TP53* null background [19]. Data suggest that loss of p53 checkpoint control is an early and important event in the carcinogenic pathway of cancer arising in *BRCA1* mutation carriers [20]. It has been postulated that the genetic instability associated with the loss of *BRCA1* functions triggers mutation of *TP53*, which in turn facilitates tumour formation. A particular spectrum of *TP53* mutations has been reported in *BRCA1* and *BRCA2*-associated breast cancer [21–24], but this has not been confirmed by others [25].

To address the question of the effect of a *BRCA1* germ-line mutation on prognosis in lymph node negative breast cancer, we studied 118 unselected Ashkenazi Jewish women with lymph node negative breast cancer diagnosed at a single institution between 1986 and 1995. We chose to study the Ashkenazi Jewish population because the majority of hereditary breast cancer can be attributed to two mutations in *BRCA1* (185delAG and 5382insC) and one mutation in *BRCA2* (6174delT) [26]. These three mutations are founder mutations and have attained a high frequency in the Ashkenazi Jewish population [27]. We have excluded *BRCA2*-positive tumours in this study. Only three *BRCA2* 6174delT mutations (2.5%) have been identified in this series of 118 women by a combination of various assays, and moreover, some studies showed clinicopathological differences between *BRCA1*- and *BRCA2*-associated tumours [24, 28, 29]. We determined *BRCA1* mutation and p53 expression status by polymerase chain reaction-based assays and immunohistochemistry (IHC), respectively. *BRCA1*-positive cases were directly sequenced for somatic *TP53* mutations in exons 5–9. The *BRCA1* status was then related to clinical outcome.

Patients and methods

Cases

Study subjects were identified in the medical records department of the Sir Mortimer B. Davis-Jewish General Hospital (SMBD-JGH), Montreal, Quebec and included all 118 women who reported themselves as being Ashkenazi Jewish by birth. All women were diagnosed with invasive breast cancer below 65 years of age between 1 January 1986 and 1 November 1995 and had undergone axillary node dissection. The routine base-line evaluation of the patients included laboratory analyses, chest radiography, mammography, liver ultrasonography and bone scanning. We selected the age of 65 as the upper age limit. Interestingly, it appears that the likelihood of observing a *BRCA1* mutation in a woman with breast cancer diagnosed after age 65 is about the same as that in the general Ashkenazi Jewish population [30]. The study was approved by the Research Ethics Committee of the SMBD-JGH. Breast cancer blocks were identified from each of these eligible women. Clinicopathological and follow-up information was obtained from chart review. The median age of patients at the time of diagnosis was 54.3 years (range 27.6–64.9). Various regimens of adjuvant chemotherapy were administered to 41 (37.3%) patients. In general, patients at the SMBD-JGH were seen on a regular basis every six months for five years, then after, on a yearly basis. The follow-up evaluation consisted of a clinical evaluation, a physical examination, radiology and serum chemistry tests. The median follow-up duration was 76 months (range 10–133). Specimens were reviewed by one pathologist (L. R. Bégin). Tumour size was less than 20 mm in 79 cases (67%). Histological tumour type and nuclear grade were determined by specimen and chart review. Tumours were pure histological variants of invasive breast carcinomas comprising 74.1% ductal, 9.3% lobular, 5.5% tubular and 11.1% other types. The specimens were then coded and DNA was extracted from the paraffin wax embedded blocks using standard techniques. Clinical, pathological and molecular data from the 118 samples were collected in a mutually blinded fashion. OS rates were calculated as the number of months from the date of primary surgery until the date of death. Eleven (9.7%) women were lost to follow-up within three years of diagnosis. Four of these women had had a breast cancer-related event and were subsequently lost to follow-up and therefore the event was included in the survival analyses and the patients were censored after this event. One of the eleven women lost to follow-up had a *BRCA1* mutation. Two *BRCA1* mutation carriers developed an ovarian cancer and were censored after this event for the breast cancer-specific survival evaluation. Nine (7.6%) patients developed an ipsilateral recurrence and ten (8.5%) a contralateral tumour. One person had both an ipsi- and a contra-lateral tumour and so was recorded once in each group. Information on family history was not available.

ER status

In 109 (95.6%) cases ER nuclear protein expression was detected using a standard streptavidin-biotin-peroxidase complex immunohistochemical technique. Positivity implies >10% of tumour cell nuclei showing immunoreactivity. In five cases (4.4%), the conventional radioimmunoassay (RIA) assays were used to determine ER nuclear protein status. A positive ER score was taken as >10 fmol/mg protein. Details of these assays have been previously described [31].

BRCA1 and *BRCA2* mutation status

Mutation analysis for the recurrent Ashkenazi Jewish *BRCA1/2* mutations (*BRCA1*: 185delAG, 5382insC; *BRCA2*: 6174delT) was carried out as described previously [9]. Briefly, the extracted DNA was amplified using the polymerase chain reaction using oligonucleotide primers specific to *BRCA1* exons 2 (185delAG) and 20 (5382insC). The products, with suitable positive controls, were electrophoresed overnight in denaturing polyacrylamide gels. After autoradiography, the sizes of the fragments were compared with the positive controls. PCR-RFLP endonuclease digestion assays, specific for the 185delAG or the 5382insC mutations were also used for all cases. Haplotype analysis was also used to confirm 5382insC mutations. We used breast cancer tissue as our source of DNA as no somatic mutations have been reported in *BRCA1* and therefore the mutations observed were assumed to be germ-line in origin. We also looked for the common Ashkenazi *BRCA2* mutation (6174delT) by single-strand conformation analysis, by a mutation-specific PCR-RFLP endonuclease digestion analysis and by direct sequencing. *BRCA2*-positive tumours (three) were excluded in the further analysis.

p53 IHC

p53 protein accumulation was detected as previously described [32], using a standard streptavidin-biotin peroxidase immunohistochemical technique with an anti-p53 (DO-7) monoclonal antibody (Dako Corp., Carpinteria, California).

TP53 sequencing

DNA isolated from paraffin-embedded tumour tissue from 14 individuals who carried *BRCA1* mutations and whose tissue was available

Table 1. Association of *BRCA1* status and clinicopathological variables.

Clinicopathological variable (number of patients)	All subjects (n = 115)	*BRCA1*-negative (n = 99)	*BRCA1*-positive (n = 16)	Odds ratio	95% CI	P-value
Age at diagnosis (years)						
Median	53.9	53.4	46.1	–	–	0.006
Range	27.6–64.9	27.6–64.9	33.2–62.1			
Tumour size (mm)						
Median	1.30	1.30	1.50	–	–	0.24
Range	0.15–5.00	0.30–5.00	0.15–5.00			
Nuclear grade (115)						
1	32	32	0	1.0		
2	45	43	2	3.7	0.2–80.5	0.51
3	38	24	14	38.5	2.2–677	<0.0001
Estrogen receptor status (114)						
Positive	68	67	1	1.0		
Negative	46	31	15	32.2	7.7–100	<0.0001
p53 status (IHC assay, 109)						
Negative	84	78	6			
Positive	25	16	9	7.3	2.5–21.2	<0.001
Adjuvant chemotherapy (108)						
No	67	60	7			
Yes	41	32	9	2.4	0.8–6.9	0.17

for analysis was screened for mutations in *TP53*. Intronic primers were designed for amplifying exons 5–9 of *TP53*. Cycle sequencing was performed directly on PCR products using both the forward and reverse primers in separate reactions using the ^{33}P-dideoxy-labelled terminators kit (Amersham Pharmacia Biotech, Uppsala, Sweden). The products of cycle sequencing were electrophoresed on glycerol tolerant 6% acrylamide gels. Samples that harbored a mutation were reamplified from the original genomic DNA sample, and the mutation was confirmed by DNA cycle sequencing.

Statistical analyses

All statistical tests were two-sided. Clopper–Pearson exact 95% confidence intervals (95% CI) were calculated for the proportion of *BRCA1* mutation carriers in the cohort. P-values were calculated for categorical variables using Fisher's exact test. For continuous variables, the non-parametric Wilcoxon's two-sample test was used. Five year and median survival rates were estimated using an actuarial approach. Significance was assessed using a log-rank test. A Cox proportional hazards model was developed for the risk of death at the median follow-up.

Results

Paraffin blocks from 118 breast cancer cases diagnosed among Ashkenazi Jewish women younger than 65 years were analyzed for the presence of two *BRCA1* mutations and one *BRCA2* mutation. Sixteen *BRCA1* mutations were identified (13.6%, 95% CI: 7.9%–21%). Eleven mutations were 185delAG and five were 5382insC. Nine of twenty-eight (32.1%) women diagnosed under the age of 45 and seven of ninety (7.7%) diagnosed between the ages of 45 and 64 carried a *BRCA1* mutation. Three individuals with the 6174delT *BRCA2* mutation (2.5%) were identified, and excluded from subsequent analyses. The histological types of *BRCA1*-related tumours were ductal invasive (14), papillary (1), and medullary (1). Clinicopathological characteristics of the studied population and association with *BRCA1* status are summarized in Table 1. *BRCA1* mutation carrier status was significantly associated with young age, high nuclear grade, ER negativity, p53 over-expression, but not with tumour size. One hundred nine tumour blocks were available for p53 IHC studies using antibody DO-7. Twenty-five (22.9%) of the breast cancers were positive by IHC. Among the 15 *BRCA1* mutation carriers who were studied for p53 over-expression, the frequency was 60% (9 of 15) compared with 17% (16 of 94) in non-carriers of *BRCA1/2* mutations (OR: 7.3, 95% CI: 2.5–21, P = 0.0009). A strong correlation between detection of p53 expression with IHC and the presence of somatic *TP53* mutations in *BRCA1* mutation carriers was demonstrated by direct sequencing of *TP53* exons 5–9 (Table 2). One breast cancer specimen did not show p53 over-expression by IHC, but was found to have a frame-shift mutation in exon 7. Two specimens were positive by IHC but no mutations could be detected in exons 5–9. It is possible that a mutation exists in other exons or non-coding regions of *TP53* for these two cases, or that p53 over-expression is occurring in the absence of a *TP53* mutation. All except one (missense A760G; Ile254Val) of the somatic *TP53* mutations identified among the *BRCA1*-positive tumours have been previously reported in breast cancer (IARC *TP53* database [33]).

Women with *BRCA1* germ-line mutations experienced a poor survival. Women who carried *BRCA1* mutations were more likely to relapse and to die of breast cancer in the first five years after diagnosis than women who were *BRCA1*-negative (five-year OS: 50% vs. 89.6%, P = 0.0001; five-year breast cancer specific survival (BCSS): 57.1% vs. 90.5%, P = 0.0001) (Figure 1). *BRCA1* mutation carriers also developed contralateral tumours significantly more frequently than non-mutation carriers

Table 2. *TP53* mutations (exons 5–9) in *BRCA1* mutation carriers.

Individual	*TP53* status by direct sequencing (exons 5–9)				p53 status by IHC[a]
	Exon	Codon	Mutation	Effect	
1	8	273	G818A	Arg273His	+
2	8	270	T809G	Phe270Cys	+
3	7	242	T724A	Cys242Ser	+
4			–		–
5			–		+
6	7	254	A760G	Ile254Val	+
7			–		–
8			–		–
9			–		+
10	5	145	T434C	Leu145Pro	+
11	7	248	G743A	Arg248Gln	+
12	7	248	G743A	Arg248Gln	+
13	7	241	722delC	Frameshift (stop 246)	–
14			Not done		–

[a] Abbreviation: IHC – immunohistochemistry. Two *BRCA1*+ individuals were not studied for *TP53* by IHC or by sequencing.

Table 3. Cox proportional hazards model for overall survival.

Variable	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P*-value	RR (95% CI)	*P*-value
Age				
⩾50 years	1.0		1.0	
<50 years	2.7 (1.1–7.2)	0.04	1.6 (0.6–4.5)	0.34
Tumour size				
<20 mm	1.0		1.0	
⩾20 mm	1.5 (0.6–3.9)	0.43	0.9 (0.3–2.4)	0.80
Nuclear grade				
1, 2, 3 (discrete)	1.0		1.0	
	3.3 (1.5–7.3)	0.003	1.6 (0.6–4.3)	0.36
Estrogen receptor				
Positive	1.0		1.0	
Negative	4.0 (1.4–11)	0.009	1.3 (0.3–5.6)	0.72
BRCA1 mutation carrier				
Negative	1.0		1.0	
Positive	8.2 (3.2–21)	0.0001	3.8 (1.3–11)	0.01
p53 expression				
Negative	1.0		1.0	
Positive	2.5 (1.0–6.7)	0.06	1.0 (0.3–3.0)	0.98

Abbreviations: RR – relative risk; 95% CI – 95% confidence interval.

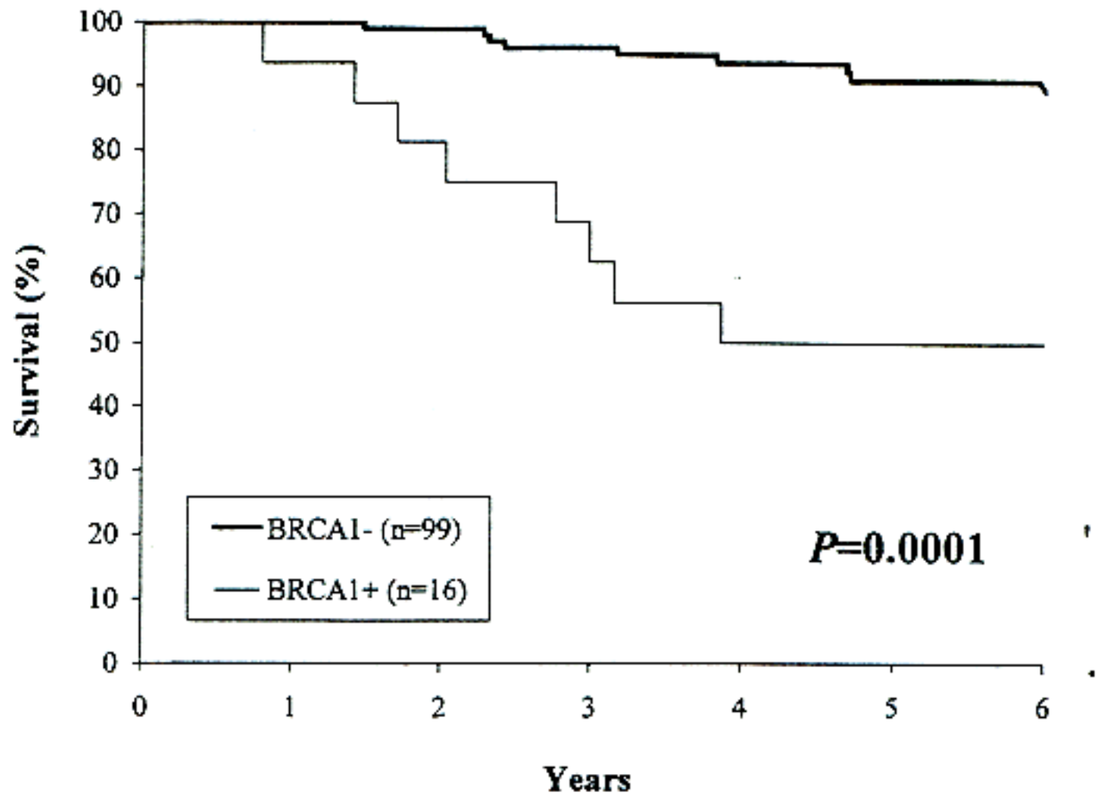

Figure 1. Kaplan–Meier survival curves with regard to survival until death are shown for *BRCA1* carriers (thin line) and *BRCA1* non-carriers (bold line).

(incidence at five years 19.3% vs. 2.0%, $P = 0.002$). By contrast, there was no significant difference in the rate of ipsilateral tumours in *BRCA1* mutation carriers compared with non-carriers (incidence at five years 4.2% vs. 12.9%, $P = 0.10$) (data not shown). Univariate analysis of OS showed that young age of onset, high nuclear grade, ER negativity, p53 over-expression, and germline *BRCA1* mutation all had prognostic value (Table 3). Similar results were seen for BCSS. Multivariate analysis showed that only *BRCA1* mutation carrier status was an independent prognostic factor (Table 3). To study this effect more specifically, we evaluated the *BRCA1* status in less favorable subgroups. Among the subpopulation of nuclear grade 3 tumours ($n = 38$), 8 out 14 *BRCA1* mutations carriers died compared with 3 out of 24 sporadic cancer cases (RR: 5.8, 95% CI: 1.5–22, $P = 0.009$). Similarly, 7 deaths occurred in the 15 ER negative *BRCA1*-positive cases compared to 5 deaths in the 31 ER negative sporadic cases (RR: 3.4; 95% CI: 1.1–11, $P = 0.038$). Only one *BRCA1* mutation carrier was ER positive: this person did not die of breast cancer. The tumour size was not significantly different between the *BRCA1*-positive and *BRCA1*-negative subgroups, but *BRCA1* mutation carrier status was a strong outcome predictor in the 79 tumours with a size <20 mm (RR: 12.5, 95% CI: 3.8–42, $P = 0.0001$). A restricted number of larger tumours did not permit analysis of the role of the *BRCA1* status in this subgroup. Interestingly, the adverse outcome for *BRCA1* mutation carriers was independent of age at diagnosis (<50 years: $P = 0.003$; ⩾50 years: $P = 0.04$). Women whose tumours over-expressed p53 had a significantly reduced five-year OS (71.8% vs. 88.4%, $P = 0.03$). However, *BRCA1* mutation status did not predict outcome for women with tumours that over-expressed p53 ($P = 0.17$). Overall, these observations suggest that *BRCA1* status is a stronger determinant of survival than *TP53* status.

Discussion

Nowadays, two-thirds of women with breast cancer have no evidence of axillary lymph node involvement at diagnosis [1]. On average, two-thirds of these women will be alive 10 years later [34]. The very good survival rate in our cohort of *BRCA1*-negative women (mostly affected by tumours of small size) is in agreement with recent studies among node negative patients [35, 36]. Numerous factors are associated with an increased risk of distant relapse. Our results suggest that in Ashkenazi Jewish women with negative axillary lymph nodes at diagnosis, the presence of a *BRCA1* mutation is an adverse prognostic factor. At 5 years follow-up, 8 of 16

BRCA1 mutation carriers (50%) had died, in contrast to 9 of 99 *BRCA1* non-carriers (9.1%). This difference was highly significant ($P = 0.0001$) (Figure 1). As previously described [28, 37, 38], we confirmed the very strong association between *BRCA1* mutation carrier status and adverse clinicopathological features of breast cancers, such as young age of onset, high nuclear grade, ER negative status, and somatic *TP53* mutations. All these characteristics are recognized as being indicators of worse prognosis in breast cancer. In Cox multivariate analyses, only germ-line *BRCA1* mutation status had an independent prognostic value for OS. Among the ER negative, nuclear grade 3 and tumour < 20 mm subpopulation, the *BRCA1*-positive status still confers a significant worse prognosis. As these data were obtained from relatively small subsets of the studied population, they need to be confirmed in larger series and should be regarded as hypothesis-generating at this point.

Previous studies have shown that p53 positivity (as demonstrated by IHC) or somatic *TP53* mutations are frequent in hereditary breast cancer [39] and particularly so in *BRCA1*-related breast cancer [17, 21, 22]. It has been suggested that *BRCA1*-related breast tumourigenesis requires a somatic *TP53* mutation [40, 41]. Here, the *TP53* mutation screening in the *BRCA1*-positive cases was performed by IHC and DNA direct sequencing, with a good correlation between the two methodologies. We identified one new missense mutation (A760G) not previously reported in the IARC breast cancer *TP53* database [33]. Another missense mutation we observed (G818A) has been already reported in a *BRCA2*-related breast cancer [25]. The other *TP53* mutations we identified were already described in the IARC *TP53* database [33]. We confirm that *TP53* mutations are significantly more common in *BRCA1*-related hereditary breast cancer than in non-*BRCA1*, non-*BRCA2*-related breast cancer ($P = 0.0009$). However, we do not find evidence for a worse prognosis for *BRCA1* mutation carriers whose tumours over-express p53 compared with those carriers whose tumours do not overexpress p53.

This study has demonstrated a survival disadvantage for node-negative women with germ-line *BRCA1* mutations. This present study includes 71 individuals from our previous study [9], where we reported that *BRCA1*-related breast cancers in Ashkenazi Jewish women were associated with a worse outcome. By identifying more *BRCA1* mutation carriers and almost doubling the median follow-up time, we have been able to generate a multivariate Cox model for survival. The key observation that the presence of a *BRCA1* mutation is an independent poor prognostic factor for lymph node negative breast cancer has extended our preliminary results.

Several previous survival studies in hereditary breast cancer have indicated that there is no clear survival disadvantage associated with a *BRCA1* mutation [7, 8, 10, 12, 14, 42, 43]. However, one study of women diagnosed with breast cancer at less than 36 years of age did find a significantly worse survival for *BRCA1* mutation carriers [11]. No studies have specifically studied lymph node negative women. The conflicting findings in these studies require further explanation. There are several sources of selection bias in clinic or pedigree-based studies, all of which favorably influence prognosis [4, 5]. This is because the biases in linkage or clinic-based studies all result in the preferential inclusion of living women (as compared to deceased women) in the study cohort. In general, to test for *BRCA1* mutation status, it is necessary to have a source of constitutional DNA. Mutation analysis is most readily performed using DNA extracted from peripheral blood as it is relatively straightforward to perform mutation analysis with this source of DNA. If the patient is deceased, it may be possible to obtain DNA from a preserved tumour specimen. However, it is generally not possible to search the entire coding region of *BRCA1* and *BRCA2* for mutations using DNA from paraffin-embedded specimens. An additional problem is that if the proband has had breast cancer, then including her in the study will result in a spurious elevation in the survival estimates. Even if the proband is excluded from the study of survivorship, as was recently carried out [8], the problem of ascertainment bias is not eliminated, because not all families with hereditary breast cancer in a population will be ascertained. Ideally, one would ascertain incident cases of breast cancer in a population, obtain information on *BRCA1* status and other prognostic factors, and follow these women forward in time for survival [11]. However, to give robust estimates, this method requires 10 or more years of follow-up. An alternate method is to use historical cases and archival specimens from ethnic groups with founder mutations, such as the Ashkenazi Jewish [44, 45] French Canadian [46] and Icelandic [47] populations. Interestingly, a recent large community-based study of breast cancer limited to the Ashkenazim [15] found no survival difference between carriers and non-carriers of *BRCA1/2* mutations. Even this study was not free of interpretative difficulties, as the retrospective cohort design did not permit the confirmation of diagnosis of breast cancer or the cause of death in the cohort. More seriously, the mutation status of all affected individuals was inferred from the tested index case. Thus, large prospective or retrospective cohort studies where all individuals in the cohort are tested for disease-associated mutations are required. In this regard, Robson et al. [48] very recently reported the results of a retrospective ethnically restricted hospital-based study similar in design to this and our previous study [9]. Among 305 breast cancer patients of Ashkenazi Jewish descent who underwent conservative treatment, 28 were identified as carriers of a founder *BRCA1* or *BRCA2* mutation. Women with mutations were more likely than non-mutation carriers to develop contralateral breast cancer ($P = 0.002$). In univariate analysis the 5- and 10-year distant disease-free survival and BCSS were significantly worse among the *BRCA1/2* mutation carriers compared with patients with mutation-negative breast cancers. In multivariate analysis, only tumour stage and nodal status retained prognostic significance, but

positive *BRCA1/2* mutation status was associated with a statistically non significant trend towards a worse outcome ($P = 0.14$). As results were not dichotomized between *BRCA1* and *BRCA2* mutation carriers or according to the nodal status, it is difficult to exactly compare the two data sets, but clearly the results point in the same direction as those reported here.

Finally, a possible explanation for the worse survival encountered by this hereditary breast cancer subgroup is that the *BRCA1* germ-line mutations found in the Ashkenazi Jewish population are in fact associated with a particular unfavorable prognosis compared to other *BRCA1* mutations. To date, no clearly documented gene modifiers or specific somatic genetic abnormalities have been reported in this population.

Other studies will be required to confirm our findings. However, we believe the methods we have employed are robust and as free from bias as is possible in follow-up studies. If our observation that small, lymph node negative breast cancers occurring in *BRCA1*-positive women have a poor prognosis is confirmed, then different detection and treatment regimens may be required for this particular subgroup of patients.

Acknowledgements

Research support: Funding: Quebec Family Cancer Network grant from the Fonds de la Recherche en Santé du Québec, Department of Defense Grant, no. DAMD17-98-1-8112, Judy Steinberg Research Fund. POC was supported by a grant from la Ligue Genevoise contre le Cancer and Cancer & Solidarité Fondation.

We would like to thank Dr F. C. Fraser for advice, Drs L. Pinsky and P. Gordon for support, M. Bourdeau and M. Cotiangco for immunohistochemistry technical assistance, M. Argiriou for secretarial assistance and Dr P. Jedlicka, C. Serruya and W. Frisell for help in preparing the manuscript.

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