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SHORT REPORT

BRCA1 and BRCA2 founder mutations in patients with bilateral breast cancer

Ruth Gershoni-Baruch[1,3], Efrat Dagan[1,3], Getta Fried[2], Ilana Kepten[1] and Eliezer Robinson[2,3]

[1]*Department of Human Genetics,* [2]*Department of Oncology, Rambam Medical Center*
[3]*Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

Bilateral breast cancer is traditionally considered an indirect indicator of inherited predisposition to cancer. To appreciate the contribution of genetic determinants to bilateral breast cancer in Jewish women we genotyped 55 such women for the three predominant mutations in BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) that account for the overwhelming majority of BRCA mutations in high-risk Jewish families. Among women with bilateral breast cancer, 17 mutation carriers (17/55; 29.6%) were identified. Individual mutation frequencies were 18.5% (10/55) for 185delAG, 3.7% (2/55) for 5382insC and 7.4% (5/55) for 6174delT. Carrier rate was significantly higher ($P < 0.0016$) in women with bilateral breast cancer whose first tumour was diagnosed at or before 42 years of age (82%; 14/17) than in women diagnosed after 42 years of age (7.9%; 3/38). Among patients with bilateral breast cancer and positive family history 45% (14/31) carried a BRCA mutation. Of these 86% (12/14) had one breast cancer diagnosed at or before 42 years of age. Our results suggest that bilateral breast cancer *per se*, in most cases, does not reflect genetic predisposition, unless associated with early age of onset (first tumour diagnosed at or before 42 years of age). Although the relationship between young age and carrier state in women with bilateral breast cancer is strong, no significant association between family history and carrier state was found. We can thus speculate that women with early onset breast cancer who carry a BRCA1 or BRCA2 mutation are prone to acquire a second breast tumour.

Keywords: BRCA1; BRCA2; bilateral breast cancer; hereditary breast/ovarian cancer

Introduction

Contralateral breast cancer, although rare, is the most common second primary tumour found in breast cancer patients. The incidence of synchronous bilateral breast cancer is 1–2%, whilst that of metachronous reaches 5–6%.[1,2] The lifetime risk of developing a second primary has been estimated to exceed 10% in women younger than 50 years.[2] It is still argued, however, whether bilaterality *per se* reflects genetic predisposition or rather results from a combination of other events.

An expanded population-based study has confirmed that BRCA1 185delAG and 5382insC mutations and BRCA2 6174delT mutation are frequent mutation alleles predisposing to hereditary breast cancer among Ashkenazi Jews[3,4] with carrier frequencies of 1.09% for 185delAG, 0.13% for 5382insC and 1.52% for

Correspondence: Dr Ruth Gershoni-Baruch, Department of Human Genetics, Rambam Medical Center, POB 9602, 31096 Haifa, Israel. Fax: 972 4 8542441; E-mail: rgershoni@rambam.health.gov.il
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6174delT.[5,6] The estimated carrier frequency of mutation 185delAG in non-Ashkenazi Jews is about 0.5%.[7]

In order to appreciate the contribution of genetic determinants to bilateral breast cancer, we genotyped 55 Jewish women with bilateral breast cancer for the three founder Jewish mutations in BRCA1 and BRCA2.

Patients and Methods

A retrospective study cohort was composed of 55 (51 Ashkenazi and 4 non-Ashkenazi) Jewish women diagnosed with bilateral breast cancer (17 synchronous and 38 metachronous). The study cohort was drawn from 126 Jewish women given the diagnosis of bilateral breast cancer between 1973 and 1998 at Rambam Medical Center and identified via the registry of the Oncology Department. Our non-Ashkenazi patients were of Jewish Iraqi and Turkish descent, ethnic subgroups known to harbour the 185delAG mutation. Of the 126 patients with bilateral breast cancer identified as eligible for the study, 32 died, 25 refused to participate, and 14 were unavailable. The remaining 55 women were interviewed at our cancer genetic clinic between February 1996 and December 1998. A comparison group of 55 Jewish women with unilateral breast cancer, matched for age of onset, ethnic origin and family history were randomly selected from a group of 245 patients who have attended our oncogenetic clinic and had been diagnosed with breast cancer 5 years or more earlier. Of these, none underwent prophylactic mastectomy. Pathological confirmation of the diagnosis and a clinical and family history were available in every case along with blood samples. Family history was considered positive if one or more first or second-degree relatives were affected with breast and/or ovarian cancer. Positive family history was further defined as strong (pre-menopausal breast cancer, ovarian cancer and male breast cancer irrespective of age at onset) or moderate (post-menopausal breast cancer). All patients signed an informed consent form approved by the Institutional Review Board (IRB).

Genetic Testing

DNA Extraction Genomic DNA was extracted from peripheral blood samples by standard methods.

Mutation Analyses Germline mutations in BRCA1 (185delAG, 5382insC) and in BRCA2 (6174delT) were detected by PCR and modified restriction analysis, as previously described.[8,9]

PCR products were separated on 8% non-denaturing polyacrylamide gel, stained by ethidium bromide, and visualised under a UV lamp.

Statistical Analysis

Study patients were divided into carriers and non-carriers; early and late onset cases (diagnosis of first tumour given before or after the age of 42 years, respectively), with or without positive family history.

Fisher's exact test was used for comparison between associations. Association between age of onset (late *vs* early) and carrier status was determined.

Logistic regression analysis was used to determine the independent effect of age of onset and family history on carrier status.

Results

The study group was composed of 55 Jewish women with bilateral breast cancer of whom 17 were diagnosed before the age of 42 years (early onset) and 38 after the age of 42 years (late onset). Positive family history was recorded in 30 patients and 25 patients had no family history of cancer (Table 1). Seventeen women (31%) had synchronous and 38 (69%) had metachronous bilateral breast cancer. The mean time intervals between both breast tumours of patients with metachronous bilateral breast cancer was 5 ± 4.2 years (range 1–16 years). No significant statistical difference in the mean time interval between both breast tumours, in women whose first tumour was diagnosed before or after 42 years of age was observed. The mean ages of diagnosis of the first and second tumour in the 55 patients included in the study were 49.18 ± 11.1 and 52.69 ± 10.8, respectively, compared with 56.19 ± 13 and 59.62 ± 13.8, respectively, recorded for the 71 patients who were not available for study.

Overall, 17 (17/55; 31%) mutation carriers were identified; ten (10/55; 18.5%) had 185delAG, two (2/55; 3.7%) had 5382insC and five (5;55; 7.4%) had 6174delT (Table 2).

Table 1 Subdivision of patients with bilateral breast cancer as regards, age of onset, family history and carrier state

Groups	*Family history*	*Carriers*	*Non-carriers*	*Total*
Total		17	38	55
Early onset	Strong	10	3[a]	13
	Moderate	2	0	2
	Negative	2	0	2
	All	14	3	17
Late onset	Strong	0	5[a]	5
	Moderate	0	10	10
	Negative	3	20[b]	23
	All	3	35	38

Early onset: first tumour diagnosed at or before 42 years of age; late onset: first tumour diagnosed after 42 years of age; [a]includes one non-Ashkenazi women; [b]includes two non-Ashkenazi women.

The prevalence of mutation carriers in women with early onset disease (14/17; 82%) was significantly higher ($P < 0.0001$) than in women with late onset disease (3/38; 7.9%).

Among women with bilateral breast cancer and positive family history, the prevalence of mutation carriers (14/30; 47%) was higher ($P < 0.01$) than among those with bilateral breast cancer and negative family history (3/25; 12%). All three carrier patients with late onset disease recorded no family history. Of the 17 mutation carriers identified, 14 (82%) had early onset disease and 14 (82%) presented with positive family history. Of these, family history was defined as strong in 10 (Table 1).

The presence of a BRCA mutation was significantly inversely correlated to age of onset of the first breast tumour ($P < 0.0016$; O.R. = 0.8255; 95% CI 0.73–0.92). No significant association between family history and carrier frequencies was noted.

The number of mutation carriers among patients with bilateral synchronous breast cancer (5/17; 29.4%) did not differ statistically from that observed among patients with bilateral metachronous breast cancer (12/38; 31.6%). The prevalence of mutation carriers was lower (9/17; $P = 0.067$) in women with unilateral breast cancer diagnosed at or before 42 years of age and matched for age of onset and family history.

Table 2 Characteristics in carrier women with bilateral breast cancer

Subject	*Mutation*	*Age of onset*		*Year of diagnosis*	*Family history*
		1st tumour	*2nd tumour*		
1	185delAG	30	32	1990	S
2	185delAG	30	41	1975	S
3	185delAG	31	31	1994	O
4	185delAG	37	39	1979	S
5	185delAG	40	40	1993	S
6	185delAG	40	42	1973	S
7	185delAG	41	41	1994	S
8	185delAG	41	50	1986	O
9	185delAG	42	46	1973	S
10	185delAG	49	49	1997	O
11	5382insC	38	44	1984	O
12	5382insC	42	47	1988	M
13	6174delT	36	40	1985	M
14	6174delT	37	49	1978	M
15	6174delT	39	39	1977	M
16	6174delT	53	58	1990	N
17	6174delT	60	69	1990	N

S: strong family history for breast and/or ovarian cancer; M: moderate family history for breast and/or ovarian cancer; O: positive family history for cancer other than breast or ovarian; N: negative family history.

Discussion

It is generally agreed that genetic predisposition to cancer is hallmarked by early age of onset, positive family history and the occurrence of bilateral breast cancer. But it is still debated whether bilaterality *per se* reflects genetic predisposition or rather represents independent sporadic events. Dawson *et al*[10] assert that lack of biological concordance among tumours and no association with positive family history, in 88 patients with bilateral breast cancer, favours the interpretation of independent tumour origins.

Adami *et al*[2] calculated that a woman with breast cancer diagnosed before 50 years of age has a four-fold increased risk of developing a contralateral breast tumour than a woman diagnosed after that age – 13% and 3.5%, respectively. Robinson *et al*[11] reported that metachronous bilateral breast cancer occurs more frequently among Ashkenazi Jewish women whose first tumour was diagnosed before 55 years of age.

In unselected Jewish Ashkenazi women with early onset breast cancer diagnosed by age 40, a high rate of the specific founder mutations in BRCA1 and BRCA2 (20 to 26%) was found.[12–14]

Using a retrospective cohort of 55 Jewish women with bilateral breast cancer, we found that 14 out of 17 patients (82%) with early onset disease carried one of three target mutations (185delAG, 5382insC in BRCA1 and 6174delT in BRCA2). In contrast, only three of 38 patients (7.9%) with bilateral breast cancer not associated with early age of onset had a BRCA mutation.

Couch *et al*[15] state that no concordance between bilateral breast cancer and the presence of BRCA1 mutations can be traced. According to our data, this is correct if bilateral breast cancer is not associated with early age of onset. However, when bilateral breast cancer and early age of onset are taken together the association with BRCA1/BRCA2 founder mutations becomes very strong (82%). This perception is strengthened by the observation that young women with unilateral breast cancer matched for family history are less likely to harbour a BRCA founder mutation.

The majority (12/14; 86%) of early onset cases with bilateral breast cancer and positive family history were mutation carriers. Of 18 patients with late onset disease and a positive family history none carried a BRCA founder mutation. Even though no strong association between family history, bilaterality and carrier state was found, the majority of carrier women with early onset disease recorded a strong family history, thus implying

that family history retains its contribution to the prediction of carrier state.

All in all the occurrence of a contralateral breast tumour *per se* does not reflect genetic predisposition, unless associated with early age of onset. The occurrence of breast cancer in a young woman who carries a mutation in either BRCA1 or BRCA2 may predict bilaterality. This observation should be seriously weighed when contemplating the best surgical option to be offered to women with early onset breast cancer who carry a BRCA1 or BRCA2 mutation.

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