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Hormone replacement therapy is more prevalent among Jewish *BRCA1/2* mutation carriers

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ARTICLE INFO

Article history:
Received 31 July 2005
Received in revised form
4 August 2005
Accepted 8 August 2005
Available online 7 February 2006

Keywords:
Breast cancer
BRCA1/2 germline mutations
Reproductive factors
HRT and OC use

ABSTRACT

The aim of this study was to compare reproductive factors, use of oral contraceptives (OC) and hormone replacement therapy (HRT) in consecutive Jewish Ashkenazi breast cancer patients, with and without *BRCA1/BRCA2* mutations. Jewish Israeli women with breast cancer ($n = 385$) were genotyped for the three predominant Jewish mutations in *BRCA1* and *BRCA2*, and data on reproductive factors, OC and HRT use, were analyzed using logistic regression analyses. Overall, 28/385 (7.3%) of participants were mutation carriers, the majority of whom were Ashkenazi ($n = 22$; 78.6%) and were diagnosed with breast cancer at or under age 49 years ($n = 18$; 64.3%). Mutation carriers were more likely than non-carriers to ever use OC (39.3% vs. 20.2%; $P = 0.053$), HRT (35.7% vs. 13.7%; $P = 0.007$), and have first menarche at or below 12 years of age (71.4% vs. 40.6%; $P = 0.03$). Multivariate analysis showed that Ashkenazi women diagnosed with breast cancer under 40 years of age, with a family history of breast/ovarian cancer, who ever used HRT were more likely to be mutation carriers. This study has shown that HRT use is more prevalent among Jewish Ashkenazi mutation carriers, but its role in modifying breast cancer risk in mutation carriers remains unknown.

1. Introduction

Breast cancer (BC) is a common female malignancy, with more than 200 000 new cases diagnosed annually in the USA[1] and about 3500 in Israel (http://www.health.gov.il/icr/99/Breast2.xls). Breast cancer is a multifactorial disease and several risk factors have been implicated in and associated with breast cancer risk in the population at large: hormonal status, reproductive history, previous benign breast disease, anthropometric measurements, and demographic characteristics.[2] Family history of breast and/or ovarian cancer is the single most important factor in determining individual BC risk.[3–5] Germline mutations in two genes *BRCA1* (MIM # 113705) and *BRCA2* (MIM # 600185) are estimated to account for about 80% of all inherited breast and ovarian cancer and less than 50% in site-specific breast cancer.[6,7] In Jewish

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doi:10.1016/j.ejca.2005.08.045

high-risk individuals of Ashkenazi (East European) decent, three predominant mutations: 185delAG and 5382insC (*BRCA1*); and 6174delT (*BRCA2*) seem to account for a substantial proportion of germline mutations detected in high-risk families with inherited breast and ovarian cancer, and about 40–50% of site-specific breast cancer families.[8,9] Of these three mutations, the 185delAG is a Jewish mutation found in Ashkenazi and non-Ashkenazi populations, the 6174delT *BRCA2* is an Ashkenazi-specific mutation, and the 5382InsC is a Baltic origin mutation that can be found in high-risk individuals, Jewish and non-Jews, with Baltic ancestors.[9] Notably other mutations in both *BRCA1* and *BRCA2* are infrequent among Jewish Ashkenazi high-risk women.[10]

It is an unsettled issue whether, and to what extent, breast cancer risk factors influence *BRCA1/BRCA2* mutation carriers (i.e., high-risk population) compared with their effects on average-risk population. Oral contraceptive (OC) use was reportedly weakly associated with risk of breast cancer after 10 years duration in the general population.[11] Grabrick and colleagues[12] suggested that women who have ever used early formulations of OC and who also have family history of breast cancer were at a significantly higher risk for breast cancer. Similarly, Ursin and coworkers[13] suggested that OC use may increase breast cancer risk in *BRCA1/BRCA2* mutation carriers more than in non-carriers, and an increased risk for breast cancer was reported in Jewish OC users who are *BRCA1* but not in *BRCA2* mutation carriers.[14]

Hormone replacement therapy (HRT) was also reported to increase breast cancer risk.[15,16] Such an increase in risk was reported for women from the general population who take combined HRT for more than 3 years, with no differences noted between women with and without family history of cancer.[17] Similarly, the effects of number of births, age at first menarche, breast feeding, and age at first birth, on breast cancer morbidity in mutation carriers compared with the effects of these factors in non-carriers are not well defined, with inconsistent results reported from ethnically diverse populations.[18–20]

To gain insight into the effects of reproductive factors, use of OC and HRT, on breast cancer, we assessed these parameters in a cohort of unselected Jewish women with breast cancer who were also genotyped for the predominant Jewish *BRCA1/BRCA2* mutations.

2. Patients and methods

2.1. Study participants

All Jewish women ("Jewish" as defined by their Israeli ID cards and the definition of their nationality status) with pathologically confirmed breast cancer who were treated in either one of the two participating medical centers (Sheba and Rabin) between January 1, 1997 and December 31, 1998 were eligible for participation. The study was approved by the Institutional review board of both medical centers, and each participant signed a written informed consent. To emphasize, all patients were unselected for age at diagnosis, ethnic origin, or family history of cancer. Ethnicity was assessed by place of birth of patients and their ancestors (up to three generations back) and was classified as "Ashkenazi" or "non-Ashkenazi" as previously described by us.[10] A positive family history of cancer was defined according to established criteria, as detailed by Lynch and coworkers.[21] The only exclusion criteria were non-Jewish origin, and unwillingness to participate. Study participants were interviewed at the Oncology institutes by a personal interview using a detailed questionnaire (available from the authors upon request in Hebrew), and the time of interview ranged from 3 to 6 months after the diagnosis of breast cancer was made.

3. Experimental methods

3.1. DNA extraction

DNA was extracted from peripheral venous leukocytes by using the PUREGene DNA extraction kit (Gentra Inc., Minneapolis, MN), according to the manufacturer's recommended protocol.

3.2. Genotyping for the predominant Jewish mutations in BRCA1 BRCA2

Three predominant Jewish mutations were tested in each participant: 185delAG and 5382insC in *BRCA1*, and 6174delT in *BRCA2*. Mutation analysis schemes were based on PCR and restriction enzyme digests that distinguish the wild type from the mutant allele, as previously described[22] and adopted by us.[10] For each of these three mutations, a known mutation carrier was used as a positive control in each experiment.

4. Statistical analysis

Differences between the mutation carrier and the non-carrier group were tested using χ^2 test for categorical variables and Fisher's exact test when needed. We used a case-only study approach to study whether HRT, OC, and reproductive factors interacted with *BRCA* mutation status with respect to risk for breast cancer.[23] This method assumes that in general there is independence between genotype and exposure.

We first estimated the association of demographic and clinical factors with mutational status by performing a multiple logistic regression model. Ethnic origin, age at diagnosis, age at first menarche, bilateral or unilateral breast cancer, past history of ovarian cancer and benign breast disease, and family history of a first degree relative with breast or ovarian cancer were introduced as independent variables. We then ran a separate analysis including demographic and hormonal variables: parity, previous abortions, oral contraceptive use, and hormone replacement therapy. Due to the limited number of the mutation carriers, a final model was designed including demographic, clinical and hormonal factors that showed an association with mutation status with a P value <0.1 in order to retain the maximum number of subjects.

5. Results

Overall, there were 524 women who were eligible for the study. Of these, 435 women with breast cancer agreed to participate (83%), and 385 (88.5%) were successfully genotyped

for the *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) mutations. Of the 50 women who were not genotyped for all three mutations, the majority ($n = 44$) were genotyped for only two mutations (185delAG in *BRCA1* and 6174delT in *BRCA2*) and the rest were genotyped for one of these mutations only. The reasons for failure of full mutational analysis were insufficient amount or poor quality DNA. Comparison of demographic parameters and relevant selected characteristics (e.g., family history of cancer, previous history of ovarian cancer, age at menarche, OC and HRT use) between the 385 women and the 50 breast cancer patients who were excluded did not reveal any significant differences in these parameters. The mean age diagnosis of the study group ($n = 385$) was 52.6 ± 12.6 years, and that of the "excluded group ($n = 50$) was 53.6 ± 12.8 years ($P = 0.6$).

Overall, 28 patients (7.3%) had one of the three founder mutations in *BRCA1* or *BRCA2*: 14 patients (3.6%) carried the 185delAG *BRCA1* mutation, 5 (1.3%) harboured the 5382insC *BRCA1* mutation, and 9 (2.3%) displayed the 6147delT *BRCA2* mutation. Of the Ashkenazi patients, 22 (8.9%) were mutation carriers. Table 1 shows prevalence of mutation carriers by the relevant characteristics: age at diagnosis, ethnic origin, family history of cancer, presence of bilateral breast cancer, or past diagnosis of ovarian cancer.

The prevalence of mutation carriers was significantly higher among women diagnosed with breast cancer under 40 years of age. The mean age at diagnosis of mutation carriers was significantly younger than the age at diagnosis of non-carriers (45.6 ± 11.3 years compared with 53.2 ± 12.5 years, respectively, [$P = 0.002$]). Almost a threefold excess of mutation carriers was observed among women with a positive family history of cancer (14.9% vs. 5.5% among positive and negative family history, respectively; $P = 0.01$). A carrier rate of 33.3% was found among women with previous ovarian cancer.

Table 2 shows comparison of hormonal characteristics between carriers and non-carriers. No statistical differences were observed between the distribution of the two study groups according to number of pregnancies, total breast feeding months, and age at menopause. Higher proportion of oral contraceptive users and individuals taking HRT was found among mutation carriers ($P = 0.03$ and $P = 0.007$, respectively). Analysis of *BRCA1* only cases ($n = 19$) showed that 10.2% of *BRCA1* mutation carriers and 4.2% of non-carriers were exposed to HRT ($P = 0.06$). Differences among *BRCA* gene mutation carriers regarding OC use were most apparent among women aged 50 years or older (18.7% *BRCA* positive among OC users compared with 3.2% among non-users [$P = 0.03$]) (data not shown).

Table 1 – Prevalence of mutation carriers by relevant selected characteristics

Characteristics	Tested	Carrier		P-value
	n	*n*	%	
Total	385	28	7.3	
Age at diagnosis				
<40	59	10	16.9	0.02
40–49	114	8	7.0	
50–59	91	6	6.6	
60–69	64	3	4.7	
70+	53	1	1.9	
Unknown	4	0	0	
Ethnic background				
Ashkenazi	247	22	8.9	0.13
Non-Ashkenazi	103	3	2.9	
Mixed ancestry	35	3	8.6	
Family history of breast or ovarian cancer[a]				
Negative	311	17	5.5	0.01
Positive	74	11	14.9	
Breast cancer side				
Unilateral	364	26	7.1	NS
Bilateral	21	2	9.5	
Previous ovarian cancer				
No	379	20	6.9	0.06
Yes	6	2	33.3	

a Positive family history of cancer was defined according to established accepted criteria as detailed by Lynch.[21]

Table 2 – Distribution of hormonal characteristics by BRCA mutation status

Characteristics	Carrier ($n = 28$)		Non-Carrier ($n = 357$)		P-value
	n	%	*n*	%	
Age at menarche (years)					
<12	5	17.9	45	12.6	
12	15	53.6	100	28.0	
13	3	10.7	87	24.4	0.04
14+	5	17.8	96	27.0	
Unknown	–		29	8.1	
Oral contraceptives					
Never	17	60.7	265	74.2	
Ever	11	39.3	72	20.2	0.03
Unknown	–	–	20	5.6	
Number of pregnancies					
0	1	3.6	16	4.5	
1–2	5	17.9	80	22.4	NS
3–4	9	32.1	148	41.5	
5+	13	46.4	104	29.1	
Unknown	–		9	2.5	
Breast feeding (months)					
None[a]	11	39.2	81	22.8	
1–6	7	25.0	96	26.9	
7–12	4	14.3	61	17.0	NS
>12	4	14.3	60	16.8	
Unknown	2	7.1	59	16.5	
HRT					
Never	18	64.3	288	80.7	
Ever	10	35.7	49	13.7	0.007
Unknown	–		20	5.6	
Age at menopause[b]					
<45	6	31.6	54	21.1	
45–49	6	31.6	82	32.0	NS
50+	5	26.3	112	43.8	
Unknown	2	10.5	8	3.1	

a Included those who never gave birth.
b Only for those who are menopause.

Table 3 – Factors associated with mutation status multivariate logistic regression analysis

Characteristic	OR[a]	95% CI	P-value
Age at onset			
<40	5.60	1.96–15.96	0.001
40–49	1.55	0.56–4.25	0.4
50+	1.0	–	–
Ethnic origin			
Asia–Africa	1.0	–	
Europe–America	3.92	1.09–14.09	0.04
Mixed	2.42	0.44–13.45	0.3
Family history of breast or ovarian cancer			
Negative	1.0		
Positive	3.11	1.32–7.35	0.01
HRT use			
No	1.0		
Yes	3.63	1.45–9.09	0.006
Oral contraceptive use			
No	1.0		
Yes	1.49	0.57–3.90	0.4

a The reference group is indicated by an OR of 1.

The first model presenting the demographic and clinical variables showed that young age at diagnosis (<40 years), Ashkenazi origin, and family history of breast or ovarian cancer were significantly associated with being a mutation carrier. Specifically, past history of ovarian cancer, age at menarche, and bilateral breast cancer were not significantly associated with *BRCA* mutation status. From the model, considering the hormonal parameters, neither parity or oral contraceptive use were found to be associated with mutation status, and only HRT use presented an OR of 4.3 with 95% CI of 1.67–10.87 for *BRCA* gene mutation carriers.

The final model showed a significant association of mutation status with Ashkenazi origin (OR = 3.92; 95% CI 1.09–14.09), age at diagnosis under 40 years (OR = 5.60; 95% CI 1.96–15.96), family history of cancer (OR = 3.22; 95% CI 1.32–7.35), and use of HRT (OR = 3.63; 95% CI 1.45–9.09) (Table 3).

No significant associations between *BRCA* mutation status and use of OC, after adjustments for age at diagnosis, ethnic origin, family history of cancer and HRT use were made.

6. Discussion

In this study, the rate of the predominant Jewish mutations in *BRCA1* and *BRCA2* genes in a hospital-based series of Jewish-Israeli women with breast cancer was 7.3% for women of all ethnic extractions, and 8.9% for Ashkenazi women. The women in this study were unselected for family history of cancer, and this result is consistent with findings from the study of Warner and coworkers[24] who reported 11.7% carrier rate among 412 consecutive Jewish Ashkenazi breast cancer patients. Similarly, in the Icelandic population, the rate of the founder Icelandic mutation (999del5 in *BRCA2*) in unselected Icelandic breast cancer patients ranges from 7.7% to 10.4%.[25,26] Among unselected Finnish breast cancer cases, the rate of the 11 predominant *BRCA1/BRCA2* mutations is reportedly 1.8%.[27]

Breast cancer diagnosed under 40 years, positive family history of cancer, Ashkenazi origin, and prior ovarian cancer diagnosis, were strongly and significantly correlated with the presence of either *BRCA1* or *BRCA2* mutations among Jewish-Israeli breast cancer patients. These results are expected, and in line with the reported phenotypic characteristics of *BRCA1/BRCA2* mutation carriers in ethnically diverse populations,[28] as well as Jewish Ashkenazi individuals.[29,30] Thus, it seems that an unselected genotyping of all Jewish women with breast cancer for harbouring a *BRCA1/BRCA2* mutation is unjustified, both ethically and in terms of low yield. However, Ashkenazi women who developed breast cancer under 40 years, in particular those with a positive family history of breast and/or ovarian cancer should be offered genetic counseling and testing.

In the present study, HRT was strongly correlated with the presence of either a *BRCA1* or *BRCA2* mutation in breast cancer patients: 35.7% of mutation carriers and 13.7% of non-carriers ever used HRT ($P = 0.007$). This correlation was significant both in uni- and multivariate analyses. Excess rate of breast cancer was observed among Swedish women who were ever users of HRT compared with never users, with increasing rates for prolonged use, without significant interaction with family history of breast cancer.[31] Subsequent studies have confirmed an increased risk for developing breast cancer associated with prolonged HRT use in other populations,[15,16] while other studies did not find significant risk with HRT and breast cancer.[32] In neither of these studies was the status of *BRCA* mutation taken into consideration. The results of the present study, taken together with the effects of estrogenes on breast cancer risk[33–35] and the recent concerns about the safety and rationale of use of HRT,[36] seem to dictate cautious short-term use of HRT in mutation carriers. These effects of HRT exposure on breast cancer risk in *BRCA1* carriers are interesting and even counter-intuitive, in light of the fact that the majority of breast cancer in *BRCA1* mutation carriers breast cancer are estrogen receptor (ER) negative.[37] The effects of HRT in these ER negative cases are speculative at best, but are probably unrelated to their direct estrogenic effects on breast tissue, but rather indirect as modulators of other hormones or affecting some other biochemical pathways.

Oral contraceptive (OC) use was more prevalent among *BRCA1/BRCA2* carriers (39.3%), compared with non-carriers (20.2%) ($P = 0.03$) in a univariate analysis. Stratified age category analysis showed that these differences were only significant for women 50 years or older. These latter data may indicate that the older OC preparations, which contained higher doses of estrogen, may have had an effect on breast cancer risk. Indeed estrogen-rich OC preparations were associated with a slight increase of breast cancer risk among Danish women.[38] OC use is considered a modest risk factor for breast cancer, with a relative risk of 1.2 in a comprehensive meta-analysis that encompassed more than 50000 BC patients and more than 100000 controls.[11,39] Grabrick and coworkers,[12] suggested that first degree relatives of women with breast cancer who have ever used OC, may be at particularly high-risk for breast cancer with a RR = 3.3. These results could not be reproduced in ethnically diverse populations.[40–42] Among Jewish Ashkenazi women, two

studies showed that long-term OC use was more prevalent among *BRCA1* or *BRCA2* mutation carriers compared with either controls[13] or ovarian cancer patients.[43] Similarly, retrospective analysis of OC use among Jewish mutation carriers who were selected from high-risk clinics, was associated with a significant increase in breast cancer risk among *BRCA1* mutation carriers and not in *BRCA2* mutation carriers.[14] Taken together, these data suggest that the issue of OC use and its effects on breast cancer risk in asymptomatic *BRCA1/BRCA2* mutation carriers needs to be addressed prospectively.

A statistically significant difference was noted in the present study between carriers and non-carriers in the age at first menarche: mutation carriers had their first menstrual period earlier than non-carriers ($P = 0.04$), whereas other reproductive factors such as age at first birth, total months of breast feeding, and number of completed pregnancies, did not statistically differ between the two groups in multivariate analysis. The effect of reproductive factors on breast cancer risk in high-risk individuals has been reported by several groups, with inconsistent results. Multiparity, early age at first birth and early age at menopause were important determinants of breast cancer risk in an Italian study.[44] A possible three way interaction between family history of cancer, parity, and patients' age or age at first full-term pregnancy was reported to have an effect in French women.[45] In another French study, age at first menarche affected breast cancer risk in pre-menopausal breast cancer patients only, age at full-term pregnancy affected pre-and post-menopausal women, and high parity in only post-menopausal women.[46] Egan and coworkers[5] reported that the protective effects of increased parity and longer breast feeding intervals were more noticeable in women with a family history of cancer, while Grabrick and coworkers[47] could not demonstrate similar differences. Notably, *BRCA* mutation status was not determined in any of the above mentioned studies. Jernstrom and coworkers[18] reported that childbearing in *BRCA1/BRCA2* mutation carriers is associated with a significantly increased risk for developing breast cancer by the age of 40 years, whereas among Swedish[19] and German[48] high-risk patients differences in reproductive factors were insignificant. Warner and coworkers[24] concluded that reproductive factors did not distinguish *BRCA1/BRCA2* mutation carriers from non-carriers in Ashkenazi Jews, and no protective effect of early age at first pregnancy was shown for Ashkenazi mutation carriers.[49] In an Icelandic study, differences were noted between mutation carriers and non-carriers with respect to the effect of number of pregnancies and total breast feeding time on breast cancer risk, whereas no differences were noted with respect to age at menarche and age at first birth.[50] If these data are confirmed in a prospective study, encompassing more mutation carriers of different germline mutations, it may indicate that the ability to affect the phenotypic expression of mutant *BRCA* alleles using simple, non-invasive means such as early child bearing or breast feeding, is limited.

The limitations of the current should be pointed out. The number of mutation carriers analyzed retrospectively from two medical centers in the central part of Israel was limited, and they are not representative of the ethnic makeup or other important confounders of all cases of breast cancer in Israel. There are no data regarding the length of HRT or OC use, an important factor in assessing the risks associated with their use. Last, this is a case-only study, and the lack of a control group of ethnically and age stratified unaffected controls, limits the applicability of the findings. Still, the unselected manner of recruitment provides an initial estimate and a reference framework for future studies.

We conclude that Jewish Ashkenazi women, diagnosed with breast before age 40 years, and/or with a family history of cancer, and/or a personal history of ovarian cancer are at higher risk for carrying a founder mutation in either one of the two *BRCA* genes, and they should be offered genetic testing and counselling. Hormone replacement therapy and age of first menarche under 12 years among breast cancer patients are associated with an increased likelihood of detecting one of the three founder Jewish mutations. HRT may serve as a modifier of breast cancer risk in Jewish *BRCA* gene mutation carriers, but this awaits further studies.

Conflict of interest statement

None declared.

Acknowledgments

This study was performed in part by a grant support to Eitan Friedman from the Tel-Aviv University, and in partial fulfillment of the requirements for a PhD degree to Revital Bruchim Bar-Sade, at the Department of Human Genetics, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

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