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ORIGINAL ARTICLE

Epidemiological and clinicopathological characteristics of *BRCA*-positive and *BRCA*-negative breast cancer patients in Greece

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Summary

Purpose: *BRCA mutation carriers can benefit from targeted clinical interventions. On the other hand, families with evident aggregation of breast cancer (BC) cases and a BRCA-negative genetic test can still be considered as of elevated risk, since the underlying genetic factor remains unidentified. In the present study, we compared clinical and demographic characteristics between BRCA1 mutation carriers (BRCA1mut) and non-carriers (non-BRCA1) in a Greek group of BC patients (n=321).*

Methods: *Data were collected and analyzed from 321 women with BC, with 131 patients screened for pathogenic mutations in the high-penetrant genes BRCA1 and BRCA2. Collected data included demographics, pedigrees, tumor histopathology and immunohistochemistry findings.*

Results: *In BRCA1mut patients, their mothers and grandmothers were diagnosed at a younger age compared to non-BRCA1-carriers. Additionally, BRCA1mut patients were diagnosed with mainly estrogen receptor (ER) negative (p<0.001), Her-2 negative (p<0.05) and triple negative (p<0.01) tumors. The youngest generation was diagnosed with familial breast cancer (FBC) 9.7 years earlier than their mothers (p<0.001). Age at BC diagnosis negatively correlated with the nuclear grade of breast tumors (r=-0.3, p<0.05). Among parous individuals, the number of full-term pregnancies significantly correlated with the age at BC onset (r=0.19, p<0.05).*

Conclusion: *Despite their similarities, FBC cases with identified BRCA1 mutations exhibit a clearly distinct profile. We have identified an anticipation effect in FBC patients, with significantly reduced age at diagnosis in younger generations. Increased parity seems to prevent early BC onset. This is the first study comparing clinical and demographic characteristics of FBC BRCA1mut and non-carriers in a Greek cohort.*

Key words: *BRCA1, BRCA2, hereditary breast cancer, mutations, triple negative breast cancer*

Introduction

BC is the most frequent neoplastic condition and the second leading cause of cancer-related deaths among women in the Western world [1]. According to European statistics, it is estimated that approximately 6,000 women are diagnosed annually with BC in Greece [2]. Among all BC

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cases, 15-30% exhibit distinct familial clustering and can be therefore attributed to genetic factors. Loss-of-function mutations in the high-penetrant genes *BRCA1* & *BRCA2* account for 5-10% of all BC cases and 20-25% of familial cases, while conferring high lifetime breast cancer risk (45-80% by the age of 70 years). To date, a number of additional BC predisposing genes have been discovered and associated with a high, intermediate and low BC risk [3]. Our current knowledge on BC genetics can define the hereditary component in approximately ~15% of the total number of cases [4-6].

As genetic testing was not widely available worldwide prior to the last decade, family history of BC remains an established major risk factor. The risk for BC increases 2- to 3-fold when at least one first-degree or two second-degree relatives have been diagnosed with BC. The risk is even higher when affected family members are premenopausal. In addition, reproductive factors are also considered as significant in BC risk estimation. The underlying mechanisms of hormone dynamics occurring during early age pregnancy, as well as multiparity seem to provide a significant protective effect against BC.

In particular, women who have their first full-term pregnancy before the age of 20 have 50% less chance of developing ER positive BC during their lifetime [7] when compared to nulliparous women or women having later onset pregnancies (i.e. after the age of 35) [8,9]. The key underlying process seems to be the mammary gland epithelial cell differentiation, which is orchestrated by the undergoing hormonal changes during pregnancy [10].

On the contrary, the protective effect of early-age first full-term pregnancy and multi-parity was not observed in a large series of *BRCA* mutation carriers [11]. The only beneficial effect –a 38% decrease in BC risk– was seen in *BRCA1* mutation carriers who gave birth to 4 children or more. On the other hand, an increased parity in *BRCA2* carriers seemed to be associated with borderline elevation of BC risk [12]. This difference between *BRCA1* and *BRCA2* mutation carriers can be partially explained by the distinct biological features of tumors observed in these patients: *BRCA1*-related tumors tend to be hormone receptor negative, while *BRCA2*-related tumors tend to be hormone-dependent [13]. A number of studies have revealed that FBC exhibits specific clinical and demographic features compared to sporadic cases, indicating that FBC patients can be considered as a separate clinical entity [14-16].

The main purpose of this study was to evaluate and compare the clinicopathological, family history characteristics, the age of first full-term pregnancy and parity in a cohort of Greek FBC patients. Importantly, the study assessed the possible differences between *BRCA1* mutation carriers (*BRCA1*mut) and non-carriers (non-*BRCA1*). Another objective of the current analysis was to compare the age of BC diagnosis between three generations of FBC patients and to investigate the presence of anticipation effects.

Methods

Patient study group

This prospective trial enrolled patients from 2008 until 2012 in collaboration with three major hospitals in Greece: (i) 1st Department of Medical Oncology, "Metaxa" Cancer hospital, (ii) 2nd Department of Medical Oncology, "Agios Savvas" Anticancer Hospital, (iii) 1st Department of Obstetrics and Gynecology, "Alexandra" Hospital. Epidemiological and clinicopathological data from 321 women aged from 19 to 80 years with family history of BC were collected. The selection criteria have been amended from the proposed National Comprehensive Cancer Network (NCCN) guidelines and are summarized in Table 1 [6]. The study was approved by the Bioethics committee of NCSR 'Demokritos' (240/EHΔ/11.3) and was in agreement with the 1983 revision of the 1975 Helsinki statement. All patients had signed an informed consent form prior to their inclusion to the study.

Progeny (Progeny Software LLC, USA) and Cyrillic (Cyrillic Software, UK) software were used to store and represent family history data and pedigrees for all 321 families. Every genealogical pedigree comprised at least three generations and included personal/family history as well as clinical, histological and immunohistochemical data.

Mutation analysis

Based on the pedigree analysis, 131 women with FBC proceeded to *BRCA1* & *BRCA2* genetic testing using a two-step approach: a) screening for 6 Greek founder mutations ("hot spots") of the *BRCA1* gene, and b) in case of a negative result, complete *BRCA1* and *BRCA2* screening was performed. Patients found to be mutation carriers received appropriate genetic counseling, while their family members were also informed in specialized counseling sessions. If consented to genetic analysis, they were also tested for the specific mutation.

BRCA1 & *BRCA2* mutation screening was performed as previously described. Genomic DNA was extracted from peripheral blood lymphocytes following the salt extraction procedure. Primer sequences and

Table 1. Patient selection criteria for BRCA genetic testing (modified from http://www.nccn.org/)

Individual with breast/ovarian cancer and one of the following
(i) Breast and/or ovarian or pancreatic cancer in at least two blood relatives
(ii) Multiple primary breast cancers or bilateral breast cancer, first diagnosed before the age of 50 years
(iii) Triple negative breast cancer diagnosed before the age of 60 years
(iv) Male breast cancer in a blood relative

protocols are available upon request [17].

Statistics

Data were expressed as mean±standard deviation (SD), median (Interquartile range) and frequency (%) for continuous, ordinal and nominal parameters, respectively. The normality of the distributions was assessed with the Kolmogorov-Smirnov test and graphical methods. Comparisons between two groups were performed with Student's t-test and Mann-Whitney's U test, as appropriate. Comparisons of BC onset age between three generations were performed with repeated measures ANOVA. Pearson's correlation coefficient and Spearman's rho were calculated in order to examine correlations between variables.

In all cases of multiple hypothesis testing, FDR was utilized in order to assess between-group differences, as well as to control family-wise error to <0.05.

All tests were two-sided. Differences were considered as statistically significant if the null hypothesis could be rejected with >95% confidence (p<0.05).

Results

From the total of 131 patients with FBC, 17 (13%) carried a deleterious mutation in *BRCA1* gene (*BRCA1*mut group, n=17), one patient carried a deleterious mutation in *BRCA2* gene, while 113 did not have a detectable mutation in *BRCA1* and *BRCA2* genes (non-*BRCA1* group, n=113). The patient carrying a deleterious mutation in *BRCA2* gene was excluded from further analysis due to lack of other similar cases.

Demographic characteristics

Patient characteristics including the mean age of BC diagnosis in three generations (patients, their mothers and grandmothers), the age of first full-term pregnancy and the number of full-term pregnancies were evaluated. As shown in Table 2, the age of BC diagnosis in *BRCA1*mut was significantly lower than in non-*BRCA1* patients (p<0.05). Furthermore, the age of BC diagnosis in mothers (Generation-2) was also significantly lower in *BRCA1*mut group (p<0.005). The same parameter could not be evaluated for grandmothers (Generation-1) as data for only two BC cases were available in the *BRCA1*mut group.

Table 2. Demographic characteristics of *BRCA1*mut and non-carriers and their blood relatives in three generations

Parameter	*Group*	*N*	*Mean±SD*	*p value*
Proband's age at BC diagnosis (Generation-3)	Non-*BRCA1*	113	45.4±10.8	<0.05
	*BRCA1*mut	17	39.1±9.1	
Mother's age at BC diagnosis (Generation-2)	Non-*BRCA1*	45	57.3±13.2	<0.005
	*BRCA1*mut	9	47.4±13.3	
Grandmother's age at BC diagnosis (Generation-1)	Non-*BRCA1*	12	59.3±16.7	NS
	*BRCA1*mut	2	59.5±10.6	
Age at first full-term pregnancy	Non-*BRCA1*	101	25.7±5.1	NS
	*BRCA1*mut	12	26.8±9.9	
Number of full-term pregnancies	Non-*BRCA1*	102	2.0±0.8	<0.005
	*BRCA1*mut	15	1.5±0.9	
Grade	Non-*BRCA1*	51	3	NS
	*BRCA1*mut	16	3	

NS: Statistically non-significant, SD: standard deviation, BC: breast cancer

Among parous women, there were no significant differences in the age of first full-term pregnancy between the two groups or the number of children. When evaluating differences in the age of BC diagnosis between three generations in the cohort of 131 women (Table 3), Generation-2 had a significantly greater age at diagnosis than that of their daughters (p<0.001). An analogous difference was observed between granddaughters (Generation-3) and grandmothers, with the later showing a significantly greater age at diagnosis (p=0.008). On the contrary, there was no significant difference in the age of diagnosis between Generation-1 and Generation-2 individuals (p=0.92).

Tumor characteristics

The most common histological BC tumor type in both groups was the invasive ductal carcinoma. There was an increased proportion of medullary breast cancer among *BRCA1*-carriers (*BRCA1*mut 11.7% vs non-*BRCA1*: 6.3%, p=0.057), but the difference did not reach (marginally) statistical

Table 3. Paired difference of breast cancer onset in different generations

Comparisons		*N*	*Mean±SD*	*p value*
Pair 1	Age at BC diagnosis (Generation-3)	54	45.9±9.5	p<0.001
	Mother's age at BC diagnosis (Generation-2)	54	55.7±13.6	
Pair 2	Age at BC diagnosis (Generation-3)	14	40.2±8.2	p=0.008
	Grandmother's age at BC diagnosis (Generation-1)	14	59.4±15.6	
Pair 3	Mother's age at BC diagnosis (Generation-2)	7	59.4±14.1	NS
	Grandmother's age at BC diagnosis (Generation-1)	7	60.9±11.9	

For abbreviations see footnote of Table 2

Table 4. Tumor characteristics and family history of *BRCA1*-carriers and non-*BRCA1*-carriers

Characteristics	*BRCA1+*		*Total*	*p value*
	No N (%)*	*Yes* N (%)*	*129*	
Cancer site				
Breast	106 (94.6)	14 (82.3)	120	0.098
Breast-Ovarian	5 (4.5)	3 (17.7)	8	
Breast-Endometrial	1 (0.9)	-	1	
Histological type				
Invasive ductal carcinoma (IDC)	49 (62.0)	13 (76.5)	62	
Invasive lobular carcinoma (ILC)	8 (10.1)	-	8	
IDC+ILC	4 (5.1)	1 (5.9)	5	0.057
Medullary	5 (6.3)	2 (11.7)	7	
In situ IDC and/or ILC	12 (15.2)	1 (5.9)	13	
Other	1 (1.3)	-	1	
Estrogen receptors				
ER negative	25 (39.7)	16 (94.1)	41	<0.001
ER positive	38 (60.3)	1 (5.9)	39	
Progesterone receptors				
PR negative	33 (53.2)	13 (76.5)	46	0.1
PR positive	29 (46.8)	4 (33.5)	33	
HER-2 overexpression				
HER-2 negative	41 (62.1)	15 (88.2)	56	<0.05
HER-2 positive	25 (37.9)	2 (11.8)	27	
Triple negative breast cancer				
Yes	16 (23.5)	10 (58.8)	26	<0.01
No	52 (76.5)	7 (41.2)	59	
Family history				
Maternal	59 (52.7)	10 (58.8)	69	0.42
Paternal	24 (21.4)	5 (29.4)	29	
No family history	29 (25.9)	2 (11.8)	31	

significance. Furthermore, even though ovarian cancer was almost 3 times more frequent in the *BRCA1*mut group, interestingly the difference was not identified (marginally) as statistically significant (p=0.068) (Table 4).

*BRCA1*mut patients were mainly ER-negative (p<0.001), Her-2 negative (p<0.05), and triple negative BC (p<0.01). These patients presented a 15.2 times higher likelihood of having ER-negative tumors (RR:15.22, 95%CI:2.1-109.4), 3.6 times Her-2 negative (RR:3.62, 95%CI:0.89-14.7) and 3.2 times triple negative breast tumors (RR:3.2, 95%CI:1.39-7.6) than non-*BRCA1* carriers. Nuclear grade 3 was the most common grade in both groups. Howev-

er, there was no statistical difference in the grade of differentiation (p=0.16) between two groups of patients, as well as in progesterone receptor (PR) status (p=0.1).

Patients with ER-positive tumors had significantly greater age at diagnosis (p<0.05) and lower grade (p=0.001), while PR-positive tumors were assessed with lower grade (p<0.05). The opposite was observed in triple negative BC patients, which presented a significantly lower age at onset (p<0.05) and significantly higher grade of differentiation (p=0.002). A negative significant correlation between age of BC diagnosis was observed in the cohort of 131 FBC patients (r=-0.3, p<0.05).

Finally, among parous patients, a significant correlation between number of children and age at BC diagnosis was also observed (r= 0.19, p<0.05). More specifically, it was observed that each additional birth increased the age at onset of FBC, thus reducing the risk of early BC onset.

Discussion

In the present study we evaluated the clinicopathological, family history characteristics, the age at first full-term pregnancy and parity in a cohort of FBC patients. We also paid specific attention to identify any possible differences between *BRCA1* mutation carriers (*BRCA1*mut) and non-carriers (non-*BRCA1*). In the studied cohort, 13% (17/131) of the individuals carried a deleterious mutation in *BRCA1* gene, while only one patient carried a deleterious mutation in *BRCA2* gene. The low rate of *BRCA2* mutations in this study can be attributed to the existence of *BRCA1* founder mutations in the Greek population [18].

The mean age at BC diagnosis in the general population is 54 years, while in *BRCA* mutation carriers the age at onset is reduced by more than a decade (39-44 years) [19-22]. In our study the mean age of non-*BRCA1* was 45.4 years, which can be attributed to the fact that these patients belong to families with increased BC aggregation and cannot be considered as sporadic. On the other hand, in our study the age at BC onset for *BRCA1* mutation carriers was 39 years, which is also comparable to the study of Armaou et al. In that study, the mean age at BC onset in Greek *BRCA* carriers was identified as 42.5 years [21].

During the evaluation of pedigrees belonging to three generations of patients with FBC, an earlier age of diagnosis for younger generations was observed. The mean reduction was 9.7 years, compared to the age at BC onset of the previous generation (Generation-2). Our findings are similar to a previously published study by Litton et al., who identified a difference of similar magnitude (7.9 years) [23]. Importantly, there was no statistically significant difference in age at BC onset between Generation-1 and Generation-2. This finding can be explained by socioeconomic differences observed between Generation-3 and the precedent two. In contemporary Greece, the majority of women have their first full-term pregnancy in the end of the third decade of their lives, while their menarche is before entering high-school (≤12 years) [24]. Early childbirth is a known protective factor for lifetime BC risk, regardless of ethnicity. Women who have undergone a first full-term pregnancy before 20-25 years of age exhibit a 50% reduced lifetime risk of developing BC when compared to nulliparous women [25,26]. The time span defined by these two milestones (menarche–first full-term pregnancy) is increased in the current generation, diminishing the protective effects of parity and late menarche. Importantly, we did not observe any differences in the age of first full-term pregnancy among parous women between *BRCA1*mut and non-*BRCA1* patients. These findings are important to assess the effect of societal and hormonal factors to BC risk, as well as to better prepare women with family history of BC in regard to the timing of screening and intervention. Currently, NCCN guidelines propose that mutation carriers should start breast and ovarian screening at the age of 20 to 25 years, or 5 to 10 years earlier than the age at the earliest diagnosis within their family [27]. Our findings suggest that such a recommendation could be expanded to all FBC patients, regardless of the identification of the culprit gene. In our study, Generation-3 FBC patients were diagnosed with BC a decade earlier than the previous generations.

Moreover, the number of full-term pregnancies seems to confer protection against sporadic breast cancer diagnosis, especially against hormone-sensitive breast tumors. More specifically, the decrease is calculated around 11-14% for each additional birth [10]. On the contrary, the picture is rather different for *BRCA* mutation carriers, since it was initially shown that parity increases the risk for BC development [28]. Most recent studies have not found a statistically significant association between childbearing and risk of BC, excepting a significant decrease in *BRCA* carriers bearing 4 children or more [12,29]. In our study, we identified a significant positive correlation between the age of diagnosis and the number of offspring (r=0.19, p<0.05).

A clear association of germline *BRCA1* mutations and triple-negative breast cancer has been established [16,30,31]. On the contrary, non-*BRCA1* carcinomas seem to have different morphological and immunohistochemical profiles resembling that of sporadic BC. In a recent study, Hines et al. [32] reported that family history was significantly associated with an increased risk of ER-negative tumors in Hispanic women, but it seems that this is population-specific. Consistent with the current data, *BRCA1* mutation carriers in the present study had mainly ER-negative, Her-2 negative disease, as well as triple-negative tumors characterized by high level of nuclear grade. An established association between young age at diagnosis and triple-negative BC, irrespective of the presence of *BRCA* mutation, has been made [33,34]. This was also observed in our study, with triple-negative BC patients diagnosed at a significantly lower age when compared to patients with hormone-sensitive tumors.

Although the current study cannot sufficiently address whether full-term pregnancies can have a protective role against BC diagnosis, the anticipation effect in younger generations that was observed can lead to optimized surveillance protocols that can be initiated on time in order to diagnose early –or even prevent– BC diagnosis.

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