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Young Onset HER2 Positive Breast Cancer is Associated with Germline *TP53* Mutations

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Abstract

Background—Germline *TP53* mutations predispose to early onset breast cancer (BC) in women and are associated with the Li Fraumeni syndrome. Published data on the pathological characteristics of breast cancer among women with *TP53* mutations is limited.

Methods—We retrospectively reviewed clinical records of women who had genetic testing for suspected germline *TP53* mutations and who were diagnosed with BC between 2000 to 2011. The pathological characteristics of the breast tumors from women testing positive (cases) for a mutation were compared to those testing negative (controls).

Results—Patients who tested positive for germline *TP53* mutations (N=30) were compared to (N=79) controls. Human epidermal growth factor receptor 2 (HER2) amplification and/or overexpression was found in 67% of the tumors from the cases, compared to 25% for the controls (p=0.0001). Among patients with a mutation, 70% had estrogen receptor and/or progesterone receptor positive tumors, compared to 68% in the control group (p= 0.87). After adjusting for age at BC diagnosis, having a HER2 positive tumor increased the odds of testing positive for a germline *TP53* mutation (OR, 6.9, 95% CI, 2.6 to 18.2). For each yearly increments in age at BC diagnosis, there was decreased likelihood of having a *TP53* mutation by 5% (OR=0.95, CI 0.91 to 0.99).

Conclusion—This study suggests an association between germline *TP53* mutations and early onset HER2 positive breast cancer. If confirmed in a larger cohort, these results could guide genetic testing strategies, lead to chemoprevention trials incorporating HER2 targeted therapies, and elucidate some of the molecular pathways involved in breast cancer.

INTRODUCTION

Germline *TP53* mutations are the primary cause of Li Fraumeni syndrome (LFS), which is a cancer predisposition syndrome primarily associated with breast cancer, sarcomas, brain

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tumors, and adrenocortical carcinoma [1]. The risk of cancer is higher for women than men and is mostly the result of the increased frequency of breast cancer among women with LFS [2–5]. Published data on the specific clinical and pathological characteristics among women with breast cancer and germline *TP53* mutations is limited. In the absence of a specific breast cancer phenotype, there remains little guidance for testing patients with early onset breast cancer besides the strict classical diagnostic criteria requiring a strong family history or personal history of LFS associated cancers [6].

Inherited mutations in *BRCA1* or *BRCA2* genes are the most important predictors of early onset breast cancer as they are found in 5% to 30% of cases unselected for family history depending on the population [7]. The American Society of Clinical Oncology Statement on Genetic Testing emphasizes the utility of genetic testing as part of a comprehensive cancer risk assessment, especially where there is a clinical benefit to the patient or family. Most recently the National Comprehensive Cancer Network (NCCN) has added breast cancer diagnosed before the age of 30 as an indication for germline *TP53* mutation testing [8–10]. Based on our preliminary observation and a recently published study, we hypothesized that patients with breast cancer and a germline *TP53* mutation have an increased preponderance of human epidermal growth factor receptor 2 (HER-2) amplified breast cancer subtype[11, 12].

To better describe the genotype-phenotype characteristics of women with breast cancer and a germline *TP53* mutation, we performed a retrospective chart review at MD Anderson Cancer Center and the University of Chicago of all women with a known history of breast cancer that have undergone genetic testing for suspected germline *TP53* mutations, and have previously tested negative for *BRCA1* and *2* mutation. The pathological characteristics of the tumors from women testing positive for germline *TP53* mutation were then compared to those of the women testing negative. We focused on specific histological features, estrogen receptor (ER), progesterone receptor (PR) and HER-2 status.

METHODS

Patients

Women who underwent genetic testing for suspected germline *TP53* mutations and carry a diagnosis of breast cancer were identified. The majority of patients were referred for genetic counseling to MD Anderson Cancer Center or the University of Chicago by their medical or surgical oncologist. A total of 109 women were evaluated between 2000 to 2011, and were included in the analysis. Most met the National Comprehensive Cancer Network (NCCN) consortium guidelines for germline *TP53* mutational analysis testing based on their personal and/or family history of cancer [8]. The following exclusion criteria were applied: male sex, incomplete pathological records, unknown ER, PR, or HER-2 status, non-invasive breast tumors, phyllodes tumors, or sarcomas of the breast, and known *BRCA1 or 2* germline mutations. The patient's electronic medical records were reviewed to extract data on clinical characteristics, including ethnicity, age, and other malignancies. The institutional review board approved the retrospective review of the medical records for the purposes of this study.

Pathology

Information regarding the histologic type of breast cancer; tumor grade using the modified Black's nuclear grading system; and ER, PR, and HER-2 status of breast cancer samples were obtained from the patients' pathology reports. Invasive breast cancer specimens that were routinely evaluated for ER, PR, using immunohistochemistry (IHC) and HER-2 status using IHC and/or Fluorescence in situ hybridization (FISH) were included. A positive HER-2 status was defined as a score of 3 + by IHC and/or a ratio of 2.2 or more by FISH. A

negative status was defined as a score of 0 or 1+ by IHC, and/or a ratio of <1.8 by FISH. A FISH analysis was not available for all patients and depended on their IHC results.

TP53 germline mutation analysis

Women seen through the clinical cancer genetics service at MD Anderson Cancer Center or the University of Chicago and who had clinical indication of germline *TP53* mutation due to personal and/or family history were primarily tested at outside CLIA certified laboratories. Their *TP53* mutation reports were reviewed and considered for this analysis. Women that were part of a LFS long-term study headed by Dr.Louise C. Strong, had their *TP53* mutation analysis performed at MD Anderson Cancer Center research laboratory as previously described [13]. This study was approved by the institutional review board. Briefly, peripheral blood samples were collected from participants and DNA was extracted and screened for mutations along all coding exons (2–11) and associated splice junctions of the *TP53* gene. Results were confirmed by a second independent sample tested at a different time. In some instances, participants had clinical testing done either prior to or after having research *TP53* mutation testing. All patients in this study consented for genetic testing.

Statistics

We used Pearson's χ^2 test (or Fisher's exact test where appropriate) to test for associations between the receptor type (ER, PR, or HER-2), tumor grade, and ethnicity, according to *TP53* mutational status. The Mann-Whitney U test was used to test for differences in age at diagnosis by *TP53* mutational status. A p value of < 0.05, using the two-sided test was considered statistically significant.

A multicovariate logistic regression model was used to estimate the odds ratio (ORs) and the corresponding 95% confidence intervals (CI) for HER2 in predicting *TP53* mutational status in patients, adjusting for age. All the statistical analyses were done using SAS 9.1.3 for Windows.

RESULTS

A total of 109 female breast cancer patients who underwent testing for germline *TP53* mutations were identified. All the patients included had invasive carcinoma. A total of 30 patients (28%) tested positive for a *TP53* germline mutation (cases). Patient characteristics were summarized for patients with and without a *TP53* germline mutation (Table1). There was a significant difference in regards to age at diagnosis of breast cancer among the two groups, with a median age of 31.5 years for the patients with a germline mutation compared to 40 years for the patients testing negative (controls) (p=0.035). The self-described ancestries of the two groups were comparable with the majority of the patients being of European ancestry (67% and 71% in cases and controls, respectively). Hispanic patients made up 27% of the group with a germline mutation compared to 16% of the control group (p =0.64). None of the 30 patients with a mutation were family members in this study.

The pathological features of the tumors are summarized (Table 2). Among the women with a germline *TP53* the prevalence of ER and/or PR positive tumors was 70 % (21/30) compared to 68% (54/79) in the controls (p= 0.87). In contrast, the presence of HER2 positive tumors was significantly different between the groups, 67% (20/30) in the cases compared to 25% (20/79) in the controls (p=0.0001). Nine (30%) patients with a germline mutation had estrogen receptor (ER) and/or progesterone receptor (PR) positive and HER2 negative tumors compared to 43 (54%) in the control group (p=0.02). We found one patient with triple negative breast cancer (TNBC) (ER−/PR−/HER2−) in the cases while 20% of the controls had TNBC (p=0.04). Among the patients with a germline mutation who had

bilateral breast cancers and HER2 positive tumors (N=4), both breasts contained HER2 positive disease in all but one patient who's tumor was equivocal by IHC (2+) and non-amplified by FISH; these were not counted as separate cases for our analysis. None of the controls with HER2 positive breast cancer had bilateral disease.

A total of 10 out of 30 patients with a germline mutation underwent contralateral prophylactic mastectomy at the time of breast cancer or LFS diagnosis, and had no evidence of contralateral disease. Of the remaining 20 patients, 75% (N=15) had bilateral breast pathology. Six patients had DCIS, 2 patients had phyllodes tumors and 7 patients had IDC in both breasts. The most common cancers diagnosed in addition to breast cancer were sarcomas (N=6), tumors of the CNS (N=3), and adrenal carcinomas (N=3).

The germline mutation for each patient with documented histology is shown in table 3. The majority were missense mutations (70%), and were mostly located in the major and minor DNA binding domains. The remainders were nonsense (13%), frameshift (7%), deletion (7%), and splice (3%) mutations.

In the multicovariate logistic regression analysis, there was a statistically significant prediction for being a carrier for a *TP53* germline mutation by age at diagnosis and HER2 status ($p=0.02$ and $p<0.0001$ respectively. For each year increase in age at breast cancer diagnosis, there is a decreased likelihood of having a *TP53* mutation by 5% (OR=0.95, 95% CI 0.91 to 0.99). In young women (average age 38.5) diagnosed with breast cancer in this study, having a tumor that is positive for HER2 increases the odds of having a *TP53* germline mutation by nearly 7 folds [OR, 6.9, 95% CI, 2.6 to 18.2 ; $P< 0.0001$].

DISCUSSION

In this case-control study, we found that patients with breast cancer and germline *TP53* mutation had significantly higher prevalence of HER2 positive tumors compared to their counterparts who lacked the mutation. This is the largest study published to date describing this association. Interestingly, in patients with HER2 positive disease and contralateral breast cancer, the contralateral primary also had HER2 overexpression in all but one case, thus providing further support for the association between germline *TP53* mutation and HER2 overexpression.

Our findings are in line with a recently published study by Wilson *et al,* where 9 patients with LFS and breast cancer were compared to a reference panel of patients with early onset breast cancer [12]. HER2 was amplified in (83%) of the breast tumors from patients with LFS compared to (16%) of their control group. The slightly higher prevalence found in their study is probably related to the evaluation of HER2 status in the breast cancers (i.e. bilateral breast cancers in individual patients were counted as separate cases) as opposed to the prevalence per patient as done in our study. Interestingly, only one case of triple negative breast cancer (TNBC) was identified in the patients with a mutation in our series, this was significantly lower than in the controls; none were found in the study by Wilson and colleagues. It is important to note that patients with a known deleterious *BRCA1 or 2* mutation were excluded from our study.

Furthermore, we found that in patients with an indication for germline *TP53* testing, the odds of finding a germline mutation increases by almost 7 folds if their breast tumor overexpresses HER2. The likelihood of encountering a germline mutation also decreases by 5% with yearly increments in age at diagnosis. If confirmed in a larger series, we anticipate that these findings could be used to refine prediction models for germline *TP53* testing in women with breast cancer.

There has been considerable interest in the role of p53 in breast cancer. Most of this work has centered on somatic *TP53* mutations which are found in about 25% of breast cancer cases and are associated with a poor prognosis[14]. Previous work by Sorlie *et al* found a strong association between somatic *TP53* mutations, HER*2* positive and basal-like breast cancer subclasses [15]. Specifically, mutated *TP53* was found in 71% of HER2 positive subclasses, and 82% of basal like breast tumors. Other studies evaluating somatic *TP53* mutations in breast tumors have also reported this interdependence between *TP53* and HER*2* [16–18]. While we did not find an association between TNBC and a germline *TP53* mutation, our findings for mutated germline *TP53* support an association with HER2 overexpression. This may be partly explained by differences between the somatic and germline *TP53* mutations. Further work examining these differences may help elucidate how mutations in *TP53* can influence the subsequent development of different breast cancer subtypes. Most recently, loss of p53 function has been shown to increase cross-talks between the estrogen receptor and EGFR/HER2 pathways in *p53* mutant cells leading to tamoxifen resistance [19].

Interestingly, both *ERBB2* (the proto-oncogene encoding HER2) and *TP53* are located on chromosome 17, (17q 21 and 17p13.1respectively) [16, 20]. How this proximity may affect the potential interaction between these two genes is largely unknown.

The international agency for research on cancer (IARC) TP53 database (http://www-p53.iarc.fr/) compiles data from published literature on germline and somatic *TP53* mutations and this information is available publically. Their analysis shows that breast cancer is the most common cancer type in women with germline *TP53* mutations. Moreover, missense mutations account for more than 70% of all cancer cases they analyzed, followed by nonsense and splice mutations [21]. As expected, the most common mutations seen in our cohort were missense mutations [3, 22]. Deletions, and frame shift mutations were also detected. Her2 amplification was encountered in all mutation types in this patient population. The same mutation at R175H, a mutation thought to be associated with gain of function of p53 was found in 4 patients[21]. Interestingly, in 2 patients it was associated with HER2 amplification, while normal and equivocal in the remaining 2 patients respectively (table 3). Further mechanistic studies evaluating the effect of a specific mutation and its ensuing effect on breast cancer phenotype are clearly needed.

Another expected finding from our study was the development of subsequent primaries, specifically, contralateral breast cancers, sarcomas and tumors of the CNS. These findings certainly provide support for aggressive screening of the contralateral breast or consideration for prophylactic mastectomy [23, 24]. Further work looking into EGFR/HER2 pathway activation in other tumors from patients with LFS is certainly warranted.

Our study is limited by its retrospective nature, small numbers, and lack of independent pathological review of all the breast cancer cases. Further work needs to be done to confirm these findings. If confirmed, they could potentially enhance genetic testing strategies for patients with limited family history and early onset breast cancer [6]. From our data, a HER2 overexpressing tumor in a young woman should be alerting to the higher probability of having a germline *TP53* mutation.

Furthermore, chemo-preventative trials incorporating anti-HER2 therapies or potentially anti-HER2 vaccines could be offered to patients with a germline *TP53* mutation [25]. Certainly this would be of great interest if similar activation of the HER2 pathway is found in tumors of the CNS, where screening and therapeutic options are limited [26]. Finally, determining how these germline mutations lead to a specific breast cancer subtype could help elucidate the role of p53 in breast cancer and help guide therapeutic strategies.

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Table 1

Summary of Study Population

	Cases n=30	**Controls n= 79**	**p value**
Median age at diagnosis (range yrs)	**31.5 years (20–59)**	**40 years (20–75)**	**0.035**
Ancestry N (%):			0.64
European	20 (67%)	56 (71%)	
Hispanic	8(26%)	13(16%)	
African American	2 (7%)	5(6%)	
Asian	-	3(4%)	
Other	-	2(3%)	

Table 2

Comparison of pathological features in patients with invasive carcinoma

	Cases n=30	**Controls n=79**	**p value**
Nuclear grade n(%)			
3	19 (63%)	42 (53%)	0.29
2	7 (23%)	28 (35%)	
1	-	4 (5%)	
Unknown grade	4 (13%)	5 (6%)	
ER+ or PR+/HER2−	**9(30%)**	**43(54%)**	**0.02**
ER±/PR±/HER2 +	**20 (67%)**	**20 (25%)**	**0.0001**
ER−/PR−/HER2−	**1(3%)**	**16 (20%)**	**0.04**

Abbreviation: ER, Estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Table 3

Specific mutation and other cancers in patients with inherited *TP53* mutation and breast cancer

Patient	*TP53* mutation			HER2 IHC/ FISH	Other cancers
	Gene bank ref seq X54156[a]	*Coding DNA sequence*[b]	*Mutation effect*		
1	g.13130	c.451C>G	missense	3+ /amplified	Bilateral breast cancer
2	g.14090_92	c.763delATC	deletion	amplified	Astrocytoma/ phyllodes
3	g.14589	c.918+1G>T	splice	3+	-
4	g.13152	c.473G>A	missense	3+	Contra DCIS
5	g.13340	c.580C>T	missense	0	Bilateral breast cancer
6	g.14070	c.743G>A	missense	3+	GBM
7	g.14516	c.847C>T	missense	0	- (Proph mast)
8	g.13203	c.524G>A	missense	2+/equivocal	liposarcoma
9	g.13419	c.659A>G	missense	0	Adrenal carcinoma
10	g.13203	c.524G>A	missense	3 +	Bilateral breast cancer
11	g.12066	c.142delG	frameshift	3+	Bilateral breast cancer
12	g.14070	c.743G>A	missense	Amplified	-
13	g.13203	c.524G>A	missense	Amplified	Multifocal breast ca/ contra DCIS
14	g.12113	c.189delTinsAGA	frameshift	3+	-
15	Del E10–11	large deletion no specific endpoint	deletion	Non-amplified	Sarcoma
16	g.13068	c.389T>A	missense	3+	Contra DCIS
17	g.17602	c.1024C>T	nonsense	3+	Contra DCIS
18	g.14501	c.832C>G	missense	Non-amplified	(Proph mast);ovarian cystadenoma
19	g.14487	c.818G>A	missense	1+	-(Proph mast)
20	g.13117	c.438G>A	nonsense	0	(Proph mast);sarcoma, endometrial ca, RCC
21	g.14468	c.799C>T	missense	3+	-
22	g.14502	c.833C>T	missense	3+	-
23	g.14513	c.844C>T	missense	3+	(Proph mast);adrenal ca, melanoma, thyroid ca
24	g.12108	c.184G>T	nonsense	2+/Amplified	Bilateral breast cancer
25	g.13116	c.437G>A	nonsense	3+	Contra DCIS, colon ca; pancreatic ca

Patient	*TP53* mutation			HER2 IHC/ FISH	Other cancers
	Gene bank ref seq X54156[a]	***Coding DNA sequence***[b]	***Mutation effect***		
26	g.13419	c.659A>C	missense	1+	Bilateral breast cancer; osteosarcoma
27	g.13203	c.524G>A	missense	Non-amplified	Contra phyllodes, rhabdomyosarcoma
28	g.14060	c.733G>A	missense	Amplified	Contra DCIS, astrocytoma, leiyomyosarcoma
29	g. 13346	c.586C>T	missense	3+/amplified	- (Proph mast)
30	g.13152	c. 473G>A	missense	3+	-

Abbreviation: aa, amino acid, HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; Contra, contralateral; DCIS, ductal carcinoma in situ; GBM, glioblastoma multiforme; proph, prophylactic; RCC, renal cell carcinoma; ca, cancer.

[a]Accession X54156 Version X54156.1 source: http://www.ncbi.nlm.nih.gov/nuccore/x54156

[b]Accession X02469 Version X02469.1 source: http://www.ncbi.nlm.nih.gov/nuccore/x02469