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Prevalence of germline *TP53* mutations in HER2-positive Breast Cancer Patients

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Abstract

Background—Breast cancer is the most frequent tumor in Li-Fraumeni syndrome (LFS), a rare inherited cancer syndrome associated with germline mutations in the *TP53* gene. Recent data show that breast cancer in germline *TP53* mutation carriers is commonly HER2-positive (63–83%). We assessed the prevalence of germline *TP53* mutations in a cohort of women with HER2+ breast cancer diagnosed age ≤ 50 years.

Material & Methods—We identified blood specimens from 213 women with primary invasive HER2+ breast cancer age ≤ 50 years from a single center. EGAN sequencing and MLPA techniques were used to screen for germline *TP53* mutations.

Results—Among 213 women with HER2+ breast cancer age ≤ 50 years, 3 (ages at diagnosis 23, 32, 44 years) were found to carry a *TP53* mutation (1.4%, 95%CI 0.3%–4.1%). ER/PR status was not uniform. Two *TP53*-carriers met Chompret criteria for LFS; none met classic LFS criteria.

Conclusion—Although two-thirds of breast cancers in women with *TP53* mutations are HER2+, we observed a low prevalence of germline *TP53* mutations among unselected young women with HER2+ breast cancer. Given the potential clinical impact, consideration of germline *TP53* testing should be given to young women with HER2+ breast cancer, especially if family cancer history is notable.

Keywords

Li-Fraumeni Syndrome; Breast Cancer; *TP53*

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Introduction

The Li-Fraumeni Syndrome (LFS) is a rare inherited cancer susceptibility syndrome associated with germline mutations in the *TP53* gene (tumor protein *TP53*, chromosome 17p13; OMIM 191170). Diverse malignancies begin in childhood and early adulthood, classically including soft tissue and bone sarcomas, breast cancers, brain tumors, adrenal cortical carcinomas, and leukemias [15]. A wide range of additional tumors have been shown to develop excessively in individuals with LFS [4, 8, 22, 28]. Breast cancer is the most frequent tumor among women with LFS [10].

Women carrying germline *TP53* mutations have a remarkable risk of developing cancer: 50% by age 30 years and 90% by age 60 years [16]. Mean age at first cancer diagnosis among females heterozygous for a germline *TP53* mutation in one prospective cohort was 29 years [9]. The reported frequency of germline *TP53* mutations in population-based series of women diagnosed with unselected invasive breast cancer before age 30 years ranges from <1% to 4% [5, 12].

Somatic mutations in the *TP53* gene are frequent in sporadic basal (83%) and HER2-amplified (70%) breast cancer subgroups [14]. Recent reports have identified an increased frequency of HER2 positive breast cancer in women with LFS [18, 20, 26]. Among young women with breast cancer, HER2 positive tumors comprise 20–25% of tumors, but in series of breast cancers in women with germline *TP53* mutations, approximately 63–83% of tumors are HER2 positive by clinical determinations [19, 20, 26]. Therefore, we investigated the frequency of germline *TP53* mutations among women aged 50 years or below at diagnosis of HER2 positive breast cancer.

Material & Methods

We searched the Clinical Operations and Research Information System (CORIS) database at the Dana Farber Cancer Institute to identify women with a diagnosis of HER2 positive invasive breast cancer seen in the Breast Oncology Clinic. All patients with a diagnosis of breast cancer, who consented to collection, storage and analysis of their samples (peripheral blood and tumor specimens) for research purposes in Institutional Review Board approved protocols, were included in this database. Blood specimens were retrieved from the annotated Dana-Farber/Harvard Cancer Center Spore Core Blood Repository.

The blood sample collection, *TP53* germline analysis and all data extraction from the CORIS database and medical records, were approved by the Institutional Review Board. The cohort was comprised of women with primary invasive breast cancer diagnosed at age 50 years or below from the CORIS database who met the following criteria: HER2 amplification on a primary breast cancer specimen by immunohistochemistry (IHC) (3+) and/or Fluorescence in Situ Hybridization (FISH) (ratio > 2.2 was considered amplified) according to ASCO/CAP guidelines [27]. Equivocal HER2 (2+ by IHC) cases were included only if they were positive by FISH.

All specimens were reviewed clinically in one of the Harvard teaching hospital pathology departments. Tumor ER, PR and HER2-status were reviewed specifically in 70% of cases;

otherwise the analysis from the referring hospital was accepted. Data were extracted from clinical pathology reports. Tumors were classified as hormone receptor positive if tumor nuclei staining for estrogen or progesterone receptors by IHC was greater than or equal to 1% according to ASCO/CAP guidelines [27].

Women with initial breast cancer diagnoses between October 1992 and April 2010 and invasive HER2 positive breast cancer diagnosed at age ≤50 years were identified from the database. Personal and family cancer history were extracted from questionnaires completed by all individuals enrolled in the CORIS database and were supplemented by review of subject medical records.

The family cancer diagnoses could not be verified with medical records under the terms of the protocol which precluded further patient contact. Family history information was missing from 6 women who were adopted, and 5 women who did not provide data. Family cancer histories, including the breast cancer in the proband were classified according to two sets of published criteria for LFS, classic and updated Chompret [15, 24].

The classic criteria for LFS families include a sarcoma before age 45 years in a proband, a first-degree relative with any cancer before age 45 years, and another first-degree relative with soft tissue sarcoma or osteosarcoma at any age, or any cancer before age 45 years [15]. The 2009 Chompret criteria, which were derived from the systematic study of LFS kindreds in France include: a proband with a tumor belonging to the narrow LFS tumor spectrum (e.g. soft tissue sarcoma, osteosarcoma, brain tumor, premenopausal breast cancer, adrenocortical carcinoma, leukemia, and lung bronchoalveolar cancer) diagnosed before age 46 years, with at least one first- or second-degree relative with an LFS tumor (except breast cancer, if the proband has breast cancer) before age 56 years or with multiple tumors; or a proband with multiple tumors, two of which belong to the narrow LFS tumor spectrum (except multiple primary breast cancers) and the first of which occurred before age 46 years; or a patient with adrenocortical carcinoma or a choroid plexus carcinoma, irrespective of family history [24].

A group of 347 women diagnosed at age ≤50 years with initial HER2 positive breast cancer between October 1992 and April 2010 was identified from the CORIS database. One hundred thirty-four subjects were excluded from the analysis for the following reasons: in 71 individuals the HER2-status could not be confirmed; in 23 subjects the carcinoma was exclusively *in-situ*; peripheral blood DNA-samples were missing from 29 cases, poor DNA quality precluded *TP53* analysis in one sample and two patients had high median absolute variation (MAD) values for control probes and were excluded from MLPA-analysis. In addition, we excluded subjects with germline mutations or a sequence variant suspected deleterious in the *BRCA1* (n=2) or *BRCA2* (n=2) genes and the *von Hippel-Lindau* gene (n=1). Subjects with other primary cancers before the diagnosis of breast cancer (n=3) were also excluded from the analysis. Therefore, the analysis was limited to 213 women with documented invasive, HER2 positive primary breast cancer as their first malignancy. Among the final cohort, 78 women had previously tested negative for germline *BRCA1* and *BRCA2* mutations.

DNA-Extraction

DNA was extracted from patient whole blood samples using a Qiagen DNA extraction kit (QIAamp DNA Blood Mini kit) according to manufacturer instructions on a Qiacube instrument. This was carried out in the Dana Farber Cancer Institute Breast Cancer SPORE CORE Laboratory.

Germline *TP53*-Mutation Analysis

Subjects were tested for *TP53* alterations using exon grouping analysis (EGAN). EGAN is based on conformation-specific gel electrophoresis (CSGE) [3, 11] and conformation-specific capillary electrophoresis (CSCE) [2, 25]. All coding exons and surrounding intronic sequences were positive and analyzed on ABI3730 sequencers with CAP polymer (Applied Biosystems, CA) using 12 primer pairs. Raw data were processed and sized using Dax software (Van Mierlo Software Consultancy, Eindhoven, NL). Polymerase chain reaction (PCR) fragments with aberrant mobility on ABI-3730XL were sequenced. In addition all subject DNA's were sequenced for Exons 2 and 3. This method permits detection of mutations and polymorphisms in all coding regions as well as splice-site mutations. Analysis of the alterations was performed using the *TP53* database at IARC (http://www-p53.iarc.fr/) [7].

MLPA-Analysis

Multiplex Ligation-dependent Probe Amplification (MLPA)-analysis was performed for the same cohort using the P056 SALSA MLPA kit (MRC-Holland, Amsterdam, NL) according to manufacturer instructions. A total of 100ng of DNA were used from each individual. PCR was done in 25μl reactions. Analysis of samples was divided into three sets and each performed in a quadrant of a 384 well plate. At least five control samples were added to each quadrant. Detection was performed on ABI3730 (Applied Biosystems, CA) sequencers. Raw data were processed and sized using Dax software (Van Mierlo Software Consultancy, Eindhoven, NL). Coffalyser software (MRC-Holland, Amsterdam, NL) was used for data analysis and visualization. For each sample median absolute variation (MAD) of control probes, ratio calls as well as standard error for each *TP53* specific probe were determined. The overall median absolute variation (MAD) of control probes in the data set was at 0.07.

Statistics

Exact 95% binomial confidence limits (CI) were calculated for the percent of women who had *TP53* germline mutations, both overall (all women < 51 years old) and in the subset of women < 40 years old and the subset ≥ 41 years (an age division that was pre-specified in the study protocol). The distribution of various characteristics was compared between patients < 40 years and patients ≥ 40 years old at diagnosis using Fisher exact tests. All test results are two-sided, but not corrected for multiple comparisons.

Results

213 women with confirmed HER2+ breast cancer diagnosed at age 50 years or below were identified from the CORIS database and were included in the analysis (Table 1). Most tumors were ductal (n=192, 90%), high grade (Grade III n=146, 69%) and hormone receptor

positive (ER+ n=144, 68%; PR+ n=121, 57%). Eighty-one patients were under 40 years at their breast cancer diagnosis. Their tumors were also most often ductal (n=73, 90%), high grade (n=59, 73%) and hormone receptor positive (ER+ n=53, 65%; PR+ n=44, 54%). As expected, the younger patients were more likely to have received neoadjuvant therapy (44% vs. 28%, p = 0.02 when patients with metastases at diagnosis were omitted) and more likely to have been tested for BRCA mutations (53% vs. 27%, p = 0.0001). Younger patients were also more likely to be HER2 IHC 3+ (99% vs 89%, p=0.01), to have no FISH done or to be negative by FISH (93% vs. 79%, p=0.01).

In three subjects, a deleterious germline *TP53* mutation was identified: two missense mutations R110H (c.329G>A) and G334R (c.1000G>C), and one nonsense mutation R196X (c.586C>T). A novel intronic change 5 base pairs upstream of exon 10 which may affect splicing (IVS994-5T>C) was detected in a woman with breast cancer at age 50 years, but was not considered a mutation for this report. All mutations have been reported previously in the IARC repository [7]. The MLPA analysis showed no significant alteration in *TP53* copy number, including insertions and deletions in the *TP53* gene in any of the samples analyzed.

The youngest mutation carrier (age of diagnosis 23 years, c.586C>T) had an invasive ductal, grade III, T1N0M0, ER/PR positive, HER2 positive unilateral breast cancer. Her family history met Chompret criteria for LFS (an uncle had a brain tumor at age 9 years). The second individual with a germline *TP53* mutation (32 years at diagnosis, c.329G>A) had an invasive ductal, grade III, T2N0M0, ER/PR negative HER2-positive breast cancer. Her family history did not meet classic or Chompret criteria for LFS, though there were other relatives with breast cancer in the family. The third mutation carrier (c.1000G>C) was diagnosed at age 44 years with a grade II, T1N0M0, mixed ductal-lobular, ER/PR positive HER2 positive breast cancer. Her family history met Chompret criteria (an aunt died of leukemia at age 14 years).

Five subjects developed second cancers after their breast cancer diagnosis including one each acute myeloid leukemia, endometrial cancer, bladder cancer, parotid cancer and papillary thyroid cancer. None of these subjects with a second cancer diagnosis was found to carry a germline *TP53* mutation.

The overall frequency of germline *TP53* mutations in the cohort of women diagnosed with HER2-positive breast cancer age 50 years or below was 3 of 213 (1.4%, 95%CI 0.3% to 4.1%). Among women younger (under 40 years) at diagnosis of HER2 positive breast cancer, the prevalence was 2/81 (2.5%, 95%CI 0.3% to 8.7%). In the total cohort of 213 patients, none met classic criteria for LFS, however 18 patients (8%) met Chompret criteria. Among women ≤ 50 years old at diagnosis whose family history was consistent with Chompret criteria, 2 of 18 (11%, 95%CI 1.4% to 34.7%) had *TP53* mutations. Among women without a known family history consistent with Chompret criteria, TP53 mutations were found in only 1/195 (0.5%, 95% CI 0.01% to 2.9%).

Discussion

Recent reports have shown that a higher proportion of invasive breast cancers in women with germline *TP53* mutations are HER2 positive than in other women. Wilson et al. compared the pathological characteristics of 12 breast cancers arising in nine subjects carrying pathogenic *TP53* mutations to a reference panel of 231 women with young onset breast tumors defined as ≤ 30 years [26] and found a significantly higher prevalence of HER2 amplification (83%) in germline *TP53* mutation carriers compared to a cohort of young onset breast cancer cases (16%, ≤ 30 years); ER and PR status were equally distributed between groups [26]. Another series from the MD Anderson Cancer Center noted similar results (67% were HER2 positive and 70% ER and/or PR positive) among 30 *TP53* mutation carriers [20]. Our group has reported similar findings on behalf of a consortium [18]. Among 43 tumors in 39 women (age range 22–60 years at diagnosis) with confirmed germline *TP53* mutations, 63% of the invasive ductal and 73% of the ductal carcinomas in situ were HER2 positive by IHC and/or FISH [18]. Given the rarity of germline *TP53* mutations, (estimated prevalence 0.06% to 0.5%), Bayes' theorem can be used to predict that between 0.4% and 3% of women diagnosed with a HER2 positive breast cancer at age < 50 years or below would carry a germline *TP53* mutation. The prevalence we found is consistent with that prediction.

In this study we identified three women with germline *TP53* mutations in a cohort of 213 women with confirmed HER2-positive invasive breast cancer from a single institution diagnosed at age 50 years or below (1.4%, 95%CI 0.3% to 4,1%). None of the women with a deleterious *TP53* mutation met classic LFS criteria; two women met Chompret criteria. Conversely, 2 (11%) of the 18 women whose family histories met Chompret criteria were found to carry germline *TP53* mutations. One of the *TP53* mutation carriers would not have been identified if family history criteria alone had been used to determine who should be tested.

Two groups have reported the prevalence of germline *TP53* mutations in population-based series of young women with breast cancer unselected for subtype. Lalloo et al. reported germline *TP53* pathogenic mutations in 4% of breast cancer patients diagnosed ≤ 30 years (n=99) [12, 13]. Chompret et al. detected 3 germline *TP53* mutations among 116 breast cancer patients (2.5%) diagnosed ≤ 35 years[1]. In our study, among women diagnosed ≤ 35 years with HER2 positive breast cancer only, we identified 2 *TP53* mutation carriers among 40 women (mutation prevalence 2/40 = 5%).

The study does have limitations. All individuals were seen at a single center; the group is not population-based. Further, the size of the cohort is small. Family-history information for some pedigrees is likely to be incomplete, as it was provided by women in a questionnaire at diagnosis and by physicians in notes created in the context of usual clinical oncology care, and could not be confirmed according to the terms of this protocol. HER2 was not always tested at time of diagnosis but sometimes tested at a later date, raising the issue of biases that could have been caused by some association of year of diagnosis, year of recurrence or metastases, survival time, and likelihood of *TP53* mutation. Despite these limitations, this is

the first study to date looking at the prevalence of *TP53* mutations in subjects with younger onset (age 50 years or below) HER2 positive breast cancer.

According to the 2011 National Comprehensive Cancer Network (NCCN) guidelines for *TP53* testing, in addition to women whose family histories meet classic LFS or Chompret criteria, it is reasonable to test individuals with breast cancer diagnosed before age 30 years with a negative *BRCA1/2* test, especially if there is a family history of tumors associated with LFS [21]. Our series suggests that this approach may be too restrictive. Family history is not always reported accurately: only 55% of other LFS-related cancers have been accurately reported in classic Li-Fraumeni families undergoing genetic testing, possibly because many Li-Fraumeni-associated tumors are uncommon, or because patients may not recall ages at cancer diagnosis in relatives [23]. The frequency of *de novo* mutations in LFS is not insignificant (7%–20%) [6].

It is potentially important to recognize women with *TP53* mutations at the time of their breast cancer diagnosis. Like women with *BRCA1/2* germline mutations, they may wish to manage the increased risk of a second primary breast cancer with prophylactic contralateral mastectomies. Further, there are data suggesting an increased risk of radiation-associated cancers in women with *TP53* germline mutations, who might therefore avoid therapeutic radiation, if possible [16, 17]. In contrast to *BRCA1/2* carriers, women with LFS will still have a significant residual risk of life-threatening malignancies at other sites over their lifetimes.

The recent observation of an increased prevalence of HER2-positive breast cancers in women with germline *TP53* mutations raises questions about the biology of breast cancer development in this cohort. The majority of basal-like and *BRCA1*-associated breast cancers have somatic mutations in *TP53*, yet are not HER2 positive. One possibility is that the order of mutation acquisition contributes to the breast cancer subtype. An alternate hypothesis to explain the increased frequency of HER2 positive breast cancers in germline *TP53* mutation carriers compared with basal-like breast cancers in *BRCA1* mutation carriers could be a different cell of origin.

In this study, we identified a low prevalence of germline *TP53* mutations among women diagnosed with HER2 positive breast cancers before age 50 years. As the field of oncology move to more comprehensive molecular analyses of patient tumor and germline tissue at the time of a new breast cancer diagnosis, we will likely be able to refine the characteristics that predict for identification of germline *TP53* mutations. Analysis of other series will be helpful in reaching more definitive estimates of the prevalence of mutation carriers among patients with HER2 positive breast cancer at young age.

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Table 1

Characteristics of the Cohort (n=213)

Age groups:	**23–39 (%)**	**40–50 (%)**	**Total (%)**
Total:	**81**	**132**	**213**
Grade:			
G1	1 (1)	1 (1)	2 (1)
G2	21 (26)	42 (32)	63 (30)
G3	59 (73)	87 (66)	146 (69)
N/A	–	2 (1)	2 (1)
Histology:			
Ductal	73 (90)	119 (90)	192 (90)
lobular	1 (1)	–	1 (1)
mixed ductal/lobular	4 (5)	9 (7)	13 (6)
mucinous	1 (1)	2 (1)	3 (1)
papillary	1 (1)	1 (1)	2 (1)
N/A	1 (1)	1 (1)	2 (1)
Neoadjuvant Therapy:			
Yes	36 (44)	37 (28)	73 (34)
No	42 (52)	86 (65)	128 (60)
metastasis at dx	3 (4)	9 (7)	12 (6)
Her2 Status:			
IHC 3+, FISH N/A	74 (91)	104 (79)	178 (84)
IHC 3+ and FISH +	5 (6)	14 (11)	19 (9)
IHC 2+, FISH +	1 (1)	12 (9)	13 (6)
FISH +	–	2 (2)	2 (1)
IHC 3+, FISH −	1 (1)	–	1 (1)
Estrogen-Receptor-Status:			
positive	53 (65)	91 (69)	144 (68)
negative	28 (35)	41 (31)	69 (32)
Progesterone-Receptor-Status:			
positive	44 (54)	77 (58)	121 (57)
negative	37 (46)	55 (42)	92 (43)
BRCA 1/2-Testing:			
not tested	38 (47)	97 (74)	135 (63)
BRCA 1/2 negative*	43 (53)	35 (27)	78 (37)

[*] 6 of the BRCA 1/2 negative patients had variants (UV and 2 polymorphisms): 5 (12%) of the patients < 40 who had BRCA 1/2 testing and 1 (3%) of the patients ≥ 40 who had BRCA1/2 testing

Table 2

Characteristics of the *TP53*-mutation carriers

	Patient 1	Patient 2	Patient 3
Age at diagnosis	23	32	44
Histology	ductal	ductal	mixed ductal-lobular
ER	+	−	+
PR	+	−	+
BRCA-Testing	not tested	negative	negative
***TP53* Mutation**	c.586C>T	c.329G>A	c.1000G>C
***TP53* Protein Change**	p.R196X	p.R110H	p.G334R