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**“A novel HER2-positive breast cancer phenotype arising from germline
TP53 mutations”**

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Abstract:

INTRODUCTION: The Li-Fraumeni Syndrome (LFS) is caused by a germline *TP53* mutation and is associated with a high risk of breast cancer at young ages. Basal (triple negative) breast cancers are now well recognized to be a typical sub-type of breast cancer developing in a large proportion of BRCA1 gene carriers. We considered whether a similar narrow sub-type of breast cancer was found in *TP53* gene mutation carriers. **OBJECTIVE:** A hypothesis generating study to investigate whether there are specific breast tumour characteristics associated with germline *TP53* mutations. **METHODS:** Pathological characteristics in 12 breast cancers arising in 9 patients carrying pathogenic *TP53* mutations were compared to a reference panel of 231 young onset breast tumours included in the POSH study. **RESULTS:** Patients carrying a *TP53* mutation showed a significantly higher likelihood of developing a breast cancer with Human Epidermal growth factor Receptor (HER2) amplification (83%) when compared to the cohort of young onset breast cancer cases (16%), ER and PR status were equivalent between groups. **CONCLUSION:** These findings suggest that breast cancer developing on a background of an inherited *TP53* mutation is highly likely to present with amplification of HER2.

Keywords: *TP53*, Li-Fraumeni, Breast Cancer, Pathology, Early Onset

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Introduction

The Li Fraumeni Syndrome (LFS) is a clinical diagnosis first described in 1969¹. In 1990 mutations in the *TP53* gene were found to be the underlying cause². The clinical guidelines for selecting cases for *TP53* mutation testing vary widely; more stringent guidelines lead to a higher rate of detection of germline *TP53* mutations²⁻⁴. Germline *TP53* mutations are rare in isolated cases of breast cancer with young ages of onset (<30 years at diagnosis), with reported incidences ranging from <1% to approximately 7%^{2,4-6}. Given that most clinical mutation testing is driven by a family history of sarcomas/other LFS-associated cancers and taking into account the high mortality of this condition at young ages⁷, it is likely that *de novo* *TP53* mutations will account for a small proportion of all isolated young onset LFS cancers.

The p53 protein has a fundamental role in sensing irreparable DNA damage and facilitating apoptosis, and so the use of cytotoxic therapies in *TP53* gene carriers might encourage the emergence of subsequent malignancies in an already highly susceptible individual. Thus, the balance of benefit and toxicity of such therapies in a patient with a germline *TP53* mutation is likely to be different from the average breast cancer patient. Recognition of a typical LFS-associated breast cancer phenotype would be very valuable for oncologists and would improve targeted genetic counselling and testing.

The aim of our study was to determine whether breast cancers arising in carriers of germline *TP53* mutations were associated with specific pathological characteristics when compared to a cohort of young onset breast tumours.

Methods

TP53 Case Selection

Group 1: All patients diagnosed with LFS associated with a pathogenic *TP53* gene mutation in one UK regional genetics service were reviewed; 5/8 family pedigrees contained at least one family member diagnosed with breast cancer where tumour tissue samples could be obtained. Formalin fixed paraffin embedded breast cancer tissue for all known gene carriers were retrieved and immunohistochemistry (IHC) for ER, PR and HER2 (Dako HERCEPTEST[®]) was repeated on whole tumour sections. When HER2 IHC gave an equivocal result (2+) then Fluorescent in-situ hybridisation (FISH - Vysis[®]) was used to determine whether the HER2 gene was amplified (ratio > 2.2 relative to a reference chromosome 17 centromeric probe).

Group 2: 234 breast cancer patients diagnosed aged 30 years or younger participating in the POSH study (Prospective study of Outcomes in Sporadic versus Hereditary breast cancer, MREC 00/6/69⁸) were selected for germline *TP53* testing. 216 of these had an available DNA sample and were included.

TP53 germline mutation analysis

For the Clinical Genetics cases, *TP53* mutation testing reports were collected. All testing was conducted by the regional genetics service in response to clinical suspicion of a diagnosis of LFS.

For the 216 POSH cases aged ≤ 30 years, DNA was extracted from peripheral blood leukocytes and screened for mutations in all coding exons (2 to 11) and associated splice junctions of the *TP53* gene. Unidirectional sequencing was performed using the BigDye Terminator Cycle sequencing kit and a 3730 automated fluorescent sequencer (ABI Perkin Elmer). Mutation surveyor software (v3.20 SoftGenetics) was used to analyse the exonic sequence, intron-exon boundaries and 10 bases of the

flanking intron for all coding exons. All mutations were confirmed by bi-directional sequencing in a fresh aliquot of the stock DNA. Systematic BRCA1 and BRCA2 mutation testing was not undertaken as part of this study.

Immunohistochemistry – POSH cases

A reference panel of 231 tumours from patients diagnosed with breast cancer under 40 years of age was available as Tissue Micro Arrays (TMA) as part of the POSH study⁸. Immunohistochemistry was performed on 4µm, formalin-fixed, paraffin-embedded serial sections of TMA. Each sample was represented on the TMA by a minimum of two x 0.6mm tumor cores. Immunohistochemistry for ER, PR and HER2 (Ventana PATHWAY®) and a standard Avidin-Biotin Complex technique was employed.

Immunohistochemical evaluation – TP53 and POSH cases

Staining with ER, PR and HER2 was evaluated using a semi-quantitative scoring system. ER and PR staining were scored on the basis of percentage of the tumor cells showing nuclear staining (0 = 0, <1=1, 1-10=2, 11-33=3, 34-66=4, >67=5) and the intensity of staining (1=weak, 2=moderate, 3=strong); the sum of these scores giving a final score range of 0-8. An overall score of 4 or more was considered to represent positive expression for each receptor. HER2 expression was assessed using a 0-3 scale (0 – no staining, 1+ – weak cytoplasmic membrane staining in any proportion of tumour cells, 2+ – moderate cytoplasmic membrane staining in at least 10% of tumour cells, 3+ – intense cytoplasmic membrane staining in at least 30% of tumour cells).

In-situ hybridisation – POSH cases panel

Chromogenic in situ hybridization (CISH) was used for all TMAs in the POSH series to determine HER2 amplification where IHC result was equivocal (2+). CISH was performed using SPoT-Light CISH Polymer Detection Kit (Invitrogen, UK) following the standard protocol.

POSH cases aged ≤30 years – immunohistochemistry

The POSH study database records the results of any clinical testing for ER, PR and HER2 and the clinical testing data for these three antibodies was retrieved for comparison with TP53 carriers and the 231 POSH cases systematically stained and scored using the TMAs.

Statistical Analysis

The chi-squared test for independence was used to investigate the relationship between TP53 and POSH cohorts with respect to ER, PR or HER2 status. Yates' correction for continuity was used to correct for frequency values <5.

Results

Twelve breast cancers arising in nine TP53 gene carriers were evaluated (table 1) and compared with the POSH reference panel. The frequency of ER and PR positive tumours was similar between the two groups. Nine out of the twelve tumours associated with a germline TP53 mutation showed strong staining using IHC for HER2 (3+). One case was unequivocally negative for HER2. Of the two cases equivocal on IHC for HER2 overexpression (2+), FISH revealed one was amplified and one was within normal limits. Thus, of the cases with confirmed pathogenic germline TP53 mutations, ten out of the twelve showed HER2 protein overexpression or gene amplification (83%). Furthermore, five of the twelve tumours (42%) were positive for all three markers (triple positive).

Table 1. Tumour features of patients in the *TP53* cohort. C = Contralateral tumour, ER = Oestrogen Receptor, PR = Progesterone Receptor, HER2 = Human Epidermal Growth Factor Receptor 2, IHC = Immunohistochemistry, FISH = Fluorescent In-Situ Hybridisation. *Reported as somatic mutation 13 times and germline once, deleterious (<http://www-p53.iarc.fr/MutationValidation.asp>).

**Reported in 340 tumours and as causative mutation in 6 LFS families, deleterious (<http://www-p53.iarc.fr/MutationValidation.asp>).

Patient	Age at onset	ER	PR	HER IHC (FISH)	Grade	<i>TP53</i> mutation
1	35	+	+	3+	3	Splice c.672+1G>T
2	26	-	-	2+ (amplified)	3	Nonsense c.38C>T, exon 4
3	24	+	+	3+	3	Missense c.724T>C (p.C242R)*
4	24	-	-	3+	3	Splice c.743G>A
4 (C)	31	-	-	3+	3	as above
5	28	+	+	3+	3	Missense c.659A>G (T220C)**
5 (C)	28	+	+	2+ (normal range)	3	as above
6	28	+	-	3+	3	Nonsense p.R209R/X
7	29	-	+	3+	3	Splice c.919+1G>G/A
8	24	+	+	3+	3	Nonsense p.R196R/X
8 (C)	27	+	-	-	2	as above
9	22	+	+	3+	3	Nonsense p.W146W/X

The germline mutation underlying each case is shown in Table 1. Four patients had nonsense mutations, three patients had splice mutations and two patients had missense mutations highly likely to be pathogenic.

We reviewed the IHC data for 231 patients identified from the POSH reference panel. In total 229 cases were successfully scored for all three markers. 58% (n=134) of cases were ER positive, not significantly different to the proportion seen in the *TP53* mutation carriers (p value = 0.77). 58% (n=132) of cases were PR positive, which was similar to the *TP53* cohort (p value = 0.75). Only 19% were scored positive for HER2 (n=44), a significantly lower figure compared to the *TP53* mutation carriers (p value = 1.2×10^{-6}). 30% (n=69) of the POSH cases were triple negative, but none of the *TP53* group were negative for all three markers (p value = 0.06). In contrast only 18 out of 229 POSH cases (8%) were triple-positive compared to 5/12 (42%) in the *TP53* cohort (p value = 9.3×10^{-5}).

Finally a review the clinically reported HER2, ER and PR status for the 216 cases diagnosed aged 18-30 years from the POSH study that underwent germline *TP53* testing in this study was included for completeness. HER2 testing was not routinely performed for clinical purposes during most of the recruiting period for the POSH study. HER2 overexpression is associated with a high chance of early relapse after breast cancer, so testing for HER2 in this clinical setting is likely to be triggered when patients relapse. HER2 status was only available for 161/234 cases and all three markers were available for only 148 cases. For the 161 cases where HER2 was reported, 67(41.6% of tested and 29% of all cases) were reported to over express HER2, still a significantly lower proportion than observed in the *TP53* associated cases ($p=0.012$). Triple-negative tumours were reported in 69/148 (47%) ($p = 0.0046$). 24/148 (16%) had triple-positive tumours, not significantly different to observed values in the *TP53* carriers ($p = 0.07$).

Discussion

The *TP53* gene has been recognised for over two decades as a key molecule in both somatic and inherited cancer^{2;9}. It has a major role in sensing DNA damage (to stall the cell cycle while DNA is repaired), or in the absence of effective repair, facilitates apoptosis. The *TP53* gene is frequently somatically mutated in many types of cancer, and mutation or dysfunction of the *TP53* gene is consistently associated with a poor prognosis. Consequently, there is considerable interest in developing therapeutics targeted at the *TP53* pathway¹⁰. In sporadic breast cancer, somatic *TP53* mutations are more frequently detected in the poorer prognosis sub-groups, particularly the basal and HER2 amplified sub types¹¹.

Perou et al first described “molecular portraits” of breast cancer, and subsequent studies have shown patterns of aberrant gene expression that correlate well with prognostic subgroups¹². Antibodies used for immunohistochemical profiling can identify similar sub-groups (including the ‘triple-negative’ group, negative for oestrogen, progesterone and HER2 receptors) that loosely equate to the sub-groups determined by molecular analyses¹³ and have been used to characterise breast cancers in genetic predisposition syndromes¹⁴. In patients with a BRCA1 germline mutation the most frequent sub-type of breast cancer is the basal type, usually ‘triple-negative’. There has been no detailed study of tumour subtypes in carriers of germline *TP53* mutations to date.

The pathological characteristics of breast cancer from immunohistochemistry are used routinely to predict prognosis. The presence or absence of ER or HER2 amplification in tumour cells has prognostic implications, and can determine best treatment options for different breast cancer subtypes¹⁵. The observation in this study of a high percentage of HER2 amplified tumours in carriers of a germline *TP53* mutation are compatible with these observations in sporadic cancers but imply that somatic *TP53* mutation may be an early oncogenic event in HER2 amplified tumours that somehow predetermines the development of the breast cancer along a specific oncogenic pathway.

HER2 amplification is uncommon in breast cancers, found in only 15-20% of all tumours^{16;17}. With a systematically reviewed cohort of cases, HER2 amplification does not appear to be more common in young onset breast cancer, 19% in the POSH cohort presented here. In the germline *TP53* mutation cohort we have observed a

remarkable difference in the proportion with HER2 overexpression/amplification (83%), significantly more tumours were positive for all three markers than expected and no triple-negative tumours were observed. Although our control group were very young at diagnosis (mean age 35 years and all under 41 years), breast cancer diagnosed under 30 years represents less than 1% of all breast cancer and may be biologically different. The review of the immunohistochemistry from the 216 patients with diagnosis ≤ 30 years showed that HER2 overexpression was possibly higher in very young onset cases, however it is important to stress that this proportion may be falsely inflated due to a bias towards testing relapsed patients for HER2 status in patients experience tumour relapse. Furthermore, despite this likely bias, the difference in HER2 over expression in tumours comparing the TP53 gene carriers and the ≤ 30 group is still significant ($p = 0.012$).

It is possible that technical differences between using whole tumour sections and tissue microarrays could lead to small differences in the designation of tumour markers. The method of assessing HER2 amplification (IHC or FISH/CISH, TMA or whole sections) may lead to small variations in proportions but the large differences observed in this study are very unlikely to have arisen as a result of either of these technical variations.

The underlying germline *TP53* mutations in our cohort included 7 out of 9 patients with truncating mutations (78%). This contrasts with both somatic and germline mutations most frequently recorded in the IARC database where missense mutations are more frequent than truncating mutations^{10;18}. The numbers of mutation carriers in this series is small but this observation may offer a further intriguing insight into possible correlations between the type of underlying *TP53* mutations and the nature of the ensuing malignancy risk.

In summary our results suggest that germline *TP53* mutation encourages breast cancer development along a fairly specific oncogenic pathway that frequently includes HER2 overexpression/amplification. Further work needs to be done to confirm our observations but if these findings are representative then for unusually young onset breast cancers with or without a family history, HER2 positive tumours and probably also ER and PR positive, should raise the possibility of an underlying germline *TP53* mutation. Specific cancer risks associated with different *TP53* mutation types needs further exploration in order to improve clinical genetic advice. Future exploration of the more detailed molecular characteristics of the *TP53* mutation-driven oncogenic pathway may reveal diagnostic and therapeutic insights into early onset HER2 positive breast cancer.

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Competing Interests

None declared.

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