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Genotype–phenotype associations among panel-based *TP53*+ subjects

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Purpose: Panel testing has led to the identification of *TP53* pathogenic/likely pathogenic (P/LP) variant carriers (*TP53*+) who exhibit a broad range of phenotypes. We sought to evaluate and compare genotype–phenotype associations among *TP53*+ panel-ascertained subjects.

Methods: Between 2012 and 2017, 317 *TP53*+ subjects (279 females and 38 males) identified through panel testing at one testing laboratory were found to have evaluable clinical histories and molecular results. Subject cancer histories were obtained from test requisition forms. P/LP variants were categorized by type and were examined in relation to phenotype.

Results: Loss-of-function (LOF) variants were associated with the earliest age at first cancer, with a median age of 30.5 years ($P = 0.014$); increased frequency of a sarcoma diagnosis ($P = 0.016$); and more often meeting classic LFS testing and Chompret 2015 criteria ($P =$ 0.004 and 0.002 respectively), as compared with dominant-negative missense, other missense, or miscellaneous (splice or in-frame deletion) P/LP variant categories.

Conclusion: Loss-of-function variants were more often associated with characteristic LFS cancer histories than other variant categories in *TP53*+ carriers ascertained through multigene panel testing. These findings require validation in other *TP53*+ cohorts. Genetic counseling for panel-ascertained *TP53*+ individuals should reflect the dynamic expansion of the Li–Fraumeni syndrome phenotype.

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INTRODUCTION

Despite its original description by Drs. Li and Fraumeni nearly 50 years ago, many questions about the phenotype of Li–Fraumeni syndrome (LFS) remain.[1] LFS is a pleiotropic hereditary cancer predisposition syndrome associated with germline pathogenic/likely pathogenic (P/LP) variants in the *TP53* gene.[2] Classically it has been associated with rare malignancies, aggressive cancers in children and adults at early ages, and multiple primary tumors, with a particular propensity for sarcoma, brain/central nervous system (CNS) tumor, breast cancer, and adrenocortical carcinoma.[1,3,4]

As multigene panel testing for hereditary cancer has gained traction, an increasing number of germline *TP53* P/LP variants have been identified in individuals who do not meet established criteria for LFS. We previously described differences between multigene panel–ascertained *TP53* P/LP variant (*TP53*+) carriers with single-gene tested *TP53*+ subjects. Panel-based *TP53*+ subjects were diagnosed with their first cancer at later ages than single-gene *TP53*+ individuals and were significantly less likely to meet Chompret or National Comprehensive Cancer Network (NCCN) criteria for *TP53* testing.[5] These findings suggested more phenotypic variability in germline *TP53*+ subjects than previously appreciated.

In parallel, questions have arisen about the higher than expected frequency of germline *TP53* P/LP variants in cohorts of patients with breast, colorectal, and other cancers.[6–10] Efforts to reevaluate the epidemiology of LFS were recently undertaken through analysis of tumor types by phase of life (childhood, early adulthood, and later adulthood) by retrospective review of the International Agency for Research on Cancer (IARC) *TP53* database.[11] Likewise, colleagues have recognized that stringent *TP53* testing guidelines miss individuals with germline *TP53*+ results and that the understanding of germline *TP53* P/LP variants would be aided by less discriminant *TP53* testing.[12] Complicating matters, however, is the identification of *TP53* P/LP variants due to clonal hematopoiesis of indeterminate potential (CHIP), which can lead to misclassification and misdiagnosis.[13] Missense variation in the *TP53* gene poses another

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longstanding challenge to diagnosing LFS, which has resulted in renewed efforts to improve in silico prediction tools for interpretation of these variants.[14]

An unanticipated *TP53*+ result on panel testing can be alarming for patients and clinicians alike. There is sustained interest in genotype–phenotype associations in *TP53*+ carriers including effects of loss-of-function or null, hotspot, founder, missense, and oligomerization domain (residues 325–355) P/LP variants.[4,15–18] To aid in risk assessment and genetic counseling, we examined the clinical characteristics and investigated genotype–phenotype associations among *TP53*+ subjects ascertained through multigene panel testing (MGPT).

MATERIALS AND METHODS

Subject and phenotype analysis

The study cohort was limited to subjects with a *TP53*+ result, defined as a P/LP variant, who also had evaluable clinical and molecular information. Subject specimens were submitted to a single laboratory (Ambry Genetics, Inc.) between March 2012 and June 2017 for panel testing including *TP53*. Twelve different panels yielded at least one positive result (Supplemental Table 1). This cohort is an expansion of a previously reported cohort in which the distribution of *TP53*+, variant of uncertain significance (VUS), and negative results by clinical phenotype and test type was described.[5]

Subjects' cancer histories were obtained from clinician-completed test requisition forms (TRFs), which accompany all specimens, along with additional clinical documents such as pedigrees and/or clinic notes when available. Each *TP53*+ subject's clinical history was also reviewed to determine if Chompret criteria (2.2017) or classic LFS criteria were fulfilled.[3,4,19,20] LFS core cancers in this study were defined as sarcoma, CNS tumor, and adrenocortical carcinoma. Breast cancer, while a classic LFS component tumor, was examined separately because of its high frequency in the cohort.

Dana-Farber/Harvard Cancer Center's (DF/HCC) Institutional Review Board (IRB) and Western IRB provided oversight in accordance with regulations. The sharing of de-identified data was determined to be exempt from the Office of Human Research Protection's Regulations for the Protection of Human Subjects.

Molecular analysis

Sequence enrichment, next-generation sequencing (NGS), targeted chromosomal microarray, and bioinformatics were performed, as previously described, of all coding exons and 5′ base pairs into introns.[21,22] Variant classification has been previously described and was based on the variant interpretation as of April 2018.[23,24] *TP53*+ results were categorized as follows: loss-of-function including genomic rearrangement (LOF), missense as either dominant-negative (DN) missense effects per the IARC *TP53* Database (http://p53.iarc.fr) or other missense (OM),[25–27] and miscellaneous (MISC) including splice variants and in-frame deletions.

Statistical analysis

Descriptive statistics for subjects stratified by *TP53*+ category (LOF, DN, OM, and MISC) are summarized as median (interquartile range [IQR]) for continuous and proportions for categorical characteristics. Differences in medians and proportions across variant category were assessed with Kruskal–Wallis tests and Fisher's exact tests, respectively. Frequencies of specific P/LP variants were summarized by phenotype group: subjects with a personal history of only breast cancer, subjects with a history of sarcoma alone or in combination with any other cancer, and all other subjects. Fisher's exact test was used to determine whether the proportion of subjects in each phenotype group differed by P/LP variant type (LOF, DN, OM, and MISC). All analyses were conducted with R v.3.3.3.

RESULTS

Cohort definition

Of the 468 subjects with heterozygous *TP53*+ results from panel testing, 141 subjects (30%) were excluded from genotype–phenotype analysis based on a *TP53* mosaic result defined as either allelic frequency <25% on NGS and/or clinical history or molecular results suspicious for somatic interference. Additional exclusions were as follows: 5 (1.07%) had a known *TP53*+ result prior to MGPT, 4 (0.85%) had an unknown personal cancer history, 1 (0.21%) had two *TP53* P/LP variants and whether they were in *cis* or *trans* was unknown. Thus, there were 317 subjects with *TP53*+ results with evaluable clinical and genetic information who were ascertained from panel testing.

TP53+ subjects with a second positive result (P/LP variant) in another cancer predisposition gene were included in the analysis ($n = 21$, 6.6%): *ATM* (4.8%), *BRCA1* (14.3%), *BRCA2* (4.8%), *BRIP1* (4.8%), *CHEK2* (9.5%), *MRE11A* (4.8%), *MSH2* (4.8%), *MSH6* (4.8%), *PALB2* (4.8%), *PMS2* (9.5%). Known low penetrance risk alleles were present in seven subjects (33.3%): four *MUTYH* heterozygotes and three *APC*, p.I1307K carriers (Supplemental Table 2). Sensitivity analysis was performed on all significant outcomes including and excluding these subjects and the results were similar (data not shown).

Demographics

Of 317 phenotype-evaluable *TP53*+ subjects, 279 (88%) were female and 38 (12%) were male (Table 1). The *TP53*+ subjects were of the following ancestries: Caucasian (64.4%), Hispanic (8.5%), African American/Black (7.6%), Asian (7.3%), and Other (12.3%). Most subjects (90.9%) had a personal history of cancer. Multiple primary tumors were noted in 89 subjects (28.1%). The median ages (IQR, range) at first cancer diagnosis and genetic testing were 36 (19, 1–77) years and 45 (22, 5–90) years respectively. Only five of the *TP53*+ subjects (1.5%) were under age 18 at the time of testing. While 214 female subjects (67.5%) had breast cancer, it was the first malignancy in 181 female subjects (57.1%). None of the 38 males were known to have breast cancer.

Table 1 *TP53*+ subject demographics

Variable	All (%)	Loss-of-function (%)	DN missense (%)	Other missense (%)	Miscellaneous[b] (%)	*P* value[c]
TP53 P/LP variant category, *n* (%)	317 (100)	57 (100)	165 (100)	75 (100)	20 (100)	
Sex						0.497
Female	279 (88.0)	49 (86.0)	148 (89.7)	66 (88.0)	16 (80.0)	
Male	38 (12.0)	8 (14.0)	17 (10.3)	9 (12.0)	4 (20.0)	
Race/ethnicity						0.246
Caucasian	204 (64.4)	29 (50.9)	111 (67.3)	50 (66.7)	14 (70.0)	
African American	24 (7.6)	6 (10.5)	11 (6.7)	6 (8.0)	1 (5.0)	
Asian	23 (7.3)	8 (14.0)	10 (6.1)	3 (4.0)	2 (10.0)	
Hispanic	27 (8.5)	4 (7.0)	11 (6.7)	10 (13.3)	2 (10.0)	
Multiple/other/unknown	39 (12.3)	10 (17.5)	22 (13.3)	6 (8.0)	1 (5.0)	
Personal history of cancer	288 (90.9)	54 (94.7)	150 (90.9)	67 (89.3)	17 (85.0)	0.514
Median (IQR) age at 1st cancer	36 (19)	30.5 (16.3)	37 (19.8)	39 (16.5)	38 (21.0)	**0.014**
Median (IQR) age at testing	45 (22.0)	36 (24.0)	47 (22.0)	46 (23.0)	46.5 (21.3)	**0.008**
Meet classic LFS criteria	6 (1.9)	5 (8.8)	1 (0.6)	0 (0.0)	0 (0.0)	**0.004**
Meet Chompret 2015 criteria	109 (34.4)	32 (56.1)	53 (32.1)	19 (25.3)	5 (25.0)	**0.002**
LFS core cancer[a] w/o BC	52 (16.4)	17 (29.8)	22 (13.3)	10 (13.3)	3 (15.0)	**0.043**
LFS core cancer[a] with BC	238 (75.1)	47 (82.5)	124 (75.2)	55 (73.3)	12 (60.0)	0.237
Multiple primaries	89 (28.1)	19 (33.3)	45 (27.3)	20 (26.7)	5 (25.0)	0.805

BC breast cancer, *DN* dominant-negative, *IQR* interquartile range, *LFS* Li–Fraumeni syndrome, *P/LP* pathogenic/likely pathogenic.
Bold *P* values indicate statistically significant differences.
[a]LFS core cancer: sarcoma, brain cancer or tumor, and adrenal cancer; this was not able to be determined for two subjects in the w/o BC category and one subject in the with BC category.
[b]Splice, indel, in-frame deletions.
[c]Derived from Fisher's exact test for differences in categorical variables, or Kruskal–Wallis test for differences in median age among groups.

P/LP variant spectrum

Among the 317 subjects, there were 133 distinct *TP53* P/LP variants identified, of which 95 (71.4%) appeared in this cohort only once. All P/LP variants (Supplemental Table 3) were uploaded to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). The frequencies of each P/LP variant in this cohort, in the IARC *TP53* database, and in gnomAD are provided, but due to small numbers and differences in ascertainment, no direct comparisons were made. The distribution of variant types was as follows in decreasing frequency: 165 dominant-negative missense, 75 other missense, 57 LOF (14 genomic rearrangements, 22 nonsense, 21 frameshifts), and 20 miscellaneous variants (17 splice, 1 indel, and 2 in-frame deletions) (Table 1).

Cancer phenotype by variant category

The median age at first cancer diagnosis and age at testing significantly differed by P/LP variant type, with LOF carriers having the earliest age at first cancer diagnosis, median 30.5 (16.25) years; $P = 0.014$, and in turn the earliest age at testing, median 36 (24) years; $P = 0.008$. Median age at first cancer diagnosis of LOF carriers was 6–8 years earlier, and median age at testing was 10 years earlier than respective median ages for carriers of non-LOF variant types. LOF carriers were also significantly more likely than non-LOF carriers to meet classic LFS (8.8%; $P = 0.004$) or Chompret 2015 (56.1%; $P = 0.002$) testing criteria and to have an LFS core cancer without breast cancer (29.8%; $P = 0.043$). P/LP variant category was not associated with the development of multiple primaries.

Sarcoma was significantly more common in subjects with LOF variants (28.1%, $P = 0.016$) than in subjects with other variant types (Table 2, Fig. 1). There were no significant differences in the prevalence of many cancers by P/LP variant category including other classically associated LFS cancers (adrenal, brain, breast, leukemia), melanoma, and cancers of the ovary, pancreas, prostate, thyroid, and uterus. The prevalence of cancers of the biliary tract and colorectum also did not differ by variant category, but gastric cancer was associated with LOF P/LP variants ($P = 0.01$). Four of five subjects with gastric cancer had additional malignancies.

Within specific cancers, variant category was generally not associated with median age at diagnosis, although the variant-specific sample size was too small to assess significance for most cancer types. The median (IQR) age of breast cancer diagnosis overall was 38 (17) years. While breast cancer median (IQR) age was earliest in LOF subjects (33 [15] years), this was only marginally lower than the median age at breast cancer diagnosis in other P/LP variant types ($p = 0.07$). When limiting analysis to breast cancer as the first cancer diagnosis and stratifying by age at diagnosis at 35 years, LOF variant carriers were more often diagnosed at ≤35 years compared with non-LOF carriers ($p = 0.042$; Table 2).

Cancer phenotypes by P/LP variant category were evaluated in aggregate, mutually exclusive groupings (Table 3): breast cancer only, sarcoma regardless of another cancer, and not otherwise specified (NOS). The NOS category contained 29 (23%) subjects with no personal history of cancer, 41 (32.5%) with breast and an additional cancer exclusive of sarcoma, 9

Table 2 Cancer type by pathogenic/likely pathogenic variant category

Variable	All *n* = 317 (%)	Loss-of-function *n* = 57 (%)	DN missense *n* = 165 (%)	Other missense *n* = 75 (%)	Miscellaneous *n* = 20 (%)	*P* value[a]
Adrenal cancer	3 (0.9)	1 (1.8)	0 (0.0)	2 (2.7)	0 (0.0)	0.113
Biliary tract cancer	2 (0.6)	0 (0.0)	2 (1.2)	0 (0.0)	0 (0.0)	1.000
Brain cancer or tumor	8 (2.5)	2 (3.5)	4 (2.4)	2 (2.7)	0 (0.0)	0.934
Colorectal cancer	21 (6.6)	4 (7.0)	10 (6.1)	6 (8.0)	1 (5.0)	0.929
Gastric cancer	5 (1.6)	3 (5.3)	0 (0.0)	1 (1.3)	1 (5.0)	**0.010**
Kidney cancer	6 (1.9)	0 (0.0)	2 (1.2)	3 (4.0)	1 (5.0)	0.170
Leukemia	7 (2.2)	1 (1.8)	2 (1.2)	3 (4.0)	1 (5.0)	0.263
Lung	9 (2.8)	0 (0.0)	5 (3.0)	3 (4.0)	1 (5.0)	0.367
Melanoma	8 (2.5)	0 (0.0)	5 (3.0)	3 (4.0)	0 (0.0)	0.505
Pancreatic cancer	10 (3.2)	0 (0.0)	8 (4.8)	1 (1.3)	1 (5.0)	0.191
Sarcoma	45 (14.2)	16 (28.1)	19 (11.5)	7 (9.3)	3 (15.0)	**0.016**
Thyroid cancer	7 (2.2)	1 (1.8)	4 (2.4)	2 (2.7)	0 (0.0)	1.000
Breast cancer	214 (67.5)	40 (70.2)	115 (69.7)	49 (65.3)	10 (50.0)	0.310
As first primary	176 (55.5)	33 (57.9)	94 (57.0)	41 (54.7)	8 (40.0)	0.600
Diagnosed <35 years	82 (25.9)	22 (38.6)	43 (26.1)	14 (18.7)	3 (15.0)	**0.042**[b]
Diagnosed ≥35 years	94 (29.7)	11 (19.3)	51 (30.9)	27 (36.0)	5 (25.0)	
Female-specific cancers	***n* = 279 (%)**	***n* = 49 (%)**	***n* = 148 (%)**	***n* = 66 (%)**	***n* = 16 (%)**	
Ovarian cancer	24 (8.6)	4 (8.2)	12 (8.1)	5 (7.6)	3 (18.8)	0.479
Uterine cancer	7 (2.5)	1 (2.0)	4 (2.7)	2 (3.0)	0 (0.0)	1.000
Male-specific cancer	***n* = 38 (%)**	***n* = 8 (%)**	***n* = 17 (%)**	***n* = 9 (%)**	***n* = 4 (%)**	
Prostate cancer	4 (10.5)	0 (0.0)	3 (17.6)	1 (11.1)	0 (0.0)	0.867

DN dominant-negative.
Bold *P* values indicate statistically significant differences.
[a]*P* value derived from Fisher's exact test. Percentages in parentheses are by column.
[b]*P* value for "Diagnosed <35 years" variable is based on percentages of patients with breast cancer as first primary (*n* = 176).

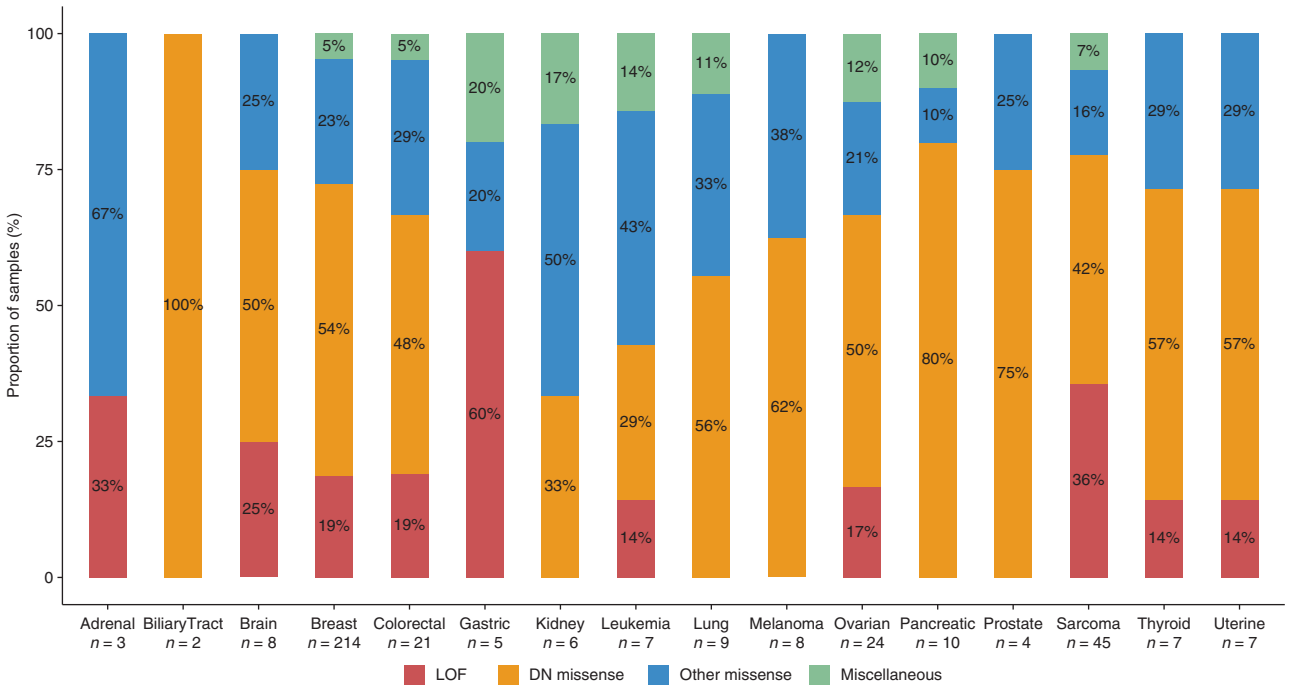

Fig. 1 Cancer type by variant category frequencies. *DN* dominant-negative, *LOF* loss-of-function.

Table 3 Pathogenic/likely pathogenic variant category by aggregate, mutually exclusive, cancer phenotype

Cancer phenotype	Loss-of-function $n = 57$	DN missense $n = 165$	Other missense $n = 75$	Miscellaneous $n = 20$	*P* value[d]
Breast cancer only[a] $n = 146$ (%)	25 (17.1)	76 (52.1)	38 (26.0)	7 (4.8)	
Sarcoma with or without other cancer diagnosis[b] $n = 45$ (%)	16 (35.6)	19 (42.2)	7 (15.6)	3 (6.7)	
NOS[c] $n = 126$ (%)	16 (12.7)	70 (55.6)	30 (23.8)	10 (7.9)	
					0.058

DN dominant-negative, *NOS* not otherwise specified.
[a]Patients with any number of primary breast cancers and no other cancer.
[b]Patients with sarcoma regardless of other cancer status.
[c]No history of breast cancer only or of any sarcoma.
[d]Fisher's exact test; % reflects row percents.

(7.1%) with ovarian cancer alone, 9 (7.1%) with colorectal cancer alone, and 38 (30.2%) with unique combinations of one or more other cancer phenotypes. Of 146 subjects with a breast cancer only, 76 (52.1%) had dominant-negative missense P/LP variants, 38 (26%) had other missense P/LP variants, 25 (17.1%) had LOF P/LP variants, and 7 (4.8%) had miscellaneous P/LP variants (Table 3). P/LP variant category was not significantly associated with the aggregate cancer phenotype ($p = 0.058$). However, dominant-negative missense P/LP variants accounted for 52.1% of the cases with breast cancer only and were followed distantly by the other P/LP variant categories: other missense variants (26%), LOF (17.1%), and miscellaneous (4.8%).

Recurrent P/LP variants

There were 24 P/LP variants identified four or more times in this cohort, accounting for 191 subjects. We also report the above aggregate, mutually exclusive cancer phenotype for these specific P/LP variants (Supplemental Table 4). The two most common recurrent P/LP variants, c.467G>A (p.R156H) and c.743G>A (p.R248Q), were each identified 17 times and there were not specific patterns of cancers associated with them. The c.473G>A (p.R158H) variant was identified 6 times, all of which were in breast cancer only cases. Except for c.1010G>A (p.R337H), none of the recurrent P/LP variants were in the *TP53* oligomerization domain. The whole-gene deletion, 5′UTR_3′UTRdel, was not identified among subjects with a personal history of breast cancer only, but instead was present in subjects with sarcoma (with or without other cancers).

DISCUSSION

Our data demonstrate considerable phenotypic heterogeneity among *TP53*+ subjects ascertained through panel testing. Compared with other P/LP variant categories, LOF variants were associated with the earliest ages of cancer diagnosis overall, and with sarcoma. Despite this, the P/LP variant category was not associated with age of onset within each cancer type. Thus, the P/LP variant category is not yet a consistent means to distinguish *TP53*+ carriers with more classic, high penetrance LFS from the less striking phenotype, which is critical for genetic counseling purposes.

The association of LOF variants with the earliest-onset cancers is consistent with findings from a previous US-based cohort ascertained through childhood cancers.[18] An IARC-based analysis also found that missense *TP53* P/LP variants representing partial deficiency transactivation alleles were associated with a milder phenotype of LFS compared with loss-of-function variants, which ostensibly represent severe deficiency due to protein truncation.[16,28] These results differ from findings among the French LFS cohort, in which DN missense variants in which the mutant transcript interferes with the transcriptional activity of the wild-type p53 were associated with earlier onset of malignancies.[4,29]

We note the age of onset of breast cancer (38 years) in this cohort is older than typically associated with Li–Fraumeni syndrome. While two-thirds of LOF variant carriers diagnosed with breast cancer as their first cancer were diagnosed under age 35, non-LOF P/LP variants were less frequently associated with a breast cancer diagnosis below age 35 years. Our recent publication found that the median age of breast cancer in *TP53*+ carriers from panel testing was 40 years and 22% of *TP53*-associated breast cancers were diagnosed after age 45 years.[5] These findings are remarkably consistent with findings from two adult cancer genetics services in Ireland, in which 18% of the breast cancers among *TP53*+ women were diagnosed after 49 years.[12] Together these data suggest that re-evaluation of age-specific penetrance of breast cancer in *TP53*+ carriers is required as is evaluation of the role of family history, hormones, and polygenic risk factors.

The incidence of ovarian cancer among *TP53*+ subjects has been poorly quantified due to the rarity of this syndrome and likely also due to competing comorbidities. While the frequency of ovarian cancer among this cohort (8.6%) is appreciably higher than in another reported *TP53*+ cohort (1%), this likely reflects the differences in ascertainment of each cohort.[4] Nonetheless, this bears investigation in other *TP53*+ cohorts.

Our findings must be interpreted in the context of the study constraints. Phenotype analysis was limited to the subject's cancer phenotype. Thus genotype–phenotype correlations did not account for family cancer history. It was prudent to limit our analysis to the subject's phenotype because this is where the most detailed, high-quality phenotypic information is

available.[30] We have previously demonstrated that among LFS families, cancers are underreported and misreported owing to the rare cancer types in the syndrome.[31] Our data are from mostly an adult, cancer-affected population, half of whom were women diagnosed with breast cancer as their first malignancy, which is reflective of the majority of probands referred for cancer genetic testing. Until recently, children suspected to have LFS were not tested with multigene panels, but rather through *TP53* single-gene testing;[32–34] thus it is possible that severe genotypes are underrepresented or not present in this cohort due to testing practices as well as survivor effects. The disproportionate cancer genetic testing of adult women with breast and/or ovarian cancer, while guideline-based, limits the generalizability of our findings.[19] While exclusions were made to account for cases with somatic interference or clonal hematopoiesis of indeterminate potential, we could not systematically exclude it through tissue analysis in all cases. Finally, we note that the variant categories (LOF, DN missense, other missense, and miscellaneous) provide a framework for analyzing the detected variation in *TP53*. However, these categories do not represent absolutes as some LOF variants may retain expression of forms of p53 and some missense variants maintain partially functional transactivation.

There were too few P/LP variants identified in the oligomerization domain (OD), which affect the tetramerization of p53, to perform genotype assessment by oligomeric status of the resultant p53 proteins. Interestingly, colleagues recently demonstrated that carriers with mutant multimeric p53 had significantly favorable survival compared with carriers of OD variant resulting in monomeric p53.[15] Monomeric p53 has been associated with the greatest reduction in p53 transcriptional activity.[15] This study highlights the importance of residue- and allele-specific functional analysis to inform the effects of genotype on phenotype.

One recent study of "hotspot" *TP53* somatic variants suggests that cancer cell lines with these variants did not demonstrate *TP53* dependency and may occur for other reasons including spontaneous deamination of the cytosine residue.[35] Likewise, a new American College of Medical Genetics and Genomics (ACMG)–approved in silico prediction tool for analysis of *TP53* missense variants may help to reconcile the challenges associated with *TP53* variants of uncertain significance.[14]

The extent to which *TP53*+ individuals identified through panel testing or their families face classic LFS cancer risks remains unknown. The challenge of counseling and managing cancer risk in nonclassic, *forme fruste* families found with *TP53*+ genetic test results is likely to increase. Careful characterization and prospective follow-up of panel-ascertained *TP53*+ carriers and their families are required to answer the most important questions. In the interim, there are measures that may help in evaluating the full spectrum of a molecular *TP53* diagnosis. Ideally, screening studies would have sufficient power to account for family history of LFS (classic vs. nonclassic) in assessing outcomes of surveillance protocols until a more personalized approach becomes available, taking into account modifier genes and other molecular markers of LFS severity.[36] Case-level de-identified data sharing of *TP53*+ subjects with long-term follow-up to measure outcomes, and incidence of interference from mosaicism or CHIP, will be necessary as germline *TP53*+ results continue to be a rare finding, although with urgent clinical implications.

It seems prudent that counseling of *TP53*+ subjects reflect the breadth of observed phenotypes and published risk estimates be contextualized with the bias of years of limiting testing to classic and other highly suggestive LFS families. Updated accurate cancer risk estimates will require long-term, multigenerational, prospective data collection on kindreds ascertained through panel testing. In the absence of validated individualized cancer risk data, clinical management of all germline *TP53*+ subjects should utilize currently recommended multifaceted protocols such as those published by NCCN or the American Association for Cancer Research (AACR).[19,37,38]

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-019-0541-y) contains supplementary material, which is available to authorized users.

DISCLOSURE

J.C., L.H., H.L., M.H.B., S.L., K.M., V.S., J.S.D., and C.-L.G. are employees of Ambry Genetics, Inc. J.E.G. has received research support from Myriad Genetics and consulting fees from Helix; her spouse has received research support and consulting fees from Novartis Oncology, and consulting fees from GTx. H.Q.R. declares no conflicts of interest.

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