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Brief Communications

Cancer risk in carriers of *TP53* germline variants grouped into different functional categories

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Abstract

Li-Fraumeni syndrome is a cancer predisposition syndrome caused by pathogenic *TP53* germline variants; it is associated with a high lifelong cancer risk. We analyzed the German Li-Fraumeni syndrome registry, which contains data on 304 individuals. Cancer phenotypes were correlated with variants grouped according to their ability to transactivate target genes in a yeast assay using a traditional (nonfunctional, partially functional) and a novel (clusters A, B, and C) classification of variants into different groups. Partially functional and cluster B or C variants were enriched in patients who did not meet clinical testing criteria. Time to first malignancy was longer in carriers of partially functional variants (hazard ratio = 0.38, 95% CI = 0.22 to 0.66). Variants grouped within cluster B (hazard ratio = 0.45, 95% CI = 0.28 to 0.71) or C (hazard ratio = 0.34, 95% CI = 0.19 to 0.62) were associated with later cancer onset than NULL variants. These findings can be used to risk-stratify patients and inform care.

Li-Fraumeni syndrome (Online Mendelian Inheritance in Man MIM No. 151623) is an autosomal dominant cancer predisposition syndrome caused by germline or postzygotic somatic mosaic pathogenic variants in *TP53*.[1-5] The gene product TP53 acts as a transcription factor and controls cell cycle, apoptosis, senescence, stem cell differentiation, metabolism, reactive oxygen species generation, mitochondrial function, and DNA repair.[6] Li-Fraumeni syndrome is associated with the "Li-Fraumeni syndrome core cancers"—namely, brain tumors, adrenocortical carcinoma, soft tissue sarcoma, osteosarcoma, and breast cancer. Also, carriers of *TP53* pathogenic variants are at increased risk of hematologic, gastrointestinal, skin, lung, prostate, pancreatic, and other cancer types. Following the discovery of the Li-Fraumeni syndrome–causative gene *TP53*, genetic testing criteria, called *Chompret criteria*, have been continuously improved.[7,8] With the widespread use of clinical panel sequencing, *TP53* pathogenic variants are identified among individuals who did not meet Chompret criteria.[9] This observation led to a Li-Fraumeni spectrum classification that distinguishes Li-Fraumeni syndrome, defined by the presence of a *TP53* pathogenic variant in a person who meets Li-Fraumeni syndrome testing criteria, from attenuated Li-Fraumeni syndrome, defined by the presence of a *TP53* pathogenic variant in a person who does not meet such criteria.[10]

Kato and colleagues[11] used site-directed mutagenesis and a yeast-based functional assay to analyze how *TP53* missense pathogenic variants disrupt the ability of TP53 to transactivate downstream target genes. Traditionally, variants were grouped into 1 of 4 categories: functional, partially functional, nonfunctional,[11] and NULL variants (defined here as variants predicted to produce no protein product). A recently published, more granular approach, however, groups variants into deviating functional classes 0 (NULL variants), A, B, C, and D.[12]

We recently conducted genotype-phenotype correlations in a cohort of 141 individuals from 94 families enrolled in the German Li-Fraumeni syndrome registry.[13] There was a statistically significant association between NULL variants and Li-Fraumeni syndrome but not with attenuated Li-Fraumeni syndrome. In addition, there was a statistically significant association between partially functional variants and fewer childhood cancers, except adrenocortical carcinoma.[13] The same cohort was included in the cluster approach mentioned above, which established that patients with *TP53* pathogenic variants grouped into classes A or 0 had earlier occurrence of cancer than carriers of pathogenic

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variants grouped into class B (intermediate) or C.[12] Here, we present data from a follow-up analysis of the German Li-Fraumeni syndrome registry, which includes data from 304 individuals from 212 families. We show that the traditional and the cluster approaches stratify patients into different cancer risk groups and propose that this information can be used to inform clinical decisions.

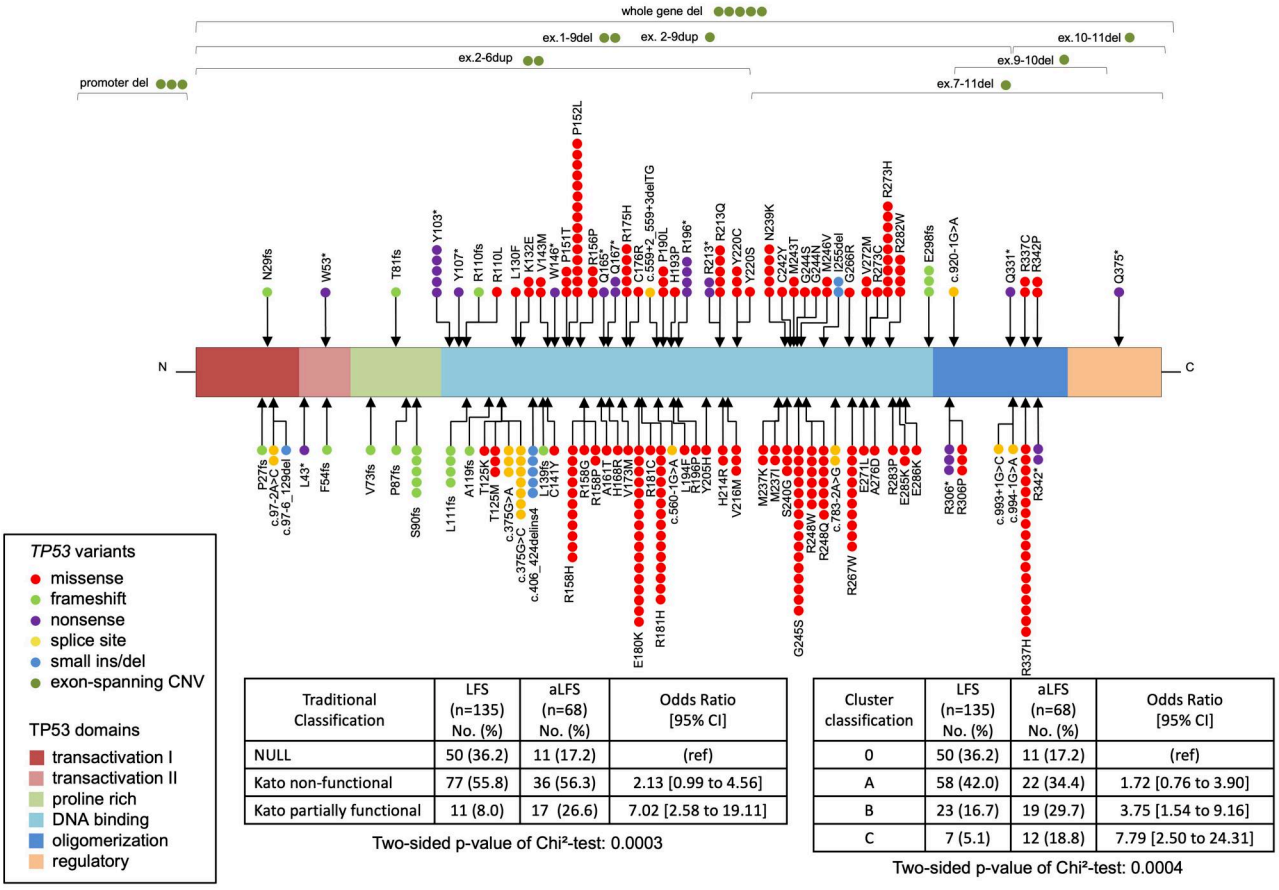
The institutional review board–approved German Cancer Predisposition Syndrome Registry (DRKS00017382) has collected information about genotypes, details on cancer diagnoses, family histories, and surveillance since August 2017. Informed consent was obtained. The cutoff date for study inclusion for the present data analysis was April 15, 2024. All variants were curated according to *TP53*-specific guidelines, and only patients with likely pathogenic variants or pathogenic variants were enrolled.[14] The Li-Fraumeni spectrum classification was assessed.[10] We used functional data from Kato that we retrieved from the *TP53* database.[11,15] The cluster classification was employed as recently described.[12] Differences between patients with Li-Fraumeni syndrome and patients with attenuated Li-Fraumeni syndrome with respect to genotype group were analyzed statistically using 2-sided χ^2-tests. Odds ratios and respective 95% confidence intervals were reported against the reference group (NULL/0). Time to malignancy for all patients was depicted using Kaplan-Meier curves, stratified by genotype group. Furthermore, hazard ratios and respective 95% confidence intervals were estimated for the genotype group by a Cox regression model. Kaplan-Meier curves were created in R, version 4.4.1, software (using the *survminer* package; R Foundation for Statistical Computing). All other statistical analyses were performed using SAS, version 9.4, software. A *P*-value below .05 was considered statistically significant.

Demographic characteristics are shown in Table S1. The cohort comprised 304 individuals from 212 families. Variants observed in this cohort are depicted in Figure 1. Specifics on individual variants, functional data, and cancer phenotypes are provided in Table S2. There were statistically significant differences in the variant spectrum in patients with Li-Fraumeni syndrome vs attenuated Li-Fraumeni syndrome. Specifically, partially functional variants and variants belonging to cluster B or C were associated with attenuated Li-Fraumeni syndrome. In contrast, NULL (cluster 0) variants and variants belonging to cluster A were associated with Li-Fraumeni syndrome (Figure 1). Kaplan-Meier and Cox regression analyses of the time to first malignancy in patients within different functional variant subgroups also showed statistically significant differences (Figure 2). Using the traditional classification, partially functional variants had the latest onset of cancer. Using the more granular cluster approach, patients with variants grouped into cluster A or 0 had the earliest cancer diagnoses, and patients with variants grouped into cluster C had the latest cancer diagnoses. Carriers of cluster B variants had an intermediate cancer phenotype.

In summary, we analyzed a cohort of 304 individuals from 212 families with Li-Fraumeni syndrome enrolled in the German Li-Fraumeni syndrome registry and correlated cancer phenotypes with the underlying variants grouped into different functional groups. We found that carriers of partially functional variants as well as carriers of cluster B and C variants had less severe cancer phenotypes than carriers of variants belonging to other functional groups. We also found that except for childhood adrenocortical carcinoma, childhood cancer was rare among carriers of partially functional or cluster C variants. These results are in agreement with our previous analysis of a smaller cohort of patients with Li-Fraumeni syndrome showing that partially functional variants were associated with fewer childhood cancers other than adrenocortical carcinoma.[13] Notably, one of the recurrent partially functional and cluster C variants studied in our analysis was the Brazilian founder variant p.(Arg337His). This variant has a prevalence of approximately 0.27% in southeastern Brazil. A newborn screening study from Brazil showed that this variant was associated with childhood adrenocortical carcinoma and, more rarely, other childhood cancers, but the penetrance during the pediatric age range was modest. Approximately 2.4% of carriers developed childhood adrenocortical carcinoma, and the incidence of other childhood cancers was substantially lower.[16] Notably, adults with this variant frequently developed breast and a range of other cancer types.[16] A recent genome-first study of the prevalence and penetrance of pathogenic or likely pathogenic variants in *TP53* showed that another partially functional and cluster C variant, p.(Arg181His), which was also detected in cancer-free adults, was the most commonly observed variant in the studied population and that penetrance was reduced for this variant.[17] Taken together, our findings support the hypothesis that in carriers of partially functional or cluster C variants, cancer is rare during the first 2 decades of life, with the exception of childhood adrenocortical carcinoma.

Our study has several limitations. First, we present data on 304 patients only; however, given that Li-Fraumeni syndrome is a rare condition, this represents a relatively large cohort. Second, the patients recruited into the study were ascertained phenotypically, but risk estimates conducted in genomically ascertained cohorts are more accurate.[17] Given that this bias affects all studied pathogenic variant functional groups, the observed penetrance differences are unlikely to be affected by this limitation. Third, our analysis focused on 1 out of several functional assays[11,18-20] and did not account for other epigenetic, genetic, immunologic, or lifestyle factors that may influence the individual cancer risk. Notably, a modifier variant in the nearby gene, *XAF1* p.(Glu134Ter), leads to a more severe cancer phenotype in *TP53* p.(Arg337His) pathogenic variant carriers,[21] but the haplotype harboring this *XAF1* variant is rare among *TP53* p.(Arg337His) pathogenic variant carriers in Germany.[22] Therefore, this modifier gene is unlikely to have influenced the results of our study.

Despite several limitations, the results presented here provide further evidence that information about the ability of TP53 mutant proteins to transactivate target genes in yeast[11] can be used to predict the clinical severity in carriers of germline *TP53* pathogenic variants. Notably, the same yeast assay[11] was used for *TP53* variant curation according to published recommendations,[14] and only patients with pathogenic or likely pathogenic variants, who tend to have mutant proteins with a stronger impairment of the transcriptional activity, were included in this study. We anticipate that the results will inform future cancer prevention and surveillance recommendations among individuals with Li-Fraumeni syndrome. For example, whole-body magnetic resonance imaging may not be required before age 18 years in carriers of group C pathogenic variants. Instead, it may be sufficient to screen for adrenocortical carcinoma in these children. Later in life, however, the full surveillance program may be necessary. The described classification provides a guide but should not be taken as an absolute prediction. The approach taken here expands our understanding of cancer risk prediction based on functional information and should be useful to clinicians working with individuals who have Li-Fraumeni syndrome. Similar



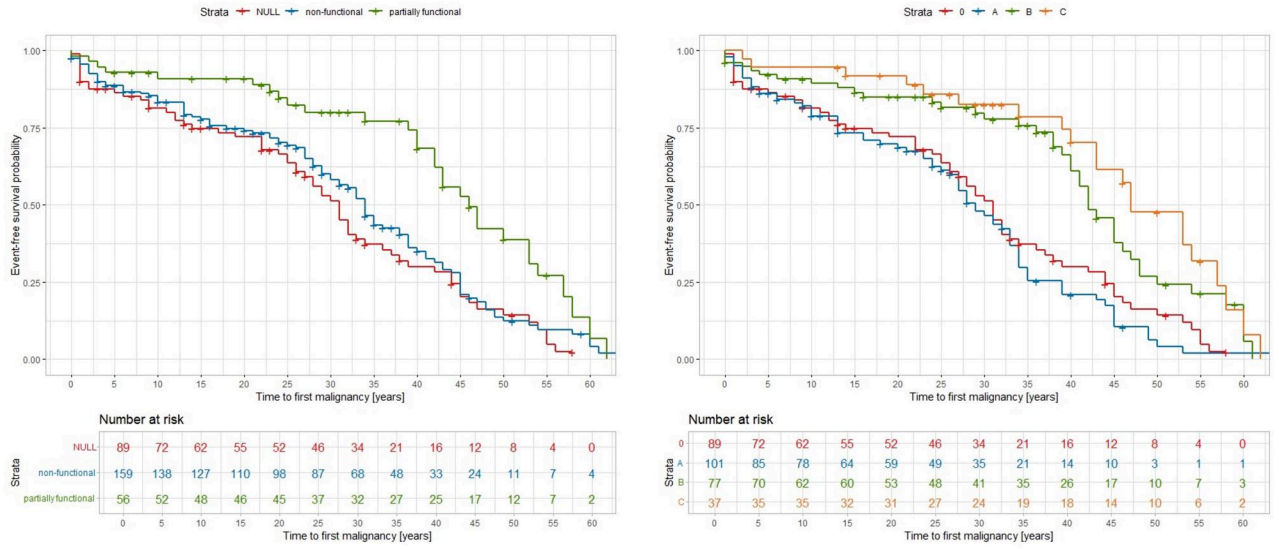
Traditional Classification	LFS (n=135) No. (%)	aLFS (n=68) No. (%)	Odds Ratio [95% CI]
NULL	50 (36.2)	11 (17.2)	(ref)
Kato non-functional	77 (55.8)	36 (56.3)	2.13 [0.99 to 4.56]
Kato partially functional	11 (8.0)	17 (26.6)	7.02 [2.58 to 19.11]

Two-sided p-value of Chi²-test: 0.0003

Cluster classification	LFS (n=135) No. (%)	aLFS (n=68) No. (%)	Odds Ratio [95% CI]
0	50 (36.2)	11 (17.2)	(ref)
A	58 (42.0)	22 (34.4)	1.72 [0.76 to 3.90]
B	23 (16.7)	19 (29.7)	3.75 [1.54 to 9.16]
C	7 (5.1)	12 (18.8)	7.79 [2.50 to 24.31]

Two-sided p-value of Chi²-test: 0.0004

Figure 1. Spectrum of *TP53* variants and statistical genotype-phenotype correlations (updated version of a previous analysis that included 141 patients[13]). Variant nomenclature refers to reference sequence NM_000546.5. Colored spheres refer to different patients harboring the corresponding variant. The genotype-phenotype correlation was based on assessed Li-Fraumeni spectrum status of individual families and not individual probands.[10] Abbreviations: aLFS = attenuated Li-Fraumeni syndrome; LFS = Li-Fraumeni syndrome.



Traditional Classification	Hazard Ratio [95% CI]	p-value
NULL	(ref)	
Kato non-functional	0.83 [0.57 to 1.22]	0.3490
Kato partially functional	0.38 [0.22 to 0.66]	0.0005

Cluster Classification	Hazard Ratio [95% CI]	p-value
0	(ref)	
A	1.20 [0.81 to 1.78]	0.3643
B	0.45 [0.28 to 0.71]	0.0007
C	0.34 [0.19 to 0.62]	0.0004

Figure 2. Kaplan-Meier analyses of time to first malignancy in patients stratified according to the results of a yeast assay that measures transactivation of target genes.[11] Left: Traditional classification.[11] Right: Cluster classification.[12]

approaches could be applied to other cancer predisposition syndromes.

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Author contributions

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Supplementary material

Supplementary material is available at *JNCI Cancer Spectrum* online.

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Conflicts of interest

None declared.

Data availability

All data used for the analysis is available online (Table S2).

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