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Genotype–Phenotype Correlations of Li–Fraumeni Syndrome in Japan Children's Cancer Group LFS20 Study Cohort

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ABSTRACT

Li–Fraumeni syndrome (LFS) is a cancer predisposition syndrome caused by germline pathogenic variants in the *TP53* gene. With the increasing use of multi-gene panel testing, *TP53* variants have been identified in individuals who do not meet established *TP53* testing criteria, such as the Chompret criteria. The term "attenuated LFS" has been proposed for some of these cases, particularly those with adult-onset cancer. We analyzed participants of the Japanese nationwide prospective clinical trial of the cancer surveillance program (Japan Children's Cancer Group LFS-20), along with clinical information including their family histories, to better understand their genotypic and phenotypic characteristics. We identified 32 distinct *TP53* variants from 41 families (45 participants), including four missense variants with conflicting classifications of pathogenicity in ClinVar. Among these families, 36 (88%) met the LFS criteria (hereafter referred to as "LFS" in contrast to attenuated LFS), while 5 (12%) were classified as attenuated LFS. Including 30 additional family members carrying the same variant, we analyzed 75 individuals with *TP53* variants. Of these, 40 with LFS and 6 with attenuated LFS had cancer. Multiple primary cancers occurred in 22 individuals (21 LFS, 1 attenuated LFS). LFS-core tumors accounted for 66% (58/88) of cancers in the LFS group and 63% (5/8) in the attenuated LFS group; of note, all core tumors in the attenuated group were limited to breast cancer. Hotspot missense variants were detected in 11 of 36 LFS families and in none of 5 attenuated LFS families, and non-hotspot null variants were found in 14 and 1, respectively. Our study revealed genotype–phenotype correlations in several respects. UMIN-CTR: UMIN000045855.

Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics and Association for Molecular Pathology; gnomAD, Genome Aggregation Database; JCCG, Japan Children's Cancer Group; LFS, Li–Fraumeni syndrome; MRI, magnetic resonance imaging; PV, pathogenic or likely pathogenic variant.

1 | Introduction

Li–Fraumeni syndrome (LFS; OMIM151623) is an autosomal dominant cancer predisposition syndrome caused by germline (or mosaic) pathogenic or likely pathogenic variants (PVs) in the *TP53* tumor suppressor gene. LFS has traditionally been defined as a specific condition associated with early-onset, multiple primary tumors, with a particular propensity for LFS-core tumors (adrenocortical carcinomas, breast cancer, central nervous system tumors, osteosarcomas, and soft-tissue sarcomas) [1, 2]. Multiple criteria were developed to diagnose clinical LFS and to determine individuals who should undergo *TP53* genetic testing, with the Chompret criteria being one of the most widely used for the latter purpose [2–5]. However, the increasing use of multigene panel testing led to the identification of germline *TP53* PVs in individuals who do not meet existing criteria for LFS [6].

This phenomenon, namely the detection of germline *TP53* PVs in individuals outside existing criteria for LFS, represents part of the considerable phenotypic heterogeneity of LFS. Such heterogeneity is influenced not only by *TP53* genotype but also by additional modifiers, including *MDM2* SNP309, *TP53* codon 72, miR-605, and broader genomic events such as telomere attrition and methylation, all of which may modulate cancer risk and age of onset [7]. To address these complexities, and to systematically investigate the contribution of TP53 genotype to phenotypic heterogeneity, a Li–Fraumeni spectrum classification was proposed, which includes: (1) phenotypic LFS, defined by the absence of germline *TP53* PVs in individuals or families meeting classic LFS criteria; (2) LFS, the presence of *TP53* germline PVs in persons/families meeting LFS testing criteria or having pediatric cancer history; (3) attenuated LFS, the presence of *TP53* germline PVs in a person/family with cancer who does not meet LFS testing criteria and lacking pediatric cancer history; and (4) incidental LFS, the presence of germline *TP53* PVs in a person/family without a history of cancer [8]. Patients with attenuated LFS tended to develop tumors at a later age and were less likely to develop some of the LFS-core tumors, such as ACC, than patients with classic LFS. Although the study was conducted using a large cohort from the International Agency for Research on Cancer database, it remains unclear whether these findings are applicable to the Japanese population. Therefore, to better understand the genotype–phenotype correlation and to assess the utility of this classification in patients with LFS in Japan, we analyzed participants and their family members with germline *TP53* PVs enrolled in the Japan Children's Cancer Group (JCCG) LFS20 nationwide surveillance trial, and characterized their genotypic and phenotypic features.

2 | Material and Methods

This study enrolled participants under 40 years of age with confirmed or suspected LFS from 38 JCCG institutions from October 2021 through February 2023. Inclusion criteria included individuals without a personal cancer history, or cancer survivors who were at least 1 year post-treatment completion (except for adjuvant hormone therapy for breast cancer). Individuals requiring sedation or anesthesia for MRI were excluded. This study collected information on genotypes, personal medical details, and family histories. Germline analysis of *TP53* in all participants was performed using Sanger sequencing of all coding exons and intron/exon boundaries and/or multiplex ligation-dependent probe amplification in blood samples, conducted by OVUS Co. Ltd. [9]. Individuals with germline or somatic mosaic *TP53* PVs were eligible to undergo protocol-defined cancer screening.

All variants were interpreted using *TP53*-specific guidelines from the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG/AMP) [10] for all non-CNV variants, while CNVs were classified according to the ACMG CNV criteria [11]. Variant interpretation incorporated functional data from Kato [12], Giacomelli [13], and Kotler [14], population data from the Genome Aggregation Database (gnomAD) [15], hotspot data from Cancerhotspots [16], and in silico functional prediction algorithms of Align-GVGD [17] and BayesDel [18]. TogoVar [19] demonstrated more in-depth information on Japanese population data. PVS1 criteria were determined based on guidance from Tayoun et al. [20]. The *TP53* database [21] was used to collect clinical, functional, and in silico data. Classic LFS criteria [2], Chompret criteria [5] were applied to evaluate previous reports. We integrated functional data, population frequencies, mutational hotspots, in silico predictions, and published literature, and applied weighted *TP53*-specific ACMG/AMP guidelines to ultimately classify the pathogenicity of each variant [10]. ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) variant classifications, as updated on April 12, 2025, were used as a reference in our interpretation. Additionally, all missense variants were assessed with a machine-learning approach named AlphaMissense [22], though these results were not incorporated into the *TP53* ACMG/AMP guidelines.

For the phenotypic evaluation of families carrying pathogenic variants, we applied the Li–Fraumeni spectrum classification [8] and categorized them as either "LFS" or "attenuated LFS." Individuals with pathogenic variants who had not developed cancer were referred to as "LFS carriers" or "attenuated LFS carriers."

We applied the Fisher's exact test to analyze whether the phenotypes LFS versus attenuated LFS were associated with specific genotypic/functional TP53 PV subgroups. A P value of <0.05 was considered statistically significant.

3 | Results

Among the 85 individuals tested in the LFS20 study, 45 were confirmed or found to carry germline *TP53* PVs, and we classified these variants according to the *TP53*-specific ACMG/AMP guidelines [10] and ACMG CNV criteria [11]. Pedigree analysis incorporated 30 family members who were already known to carry PVs, resulting in a total of 75 individuals with germline *TP53* PVs from 41 families (Figure S1). Table S1 shows individual characteristics. In total, 32 distinct PVs were identified across the 41 families (Figure 1, Tables S2 and S3). These included 19 missense, 4 nonsense, 2 frameshift, 2 large deletion, and 5 splicing variants. A case of somatic mosaicism was reported.

Of the 32 PVs, four had not yet been recorded in ClinVar (as of April 12, 2025). They consisted of 1 frameshift, 2 large deletions, and 1 splicing. The 24 variants (15 missense, 4

nonsense, 1 frameshift, and 4 splicing) were pathogenic/likely pathogenic in ClinVar. The remaining four missense variants (NM_000546.6:c.394A>G [p.Lys132Glu], NM_000546.6:c.413C>T[p.Ala138Val],NM_000546.6:c.514G>T [p.Val172Phe], NM_000546.6:c.843C>G [p.Asp281Glu]) were conflicting classifications of pathogenicity. All 32 variants, except one (NM_000546.6:c.1010G>A [p.Arg337His]), were classified as pathogenic in AlphaMissense.

According to the Li–Fraumeni spectrum classification [8], 36 of 41 families (88%) met the "LFS" (in contrast to attenuated LFS) criteria, whereas 5 families (12%) did not and were classified as "attenuated LFS" (Figure 2A). Among the 75 individuals with PVs, 46 had a history of malignancy and were categorized as LFS patients, 17 were LFS carriers (with no cancer history), 6 were attenuated LFS patients, and 6 were attenuated LFS carriers. Multiple primary cancers (ranging from 2 to 7) were reported in 21 (47%) of the LFS patients and in 1 (17%) of 6 attenuated LFS patients. Among individuals who developed cancer, 19 were male and 27 were female (59% female) in the LFS group, with a median age of 30 years. In the attenuated LFS group, 1 male and 5 female (83% female) were affected, with a median age of 40.5 years. A total of 88 tumors in the 46 LFS patients and 8 tumors in 6 attenuated LFS patients were observed

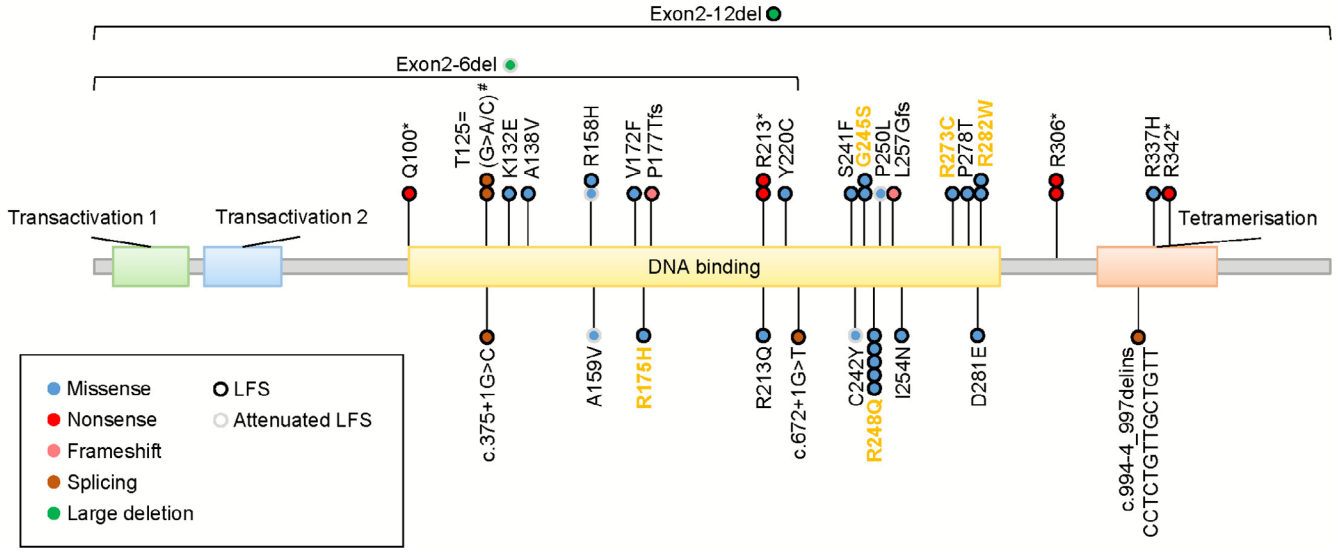

FIGURE 1 | Distribution of *TP53* variants identified in families with LFS and attenuated LFS. Lollipop plot showing the location of 32 distinct germline *TP53* pathogenic or likely pathogenic variants identified across the study population. Variants are mapped along the *TP53* coding sequence, with orange indicating hotspot missense variants. Recurrent variants were observed at several positions, including known hotspots. Variants are annotated according to their protein-level changes. T125 = represents a synonymous variant. Two different variants (c.375G>A and c.375G>C) were observed at this position.

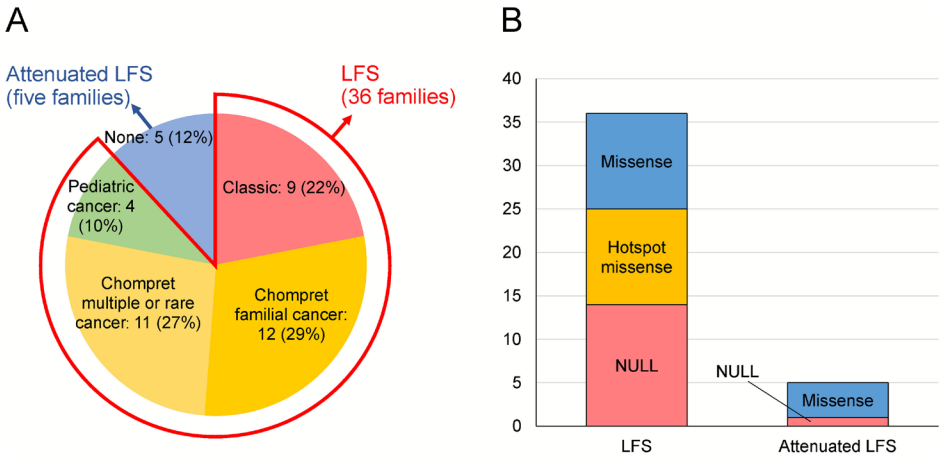

FIGURE 2 | Clinical classification and *TP53* variant types in families with LFS and attenuated LFS. (A) Distribution of clinical diagnostic categories among 41 families with germline *TP53* pathogenic variants. Thirty-six families met the criteria for LFS, while five were classified as attenuated LFS. The pie chart shows mutually exclusive diagnostic categories assigned according to a hierarchical classification: Classic LFS, Chompret criteria (familial or rare/multiple cancers), and pediatric cancer. When a family met multiple criteria, only the highest-priority category was counted (e.g., families fulfilling both classic and Chompret criteria were counted under classic). (B) Distribution of *TP53* variant types in families with LFS and attenuated LFS. Hotspot missense variants were observed only in LFS families. Null variants (nonsense, frameshift, large deletions, or splicing) were more frequent in LFS than in attenuated LFS.

(Table S4). Over half of the tumors were LFS-core tumors in individuals with LFS (58 of 88 tumors: breast cancer 19, non-rhabdomyosarcoma soft tissue sarcoma 10, osteosarcoma 9, adrenocortical carcinoma 8, rhabdomyosarcoma 7, brain tumor 5), as well as in those with attenuated LFS (5 of 8 tumors: all breast cancer) (Table S4).

Next, to investigate genotype–phenotype correlations, we focused specifically on hotspot variants, which were defined as amino acid positions 175, 245, 248, 249, 273, and 282, based on a previous report [10]. Hotspot missense variants were detected in 11 of 36 LFS families and in none of 5 attenuated LFS families, and non-hotspot null variants were found in 14 and 1, respectively. Because hotspot missense and non-hotspot null variants are considered to have stronger functional impact than non-hotspot missense variants, they were analyzed together. This combined category was observed in 25 (69%) of 36 LFS families and in 1 (20%) of 5 attenuated LFS families ($p = 0.051$) (Figure 2B). All four missense variants of conflicting pathogenicity classification in ClinVar were detected in LFS families. Among the four variants not listed in ClinVar, three were observed in LFS families and one large deletion, encompassing exons 2–6, with unequivocal pathogenicity, was identified in an attenuated LFS family (Table S2).

4 | Discussion

In this study, we observed a higher prevalence of hotspot missense and non-hotspot null variants among individuals with LFS compared to attenuated LFS. A previous report indicates that carriers of truncating and hotspot variants are more likely to present with LFS core cancers and tend to experience an earlier onset of first cancer compared to carriers of other variant types [23]. Furthermore, individuals with hotspot variants are more likely to fulfill classic LFS criteria, to develop breast cancer at a younger age, and to receive an earlier diagnosis of any cancer than those with non-hotspot variants [24]. Null variants were significantly more frequent in families with LFS than in those with attenuated LFS [25]. Our results are consistent with these published results.

This study has several limitations. The sample size was relatively small, and there may be selection bias, as the study eligibility criteria restricted enrollment to participants younger than 40 years of age, who were recruited from a prospective cancer surveillance trial. Nonetheless, our findings provide supportive evidence for genotype–phenotype correlations in this Japanese LFS cohort. The recruitment for the LFS20 trial concluded in 2024, and a subsequent study is currently being planned to evaluate the efficacy of cancer surveillance. Continued accumulation of genotype, phenotype, and surveillance outcome data will be essential to further optimize cancer screening strategies based on specific *TP53* variant profiles.

Author Contributions

Fumito Yamazaki: conceptualization, data curation, formal analysis, investigation, writing – original draft, writing – review and editing. **Yoshiko Nakano:** formal analysis, writing – review and editing. **Masashi Sanada:** formal analysis, writing – review and editing. **Shunsuke Miyai:** formal analysis, writing – review and editing. **Hiroki Kurahashi:** formal analysis, writing – review and editing. **Arisa Ueki:** investigation, writing – review and editing. **Yuko Watanabe:** investigation, writing – review and editing. **Daisuke Hasegawa:** investigation, writing – review and editing. **Shuhei Karakawa:** investigation, writing – review and editing. **Toshifumi Ozaki:** investigation, writing – review and editing. **Akira Hirasawa:** investigation, writing – review and editing. **Akiko M. Saito:** methodology, writing – review and editing. **Eisuke Inoue:** methodology, writing – review and editing. **Motohiro Kato:** formal analysis, writing – review and editing. **Hiroyoshi Hattori:** conceptualization, formal analysis, funding acquisition, investigation, supervision, writing – review and editing.

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Ethics Statement

This study was registered in the UMIN Clinical Trial Registry as #UMIN000045855. Ethics approval was obtained from the Central Institutional Review Board at NHO Nagoya Medical Center for clinical research, and written informed consent was obtained from all participants prior to enrollment. No animal studies were conducted as part of this research.

Conflicts of Interest

Hiroki Kurahashi has received consultant fees from OVUS. Arisa Ueki has received an honorarium from Myriad Genetics G.K. and a medical education grant from Pfizer Japan INC. Other authors do not have a conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Study flow diagram of participants with germline *TP53* pathogenic variants (PVs) in the JCCG LFS20 cohort. **Table S1:** Characteristics of individuals with germline pathogenic variants in *TP53* gene. **Table S2:** Summary of data for 45 participants with *TP53* germline variants. **Table S3:** Summary of data for each *TP53* germline variants. **Table S4:** Characteristics of individuals with LFS and attenuated LFS.