Cancer incidence, patterns, and genotype–phenotype associations in individuals with pathogenic or likely pathogenic germline *TP53* variants: an observational cohort study

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Summary

Background Li-Fraumeni syndrome, caused primarily by pathogenic or likely pathogenic germline *TP53* variants, is a rare, variably penetrant, cancer predisposition syndrome with very high risks of cancer starting in childhood, including the risk of multiple primary malignancies over an individual's lifespan. We aimed to characterise and quantify cancer incidence, patterns, and genotype–phenotype associations in individuals with pathogenic or likely pathogenic germline *TP53* variants.

Methods This observational cohort study was done in 480 carriers of pathogenic or likely pathogenic germline *TP53* variants enrolled in the National Cancer Institute's referral-based longitudinal Li-Fraumeni syndrome study between Aug 1, 2011, and March 24, 2020. Data on personal and family history of cancer were obtained through study questionnaires and validated by medical records. Variants were categorised on the basis of both loss-of-function (LOF) and dominant-negative effect (DNE) properties. Cancer incidence associated with Li-Fraumeni syndrome was compared with that of the general population using the Surveillance, Epidemiology, and End Results (SEER) 1975–2017 registry. Cancer incidence was evaluated with family-clustered Cox regression models and competing risk methods. This study is registered with ClinicalTrials.gov, NCT01443468.

Findings Individuals with Li-Fraumeni syndrome had a nearly 24 times higher incidence of any cancer than the general population (standardised incidence ratio 23·9; 95% CI 21·9–26·0), with the highest comparative incidence from childhood to 30 years of age. The overall cancer incidence remained 10·3 (95% CI 7·9–13·2) times higher than that of the general population after age 50 years. In women, when considering breast cancer as a competing risk, the probability of a first diagnosis of a non-breast cancer malignancy was substantially lower than that of any first cancer (24·4% [95% CI 19·6–30·5] *vs* 50·4% [43·5–56·5] by age 33·7 years). Overall, DNE_LOF and notDNE_LOF variants were associated with earlier age at first and second cancer compared with notDNE_notLOF and DNE_notLOF variants. The time interval from first to second cancer was shorter among carriers whose first cancer diagnoses were later in life. Multiple cancers were diagnosed within a short timeframe in some individuals, regardless of the order of cancer occurrence.

Interpretation This study adds granularity to the understanding of cancer incidence and patterns in individuals with pathogenic or likely pathogenic germline *TP53* variants. Integration of age range-specific cancer incidence estimates, cancer-free survival by functional variant group, the potential impact of risk-reducing mastectomy on female cancer incidence, and data on subsequent malignancies will be important for the development of strategies to optimise cancer screening and management for these individuals.

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Introduction

Li-Fraumeni syndrome is an autosomal dominant, variably penetrant, inherited cancer predisposition disorder characterised by elevated risks of cancers beginning in infancy. The core cancers associated with Li-Fraumeni syndrome are premenopausal breast cancer, osteosarcoma and soft-tissue sarcomas, brain tumours, and adrenal cancer, among many others, with most cancers occurring at ages earlier than expected.[1,2] The median age at first cancer diagnosis in Li-Fraumeni syndrome is approximately 31 years in women and 46 years in men. Individuals with Li-Fraumeni syndrome are also at risk of developing multiple primary malignancies.[3] Li-Fraumeni syndrome is a clinical diagnosis based on personal and family history of cancer. Multiple criteria exist to diagnose Li-Fraumeni syndrome and to identify individuals who should be tested for pathogenic or likely pathogenic germline *TP53* variants—the only known genetic cause of Li-Fraumeni syndrome to date.[1,2] Because of the high cancer risks and heterogeneity

Research in context

Evidence before this study

Li-Fraumeni syndrome is a variably penetrant autosomal dominant cancer predisposition disorder, primarily caused by pathogenic or likely pathogenic germline *TP53* variants, resulting in extremely high risks of multiple primary malignancies over the individual's lifespan. A PubMed search from database inception through to March 24, 2020, using the terms ("genotype–phenotype" or "cancer incidence" or "cancer pattern" or "cancer risk") and ("Li-Fraumeni "or "germline *TP53*") primarily identified literature reviews or studies with variable sample sizes and inconsistent approaches to genotype–phenotype characterisation. About half of first cancer diagnoses associated with Li-Fraumeni syndrome occur in women by the time they are in their early 30s and in men by the time they are in their mid-40s. The very high risk of cancer in Li-Fraumeni syndrome has led to the development of an internationally adopted intensive annual cancer screening regimen, aimed at early detection and centred around whole-body MRI. However, this one-size-fits-all approach does not take into account the germline *TP53* variant, or personal or family history of cancer.

Added value of this study

This cohort study quantifies the exceedingly high burden of cancer in individuals with Li-Fraumeni syndrome in comparison with their counterparts in the general population. It refines and expands on previous genotype–phenotype correlation studies by categorising variants on the basis of both loss-of-function and dominant-negative functional properties. Furthermore, it shows that risk-reducing mastectomy substantially delays the onset of any first malignancy in women with Li-Fraumeni syndrome. This study also provides previously undescribed granularity on second and subsequent malignancies in individuals with Li-Fraumeni syndrome and highlights the temporality of second cancer onset based on age at first cancer diagnosis.

Implications of all the available evidence

Individuals with Li-Fraumeni syndrome have a nearly 90% lifetime risk of developing at least one cancer and often develop multiple cancers. Cancer screening strategies offer the benefit of early cancer detection, but are emotionally and resource intensive. By putting the cancer burden in Li-Fraumeni syndrome into context with that of the general population, examining genotype–phenotype associations based on *TP53* variant functional group, and evaluating the temporality of subsequent malignancies in Li-Fraumeni syndrome, this study, together with the existing published literature, creates a foundation for the development of tailored cancer screening and personalised risk assessment in Li-Fraumeni syndrome.

associated with this disorder, current cancer screening recommendations aimed at early cancer detection are multimodal, high-frequency evaluations centred around whole-body MRI.[4,5] Cancer screening in Li-Fraumeni syndrome has been shown to improve survival but can induce anxiety, uncertainty, and screening burden in individuals with Li-Fraumeni syndrome.[6,7]

Variants in *TP53* affect different functions of the p53 protein and, consequently, its tumour-suppressive activity. *TP53* variants can be grouped on the basis of their functional consequences: variants associated with loss of function (LOF) leading to haploinsufficiency; variants that endow the p53 protein with gain of functions (GOF); and variants associated with a dominant-negative effect (DNE) and impairment of transactivation activities.[8] Variable phenotypes, expressivity, and penetrance of cancer are frequent in carriers of pathogenic or likely pathogenic germline *TP53* variants.[9] The shift from single-gene testing to large multigene cancer panels (almost of all which include *TP53*) has led to the unexpected identification of individuals with pathogenic or likely pathogenic germline *TP53* variants in the absence of a typical cancer family history.[10] A small number of genotype–phenotype studies have evaluated how functional properties of *TP53* variants affect cancer risk.[8] For example, DNE variants are typically associated with earlier age at cancer onset and considered highly penetrant,[11,12] while another study found that LOF variants are more likely to occur in families meeting classic clinical Li-Fraumeni syndrome criteria.[13] Although previous studies have directly compared DNE with LOF variants on the basis of functional assay-specific characteristics, these properties are not mutually exclusive. A recent assay investigated these properties for *TP53* variants, stratifying variants on the basis of DNE and LOF properties, allowing assessment of both features simultaneously.[14]

Further refinement and quantification of cancer risks and patterns are needed to optimise the care of pathogenic or likely pathogenic germline *TP53* variant carriers. We aimed to comprehensively evaluate cancer incidence, patterns, and genotype–phenotype associations by functional *TP53* groups in a large cohort of individuals with pathogenic or likely pathogenic germline *TP53* variants, to facilitate the development of personalised cancer risk assessments.

For more on the **Li-Fraumeni Syndrome Study** see http://lfs.cancer.gov

Methods

Study participants and data collection

Individuals in this cohort study were part of the longitudinal Li-Fraumeni Syndrome Study (NCT01443468) approved by the institutional review board of the National Cancer Institute (NCI) and enrolled between Aug 1, 2011, and March 24, 2020. Participants or their legal guardians provided written, informed consent, completed questionnaires, and provided medical records, including pathology and genetic testing reports.[3] Metastatic cancers and recurrences were excluded. We included confirmed and

obligate carriers of germline *TP53* variants classified as pathogenic or likely pathogenic (hereby referred to as individuals with Li-Fraumeni syndrome) in ClinVar[15] by one or more major genetic testing laboratories, or by the ClinGen *TP53* Variant Curation Expert Panel (VCEP).[16] Exclusion criteria were confirmed germline mosaics or individuals with a low variant allele fraction suggestive of mosaicism or clonal haematopoiesis; individuals with pathogenic or likely pathogenic germline *TP53* variants and personal or familial cancer history (or both) suggestive of another hereditary cancer predisposition syndrome; and individuals who were confirmed to carry an additional pathogenic or likely pathogenic germline variant or variants in a different high-penetrance cancer susceptibility gene.

TP53 variant categorisation

Missense and nonsense variants were classified into four groups on the basis of a recently published systematic functional assay.[14] Thresholds to determine DNE (p53WT_Nutlin, Z score≥0·61) and LOF (p53NULL_Etoposide, Z score≤–0·21) properties were utilised as currently adopted by the International Agency for Research on Cancer (IARC) *TP53* database (version R20; July, 2019)[17] and by the ClinGen *TP53* Variant Curation Expert Panel revised classification criteria.[16] Based on the combination of scores, the following functional groups were designated: DNE_LOF, notDNE_LOF, notDNE_notLOF, and DNE_notLOF. A fifth group designated as "not included" consisted of variants that were not investigated in the original assay (eg, insertions, deletions, frameshifts, and splice-site variants). For completeness, additional analyses comparing DNE versus notDNE, and LOF versus notLOF were done for time-to-event analyses examining age at first cancer diagnosis and the time interval between the first and second cancer.

Statistical analysis

Outcomes assessed included order of cancer occurrence; cancer incidence compared with the general population; overall survival; cumulative incidence of first cancer diagnoses; and cancer patterns including age at first cancer diagnosis, age at second cancer diagnosis, time interval between first, second, and subsequent cancers, and probability of death after first cancer before the development of a second cancer. Cancer types were grouped into 16 morphological categories. Non-melanoma skin cancers and human papillomavirus (HPV)-associated high-grade dysplasia of the anus, cervix, and vulva were excluded. Synchronous cancers in the same individual were treated as one event for all non-cancer-specific time-to-event analyses, and as independent events for order of cancer occurrence, cumulative and standardised incidences, and first cancer-specific survival analyses. For the overall survival analyses, participants were censored at last follow-up. For time-to-event analyses of age at first cancer diagnosis, individuals were censored at death or last follow-up. In some time-to-event analyses, sex-specific cancers (including breast, gynaecological, and prostate cancers) were jointly considered as a competing event to other first-cancer diagnoses for direct comparisons of women with men, and to ensure that the proportionality of cause-specific hazards was a reasonable assumption between groups (assessed with χ^2 tests; appendix p 1). For cancer-specific estimates, other first cancer diagnoses were treated as competing risks to avoid any potential treatment-associated bias. For breast cancer-specific analyses, prophylactic bilateral mastectomy was considered as an additional competing risk, when data were available. Sensitivity analyses were done for time-to-event analyses for breast cancer as a first malignancy, excluding the women for whom mastectomy data were incomplete. Time-to-event analyses examining risk of second cancer included analysis of the time interval from the first to second cancer diagnosis, as well as age at second cancer diagnosis. Analyses of age at second cancer diagnosis used delayed entry (entry at first cancer diagnosis) methods adapted for left-truncated and right-censored survival data, and all analyses accounted for death before second cancer as a competing risk. Correspondingly, analyses of the estimated probability of death after first cancer diagnosis but before second cancer diagnosis were done accounting for second cancer diagnosis as a competing risk. All non-parametric survival or cumulative incidence estimates also accounted for family clustering due to potential shared risk factors within families. Family-clustered proportional cause-specific hazards models were used to compare survival between groups. Baseline groups were established either on the basis of the largest category (ie, DNE_LOF) or the ease of interpretation (ie, 0–17 years age-range category). Survival curves were created with the Kaplan-Meier method.[18] Cumulative incidence with competing risks[19] was calculated with the Aalen-Johansen estimator.[20]

See Online for appendix

Standardised incidence ratios (SIRs) were obtained by comparing observed cancer incidences in the cohort against expected incidences based on the Surveillance, Epidemiology, and End Results (SEER) 1975–2017 data. Individuals were stratified by sex, 5-year attained age group, and 5-year attained calendar year group. Individuals with missing date of birth or date of last follow-up were excluded from analyses (appendix p 2 includes International Classification of Diseases for Oncology [ICD-O] definitions for each cancer category).

For more on **SEER** see https://seer.cancer.gov/

p values less than 0·05 were considered statistically significant. Analyses and plotting were done with Python 3 (version 3.8.3) programming language using the Lifelines library (version 0.25.8) and R (version 4.0.2) with the survival package (version 3.1.12) and tidyverse.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

	DNE_LOF		Not included		notDNE_notLOF		notDNE_LOF		DNE_notLOF		Total (n=480)
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	
Demographics and cancer status											
Number of unaffected individuals	40	27	22	25	28	20	1	4	5	3	175
Median age at last follow-up, years (IQR)	20·4 (12·7–31·8)	19·0 (7·8–32·6)	18·4 (9·5–28·6)	20·9 (12·8–42·6)	30·2 (7·2–39·3)	31·4 (15·3–40·5)	13·9 (13·9–13·9)	17·3 (16·5–24·9)	44·6 (41·8–46·4)	42·3 (41·7–44·4)	21·8 (11·3–38·0)
Number of affected individuals	100	44	43	27	32	20	20	4	8	7	305
Median age at last follow-up, years (IQR)	37·8 (29·3–50·2)	29·0 (19·3–45·4)	40·3 (34·0–50·9)	41·1 (21·5–56·8)	47·0 (35·7–56·7)	60·5 (48·4–66·1)	43·0 (33·9–45·7)	41·7 (30·3–50·6)	48·9 (45·5–65·5)	65·2 (61·6–69·4)	41·6 (29·9–55·5)
Total number of cancers*											
Breast	92 (n=67)	2 (n=2)	40 (n=30)	..	25 (n=22)	..	23 (n=15)	..	8 (n=6)	..	190 (n=142)
Soft-tissue sarcoma	38 (n=27)	18 (n=13)	21 (n=17)	12 (n=10)	10 (n=8)	8 (n=5)	12 (n=10)	2 (n=2)	2 (n=1)	1 (n=1)	124 (n=94)
Brain	16 (n=16)	25 (n=25)	3 (n=3)	6 (n=5)	4 (n=4)	5 (n=5)	1 (n=1)	..	..	2 (n=2)	62 (n=61)
Osteosarcoma	13 (n=10)	8 (n=7)	7 (n=7)	8 (n=8)	1 (n=1)	2 (n=2)	2 (n=2)	..	..	..	41 (n=37)
Haematological	11 (n=10)	6 (n=6)	3 (n=3)	5 (n=4)	1 (n=1)	4 (n=4)	1 (n=1)	1 (n=1)	..	1 (n=1)	33 (n=31)
Lung	11 (n=9)	1 (n=1)	3 (n=3)	..	4 (n=4)	1 (n=1)	2 (n=2)	..	2 (n=2)	..	24 (n=22)
Colorectal	9 (n=9)	6 (n=6)	3 (n=3)	2 (n=2)	1 (n=1)	2 (n=2)	1 (n=1)	..	..	..	24 (n=24)
Melanoma	7 (n=4)	..	4 (n=3)	2 (n=2)	2 (n=2)	3 (n=3)	3 (n=3)	..	..	1 (n=1)	22 (n=18)
Thyroid	9 (n=8)	2 (n=2)	..	2 (n=2)	1 (n=1)	..	1 (n=1)	1 (n=1)	..	..	16 (n=15)
Prostate	..	4 (n=4)	..	1 (n=1)	..	7 (n=7)	..	1 (n=1)	..	2 (n=2)	15 (n=15)
Other	7 (n=6)	2 (n=2)	1 (n=1)	3 (n=3)	..	1 (n=1)	..	..	1 (n=1)	..	15 (n=14)
Pancreatic or liver	3 (n=3)	1 (n=1)	..	3 (n=3)	..	2 (n=2)	2 (n=1)	..	1 (n=1)	1 (n=1)	13 (n=12)
Adrenal	6 (n=6)	3 (n=3)	..	1 (n=1)	..	1 (n=1)	..	..	..	..	11 (n=11)
Upper gastrointestinal	2 (n=2)	5 (n=5)	..	1 (n=1)	..	1 (n=1)	1 (n=1)	1 (n=1)	..	..	11 (n=11)
Gynaecological	5 (n=4)	..	2 (n=2)	..	1 (n=1)	..	2 (n=2)	..	..	..	10 (n=9)
Kidney	1 (n=1)	2 (n=2)	..	1 (n=1)	..	1 (n=1)	2 (n=2)	..	..	1 (n=1)	8 (n=8)
Total	230 (n=100)	85 (n=44)	87 (n=43)	47 (n=27)	50 (n=32)	38 (n=20)	53 (n=20)	6 (n=4)	14 (n=8)	9 (n=7)	619 (n=305)

Data as of study close date (March 24, 2020). Ten cancer types were categorised as "Other" and include: unknown primary cancer (n=3), head and neck cancer (n=2), carcinoid tumour (n=2), thymus cancer (n=2), bladder cancer (n=1), parotid gland cancer (n=1), neuroblastoma (n=1), malignant peripheral nerve sheath tumour (n=1), mesothelioma (n=1), and chordoma (n=1). LOF=loss of function. DNE=dominant-negative effect. *Data shown are number of cancers; number of individuals are shown in parentheses.

Table: **National Cancer Institute Li-Fraumeni syndrome cohort demographics stratified by functional variant group, sex, and cancer status**

Results

480 individuals with pathogenic or likely pathogenic germline *TP53* variants from 143 families were included in this cohort study, after exclusion of nine individuals with low allele fraction, one individual with a family history suggestive of another hereditary cancer predisposition syndrome, and one individual with an additional pathogenic or likely pathogenic germline variant in *BRCA1*. 299 (62·3%) of 480 participants were female. 33 (23·0%) of 143 families met classic[21] Li-Fraumeni syndrome criteria, 56 (39·2%) met Chompret criteria,[22,23] 11 (7·7%) met revised Chompret 2015 criteria,[11] ten (7·0%) met Eeles criteria,[24] one (0·7%) met Birch criteria,[25] and no clinical familial criteria were met in 32 (22·4%) families. The majority of participants for whom information was available were of European ancestry (307 [95·3%] of 322).

95 individuals were aged 0–17 years, 84 were aged 18–29 years, 141 were aged 30–44 years, and 148 were older than 45 years at last known follow-up or death; data on age at last follow-up or death were not available for 12 participants. 28 (29·5%) of 95 individuals aged 0–17 years, 49 (58·3%) of 84 aged 18–29 years, 99 (70·2%) of 141 aged 30–44 years, and 123 (83·1%) of 148 individuals older than 45 years had been diagnosed with at least one cancer. The median age at last follow-up or death was 36·7 years (IQR 25·4–47·2) for females and 34·3 years (17·8–52·5) for males. 305 (63·5%) of 480 individuals had at least one cancer (table). Of the 619 cancers diagnosed, 384 (62·0%) were validated by medical records and 334 (54·0%) were confirmed by pathology. The most commonly observed cancers were female breast cancer (188 [30·3%] of 619 cancers), soft-tissue sarcomas (124 [20·0%]), brain cancer (62 [10·0%]), osteosarcoma (41 [6·6%]), and haematological cancers (33 [5·3%]; table). 117 individuals (64 women and 53 men) had died at the end of the study period. The median overall survival of the cohort was 65·5 years (95% CI 60·8–68·9), with a median overall survival of 66·0 years (62·4–71·3) in women and 63·3 years (57·0–68·9) in men (appendix p 3).

There were 83 unique pathogenic or likely pathogenic germline *TP53* variants in the study. One family had

two pathogenic or likely pathogenic germline *TP53* variants, p.E298* and p.G245A, identified in different individuals. Most variants were in the "not included" or DNE_LOF categories (31 in the "not included" category and 30 in the DNE_LOF category), in addition to nine notDNE_LOF, eight notDNE_notLOF, and five DNE_notLOF variants. DNE_LOF variants were the most frequent, seen in 211 (44·0%) of 480 individuals (appendix pp 4–5).

The order of cancer occurrence was confirmed for 582 cancers. In women (appendix p 6), breast cancer was the most common first (123 [56·9%] of 216) and second primary malignancy (40 [40·4%] of 99). In men, brain cancer (27 [26·0%] of 104) was the most frequent first cancer and soft-tissue sarcomas (nine [23·7%] of 38) were the most frequent second cancer (appendix p 6). 30 synchronous pairs of primary cancers (60 cancers) were detected among women (20 as first primary cancers, of which 14 were bilateral breast cancers) and eight synchronous pairs (16 cancers) detected among men (seven first primary cancers, including three pairs of soft-tissue sarcomas).

The cumulative incidence of first cancer accounting for competing risks in women confirmed breast cancer as the most frequent first cancer (cumulative incidence of 56·0% by age 60 years), followed by soft-tissue sarcomas (lifetime cumulative incidence of 15·0%; appendix p 7). In men, brain cancer and soft-tissue sarcomas each had a lifetime first-cancer cumulative incidence of around 20%, with soft-tissue sarcomas showing bimodal incidence in early childhood, and after age 40 years. The cumulative incidence of male osteosarcoma steadily increased to plateau at approximately 10% by the mid-late 30s. The cumulative incidence of other male cancers increased after age 40 years, with prostate, colorectal, and haematological cancers each exceeding a lifetime probability of 5% as a first malignancy (appendix p 7).

Individuals with Li-Fraumeni syndrome had a nearly 24 times higher incidence of any cancer (excluding ductal carcinoma in-situ) than the general population (SIR 23·9; 95% CI 21·9–26·0), with the highest comparative incidence from childhood to 30 years of age (figure 1, appendix p 8). Although SIRs declined with age, the overall cancer incidence was still 10·3 (95% CI 7·9–13·2) times higher than that of the general population after age 50 years (figure 1A). The core Li-Fraumeni syndrome cancers had the highest incidence magnitudes: up to 1000 times higher for adrenal cancer (95% CI 530·1–1902·7; figure 1B), more than 700 times higher for osteosarcoma (498·1–966·3; figure 1M), more than 200 times higher for soft-tissue sarcomas (186·6–268·7; figure 1P), more than 100 times higher for brain cancer (78·5–133·0; figure 1C), and more than 36 times higher for breast cancer (31·2–41·5; figure 1D). The elevated mastectomy-agnostic incidence of female breast cancer approached the population level after age 60 years (SIR 0·9 [95% CI 0·0–5·1]; figure 1D).

Among the 457 individuals with complete information for this analysis, the median age at first cancer diagnosis (50% probability of cancer) was 36·1 years (95% CI 34·4–38·2) overall, 33·7 years for women (31·4–35·6), and 45·0 years for men (40·6–50·2; figure 2A). When breast cancers were treated as a competing risk, the probability of a first diagnosis of a non-breast cancer malignancy for women decreased from 50·4% (95% CI 43·5–56·5) to 24·4% (19·6–30·5) by age 33·7 years (figure 2B). For men, the median age at first cancer diagnosis only minimally increased to 45·7 years (95% CI 40·8–50·7; figure 2B) when considering male-specific cancers as a competing risk. Overall, the risk of non-sex-specific cancers was significantly higher for men than for women (p=0·0027; figure 2B).

Analyses stratified by *TP53* variant functional classes showed that the notDNE_LOF, DNE_LOF, and "not included" groups had overall earlier median ages at first cancer, with a difference of up to 30·9 years between the notDNE_LOF and DNE_notLOF groups (figure 2C). Similar findings were observed when treating sex-specific cancers as a competing risk (figure 2D) or when stratifying by sex (appendix p 9).

Cancer-specific analyses estimated a 25% probability of diagnosis of breast cancer as a first malignancy by age 33·7 years in women (95% CI 31·1–35·6; appendix p 10); five women underwent prophylactic bilateral mastectomy before diagnosis of a first cancer of any type (considered as a competing event; appendix p 10). Sensitivity analyses, excluding 78 women for whom mastectomy information was incomplete, showed similar cumulative incidence curves (data not shown). There was no significant difference between sexes for age at diagnosis of soft-tissue sarcomas as a first cancer (p=0·65, appendix p 10). As first cancers, brain cancer and osteosarcoma developed earlier in men than in women (p=0·0018 for brain cancer and p=0·0056 for osteosarcoma; appendix p 10). For breast cancer and soft-tissue sarcomas, the differences by functional variant groups were similar to those of all cancers, with the notDNE_LOF, DNE_LOF, and "not included" classes showing earlier age at first cancer diagnosis (appendix p 10). However, the DNE_LOF group was associated with earlier age at brain cancer diagnosis (appendix p 10) and the "not included" group associated with earlier osteosarcoma (appendix p 10). Parameter estimates and p values for all between-functional group comparisons are shown in the appendix (pp 11–13).

Of 276 individuals with complete data and a first cancer diagnosis, 129 (46·7%) developed second primary cancers. Our data showed that, by 20 years after the first cancer diagnosis, the risk of death before a second cancer was 22·2% (95% CI 17·3–28·7; appendix p 14); our results suggest that the risk of death after first cancer but before a second cancer was higher in men than in women (appendix p 14). The DNE_notLOF group had the shortest time from first cancer to death before a second cancer (appendix p 14), probably due primarily to this group's

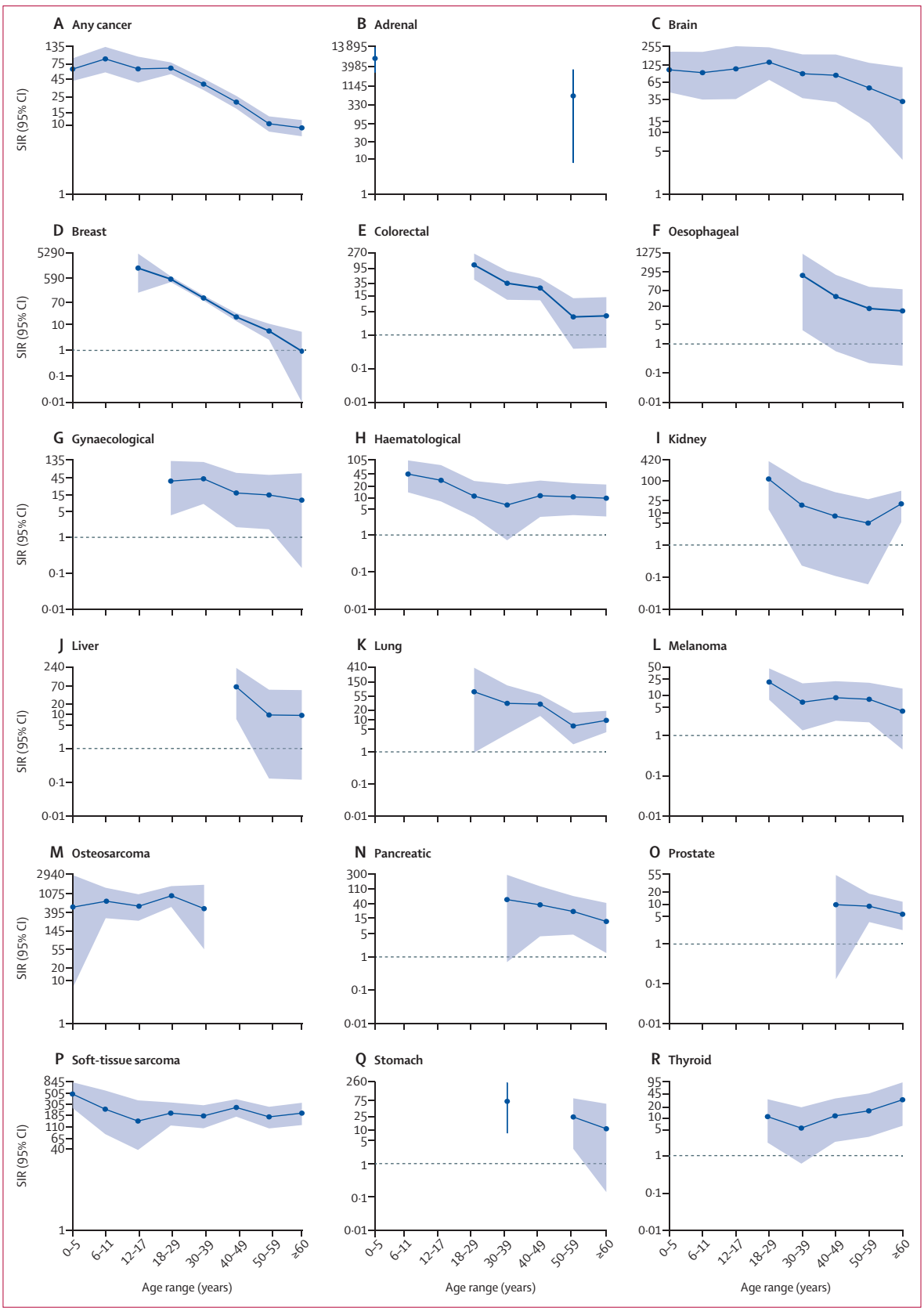

Figure 1: **Standardised incidence ratios for the main cancer types included in the analysis**

Synchronous cancers were counted independently. The dashed line at 1 represents the expected standardised incidence ratio (SIR) associated with Surveillance, Epidemiology, and End Results (SEER) data. The shading represents 95% CIs. Results for any cancer (plus breast in situ), leukaemia, lymphoblastic leukaemia, myelodysplastic syndrome, acute myeloid leukaemia, lymphoma, leiomyosarcoma, liposarcoma, and rhabdomyosarcoma are provided in the appendix (p 8). Adrenal cancer occurred only in two age ranges (0–5 years and 50–59 years) in this dataset, hence only two data points are shown.

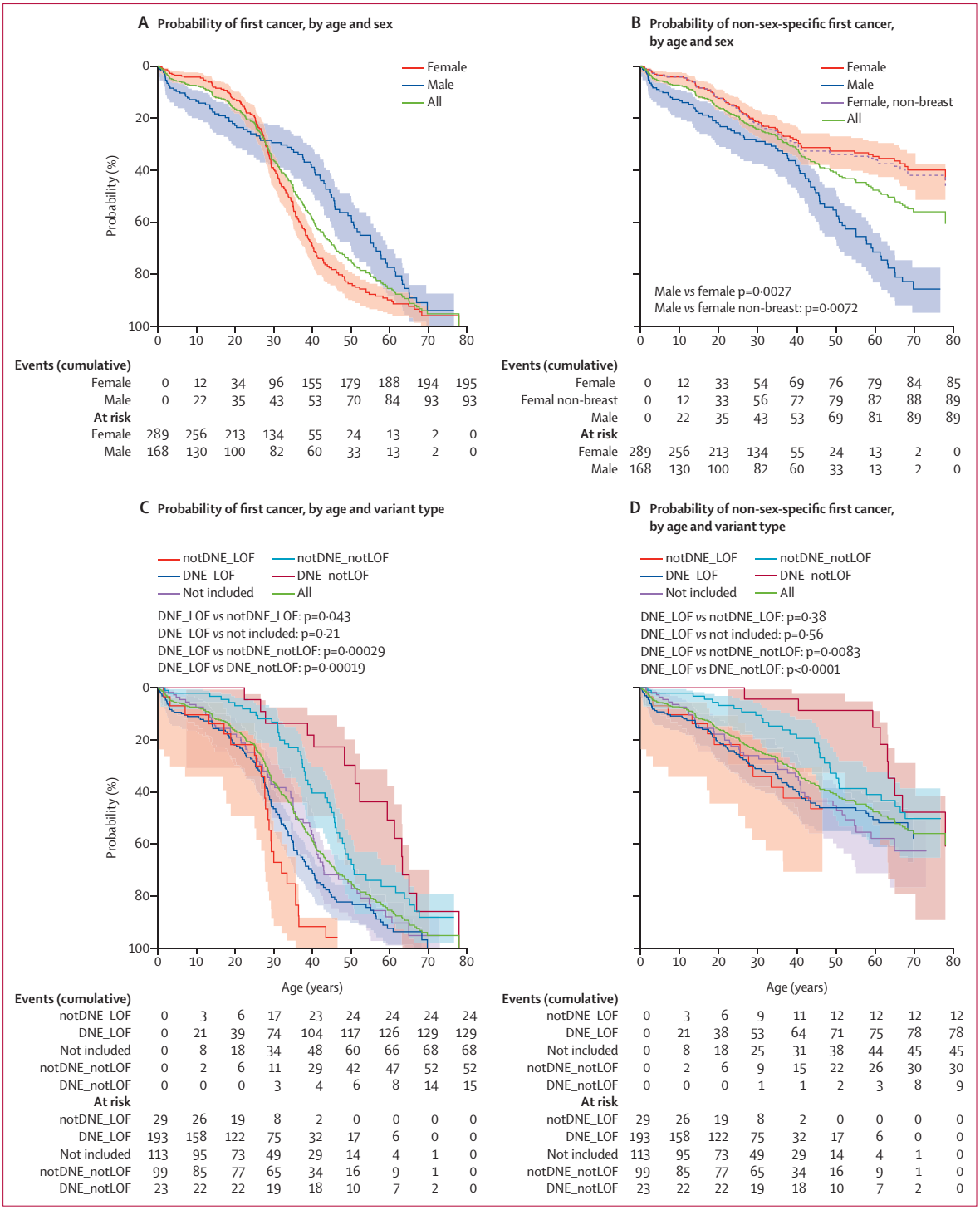

***Figure 2*: Probability of diagnosis of a first cancer by age, stratified by sex and by functional variant group**

(A) Any first cancer, stratified by sex. (B) Any non-sex-specific or non-breast first cancer, stratified by sex, considering sex-specific (breast, gynecological, and prostate) or breast cancers only as a competing risk. (C) Any first cancer, stratified by variant type. (D) Any non-sex-specific or non-breast first cancer, stratified by variant type, considering sex-specific (breast, gynecological, and prostate) or breast cancers only as a competing risk. Synchronous cancers of the same category were counted as single events; synchronous cancers of different categories were counted independently. DNE_LOF class was considered as a baseline. p values calculated with family-clustered Cox proportional hazards (A, C) and proportional cause-specific hazard (B, D) models. The shading represents 95% CIs. LOF=loss of function. DNE=dominant-negative effect.

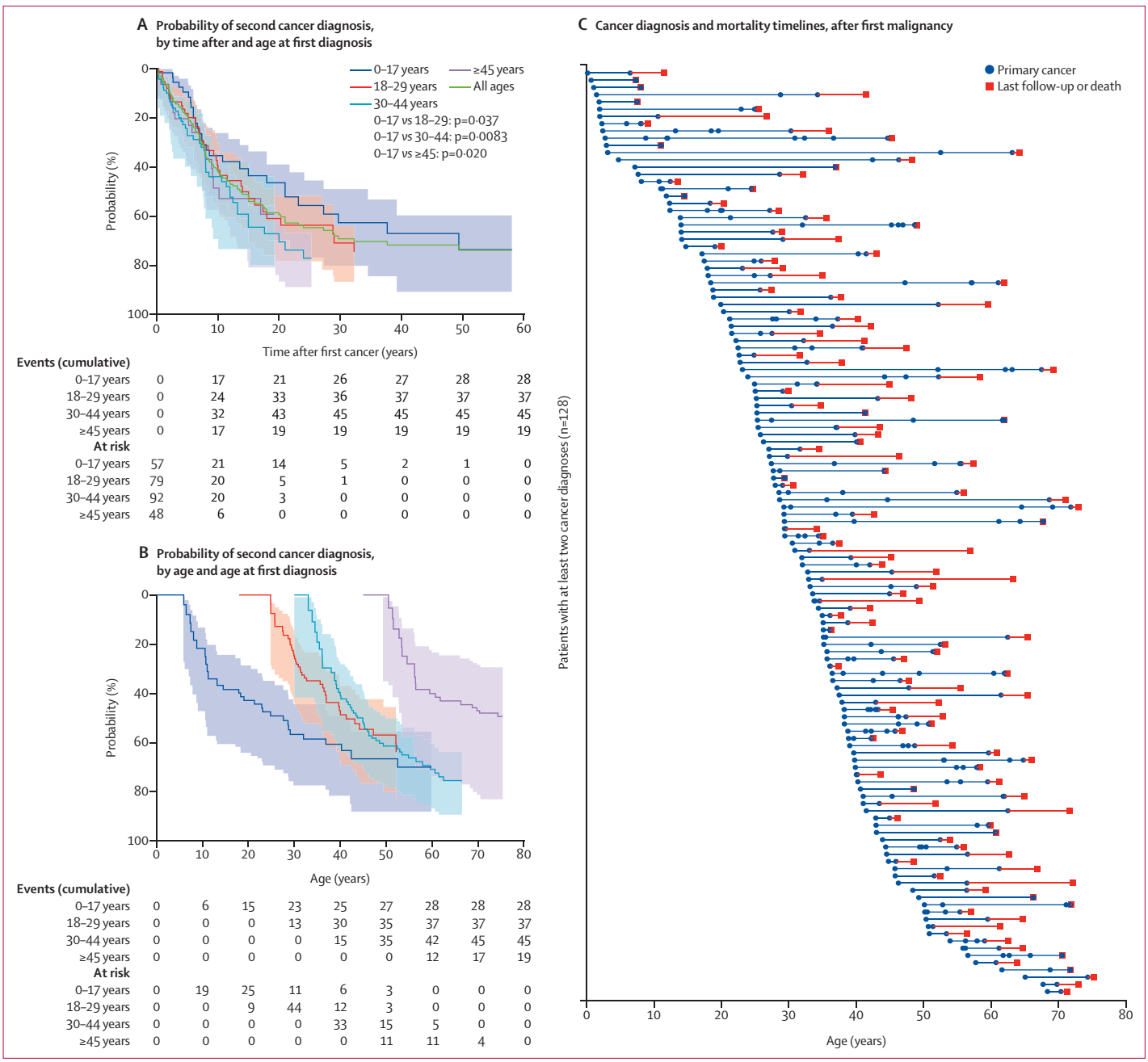

Figure 3: **Probability of diagnosis of a second cancer, by time after first cancer diagnosis and age at second cancer diagnosis, accounting for death before second cancer diagnosis as a competing risk**

Probability of diagnosis of a second cancer, by time after first cancer diagnosis (A), and age at second cancer diagnosis (B), accounting for death before second cancer diagnosis as a competing risk. Stratification by age at first cancer diagnosis. Individuals with a cancer diagnosis at age 0–17 years were considered as the baseline. p values calculated with family-clustered proportional cause-specific hazard models. The shading represents 95% CIs. (C) Timeline plot showing time intervals between primary cancers and last follow-up or death, restricted to the 128 individuals with at least two primary cancers and known age at diagnosis for all cancers. Synchronous cancers were counted as single events. Blue lines represent time intervals between primary cancers; red lines represent the time interval between the last diagnosed primary cancer and either last follow-up or death.

older age at first cancer diagnosis (figure 2C); a shorter time from first cancer to death before a second cancer was also noted among individuals with a later first cancer diagnosis (appendix p 14). After accounting for death before second cancer as a competing risk, women had a shorter interval between first and second cancer diagnoses than men, although this difference was not significant when comparing cause-specific hazards (50% risk of second cancer by 12·0 years [95% CI 9·14–15·0] after the first cancer in women and by 28·9 years [10·0–37·7] in

men, p=0·19; appendix p 15). Based on these analyses, women have an estimated probability of 66% of developing a second cancer, and men have a probability of 45·4% of developing a second cancer, within 20 years after the first cancer (appendix p 15). There was no significant difference between men and women in the time to second cancer when sex-specific cancers were considered as an additional competing risk (p=0·66; appendix p 15). Analysis by functional variant group showed that, although the differences in time to second cancer were not considerably pronounced, DNE_LOF variants were associated with the shortest intervals between first and second cancers (appendix p 15). Comparisons between all functional groups are shown in the appendix (pp 11–13).

When accounting for death before second cancer as a competing risk, the probability of developing a second cancer by 7·5 years after the first was similar when stratifying by age group at first cancer diagnosis (32·0% at 0–17 years, 29·4% at 18–29 years, 32·0% at 30–44 years, and 29·6% at ≥45 years). After this period, individuals whose first cancers were diagnosed before 17 years of age developed a second cancer after a longer time interval (median interval 21·0 years; 95% CI 8·6–37·7) than those with later first cancers (figure 3A). Comparisons between all age-range groups are shown in the appendix (pp 11–13). Similar analyses with delayed entry based on age at first cancer diagnosis showed an estimated 25% probability of developing a second cancer by age 10·6 years (95% CI 5·9–17·9) for those with a first cancer between 0–17 years of age, by age 30·0 years (24·8–36·2) for those with a first cancer between 18–29 years of age, by age 36·1 years (33·0–39·6) for those with a first cancer between 30–44 years of age and by age 54·6 years (50·5–66·3) for those with a first cancer after 45 years of age (figure 3B). Corresponding graphs for age at death before second cancer, stratified by age at first cancer, are shown in the appendix (p 14).

Analysis of the timeline of 128 individuals who developed at least two primary cancers, with known age at diagnosis for all of their cancers, shows that some carriers develop multiple cancers within a short time frame regardless of the order of cancer occurrence (figure 3C).

Time-to-event analyses examining age at first cancer diagnosis and the time interval between the first and second cancer, by individual variant functional groups (DNE *vs* notDNE and LOF *vs* notLOF), are provided in the appendix (p 16).

Discussion

A detailed understanding of cancer incidence, risks, and patterns is required to develop personalised risk assessment and tailored screening for individuals with pathogenic or likely pathogenic germline *TP53* variants. This study not only validates previous important studies of cancer occurrence in Li-Fraumeni syndrome, but further refines them, expands on the understanding of second and subsequent malignancies, and genotype–phenotype associations, and puts cancer incidence associated with Li-Fraumeni syndrome into context with that of the general population.

The exceedingly high overall and age range-specific incidence of cancer in individuals with Li-Fraumeni syndrome compared with that of the general population (ie, based on SEER data) quantifies the substantial cancer burden these individuals experience throughout their lifespan, which continues into the fifth and sixth decades of life. By contrast with other studies of Li-Fraumeni syndrome that focused on comparisons with the general population on a single cancer type,[26] or studies that focused on median age at cancer onset in the published literature (IARC database),[27] we calculated age-specific and cancer-specific SIRs for 24 different cancer types and subtypes from a single, large, clinically curated Li-Fraumeni syndrome cohort. Our data showed the temporality of age distribution similar to the IARC comparison by Amadou and colleagues,[27] with the age at onset of many cancers being relatively similar to those in their sporadic counterparts.[28,29] One notable exception is female breast cancer, which occurs substantially earlier in women with Li-Fraumeni syndrome than in the general population.[30] The incidence of female breast cancer associated with Li-Fraumeni syndrome reached population levels after age 60 years, which could either represent a true decline in risk or be due to the small numbers of older at-risk women with intact breast tissue. These data suggest that risk-reducing mastectomy might not substantially change the breast cancer risk in women with Li-Fraumeni syndrome older than 60 years of age who have not yet developed breast cancer and have intact breast tissue, although mastectomy counselling at any age should take multiple factors into consideration, including near-term and remaining lifetime risk, family history, screening adherence and accessibility, and individual concerns.

It is notable that treating first primary breast cancers as a competing event significantly increased the age at first cancer diagnosis in women, with the risk dropping by 26% by age 33·7 years. There were also no appreciable differences in the time interval to second cancer or age at second cancer between men and women when sex-specific cancers were treated as an additional competing risk. These findings highlight the importance of early discussions between health-care providers and patients about cancer risk-reduction strategies, and the potentially significant impact that risk-reducing mastectomy might have in delaying cancer onset in women with Li-Fraumeni syndrome.

There are few published quantitative data on second and subsequent malignancies in Li-Fraumeni syndrome. Bougeard and colleagues[11] reported on the number of patients with multiple primary tumours and also within each time interval range to second cancer diagnosis. Our family-clustered time-to-event analysis, which estimated

second-cancer-free survival as well as the time interval to second cancer based on the age of the first cancer diagnosis, is a different approach and further refines the previously published estimates. We report on the risk of death being just higher than 20% before a second cancer is diagnosed. Regardless of age at first cancer diagnosis, we observed that second cancers develop more frequently in the first 10 years after the first cancer. 7·5 years after the first cancer diagnosis, individuals with the first cancer diagnosis at a later age had a shorter interval to a second cancer than did those with a diagnosis during childhood. In a novel approach, the timeline plot also allowed us to visually show the time variability between subsequent cancers, beyond second cancers, showing that several cancers could arise within short time intervals regardless of the order of cancer occurrence. Based on this observation, we hypothesise that a trigger effect might occur in some individuals with two or more cancers, leading to the development of multiple malignancies in relatively short time intervals. This trigger effect might be a combination of biological and therapy-related phenomena. The relationship between chemotherapy and subsequent cancers is unknown in Li-Fraumeni syndrome and warrants further study.

Genotype–phenotype associations were analysed with a functional assay that allowed assessments of both DNE and LOF properties simultaneously,[14] in contrast to previous publications comparing DNE variants to LOF or truncating variants.[8,11] Our data show that specific functional groups could be used to characterise cancer incidence estimates among carriers of pathogenic or likely pathogenic germline *TP53* variants. Overall, DNE_LOF variants were associated with earlier ages at first and second cancer diagnoses than notDNE_notLOF variants. Our results also suggest that the variants from the "not included" category had similar effects to those from the DNE_LOF functional group. Individual analyses of functional groups suggest that LOF status has a more pronounced impact than DNE in all time-to-event analyses, and, in fact, when controlling for LOF status, more severe effects of DNE in any DNE versus notDNE analyses seemed to be driven by the excess of DNE_LOF variants in our dataset. Similarly, when controlling for LOF status, the presence of DNE actually appeared to decrease severity of cancer onset. This finding is the opposite of what would be expected biologically and should be interpreted with caution given the specific variants and the relatively lower number of participants in the notDNE_LOF and DNE_notLOF functional variant groups, as well as the strong correlation between LOF and DNE properties reflected by the pathogenic or likely pathogenic germline *TP53* variants included in this study. The later median age at first cancer diagnosis in the notDNE_notLOF group might be skewed because of over-representation of two of the eight included variants, p.R337H and p.A347D, which each account for approximately 30% of the 100 individuals in this group. The few variants within the DNE_notLOF group, generally associated with the highest median ages at cancer diagnoses, have counter-intuitive functional properties and these results should be interpreted carefully. Due to the high incidence of breast cancers in Li-Fraumeni syndrome, it is still uncertain whether these rankings are consistent across different first primary cancer types or if it is mainly driven by the breast cancer-specific incidence estimates in women. We noted a relative enrichment for prostate cancer in carriers of notDNE_notLOF variants that was likely to be driven by a single family in which there were four men with prostate cancers (who were negative for other known prostate cancer predisposition genes) and additional cases of prostate cancer in non-carriers of germline *TP53* variants, suggesting the potential for additional uncharacterised predisposition factors in this family. Some differences in our genotype–phenotype findings might reflect the fact that other studies used different assays to define the effect of variants on *TP53* function and had different sample sizes and analytical approaches. Future large consortia studies will be helpful to understand variant-specific effects, and to characterise variant effects from the "not included" group (ie, splice-site, frameshift, and insertion or deletion variants).

The present study expands on and refines existing knowledge of Li-Fraumeni syndrome and provides a foundation upon which to build future cancer risk modelling for personalised management. Variables to consider integrating into future studies of cancer risk modelling in pathogenic or likely pathogenic germline *TP53* variant carriers include our data on age range-based cancer incidence compared with the general population, cancer incidence patterns among carriers, cancer diagnosis chronology, risk of first and subsequent cancers, and variant functionality. For example, based on SIRs and cumulative incidence curves, a woman aged 25 years with a pathogenic or likely pathogenic germline *TP53* variant has very high cancer risks compared with the general population and a specifically high risk of breast cancer, in addition to that of other cancers. Regardless of her specific pathogenic or likely pathogenic germline *TP53* variant, a discussion about risk-reducing mastectomy would be warranted on the basis of current recommendations.[31] If the woman has a DNE_LOF variant, history of a cancer in the past 5 years, and intact breast tissue, she is likely to develop a breast cancer before age 30 years or develop a second cancer in the next 5 years, or both. By contrast, if a woman aged 25 years has no previous cancer history, has a notDNE_notLOF variant, and has already undergone risk-reducing mastectomy, her overall cancer-free survival would be similar to that of men, and her functional variant group also seems less prone to developing sarcomas. These two women could potentially be approached differently for their screening management in the future. These hypothetical examples illustrate

some of the complexities of personalised cancer risk management that the data presented in this study can begin to address.

This study has many strengths, including being one of the largest clinically curated Li-Fraumeni syndrome cohorts in the world, with extensive data on second and subsequent primary malignancies. To our knowledge, our study is the first to implement a family-clustered Cox regression analysis in Li-Fraumeni syndrome to account for intrafamilial shared risk factors in our time-to-event analyses, and the first to comprehensively account for competing risks throughout the analyses. The ability to do delayed-entry second cancer analyses is novel and provides data for those truly at risk of developing a second cancer. Additionally, we not only accounted for mortality risk in our analysis of second cancer but also provided estimates of that risk, stratified by the same factors. We acknowledge the limitations of this study, including the referral-based enrolment that might contribute to ascertainment bias and the high proportion of individuals of European ancestry with lack of representation of underrepresented minorities. Although 62% of cancer diagnoses were validated by review of medical records, including pathology reports, this measure is not always reported but is similar to or higher than that of other large studies of Li-Fraumeni syndrome cohorts.[3,11,22,32,33] Survival bias allowing particular individuals to be tested might inflate some of the adult cancer estimates and might explain why there were relatively fewer paediatric than adult cancer diagnoses in our cohort. Our study was not statistically powered to properly answer specific questions about second and subsequent cancers and was not able to account for previous cancer treatment. We acknowledge that the presented Li-Fraumeni syndrome cohort, similar to others, has female over-representation due to the high incidence of breast cancer. We dealt with this over-representation by stratifying our analyses by sex whenever possible, in addition to separate breast cancer-specific analyses when appropriate.

In conclusion, our study provides new data that could help in the future development of personalised cancer risk modelling and tailored screening approaches for individuals with Li-Fraumeni syndrome.

Contributors

SAS and PPK contributed to conceptualisation of the study. KCA, PPK, JNH, MNF, and AFB contributed to data curation and verification. KCA and AFB contributed to the formal analysis. SAS contributed to acquisition of funding. KCA, PPK, JNH, and MNF contributed to the investigation. KCA, PPK, JNH, TWO, AFB, and SAS contributed to the methodology. KCA, PPK, AFB, and SAS contributed to project administration. PPK, JNH, MNF, PLM, and SAS contributed to resources. SAS was responsible for supervision. KCA and AFB contributed to visualisation. KCA, PPK, JNH, MNF, TWO, PLM, AFB, and SAS contributed to writing of the original draft. KCA, PPK, JNH, MNF, TWO, PLM, AFB, and SAS contributed to review and editing of the manuscript. All authors had access to the data in the study and KCA, PPK, JNH, MNF, AFB verified the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Declaration of interests

KCA, JNH, MNF, PLM, and SAS are unpaid members of the ClinGen *TP53* Variant Curation Expert Panel. MNF is a co-developer of CancerGene Connect and a member of the National Accreditation Program for Breast Centers Board, representing the National Society of Genetic Counselors. All other authors declare no competing interests.

Data sharing

Upon publication of the manuscript, participant data including germline *TP53* variant data, age, sex, and cancer history will be available, after appropriate de-identification. These data dictionaries will be shared after data sharing agreements are established between the NCI and requesting institution, for scientists with relevant expertise and hypotheses to test using these data, as long as the request is consistent with study consent forms.

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