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TP53 germline testing and hereditary cancer: how somatic events and clinical criteria affect variant detection rate

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Abstract

Background Germline heterozygous pathogenic variants (PVs) in *TP53* cause Li-Fraumeni syndrome (LFS), a condition associated with increased risk of multiple tumor types. As the associated cancer risks were refined over time, clinical criteria also evolved to optimize diagnostic yield. The implementation of multi-gene panel germline testing in different clinical settings has led to the identification of *TP53* PV carriers outside the classic LFS-associated cancer phenotypes, leading to a broader cancer phenotypic redefinition and to the renaming of the condition as "heritable *TP53*-related cancer syndrome" (hTP53rc). Germline *TP53* variant interpretation is challenging due to the diverse nature of *TP53* PVs, variable penetrance of the syndrome, possible occurrence of *TP53* somatic mosaicism, and *TP53* involvement in clonal hematopoiesis of indeterminate potential (CHIP). Here we aim to assess the relevance and impact of these issues on the diagnostic routine, and to evaluate the sensitivity of the different LFS clinical criteria to identify hTP53rc.

Methods *TP53* was analyzed in 6161 suspected hereditary cancer non-related patients categorized into three subgroups: (1) 495 patients fulfilling any LFS/Chompret clinical criteria; (2) 2481 patients diagnosed with early-onset breast/colorectal cancer; (3) 3185 patients without clinical criteria suggestive of hTP53rc. Ancillary tests were performed when *TP53* PVs were identified in individuals not meeting LFS/Chompret criteria and/or when the variant was identified at low variant allele frequency (VAF).

Results *TP53* PVs were identified in blood DNA of 45 probands. Variant origin was elucidated in 39 of these: 72% patients had a constitutional PV, 10% were mosaics, and 18% had CHIP-associated PVs. Notably, two of the seven CHIP-*TP53* PVs identified were detected at high allelic frequencies (VAF > 35%). Twenty-nine percent of germline *TP53* PV

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did not meet any of the LFS clinical criteria. Among the clinical criteria, Chompret 2009 showed the highest sensitivity in our cohort (68% vs. 54% for Chompret 2015), highlighting the relevance of considering lung cancer in the criteria.

Conclusions Our data supports performing *TP53* ancillary testing for the identification of potential mosaicisms and CHIP-associated PVs, particularly in patients not meeting clinical criterial for LFS, irrespective of the VAF, and the application of clinical criteria that include lung cancer diagnosis.

Keywords *TP53*, Li-Fraumeni syndrome, Heritable *TP53*-related cancer syndrome, Hereditary cancer, Clonal hematopoiesis, Mosaicism

Background

TP53 is a tumor suppressor gene involved in multiple cellular processes that coordinate DNA damage response [1]. Somatic *TP53* pathogenic variants (PVs) occur in most tumor types, and when present in the germline, they cause an autosomal dominant cancer susceptibility condition called Li-Fraumeni syndrome (LFS). LFS is characterized by a strong predisposition to multiple primary early-onset tumors. Lifetime cumulative cancer risk is estimated to reach nearly 100% both in men and women [2]. Osteosarcomas, soft-tissue sarcomas, central nervous system tumors, adrenocortical carcinomas (ACC), and premenopausal breast cancer are the main LFS-associated malignancies, conforming the "core tumors" or "narrow spectrum" of the syndrome [3–6].

The clinical diagnosis of LFS is based on personal and family history of cancer. Since it was first described in 1969, clinical criteria have evolved over time from the most restrictive and highly specific classic criteria, defined in 1988 [7], to the currently used Chompret criteria, proposed in 2001 and last updated in 2015 [4–6] (summarized in Additional file 1: Table S1). These refinements of the clinical criteria aimed to improve diagnosis by accounting for the wide heterogeneity of observed phenotypic manifestations. Moreover, the implementation of multi-gene panel testing has resulted in an exponential increase of germline *TP53* testing among individuals outside the LFS narrow spectrum, in particular, those diagnosed with early-onset breast cancer. The general acknowledgment of a broader phenotypic cancer spectrum and variable penetrance associated with germline *TP53* PVs has led to redefining and renaming this genetic condition as "heritable *TP53*-related cancer syndrome" (hTP53rc), which expands the classical boundaries of LFS [8, 9].

Germline *TP53* PVs show variable penetrance, related to the variant's effect on the tumor suppressor activity of the protein. Most disease-causing variants are missense, and those with a dominant-negative effect (DNE) result in malfunctioning or non-functioning p53 tetramers, which results in reduced transcriptional activity of wild-type protein [10]. DNE PVs are usually associated with the most severe phenotypes, such as very early ages of cancer onset. In fact, they are frequently identified in pediatric cancer patients. In contrast, loss-of-function (LOF) variants (i.e. nonsense, frameshift, splicing and genomic rearrangements) are typically associated with later tumor onsets, representing the least severe PVs. Non-DNE missense PVs represent an intermediate class in terms of clinical impact [6].

Interpretation of germline *TP53* testing involves additional layers of complexity that translate into relevant challenges. De novo *TP53* PVs occur in up to 20% of diagnosed patients. These cases often lack family cancer history and present incomplete penetrance [11]. In the context of de novo cases, somatic mosaicisms are increasingly being reported, as occurs for other hereditary cancer tumor suppressor genes that show a high mutation rate, such as *APC*, *NF1*, or *PTEN* [12–14]. Nevertheless, the prevalence of *TP53* mosaicism remains undetermined. On the other hand, *TP53* is one of the most frequently mutated genes as consequence of clonal hematopoiesis of indeterminate potential (CHIP). CHIP refers to the age-related acquisition and expansion of mutations in hematopoietic stem cells, although it is also associated with tobacco consumption and chemo- or radiotherapy exposure [15]. This phenomenon affects 5% of individuals aged > 60, and results in increased risks of hematological malignancies and coronary artery disease [16, 17]. Since germline testing is mainly performed in blood samples, interpreting *TP53* PVs becomes challenging, as variants derived from both mosaicism and CHIP are generally detected at low variant allele frequency (VAF) and may be overlooked.

In this article, we sought to perform a comprehensive analysis of the *TP53* PVs identified in blood DNA of 45 out of 6161 patients referred to a hereditary cancer program. We assessed the prevalence and impact in germline testing of somatic *TP53* variation associated with mosaicism and CHIP. Among 105 germline variant carriers (including index cases and relatives), we evaluated genotype–phenotype correlations and performance of the different *TP53* clinical criteria versions.

Methods

Patients

A total of 6161 unrelated patients were included in this study. All individuals were referred to the genetic counseling unit at the Catalan Institute of Oncology (ICO) between January 2000 and June 2022 due to personal and/or family history suggestive of germline cancer susceptibility. For this study, three different patient cohorts (C1–C3) were established based on *TP53* testing eligibility: C1 comprises patients fulfilling any LFS or Chompret clinical criteria ($N=495$; criteria listed in Additional file 1: Table S1); C2 includes patients diagnosed with breast or colorectal cancer before age 50 ($N=2481$), meeting eligibility for *TP53* testing in Catalonia region according to the 2022 Catalan Consensus on Clinical Criteria and Gene Panels for the Study of Hereditary Cancer Syndromes [18]; and C3 comprises patients with hereditary cancer suspicion, eligible for testing of other genes based on their tumor type or family history, but who did not meet the criteria for *TP53* testing ($N=3185$). *TP53* gene was studied for research purposes in adherence to ACMG guidelines, which recommend reporting *TP53* PVs as secondary findings (SF v3.2 list) [19]. Informed written consent for both diagnostic and research purposes was obtained from all participants, and the ethics committee of Bellvitge Biomedical Research Institute (IDIBELL) approved the study protocol (PR278/19).

Genetic testing

Before 2016, genetic testing for hereditary cancer syndromes was conducted on a gene-by-gene basis based on each patient's clinical manifestations. For patients meeting classic LFS or Chompret criteria, *TP53* analysis was performed using Sanger sequencing of coding regions and MLPA ($N=105$). From 2016 onward, genetic testing was standardized using our custom NGS panel I2HCP, which comprises up to 168 genes associated with hereditary cancer [20]. Copy-number variants were retrieved from NGS data using DECoN software [21] with parameter optimization [22]. *TP53* was sequenced in all patients ($N=6056$), although the initial diagnostics analysis of the panel was limited to a subset of clinically actionable genes ($N=7–15$) selected based on each patient's specific phenotype [23]. *TP53* analysis included all exons and intron–exon boundaries (± 20 bp).

All the variants identified in the *TP53* gene (NM_000546.5) at VAF ≥ 5% were classified following ClinGen *TP53* Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for *TP53* Version 1.4.0 [24, 25]. Single-nucleotide variants and indels classified as likely pathogenic or pathogenic (pathogenic variants onwards, PVs) were orthogonally confirmed by Sanger sequencing. Copy-number variants were confirmed using SALSA MLPA Probemix P056 (MRC Holland, Amsterdam, Netherlands).

Adherence to clinical criteria

An in-house bioinformatics pipeline was developed in order to assess the adherence to LFS/Chompret criteria. Briefly, the pipeline extracts clinical data and pedigree information from our internal database. It first defines familial relationships by assigning degrees of kinship (e.g., first-degree, second-degree relatives). Next, it evaluates cancer occurrences within the family, including tumor types and ages at diagnosis, and determines whether LFS/Chompret criteria are met (as defined in Additional file 1: Table S1). Fourteen families fulfilled 1988 LFS criteria, 62 fulfilled Chompret 2001, 212 fulfilled Chompret 2009, and 407 fulfilled Chompret 2015.

Variant origin assessment

Whenever possible, *TP53* variants were re-tested in non-hematopoietic, non-malignant tissue in those patients who did not meet LFS or Chompret clinical criteria (subgroups C2 and C3) and those harboring *TP53* variants at VAF < 35%. Tissue samples were selected in compliance with the following criteria to ensure the reliability of results: (1) non-malignant tissue with no lymphocyte content was chosen (saliva or buccal swabs were disregarded); (2) non-malignant tissue adjacent to tumorous tissue was discarded, in order to avoid tumor cell infiltration. DNA was extracted from FFPE blocks using Cobas DNA Sample Preparation kit (Roche, Basel, Switzerland), according to the manufacturers' protocols. Sanger sequencing was used in variants identified at VAF ≥ 10% and SNuPE in variants detected at VAF < 10%. VAF was calculated as follows: VAF (%) = V/(V + R)*100, being V the peak area of the variant allele and R the peak area of the reference allele [26]. All experiments were performed with three replicates.

Cascade testing

Cascade testing of relatives of cases harboring a PV was performed by Sanger sequencing or MLPA when their samples were available ($N=161$).

TP53 variant categorization

Variants were classified into three groups based on the functional assay performed by Giacomelli et al. [27]. The following variant types were designated: DNE_LOF, notDNE_LOF, and notDNE_notLOF. A fourth group including truncating variants with a predicted LOF nature (i.e., frameshift, nonsense, large rearrangements) not investigated by Giacomelli et al. was designated as "Predicted_LOF". A fifth group designated as "Unknown" consisted of variants not investigated

in the original assay or variants with conflicting functional evidences (Additional file 2: Table S2).

mRNA assays

Total RNA was isolated using TRIzol reagent from cultured peripheral blood lymphocytes treated with/without puromycin, and reverse transcribed with iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). cDNA amplification was performed using exonic primers that encompassed the region of interest. Transcriptional profiles from variant carriers were compared to ten controls (cancer-free individuals with no PVs identified) by agarose gel analysis and Sanger sequencing.

In order to evaluate the extent of the splicing effect, bands corresponding to the full-length transcript were excised from agarose gels and single-nucleotide primer extension (SNuPE) assay was performed to assess allele-specific expression (ASE) using a tag-SNP, as previously described [28]. ASE was calculated by dividing the proportion of variant/reference allele in cDNA by the proportion of variant/reference allele in gDNA. All experiments were performed with three replicates.

All primer sequences and PCR conditions are available upon request.

Statistical analyses

All statistical analyses were performed using GraphPad Prism v6.0 (GraphPad Software, Boston, MA, USA). Statistical significance was set at $p < 0.05$.

Results

Identification of *TP53* PVs through blood testing of suspected hereditary cancer patients

The study comprises a total of 6161 unrelated patients suspected of having a hereditary cancer syndrome based on young/early age at cancer diagnosis and/or their personal/family history of cancer or specific non-malignant tumors. Patients were categorized in three cohorts (C1–C3), according to the fulfillment, or not, of specific clinical criteria (Fig. 1A).

A total of 45 *TP53* PVs in blood DNA were identified: 21 in 495 (4.2%) C1 patients, who fulfilled either the classic LFS criteria [7] and/or any of the Chompret criteria [4–6] (Additional file 1: Table S1); 7 in 2481 (0.3%) C2 patients, who were diagnosed with breast or colorectal cancer before age 50; and 17 in 3185 (0.5%) C3 patients, for whom *TP53* germline testing was not clinically indicated (Table 1).

Germline or somatic origin of the identified *TP53* PVs

TP53 PVs were presumed to be of germline origin (constitutional) when detected at a VAF of around 50% in

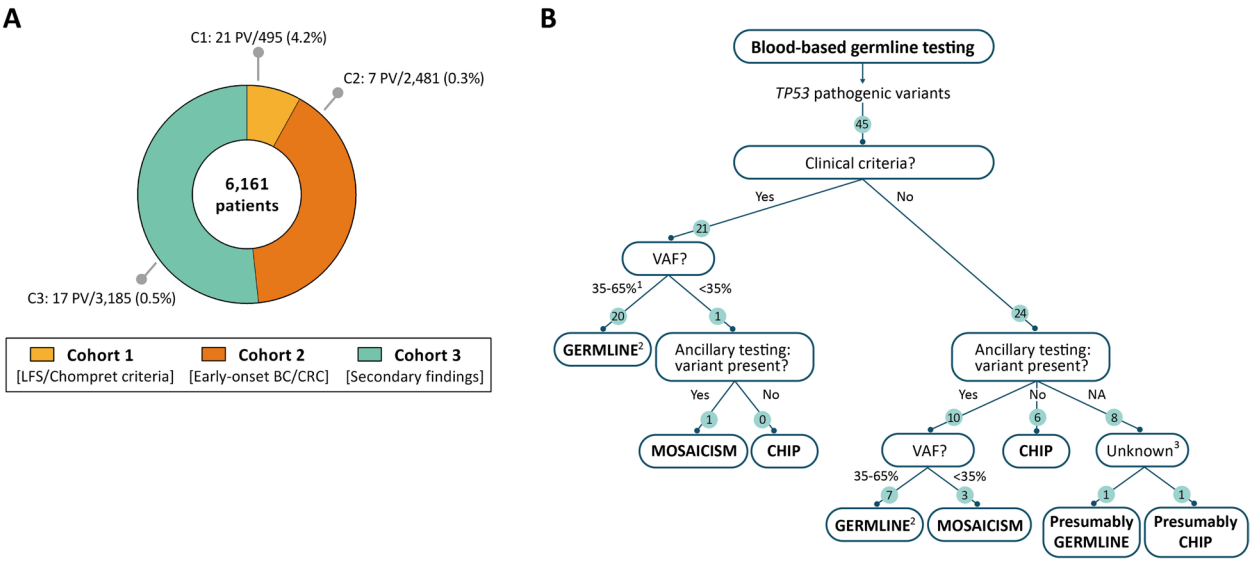

Fig. 1 Patient cohorts and workflow for the evaluation of *TP53* variant origin. **A** Pie chart displaying the distribution of 6161 patients in three different cohorts, based on the fulfillment (or not) of *TP53* clinical testing criteria. *TP53* mutational rate is indicated by cohort. **B** Workflow summarizing the assessment of the origin of 45 *TP53* pathogenic variants identified in our cohort. Considerations: [1]One biallelic carrier was identified in our cohort (VAF = 100%); [2]The identification of an additional heterozygous carrier within the family by cascade testing also confirms germline origin. [3]Variant origin could not be assessed for 8 *TP53* pathogenic variants. For patients 28 and 43, origin was presumed from genotype/clinical data. Abbreviations: BC: breast cancer; CHIP: clonal hematopoiesis of indeterminate potential; CRC: colorectal cancer; LFS: Li-Fraumeni syndrome; NA: Not assessed; PV: pathogenic variant; VAF: variant allele frequency

Table 1 Characteristics of the *TP53* PVs identified in 45 patients of the three clinical cohorts evaluated

Patient ID	*TP53* testing indication	Proband's cancer diagnosis (age)	Variant (c.)	Variant (p.)	Type	Class	Effect[a]	Detection method	Variant origin	Blood VAF (%)	VAF in other tissues (%)	Germline (likely) pathogenic variants in other cancer-susceptibility genes
Cohort 1 (C1)												
1	Chompret 2001, 2009, 2015	Breast (33)	c.332 T > C	p.(Leu111Pro)	Missense	LP	DNE_LOF	Multi-gene panel	Germline	45.1	Not tested	Not identified
2	Chompret 2015	Breast (17); Gastric (33)	c.374C > G	p.(Thr125Arg)	Missense	LP	DNE_LOF	Multi-gene panel	Germline	46.0	Not tested	Not identified
3	Chompret 2009	Bladder (46); Prostate (49); Colorectal (50); Lung (51)	c.374C > T	p.Thr125Met	Missense	LP	notDNE_not-LOF	Multi-gene panel	Germline[d]	99.7	Not tested	Not identified
4	Chompret 2001, 2009, 2015	Brain (34)	c.375G > A	p.Gly59Valfs*23	Splice	P	Predicted_ LOF[c]	Multi-gene panel	Germline	44.6	Not tested	Not identified
5	Chompret 2001, 2009, 2015	Brain (25); Breast (31); Sarcoma (41)	c.375G > A	p.Gly59Valfs*23	Splice	P	Predicted_ LOF[c]	Multi-gene panel	Germline	57.9	Not tested	Not identified
6	Chompret 2001, 2009, 2015	Sarcoma (24); Breast (bilateral) (31,31); Colorectal (32)	c.452C > G	p.(Pro151Arg)	Missense	LP	DNE_LOF	Sanger sequencing	Germline	~50[f]	Not tested	Not analyzed
7	Chompret 2009	Lung (38)	c.473G > A	p.(Arg158His)	Missense	P	DNE_LOF	Multi-gene panel	Germline[d]	53.9	Not tested	Not identified
8	Chompret 2001, 2009, 2015	Sarcoma (20); Breast (bilateral) (43,66)	c.473G > A	p.(Arg158His)	Missense	P	DNE_LOF	Multi-gene panel	Germline	46.5	Not tested	Not identified
9	Chompret 2001, 2009, 2015	Breast (36); Sarcoma (48)	c.542G > A	p.(Arg181His)	Missense	LP	Unknown	Multi-gene panel	Germline[d]	52.2	Not tested	Not identified
10	Chompret 2015	Breast (27)	c.724 T > G	p.(Cys242Gly)	Missense	LP	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
11	Chompret 2009	Desmoid (15); Lung (30)	c.725G > A	p.(Cys242Tyr)	Missense	LP	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed

Table 1 (continued)

Patient ID	*TP53* testing indication	Proband's cancer diagnosis (age)	Variant (c.)	Variant (p.)	Type	Class	Effect[a]	Detection method	Variant origin	Blood VAF (%)	VAF in other tissues (%)	Germline (likely) pathogenic variants in other cancer-susceptibility genes
12	Chompret 2001, 2009, 2015	Breast (39); Ovary (44); Colorectal (46)	c.733G > A	p.(Gly245Ser)	Missense	P	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
13	Chompret 2009	Breast (39); Lung (63)	c.742C > T	p.(Arg248Trp)	Missense	P	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
14	Chompret 2001, 2009, 2015	Skin non-melanoma (27); Breast (30); Sarcoma (32)	c.742C > T	p.(Arg248Trp)	Missense	P	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
15	Chompret 2001, 2009, 2015	Uveal melanoma (36); Breast (44); Lung (54)	c.743G > A	p.(Arg248Gln)	Missense	P	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
16	LFS; Chompret 2001, 2009, 2015	Sarcoma (2)	c.783-1G > A	p.[Gly262_Ser269del, Ser261_Gly262insAsn, Ser261Argfs*64]	Splice	LP	Unknown[b]	Multi-gene panel	Germline[d]	~50[f]	Not tested	Not identified
17	LFS; Chompret 2001, 2009, 2015	Breast (29,32); Lung (35); Brain (35)	c.844C > T	p.(Arg282Trp)	Missense	P	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
18	LFS; Chompret 2001, 2009, 2015	Breast (30)	c.844C > T	p.(Arg282Trp)	Missense	P	DNE_LOF	Sanger sequencing	Germline[d]	~50[f]	Not tested	Not analyzed
19	Chompret 2009	Cervix (26); Breast (51)	c.1010G > A	p.(Arg337His)	Missense	P	notDNE_not-LOF	Multi-gene panel	Germline[d]	~50[f]	Not tested	Not identified
20	Chompret 2009	Breast (49)	c.1024C > T	p.(Arg342*)	Nonsense	LP	notDNE_LOF	Multi-gene panel	Mosaicism	5.4	3.9 (normal[g])	*RAD51D* c.694C > T; p.(Arg232*) (LP)
21	Chompret 2009, 2015	Breast (54); Sarcoma (63); Colorectal (66)	Exon 1 deletion	p.?	Large rearrangement	P	Predicted_LOF	MLPA	Germline[d]	~50[f]	Not tested	Not analyzed

Table 1 (continued)

Patient ID	*TP53* testing indication	Proband's cancer diagnosis (age)	Variant (c.)	Variant (p.)	Type	Class	Effect[a]	Detection method	Variant origin	Blood VAF (%)	VAF in other tissues (%)	Germline (likely) pathogenic variants in other cancer-susceptibility genes
Cohort 2 (C2)												
22	Early-onset BC	Breast (47)	c.245del	p.(Pro82Argfs*41)	Frameshift	LP	Predicted_ LOF	Multi-gene panel	CHIP	15.7	0	Not identified
23	Early-onset BC	Breast (42)	c.374C>T	p.Thr125Met	Missense	LP	notDNE_not-LOF	Multi-gene panel	Germline[d]	50.3	Not tested	Not identified
24	Early-onset BC	Breast (43)	c.542G>A	p.(Arg181His)	Missense	LP	Unknown	Multi-gene panel	Germline[d]	~50[f]	Not tested	Not identified
25	Early-onset BC	Breast (35)	c.659A>G	p.(Tyr220Cys)	Missense	P	DNE_LOF	Multi-gene panel	Germline	48.0	~50[e] (normal[g])	Not identified
26	Early-onset CRC	Colorectal (47)	c.844C>T	p.(Arg282Trp)	Missense	P	DNE_LOF	Multi-gene panel	Mosaicism	27.8	17 (normal[g]); 57 (tumor)	Not identified
27	Early-onset BC	Breast (34)	c.949C>T	p.(Gln317*)	Nonsense	LP	notDNE_LOF	Multi-gene panel	Not tested	6.9	Not tested	Not identified
28	Early-onset BC	Burkitt lymphoma (34); Breast (45); Sarcoma (47)	c.1010G>A	p.(Arg337His)	Missense	P	notDNE_not-LOF	Sanger sequencing	Presumably germline	~50[f]	Not tested	Not analyzed
Cohort 3 (C3)												
29	No	Breast (56)	c.90del	p.(Asn30Lysfs*14)	Frameshift	LP	Predicted_ LOF	Multi-gene panel	Mosaicism	16.1	12 (normal[g]) 7 (tumor)	Not identified
30	No	Kidney (53); Prostate (55)	c.374C>T	p.Thr125Met	Missense	LP	notDNE_not-LOF	Multi-gene panel	Germline	50.7	~50[e] (normal[g])	Not identified
31	No	Colorectal polyp (80)	c.537 T>A	p.His179Gln	Missense	LP	DNE_LOF	Multi-gene panel	CHIP	37.0	0	Not identified
32	No	Leukemia (11)	c.638G>A	p.(Arg213Gln)	Missense	LP	DNE_LOF	Multi-gene panel	Germline[d]	~50[f]	Not tested	Not identified
33	No	Ovary (45)	c.659A>G	p.(Tyr220Cys)	Missense	P	DNE_LOF	Multi-gene panel	Not tested	24.8	Not tested	*BRCA1* c.3607C>T; p.(Arg1203*) (P)
34	No	Ovary (70)	c.659A>G	p.(Tyr220Cys)	Missense	P	DNE_LOF	Multi-gene panel	CHIP	83.0	0	Not identified
35	No	Ovary (60)	c.659A>G	p.(Tyr220Cys)	Missense	P	DNE_LOF	Multi-gene panel	CHIP	8.5	0	Not identified
36	No	Ovary (78)	c.700 T>C	p.(Tyr234His)	Missense	P	DNE_LOF	Multi-gene panel	CHIP	20.5	0	Not identified

Table 1 (continued)

Patient ID	*TP53* testing indication	Proband's cancer diagnosis (age)	Variant (c.)	Variant (p.)	Type	Class	Effect[a]	Detection method	Variant origin	Blood VAF (%)	VAF in other tissues (%)	Germline (likely) pathogenic variants in other cancer-susceptibility genes
37	No	Colorectal (83)	c.733G>T	p.(Gly245Cys)	Missense	P	Unknown	Multi-gene panel	Not tested	16.3	Not tested	Not identified
38	No	Ovary (62)	c.742C>T	p.(Arg248Trp)	Missense	P	DNE_LOF	Multi-gene panel	Mosaicism	6.6	1% (normal[g])	Not identified
39	No	Leukemia (45); Colorectal (52); Ovary (64)	c.743G>A	p.(Arg248Gln)	Missense	P	DNE_LOF	Multi-gene panel	Not tested	8.9	Not tested	Not identified
40	No	Breast (56)	c.746_747dup	p.(Pro250Glyfs*96)	Frameshift	LP	Predicted_LOF	Multi-gene panel	CHIP	18.8	0	*ATM* c.1110C>G; p.(Tyr370*) (P) *PALB2* c.1192del; p.(Val398Cysfs*26) (LP)
41	No	Colorectal (56); Ovary (56)	c.783-1G>A	p.[Gly262_Ser269del, Ser261_Gly262insAsn, Ser261Argfs*64]	Splice	LP	Unknown[b]	Multi-gene panel	Germline	52.0	~50[e]	Not identified
42	No	Unknown	c.817C>T	p.(Arg273Cys)	Missense	P	DNE_LOF	Multi-gene panel	Not tested	7.2	Not tested	Not identified
43	No	Ovary (58); Leukemia (72)	c.988del	p.(Leu330Phefs*15)	Frameshift	LP	Predicted_LOF	Multi-gene panel	Presumably CHIP[e]	52.2	Not tested	Not identified
44	No	Pancreas (72)	c.1010G>A	p.(Arg337His)	Missense	P	notDNE_not-LOF	Sanger	Germline[d]	~50[f]	Not tested	Not analyzed
45	No	Breast (64)	c.1006G>T	p.(Glu336*)	Nonsense	LP	notDNE_LOF	Multi-gene panel	Not tested	5.9	Not tested	Not identified

TP53 transcript: NM_000546.5

Abbreviations: BC Breast cancer,*CHIP* Clonal hematopoiesis of indeterminate potential, *CRC* Colorectal cancer, *LFS* Li-Fraumeni syndrome clinical criteria (defined in 1988), *LP* Likely pathogenic variant, *MLPA* Multiplex ligation-probe amplification, *P* Pathogenic variant, *VAF* Variant allele frequency

[a] Variant effect was categorized according to variant type and functional studies by [27] (PMID: 30224644). See Table S2 for more details

[b] Unknown variant effect was assigned to c.783-1G>A based on the conflicting mRNA assay results and the highly variable penetrance and clinical manifestations of the identified heterozygous carriers

[c] c.375G>A variant effect was addressed as "Predicted_LOF" based on functional assay evidence demonstrating aberrant splicing, warranting reinterpretation from Giacomelli's "notDNE_notLOF"

[d] The germline origin of the variant was demonstrated based on the identification of the variant in other family members

[e] CHIP was not experimentally demonstrated (non-hematological tissues were not tested), but it is compatible with the patient's clinical history

[f] Exact VAF not available, apparently heterozygous by Sanger sequencing

[g] Normal refers to non-malignant, non-hematopoietic tissue

blood in patients fulfilling LFS/Chompret clinical criteria (C1, cases 1–19 and 21) [9]. In fact, cascade testing in relatives of 14 of the 21 C1 patients (67%) with a *TP53* variant detected in blood confirmed the constitutional nature of the PV (Table 1).

Patient 3 was a biallelic carrier of the *TP53* c.374C > T (p.Thr125Met) variant (VAF = 99.7%) with no core tumors from the LFS narrow spectrum. He developed a high-grade pT1 urothelial carcinoma at age 46, followed by a right colon adenocarcinoma at age 50 (pT3pN0) and a lung adenocarcinoma at age 51 (pT1b) [29], despite being a non-smoker. The lung cancer was incidentally diagnosed during colorectal surveillance. Additionally, a Gleason 3 + 3 prostate adenocarcinoma was identified at age 49 during urothelial follow-up. He was first referred to the Genetic Counseling Unit in 2019 due to fulfilling the Bethesda criteria (colorectal cancer diagnosed ≤ 50), and germline *TP53* testing was performed in accordance with the Chompret 2009 criteria.

The presence of *TP53* mosaicism was confirmed in patient 20, who fulfilled Chompret 2009 criteria, as the variant was identified at a VAF of 5.4% (26/481 reads) in blood and of 3.9% in unaffected tissue from the gallbladder. This patient also harbored a germline heterozygous *RAD51D* PV, presumably related to her triple negative breast cancer diagnosis at the age of 49.

Ancillary testing in non-malignant, non-hematopoietic tissue was performed whenever possible, irrespective of VAF, in patients with blood *TP53* PVs from C2 (test performed in 3/7 patients), and from C3 (9/17 patients), in order to assess whether those variants were constitutional or somatically acquired. In addition, cascade testing in relatives was available for 2/7 C2 patients and 2/17 C3 patients. In all, the origin of the variant was depicted in 16/24 C2 and C3 patients.

Available evidence supported a germline origin of the *TP53* PV in eight of the 24 C2 or C3 patients, all of whom had a *TP53* PV VAF in blood of ~ 50% (Table 1). The constitutional nature of the variant was confirmed in four of these eight cases through cascade testing in other family members, and in three cases by identifying the variant in heterozygosis in non-hematological, unaffected tissue. The eighth case, patient 28 (C2), diagnosed with young-onset sarcoma and breast cancer, did not meet any clinical criteria because in situ breast cancers were excluded from "core" tumors. The variant was presumed to be germline based on the 50% VAF in blood, along with the strongly suggestive clinical context.

Somatic mosaicism was demonstrated in three of the 24 C2 or C3 patients by detecting the variant at low VAF (range 6.6–27.8%) in non-hematologic, non-neoplastic tissues: one C2 case (patient 26), diagnosed with early-onset breast cancer, and two C3 cases (patients 29 and 38), diagnosed with breast and ovarian cancer, respectively. While the three patients reported family history, cancer diagnosis belonged to the LFS spectrum only in family 29 (post-menopausal breast cancer and high grade glioma at age 43 in the proband's maternal aunt and cousin, respectively; cascade testing not available).

In the remaining six of the 24 C2 or C3 patients who underwent ancillary testing, all of whom had *TP53* PVs in blood samples (VAF range 8.5–83.0%), the variant was exclusively detected in blood, suggesting CHIP. These cases included one patient from the C2 subgroup, diagnosed with early-onset breast cancer, and five patients from C3 with variable cancer diagnoses (Table 1).

Even though ancillary testing could not be performed in patient 43 (C3 subgroup), the absence of an h*TP53*rc-associated clinical phenotype, the age at sample collection (> 70 years old), and the history of oncologic treatment of the patient suggested a CHIP origin. The patient, diagnosed with high-grade serous ovarian carcinoma at age 70, was exposed to adjuvant chemotherapy with an alkylating agent and to Olaparib. Seventeen months after starting the treatment she was diagnosed with high-risk myelodysplastic syndrome, likely as consequence of the treatment with the PARP1-inhibitor [30], and she was deceased a few months later. As germline testing was performed after the treatment of the ovarian cancer, it is likely that the exposure to both alkylating agents and Olaparib could explain the clonal expansion of hematopoietic cells harboring *TP53* p.(Leu330Phefs*15) [31], resulting in a VAF comparable to a germline variant. Predictive testing in two of her three children did not identify the variant in any of them, further supporting a non-germline origin.

Taken together, the origin of the *TP53* variant was elucidated or presumed in 39 out of 45 patients harboring *TP53* PVs in blood DNA with VAFs ≥ 5%: 28 (71.8%) patients had a constitutional *TP53* variant, four (10.3%) harbored a *TP53* PV in mosaicism, and seven (17.9%) acquired the corresponding PV through CHIP (Fig. 1B). By clinical groups, 95.2% (20/21) of *TP53* PVs in C1 patients were constitutional, compared with 66.7% in C2 and 36.4% in C3; 4.8% (1/21) in C1 were mosaicisms, 16.7% in C2 and 18.2% in C3; and 0% in C1 arose as consequence of CHIP, 16.7% in C2 and 46.2% in C3 patients.

Performance of LFS and Chompret criteria to identify germline *TP53* PVs

Of the 28 families harboring a germline *TP53* PV, 20 (71.4%) fulfilled any of the clinical criteria defined over time to select patients for germline testing (subgroup C1) (Table 1).

According to our data, the positive predictive value per clinical criteria was 21.4% (3/14) for the classic LFS

criteria, 19.4% (12/62) for the 2001 Chompret criteria, 9.0% (19/212) for 2009 Chompret, and 3.7% (15/407) for 2015 Chompret. Conversely, sensitivity increased from the 10.7% (3/28) of the 1988 LFS clinical criteria to 42.9% (12/28) of Chompret 2001, 67.9% (19/28) of Chompret 2009, and 53.6% (15/28) of Chompret 2015. Figure 2 shows the fulfillment of different testing criteria for each patient, including the overlap between these criteria.

Characteristics of the *TP53* PVs

Regardless of their germline or somatic origin, 29 unique *TP53* PVs were identified in 45 index cases from our complete cohort: nineteen missense, two affecting splicing, four frameshift, three nonsense variants, and one large rearrangement (Fig. 3; Additional file 2: Table S2).

TP53 missense PVs were identified as either germline PVs, as mosaics, or associated with CHIP. Except for one recurrent germline variant, c.1010G > A (p.Arg377His), located in the tetramerization domain, the other 18 missense variants were all in the DNA-binding domain (Fig. 3).

Two germline splice variants were identified in four patients of our cohort: c.375G > A, which involves the last nucleotide of exon 4 (patients 4 and 5); and c.783-1G > A (patients 16 and 41). As previously demonstrated by our group (mRNA analysis), c.375G > A causes the partial skipping of 200 nucleotides at the 3' end of exon 4 (r.176_375del), predicted to generate a truncated protein (p.Gly59Valfs*23) [28]. Through mRNA analysis we observed that c.783-1G > A results in three aberrant splicing transcripts: (1) partial in-frame skipping of exon 8 (r.783_806del; p.Gly262_Ser269del); (2) partial in-frame insertion of intron 7 (r.782_783ins[783-3_783-2;a]; p.Ser261_Gly262insAsn); and (3) total intron 7 retention (r.782_783ins[782 + 1_783-2;a]), presumably resulting in a truncated protein (p.Ser261Argfs*64) (Additional file 3:

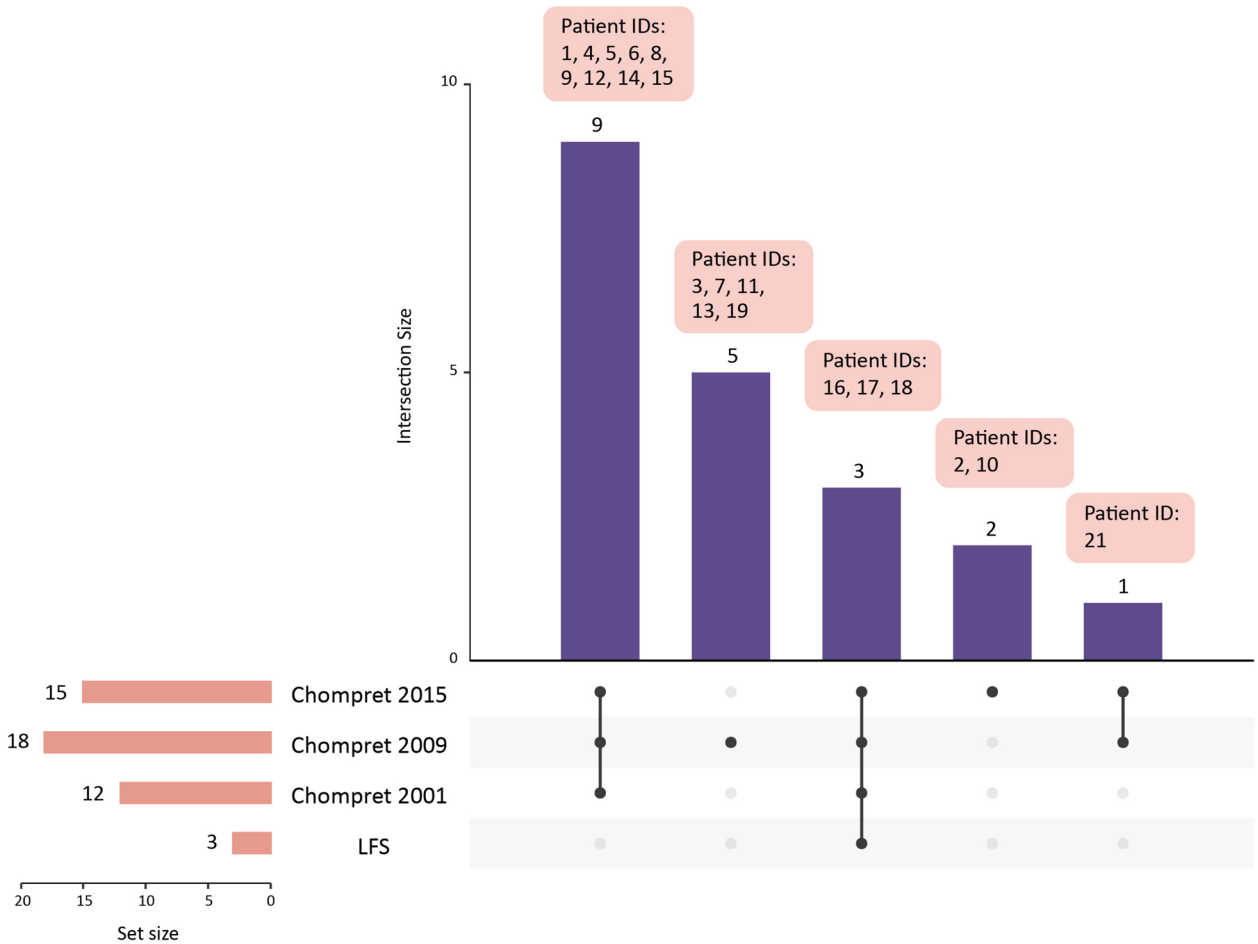

Fig. 2 Upset plot illustrating the overlap of *TP53* testing criteria met by germline pathogenic variant carriers. Four classification criteria are defined: classic 1988 Li-Fraumeni syndrome, Chompret 2001, Chompret 2009, and Chompret 2015. Horizontal bars represent the total number of patients for each category (set sizes). Vertical bars display the intersection sizes, with dots and connecting lines below indicating the criteria involved in each intersection. Patient IDs for each intersection are labeled above the bars. Abbreviations: LFS: Li-Fraumeni syndrome

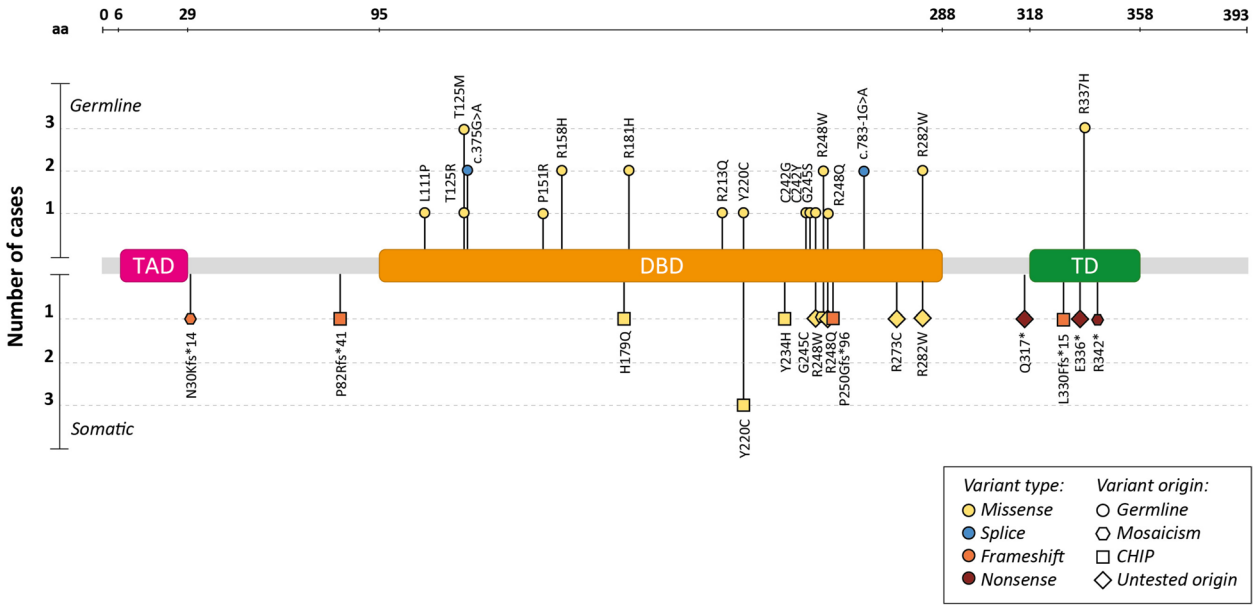

Fig. 3 Spectrum of germline and somatic *TP53* pathogenic variants identified in our cohort. Variant location is displayed by lollipop structures. Color and shape coding correspond to variant consequence and origin as indicated in the figure key. A copy-number variant encompassing exon 1 (non-coding), found in one index case, is not represented in the scheme. TP53 protein domains are shown in colored boxes with an amino acid numbered scale on top. *TP53* transcript: NM_000546.5. Abbreviations: aa: amino acid; CHIP: clonal hematopoiesis of indeterminate potential; DBD: DNA binding domain; TAD: transactivation domain; TD: tetramerization domain

Fig. S1). For both variants RNA assays demonstrated that the variant allele did not produce any full-length transcript. Following *TP53* variant classification guidelines, c.375G > A was classified as pathogenic and c.783-1G > A as likely pathogenic (Additional file 2: Table S2) [25].

Four frameshift and three nonsense variants were identified, distributed throughout the *TP53* gene. Interestingly, all of them, identified in single index cases, had a somatic origin (two mosaics, two associated with CHIP, three unknown).

A large deletion of the genomic region encompassing *TP53* exon 1 (non-coding) was identified in patient 21. Allele-specific expression analysis using a polymorphism present in the patient, c.215C > G, revealed that only the G allele was detected in the patient's RNA (Additional file 4: Fig. S2), supporting the lack of expression of the allele affected by the exon 1 deletion, and leading to the classification of the variant as pathogenic (Additional file 2: Table S2).

Several germline *TP53* variants were recurrently found in our cohort, being c.374C > T, p.(Thr125Met); c.659A > G, p.(Tyr220Cys); c.742C > T, p.(Arg248Trp); c.844C > T, p.(Arg282Trp), and c.1010G > A, p.(Arg377His) the most recurrent, each identified in at least three index cases. p.Arg337His, identified in patients 19, 28, and 44, has been reported as a founder variant in southern Brazil [32]. Interestingly, patients 19 and 44 are originally from Spanish region adjacent to Portugal, and patient 28's family name is Portuguese. These data might suggest that the origin of the founder Brazilian variant is in Portugal, and was also spread to adjacent regions in Spain. Haplotype analysis in Brazilian and Spanish heterozygote patients would be necessary to confirm this hypothesis. On the other hand, several of the identified variants, regardless of their germline or somatic origins, were located in known *TP53* mutation hotspots, affecting residues 245, 248, 273, and 282, but not hotspots 175 and 249 [24] (Fig. 3).

Phenotypic features of germline *TP53* carriers

Due to the high cancer risk in *TP53* heterozygotes, particularly at early age, current guidelines recommend cascade testing in all relatives once a *TP53* PV is identified in the family [8, 9]. In our cohort of germline carriers, we performed cascade testing in 155 family members, identifying 77 additional heterozygotes. Since patient 3 was a biallelic carrier of a *TP53* PV, he was excluded from the following calculations.

A total of 104 germline heterozygous *TP53* PV carriers, 62 (59.6%) females and 42 (40.4%) males, were identified: 27 index cases and 77 additional family members. At the time of assessment, 67 (64.4%) had developed at least one malignancy (median age at diagnosis: 39 (range 2–77). Multiple primary cancers were diagnosed in 22

individuals (21.2%). Most cancer-free heterozygotes were male: 54.8% vs. 22.5% female cancer-free individuals. Female carrier patients presented a younger median age at first cancer diagnosis compared to male carriers (38 vs. 44 years).

Among the 67 cancer-affected germline *TP53* PV carriers, a total of 103 cancers were diagnosed, being the most recurrent: breast cancers ($N=37$), sarcomas ($N=12$), lung cancers ($N=9$), brain tumors ($N=8$), colorectal ($N=6$), and prostate cancers ($N=3$) (Table 2; Additional file 5: Table S3). Lung cancers for which histological and molecular data were available ($N=7/9$) were all adenocarcinomas (7/7), and 71.4% (5/7) harbored somatic *EGFR* mutations (exon 19 deletion in four cases and L858R in one).

Table 2 Tumor diagnoses in germline heterozygous *TP53* PV carriers

Tumor type	Number of tumors	% of cancer-affected carriers (total no. of carriers: 104[a])	Mean/median age at tumor diagnosis (range)
Any cancer	103	65.4% (68/104)	39/39 (2 – 77)
Breast	37	50% (31/62[b])	41/42 (17 – 68)
Sarcoma[c]	12	10.6% (11/104)	33/31 (2 – 63)
Lung	9	8.7% (9/104)	49/47 (30 – 77)
Brain	8	7.7% (8/104)	37/35 (14 – 59)
Colorectal	6	5.8% (6/104)	48/51 (30 – 66)
Prostate	3	3.8% (4/104)	59/56 (55 – 65)
Kidney	3	2.9% (3/104)	51/53 (42 – 57)
Leukemia	3	2.9% (3/104)	15/14 (11 – 21)
Biliopancreatic[d]	3	2.9% (3/104)	53/48 (38 – 72)
Lymphoma	2	1.9% (2/104)	42/42 (34 – 51)
Germ cell tumor	2	1.9% (2/104)	18/18 (17 – 20)
Thymoma	2	1.9% (2/104)	34/34 (30 – 38)
Ovary	2	1.9% (2/104)	50/50 (44 – 56)
Skin melanoma[e]	2	1.9% (2/104)	48/48 (47 – 48)
Skin non-melanoma	2	1.9% (2/104)	38/38 (27 – 49)
CUP	2	1.9% (2/104)	Unknown
Bladder	1	1% (1/104)	57
Uveal melanoma	1	1% (1/104)	36
Head and neck	1	1% (1/104)	49
Anal canal	1	1% (1/104)	39
ACC	1	1% (1/104)	23

ACC adrenocortical carcinoma, *CUP* carcinoma of unknown primary

[a] One biallelic carrier was excluded from the analysis

[b] Considering only female carriers. No male breast cancer cases were diagnosed in the reported germline *TP53* PV carriers

[c] The term "sarcoma" includes osteosarcomas and soft-tissue sarcomas

[d] The biliopancreatic tumors identified include two individuals with biliary tract cancer from the same family, and one individual with pancreatic cancer from another family

[e] The two patients diagnosed with skin melanoma belonged to the same family

Tumor aggregation within families was analyzed. After excluding bilateral affectation in pair organs (only counted once), major cancer aggregation was observed for breast cancer (two families with more than three affected members each, and two families with two affected members), followed by sarcoma (two families with two affected members), brain (one family with three affected members), lung (one family with three affected members), and pancreas/biliary tract cancer (one family with two affected members).

Genotype–phenotype correlations among germline *TP53* carriers

The effect of *TP53* PVs was categorized in five groups: DNE_LOF, notDNE_LOF, notDNE_notLOF, Predicted_LOF, or Unknown (Table 1). Since DNE_LOF missense variants have been associated with more severe clinical features, we sought to investigate the clinical implications according to the functional effect of variants in terms of age at first tumor diagnosis, overall survival, multiplicity of tumors, and proportion of healthy carriers.

Of the 28 germline index cases, 15 *TP53* PVs were categorized as DNE_LOF, six as notDNE_notLOF, three as Predicted_LOF, and four as Unknown [27]. No germline variants belonged to group notDNE_LOF. Among the 67 cancer-affected *TP53* PV carriers, the proportion patients with DNE_LOF, notDNE_notLOF, and Predicted_LOF variants was 53.7%, 26.9%, and 10.4% respectively. Of the 37 cancer-free *TP53* PV carriers, the proportions were 37.8%, 29.7%, and 13.5% respectively.

The median age at first tumor diagnosis in DNE_LOF variant carriers was 31.5 years, 51.0 years in notDNE_notLOF variant carriers, and 47.0 years in Predicted_LOF variant carriers ($p=0.0005$, Kruskal–Wallis test). Subsequent pairwise comparisons revealed a significant difference between DNE_LOF and notDNE_notLOF variant carriers ($p<0.0001$, Mann–Whitney test) and a tendency, although non-significant, between DNE_LOF and Predicted_LOF variant carriers ($p=0.07$, Mann–Whitney test) (Fig. 4A).

Regarding overall survival, differences were statistically significant between DNE_LOF, notDNE_notLOF, and Predicted_LOF variant carriers (median survival: 52.0, 75.2, and 70.1 years respectively, $p=0.007$, Mantel-Cox test) (Fig. 4B).

No significant differences were detected among DNE_LOF, notDNE_notLOF, and Predicted_LOF variant carriers in terms of multiplicity of tumors and prevalence of healthy carriers in our cohort ($p=0.3$ and $p=0.5$ respectively, chi-square test).

Finally, we investigated the correlation between variant effect and the fulfillment or not of clinical criteria,

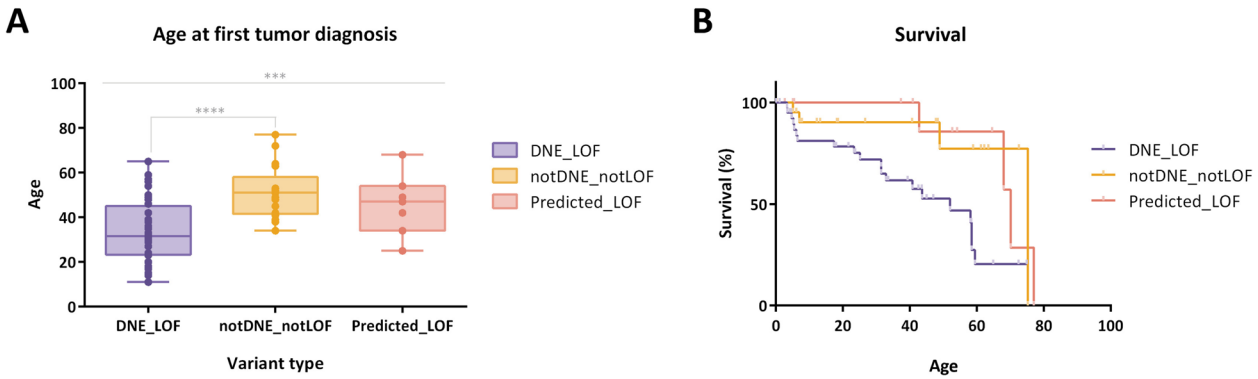

Fig. 4 Age at first tumor diagnosis (panel **A**) and overall survival (panel **B**) categorized by *TP53* variant effect. Variants are categorized into five groups (see Methods section for further details). Color coding corresponds to the variant effect categories as shown in the figure key. **A** The *x*-axis represents the categories of *TP53* pathogenic variants, while the *y*-axis shows the age at first tumor diagnosis. The central line in each box represents the median, and each patient is depicted as an individual point. Statistical significance was determined using the Kruskal–Wallis or the Mann–Whitney test. **p*-value < 0.05; ***p*-value < 0.01; ****p*-value < 0.001; *****p*-value < 0.0001. **B** The *x*-axis represents the overall survival in years, while the *y*-axis shows the survival rate. Statistical significance was determined using the Mantel-Cox test. Abbreviations: DNE: dominant negative; LOF: loss-of-function

finding a statistically significant association ($p = 0.02$, chi-square test).

Discussion

The aim of this study was to provide a comprehensive characterization of *TP53* in a large cohort of patients suspected of hereditary cancer. Since *TP53* is included in virtually all multi-gene cancer panels, genetic testing has been performed on a wide variety of individuals who do not meet the classical LFS or Chompret criteria. Germline *TP53* testing faces challenges related to the interpretation of somatic events and the use of blood samples. We defined three different patient groups based on the fulfillment of clinical indications for *TP53* genetic testing, and, whenever possible, we assessed the origin (somatic or germline) of the *TP53* PVs detected at VAF ≥ 5% (Fig. 1A, B). The highest prevalence of germline *TP53* PVs was detected among patients fulfilling either LFS and/or Chompret clinical criteria (71.4%), compared to those without clinical criteria (28.6%). To assess the prevalence of somatic events such as mosaicism and CHIP, ancillary testing and/or cascade testing in relatives were performed.

Somatic *TP53* variants

In the context of inherited cancer susceptibility, distinguishing germline and somatic variants is of paramount importance in terms of cancer risk estimation and patient management recommendations. Concerning *TP53*, several studies have reported that up to 40% of all variants detected by blood panel testing are somatic [33, 34]. Somatic origin may be suspected when identifying variants with low VAF, which could correspond to post-zygotic mosaicism, CHIP, circulating tumor DNA, or amplification artifacts. Several cases of *TP53* mosaicism have already been reported, accounting for up to 20% of all de novo variants [11, 35–37].

In our cohort, four out of 45 (10.5%) *TP53* PV carriers were mosaics, while seven out of 45 (15.8%) *TP53* PVs were related to CHIP (Table 1). Of the latter, six had received chemotherapy based on alkylating agents, which could underlie the clonal expansion. The other patient with a CHIP event was one of the oldest at testing (80 years old, patient 31).

The clinical implications and risk of transmission to offspring of somatically acquired variants depend on several factors. Variants found in mosaicism may or may not affect germ line cells. The fact that the ones identified in our study were detected in two different tissues might indicate that they occurred early in the embryonic development, and thus, might have affected germ cells [12]. Considering the difficulties of inferring the degree of mosaicism in the germ line, predictive testing should be performed in the progeny of suspected and confirmed mosaic individuals, who might be constitutional carriers of the same variant. In contrast, CHIP is a form of mosaicism restricted to the hematopoietic compartment, which appears during adulthood and entails no risk of intergenerational transmission. However, CHIP events may have clinical implications for the individual, due to the increased risk of hematologic and cardiovascular malignancies. This may justify increased surveillance protocols, which may be tailored according to the *TP53* VAF and the individual's clinical manifestations [38].

Constitutional heterozygosity is usually presumed at VAFs ≥ 30% [9, 15, 33, 34, 39, 40]. Interestingly, in our

study, some CHIP-derived *TP53* PVs were detected at VAFs ranging from 37 to 83% (cases 31, 34, and 43) (Table 1). These findings illustrate the importance of performing ancillary testing in all individuals who do not meet *TP53* testing criteria, irrespective of the VAF value. As per current clinical guidelines [9], *TP53* variants of cases 34 and 43 would have been misclassified as germline, resulting in unnecessary intensive cancer surveillance, useless cascade testing, and high psychological stress within families.

TP53 germline carriers: lung cancer awareness

The cohort of germline carriers was constituted by 105 individuals: 28 index cases and 77 relatives identified by cascade testing. LFS core tumors accounted for the majority of cancer occurrences among germline carriers, presenting with strong familial aggregation (Table 2; Additional file 2: Table S2). Breast cancer, in particular, occurred in 82.1% of our families (23/28), being HER2-positive the most common subtype, as previously reported [41]. However, cancer diagnoses per family were rarely restricted to this narrow spectrum of tumors. Other frequently observed tumors included leukemia, lung, prostate or kidney cancers, which further reinforced the idea that LFS cancer risk goes beyond core tumors [11].

Notably, lung cancer stood out as the third most common tumor in our cohort (9.5% of affected carriers). A recent multicenter study concluded that lung cancer risk was 4 to 9 times higher in male and female *TP53* PV carriers compared to the general population (HR = 4.8, 95% CI [1.5–15.6]; HR = 9.1, 95% CI [2.3–36], respectively) [42]. The strong familial aggregation and similar molecular features among all our patients (62.5% harbored somatic driver *EGFR* mutations) are noteworthy. Further studies are needed to determine whether *TP53* germline carriers are more likely to have *EGFR*-mutant lung cancer.

Although *TP53* remains the only gene associated with LFS, even after more than 50 years since it was first described, clinical criteria and tumor spectrum have been under continuous revision. Clinical criteria have evolved by compromising specificity to enhance sensitivity, aiming to improve the diagnosis of the syndrome. However, Chompret 2009 showed the highest sensitivity in our cohort. This is explained by the incorporation of lung cancer into the extended LFS spectrum of 2009 Chompret criteria [5], which was later removed in the 2015 revision [6]. In our series, families 3, 7, 11, 13, and 19 fulfilled only Chompret 2009 criteria due to the occurrence of lung carcinomas within the family, but would not be eligible for genetic testing according to the most recent guidelines (Chompret 2015) (Fig. 2). In line with all these observations, awareness should be raised regarding lung cancer predisposition among germline *TP53* PV carriers.

It is remarkable that eight out of 28 (28.6%) index cases harboring a germline *TP53* PV did not meet any clinical criteria for testing. Factors such as the broad phenotypic diversity beyond the narrow LFS tumor spectrum defined in the guidelines (cases 23, 28, 32, 41, and 44), ages of cancer onset above 46 years (case 30), or the absence of family aggregation, attributable to de novo variants (case 25) or lower disease penetrance of some variants (case 24), might be affecting the sensitivity of the clinical criteria [11].

TP53 variant nature has major clinical implications

Genotype–phenotype correlations were analyzed by assessing simultaneously the DNE and LOF properties of *TP53* PVs. Among germline heterozygous carriers, we replicated the strong association of DNE_LOF variants with earlier ages at first tumor onset compared to notDNE_notLOF variants, consistent with previous findings (Fig. 4A) [43]. While the association between DNE_LOF and Predicted_LOF variants was not significant, a trend was observed. This discrepancy with prior observations, suggesting that non-DNE variants may act as an intermediate class between DNE and LOF variants, may be partially attributed to the small number of notDNE_notLOF and Predicted_LOF variants in our cohort, limiting statistical power. Similarly, overall survival was significantly reduced among DNE_LOF variant carriers (Fig. 4B). However, these results should be interpreted with caution due to the low frequency or absence of notDNE_notLOF and notDNE_LOF germline variants in the analysis, as well as potential biases from losses to follow-up, treatment heterogeneity, and the differing prognoses of the tumors included in the study.

Our data highlighted a significant association between variant type and the fulfillment of clinical criteria, with most families meeting any clinical criteria harboring germline DNE_LOF variants compared to those who did not (65% vs. 25%). Despite the limited sample size, this finding suggests that *TP53* testing based on clinical criteria may be biased, as many LFS cohorts are enriched in DNE_LOF variants. This bias could lead to an overestimation of penetrance, underscoring the need to explore testing beyond clinical criteria in larger patient cohorts.

Among variants not included in the original assay by Giacomelli et al., one intriguing finding was the splice variant c.783-1G > A (detected in families 16 and 41). This rare variant is reported only in one non-Finnish European in gnomAD database. Its presumed LOF nature would make one expect a milder phenotype, at least in terms of age at cancer onset and clinical criteria fulfillment. As presumed, patient 41 did not fulfill clinical criteria and

the finding was incidental. In contrast, patient 16 fulfilled the most restrictive classic LFS 1988 criteria (Table 1). This variant is reported in three additional families in the literature, all presenting clinical features of LFS: one child diagnosed with ACC, one breast cancer patient diagnosed before age 30, and one lung cancer patient with family history of sarcoma and ACC [6, 44, 45]. An mRNA assay in patient 41 revealed three different aberrant transcripts: two in-frame (compatible with a DNE effect) and one out-of-frame (associated with a LOF effect) (Additional file 3: Fig. S1). We hypothesized that differences in transcript proportions might explain the phenotypic variety of c.783-1G > A carriers. Although we could not quantify transcript proportions, qualitative analysis suggested that the in-frame transcripts were more prevalent in patient 41. In contrast, Bonache et al. found the out-of-frame isoform predominantly expressed (54.4%), with one in-frame isoform at 14.5% [44]. Other unknown modifying factors may underlie this phenotypic variability. Due to its inconclusive effect, variant c.783-1G > A was excluded from statistical calculations.

Limitations and future steps

Our study represents a comprehensive analysis of *TP53* in a substantial cohort of suspected hereditary cancer patients, providing a thorough molecular and clinical characterization. The study underscores the importance of unraveling the origin of *TP53* variants by performing ancillary testing in as many cases as possible, irrespective of the VAF. We describe the tumor spectrum of *TP53* variant carriers and the mutational landscape at both germline and somatic levels. Despite these valuable insights, it is necessary to acknowledge the inherent limitations of our work. First, an ascertainment bias due to referral-based enrollment of adult patients suspected of hereditary cancer might have influenced tumor occurrences, ages of onset, and the performance of clinical criteria. Second, we have not considered the interplay of other genetic and environmental factors that may act as modifiers of the expressivity and penetrance of *TP53* variants. These elements may vary between unrelated individuals sharing a genetic variant or even within the same family, and could significantly impact the observed outcomes. Third, our decision to retrieve variants at a VAF ≥ 5% instead of the ≥ 2% recommended by the World Health Organization for CHIP [46] raises the possibility that some variants may have been overlooked. This decision was aimed at minimizing false-positive calls and simplifying the analysis, although it may hinder the true incidence of CHIP. In conclusion, we hope our study encourages further comprehensive analyses in larger cohorts, addressing the questions that were raised here. This collaborative effort could lead to a deeper understanding of hereditable *TP53*-related cancer syndrome and its related malignancies.

Conclusions

Our study underscores the relevance of somatic mosaicism and CHIP-derived variants in *TP53* germline genetic testing interpretation. We advocate for assessing the origin of *TP53* variants through ancillary testing in all individuals who do not meet *TP53* testing criteria, irrespective of the VAF. Additionally, we raise the question of whether lung cancer should be reincorporated in the LFS tumor spectrum, given the superior sensitivity of the Chompret 2009 criteria compared to Chompret 2015. Finally, we demonstrate the association of DNE_LOF variants with earlier tumor onset and reduced survival, and their increased prevalence among families meeting any clinical criteria.

Abbreviations

ASE	Allele-specific expression
CHIP	Clonal hematopoiesis of indeterminate potential
CNV	Copy-number variant
DNE	Dominant-negative effect
hTP53rc	Heritable TP53-related cancer syndrome
LFS	Li-Fraumeni syndrome
LOF	Loss-of-function
MLPA	Multiplex ligation-dependent probe amplification
NGS	Next-generation sequencing
PV	Pathogenic variant
SNuPE	Single-nucleotide primer extension
VAF	Variant allele frequency

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13073-025-01429-5.

Additional File 1: Table S1: Evolution of hereditable *TP53*-related cancer syndrome clinical criteria, from classical Li-Fraumeni to Chompret (2001, 2009 and 2015 versions).

Additional File 2: Table S2: Classification and functional effect of 29 pathogenic and likely pathogenic *TP53* variants identified in a cohort of 6,161 suspected hereditary cancer patients.

Additional File 3: Figure S1: mRNA splicing assay of variant *TP53* c.783-1G>A in patient 41.

Additional File 4: Figure S2: mRNA assay of *TP53* exon 1 deletion in patient 21.

Additional File 5: Table S3: Complete clinical data of families harboring *TP53* (likely) pathogenic variants.

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Authors' contributions

P.R., C.C., M.M., A.T., J.V., and C.L. conceived, designed, and planned the study; P.R., C.C., M.M., E.M., M.R-M., C.G., E.T., E.M., L.V., M.P., L.F., J.V. and C.L. contributed to the acquisition, analysis, and/or interpretation of the molecular data; C.C., A.T., S.I., E.D., G.C. and J.B. provided samples and clinical data; P.R. and C.C. drafted the manuscript. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article and its additional information files. Germline *TP53* (likely) pathogenic variants identified in this study were submitted to ClinVar database. Molecular Diagnostics Laboratory, Catalan Institute of Oncology. ClinVar—NCBI. SUB14877873. 2024. Available from: https://www.ncbi.nlm.nih.gov/clinvar/?term=SUB14877873.

Declarations

Ethics approval and consent to participate
The research was conducted in accordance with the principles of the Declaration of Helsinki, and ethical approval was obtained from the ethics committee of Bellvitge Biomedical Research Institute (IDIBELL; PR278/19). Informed written consent for both diagnostic and research purposes was obtained from all participants.

Consent for publication
Written informed consent for publication was obtained from all study participants.

Competing interests
The authors declare no competing interests.

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