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RESEARCH ARTICLE

Diagnosis, Follow Up and Clinical Management of Individuals Identified with a *TP53* Pathogenic Variant

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ABSTRACT

Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome. Germline pathogenic/likely pathogenic variants (P/LPVs) in the *TP53* gene are the only known genetic cause of this entity. Due to the severe phenotype and controversy over increasing surveillance and risk-reducing measures, *TP53* testing has traditionally only been offered when strict criteria were met. However, with the application of next generation sequencing (NGS) to multigene testing (MGT), such as hereditary breast cancer panels, *TP53* variants are being increasingly detected. In our multidisciplinary program, 2389 *TP53* molecular tests were performed between January 2000 and December 2021, resulting in the identification of 29 carriers harboring 20 different *TP53* P/LPVs, including one not previously described [c.242del p.(Thr81Asnfs*42)] and another of variable penetrance [c.799C>T p.(Arg267Trp)]. Two molecular findings with low allele frequencies (LAF) required additional diagnostic workup. Family phenotypes fulfilled Chompret (n=14), classic (n=4), or none of any previously described clinical criteria (n=4). For all cancers registered, patients had a first cancer diagnosis earlier when harboring DNE_LOF (DNE_LOF: dominant negative_loss of function), notDNE_LOF, frameshift and splicing variants ($p<0,05$), in contrast with notDNE_notLOF and unclassified variants. Breast (either as first or subsequent diagnosis) and cancers other than sarcomas and CNS, were diagnosed earlier in patients with notDNE_LOF variants ($p<0,05$).
For a follow-up of 51,5 months (2-118,9), we registered 11 deaths, 9 new cancers (all in previous cancer survivors), and 6 relapses (50% sarcoma cases). Radiotherapy-associated cancer was observed in one new cancer diagnosis. One healthy male underwent preimplantation genetic testing. With this study, we reinforce the need to provide multidisciplinary programs, even for a rare patient population, to avoid clinical mismanagement.

Keywords: Li-Fraumeni Syndrome, *TP53* gene, Breast Cancer, Multigene Testing, Mosaicism, Whole-body magnetic resonance imaging

Introduction

Li-Fraumeni Syndrome (LFS; MIM#151623 ORPHA:524), first described in 1969,[1] is an autosomal dominant cancer predisposition syndrome, associated with a wide spectrum of tumors such as adrenocortical carcinoma, breast cancer (BC), central nervous system tumor and soft-tissue sarcoma, among others. Germline *TP53* P/LPV are the only known cause of this entity. The *TP53* gene, which encodes a p53 protein, was initially described as an oncogene[2,3,4] but later reclassified as a tumor suppressor gene,[5,6] known as "the guardian of the genome". The p53 protein is involved in cell cycle regulation, genome integrity and cell proliferation.

Germinal *TP53* P/LPVs are rare. A recent study suggests that their prevalence in the general population is about 1:3555 to 1:5476[7]. However, this may vary according to specific populations, like the case of the founder variant c.1010G>A p.(Arg337His), described in 1:375 of the southern Brazilian population[8].

Different types of *TP53* P/LPVs include missense, nonsense, frameshift, splicing and large genomic rearrangements.[9] Missense P/LPVs can also be subclassified as dominant-negative (associated with malfunctioning or non-functioning p53 tetramers) that have been described as related to a more severe LFS phenotype.[10]

In recent years, new clinical and molecular studies changed the landscape of LFS. Clinical diagnoses evolved from classic clinical criteria with *TP53* P/LPVs identified in 70-80% of the cases[11,12] to a lower molecular detection rate, 14-35%, with Chompret criteria.[13,14] As for counselling and test prescription a change was observed, from single gene test (SGT) to MGT that include the *TP53* gene[15-17]. Thus, the number of atypical LFS families, not fulfilling clinical diagnostic criteria, is foreseen to increase, posing complex questions regarding genetic counselling as well as the management of these individuals and their families. Indeed, it is reported that *TP53* P/LPVs carriers identified by MGT are older and less likely to meet LFS clinical diagnostic criteria[18,19]. Studies reporting the changing phenotype associated with *TP53* P/LPVs led to the consideration of LFS as a wider cancer predisposition syndrome, designated as heritable *TP53*-related cancer (hTP53rc)[20].

There is controversy regarding the possibility of a genotype-phenotype correlation that would allow for the tailoring of the intensity and complexity in cancer risk management of *TP53* carriers. For LOF variants, previous studies reported the association with adult cancers and lower disease burden[10], but also with an earlier onset of the first cancer and more often meeting classic LFS and Chompret criteria[21,22]. On the other hand, dominant-negative variants have been correlated with high penetrance phenotypes and pediatric cancers[10,20]. A recent study elaborated on a different *TP53* variant categorization for missense and nonsense variants[23] that was also adopted by the International Agency of the Research on Cancer (https://tp53.isb-cgc.org/), reported that the earliest median age of first and second cancers were lower with DNE_LOF and notDNE_LOF variants, compared with notDNE_notLOF and DNE_notLOF variants[24]. Due to the inconsistency of data across several studies, at present, the genotype-phenotype correlation does not impact individual patient management.

Studies including whole-body magnetic resonance imaging (WBMRI) have been shown to improve long-term survival and early tumor detection[20,25,26]. Additionally, presymptomatic cancer surveillance was demonstrated to be cost-effective[27] and although psychosocial studies are scarce, screening protocols may have a positive effect on the psychosocial well-being of individuals belonging to LFS families[28].

In this study, we report molecular and clinical data from our prospective cohort of *TP53* families. Data collection started when clinical criteria and single gene testing were the paradigm and has evolved through the years, reflecting the evolution in counselling, molecular testing, and risk management as well as treatment decisions.

Materials and Methods

Patients: Review of genetic and clinical records of patients (pts) and family relatives diagnosed with *TP53* P/LPV through our program, between January 2000 and December 2021. These records include patient demographics, reason for referral, personal clinical history, DNA test results, and a pedigree with cancer and genetic information from at least three generations.

Genetic counselling: In the pre-NGS era, a single *TP53* genetic test was considered when a clinical diagnosis of LFS was made (classic or

Chompret criteria). Since September 2014, the introduction of NGS MGTs including the *TP53* gene have been increasingly prescribed. During the counselling and informed consent process, patients had the option to opt out of *TP53* testing, and this was weighed against the potential impact on RT treatment decisions (especially for early-stage BC patients) and risk management. If a patient was confirmed to be a carrier of a *TP53* P/LPV, genetic testing and counselling were offered to all relatives at risk.

Molecular diagnoses: DNA was extracted from peripheral blood. Sanger sequencing of all exons and adjacent intronic regions of the *TP53* gene was performed using the ABI Prism 3130/3500 Genetic Analyzer (Applied Biosystems, Foster City, USA). Starting in 2014, analysis of germline *TP53* single nucleotide variants (SNVs) and small indels was performed by next-generation sequencing (NGS) using either the Trusight Cancer Sequencing Panel (Illumina, San Diego, USA), the BRCA Hereditary Cancer MASTR™ Plus (BRCA plus) assay kit (Multiplicom NV, Agilent), or the Hereditary Oncokit DX (Imegen, Valencia, Spain) on a MiSeq platform (Illumina, San Diego, USA) was used according to the manufacturer's instructions. All P/LPV detected by NGS were confirmed by Sanger sequencing. Large deletions/insertions in the *TP53* gene were evaluated by multiplex ligation-dependent probe amplification (MLPA) analysis (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. Analysis was performed using the ABI Prism 3130/3500 Genetic Analyzer (Applied Biosystems, Foster City, USA) and results were obtained using Coffalyzer.Net software (MRC-Holland, Amsterdam, The Netherlands). Copy number variants (CNV) identified with Hereditary Oncokit DX were also confirmed with MLPA. Variants were named according to HGVS (version 15.11) and classified as P/LP according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines.[29] The *TP53* reference sequence is LRG_321t1.

Prospective follow-up: All *TP53* carriers were invited to participate in a prospective surveillance program. This follow-up included clinical and symptom surveillance, colorectal colonoscopy, breast and CNS MRI, and, since 2018, WBMRI. For each patient, the duration of follow-up was defined as the period since the post-test counselling visit to the last visit during the study period. Data collected included new cancer diagnoses and possible RT associated cancers and survival, which are considered events of interest. The institution approved protocol is the basis for risk management.

Statistics Descriptive statistics were obtained for the distribution of study variables using Microsoft Excel®.

Results

Of a total of 2389 molecular diagnostic *TP53* analyses (either single gene or MGT), 29 carriers from 22 unrelated families were identified with a *TP53* P/LPV (Figure 1). Fourteen (0.6%) pts opted out (chose not to undergo) *TP53* testing.

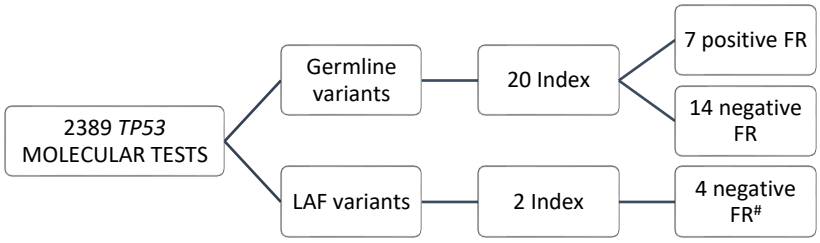


Figure 1. Identification of *TP53* carriers. (LAF - low allele frequency, FR - family relatives, #anciliary tests for results interpretation of index testing)

Phenotype: Twenty index patients (16 females, 4 males) were diagnosed with a germline *TP53* P/LPV (Table 1). In two female cancer pts, LAF *TP53* P/LPV were identified, and because these variants have a different diagnostic approach, these two families will be discussed separately. Twenty-five relatives were tested for the familial variant, resulting in the identification of 7 additional carriers. (3 healthy males and 4 affected females). All female carriers (20/27) in

our cohort were cancer survivors, mostly a breast cancer diagnosis (19/20). Among male survivors, sarcoma was the most diagnosed cancer (57%). Reverse phenotyping revealed that seventeen families (85%) met at least one of the diagnostic clinical criteria (classic or the 2015 Chompret criteria). The remaining families were identified only during MGT for BC patients.

Table 1. Characterization of the *TP53* cohort.

Cohort	**Germline *TP53* P/LPV (Families 1 to 20)**	**LAF *TP53* P/LPV (Families 21 and 22)**
Gender	**Total**	
Female	20	2
Male	7	0
Family phenotypes	**Total**	
Classic Criteria	4	0
2015 revised Chompret criteria	13	1
None	3	1
Age at genetic testing	**Median age (Min-Max)**	
Female	40 (20-71)	39 (21-57)
Male	30 (15-53)	0
Primary cancers	**Total**	
Female	median 2 (1-4)	1
Male	median 1,5 (1-4)	0
Breast Cancer	**Total**	
	26	1
Sarcoma	**Total**	
	12	0
CNS Tumors	**Total**	
	2	0
Pediatric Tumors (<18yrs old)	**Total**	
	6	0

Breast cancer and sarcomas were the most common cancers diagnosed in adults, while sarcomas and central nervous system (CNS) tumors predominated in those under 18 years of age. Among adults, in addition to breast and sarcoma, individual cases of the following tumors were registered: Glioblastoma, kidney, lung, colon, gastric, squamous cell carcinoma, melanoma, and serous endometrial carcinoma. Relevant benign tumors included multiple colorectal adenomas in at least 4 carriers during surveillance (we could not access all pathology reports for colorectal polyps), one case of osteoma, and one meningioma. In the 2 families with a LAF *TP53* P/LPV, one patient was diagnosed with breast cancer at the age of 21 years, which fulfilled the Chompret criteria. The other patient was diagnosed with endometrioid carcinoma of the fallopian tube at the age of 57 years. Family phenotype is shown in Table 2.

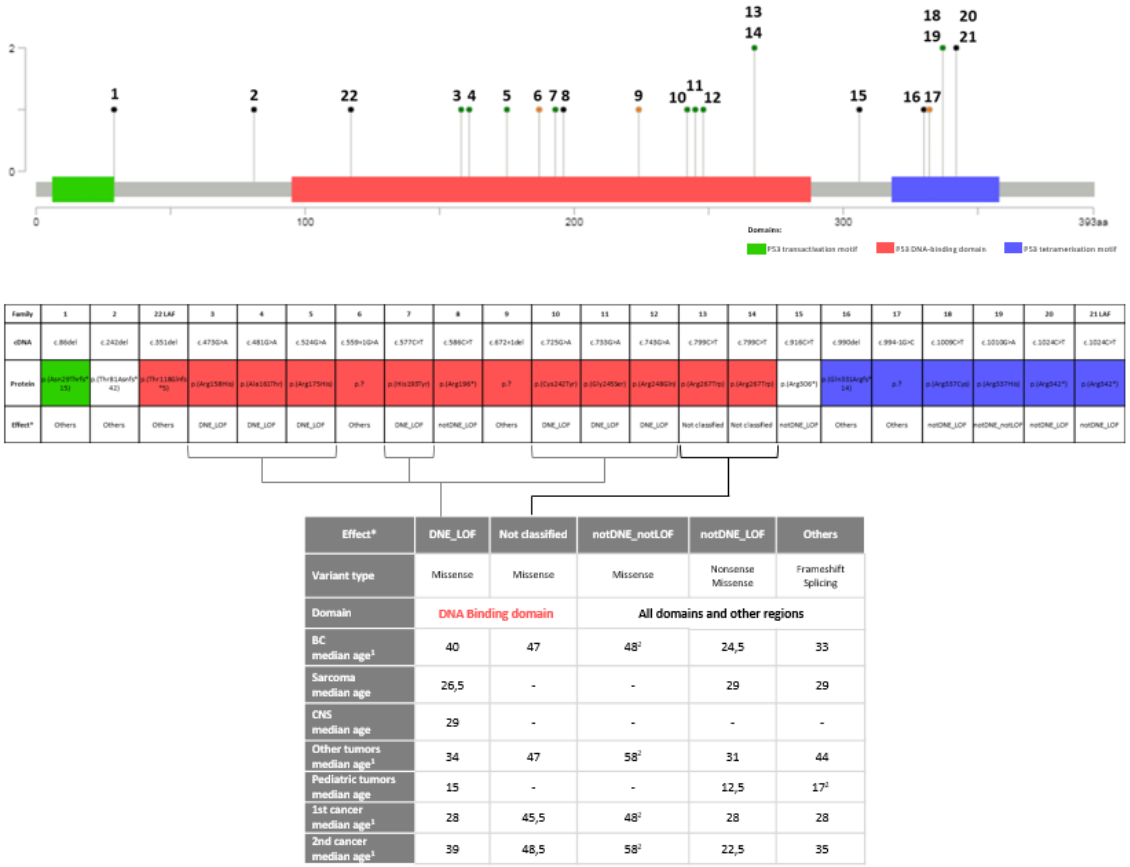
Table 2. Phenotype description of index pts and their families

Family	Index (gender, tumor, and age of diagnosis)	Relatives (tumor and age of diagnosis)
1	F - Osteosarcoma (17), breast (32), breast (37), stomach (42)	Choroid plexus (23), leukemia (37), hepatocellular (55), thyroid (60)
2	F - Breast (28), breast (32), sarcoma (41)	Hypophysis, breast (40), central nervous system (6)
3	M - Glioblastoma (20)	F - Breast (59)*, F - mucinous adenocarcinoma of the appendix, low grade glioma (34, 38)*, lung (45), stomach (50)
4	F - Breast (26), sarcoma (38)	F - Breast (45, 54)*, colorectal (48), pancreas (54), oligodendroglioma (20), sarcoma (4), central nervous system (5), breast bilateral (26, 38)
5	F - Bilateral breast (30)	Central nervous system (32), lung (51)
6	F - Breast (28)	Stomach (29)
7	M - Rhabdomyosarcoma (1), osteosarcoma (15)	Lung (52)
8	F - Bilateral breast (28), sarcoma (34)	Stomach (34), stomach, lung (64, 65), pancreas (65), lung (38)
9	F - Breast (28), breast (41)	-
10	F - Breast (24)	Breast (35), breast (40), breast (40), breast (42), breast (50)
11	F – Bilateral breast (34, 40)	Lymphoma (81)
12	F - Chondrosarcoma (15), breast (50), sarcoma (51, 53)	Leukemia (61), ovary (60)
13	F - Breast (59), melanoma (62)	Central nervous system (55), stomach (57), breast (60), leukemia (52)
14	F - Melanoma (32), breast (35)	Ovary (40), breast, sarcoma (30,53)
15	M - Hodgkin lymphoma (11), osteosarcoma (14, 24), colorectal (39)	-
16	F - Breast (34), squamous cell carcinoma (44)	Bilateral breast (31,35), sarcoma (15), leiomyosarcoma (38)
17	F - Bilateral breast (37, 38), lung adenocarcinoma (44)	Breast (36), breast (73), colorectal (78)
18	F - Breast (31), kidney (31)	F - Breast (19)*, osteosarcoma (36), central nervous system (11), rhabdomyosarcoma (1)
19	F - Breast (48), endometrial serous carcinoma (58)	Breast (29), pancreas (61), melanoma (41)
20	M - Leiomyosarcoma (53)	Sarcoma (20)
21	F - Breast (21)	-
22	F - Fallopian tube endometrioid carcinoma (57)	-

F - female, M – male, *relatives also confirmed to be carriers

Molecular characterization: Twenty different *TP53* P/LPVs were identified in this cohort (Figure 2), including one not previously described [c.242del p.(Thr81Asnfs*42)]. Two recurrent variants were diagnosed in unrelated families: the missense c.799C>T p.(Arg267Trp) in Families 13 and 14 and the nonsense c.1024C>T p.(Arg342*) in Families 20 and 21. Most of the observed *TP53* P/LPV were missense (n=10), 8 of which located in the DNA Binding Domain. The remaining variants were frameshifts (n=4), nonsense (n=3), and splicing (n=3). We were able to confirm two de novo cases by negative tests in both parents (Families 15 and 21). Regarding LAF variants, the nonsense c.1024C>T p.(Arg342*) variant was identified with an allele frequency of 34% in two different samples from the peripheral blood DNA of a

patient with breast cancer aged 21 years (Family 21). This patient has no offspring. Her phenotype (breast cancer before age 31) is consistent with Li-Fraumeni Syndrome and constitutional mosaicism was considered. The variant c.351del p.(Thr118Glnfs*5) was identified with an allele frequency of 20% in a patient diagnosed with fallopian tube endometrioid carcinoma (Family 22). The same variant was found on examination of DNA from a buccal swab. Because her only son tested negative, another tissue was to be tested to confirm constitutional mosaicism, but the patient eventually died of disease progression.



Effect*	DNE_LOF	Not classified	notDNE_notLOF	notDNE_LOF	Others
Variant type	Missense	Missense	Missense	Nonsense Missense	Frameshift Splicing
Domain	DNA Binding domain		All domains and other regions		
BC median age[1]	40	47	48[2]	24,5	33
Sarcoma median age	26,5	-	-	29	29
CNS median age	29	-	-	-	-
Other tumors median age[1]	34	47	58[2]	31	44
Pediatric tumors median age	15	-	-	12,5	17[2]
1st cancer median age[1]	28	45,5	48[2]	28	28
2nd cancer median age[1]	39	48,5	58[2]	22,5	35

Figure 2. Schematic representation of p53 protein and its domains [adapted from https://www.cbioportal.org/mutation_mapper]. Numbers represent family different P/LP variants characterized below according to its effect as described in the IARC database; [1]p-value with statistical significance; [2]only one case; DNE: dominant negative; LOF: loss of function.

Genotype-Phenotype correlation: The median age at breast cancer diagnosis was 24,5 (19-28) years in patients with notDNE_LOF variants, whereas it was 40 (24-59) years in DNE_LOF variants. Sarcomas were diagnosed at a median age of 26,5 (1-53) years in patients with DNE_LOF variants and at a median age of 29 (14-53) years in notDNE_LOF and other variants. Regarding all cancers registered in this cohort, median age at the first cancer diagnosis was earlier in patients with DNE_LOF, notDNE_LOF, frameshift and splicing variants ($p<0,05$), in contrast with notDNE_notLOF and unclassified variants. Breast cancer (either as first or subsequent diagnosis) and other tumors (excluding sarcomas and CNS) were diagnosed earlier in patients with notDNE_LOF variants ($p<0,05$).

Familial phenotype of c.799C>T p.(Arg267Trp),reported as a variable penetrance variant, was associated with diagnoses after 50 years only, in Family 13, while earlier age at diagnosis, including sarcoma, was observed in Family 14 (Table 2).

Follow up: All *TP53* carriers except index patients from Families 7 (who did not consent to follow-up) and 22 (because constitutional

mosaicism was not confirmed) were included in prospective follow-up. All patients were cancer survivors, except for 3 asymptomatic men (ages 26, 41, and 50). For a follow-up of 51,5 months (2-118,9), 59,7% of patients are still alive. Eleven deaths (40,3%) were observed, all but one related to cancer. During this period, 9 new cancers (all in previous cancer survivors) and 6 recurrences (3 in sarcoma patients) were diagnosed. One carcinosarcoma in the remaining breast was associated with radiotherapy. Most patients underwent regular colonoscopy surveillance, and multiple polypectomies of potential colorectal precursor lesions were performed in 4 of them. In this cohort, one asymptomatic individual opted for preimplantation genetic diagnosis (PGD). None of the carriers opted for prenatal diagnosis.

Discussion

In this study, we report comprehensive data from a cohort of *TP53* carriers identified through a multidisciplinary program at a national cancer center. The main findings are the increasing prescription of *TP53* tests for patients without a clinical diagnosis of LFS, allowing the identification of patients requiring specific treatment and surveillance. MGT was the single most important factor facilitating *TP53* molecular diagnosis. However, classification of a variant as clinically actionable required integration of phenotypic and molecular data and, for diagnosis of *de novo* and LAF variants, additional clinical and molecular work up, including the collaboration of family relatives.

For all cancers registered, patients had a first cancer diagnosis earlier when harboring DNE_LOF, notDNE_LOF, frameshift and splicing variants, in contrast with notDNE_notLOF and unclassified variants. Breast (either as first or subsequent diagnosis) and cancers other than sarcomas and CNS, were diagnosed earlier in patients with notDNE_LOF.

While some studies are still exploring different genetic associations of the LFS phenotype[30], our cohort includes only patients with a *TP53* P/LPV, the only genetic cause clearly associated with this syndrome[31]. Increasing test prescription facilitated by MGT has identified previously overlooked *TP53* patients[19]. However, in our group, most families identified after the introduction of NGS still meet at least the Chompret criteria. The reasons for this observation are likely related to the prospective nature of this cohort, which began enrolling patients before the routine use of NGS methodologies; missed patients due to opt out; and the lack of effective surveillance and international guidelines for the clinical management of *TP53* carriers [20,25,32] until recently. Excess of breast cancer patients explains the gender differences in our data. This is the most common cancer in female *TP53* carriers[33]. Germline *TP53* testing has been proposed over the years, initially for breast cancer patients diagnosed before 31 years of age [10,34] or with a clinical diagnosis of LFS, and more recently as part of most MGT[15,16,17]. All factors combined, we are currently unable to reliably calculate the prevalence of LFS in our population.

Because NGS is a methodology with high sensitivity, variant detection increases, but discordant reports[35] or classification of somatic variants as germinal[36,37] may influence clinical decisions. Pathogenic and likely pathogenic variants are considered clinically actionable[29] but in one study, discordant germline *TP53* interpretations were found in 39% of families studied, 11% of which have the potential to significantly influence medical management[35]. To avoid discordance, our program promotes close collaboration between clinicians and molecular biologists and the integration of specialized networks[38,39]. Prospective follow-up also allows for regular updates of individual and family cancer diagnoses, and this information is considered in the periodic review of *TP53* variant classification. This is particularly important, not only for variants of unknown significance[40,41], but also for the diagnosis of *de novo* and LAF variants.

The diagnosis of *TP53 de novo* carriers and possible constitutional *TP53* mosaicism[36,42] poses immediate problems in communicating a timely diagnosis to patients and their families. In our cohort, a 21-year-old BC patient (who met Chompret criteria) was diagnosed with an allele frequency of 34%. Additional confirmatory testing included a new DNA collection to confirm the diagnosis and a review of the familial phenotype and genotype. Allele frequencies of 30-70% are considered to correspond to non-mosaic heterozygous PV[37]. Constitutional mosaicism resulting from an early postzygotic

somatic mutation are inherently *de novo* mutations. The frequency of *de novo* PVs in LFS patients is estimated to be 7% to 20%[43,44]. In our cohort, we confirmed 2 cases (12%). Genetic testing of other tissues, preferably cultured skin fibroblasts, hair follicles, or nail sections, is required to confirm mosaicism for LAF of less than 30%[36,37]. A patient from family 22 was first diagnosed with an allele frequency of 20% in DNA extracted from peripheral blood. The same result was found when buccal swabs was examined, but further tissue testing was not possible because the patient eventually died of cancer progression. Her only offspring (a son) tested negative for this PV. Overall, this case of a 57-year-old fallopian tube carcinoma patient cannot be diagnosed as a mosaic: somatic *TP53* PV are frequent in ovarian and fallopian tube carcinomas, and our finding is most likely related to circulating tumor DNA[10] or clonal hematopoiesis[45].

TP53 is a gene with very high penetrance, but other factors (genetic and/or environmental) may act as modifier factors[46]. One such example is the previously described c.799C>T p.(Arg267Trp) with variable penetrance[46]. In our study, this was one of the two recurrent variants diagnosed. While one of the phenotypes included only cancers diagnosed after 50 years, the other included earlier diagnoses, including sarcomas. This variable penetrance of some *TP53* variants should be recognized, and clinicians need to be cautious in generalizing risk management of these carriers. In addition to the possibility of variable penetrance, there have also been studies examining a possible genotype/phenotype correlation. With this correlation[47], the predictive impact of each variant on the severity of the individual or family phenotype would allow for individualized risk management. Conflicting results have been described regarding the impact of missense and LOF variants[10,21].

The application of the recently adopted IARC categorization of *TP53* variants to our data revealed that median age at the first cancer diagnosis was earlier in patients with a DNE_LOF, notDNE_LOF, frameshift and splicing variants, in contrast with notDNE_notLOF and unclassified variants, whereas the median at the second cancer diagnosis was earlier in patients with a notDNE_LOF. Breast and tumors other than sarcomas and CNS were diagnosed at an earlier age in patients with a notDNE_LOF variants. Although the numbers are small, our data adds to previously described studies suggesting that LOF variants have impact in phenotype severity even if not classified as dominant negative. Future studies, ongoing recruitment, and additional follow-up will also help confirm these findings

Counselling for germline *TP53* testing in the era of MGT is increasingly complex. This process should guide patients through their informed decision to undergo genetic testing while avoiding information overload that could increase anxiety[28]. The possibility of opting out has been proposed by some authors to avoid excessive anxiety associated with *TP53* testing[48]. Nowadays, opt out should be weighed with the potential contribution of a positive result to treatment decisions, such as total mastectomy versus conservative surgery in BC patients[49]. Radio-induced tumors have been reported in LFS[50,51] highlighting the importance of *TP53* testing in high-risk individuals prior to the use of medical radiation[10,50,52]. This is also an issue deserving further research, as conflicting data suggest a lower risk of RT-induced secondary malignancies in LFS breast cancer patients than previously reported[52,53]. In our series, we considered as RT induced cancer a carcinosarcoma diagnosed in the remaining breast of a patient with ductal carcinoma eight years earlier. A complex case was a newly diagnosed sarcoma, 3 years after local therapy. Because the pathologic diagnosis was similar in this case, recurrence could not be ruled out. The potential risk for locoregional recurrence without RT must be weighed against the long-term risk for RT -induced malignancies. The impact of chemotherapy on second cancers in *TP53* carriers[24] and the role of potential chemoprevention have not been determined[54,55].

During follow-up, we observed a high mortality rate, several new cancers, and recurrences that eventually led to death (only one death was noncancer-related). Our institutional protocol has included WBMRI since 2018, but analysis of the impact of this surveillance on our patient management needs longer follow up. It is not yet possible to compare mortality or cancer diagnoses before and after implementation of WBMRI. Quality of life may be as important to LFS patients as early cancer detection and

survival. The question of how early to offer pre-symptomatic testing and surveillance remains unresolved[26]. Although some groups reported that WBMRI is useful as part of routine baseline screening for children and adults who are *TP53* carriers, and annual screening is suggested[20,25,32,56] the risks associated with anesthesia, false-negative results, and psychosocial impact, especially in children, still require further research[27,28]. In addition to clinical examination, breast, and CNS MRI and WBMRI and colonoscopy are included in most LFS surveillance protocols[20].

Preimplantation and prenatal genetic diagnosis may be discussed during counselling of LFS individuals. Although these procedures are technically feasible, ethical or legal restrictions may vary by region/country. In this cohort, one asymptomatic individual opted for preimplantation genetic diagnosis (PGD) and none opted for prenatal diagnosis during the reporting period.

In conclusion, our study adds to the findings of previous reports on the increasing detection of *TP53* variants with MGT, which are prescribed to hereditary cancer patients, and the complexity of a correct molecular diagnosis. Our data underscore the need to develop multidisciplinary programs, even for a rare patient population, to avoid clinical mismanagement. These programs should cover genetic counseling, diagnosis, surveillance, reproductive options, treatment decisions, and socio-psychological support.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. All genetic records are protected with restricted access as per Portuguese law. All forms related to this study were approved by the Ethics Committee of our Institute.

Additional Information: All authors were involved in (1) conception and design, or analysis and interpretation of data and (2) drafting the article or revising it critically for important intellectual content.

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