Carcinogenesis, 2017, Vol. 38, No. 9, 938–943

doi:10.1093/carcin/bgx069
Advance Access publication July 7, 2017
Original Article

ORIGINAL ARTICLE

Whole-exome analysis of a Li–Fraumeni family trio with a novel TP53 PRD mutation and anticipation profile

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Abstract

Li–Fraumeni syndrome is a clinically heterogeneous familial cancer predisposition syndrome with autosomal-dominant inheritance caused by heterozygous germline mutations in the TP53 gene. We here analyze the genetic background of a family with a 4-year-proband presented with a Li–Fraumeni tumor. The mother developed breast cancer at age 37 and the proband died at age 8. We performed Sanger sequencing and whole-exome sequencing on peripheral blood DNA from proband and relatives. Data analysis selected only high-quality score and depth reads, rare variants and protein impact involving missense, non-sense, frameshift and splice disrupt mutations. Disease implicated variants and predicted deleterious alterations were also chosen. TP53 genetic testing revealed a never reported TP53 deletion arose as de novo mutation in the mother and inherited by the proband. We then performed whole-exome analysis of the trio to uncover inherited variants from the father that potentially worsen the already altered genetic background in the proband. No pathogenic variants were inherited in autosomal recessive, de novo dominant or X-linked recessive manner. Comparing proband and father exome we detected 25 predicted deleterious variants including a nonsense mutation in ERCC3. Those inherited mutations are possible candidate modifiers linked to TP53, explaining the proband accelerated tumor onset compared to the mother and providing a possible explanation of the genetic anticipation event in this Li–Fraumeni family.

Introduction

Li–Fraumeni syndrome (LFS; OMIM 151623), as well as its variant form, Li–Fraumeni-like, is a clinically heterogeneous familial cancer predisposition syndrome with autosomal-dominant inheritance (1). LFS is not associated with a specific cancer, like other hereditary cancer syndromes, but with a broad spectrum of early-onset tumors: bone and soft-tissue sarcomas, central nervous system tumors, leukemia, adrenocortical carcinoma (ACC) and breast cancer (2). The most characteristic tumor phenotypes are rhabdomyosarcoma, ACC and choroid plexus carcinoma in early childhood, defining an LFS-specific cancer triad (1). The clinical definition of LFS known as 'classic LFS' includes three clinical criteria: a proband with a sarcoma diagnosed in childhood/young adulthood (≤45 years), a first-degree relative with any cancer in young adulthood (≤45 years) and a first- or second-degree relative with any cancer diagnosed in young adulthood (≤45 years) or sarcoma diagnosed at any age (3).

Currently, TP53 is the only gene that has been associated with LFS/Li–Fraumeni-like and no other recurrent germline alteration has been associated with this disease (1). Germline TP53 pathogenic variants have been identified in about 80% of families who meet classic LFS criteria (4). Partial or complete loss of p53 protein function may lead to decreasing in its tumor suppressive role, thus

Received: February 16, 2017; **Revised:** May 23, 2017; **Accepted:** June 23, 2017

Abbreviations

ACC	adrenocortical carcinoma
LFS	Li–Fraumeni syndrome
PRD	proline-rich domain
WES	whole-exome sequencing

predisposing carriers of germline TP53 mutations to a defined spectrum of cancers (1). In the germline, over 250 mutations have been described throughout the TP53 gene and a large number of mutations are missense mutations that cause codon changes, leading to a demanding functional interpretation of new variants (5).

Families with LFS do appear to display genetic anticipation resulting in increasingly earlier age at onset of cancer in successive generations of carriers of germline TP53 mutations (4). Unpredictable and disparate age of cancer onset is a major challenge in the management of LFS; this variability has driven to the assumption that, away from differences in germline TP53 mutations, genetic or epigenetic features may act as modifiers in determining age of disease onset increasing the severity of the already abnormal genetic pathway (6).

So far, the presence of the MDM2 promoter polymorphisms SNP309T>G (rs2279744) and shortened telomere length have been identified as two genetic modifiers associated with accelerated tumor development in families with LFS (4).

Whole-exome sequencing represents a powerful method for exploring the extent to which rare mutations may explain the heritability of complex diseases, including several types of cancer as it is able to identify single-nucleotide variants, small insertion/deletions (indels) and copy number abnormalities across the whole coding genome (7,8).

We applied this technology to a proband–father–mother trio with a 4-year-old boy presented with a LFS tumor. There was no family history of the disease and the proband presented a novel frameshift mutation in the TP53 proline-rich domain sequence inherited from the mother. Few years later the mother developed breast cancer when she was 37 and the proband died at the age of 8. We used WES to uncover inherited variants from the father that potentially worsen the already altered genetic background in the proband, explaining the accelerated tumor onset compared to the mother and providing a possible explanation of the genetic anticipation event in this LFS family.

Methods

Patients

The proband was identified in the Department of Cytogenetics and Molecular Genetic Unit at University Hospital of Pisa, Italy. The study was approved by the Ethics Committee of the University Hospital of Pisa and all methods were performed in accordance with approved guidelines. Patient's data and sample has been completely anonymised.

DNA isolation

DNA isolation from proband and relatives was performed using 200 µl of peripheral blood according to the QIAamp DNA Blood Mini Kit (QIAGEN, Venlo, Netherlands) protocol. The quantity and quality of extracted DNA was estimated with Qubit 2.0 Fluorometer (Life Technologies, Foster City, CA) by using 2 µl of undiluted DNA solution.

TP53 sequencing

All the exons and intron–exon boundaries of TP53 gene were analyzed. Primer sequences are available on request. PCR amplification was performed in a volume of 25 µl according AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA) protocol and sequenced by BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems) using an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems).

Multiplex ligation-dependent probe amplification (MLPA) analysis of the subtelomeric regions

Telomere lenght shortening was evaluated in DNA from peripheral blood of proband by using the MLPA probemix P036-E2 Subtelomeres Mix (MRC Holland, Amsterdam, The Netherlands). MLPA products were separated by capillary electrophoresis on the ABI3130 genetic analyzer (Life Technology, Applied Biosystems) and analyzed using the Coffalyser.NET software (MRC-Holland). The peak heights of the samples were compared with control probes and the ratio of peaks were calculated for all probes. If the dosage quotient was 1.0, the results were considered as normal. Thresholds for deletions and duplications were set at 0.5 and 1.5, respectively.

Whole-exome sequencing

For each sample, 1 µg high-quality genomic DNA was used to prepare the Exome library. Randomly fragmented genomic DNA underwent adapters ligation, nick repairing and purification prior to size selection according to the Ion TargetSeq Exome Enrichment Kit (Life Technologies). The 270-bp size-selected fragment library was then amplified and cleaned with the Agencourt AMPure XP Reagent (Beckman Coulter, Inc., Fullerton, CA). Library DNA was then quantitated and qualitatively assessed on the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). A total of 500 ng of each size-selected fragment library was hybridized with pooled solution-phase DNA probes from the Ion TargetSeq Exome Enrichment Kit (Life Technologies) for 72 h, then the DNA was recovered, amplified and purified according to the protocol's instructions. The enriched DNA was sequenced by the Ion Proton sequencer according to the Ion PI Sequencing 200 kit (Life Technologies). Sequencing templates were prepared on Ion OneTouch 2 and Ion OneTouch ES stations, then loaded onto the Proton PI Chip prior to sequencing.

ERCC3 sanger sequencing

For the detection of ERCC3 rs768687646 (ENSP00000285398.2:p.Arg574*), the following forward and reverse primers, respectively, were used in PCR experiments: 5′-TTGCTGTACACCATGAACCC-3′, 5′-ggaagagaaactggcctgga-3′. PCR amplification was performed in a volume of 25 µl according AmpliTaq Gold DNA Polymerase (Applied Biosystems) protocol and sequenced by BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems) using an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems).

Bioinformatic analysis

The analysis was performed automatically in Torrent Browser using Torrent Suite. Briefly, the reads were aligned to the GRCh37/hg19 reference sequence with TMAP aligner and then visualized using IGV browser (http://www.broadinstitute.org/igv/). The variant caller was done with Torrent variant caller plugin with high stringency parameters. The generate bam and vcf files were exported. Variant annotation and filtration of multi-allelic substitutions and indels was performed on each individual sample using the Enlis Genome Research (Enlis, LLC, Berkeley, CA). The software is also able to compare exomes coming from different individuals. DANN tool (9) was used to predict the pathogenicity of missense genetic variants. OMIM database (10) was used to discover associations between genes and diseases.

Results

Subjects and clinical evaluation

The proband, a 4-year-old boy, was the first component to develop a cancer typical of LFS tumor spectrum. He was diagnosed with an ACC with retrocaval lymphnodes metastasis and treated by hormonal therapy and surgery (right nephro-surrenectomy). Two years later, he developed lymphnodes metastasis spread out through abdominal cavity and died at age 8. In the same year, the mother was diagnosed with microinfiltrating in situ carcinoma of the right breast at age 37, for which she underwent to bilateral mastectomy; she is currently under surveillance with mammographic screening every 6 months, and she is disease free (3-year follow-up). The father of the proband was healthy.

TP53 mutation in the family

According to Chompret criteria, the ACC affected proband was indicated for genetic testing for whole TP53 gene sequence. A never-reported deletion of four bases was detected in the exon 4: c.266_269delCCTC (chr17:7579418_7579421delGAGG, Ser90fs*32) (Figure 1). Screening for TP53 germline mutations was performed to relatives. The Ser90fs*32 mutation was detected in germline DNA of the mother whereas it was absent in the father. Moreover, grandparents DNA was tested but no mutation was found. Therefore, we concluded that the Ser90fs*32 is a de novo mutation in the mother. The aberrant transcript gives rise to a mutant protein of only 120 amino acids (instead of 393 in the wild type) that carries 31 incorrect amino acids at the C-terminus (Figure 2). This mutation encodes for a truncated form of TP53 protein, which completely abrogates the majority of crucial domains of TP53 protein such as DNA binding and tetramerization domains.

Telomere length analysis

MLPA analysis was performed to detect proband's deletions and/or duplications in subtelomeric regions. Telomere length was not significantly different from controls, and a relatively normal rate of telomere shortening was observed.

Exome analysis

We performed whole-exome analysis of the trio to uncover inherited variants from the father that potentially worsen the already altered genetic background in the proband (QC parameters presented in Supplementary Table 1). After variant call and annotation, we used Enlis Genome Research to filter the VCF file from exome analysis with following filters: quality score ≥15, read depth ≥ 10, allele frequency (as 1000 Genome Project and Exome Aggregation Consortium) <1% and protein impact involving missense, non sense, frameshift and splice disrupt mutations. We first applied the 'family trio analysis' filter to investigate if the proband inherited pathogenic variation in autosomal recessive, de novo dominant or X-linked recessive manner. No variants were found even increasing the allele frequency to 10%. To compare proband and father exomes we add mammalian conservation, DANN model predicted deleterious alterations and absence in dbSNP filters, finding 25 mutations (Table 1) including 24 missense variants and an ERCC3 truncating mutation (confirmed by Sanger sequencing). TP53 and MDM2 gene sequences were screened in all three relatives. TP53 Ser90fs*32 mutation were confirmed in both mother and proband. Two TP53 polymorphisms were present in all three family members (rs1625895 and rs1042522), both with benign clinical significance, and one intronic mutation was present exclusively in the mother (NC_000017.10:g.7590179A>C). No evidence of a mutation was found in the MDM2 gene.

Discussion

Li–Fraumeni syndrome is an autosomal dominant cancer syndrome caused by heterozygous germline mutations in the TP53 gene (11). The most common tumor types that occur in LFS are bone and soft-tissue sarcomas, central nervous system tumors, leukemia, ACC and breast cancer (2). ACC have been reported to occur in 3–4% of patients with LFS, often under the age of 20, and 70% of LFS cases are a result of a germline mutation in the TP53 gene (12,13).

In this study, we applied WES to analyze the genetic background of an Italian family trio with a 4-year-proband presented with an ACC tumor. Whole TP53 genetic testing was indicated for the proband and his relatives. A never-reported TP53 deletion of four bases was detected in the proband and his mother, whereas was absent in the father and in grandparents DNA. The proband died at the age of 8 and in the same year the mother was diagnosed with microinfiltrating in situ carcinoma at age 37 and she is currently under surveillance. Whole-exome sequencing of the family trio was performed to reveal proband inherited variations and provide a possible explanation of genetic anticipation.

'Family trio analysis' filter revealed that no pathogenic variants in the proband were inherited in autosomal recessive, de novo dominant or X-linked recessive manner. Moreover, WES analysis detected and confirmed the TP53 Ser90fs*32 frameshift as the only one pathogenic variant inherited from the mother. To the best of our knowledge, this is the first work that describe

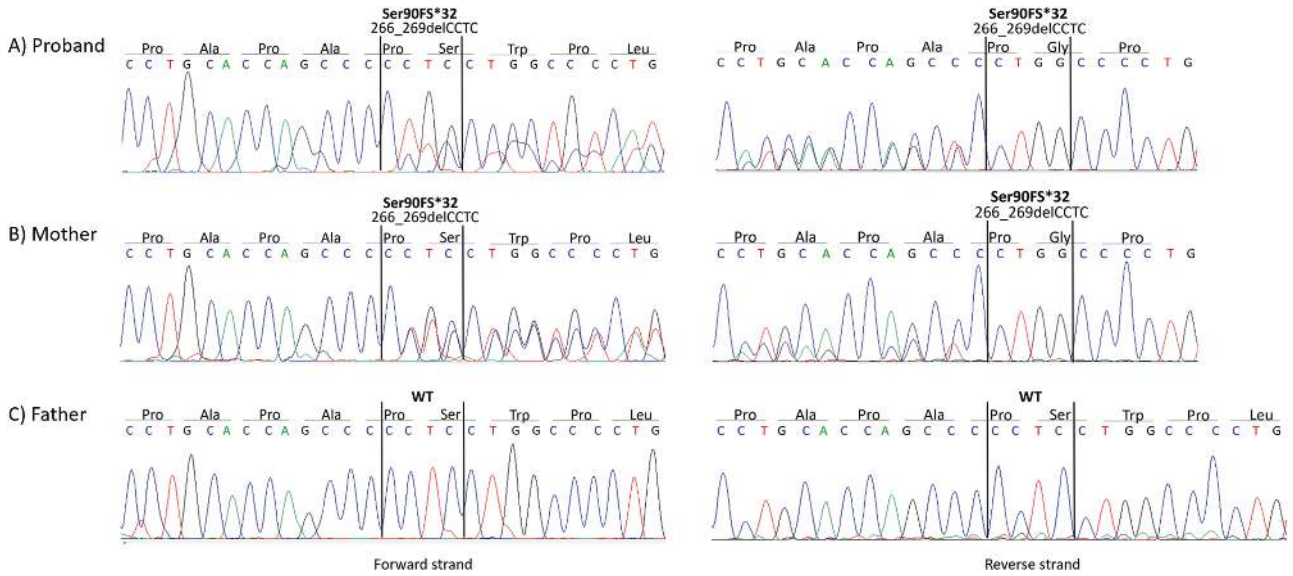

Figure 1. Frameshift deletion (Ser90fs*32) identified in exon 4 of the TP53 gene with DNA Sanger sequencing analysis. (**A**) Proband TP53 Ser90fs*32, (**B**) Mother TP53 Ser90fs*32 and (C) Father WT TP53.

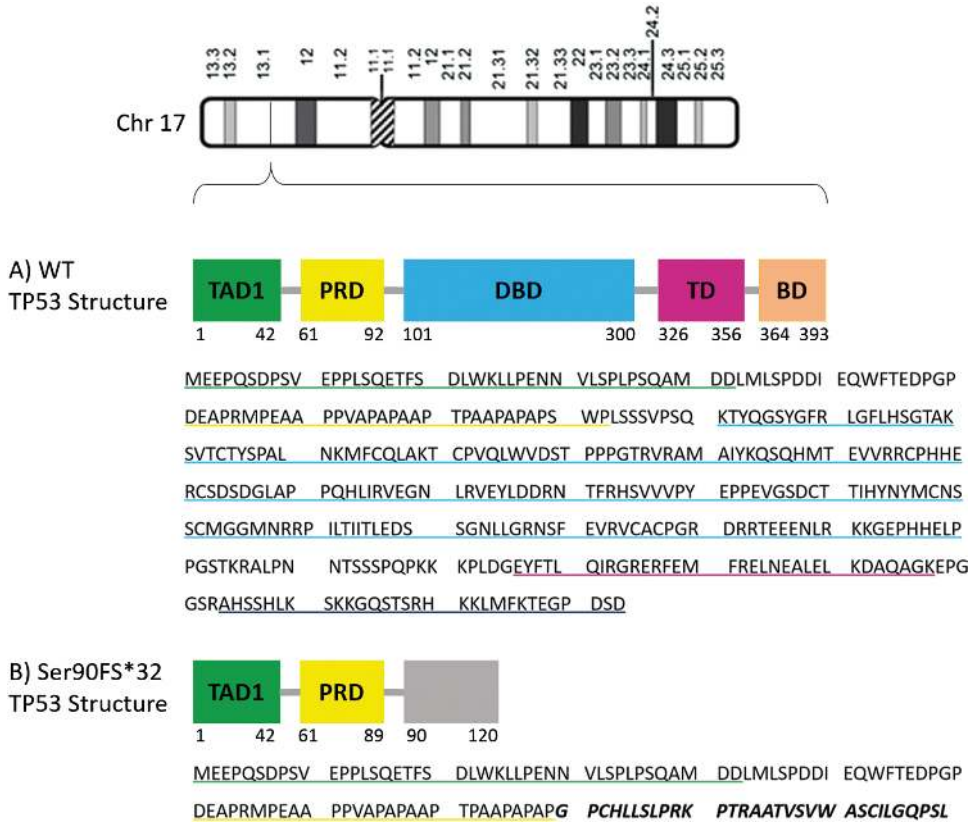

Figure 2. Schematic structure and functional domains of wild-type (**A**) and mutated (**B**) TP53. WT TP53 consists of five main domains: the N-terminal transactivation domain (TAD; 1–42, green), the proline-rich domain (PRD; 61–92, yellow), the central DNA binding domain (DBD; 101–300, blue), the tetramerization domain (TD; 326–356, magenta) and the C-terminal basic domain (BD; 364–393, orange). The aberrant TP53 Ser90fs*32 transcript gives rise to a mutant protein of only 120 amino acids (instead of 393 in the wild type) that carries 31 incorrect amino acids at the C-terminus (90–120, grey) and results in a lack of DBD, TD and BD domains.

Table 1. Proband predicted deleterious variants inherited from the father

Chr	Chr position	Ref	Var	Gene	Type	aaChange	Freq
1	87025639	T	C	CLCA4	MISSENSE	Y-62-H	0
1	158747319	A	G	OR6N2	MISSENSE	L-36-P	0
1	197316510	C	T	CRB1	MISSENSE	H-297-Y	0.01
2	99155421	G	A	INPP4A	MISSENSE	R-216-Q	0.01
2	128036759	G	A	ERCC3	NONSENSE	R-574-*	0.01
2	158157312	G	C	GALNT5	MISSENSE	R-747-P	0
3	33114075	A	G	GLB1	MISSENSE	L-69-P	0
3	52812452	G	A	ITIH1	MISSENSE	A-79-T	0.01
4	9784348	T	G	DRD5	MISSENSE	I-232-S	0
5	86697531	C	G	CCNH	MISSENSE	D-250-H	0.01
6	32157533	C	T	PBX2	MISSENSE	G-54-R	0
6	135265013	G	A	ALDH8A1	MISSENSE	A-77-V	0.01
7	16729457	G	T	BZW2	MISSENSE	A-193-S	0
7	158590647	C	T	ESYT2	MISSENSE	D-213-N	0.01
9	36671092	G	A	MELK	MISSENSE	D-535-N	0.01
9	115649657	G	A	SLC46A2	MISSENSE	T-389-I	0
10	35333722	T	C	CUL2	MISSENSE	Y-213-C	0
11	33047406	G	A	DEPDC7	MISSENSE	R-92-Q	0.01
11	62567898	C	T	NXF1	MISSENSE	D-323-N	0.01
12	69120345	G	T	NUP107	MISSENSE	R-548-L	0
12	97337476	A	G	NEDD1	MISSENSE	D-485-G	0
14	94732149	C	A	PPP4R4	MISSENSE	S-768-Y	0
15	42987472	A	T	STARD9	MISSENSE	E-4366-V	0
20	60511930	G	A	CDH4	MISSENSE	D-820-N	0.01
22	26422518	C	G	MYO18B	MISSENSE	S-2193-C	0

aaChange, aminoacid change; Chr, Chromosome; Chr Position, start chromosome position of the variant; Freq, allele frequency; Ref, ancestral allele; Var, variant allele; Gene, gene name; Type, variant type.

a TP53 Ser90fs*32 frameshift. The aberrant TP53 transcript, present in the proband and his mother, gives rise to a mutant protein of only 120 amino acids (instead of 393 in the wild type) that carries 31 incorrect amino acids at the C-terminus. This results in a truncated form of TP53 protein, which completely abrogates the majority of crucial domains such as the central DNA-binding domain, the tetramerization domain and the C-terminal basic domain, leading to loss of normal protein functions (Figure 2). Other two TP53 mutations were present in all three family members (rs1625895 and rs1042522), but these are known polymorphism with a benign clinical significance. No mutations were found in the MDM2 gene and no telomere shortening was observed in the proband.

Furthermore, exome data analysis reveals 25 predicted deleterious inherited variants in the proband from the father. Among these genes, PBX2, CCNH, CLCA4, CUL2, INPP4A, MYO18B and ITIH1, have been previously linked to cancer (14–20) and presented a missense mutation. A missense mutation was also detected in CDH4, GLB1 and MELK in their protein functional domain. The cadherin-4 gene (CDH4) of the cadherin family, encodes for a calcium-dependent cell–cell adhesion glycoprotein and has an important role in cell migration and cell adhesion, sorting, tissue morphogenesis and tumor genesis (21). The detected c.2546G>A variation (transcript ENST00000614565.4) causes the p.Asp820Asn aminoacidic change in the cytoplasmic domain important for the cell–cell-binding function (22). Galactosidase Beta 1 (GLB1) gene encodes a member of the glycosyl hydrolase 35 family, which cleaves beta-linked terminal galactosyl residues from gangliosides, glycoproteins and glycosaminoglycans.GLB1 was described to be involved in cell senescence and cancer development (23). We observed a c.351T>C mutation (p.Leu69Pro aminoacidic change) in the catalitc domain that causes a β-galactosidase product with abnormal function (24). Maternal embryonic leucine zipper kinase is an enzyme that in humans is encoded by the MELK gene and recently it has been widely correlated with cancer (25–29). We found a c.1787G>A that results in a p.Asp535Asn aminoacidic change in the C-terminal kinase associated domain 1 (KA1 domain). Several reports have suggested that the C-terminal tail of the kinase (which includes the KA1 domain) plays a role in reversible autoinhibition of kinase activity (30).

A nonsense mutation in ERCC3 was also identified in the proband's variations inherited from the father. The excision repair cross-complementation group 3 (ERCC3) gene encodes an ATP-dependent DNA helicase that functions in excision DNA repair and initiation of basal transcription as a subunit of basal transcription factor 2 (TFIIH) a larger complex implicated in DNA denaturation prior to damage excision. The full-length ERCC3 helicase consists of 782 amino-acid residues containing seven conserved helicase motifs (I, Ia and II–VI) in the middle of the polypeptide (31). The detected ERCC3 c.1720C>T variation (transcript ENST00000285398.6) encodes a truncated protein of 573 amino acids rather than 783 amino acids resulting in a loss of 210 amino acid residues.

ERCC3 has been identified as putatively pathogenic gene related to hereditary cancer susceptibility without a well-known associated risk (32,33). Moreover, it was previously shown that a frameshift mutation at the 3′-terminus of the ERCC3 gene leads to an inactivation of the nucleotide-excision repair pathway and to a DNA repair deficiency (34–38). Further studies in yeast, confirmed that the functions of ERCC3 yeast homologous (SSL2) helicase are essential for viability (39,40). In particular, we identified the yeast c.1861A>T mutation as the corresponding human ERCC3 c.1720C>T variant (http://crimetoyhu.ifc.cnr.it/). This c.1861A>T variation encodes a truncated protein of 620 amino acids rather than 843 amino acids resulting in a lethal mutation, since the lost domains are essential for nucleotide-excision repair activity and survival of yeast cells (40).

Genetic anticipation in this family, resulting in a proband LFS tumor at the age of 4, could be explained by the co-presence of the TP53 Ser90fs*32 frameshift inherited from the mother, and those predicted deleterious variants inherited from the father. In particular, the truncating mutation in the ERCC3 gene led to hypothesize that ERCC3 could be a potential candidate modifier linked to TP53, responsible for proband accelerated tumor onset compared to the mother, carrying the same TP53 mutation.

Supplementary Material

Supplementary material is available at *Carcinogenesis* online.

Acknowledgements

S.F. and L.S. prepared the manuscript. S.F., L.S., F.L. and R.S. performed the experiments. P.A. conducted all bioinformatics analyses. C.C. provided and cared for study patients. G.B. supervised the experiments. M.A.C. and C.M.M. designed the study and edited the manuscript.
Conflict of Interest Statement: None declared.

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