A Novel *TP53* Tandem Duplication in a Child with Li-Fraumeni Syndrome

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ABSTRACT

Li-Fraumeni syndrome (LFS) is one of the most common cancer predisposition syndromes that affects both children and adults. Individuals with LFS are at an increased risk of developing various types of cancer over their lifetime including soft tissue sarcomas, osteosarcomas, breast cancer, leukemia, brain tumors, and adrenocortical carcinoma. Heterozygous germline pathogenic variants in the tumor suppressor gene *TP53* are the known causal genetic defect for LFS. Single nucleotide variants (SNVs) including missense substitutions that occur in the highly conserved DNA binding domain of the protein are the most common alterations, followed by nonsense and splice site variants. Gross copy number changes in *TP53* are rare and account for less than 1% of all variants. Using next-generation sequencing (NGS) panels, we identified a paternally inherited germline intragenic duplication of *TP53* in a child with metastatic osteosarcoma who later developed acute myeloid leukemia (AML). Transcriptome sequencing (RNA-Seq) demonstrated the duplication was tandem, encompassing exons 2-6 and 28 nucleotides of the untranslated region (UTR) upstream of the start codon in exon 2. The inclusion of the 28 nucleotides is expected to result in a frameshift with a stop codon 18 codons downstream of the exon 6, leading to a loss-of-function allele. This case highlights the significance of simultaneous identification of both significant copy number variants as well as SNVs/indels using NGS panels.

CASE PRESENTATION

The patient was a 15-year-old male who was first diagnosed with metastatic osteosarcoma and later developed AML. He presented with leg pain and difficulty walking at the age of 14. X-ray examination showed a tumor of the right proximal tibia. MRI displayed a large aggressive tumor mass infiltrating the meta-diaphyseal region with aggressive periosteal reaction and cortical destruction. The tumor extended into surrounding muscles and soft tissues. PET scan exhibited skip metastasis in the mid-right tibial shaft and external iliac node. Histologic evaluation showed proliferation of large pleomorphic cells with enlarged, hyperchromatic nuclei and frequent atypical mitotic figures associated with osteoid matrix findings, consistent with a diagnosis of osteosarcoma (Fig 1A). After initial chemotherapy, the patient was transferred to Children's Hospital of Philadelphia (CHOP) for local surgical management and concurrent chemotherapy. Seven months from the final dose of the chemotherapy, the patient was found to have pancytopenia with peripheral blasts. Bone marrow biopsy found sheets of blasts with irregular nuclei, speckled chromatin, and prominent nucleoli (Fig 1B). Immunohistochemical stains and flow cytometry showed that the blasts were positive for CD33, MPO, lysozyme, CD117, CD34, indicating a diagnosis of AML.

TECHNICAL ANALYSIS

Next-Generation Sequencing (NGS) panels

The CHOP Comprehensive Hematological Malignancy Panel (CHMP) evaluates 117 genes associated with pediatric hematological malignancies (Surrey et al. 2019). CHOP Comprehensive Hereditary Cancer Panel (CHCP) is a custom-designed NGS panel that interrogates 130 genes associated with cancer predisposition. Both panels cover all coding exons and at least 10 bp of flanking intronic sequences, certain promoter regions, and known pathogenic intronic variants. An additional 1038 common SNPs (single nucleotide polymorphism) were added to the CHMP and CHCP to mimic a low-density SNP array to facilitate CNV analysis. All custom-designed probes were synthesized and biotinylated to allow for target enrichment using streptavidin-conjugated beads (Agilent Technologies, Santa Clara, CA, USA). Illumina RNA Exome kit was utilized to capture the transcriptome and generate RNA-Seq libraries (Illumina, San Diego, CA, USA). Libraries were sequenced using Illumina HiSeq 2500 with 150 bp paired end reads. Sequence data were analyzed using a homebrew NGS Analysis Pipeline, named ConcordR V1.4.2, which utilizes RSEM package for gene expression evaluation and for calculation of fold changes for gene expression levels (Li and Dewey 2011).

The CHCP test on the patient's blood sample identified a heterozygous duplication of exons 2-6 of the *TP53* gene (NM_000546.5) (Fig 1C) along with two pathogenic SNVs, each in the *HFE* and *SBDS* genes (Table 1). RNA-Seq demonstrated that the *TP53* duplication was tandem involving exons 2-6 and the mRNA transcripts contained 28 nucleotides of the 5'UTR located upstream of the start codon in exon 2 (Fig 1D). RNA-Seq also discovered reduced *TP53* expression in blood cells (fold change: 69% compared to the control). Due to the later development of AML, a CHMP test was performed and disclosed a near triploid genome with copy number gains involving almost every chromosome. Copy-neutral loss of heterozygosity was observed for chromosomes 7 and 17p, including the *TP53* gene which showed 4 copies of exons 2-6 (data not shown). A somatic frameshift variant in the *NFI* gene was observed (Table 1). *TP53* MLPA analysis on family members revealed that the *TP53* duplication was found in the 46-year-old asymptomatic father, suggesting the paternal inheritance.

Table 1. Clinically significant variants identified by CHOP NGS panels.

Gene	Chr #	Transcripts	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	Allele fraction	Germline /Somatic	Target Coverage
<i>TP53</i>	17	NM_000546	c.-28_672dup	p.?	CNV*	LoF	non-mosaic	Germline	811x
<i>HFE</i>	6	NM_000410	c.187c>g	p.His63Asp	SNV	Missense	48%	Germline	562x
<i>SBDS</i>	7	NM_016038	c.258+2T>C	p.?	InDel	Splicing	40%	Germline	345x
<i>NFI</i>	17	NM_001042492	c.3884_3887dup	p.Gln1298Serfs*17	Dup	Frameshift	64%	Somatic	616x

*Chr: Chromosome; *CNV: copy-number variant; SNV: single-nucleotide variant; LoF: loss of function

VARIANT INTERPRETATION

The tandem duplication of *TP53* exons 2-6 identified in this patient led to an extra copy of exons 2-6 and the inclusion of 28 nucleotides of the 5'UTR in mRNA transcripts. This inclusion is expected to generate a stop codon 18 codons downstream of the exon 6, resulting in a loss-of-function allele. The reduced expression of *TP53* in blood tissue is presumed to be the result of nonsense-mediated mRNA decay (Chang et al. 2007). These findings, as well as developing osteosarcoma and AML, support the diagnosis of LFS. The patient is also a carrier of two pathogenic variants, *HFE* p.His63Asp (low penetrance allele) and *SBDS* c.258+2T>C, associated with autosomal recessive hereditary hemochromatosis and Shwachman-Diamond syndrome, respectively.

SUMMARY

Here we report the first fully characterized tandem duplication of exons 2-6 of *TP53* including part of the 5'UTR in a pediatric patient with two different cancers using CHOP NGS panels, leading to the diagnosis of LFS. An exon 2-6 tandem duplication of the *APC* gene including 18 nucleotides of the 5'UTR was reported in a patient with familial adenomatous polyposis (Pedace et al. 2008). The inclusion of part of the 5'UTR introduced a premature stop codon, leading to a loss of function allele and a truncated protein. In addition, the *TP53* tandem duplication in our case potentially affects multiple important p53 functional domains including the p53 tumor suppressor family domain and DNA-binding domain. The duplication was also identified in his asymptomatic father by MLPA analysis. *TP53*-related LFS is inherited in an autosomal dominant manner. The core LFS cancers include osteosarcomas, soft-tissue sarcomas and CNS tumors in children, and breast carcinoma in adults. Missense substitutions in *TP53* concentrated in the highly conserved DNA binding domain of the protein are the most reported alterations, followed by nonsense and splice-site mutations (Varley et al. 1997; Olivier et al. 2003; Varley 2003; Ruijs et al. 2010; Giacomazzi et al. 2013; Guha and Malkin 2017; Kratz et al. 2021). Additionally, identification of *TP53* exons 2-4 duplication by array CGH was previously reported in an individual with LFS (Aury-Landas et al. 2013). LFS with secondary malignancy has been reported due to radiation therapy or chemotherapy (Shimatani et al. 2019; Hendrickson et al. 2020). Our patient had no prior radiation therapy, and his chemotherapy did not include alkylating agents and topoisomerase II inhibitors. His development of AML occurred approximately one year after the onset of chemotherapy, suggesting a second primary cancer. However, it could also be secondary leukemia resulting from the cooperation of cytotoxic osteosarcoma chemotherapy and the underlying cancer predisposition. The complexity of the genetic alterations and the biallelic loss of *TP53* in the leukemia cells portend a poor prognosis. Stem cell transplantation is currently being considered as a potential cure. This case also highlights the importance of combined identification of both significant copy number variants as well as SNVs/indels using NGS panels.

Figure 1 (A) Histologic section of the osteosarcoma prior to chemotherapy stained with hematoxylin and eosin demonstrates proliferation of large pleomorphic cells with enlarged, hyperchromatic nuclei and frequent atypical mitotic figures (arrows) associated with osteoid matrix (arrowhead). (B) Bone marrow biopsy shows hypercellular marrow for age (>95%) with variably sized atypical mononuclear cells with irregular nuclei and ample cytoplasm. Maturing myeloid and erythroid elements are markedly decreased. Small hypolobated dysplastic megakaryocytes are seen throughout the marrow (black arrows) and underlying fibrosis is suggested by the overall streaming pattern of the cells and the dilated bone marrow sinuses (white arrow). An immunohistochemical stain for myeloperoxidase is positive in a subset of the atypical cells (inset). (C) NGS copy number analysis displays a heterozygous duplication of partial *TP53* in the patient's blood sample. The green dots in the logR ratio plot represent the *TP53* exon 2-6 duplication with increased logR ratio. The grey dots represent the rest of Chr17 with normal logR ratio. In the B-allele frequency plot, green dots show the SNP patterns of 3 copies of the duplication as AAA, AAB, ABB and BBB while the rest of the SNPs in grey are two copies delineated as AA, BB or AB alleles. (D) Schematic illustration of the tandem duplication of *TP53* exons 2-6. Partial cDNA sequence identified by RNASeq is illustrated in red with red underline (exon 6), blue with grey underline (28 nucleotides of 5'UTR upstream of the start codon of exon 2), and blue with blue underline (exon 2). The start codon is marked in green, and the stop codon is marked in red.

ADDITIONAL INFORMATION

Ethics Statement

A case report does not constitute human subjects' research. Per CHOP policy, it therefore does not require IRB review.

Database Deposition and Access

The interpreted variant has been deposited in ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) as SCV002038503, SCV002038504 and SCV002038505.

Author Contributions

M.M.L., Y.Z., and F.X. designed the study. F.X., E.A., J.S. M.M.L and Y.Z. wrote the manuscript. F.X., J.W., J.S., M.P., K.C., A.L., F.Z., E.H.D, E.A.F, D.M.W, M.L, and L.K.C collected and analyzed the data. G.W., T.B., J.P., A.S.D, K.Z., S.B., N.B., and S.M. provided clinical data. All authors reviewed the manuscript.

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