Liquid Biopsy Detection of a *TP53* Variant in a "Disease-Free" Pediatric Patient with a History of *TP53*-Mutant Adrenocortical Carcinoma

Patrick R. Blackburn,[a,*] Shaohua Lei,[a,b] Sujuan Jia,[a,c] Ruth G. Tatevossian,[a,c] and Selene C. Koo [a,*]

[a] Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, United States; [b] Center of Excellence for Leukemia Studies, St. Jude Children's Research Hospital, Memphis, TN, United States; [c] Clinical Biomarkers Laboratory, St. Jude Children's Research Hospital, Memphis, TN, United States.

*Address correspondence to: P.R.B. at Department of Pathology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, United States. E-mail Patrick.blackburn@stjude.org. S.C.K. at Department of Pathology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, United States. E-mail Selene.koo@stjude.org.

CASE DESCRIPTION

The proband is a now 4-year-old female, who presented at 9 months of age following a 6-week history of virilization including development of body odor, facial acne, fine oily hair and scalp, and pubic hair. Laboratory findings showed elevated adrenal androgens and hypercortisolism with suppressed adrenocorticotropic hormone. Renal ultrasound showed a well-marginated solid mass measuring 36 × 34 × 29 mm in the right suprarenal space, worrisome for adrenocortical carcinoma.

A laparoscopic right adrenalectomy was performed to remove the tumor with negative margins. Histologically concerning features included elevated mitotic count (20 per 50 high-power fields), multiple atypical mitoses, and tumor necrosis. Her labs normalized postoperatively, consistent with stage 1 adrenocortical carcinoma. The family initially declined germline variant reporting. Comprehensive whole genome sequencing and whole exome sequencing (WES) with germline subtraction to remove false positives, and tumor-only whole transcriptome sequencing (RNA-seq) were performed on frozen tissue from the resected tumor specimen and the patient's peripheral blood specimen (1). Only variants that had mutant allele present in >10% of total reads in the germline sequence data were retained; variants at lower levels were filtered out, since they usually represent low-quality calls or tumor-in-normal contamination. The sequencing data underwent analysis using internal bioinformatics software on a secure HIPAA-compliant high-performance computing system. The alignment was performed against the hg19 (GRCh37) human genome reference sequence.

Sequencing analysis identified a *TP53* variant (NM_000546.5: c.657_668del; p.Tyr220_Pro223del) in a region of copy-neutral loss of heterozygosity on the p-arm of chromosome 17, with a variant allele frequency (VAF) of approximately 69% in whole genome sequencing, approximately 60% in WES, and approximately 59% in RNA-seq (Fig. 1). Additional abnormalities included a focal amplification including *RAC1* [7p22.1(5645827_6605937)x∼9] and a complex genome with several whole and partial chromosomal gains and losses and copy-neutral loss of heterozygosity (cnLOH) (Fig. 2A).

Plasma cell-free DNA (cfDNA) testing was performed on a sample collected 5 months after complete suprarenal mass resection. cfDNA was extracted from 1 mL plasma with the NucleoSnap® cfDNA kit and quantified with the Qubit 1xds DNA HS assay kit. The ThruPLEX® Tag-Seq HV kit was used to prepare the library. A clinically validated targeted DNA sequencing capture panel [St. Jude Pediatric Panel (2)] was used to enrich the library, following the standard manufacturer's protocol. The enriched library was sequenced on the NovaSeq platform and analyzed using in-house developed bioinformatic software (3). Analysis of the plasma cfDNA sample detected the *TP53* c.657_668del variant identified in the primary tumor at approximately 2.5% VAF. No other shared single nucleotide variants, gross or focal copy number

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alterations, or loss of heterozygosity were detected in the cfDNA sample compared to the original tumor resection (Fig. 2B).

At approximately 16 months of age (about 2 months after collection of the blood sample for cfDNA testing), the patient again developed signs of virilization with similar associated lab findings and respiratory findings. Computed tomography with positron emission tomography scan showed an approximately 1.2 cm avid lung nodule, for which she underwent a lung wedge resection; pathologic examination confirmed metastatic adrenocortical carcinoma. WES and RNA-seq of the formalin-fixed paraffin-embedded tumor tissue from the resection specimen was performed. The sequencing analysis showed the same *TP53* c.657_668del variant at 33% VAF in WES and 36% VAF by RNA-seq (Fig. 1). Similar copy number alterations and regions of cnLOH to the primary tumor were noted in the metastatic lesion (Fig. 2C).

As part of her diagnostic workup, the patient was referred for genetic evaluation with the cancer predisposition team. The patient's family history was largely noncontributory. Given the patient's history of adrenocortical carcinoma, germline genetic testing was ordered for Beckwith–Wiedemann syndrome (OMIM #130650), including methylation analysis with high-resolution copy number analysis for chromosome 11p15 (University of Pennsylvania), as well as a targeted custom exome-based sequencing panel with copy number variant detection (Prevention Genetics; genes tested: *APC*, *CDKN1C*, *EPCAM*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *NF1*, *PMS2*, *PRKAR1A*, *RET*, and *TP53*), all of which were negative.

QUESTIONS TO CONSIDER	
1.	Does the *TP53* variant identified by cfDNA testing correspond to tumor minimal residual disease (early evidence of metastatic lesion later detected in the lung)?
2.	What might differences in the VAF found in plasma cfDNA vs the tumor specimen at different timepoints indicate?
3.	How are we able to determine whether an alteration detected on tumor sequencing is germline or somatic?
4.	How might germline mosaicism affect the interpretation of cfDNA results?

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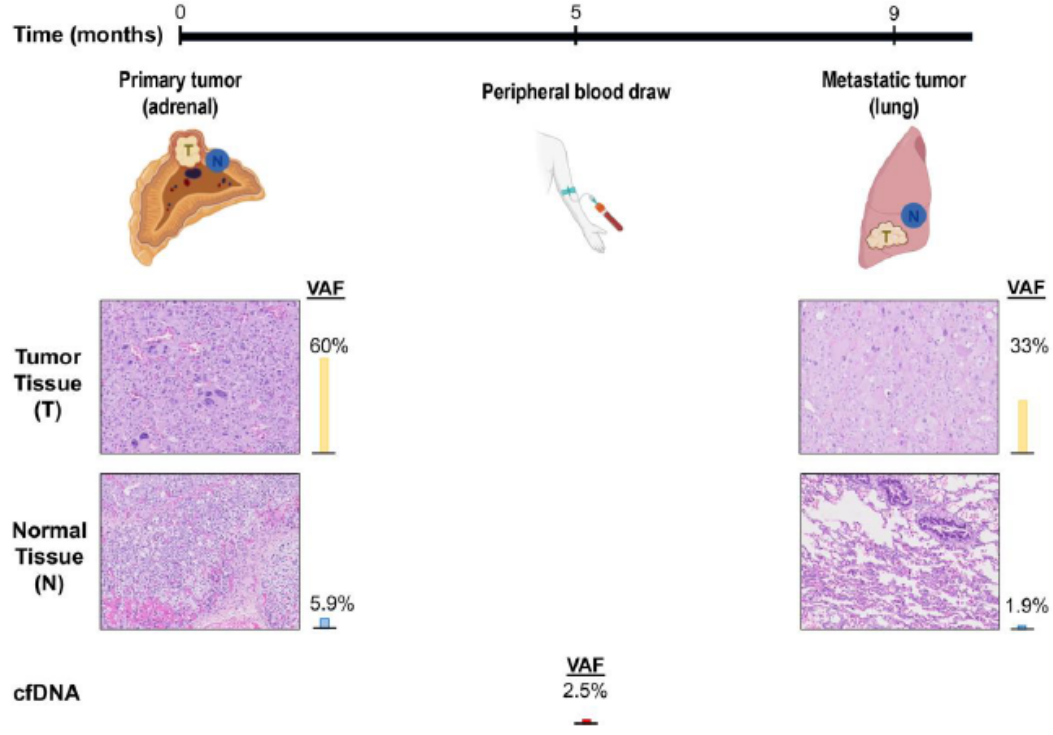

Fig. 1. Clinical course and evolution of *TP53* c.657_668del variant. Time of specimen collection is indicated at the top, with initial surgical resection of adrenal gland indicated as time 0; peripheral blood draw for cfDNA, 5 months later; and metastatic lung tumor resection at 9 months after initial adrenalectomy. Images show histologic features of this patient's adrenocortical tumor (middle) and adjacent normal tissue (below). VAFs are derived from sequencing as described in the text. T, tumor tissue; N, normal tissue. Created in BioRender. Koo, S. (2024) https://BioRender.com/d87j940. Color figure available at https://academic.oup.com/clinchem

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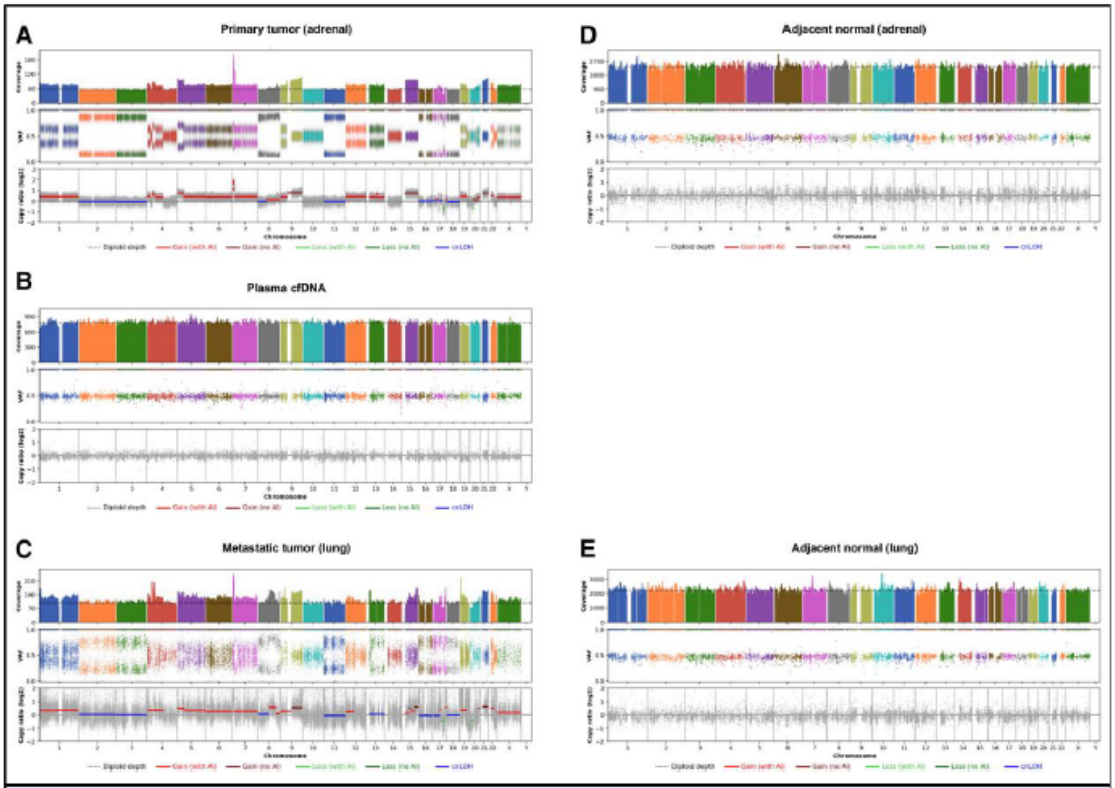

Fig. 2. Genome-wide profiles of copy number variation and copy-neutral loss of heterozygosity. (A), Primary tumor was sequenced by WES. In addition to the *RAC1* amplification, there was complex gain of chromosomes X, 1, 5, 6, 7; subclonal gain of 8q, 9; additional gain of 9q, 12, 13; 2 copy gain of 15, 20q; and 2-copy gain of 21. Complex focal gains were also noted at 4p16.3-q24, 5p15.33-p13.1, 7p22.3-7p21.1, 8q24.13-q24.21, 17q11.2-q12, 17q21.2-q22, 19p13.3-q13.32, and 22q11.1-q12.3. Two partial regions of loss were noted on 17q12-q21.2 and 17q22-q25.1. The tumor also had copy-neutral loss of heterozygosity of chromosomes 2, 3, 8, 11, 16, 17, and 18; (B), Plasma cfDNA was sequenced using SJPedPanel; (C), Metastatic tumor in lung was sequenced by WES and showed similar abnormalities to the primary adrenal tumor; (D), Adjacent normal (adrenal) was sequenced using SJPedPanel; (E), Adjacent normal (lung) was sequenced using SJPedPanel. The top panel of each plot, sequencing coverage; middle panel, the distribution of the VAF; bottom panel, log2 ratio values for copy number. Events of copy gain, copy loss, and copy-neutral loss of heterozygosity are indicated by different colors in the bottom panel. AI, allelic imbalance; SJPedPanel, St. Jude Pediatric Panel. Color figure available at https://academic.oup.com/clinchem

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Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the January 2025 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/71/1 and follow the link to the Clinical Case Study and Commentaries.

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