Letter to the Editor
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The First Korean Case of *NUP98-NSD1* and a Novel *SNRK-ETV6* Fusion in a Pediatric Therapy-related Acute Myeloid Leukemia Patient Detected by Targeted RNA Sequencing

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Dear Editor,

Targeted RNA-sequencing (RNA-seq) using next-generation sequencing (NGS) technology is a highly accurate method for selecting and sequencing specific transcripts of interest [1]. We routinely applied a customized targeted RNA-seq system during the diagnostic phase of hematologic malignancies. Our system detected the first Korean case of *NUP98-NSD1* and a novel *SNRK-ETV6* fusion with therapy-related acute myeloid leukemia (t-AML) showing a dismal clinical course. *NUP98-NSD1* accounts for approximately 4% of pediatric AML cases and shows a poor prognosis [2, 3]. It could be created by a cryptic t(5;11)(q35;p15.5) and exerts a leukemogenic function by binding near the *HOX* locus and *MEIS1* to increase expression via histone modifications [4]. The Institutional Review Board of Chonnam National University Hwasun Hospital (CNUHH), Hwasun, Korea (CNUHH-2020-091) approved this study and granted a waiver of consent due to its retrospective nature. This report highlights the role of high-throughput parallel targeted RNA-seq in enhancing the diagnostic yield of hematologic malignancies.

In April 2020, a 14-year-old girl visited the outpatient clinic of CNUHH 1.5 years and 1.9 years after a matched unrelated peripheral blood stem cell transplantation and initial diagnosis of AML, respectively, for a follow-up bone marrow (BM) examination At initial diagnosis, the Korean AML 2012 regimen (double-induction strategy with idarubicin or mitoxantrone plus cytarabine, followed by consolidation therapy with cytarabine and etoposide) was administered and complete remission was achieved 28 days after the second induction. The laboratory findings showed a leukocyte count of 3.1×10^9/L, absolute neutrophil count of 0.58×10^9/L, hemoglobin of 114 g/L, and platelet count of 37×10^9/L. BM aspirates revealed 28% leukemic blasts corresponding to French-American-British (FAB) type M2. The BM karyotype was 45,XX,add(3)(p25),del(5)(q?),-12,add(12)(p13)[8]//46,XY[12], and the multiplex reverse transcription (RT)-PCR (HemaVision kit; DNA Technology, Aarhus, Denmark) finding was negative.

Targeted RNA-seq (HEMEaccuTest RNA; NGeneBio, Seoul, Korea) of the BM sample using STAR-Fusion (ver 1.8.1) and

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FusionCatcher (ver 1.20) revealed *NUP98-NSD1* and a novel *SNRK-ETV6* fusion, which were confirmed by direct sequencing (Fig. 1). DESeq2 (ver 1.18.1) analysis showed that *WT1, ERG,* and *BAALC* expression increased 7.1, 5.6, and 4.1-log_2-fold, respectively, compared with 14 normal controls (Table 1). An additional tier II variant of *WT1*, NM_024426.3:c.1142C>A (p.Ser381*), and three tier III variants were detected by FreeBayes (ver 1.3.1) [5]. Further targeted DNA NGS (HEMEaccuTest DNA) confirmed the variants in targeted RNA-seq and additionally detected a tier II variant of *KRAS*, NM_033360.4:c.38G>A (p.Gly13Asp), and five tier III variants. However, no significant variant of *FLT3*, including *FLT3*-ITD, was detected. Donor lymphocyte infusion (DLI) was conducted on day 7 after the diagnosis; however, the BM blasts increased to 88% on day 29. The combination of fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor chemotherapy was started on day 35 and the BM blasts decreased to <5% on day 71 with sustained thrombocytopenia; however, the condition repeatedly relapsed on day 134 and the patient expired on day 223.

NUP98-NSD1$^+$ AML is characterized by frequent FAB-type M4/M5, a normal karyotype, and *HOXA/B* upregulation [2]. Further, *NUP98-NSD1* is mutually exclusive with other type II variants, but often co-occurs with type I variants such as *FLT3*-ITD or *WT1* variants [2, 3]. *FLT3*-ITD is the most common variant in *NUP98-NSD1*$^+$ AML (unlike our case), and its prognosis is dismal. Recent studies showed the promising therapeutic effects of dasatinib and navitoclax combination therapy and preemptive DLI based on minimal residual disease for *NUP98-NSD1*$^+$/*FLT3*-ITD$^+$ AML [6, 7]. Regarding the novel *SNRK-ETV6* fusion, the defect in *ETV6* is pathogenic in hematologic malignancies caused by rearrangement or deletions [8]. However, the partner *SNRK* gene defect at 3p22.1 has rarely been studied in hematologic malignancies but reportedly impacts hematopoietic cell proliferation and differentiation [9]. Further studies are needed to clarify the role of this novel fusion. This case also meets the criteria of t-AML, representing del(5q) with a complex karyotype and prior cytotoxic chemotherapy; both *NUP98*- and *ETV6*- rearrangements were reported in t-AMLs [10]. Additionally, the patient has a variant in *TP53* (rs1042522), known to increase the risk of developing therapy-related myeloid neoplasms. Owing to

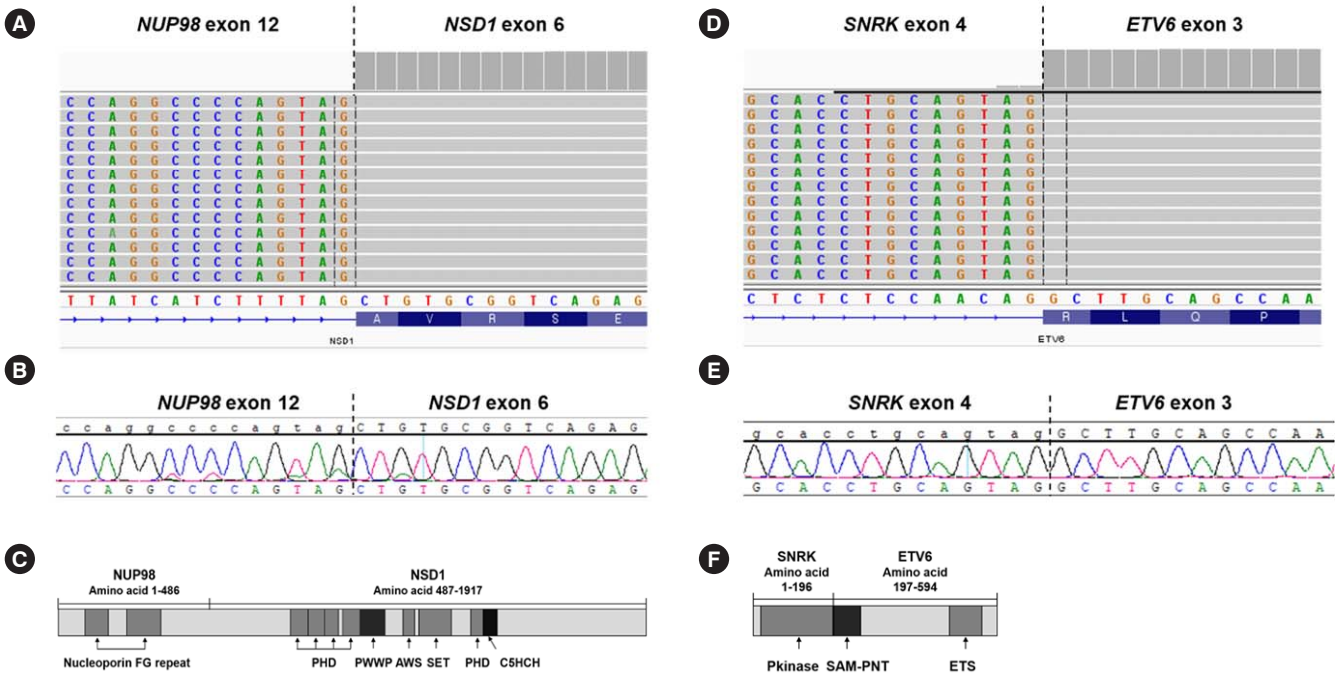

Fig. 1. Schematic representation of the *NUP98-NSD1* (A–C) and novel *SNRK-ETV6* (D–F) gene fusions and proteins. (A) Integrative genomics viewer (IGV) image showing the *NUP98-NSD1* breakpoints with 171 supporting junction read counts. (B) Direct sequencing confirmed the identical breakpoint causing an in-frame fusion of *NUP98-NSD1*. (C) The predicted fusion protein translated from the *NUP98-NSD1* transcript based on a merged sequence produced by STAR-Fusion (ver 1.8.1), which contains domains similar to a previous report [2] but is shorter. (D) IGV image showing the novel *SNRK-ETV6* fusion breakpoints with 484 supporting junction read counts. (E) Direct sequencing confirmed the identical breakpoint causing a novel in-frame fusion of *SNRK-ETV6*. (F) The predicted fusion protein translated from the *SNRK-ETV6* transcript based on the merged sequence produced by STAR-Fusion (ver 1.8.1).

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Table 1. Morphological, phenotypic, cytogenetic, and molecular characteristics of the initial and present phase of the case

	Initial phase			Present phase‡		
	Finding	VAF (%)	Method	Finding	VAF (%)	Method
WHO classification	AML, NOS			t-AML		
FAB, type	M2		Microscopic observation	M2		Microscopic observation
Expressed marker	CD33, CD34, CD117, HLA-DR, and MPO		Immunophenotyping or cytochemical stain	CD33, CD34, CD117, and MPO		Immunophenotyping or cytochemical stain
Karyotype	46,XX[20]		Karyotyping	45,XX,add(3)(p25),del(5)(q?),-12,add(12)(p13)[8]//46,XY[12]		Karyotyping
Gene fusion	Negative		Multiplex RT-PCR	*NUP98-NSD1*		Targeted RNA-seq
				SNRK-ETV6		
Upregulated gene	*WT1*		Real-time PCR*	*WT1*		Targeted RNA-seq
				ERG		
				BAALC		
				TP63		
				FGFR3		
				CCND1		
				CRLF2		
Variants	NM_024426.3(*WT1*):c.1142C>A (p.Ser381*)†	40.97	DNA NGS	NM_024426.3(*WT1*):c.1142C>A (p.Ser381*)†	12.61	Targeted RNA-seq and DNA NGS
	NM_016320.4(*NUP98*):c.3557T>G (p.Leu1186Trp)	47.15		NM_001166693.2(*AFF1*):c.1021A>G (p.Lys341Glu)§	40.58	
	NM_006197.3(*PCM1*):c.4148A>G (p.Asp1383Gly)	47.50		NM_016320.4(*NUP98*):c.3557T>G (p.Leu1186Trp)	10.45	
	NM_033360.4(*KRAS*):c.38G>A (p.Gly13Asp)†	17.39		NM_006197.3(*PCM1*):c.4148A>G (p.Asp1383Gly)	9.47	
	NM_002834.3(*PTPN11*):c.227A>C (p.Glu76Ala)	3.89		NM_033360.4(*KRAS*):c.38G>A (p.Gly13Asp)†	10.79	DNA NGS
				NM_018036.6(*ATG2B*):c.1586C>T (p.Thr529Met)§	38.94	
				NM_000051.3(*ATM*):c.2117C>T (p.Ser706Leu)	10.85	
				NM_022552.4(*DNMT3A*):c.920C>T (p.Pro307Leu)	3.11	
				NM_000215.3(*JAK3*):c.2062A>T (p.Ile688Phe)§	34.82	
				NM_017617.5(*NOTCH1*):c.4627G>A, (p.Gly1543Arg)§	35.32	

*Using the WT1 ProfileQuant kit (Ipsogen, Marseille, France). †Tier II variants classified by the grading system according to the levels of evidence required to determine significance [5]. ‡Increased gene expression was defined as a >2-$\log_2$-fold increase compared with 14 normal controls. *HOXA/B* expression could not be determined in the present case owing to the lack of a target RNA-seq panel. §Variants with a VAF of approximately 35% to 40% that could possibly be donor-derived germline variants rather than clonal evolution.
Abbreviations: FAB, French-American-British; AML, acute myeloid leukemia; NOS, not otherwise specified; VAF, variant allele frequency; RT-PCR, reverse transcription-PCR; NGS, next-generation sequencing; t-AML, therapy-related AML; RNA-seq, RNA-sequencing.

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the retrospective nature of this study, the *NUP98-NSD1* and *SNRK-ETV6* status at the initial diagnostic phase could not be ascertained.

Compared with previous studies using multiple diagnostic methods to characterize *NUP98-NSD1*$^{+}$ AML [2], the advantage of the present case was the use of RNA-seq, representing a simplified diagnostic step for gene fusion, expression, and gene variant analyses. Additionally, this system might help uncover novel genetic characteristics in leukemias in future larger-scale studies.

AUTHOR CONTRIBUTIONS

Lim HJ and Lee JH conceived and designed the study and collected and analyzed the data; Baek HJ and Kook H contributed to the data; Lim HJ and Shin MG wrote the final manuscript; Lee YE, Park JH, Lee SY, Choi HW, Choi HJ, Kee SJ, and Shin JH participated in coordination and discussion. All authors have accepted their responsibility for the entire content of this manuscript and approved the submission.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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