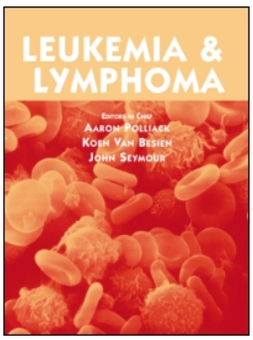

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Chimeric *NUP98–NSD1* transcripts from the cryptic t(5;11)(q35.2;p15.4) in adult de novo acute myeloid leukemia

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ABSTRACT

The t(5;11)(q35;p15.4) is a clinically significant marker of poor prognosis in acute myeloid leukemia (AML), which is difficult to detect due to sub-telomeric localization of the breakpoints. To facilitate the detection of this rearrangement, we studied *NUP98–NSD1* transcript variants in patients with the t(5;11) using paired-end RNA sequencing and standard molecular biology techniques. We discovered three *NUP98–NSD1* transcripts with two fusion junctions (*NUP98* exon 11-12/*NSD1* exon 6), alternative 5′ donor site in *NUP98* exon 7, and *NSD1* exon 7 skipping. Two of the transcripts were in-frame and occurred in all t(5;11) samples ($N = 5$). The exonic splicing events were present in all samples ($N = 23$) regardless of the *NUP98–NSD1* suggesting that these novel splice events are unassociated with t(5;11). In conclusion, we provide evidence of two different *NUP98–NSD1* fusion transcripts in adult AML, which result in functional proteins and represent suitable molecular entities for monitoring t(5;11) AML patients.

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NUP98; *NSD1*; 11p15.5 translocation; acute myeloid leukemia; alternative splicing

Introduction

In *de novo* and therapy-related lymphoid and myeloid malignancies, 11p15.5 rearrangements with the nucleoporin-98-kDa (*NUP98*) gene involve more than 30 different partner genes [1–3]. Within the diverse spectrum of *NUP98* rearrangements, the nuclear receptor-binding SET-domain protein 1 (*NSD1*) gene, located at 5q35, is the most commonly observed *NUP98* fusion partner in acute myeloid leukemia (AML) [4,5]. The relative frequency of the *NUP98–NSD1* fusion in cytogenetically normal pediatric and adult AML (CN-AML) was previously shown to be 16% and 2%, respectively. Beyond AML, *NUP98–NSD1* has been reported in rare cases of myelodysplastic syndromes (MDS), and acute biphenotypic leukemia [6–8]. Several studies have observed that *NUP98–NSD1* characterizes a clinically significant group of AML patients [4,8–11]. Furthermore, over 80% of NUP98–NSD1 patients co-express internal tandem duplications in the *FLT3* gene (*FLT3-ITD*), and co-occurrence of these two aberrations has been shown to result in especially high induction failure and poor survival in AML [8,10].

At the chromosomal level, the t(5;11) juxtaposes the N-terminus of NUP98 to the C-terminus of NSD1 [12] (5′-*NUP98–NSD1*-3′), and occasionally the N-terminus of NSD1 to the C-terminus of NUP98 (5′-*NSD1–NUP98*-3′) [8]. The rearrangement leads to haploinsufficiency of the *NUP98* and *NSD1* genes, which participate in the nucleocytoplasmic transport of small molecules [13] and in chromatin-mediated transcriptional regulation [14], respectively. NSD1 is required for normal growth and development, thus haploinsufficiency of NSD1 results in Sotos syndrome [15] that is characterized by abnormal excessive growth [15]. Haploinsufficiency of NUP98 is associated with premature separation of sister chromatids, severe aneuploidy and ill-timed degradation of securin [16]. The NUP98–NSD1 fusion protein is known to accumulate in the nucleus [8,17] and transform hematopoietic precursors through epigenetic changes that prevent myeloid cell differentiation [18]. Thus far, two

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Supplemental data for this article can be accessed here.

chimeric *NUP98–NSD1* transcripts have been described in MDS, joining *NUP98* exon 12 to *NSD1* exon 6 and *NUP98* exon 11 to *NSD1* exon 6 [6]. While the *NUP98* exon 12/*NSD1* 6 transcript is well-known aberration in AML, the sole expression of *NUP98* exon 11/*NSD1* exon 6 fusion transcript has only been described in one pediatric AML patient with a *NUP98–NSD1*-like gene expression signature [11]. To date, co-expression of these two *NUP98–NSD1* transcripts in AML has not yet been reported.

Considering the sub-telomeric location of the breakpoints, detection of t(5;11) by traditional chromosome banding can be challenging. In addition, the potential for alternative spliced versions of the *NUP98–NSD1* transcripts can complicate minimal residual disease monitoring. Application of newer technologies such as next-generation genome and RNA sequencing (RNA-seq) could overcome these challenges. By applying paired-end RNA-seq to samples from de novo AML patients and analyzing the data with a bioinformatic pipeline designed to detect fusion transcripts, we were able to efficiently detect *NUP98–NSD1* transcripts in t(5;11) positive samples that included alternatively spliced versions with novel splice junctions. In this study, we show that whole transcriptome sequencing is an informative method for identifying clinically significant fusion transcripts and novel splice junctions present in t(5;11) AML patients that can be followed by targeted methods for molecular detection.

Materials and methods

Study patients

Bone marrow (BM) aspirates were collected after written informed consent from three adult de novo AML patients with *NUP98–NSD1* fusion (*NUP98–NSD1*$^+$), ten patients without *NUP98–NSD1* (*NUP98–NSD1*$^-$) and two healthy donors. The karyotypes of all study patients' BM metaphase cells were obtained by standard G-banding. The present study was approved by the Local Institutional Review Board at the Helsinki University Central Hospital (permit numbers 239/13/03/00/2010, 303/13/03/01/2011, Helsinki University Hospital Ethics Committee) and conducted in accordance with the Declaration of Helsinki. The clinical and demographic characteristics of the *NUP98–NSD1*$^+$ study patients are shown in Table 1.

Sample preparation

Bone marrow mononuclear cells (MNCs) were isolated by gradient centrifugation (Ficoll-Paque; GE Healthcare, Little Chalfont, UK). Total RNA was extracted from the BM MNCs with the RNeasy Mini Kit (Qiagen, Hilden, Germany) or Total RNA Purification Kit (Norgen Biotek, Thorold, Canada). Genomic DNA was isolated from BM MNCs using the DNeasy Blood & Tissue Kit or QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA and RNA samples were quantified with the Qubit® Fluorometer (Thermo Fisher Scientific, Carlsbad, CA, USA) and the quality of the RNA assessed using 2100 Bioanalyzer instrument and RNA Nano chips (Agilent Technologies, Santa Clara, CA, USA).

Array-based comparative genomic hybridization

Array-CGH was used to explore the presence of small genomic deletions that could indicate sites for DNA breakpoints associated with genomic rearrangements. For this, three micrograms of digested genomic DNA was labeled and processed as previously described [19]. Sample processing and hybridization to 244K Human CGH Microarrays (Agilent Technologies, Santa Clara, CA, USA) containing up to 1×10^6 highly sensitive 60-mer oligonucleotide probes was performed according to a standard protocol from the manufacturer. The array-CGH data was analyzed with Genomic Workbench Standard Edition 5.0 (Agilent Technologies, Santa Clara, CA, USA).

RNA sequencing

One to three micrograms of DNase-treated (RNase-Free DNase Set; Qiagen, Hilden, Germany) total RNA (RIN >8) from each patient was sequenced. The RNA samples were depleted of ribosomal RNAs (Ribo-Zero

Table 1. Clinical and demographic characteristics of the *NUP98–NSD1*$^+$ patients.

Patient	Gender	Age	Disease	FAB subtype	Survival from Dg (days)	BM blast count at Dg (%)	WBC count at Dg (×E9/L)	B-neut at Dg (×E9/L)	Karyotype
600	Male	54	Acute monocytic leukemia	M5b	244	20	223.0	22.0	46 XY, t(5;11)(q35;p15.4) del (3q), t(12;15)
3600	Female	39	Acute myeloblastic leukemia with maturation	M2	1175[a]	40	13.1	3.3	46 XX, t(5;11)(q35;p15.4)
3660	Male	58	Acute monocytic leukemia	M5	545	81	32.0	10.0	46 XY, t(5;11)(q35;p15.4) del (9)(q21–22)

Dg: diagnosis; FAB: French–American–British morphological classification of acute leukemias; WBC: white blood cell; B-neut: peripheral blood neutrophils.
[a]The patient remains in complete remission.

rRNA Removal Kit; Epicentre, Madison, WI, USA), purified (RNeasy® MinElute® Cleanup Kit; Qiagen, Hilden, Germany), and reverse transcribed to double stranded cDNA using SuperScript® Double-Stranded cDNA Synthesis Kit (Thermo Fisher Scientific, Carlsbad, CA, USA) with random hexamers (New England BioLabs, Ipswich, MA, USA). The RNA-seq libraries were prepared using Illumina-compatible Nextera technology (Epicentre, Madison, WI, USA), bar-coded, and enriched by ligation PCR according to the manufacturer's instructions (Illumina, San Diego, CA, USA). PCR products were column purified and sequenced to an average length of 317 (104–530) nucleotides per mate using Illumina HiSeq™ 2000 instrument. Sequenced reads were extracted from TopHat v2.0.3 [20] alignment using in-house Perl scripts and executed in FusionCatcher [21]. Fusion gene supporting reads were aligned against a reference sequence and visually inspected in integrative genomics viewer (IGV) version 2.3.59 [22]. Initially, the sequenced FASTQ reads were aligned against human reference genome (HG18), but to improve the visualization and quantification of reads, we created FASTA format fusion constructs containing all exons of both partner genes. The 5′-*NUP98–NSD1*-3′ and 5′-*NSD1–NUP98*-3′ constructs were generated comparably by extracting *NUP98* (NM 139131.3) and *NSD1* (NM 022455.4) sequences from PubMed (NCBI nucleotide database), and by annotating them using a general feature format (GFF) annotation file. The GFF file was created using annotation files from GenBank (NCBI) and Perl scripts. The start and end exon positions for *NUP98–NSD1* and the reciprocal *NSD1–NUP98* were annotated accordingly. The GFF-file was needed for TopHat2 alignment between FASTQ reads and the fusion. After alignment, all reads (single and paired) aligned to the fusion were extracted and processed. PCR duplicates, non-primary reads, and reads with low mapping quality (>10) were filtered out before acquiring the read counts with SAMtools [23].

Cloning and Sanger sequencing

One microgram of total RNA was reverse transcribed to cDNA using SuperScript™ III First-Strand Synthesis System for RT-PCR (Thermo Fisher Scientific, Carlsbad, CA, USA) with Oligo (dT) primers. *NUP98-NSD1* was amplified from the cDNA template using Phusion™ high-fidelity (HF) DNA polymerase (Thermo Fisher Scientific, Carlsbad, CA, USA), 5 × Phusion HF buffer, dNTP mix (10 mM each), and oligonucleotide primers: NUP98 5′-ATGTTTAACAAATCATTTGG-3′ and NSD1 5′-CTACTTCTGTTCTGATTCTG-3′. The cycling conditions for PCR were as follows: an initial denaturation at 95 °C for 2 min, 35 cycles of denaturation for 20 s at 95 °C, annealing for 10 s at 54 °C, and elongation for 100 s at 72 °C with final extension time of 5 min. PCR fragments were separated on 1% agarose gel in the presence of SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific, Carlsbad, CA, USA) and gel-purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Duren, Germany). The purified fragments were cloned into the pCR® 2.1-TOPO® vector and introduced into One Shot™ StbI3™ Chemically Competent *Escherichia coli* cells according to TOPO® TA-cloning protocol (Thermo Fisher Scientific, Carlsbad, CA, USA). *NUP98–NSD1*$^{+}$ colonies were identified with colony-PCR, and propagated overnight in Luria broth supplemented with ampicillin (100 μg/ml). The plasmids were extracted with the NucleoSpin® Plasmid Easy Pure Kit (Macherey-Nagel) and sequenced. Bidirectional Sanger sequencing using oligonucleotide primers (Table S2) spanning across the full-length *NUP98–NSD1* was performed in ABI3730xl DNA Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, South San Francisco, CA, USA). Base calling was done using Sequencing Analysis v5.2 software, while the generated trace files were inspected with Sequencher™ 5.2 (Gene Codes Corporation, Ann Arbor, MI, USA) and BLAST algorithm (National Center for Biotechnology Information [NCBI] nucleotide database). The *NUP98* (NM 139131.3) and *NSD1* (NM 022455.4) sequences were used as reference for the alignment.

Statistical analysis

Statistical analysis was performed with Prism software version 6.0 (GraphPad Software, La Jolla, CA, USA). The novel splice events were compared between *NUP98–NSD1*$^{+}$ versus *NUP98–NSD1*$^{-}$ samples using unpaired nonparametric Mann–Whitney rank comparison test. The *t*-tests between groups were two-tailed, and exact *p* values below .05 were considered statistically significant.

Results

Detection of NUP98–NSD1 transcripts and exonic rearrangements

Array-CGH showed that the index patient carried narrow cryptic deletions within *NUP98* and *NSD1* genes at positions 11p15.4 and 5q35.2, respectively (Figure S1). The 0.11 Mb (chr11: 3,617,261–3,723,126) and 0.05 Mb (chr5: 176,546,244–176,595,458) microdeletions indicated

unbalanced t(5;11)(q35.2;p15.4)/*NUP98–NSD1* translocation. The chromosomal breakpoints putatively occurred within one oligonucleotide probe length (60-mer) from positions chr11: 3,723,126 and chr5: 176,595,458 (NCBI36/HG18). To verify the *NUP98–NSD1* fusion sequence, we cloned it from the index patient's leukemic cells and identified two in-frame (*NUP98–NSD1_v1-v2*) and one out-of-frame fusion (*NUP98–NSD1_v3*) transcripts with mRNA lengths of 5,676, 5,562, and 5,432 base pairs (GenBank® accession numbers: KU695532, KU695533, KU695534). The Sanger sequenced fusion transcripts revealed fusion junctions between *NUP98* exon 12/*NSD1* exon 6 and *NUP98* exon 11/*NSD1* exon 6, alternative 5′ donor site in *NUP98* exon 7 (nucleotides 757-783), and *NSD1* exon 7 skipping (nucleotides 3922-4192) (Figure 1(A–C)). The nucleotide positions were counted from the first nucleotide of ATG start codon in the corresponding NCBI reference sequence (NM 139131.3 and NM 022455.4).

Nucleotide to amino acid conversion (Figure 1(D)) indicated that the phenylalanine (F), glycine (G), and leucine (L) possessing nontandem repeats and Gle2p-binding (GLEBS)-like motif at the NH_3 terminus of NUP98 were consistent and functional in all three *NUP98–NSD1* transcripts. Each fusion transcript had eight GLFG-repeats, FGFG-repeat, GLFGFG-repeat, and non-tandem FG-repeats. The FG-repeat counts in *NUP98–NSD1_v1*, *NUP98–NSD1_v2*, and *NUP98–NSD1_v3* were 38, 36 and 39, respectively. The C-terminal functional domains of NSD1 within the hybrid proteins remained unchanged in all except in *NUP98–NSD1_v3*, which was truncated due to exon skipping related frame shift.

We re-analyzed the RNA-seq data from patient 600 using a modified alignment strategy and confirmed that the novel splice events identified through cloning and Sanger sequencing were neither PCR-introduced artifacts nor RNA-seq library preparation errors (Figure 2). In sample 600_2, 93% (75/81) of the *NUP98–NSD1* supporting reads supported the fusion between *NUP98* exon 12 and *NSD1* exon 6 (*NUP98–NSD1_v1*), and 7% (6/81) the *NUP98* exon 11/*NSD1* exon 6 fusion (*NUP98–NSD1_v2*). The percentages were similar (91% and 9%) in the relapse sample 600_3 collected 2 months later. The reciprocal 5′-*NSD1–NUP98*-3′ was undetectable in both samples.

Validation of fused exons and exonic rearrangements with paired-end RNA seq

We performed RNA-seq on additional samples with ($N = 4$) and without ($N = 16$) t(5;11) to acquire more evidence for chimeric *NUP98–NSD1* fusion transcripts and exonic rearrangements. As shown in Figure 3(A), all positive samples co-expressed the two in-frame *NUP98–NSD1* transcripts. In these samples, *NUP98–NSD1_v1* was the predominant transcript, however, 11% (95% confidence interval 6.0–16.6%) of the *NUP98–NSD1* supporting FASTQ reads supported the *NUP98–NSD1_v2* fusion. Its relative frequency increased during disease progression from 9.4% (9/96) to 18.1% (13/72) in patient 3660 and from 7% (6/81) to 9% (7/78) in patient 600. Interestingly, *NSD1* exon 7 skipping and alternative 5′ donor site of *NUP98* exon 7 were detected from all study samples regardless of *NUP98–NSD1* (Figure 3(B,C)). In the *NUP98-NSD1*$^+$ group, 8% (95% CI 5.3–9.9%) of the reads supported an alternative 5′ donor site of *NUP98* exon 7, while it was 6% (95% CI 5.3–7.4%) in the negative group (OR 1.183). In both groups, 6% (95% CIs 0–13.1% and 3.4–9.4%) of the reads spanning across *NSD1* exon 7 supported its skipping. No statistically significant differences in *NSD1* exon 7 skipping or alternative 5′ donor site was found between the *NUP98–NSD1*$^+$ and *NUP98–NSD1*$^-$ groups (*p*-values .33 and .38) suggesting these are previously unknown normal splicing events of the wild type *NUP98* and *NSD1* genes.

Discussion

In this study, our objective was to facilitate the molecular detection of *NUP98–NSD1* by investigating *NUP98–NSD1* fusion transcripts in adult de novo AML. We acquired experimental evidence of three chimeric *NUP98–NSD1* transcripts and demonstrated that the two in-frame transcripts previously reported in MDS [6] are also co-expressed in adult AML. The co-expression was found in serial samples from two different patients, indicating that both *NUP98–NSD1* transcripts are clonally stable during disease progression. The clonal stability and clinical relevance of *NUP98–NSD1* is well established [8,9]. The absence of reciprocal *NSD1–NUP98* in our study patients is in agreement with previous studies, which have suggested *NUP98–NSD1* as the sole initiating oncoprotein [6,24]. Our results showed that *NUP98–NSD1_v1,* joining *NUP98* exon 12 to *NSD1* exon 6, is the predominant transcript. However, an alternative transcript *NUP98–NSD1_v2* with *NUP98* exon 11 joined to *NSD1* exon 6 was also detected in all t(5;11) samples. Nevertheless, both fusion transcripts result in in-frame fusion proteins that retain the same functional domains of NUP98 and NSD1. Additional studies are required to assess the functional activity of the different transcript variants.

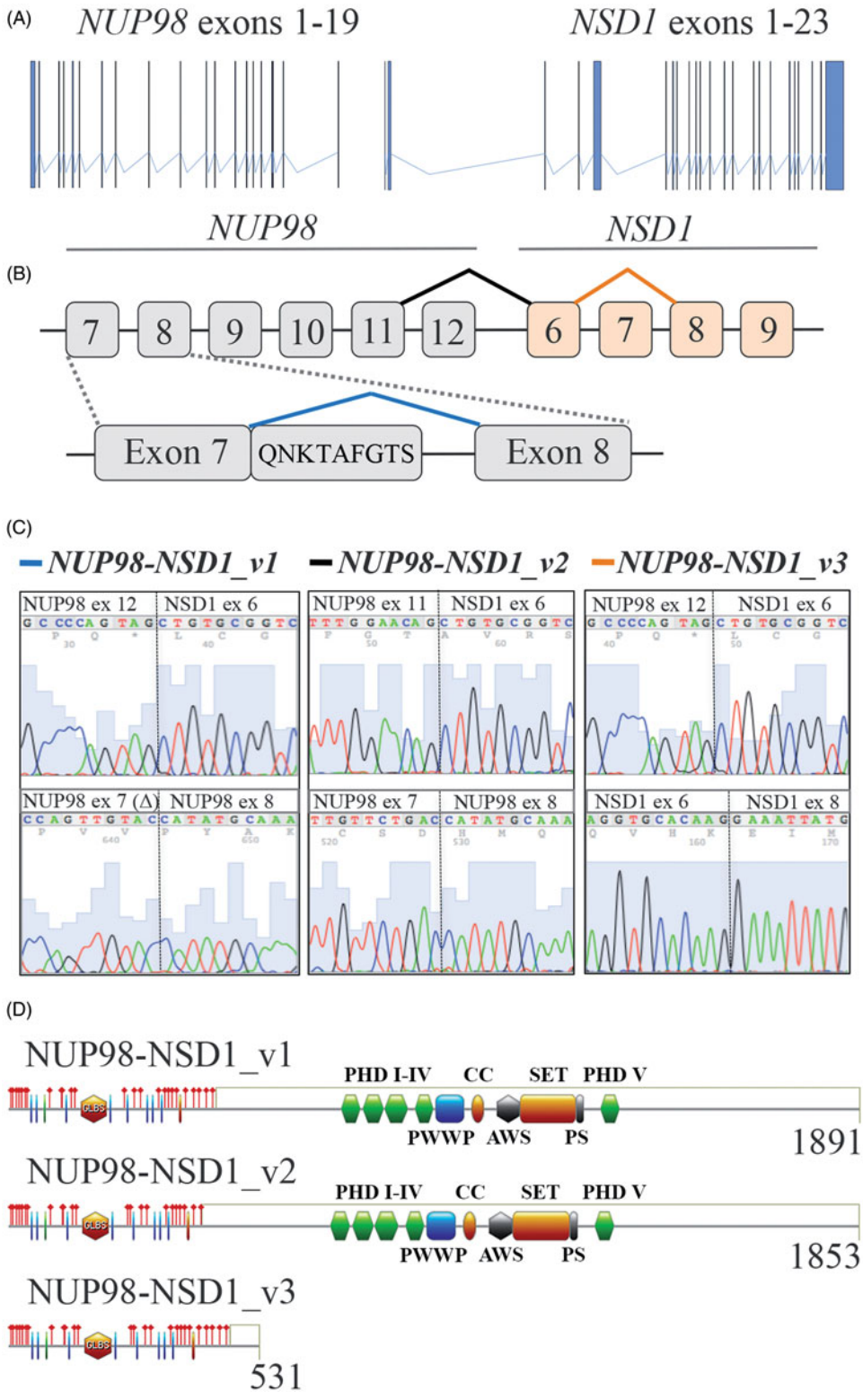

Figure 1. *NUP98–NSD1* transcripts detected from the index patient. (A) Exon structures of *NUP98* (NM 139131.3) and *NSD1* (NM 022455.4), plotted with GenomeGraphs version 3.1 [26]. Bar thickness represents the exon size in base pairs and line lengths the distance between exons. (B) The schematic illustrates three *NUP98–NSD1* transcripts detected by sequence analysis of plasmids with full-length *NUP98–NSD1*. (C) The fluorescent peak trace chromatograms revealed two fusion junctions and alternatively spliced exonic regions. (D) The cDNA sequences were translated using EMBOSS Transeq and analyzed with Simple Modular Architecture Research Tool (SMART) [27]. The structural domains and repeats, including FG- (red), FGFG- (green), GLFG- (blue), and GLFGFG-repeats (orange) were drawn to scale using MyDomains-Image creator. The domain structure abbreviations are: *Drosophila* Su(var)3-9 and 'Enhancer of zeste' proteins (SET), post-SET domain (PS), associated with SET domain (AWS), coiled-coil domain (CC), plant homeodomain (PHD), and Gle-2 binding sequence (GLBS).

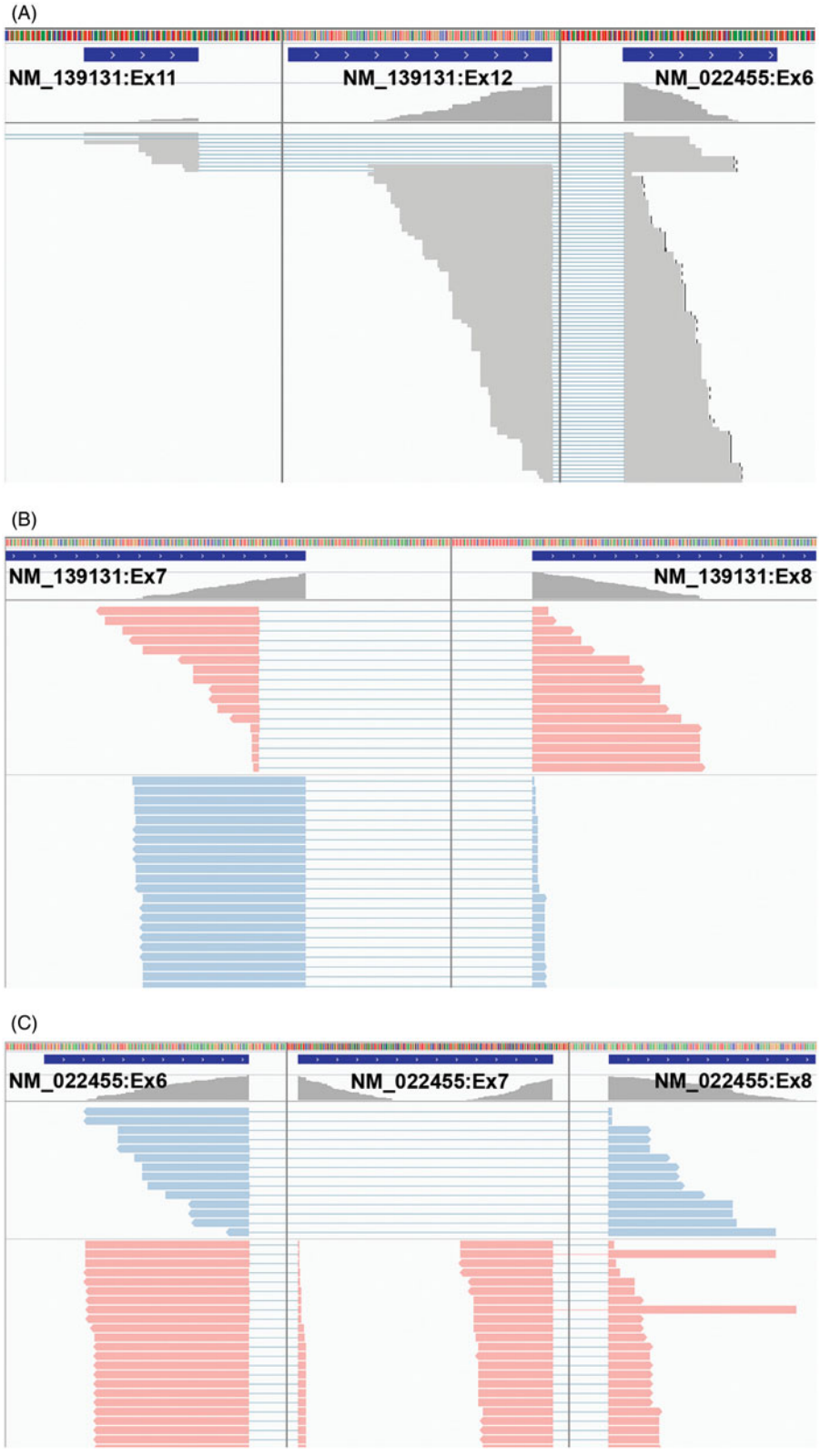

Figure 2. IGV views of two *NUP98–NSD1* fusion junctions and alternatively spliced regions in the index patient detected by paired-end RNA sequencing. The figure shows supporting FASTQ-reads for fusion junctions between *NUP98* exon 11/*NSD1* exon 6, and between *NUP98* exon 12/*NSD1* exon 6 (A), alternative 5′ donor site of *NUP98* exon 7 (B), and exon skipping of *NSD1* exon 7 (C). The color bars on top represent different nucleotides: A (green), C (blue), T (red), and G (orange). The middle panel (grey) show RNA-sequencing data coverage. *NUP98* (NM 139131) is transcribed from the − strand, and *NSD1* (NM 022455) from the + strand.

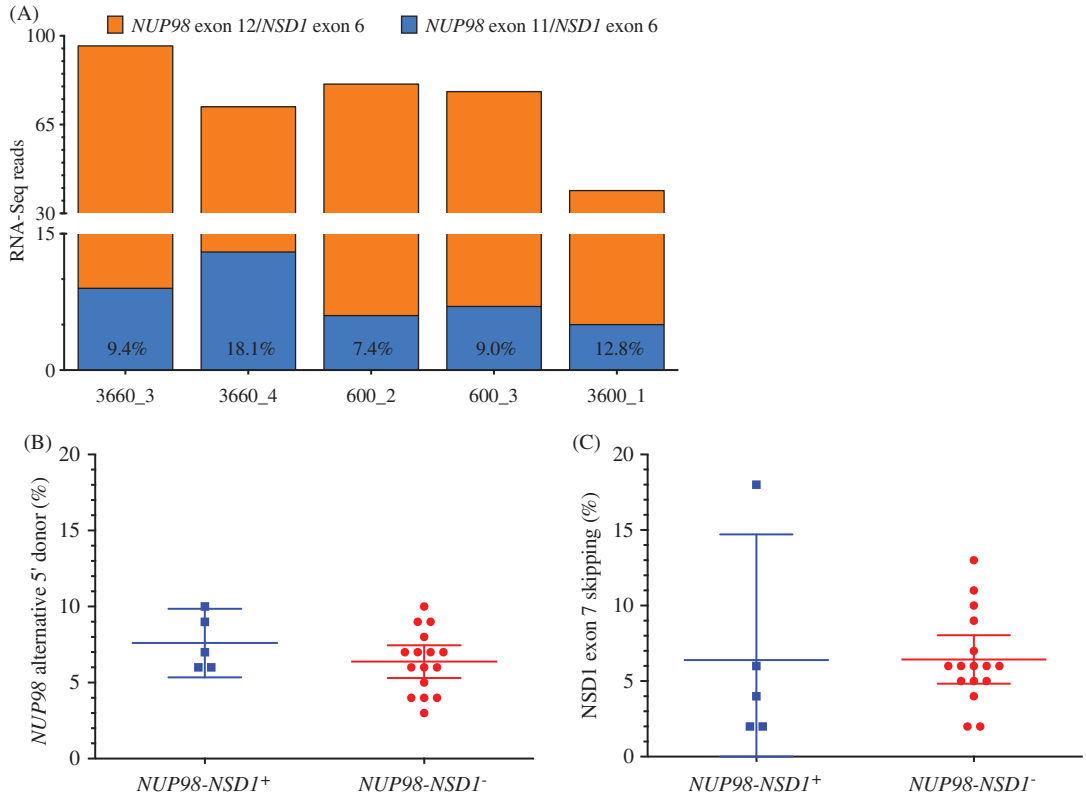

Figure 3. RNA-seq supporting read counts for the *NUP98–NSD1* fusion junctions and splicing events. (A) The stacked bars show total number of reads supporting the two *NUP98–NSD1* fusion junctions in five t(5;11)(q35.2;p15.4) positive samples from three adult *de novo* AML patients. The scatter plots visualize the percentage of RNA-seq reads supporting the alternative 5′ donor site of *NUP98* exon 7 (B) and *NSD1* exon 7 skipping (C) in *NUP98–NSD1*$^{+}$ ($N = 5$) and *NUP98–NSD1*$^{-}$ patient samples ($N = 16$). The error bars illustrate the mean percentage of reads with 95% confidence interval.

Increased *NUP98–NSD1* expression has previously been shown to correlate with hematological relapse [8,9]. In the largest AML cohort screened for *NUP98-NSD1* thus far [10], most patients remained positive for the fusion at the end of cycle 1 of induction chemotherapy (EOI-1), which strongly indicates that *NUP98–NSD1* is an important tool for evaluating treatment response and MRD, either after chemotherapy or stem cell transplantation. As recommended previously by Grimwade and colleagues [25], sequential RT-qPCR monitoring coupled with preemptive therapy may eventually lead to individualized management of patients with rare gene fusions such as *NUP98–NSD1*. Due to emerging clinical significance of *NUP98–NSD1*, especially among pediatric AML patients [8], it is advisable that all newly diagnosed AML patients without favorable genetic abnormalities should be screened upfront for t(5;11) by fluorescence in situ hybridization (FISH) and followed by monitoring of *NUP98–NSD1* transcripts by RT-PCR. Here, we show that RNA-seq is a sensitive method to detect *NUP98–NSD1* fusion transcripts as well as the different fusion splice variants. Although data from additional t(5;11) patients should be analyzed, our initial findings suggest that the fusion joining exon 11 of *NUP98* to exon 6 of *NSD1* should be included to the molecular diagnostics panel in AML. This is supported by a previous study, which indeed detected the sole expression of *NUP98* exon 11/*NSD1* exon 6 fusion in a pediatric AML patient with *NUP98–NSD1*-like gene expression signature [11]. In addition, as next generation sequencing applications are incorporated into clinical laboratory diagnostic practice, cryptic translocations such as *NUP98–NSD1* should be more readily detected. To address the therapeutic needs of *NUP98–NSD1*$^{+}$ myeloid malignancies, further intensive studies of patients with t(5;11) are warranted.

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References

[1] Kobzev YN, Martinez-Climent J, Lee S, et al. Analysis of translocations that involve the NUP98 gene in patients with 11p15 chromosomal rearrangements. Genes Chromosomes Cancer. 2004;41:339–352.

[2] Gough S, Slape C, Aplan P. NUP98 gene fusions and hematopoietic malignancies: common themes and new biologic insights. Blood. 2011;118:6247–6257.

[3] Soler G, Kaltenbach S, Dobbelstein S, et al. Identification of GSX2 and AF10 as NUP98 partner genes in myeloid malignancies. Blood Cancer J. 2013;3:e124.

[4] Struski S, Lagarde S, Bories P, et al. NUP98 is rearranged in 3.8% of pediatric AML forming a clinical and molecular homogenous group with a poor prognosis. Leukemia. 2017;31:565–572.

[5] Bisio V, Zampini M, Tregnago C, et al. NUP98-fusion transcripts characterize different biological entities within acute myeloid leukemia: a report from the AIEOP-AML group. Leukemia. 2017;31:974–977.

[6] La Starza R, Gorello P, Rosati R, et al. Cryptic insertion producing two NUP98/NSD1 chimeric transcripts in adult refractory anemia with an excess of blasts. Genes Chromosomes Cancer. 2004;41:395–399.

[7] Romana SP, Radford-Weiss I, Ben Abdelali R, et al. Groupe Francophone de Cytogenetique H. NUP98 rearrangements in hematopoietic malignancies: a study of the Groupe Francophone de Cytogenetique Hematologique. Leukemia. 2006;20:696–706.

[8] Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, et al. NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. Blood. 2011;118:3645–3656.

[9] Akiki S, Dyer SA, Grimwade D, et al. NUP98-NSD1 fusion in association with FLT3-ITD mutation identifies a prognostically relevant subgroup of pediatric acute myeloid leukemia patients suitable for monitoring by real time quantitative PCR. Genes Chromosomes Cancer. 2013;52:1053–1064.

[10] Ostronoff F, Othus M, Gerbing RB, et al. NUP98/NSD1 and FLT3/ITD coexpression is more prevalent in younger AML patients and leads to induction failure: a COG and SWOG report. Blood. 2014;124:2400–2407.

[11] Shiba N, Ichikawa H, Taki T, et al. NUP98-NSD1 gene fusion and its related gene expression signature are strongly associated with a poor prognosis in pediatric acute myeloid leukemia. Genes Chromosomes Cancer. 2013;52:683–693.

[12] Jaju RJ, Haas OA, Neat M, et al. A new recurrent translocation, t(5;11)(q35;p15.5), associated with del(5q) in childhood acute myeloid leukemia. Blood. 1999;94:773–780.

[13] Griffis ER, Xu S, Powers MA. Nup98 localizes to both nuclear and cytoplasmic sides of the nuclear pore and binds to two distinct nucleoporin subcomplexes. Mol Biol Cell. 2003;14:600–610.

[14] Qiao Q, Li Y, Chen Z, et al. The structure of NSD1 reveals an autoregulatory mechanism underlying histone H3K36 methylation. J Biol Chem. 2011;286:8361–8368.

[15] Kurotaki N, Imaizumi K, Harada N, et al. Haploinsufficiency of NSD1 causes Sotos syndrome. Nat Genet. 2002;30:365–366.

[16] Jeganathan KB, Malureanu L, van Deursen JM. The Rae1–Nup98 complex prevents aneuploidy by inhibiting securin degradation. Nature. 2005;438:1036–1039.

[17] Fahrenkrog B, Martinelli V, Nilles N, et al. Expression of leukemia-associated Nup98 fusion proteins generates an aberrant nuclear envelope phenotype. PLoS One. 2016;11:e0152321.

[18] Wang GG, Cai L, Pasillas MP, et al. NUP98–NSD1 links H3K36 methylation to Hox-A gene activation and leukaemogenesis. Nat Cell Biol. 2007;9:804–812.

[19] Edgren H, Murumagi A, Kangaspeska S, et al. Identification of fusion genes in breast cancer by paired-end RNA-sequencing. Genome Biol. 2011;12:R6. doi: 10.1186/gp-2011-12-1-r6

[20] Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics. 2009;25:1105–1111.

[21] Nicorici D, Satalan M, Edgren H, et al. FusionCatcher – a tool for finding somatic fusion genes in paired-end RNA-sequencing data. bioRxiv. 2014;011650. doi: https://doi.org/10.1101/011650

[22] Robinson JT, Thorvaldsdottir H, Winckler W, et al. Integrative genomics viewer. Nat Biotechnol. 2011;29:24–26.

[23] Li H, Handsaker B, Wysoker A, et al. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25:2078–2079.

[24] Cerveira N, Correia C, Doria S, et al. Frequency of NUP98–NSD1 fusion transcript in childhood acute myeloid leukaemia. Leukemia. 2003;17:2244–2247.

[25] Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116:354–365.

[26] Durinck S, Bullard J, Spellman PT, et al. GenomeGraphs: integrated genomic data visualization with R. BMC Bioinformatics. 2009;10:2

[27] Schultz J, Copley RR, Doerks T, et al. SMART: a web-based tool for the study of genetically mobile domains. Nucleic Acids Res. 2000;28:231–234.