Clinical whole-genome sequencing identifies NSD3 as the correct fusion partner of NUP98 in a patient with acute myeloid leukemia and t(8;11) (p11.2;p15): a case report

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Case Report

Keywords: Acute myeloid leukemia, NUP98/NSD3 rearrangement, targeted sequencing, whole-genome sequencing, fluorescence in situ hybridization, MyeloSeq, ChromoSeq.

Posted Date: February 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1374871/v1

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Table 1. Genomic Aberrations Detected by Various Assays

| Assay          | Detected Alterations                                                                 |
|----------------|--------------------------------------------------------------------------------------|
| NUP98          | Detected with NUP98/NSD3 rearrangement                                               |
| FGFR1          | Detected with FGFR1 rearrangements                                                   |
| ChromoSeq      | Identified 73bp in 40 genes or gene hotspots, 612 pre-defined structural variants     |

**Abstract**

**Background:** Only rare cases of acute myeloid leukemia (AML) have been shown to harbor a t(8;11)(p11.2;p15.4). This translocation is believed to involve the fusion of **NSD3** or **FGFR1** with **NUP98**; however, from targeted mRNA quantitative PCR analysis, no molecular approaches have been utilized to define the chimeric fusions present in these rare cases.

**Case Presentation:** Here we present the case of a 51-year-old female with AML with myelodysplastic-related morphologic changes, 13q deletion and t(8;11), where initial fluorescence in situ hybridization (FISH) assays were consistent with the presence of **NUP98** and **FGFR1** rearrangements, and suggestive of **NUP98/FGFR1** fusion. Using a streamlined clinical whole-genome sequencing approach, we resolved the breakpoints of this translocation to intron 4 of **NSD3** and intron 12 of **NUP98**, indicating **NUP98/NSD3** rearrangement as the underlying aberration. Furthermore, our approach identified small variants in **WT1** and **STAG2**, as well as an interstitial deletion on the short arm of chromosome 12, which was cryptic in G-banded chromosomes.

**Conclusions:** **NUP98** fusions in acute leukemia are predictive of poor prognosis. The associated fusion partner and the presence of co-occurring mutations, such as **WT1**, further refine this prognosis with potential clinical implications. Using a clinical whole-genome sequencing analysis, we resolved t(8;11) breakpoints to **NSD3** and **NUP98**, ruling out the involvement of **FGFR1** suggested by FISH while also identifying multiple chromosomal and sequence level aberrations.

**Background**

The presence of t(8;11)(p11.2;p15.4) has been described in rare cases of acute myeloid leukemia (AML) as an isolated aberration [1, 2], with an additional chromosomal aberration [3–5], or in the setting of a complex karyotype [6]. Previously reported FISH studies had suggested the involvement of either **FGFR1** [6] or **NSD3** [1] in the formation of a chimeric protein with **NUP98**. However, when performed, targeted RT-PCR [1, 4] and Southern hybridization [5] analyses have revealed expression of **NUP98**/**NSD3** chimeric fusion transcripts. Here we present a case of AML with t(8;11)(p11.2;p15), which initially appeared to be an isolated **NUP98**/**FGFR1** rearrangement as detected by fluorescence in situ hybridization (FISH). However, a subsequent clinical whole-genome sequencing assay [7] identified **NSD3** as the correct fusion partner, while also revealing the presence of sequence variants in **WT1** and **STAG2**, as well as an interstitial deletion on the short arm of chromosome 12. To our knowledge, this represents only the seventh reported case of t(8;11), a translocation which has not been documented in the literature or publicly accessible databases since 2009 [5]. Furthermore, this report illustrates the potential clinical utility of whole-genome sequencing in AML and myelodysplastic syndrome (MDS).

**Case Presentation**

This is a case of a 51 year-old female who had received a heart transplant with basiliximab induction therapy 6 years prior to nonischemic cardiomyopathy. She presented with 1-week of exertional chest pain associated with nausea, shortness of breath and dizziness. She had no prior history of cardiac graft rejection and was on stable doses of tacrolimus and mycophenolate mofetil, as well as infectious prophylaxis with acyclovir and trimethoprim/sulfamethoxazole. Initial work-up for a primary cardiopulmonary etiology, including electrocardiogram (ECG), troponins, transthoracic echocardiogram (TTE) and chest radiograph (CXR), was unremarkable.

Peripheral blood smear revealed pancytopenia with red blood cell anisopoikilocytosis, hypogranular neutrophils and circulating blasts. Bone marrow biopsy demonstrated findings consistent with AML with myelodysplastic related morphologic changes, including 50% cellularity with 59% blasts (enzyme cytochemistries: alpha naphthyl butyrate esterase negative, myeloperoxidase positive), decreased megakaryocytes, multi-lineage dyspoiesis and increased iron with normal sideroblasts. Flow cytometry identified an immunophenotypically abnormal blast population consistent with myeloid differentiation comprising 19.46% of total cellular events. Blasts expressed CD45 (dim), CD33, CD34, CD13, CD117, CD38, HLA-DR and CD123, but were negative for CD19, CD15, TdT, CD16, CD11b, CD7, CD10, CD14 and CD64.

Complete blood count (CBC) revealed new-onset pancytopenia with a white blood cell count (WBC) of 0.9 x 10^9/L, absolute neutrophil count (ANC) of 0.2 x 10^9/L, hemoglobin of 7.4 g/dL, mean corpuscular volume (MCV) of 111.2 fL and platelets of 101 x 10^9/L (previous CBCs had been entirely within normal limits). Further evaluation for secondary causes of pancytopenia was unrevealing, including infectious and nutritional testing. The bone marrow aspirate was sent for comprehensive genomics analyses. Interphase FISH analyses for MDS (chromosomes 5 and 7) and AML (MECOM, RUNX1/T11/RUNX1, PML/RARA, CBFB) probes were negative (Table S1; Fig. S1a-f). However, karyotype analysis (Table 1; Fig. 1a) revealed two abnormal, related clones; 20% with t(8;11) only, and 50% with 13q deletion in addition to t(8;11), with the rest of the metaphase cells being normal. Sequential metaphase FISH studies were performed to determine the possible involvement of **FGFR1** (8p11) and **NUP98** (11p15) loci. Results showed 23% with 3′ **FGFR1** on derivative 11p and 36.5% with 5′ **NUP98** on derivative 8p, indicative of **FGFR1** and **NUP98** rearrangements (Table 1; Fig. 1b-c).

Subsequent clinical genome-wide sequencing was performed via ChromoSeq (Methods S1) [7], a next generation sequencing (NGS)-based assay which detects SNVs and indels (≥73bp) in 40 genes or gene hotspots, and 612 pre-defined structural variants including 624 genes, and genome-wide copy number alterations (≥5Mb). The ChromoSeq assay identified **NSD3** (Nuclear Receptor Binding SET Domain Protein 3), a gene 27 kb downstream of **FGFR1**, as the fusion partner of **NUP98** (Table 1; Fig. 1d), specifically between intron 4 of **NSD3** (Fig. 1e) and intron 12 of **NUP98**.

Table 1. Genomic Aberrations Detected by Various Assays
the WHO (2016) showed that fusion, however, was not verified by other techniques, leaving the possibility of a different partnership with a different gene at an adjacent locus. Of note, in our review of the literature, we identified only six documented cases of t(8;11) prior to this report occurring in AML and MDS. 

**Discussion And Conclusions**

In our review of the literature, we identified only six documented cases of t(8;11) prior to this report occurring in AML and MDS [1–6]. One of these cases showed FGFR1 and NUP98 rearrangements by FISH [6], findings similar to what we initially observed in this AML patient. The suspected NUP98/FGFR1 rearrangement (0.5%) detected by FISH, with no evidence of deletion in the long arm of chromosome 13. The patient was subsequently treated with 2 consolidation cycles of VYXEOS® with a sustained remission and the ultimate plan to pursue consolidation allogeneic hematopoietic cell transplantation, if possible.
which is also known as 8p11 myeloproliferative syndrome. This diagnosis represents a rare, generally aggressive, and clinically heterogeneous class of hematologic malignancies unified by the presence of the FGFR1 rearrangement. Neither our patient nor the patient reported by Sohal et al. [6] showed features of an MPN. Based on combined FISH and RT-PCR results, the five remaining documented cases were ultimately determined to have NUP98/NSD3 chimeric fusions, similar to our current case. However, the breakpoints reported by previous studies were intron 3 and intron 11 [1], while in our patient the breakpoint occurred in intron 4 of NSD3. Therefore, it is likely that none of the reported cases of AML and MDS contained an FGFR1 rearrangement. Moreover, it is possible that additional unreported cases classified as “myeloid and lymphoid neoplasms with FGFR1 abnormalities” may, in fact, represent a genetically heterogenous cohort that does not have an FGFR1 abnormality and in which at least some of the clinical variability and poor prognosis may be attributable to chromosomal aberrations which go unidentified and uncharacterized in the absence of broader, more precise genomic profiling.

This case highlights the biologically relevant implications of identifying and resolving such aberrations. The gene NUP98 (nucleoporin 98 kDa) encodes a central component of the multi-peptide nuclear pore complex, which regulates RNA trafficking between the nucleus and cytoplasm, as well as playing a role in transcriptional regulation and cell-cycle progression [15–17]. To date, >30 NUP98/NSD1 fusion genes have been described in association with a number of myeloid neoplasms, including AML and MDS [18]. Additional studies have confirmed the presence of a NUP98 gene fusion defines a high-risk subset of leukemia with a poor prognosis [19]. Variation within the fusion partner may further modulate this risk, as does the co-occurrence of additional mutations including FLT3 internal tandem duplication (ITD) and WT1. Interestingly, the frequency and types of NUP98 fusion partners also varies by disease subset. For example, NUP98/NSD1 fusions have been associated with myelomonocytic leukemias while NUP98/KDM5A are more commonly observed in monocytic, erythroid and megakaryoblastic leukemias [20, 21]. Meanwhile, NSD3, the fusion partner identified in this case, encodes a histone methyltransferase which has been found to be transcriptionally activated with NUP98, and has been associated with MDS-related AML as well as leukemogenic transformation of de novo and treatment-related AML [1] (reviewed by Alexander et al, 2016 [22]). Thus, identification of the specific NUP98/NSD3 fusion in this case reveals key biologic upregulations of the disease and may allow greater ability to risk stratify and manage patients such as this in the future.

Furthermore, identifying the presence of a NUP98 fusion AML may also allow for the development of rational, targeted treatment approaches that leverage our growing understanding of the drivers of disease biology. Given this patient’s MDS-related changes and complex karyotype, in addition to the poor prognosis associated with NUP98 gene fusions, an induction and consolidation treatment approach for adverse-risk AML was undertaken with ultimate plans for an allogeneic hematopoietic cell transplantation in first remission, if possible. However, there are a number of potential agents which may be relevant in targeting the disease biology of NUP98 fusion AML. Based on the role of NUP98 in regulating RNA and peptide trafficking to and from the nucleus, as well as the importance of transcriptional regulation of additional genes by the NUP98-fusion product, the use of selinexor, an XPO1 inhibitor, may disrupt the nuclear trafficking and impair expression of gene products relevant to the leukemogenesis driven by the NUP98 gene-fusion product [23]. Additional pre-clinical studies using various sequencing techniques have also suggested the therapeutic potential for BCL2 inhibition, and JAK-STAT inhibition, in targeting key signaling pathways operative in the disease biology of NUP98-fusion AML [24, 25]. Meanwhile, the observation of increased co-occurrence of FLT3 ITD alterations in the context of NUP98 fusion AML, the use of any number of FLT3 inhibitors may be a consideration [25, 26]. In addition, in the setting of NSD3 rearrangements, the development of histone methyltransferase inhibitors or the use of currently available histone deacetylase inhibitors (HDACI) may represent another potential treatment approach [27].

In summary, although NUP98-NSD3 chimeric mRNA had been previously reported in rare cases of AML, to the authors’ knowledge, this case represents the first report of the detection of this fusion using a genome sequencing approach. As such, this case highlights the ability of clinical genome sequencing to collectively provide a comprehensive understanding of the AML genetic architecture consistent with conventional approaches while also allowing base-pair level resolution of the t(8;11) with precise identification of the translocation partners. Notably, this observation may have particular relevance in cases of AML or MDS when FISH is consistent with the presence of an FGFR1 rearrangement, which may in fact be an NSD3 rearrangement. More broadly, the application of genome sequencing may result in a more complete understanding of genetic underpinnings of each patient’s disease, and thus allow clinicians the opportunity to more accurately risk stratify and tailor treatment strategies [10].

**Abbreviations**

AML  
acute myeloid leukemia  
MDS  
myelodysplastic syndrome  
MPN  
myeloproliferative neoplasm  
FISH  
fluorescence in situ hybridization  
ITD  
internal tandem duplication  
NGS  
next generation sequencing  
RT-PCR
Declarations

Consent for publication
The patient provided written informed consent for the use of her health information in this publication, which is available upon request.

Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. Any patient specific information or data is not publicly available due to patient privacy and protection of health information.

Competing interests
The authors declare that they have no competing interests.

Funding
No funding sources.

Authors’ contributions
BAM analyzed/interpreted patient data, and assisted in the conceptual planning, wrote the manuscript with contributions from ZDC, IA, JS and MCS.

ZDC participated directly in the clinical care of the patient, ZDC analyzed/interpreted patient data, and assisted in the conceptual planning, drafting and final editing of the manuscript.

MCS analyzed and interpreted patient data, participated in drafting and final editing of the manuscript.

JV participated directly in the clinical care of the patient, edited, and approved the final manuscript.

ZX participated directly in the clinical care of the patient and final editing of the manuscript.

JS participated in the clinical care of the patient, analyzed/interpreted patient data, and assisted in the conceptual planning, edited, and approved the final manuscript.

JF participated in the clinical care of the patient, edited, and approved the final manuscript.

ED analyzed/interpreted patient data, read and approved the final manuscript.

DS analyzed/interpreted patient data, read and approved the final manuscript.

JN analyzed and interpreted data, edited, and approved the final manuscript.

KL participated in the clinical care of the patient, read and approved the final manuscript.

IA conceptualized and designed the study, analyzed and interpreted data, participated in drafting and final editing of the manuscript.

Acknowledgements
The authors would like to acknowledge the patient presented, who consented to the sharing of her story with the altruistic wish that her experience would positively contribute to the scientific understanding of the biology of AML. The authors would also like to thank Shelia Rackers for performing sequential metaphase FISH and providing high quality chromosome and FISH images.

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Results from karyotype, interphase and sequential metaphase FISH, and ChromoSeq analyses.

a. One of the two clones revealed by karyotype analysis with co-occurring t(8;11)(p11.2;p15.4) and deletion 13q14.11q14.3 (band level 400).

b-c. Sequential metaphase FISH revealed 3'\textit{FGFR1} on derivative 11p (b) and 5'\textit{NUP98} on derivative 8p (c) indicative of \textit{FGFR1} and \textit{NUP98} rearrangements, and suggestive of an \textit{FGFR1}/\textit{NUP98} fusion.

d. ChromoSeq revealed t(8;11), deletion of 12p12.3p13.31, and 13q14.11-q14.3 deletion (more distal than the band interval reported by karyotype analysis).

e. 8p11.23 breaks at intron 4 of \textit{NSD3} and \textbf{NOT} within \textit{FGFR1}. (\textbf{Note}: Kreatech has \textit{FGFR1} on 8p12 and not 8p11)

**Supplementary Files**

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