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Rare Non-Cryptic *NUP98* Rearrangements Associated With Myeloid Neoplasms and Their Poor Prognostic Impact

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Background: *NUP98* rearrangements (*NUP98*r), associated with various hematologic malignancies, involve more than 30 partner genes. Despite their clinical significance, reports on the clinicopathological characteristics of rare *NUP98*r remain limited. We investigated the characteristics of patients with myeloid neoplasms harboring *NUP98*r among those identified as having 11p15 translocation in chromosomal analysis.

Methods: We retrospectively reviewed results from bone marrow chromosomal analyses conducted between 2011 and 2023 and identified 15 patients with 11p15 translocation. Subsequently, *NUP98*r were evaluated using FISH and/or reverse transcription PCR, and clinical and laboratory data of the patients were analyzed.

Results: *NUP98*r were identified in 11 patients initially diagnosed as having AML (N=8), myelodysplastic syndrome (N=2), or chronic myelomonocytic leukemia (N=1), with a median age of 44 yrs (range, 4–77 yrs). Three patients had a history of chemotherapy. In total, five *NUP98* fusions were identified: *NUP98*::*DDX10* (N=3), *NUP98*::*HOXA9* (N=2), *NUP98*::*PSIP1* (N=2), *NUP98*::*PRRX1* (N=1), and *NUP98*::*HOXC11* (N=1). Patients with *NUP98*r exhibited a poor prognosis, with a median overall survival of 12.0 months (95% confidence interval [CI], 3.4–29.6 months) and a 5-yr overall survival rate of 18.2% (95% CI, 5.2%–63.7%).

Conclusions: Our study revealed the clinical and genetic characteristics of patients with myeloid neoplasms harboring rare and non-cryptic *NUP98*r. Given its association with poor prognosis, a comprehensive evaluation is crucial for identifying previously underdiagnosed *NUP98*r in patients with myeloid neoplasms.

Key Words: Gene fusion, Hematologic neoplasms, *NUP98*, Prognosis, Translocation, 11p15

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INTRODUCTION

The *NUP98* gene, located on chromosome 11p15, encodes a protein of the nucleoporin family of carriers that transport substrates into the nucleus and are responsible for regulating transcription and mitotic progression [1]. It is involved in various chromosomal translocations, and proteins produced by *NUP98* fusions aberrantly regulate gene expression, leading to uncon-

trolled cell proliferation and impaired differentiation, ultimately resulting in oncogenesis [2].

Since the description of the translocation involving chromosome 11p15 in the blast phase of chronic myeloid leukemia in 1982, *NUP98* rearrangements (*NUP98*r) have been reported as fusions with more than 30 partner genes in hematological malignancies, including myeloid and lymphoid neoplasms [3-6]. *NUP98*r are largely divided into two categories depending on whether they are fused with homeobox (HOX) genes (e.g., *HOXA9*, *HOXA11*, *HOXA13*, *HOXC11*, *HOXC13*, and *PRRX1*) or non-HOX genes (e.g., *NSD1*, *KDM5A*, *DDX10*, and *PSIP1*) [7, 8]. These partners share similar leukemic phenotypes, often resulting in poor outcomes, particularly in pediatric patients [9]. The recent expansion of RNA-based whole-transcriptome sequencing has led to increased detection of *NUP98*r, even in cases with cytogenetically cryptic fusions (e.g., *NUP98*::*NSD1* and *NUP98*::*KDM5A*), underscoring their clinical significance.

Considering their distinctive clinicopathologic characteristics, *NUP98*r were recently included as recurrent genetic abnormalities defining AML in the 2022 WHO classification as well as the International Consensus Classification (ICC) [10, 11]. However, until recently, the 11p15 translocation identified through chromosomal analysis was often overlooked, and thorough evaluation for *NUP98*r has not been frequently conducted. Moreover, data regarding the laboratory and clinical characteristics associated with rare *NUP98*r partner genes are insufficient. Therefore, we investigated the clinical and genetic characteristics of patients with myeloid neoplasms who showed *NUP98*r among those identified as having 11p15 translocation through chromosomal analysis.

METHODS

Patients

We retrospectively reviewed the results of bone marrow (BM) chromosomal analyses conducted between 2011 and 2023 and identified 15 patients showing 11p15 translocation. Subsequently, *NUP98*r were evaluated in these patients using FISH and/or reverse transcription (RT-)PCR. All patients were diagnosed or revised according to the 2016 WHO classification of myeloid neoplasms [12], and patient clinical and laboratory information was obtained from electronic medical records. For survival analysis, patients with AML aged ≥18 yrs who were diagnosed between July 2018 and December 2022 were used as control groups, including patients with AML with a normal karyotype (NK) (N=26), AML with myelodysplasia-related (MR) (N=58), and AML with mutated *TP53* (N=33), with the latter two groups reclassified according to the ICC [11, 13]. This study was approved by the Institutional Review Board of Samsung Medical Center, Seoul, Korea (approval No.: 2023-08-152), and informed consent was waived.

Cytogenetic studies

Conventional chromosomal analysis was performed on heparinized BM aspirates using a standard G-banding technique following short-term culturing without mitogen, and at least 20 metaphases were analyzed for karyotyping. FISH was performed on BM aspirates using a *NUP98* break-apart FISH probe (MetaSystems, Altlussheim, Germany) according to the manufacturer's instructions. Briefly, after spreading cells onto slides, the cells and probe were denatured at 73±1°C for 5 mins and incubated at 37°C overnight. The cells were incubated in 50% formamide/2XSSC solution and counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). FISH signals were analyzed using DAPI, FITC, and rhodamine triple-band pass filter sets, and at least 200 interphase cells were analyzed.

Molecular genetic studies

RNA was extracted from BM aspirates using a QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized using a HiSenScript RH(–) RT PreMix Kit (Intronbio, Seongnam, Korea) according to the manufacturer's instructions. For each chromosomal abnormality observed in individual patients, candidate exons of partner genes were selected based on previous reports [8, 14-19], and specific primers were designed. Selected *NUP98* partner genes included *DDX10* (11q22), *HOXA9/HOXA11/HOXA13* (7p15), *PSIP1* (9p22), *PRRX1* (1q24), and *HOXC11/HOXC13/RARG* (12q13). In cases where the partner gene was undisclosed or where insufficient sample was available, molecular analysis was not performed. After confirmation of PCR amplification, the breakpoint of each fusion transcript was verified through Sanger sequencing, and data were analyzed using Sequencher (v.5.4; Gene Codes, Ann Arbor, MI, USA).

Genomic DNA was extracted from BM aspirates using a DNA extraction kit (Promega, Madison, WI, USA). Fragment analysis for *FLT3*-internal tandem duplication (ITD) was performed on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using in-house primers, and the data were analyzed using GeneMapper Software 4.0 (Applied Biosystems). Targeted next-generation sequencing (NGS) of 49 myeloid neoplasm-related genes was conducted using IDT xGen pre-designed/custom probes (Integrated DNA Technologies, Coralville,

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USA) on a NextSeq 550Dx instrument (Illumina, San Diego, CA, USA). Reads were aligned to the GRCh37/hg19 human reference genome using Burrows–Wheeler Aligner-MEM, and variant calling, annotation, and analysis were carried out using the in-house pipeline.

Statistical analysis

Statistical analyses were performed using the Statistical Software Package for Social Sciences (IBM SPSS Statistics v.27; IBM, Armonk, NY, USA). Relapse-free survival was determined from the time of morphologic complete remission of AML to disease relapse, death from any cause, or last follow-up. Overall survival (OS) was determined from the time of initial diagnosis to death from any cause or last follow-up. Survival analysis was performed using Kaplan–Meier plots, and differences in survival were compared using the log-rank test. Values are expressed as the median with interquartile range (IQR). Statistical significance set at $P<0.05$.

RESULTS

Characteristics of patients with *NUP98*r

Among the 15 patients with 11p15 translocation, 11 had *NUP98*r, and their clinicopathological characteristics are summarized in Table 1. The median patient age was 44 yrs (range, 4–77 yrs), and 64% of patients were female. The median white blood cell count, Hb level, and platelet count were 6.68×10^9/L (IQR, 2.93–88.79 $\times10^9$/L), 85 g/L (IQR, 71–98 g/L), and 40×10^9/L (IQR, 30–83 $\times10^9$/L), respectively. All patients exhibited anemia, and nine (82%) showed thrombocytopenia.

At initial diagnosis, eight (73%) patients had AML, one (9%) had chronic myelomonocytic leukemia (CMML)-2, and two (18%) had myelodysplastic syndrome (MDS); one with MDS-excess blasts (EB)-2, and the other diagnosed as having MDS based on cytogenetics despite the absence of dysplasia. The frequency of non-cryptic *NUP98*r was 0.81% in AML, 0.40% in MDS, and 1.67% in CMML. Three (27%) patients developed the diseases as a result of prior chemotherapy for ovarian or breast cancer. Among non-AML patients, those with CMML-2 and MDS-EB-2 progressed to AML after 28 months and 4 months, respectively. Ultimately, 10 (91%) patients were diagnosed as having AML. In the French-American-British classification of AML, M5 (acute monoblastic/monocytic leukemia) was the most common subtype (N=5), followed by M1 (AML without maturation) (N=3) and M4 (acute myelomonocytic leukemia) (N=2), and half of the patients with AML exhibited negative or dim CD34 expression. Dysplasia in at least one lineage was observed in seven (63.6%) patients.

Genetic characteristics of *NUP98*r

NUP98::*DDX10* was the most common (N=3), followed by *NUP98*::*HOXA9* (N=2), *NUP98*::*PSIP1* (N=2), *NUP98*::*PRRX1* (N=1), and *NUP98*::*HOXC11* (N=1). Representative FISH, RT-PCR, and sequencing results of patients with *NUP98*r are presented in Fig. 1, and detailed cytogenetic and molecular genetic characteristics are provided in Table 2.

All three patients with inv(11)(p15q22) harbored a fusion between *NUP98* exon 14 and *DDX10* exon 7 (Fig. 1). Among them, two were diagnosed as having AML, and one as having MDS. t(7;11)(p15;p15) was detected in three patients, with two diagnosed as having AML and one as having CMML-2. In the 7p15 region, known partner genes of *NUP98* included *HOXA9*, *HOXA11*, and *HOXA13*, and a fusion between *NUP98* exon 12 or 11 and *HOXA9* exon 1 was identified in two patients. However, in one patient, the partner gene could not be identified because of insufficient samples. Two patients with t(9;11)(p22;p15) were diagnosed as having MDS-EB2 and AML, respectively. They had a fusion between *NUP98* exon 9 and *PSIP1* exon 5 or 7. Lastly, in one patient each with AML with t(1;11)(q24;p15) and t(11;12)(p15;q13), *NUP98*::*PRRX1* and *NUP98*::*HOXC11* were detected, respectively. In a patient with t(11;16)(p15;q23), *NUP98*r were confirmed via FISH. However, there was no previously known partner gene of *NUP98* in the 16q23 region, suggesting a potential novel partner gene. Unfortunately, further evaluation could not be conducted because of insufficient sample.

Besides *NUP98*r, three patients exhibited additional cytogenetic abnormalities, such as 5q deletion, +4, +8, +15, or +21; and *FLT3*-ITD was detected in only one out of nine patients with AML. Targeted NGS was performed for four patients with AML. A *WT1* variant was observed in one patient with *NUP98*::*DDX41*, accompanied by a *KRAS* variant. Additionally, one patient with *NUP98*::*PSIP1* had a *PHF6* variant, and the other patient with *NUP98*r involving 16q23 harbored five variants in *ASXL1*, *DNMT3A*, *IDH2*, and *STAG2*.

Prognostic impact of *NUP98*r

During the median follow-up of 12 months (range, 0–107 months), four (36%) patients had relapsed, and nine (82%) had expired. The median OS of patients with *NUP98*r was 12.0 months (95% confidence interval [CI], 3.4–29.6 months), and the 5-yr OS was 18.2% (95% CI, 5.2%–63.7%) (Fig. 2). When considering only patients with *de novo NUP98*r, the median OS

Table 1. Clinicopathological characteristics of patients with non-cryptic *NUP98* rearrangement

Case No.	Age (yrs)	Sex	WBC ($\times 10^9$/L)	Hb (g/L)	PLT ($\times 10^9$/L)	Diagnosis	Etiology	FAB classification	Immunophenotype	Dysplasia (lineage)	CR	Allogeneic PBSCT	RFS	Outcome (OS)
1	4	M	5.75	68	34	AML	*de novo*	M5	CD11c+, CD13+, CD33+, CD34+^{dim}, CD64+^{dim}, CD117+, cMPO+, HLA-DR+	+ (E/M)	+	+	+ (12 mo)	D (23 mo)
2	45	M	95.13	84	40	AML	*de novo*	M5	CD13+, CD33+, CD64+, CD117+^{dim}, cMPO+, HLA-DR+	+ (M)	+	+	– (41 mo)	A (42 mo)
3	50	F	2.47	100	56	MDS	therapy-related (OC)	NA	NA	–	NA	–	–	D (5 mo)
4	37	F	214.37	74	38	CMML-2; AML (28 mo)*	*de novo*	M5	CD11c+^{dim}, CD13+, CD33+, CD34+	+ (E/G/M)	+	+	+ (12 d)	D (32 mo)
5	77	F	15.68	61	4	AML	therapy-related (OC)	M5	CD13+, CD33+, CD64+, CD66c+, CD117+, cMPO+	+ (E)	NA	–	–	D (4 mo)
6	58	F	2.54	98	96	AML	*de novo*	M1	CD13+, CD33+, CD34+, CD117+, cMPO+, HLA-DR+	–	+	+	– (106 mo)	A (107 mo)
7	55	F	3.31	98	154	MDS-EB2; AML (4 mo)*	*de novo*	M5	CD4+^{dim}, CD11c+^{dim}, CD13+, CD33+, CD64+, CD117+, cMPO+, HLA-DR+, nTdT+^{dim}	+ (E/G)	+	–	– (6 mo)	D (12 mo)
8	14	M	82.44	121	26	AML	*de novo*	M1	CD7+, CD13+, CD33+, CD34+, cMPO+	–	NA	–	–	D (3 d)
9	60	F	1.29	67	5	AML	therapy-related (OC)	M1	CD7+, CD13+, CD33+, CD34+^{dim}, CD64+, CD117+, cMPO+, HLA-DR+	–	NA	–	–	D (30 d)
10	29	F	233.81	98	70	AML	*de novo*	M4	CD2+^{dim}, CD11c+, CD13+, CD19+^{dim}, CD33+, CD34+, CD64+^{dim}, cCD79a+^{dim}, CD117+, cMPO+, HLA-DR+, nTdT+^{dim}	+ (M)	+	+	+ (4 mo)	D (8 mo)
11	58	M	6.68	85	573	AML	*de novo*	M4	CD13+, CD14–/+, CD33+, CD34+/+^{dim}, CD64–/+, CD117+/–, cMPO+, HLA-DR+	+ (G/M)	+	+	+ (5 mo)	D (13 mo)

*Time to leukemic transformation.
Abbreviations: WBC, white blood cell; PLT, platelet; FAB, French-American-British; CR, complete remission; PBSCT, peripheral blood stem cell transplantation; RFS, relapse-free survival; OS, overall survival; M, male; F, female; aE, erythroid; M, megakaryocytic; G, granulocytic; D, dead; A, alive; mo, months; d, days; NA, not available; MDS, myeloid dysplastic neoplasm; CMML, chronic myelomonocytic leukemia; MDS-EB2, MDS with excess blasts; OC, ovarian cancer; BC, breast cancer.

was 13.0 months (95% CI, 0–28.2 months) and the 5-yr OS was 25.0% (95% CI, 7.5%–83.0%) (Supplemental Data Fig. S1). Notably, one pediatric patient with AML with *NUP98*::*PSIP1* expired only 3 days after the diagnosis because of rapidly evolving disseminated intravascular coagulation (DIC) (i.e., brain infarction and hemorrhage).

Finally, we compared the survival of patients with *NUP98*r with that in other AML subgroups (Fig. 2). Patients with AML with

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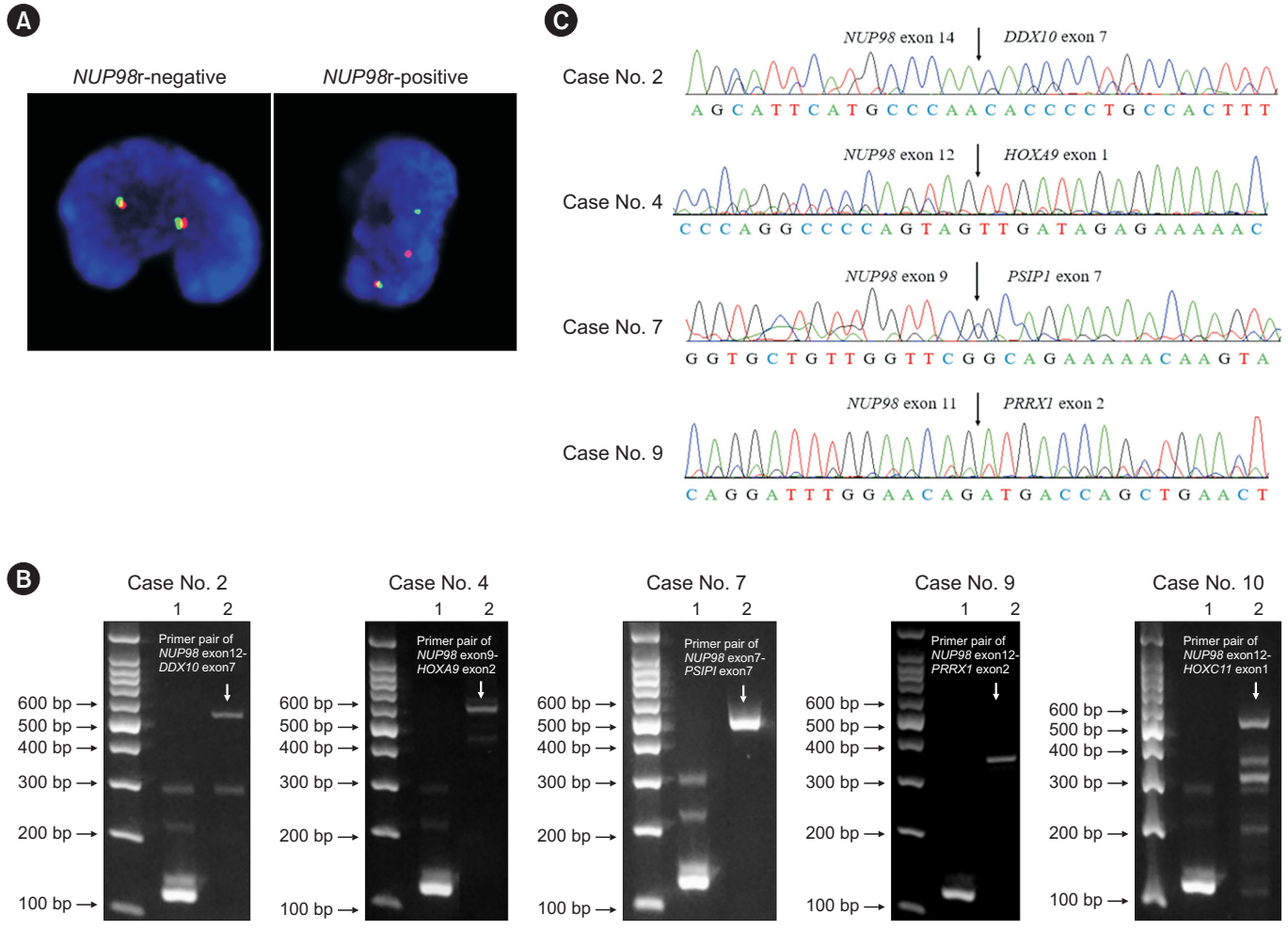

Fig. 1. Representative FISH and molecular analysis results for various *NUP98*r. (A) FISH analysis using a *NUP98* break-apart probe showed negative (two fusions) and positive (one red, one green, and one fusion) signals in case No. 11. (B) Agarose gel electrophoresis of fusion transcripts between *NUP98* and each partner gene (lane 1, internal control using *GAPDH* [amplicon size, 131 bp], lane 2, fusion transcripts using primer pairs of *NUP98* and partner genes). (C) Confirmation of the breakpoint of each fusion transcript via Sanger sequencing.
Abbreviation: *NUP98*r, *NUP98* rearrangement.

mutated *TP53* had the poorest survival ($P=0.025$), and the survival rate of patients with *NUP98*r was similar to that of patients with AML with MR ($P=0.997$). Although not statistically significant, the survival rate of patients with *NUP98*r appeared to be inferior to that of patients with AML with NK ($P=0.188$).

DISCUSSION

We identified various rare non-cryptic *NUP98*r in patients with myeloid neoplasms and demonstrated their poor prognostic impact. *NUP98*r are most frequently reported in pediatric AML, occurring in 4%–7% of patients, with a slightly higher incidence in male patients [9, 20, 21]. Although research on *NUP98*r in adult AML is limited, the reported frequency of *NUP98*r is 2.5% [1]. We found a significantly lower frequency of *NUP98*r, which appeared more frequently in adults, suggesting that it is a characteristic of non-cryptic *NUP98*r.

The disease phenotypes associated with *NUP98*r encompass various hematologic malignancies, including AML, MDS, chronic myeloid leukemia (CML), CMML, mixed-phenotype acute leukemia, and T-cell acute lymphoblastic leukemia [8]. Regarding the phenotypes of AML, in a recent large-scale pediatric AML study, *NUP98*::*KDM5A* was found to be associated with erythroid (M6) and megakaryocytic (M7) phenotypes, but no consistent immunophenotypic correlations were observed [9]. In contrast, Hollink, *et al*. [7] found an association between *NUP98*::*NSD1* and

Table 2. Cytogenetic and molecular genetic characteristics of patients with non-cryptic *NUP98* rearrangement

Case No.	Dx	Karyotype	*NUP98* FISH (%)	Candidate partner gene	Identified partner gene	Breakpoint	*FLT3*-ITD	Targeted NGS
1	AML	46,XY,inv(11)(p15q22)[19]/46,XY[1]	NA	*DDX10*	*DDX10*	*NUP98* exon 14::*DDX10* exon 7	ND	NA
2	AML	46,XY,inv(11)(p15q22)[12]/46,idem,del(5)(q22q33)[5]/46,XY[3]	1F1R1G (59.5%)	*DDX10*	*DDX10*	*NUP98* exon 14::*DDX10* exon 7	ND	*KRAS* c.35G>T, p.G12V (33.8%); *WT1* c.1356_1358delinsTTC, p.Q452_C453delinsHS (30.0%)
3	MDS	46,XX,inv(11)(p15q22)[14]/46,XX[6]	1F1R1G (58.0%)	*DDX10*	*DDX10*	*NUP98* exon 14::*DDX10* exon 7	NA	NA
4	CMML-2; AML	46,XX,t(7;11)(p15;p15)[19]/46,XX[1]	1F1R1G (91.5%)	*HOXA9*, *HOXA11*, *HOXA13*	*HOXA9*	*NUP98* exon 12::*HOXA9* exon 1	ND	NA
5	AML	46,XX,t(7;11)(p15;p15)[20]	1F1R1G (92.5%)	*HOXA9*, *HOXA11*, *HOXA13*	*HOXA9*	*NUP98* exon 11::*HOXA9* exon 1	ND	ND
6	AML	49,XX,+4,t(7;11)(p15;p15),+8,+15[20]	1F1R1G (83.5%)	*HOXA9*, *HOXA11*, *HOXA13*	NA*	NA	ND	NA
7	MDS-EB2; AML	46,XX,t(9;11)(p22;p15)[20]	1F1R1G (78.5%)	*PSIP1*	*PSIP1*	*NUP98* exon 9::*PSIP1* exon 7	NA	NA
8	AML	46,XY,t(9;11)(p22;p15)[19]/46,XY[1]	1F1R1G (94.0%)	*PSIP1*	*PSIP1*	*NUP98* exon 9::*PSIP1* exon 5	ND	*PHF6* c.902_903insGGGATAA, p.Y301* (90.0%)
9	AML	46,XX,t(1;11)(q24;p15)[19]/46,XX[1]	1F1R1G (82.0%)	*PRRX1*	*PRRX1*	*NUP98* exon 11::*PRRX1* exon 2	ND	NA
10	AML	46,XX,t(11;12)(p15;q13)[5]/46,XX[15]	1F1R1G (80.5%)	*HOXC13*, *HOXC11*, *RARG*	*HOXC11*	Inconclusive†	D	NA
11	AML	47,XY,t(11;16)(p15;q23),+21[17]/46,XY[3]	1F1R1G (43.5%)	Unknown	NA‡	NA	ND	*ASXL1* c.1934dup, G646Wfs*12 (31.9%); *DNMT3A* c.917G>A, p.W306* (41.8%); *DNMT3A* c.2111T>G, p.V704G (39.6%); *IDH2* c.419G>A, p.R140Q (38.4%); *STAG2* c.1416+2T>C, p.? (81.2%)

*RT-PCR was not available because of the lack of a sample.
†Multiple bands were observed on RT-PCR electrophoresis, and sequencing yielded inconclusive results.
‡Targeted RT-PCR could not be performed for candidate partner genes because the partner gene of the *NUP98* rearrangement at the 16q23 locus was unknown.
Abbreviations: Dx, diagnosis; *FLT3*-ITD, *FLT3*-internal tandem duplication; NGS, next-generation sequencing; MDS, myeloid dysplastic neoplasm; CMML, chronic myelomonocytic leukemia; MDS-EB2, MDS with excess blasts; NA, not available; ND, not detected, D, detected.

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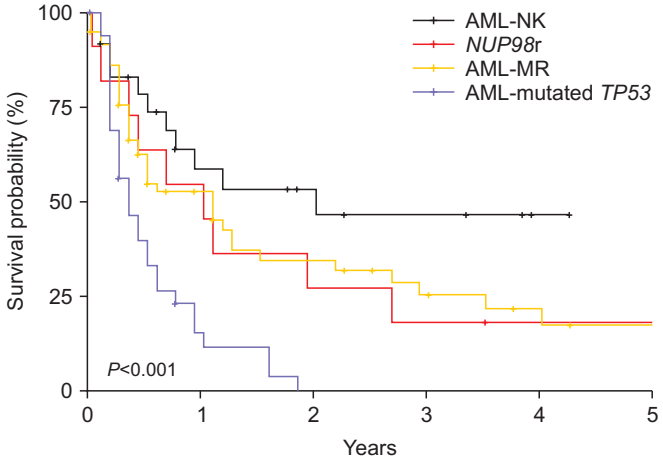

Fig. 2. Kaplan–Meier plot showing the survival probability of patients with *NUP98*r and other AML subgroups.
Abbreviations: AML-NK, AML with normal karyotype; AML-MR, AML with myelodysplasia-related; AML-mutated *TP53*, AML with mutated *TP53*.

monocytic differentiation (M4 and M5) in adult patients with AML but not in pediatric patients. In our study, patients predominantly exhibited monocytic differentiation. Moreover, approximately half of our patients had accompanying leukocytosis. Notably, previous studies have reported leukocytosis in 78%–85% of *NUP98*r patients [1, 7], suggesting it is a characteristic feature of *NUP98*r. *NUP98*r were also frequently reported in therapy-related myeloid neoplasms, which were observed in 27% of patients in our study.

The most common partner genes involved in *NUP98*r are *NSD1* and *KDM5A*, collectively accounting for 6.2% of pediatric patients with AML [9]. As these genes are located at the distal ends of chromosome 5q and chromosome 12p, respectively, they are challenging to detect using conventional cytogenetics [8, 22]. Conversely, *NUP98*r other than *NUP98*::*NSD1* and *NUP98*::*KDM5A* are rare and are mostly identified through conventional cytogenetics, with *NUP98*::*HOXA9* being frequently reported [8, 9]. Therefore, we focused on characterizing these rare and non-cryptic *NUP98*r, excluding common *NUP98*::*NSD1*, *NUP98*::*KDM5A*, and other cryptic *NUP98*r. *NUP98*::*DDX10* was the most common, followed by *NUP98*::*HOXA9* and *NUP98*::*PSIP1*.

RNA-sequencing has revealed various *NUP98* fusion exon junctions. Bertrums, *et al*. [9] suggested an association between exon junctions and fusion partners, reporting that breakpoints of *NUP98* fusions were located at *NUP98* exons 12 and 13 in 85% of patients, with additional breakpoints spanning from exon 11 to exon 29. Notably, they observed an enrichment of breakpoints at *NUP98* exon 12 for *NUP98*::HOX gene fusions. In our study, *NUP98*::HOX gene fusions (i.e., *NUP98*::*HOXA9* and *NUP98*::*PRRX1*) exhibited breakpoints at *NUP98* exons 11 and 12, whereas *NUP98*::non-HOX gene fusions (i.e., *NUP98*::*DDX10* and *NUP98*::*PSIP1*) showed breakpoints at *NUP98* exons 9 and 14. Interestingly, in pediatric patients with AML with rare *NUP98*r excluding *NUP98*::*NSD1*, breakpoints at *NUP98* exon 13 and improved outcome trends have been reported [9]. However, in our study, none of the patients exhibited breakpoints at exon 13 of *NUP98*.

FLT3-ITD has been reported to be associated with *NUP98*::*NSD1* in AML, with a frequency of 48%–91%, predicting poor prognosis when observed with *NUP98*::*NSD1* and/or *WT1* variants [7-9, 20]. *WT1* variants have also been frequently reported in AML with various *NUP98*r [8]. In our study, *FLT3*-ITD and *WT1* variants were observed in one out of nine and one out of four patients with AML, respectively. As expected, *FLT3*-ITD appeared to be uncommon in *NUP98*r other than *NUP98*::*NSD1*.

In the 2022 WHO classification and ICC for myeloid neoplasms, *NUP98*r has been newly added as a defining genetic abnormality of AML [10, 11]. Specifically, the 2022 WHO classification has conferred significant clinical significance by diagnosing AML regardless of the blast percentage when *NUP98*r is present [10]. In this regard, all patients in our study could be classified as AML with *NUP98*r from the initial diagnosis. However, according to the ICC, AML with *NUP98*r is diagnosed when blasts comprise ≥ 10% [11], and in our study, 10 out of 11 patients met this criterion. Especially in our study, the clinical utility of the criterion of ≥ 10% blasts was supported by the leukemic transformation of both patients with MDS-EB2 and CMML-2. However, there was uncertainty regarding the diagnosis of one patient with low-grade MDS harboring *NUP98*::*DDX10* as having AML according to the 2022 WHO classification, warranting caution in diagnosis. This patient, undergoing treatment for metastatic ovarian cancer, showed no evidence of dysplasia or blast increase and died because of ovarian cancer progression [23].

*NUP98*r is associated with a dismal prognosis in AML [1, 7-9, 14, 20, 21]. While most studies were based on high-frequency *NUP98*r, such as *NUP98*::*NSD1* and *NUP98*::*KDM5A*, our study added prognostic significance to rare *NUP98*r. In two studies of pediatric AML, the 5-yr OS rates were 35% and 48% for patients with *NUP98*r, respectively, versus 64% and 72% in those without *NUP98*r [9, 20]. In adult AML with *NUP98*r, although studies are limited, the median OS has been reported as 13 months [1, 14], which is lower than the median survival of 33 months and the 5-yr OS of 63% in *de novo* patients with AML aged 40–59 yrs [24]. Similarly, our study demonstrated a poor prognosis in pa-

tients with rare *NUP98*r, with a median OS of 12 months and a 5-yr OS rate of 18.2%. These results are in line with previous findings in patients with AML with MR, who are known to have a poor prognosis [25]. In one patient, the concurrent presence of DIC was associated with an extremely poor prognosis, highlighting the need for caution and further research into its relevance.

Our study has several limitations. We did not comprehensively investigate the characteristics of cryptic *NUP98*r, and only a few patients were included because of the rarity of non-cryptic *NUP98*r. Additionally, partner genes in two patients with *NUP98*r were not identified, and NGS was conducted in only one-third of the patients.

In conclusion, our study identified the clinical and genetic characteristics of patients with myeloid neoplasms harboring rare and non-cryptic *NUP98*r. Currently, patients with *NUP98*r are believed to be underdiagnosed. As cancer therapy advances toward personalized treatment tailored to individual genetic profiles, the importance of comprehensive assessment for identifying *NUP98*r in patients with myeloid neoplasms is emphasized, particularly given its association with poor prognosis.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0190

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AUTHORS CONTRIBUTIONS

Park MS and Kim BR analyzed the data and drafted the manuscript; Jang JH and Jung CW provided clinical information; Kim HJ provided expertise regarding the laboratory data and supervised the study; Kim HY conceptualized the study and edited the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared

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