Screening for *NUP98* rearrangements in hematopoietic malignancies by fluorescence *in situ* hybridization

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Background and Objectives. The aim of this study was to determine the incidence of rearrangements of *NUP98* (the gene coding for nucleoporin 98kDa protein) in childhood acute myeloid leukemia (AML) and selected patients with 11p13-15 rearrangements. This aim was achieved using a fluorescence *in situ* hybridization (FISH) assay that allows the detection of *NUP98* aberrations independently of the partner gene involved.

Design and Methods. Screening of 59 consecutive patients enrolled in the Austrian AML-BFM93 clinical trial was performed by dual-color FISH. In addition, 14 selected cases with various hematologic malignancies and 11p13-15 aberrations were analyzed. *NUP98*-positive cases were further investigated by fusion gene-specific FISH and reverse transcription polymerase chain reaction assays.

Results. Among the 59 AML patients, one *NUP98-NSD1* positive case (1.7%) was detected. Among the 14 selected patients, five new *NUP98* positive cases were determined. Two cases showed an inv(11)(p15q22)/*NUP98-DDX10* fusion, one each displayed a t(5;11)(q35;p15)/*NUP98-NSD1* and a t(11;20)(p15;q12)/*NUP98-TOP1* fusion, and one case with a putative new fusion partner gene at 3p24 was identified.

Interpretation and Conclusions. The observed frequency of 1.7% confirmed the low incidence of *NUP98* rearrangements in childhood AML. The low occurrence of *NUP98* rearrangements in selected samples with 11p13-15 alterations suggests the existence of variable chromosomal breakpoints and affected genes in this region. The identification of a new *NUP98* fusion partner region confirms the evident promiscuity of *NUP98*. Thus, analysis of *NUP98* aberrations by FISH seems to be the method of choice for determining the presence of these genetic lesions in unselected patients, and to confirm the involvement of *NUP98* in cases with 11p15 aberrations.

Key words: NUP98 rearrangements, FISH screening, *NSD1*, *DDX10*, *TOP1*.

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Leukemia is associated with a wide spectrum of recurrent non-random chromosomal translocations. Molecular analysis of the genes involved in these translocations has led to a better understanding of both the causes of chromosomal rearrangements and the mechanisms of leukemic transformation. *NUP98* (nucleoporin 98kD) rearrangements occur in a broad range of hematopoietic malignancies including *de novo* and therapy-related myeloid as well as lymphoid malignancies, such as acute myeloid leukemia (AML), chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), and T-cell acute lymphoblastic leukemia (T-ALL). The clinical course of patients with *NUP98* rearrangements seems quite aggressive and the treatment outcome disappointing, thus further evaluation of the incidence and prognostic significance of *NUP98* fusions is needed.[1] *NUP98* is localized on chromosome region 11p15.5 and encodes the 98kDa nucleoporin protein NUP98, which is part of the nuclear pore complex (NPC), through which communication between the nucleus and the cytoplasm is mediated in eukaryotic cells.[2] Involvement of *NUP98* in hematologic malignancies was first described in patients with a t(7;11)(p15;p15.5) resulting in expression of a *NUP98-HOXA9* chimeric transcript.[3,4] So far, 17 different fusion partner genes have been identified and partially characterized. The *NUP98* fusion partner genes can be divided into two groups: *HOX* partner genes *HOXA9*, *HOXA11*, and *HOXA13*, *HOXC11* and *HOXC13*, *HOXD11* and *HOXD13*, and *PRRX1/PMX1* and *PRRX2*.[5,6] The non-HOX partner genes comprise *FN1*, *RAP1GDS1*, *NSD1*, *WHSC1L1/NSD3*, *PSIP2/LEDGF*, *ADD3*, *DDX10* and *TOP1*.[5,7] The *HOX* genes are a family of

transcription factors containing a homeobox domain, which is a highly conserved helix-turn-helix motif. They provide an evolutionary conserved elementary regulatory mechanism for major differentiation programs from the embryo to the adult, and are also involved in hematopoietic processes.[8] Most of the *NUP98* fusion partner genes belong to the class-I-*HOX* group and only two, *PRRX1/PMX1* and *PRRX2*, are members of the class-II or orphan *HOX* genes. The non-*HOX* fusion partner genes are either transcription factors or factors indirectly contributing to transcriptional control. The unifying feature of these *NUP98* fusion partners is a significant probability of a coiled-coil conformation being adopted.[9] Intriguingly, all *NUP98* fusions reported so far retain the FG repeat motifs at the N-terminus and these are fused to the homeobox domain of the *HOX* partner genes or to the regions that encompass the predicted coiled-coil domain of the non-*HOX* partner genes.[1,5,10] So far, most studies regarding *NUP98* rearrangements have been performed either on selected patient samples, thus provided limited information about the true incidence of these rearrangements or were focused on the detection of specific *NUP98* rearrangements.[11-13] In this study, a *NUP98* FISH assay that allows the detection of all *NUP98* rearrangements, regardless of the *NUP98* fusion partner gene involved was used to screen unselected consecutive childhood AML cases. In addition, 14 patients with various cytogenetically determined 11p13-15 rearrangements were analyzed. All cases that were positive for a *NUP98* rearrangement were further evaluated to identify the specific affected partner gene.

Design and Methods

Patients

Out of 67 patients registered in the Austrian AML-BFM93 (Acute Myeloid Leukemia - Berlin-Frankfurt-Münster) study, 59 patients were analyzed by FISH for *NUP98* rearrangements. These childhood AML and transient myeloproliferative disorder patients had been previously analyzed using a multiplex reverse transcription polymerase chain reaction (RT-PCR) approach that, however, did not cover *NUP98* fusions.[14] In addition, 14 childhood and adult patients with various 11p13-15 rearrangements (Table 1), who were referred to the cytogenetic laboratory of the Children's Cancer Research Institute from different hospitals for cytogenetic analysis, were screened for *NUP98* rearrangements.

Cytogenetic and FISH analysis

Cytogenetic analysis was performed on GTG-banded metaphases and karyotypes are described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).[15] For the detection of *NUP98* rearrangements PAC 1173K1[16] (encompassing exons 10-20 of *NUP98*) was combined with the 11p-subtelomere-specific clone, PAC 908H22[17] (kindly provided by Lyndal Kearney, Medical Research Council, UK). *NUP98-DDX10* fusions were detected using a pool of 3 different *DDX10*-specific clones: RP11-35N19 (AP003384), RP11-700F9 (AP003387), and RP11-25I9 (AP003027), in combination with PAC 1173K1. Depending on the resolution, in case of a *NUP98-DDX10* fusion this probe mixture resulted in one or two fusion signals. *NUP98-NSD1* rearrangements were detected with the *NUP98* probe and the *NSD1*-specific RP11-265K23 (AC110005) clone, which encompasses exons 2-23 of the gene. In addition, the *MLL*-specific probes PAC 217A21 and PAC 167K13 (kindly provided by Ed Schuuring, Leiden University Medical Center, The Netherlands),[18] and clones specific for *ATM* (RP11-241D13, AP001925), *LMO1* (11p15, proximal to *NUP98*; RP11-379P15, AC091013), and *WT1* (11p13; RP1-74J1, AL049692.13 Research Genetics, Invitrogen) were used. All BAC clones derived from the RP11 library and PAC 1173K1 were obtained from Mariano Rocchi, Resources for Molecular Cytogenetics, University of Bari, Italy. FISH was performed as previously described.[19] In brief, probes were differentially labeled by nick translation, either with biotin-16-dUTP or digoxigenin-11-dUTP (Roche Diagnostics). Immunodetection was performed using CY3-conjugated mouse anti-biotin (Jackson Immuno Research), Fluorescein isothiocyanate (FITC)-conjugated sheep anti-digoxigenin, and rabbit anti-sheep FITC (Roche Diagnostics) antibodies. Samples were evaluated using an Axioplan fluorescence microscope (Zeiss) equipped with the appropriate filter sets for FITC, CY3, and DAPI and images were taken with a CCD camera (Photometrix) using the IPLab software (Vysis).

RT-PCR analysis

Total RNA was isolated from methanol/acetic acid fixed cells as previously described.[14,19] RNA was reverse transcribed using random hexamers and 200 units Moloney-murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen) at 42°C for 60 min. The *NUP98-DDX10*-specific RT-PCR was carried out using sense primer *NUP98*-F2 (5'-TGTTTGGGAA-CAACCAACCTAAG-3'), localized in exon 12 and antisense primer *DDX10*-R1 (5'-TGCTCTTCTTCT-TCAGATGGCTTC-3'), localized in exon 7. RT-PCR specific for the *NUP98-NSD1* fusion transcripts was performed using sense primer *NUP98*-F1 (5' AGC-CACTTTGGGCTTTGGAG-3'), located in exon 12 and antisense primer *NSD1*-R1 (5'-TGCACCT-GCTCCTGTACCTTC-3'), located in exon 5. The *NUP98-TOP1*-specific RT-PCR was carried out using

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Table 1. Clinical characteristics, cytogenetics, FISH, and RT-PCR data of 14 patients with 11p13-15.

No	Age (yr.)	Sex	Diagnosis	Karyotype	FISH[f]			RT-PCR
					NUP98/11p	NUP98/DDX10	NUP98/NSD1	
1	74,3	M	AML	46,XY,inv(11)(p15q22)[20]	13%	1%	ND	ND
2	6,4	M	AML	46,XY,inv(11)(p15q21)[3]/46,XY,idem, der(17)t(17;?)(q?11;?)[10]/ 46,XY,idem,del(2)(p?22), der(17)t(17;?) (p?11;?)[5]/46,XY[2]	62%	65%[1]	ND	NUP98-DDX10
3	18,3	M	t-AML	46,XY,inv(11)(p15q22)[20]	ND	80%[2]	ND	NUP98-DDX10
4	69,7	M	MDS	46,XY,inv(11)(p15q23)[2]/46,XY[18]	3%	DDX10 deletion	ND	ND
5	48,6	F	AML M1	46,XX,inv(11)(p15q23)[18]	8.7%	0%	ND	ND
6	56,5	F	t-AML	46,XX,t(11;20)(p15;q12)[16]/45,XX, idem,-21[4]	56%	ND	ND	NUP98-TOP1
7	78,2	M	AML M0/1	47,X,-Y,+13,+mar[16]/46,X,-Y, der(11)t(11;13)(p?15;q?11),+mar[4]	13%	ND	ND	ND
8	65	M	AML	46,XY,t(7;11)(q22;p15)[2]/46,XY[4]	4.2%	ND	ND	ND
9[a]	3,9	F	AML M2	46,XX[17]	ND	ND	75%[3]	NUP98-NSD1
9[b]				46,XX,t(1;18)(q32;q21),del(11) (p13)t(5;11)(q35;p15)[20][d]	40%	ND	ND	ND
10	39,1	F	AML M2	45,XX,-7,del(11)(p13),del(17)(p11)[18]/ 43,XX,der(1)t(1;?)(p11;?),-7,-16,-17,-18,+mar[4]	4%	ND	ND	ND
11[a]	63,8	M	AML M5a	46,XY,t(3;11)(p24;p15)[20]	72%	ND	ND	ND
11[b]				46,XY,t(3;11)(p24;p15)[7]/45,X,idem,-Y,[12]	63%			
12[c]	31,9	M	MDS	45,XY,-7,der(11)(p15)dup(11)(q13q25)[16]/ 47,XY,+8[3]	8%	ND	ND	ND
13	72,8	F	AML M1	46,XX,der(11)t(1;11)(q21;p15), add(11)(q?21)[20]	13%	ND	ND	ND
14	71	F	MDS	45,XX,der(1)t(1;7;11)(p13;?;p12p15), der(11)t(1;7;11)(p13;?;p12), der(17)t(11;17)(p15;p13),-7[15][e]	9.5%	ND	ND	ND

Patients are grouped in inv(11) cases and in cases with other 11p13-15 aberrations. [a]Sample from diagnosis and [b]sample from relapse of the same patient. [c]Relapse sample. [d]FISH analysis using different probes specific for chromosomes 1, 5, 11, 18, the Spectra-Vysion 24-Color-Chromosome-Painting-Kit (Vysis), and the NUP98/11p assay revealed a t(5;11)(q35;p15) and a t(1;18)(q32;q21). [e]Karyotype was established after 24-Color-Chromosome-Painting and NUP98/11p FISH; the breakpoint at 11p15 is localized telomeric to NUP98. [f]Percentages of FISH patterns suggestive for NUP98 rearrangements: NUP98/11p – 3G/2R; NUP98/DDX10 and NUP98/NSD1 – fusion signals. [1]13% and 52% of the cells displayed one and two fusion signals, respectively. [2]16.5% and 63.5% of the cells displayed one and two fusion signals, respectively. [3]17% and 58% of the cells displayed one and two fusion signals, respectively. AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; ND: not determined; t-AML: therapy-related AML.

sense primer *NUP98*-F3 (5'-GACAGCCACTTT-GGGCTTTG-3') (exon 12) and antisense primer *TOP1*-R1 (5'-CCTCTGCTTTGGGGCTCAG-3') (exon 8). All RT-PCR reactions were carried out using Hot Start Taq (Qiagen). The cycling conditions were: initial activation 95°C for 14 min; denaturation at 95°C for 30 sec, annealing at 63-64°C for 30 sec, elongation at 72°C for 30-45 sec carried out for 35-45 cycles; final extension at 72°C for 7 min. RT-PCR products were sequenced by MWG-Biotech (Germany). The exon nomenclature of all genes followed that described at *http://www.ensembl.org/*.

Results

Detection of NUP98 rearrangements

To determine the presence of *NUP98* rearrangements, independently of the partner gene involved, FISH was performed using the *NUP98*-specific PAC 1173K1 which splits if a rearrangement is present. In order to avoid misinterpretation of the *NUP98* signal pattern due to chromosome 11 or 11p trisomies, the *NUP98*-specific clone was combined with an 11p-subtelomere-specific clone. This probe combination

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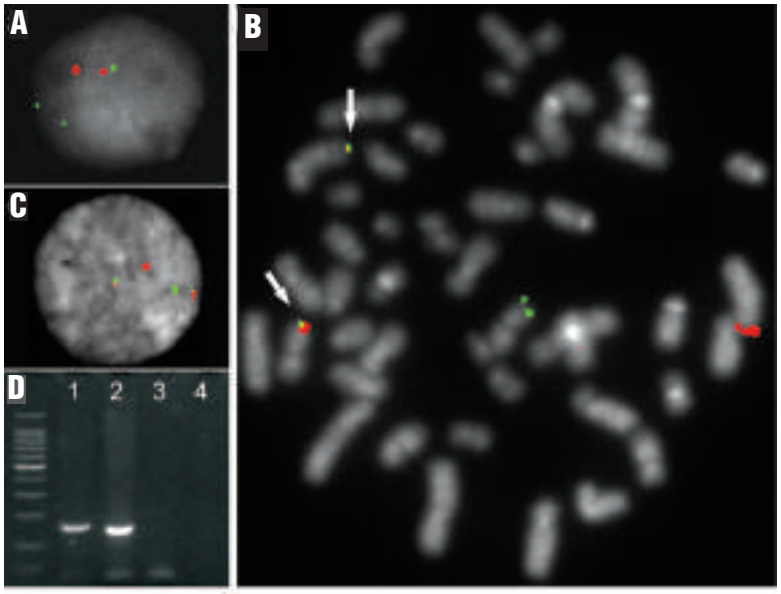

Figure 1. *NUP98-NSD1* analysis (A) Positive *NUP98* interphase nucleus of patient no. 20[14] displaying the 3G/2R - *NUP98/11p* pattern resulting from a splitting of the *NUP98* FISH probe; *NUP98* PAC 1173K1 (green) and 11p-subtelomere PAC 908H22 (red). (B) and (C) *NUP98-NSD1* positive metaphase and interphase of patient #9 showing two *NUP98-NSD1* fusion signals; *NUP98* PAC 1173K1 (green) and *NSD1* RP11-254K23 (red); white arrows are pointing to rearranged chromosomes. (D) *NUP98-NSD1* RT-PCR; Molecular weight marker, 100bp ladder; patient #20[14] (lane 1); patient #9 (lane 2); normal control (lane 3); negative control (H_2O) (lane 4). (E) Partial nucleotide and amino acid sequence of the NUP98-NSD1 fusion.

resulted in 2 green and 2 red (2G/2R-*NUP98*/11p) signals for each probe in normal cells and, in the case of a *NUP98* rearrangement, in a pattern of 3 signals for *NUP98* and 2 subtelomeric signals (3G/2R). In normal peripheral blood cells the cut-off value for false positive nuclei displaying the 3G/2R pattern was determined as 7.5% (mean±3×sd).

NUP98 rearrangements in consecutive childhood AML patients

Using the *NUP98*/11p assay, in 58 out of 59 consecutive childhood AML cases a 3G/2R-*NUP98*/11p FISH pattern suggestive of a *NUP98* rearrangement was observed in 1-16% of the nuclei. Probably due to the prolonged storage of the fixed bone marrow samples with ≥40% blast cells in almost all cases the hybridization was slightly less specific, and therefore these samples were considered as *NUP98* negative. Moreover, taking into consideration possible 3' deletions of *NUP98*, when no additional signal was observed, special attention was paid to the cytogenetics and the location of the signals on the metaphase chromosomes. Only one patient showed a 3G/2R-*NUP98*/11p pattern in a significant number of nuclei (37%), suggesting the presence of a *NUP98* rearrangement (Figure 1A). This particular patient displayed a del(5)(q13q33) and has been described previously as t(5;11)(q35;p15)*/NUP98-NSD1* positive [*case #20 in Strehl et al., 2001; note: erroneously the footnote to case 20 was placed at case #2*].[14,20] The childhood AML cases included one with a cytogenetically determined t(7;11)(p15;p12) (*case #45 in Strehl et al., 2001*) suggesting the presence of a *NUP98-HOXA* fusion. However, FISH analysis with 11p-specific probes and whole chromosome painting revealed a more complex karyoptype and no involvement of 11p (*data not shown*).

NUP98 rearrangements in selected cases with 11p13-15 rearrangements

Based on cytogenetic data 14 cases with various 11p13-15 alterations (Table 1) were analyzed for *NUP98* rearrangements. In nine samples the 3G/2R-*NUP98*/11p FISH pattern was observed in merely 3-13% of the nuclei. Five patients (#2, 3, 6, 9, and 11) analyzed using the *NUP98*/11p or fusion gene-specific FISH assays were positive for a *NUP98* aberration (Table 1).

Five patients (#1-5) were karyotyped as having inv(11)(p15q21-q23) suggesting a fusion between the *NUP98* and *DDX10* genes. Using a *NUP98-DDX10* dual-color FISH assay, two of the five patients (#2 and 3) showed *NUP98-DDX10* fusion signals (Figure 2A; Table 1). Sequence analysis of the *NUP98-DDX10* RT-PCR products revealed an in-frame fusion of *NUP98* exon 14 with *DDX10* exon 7 in both cases (Figures 2B,D). Patient #4 showed only one *DDX10* and two *NUP98* signals, and thus displayed a hemizygous deletion of *DDX10*. More detailed FISH analyses using probes specific for *MLL* and *ATM*, which is localized just 400 kb centromeric to *DDX10*, also revealed a hemizygous deletion of *ATM*, whereas *MLL* was not affected. In the remaining two inv(11) cases, FISH analysis revealed inver-

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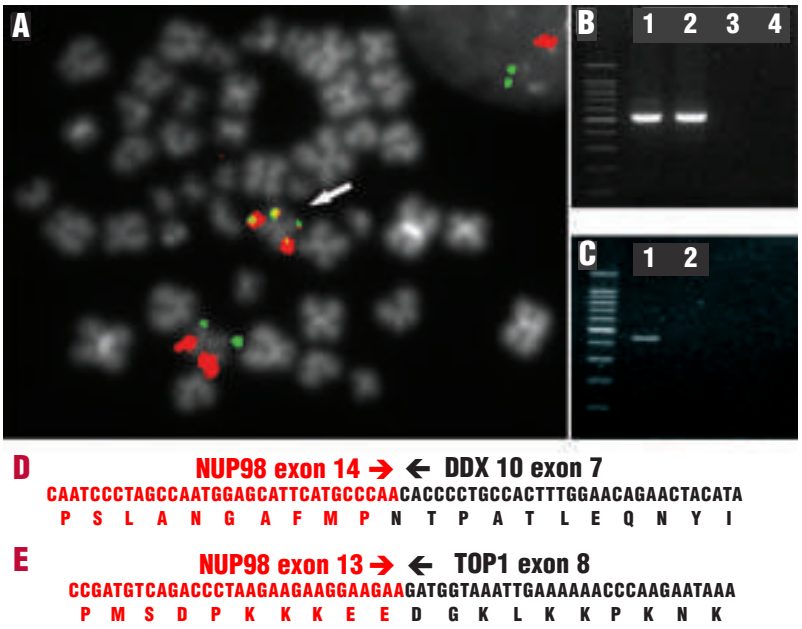

Figure 2. *NUP98-DDX10* and *NUP98-TOP1* analysis (A) *NUP98-DDX10* positive metaphase of patient no. 2; NUP98 PAC 1173K1 (green) and *DDX10* (RP11-35N19, RP11-700F9, and RP11-25I9) (red); the white arrow indicates the rearranged chromosome. (B) NUP98-DDX10 RT-PCR; Molecular weight marker, 100bp ladder; patient no. 3 (lane 1); patient no. 2 (lane 2); normal control (lane 3); negative control (H_2O) (lane 4). (C) NUP98-TOP1 RT-PCR; Molecular weight marker, 100bp ladder; patient no. 6 (lane 1); negative control (H_2O) (lane 2). Partial nucleotide and amino acid sequences of (D) NUP98-DDX10 fusion and (E) NUP98-TOP1 fusion.

sion breakpoints distal to *NUP98* and distal to *MLL* in case #1, whereas in case #5 FISH with several chromosome 11 locus-specific probes could not confirm an inversion event (*data not shown*).

In patient #6, whole chromosome painting confirmed the t(11;20)(p15;q12) and a *NUP98* rearrangement was detected (Table 1). RT-PCR and subsequent sequencing revealed an in-frame fusion of *NUP98* exon 13 and *TOP1* exon 8 (Figures 2 C,E).

In patient #9, at diagnosis cytogenetics revealed a 46,XX karyotype but at relapse the presence of a del(11)(p13) suggested involvement of *NUP98*. FISH analysis revealed a cryptic t(5;11) (Figures 1B-C; Table 1). The presence of a *NUP98-NSD1* fusion was confirmed by RT-PCR (Figure 1D) and resulted in an in-frame fusion of *NUP98* exon 12 and *NSD1* exon 5 (Figure 1E). Interestingly, at diagnosis and relapse metaphases displayed *NUP98* signals on both derivative chromosomes including the del(11)(p13). At diagnosis both the *LMO1* and the *WT1* loci were retained on both chromosomes 11, whereas at relapse only *LMO1* was present on the del(11)(p13) but an interstitial deletion eliminated the *WT1* locus (*data not shown*). Patient #11 displayed a t(3;11)(p24;p15) that has not been described as a recurrent aberration. FISH analysis showed rearrangement of the *NUP98* gene (Table 1). Involvement of *NUP98* in a t(3;11)(p24;p15) has not been reported until now, and therefore a new *NUP98* partner gene seems to be affected by this translocation.

Discussion

In this study the most comprehensive group of consecutive childhood AML patients was analyzed so far for *NUP98* rearrangements. Among 59 patients, only one patient (1.7%) was positive for this specific genetic alteration confirming the overall low incidence of *NUP98* rearrangements.[11-13] In a previous study, 20 consecutive childhood AML cases were screened for *NUP98-NSD1* fusions and one (5%) positive case was found.[11] Also in a significant larger series of more than 200 adult AML cases a *NUP98-HOXA9* fusion was observed in merely 1.5% of the cases,[12] and in a survey of 43 adult T-ALL cases 5% were *NUP98-RAP1GDS1* positive.[13] However, due to selection bias of patient cohorts and screening for specific *NUP98* fusions the overall incidence of *NUP98* rearrangements remains unclear and further comprehensive studies on larger series of well-defined patient groups are needed to determine their occurrence as well as any clinical correlations.

In general, *NUP98* fusions appear to be associated with a poor prognosis; however, there are few comprehensive data sets available. It seems that the *NUP98-NSD1* fusion is strongly associated with childhood AML and so far, including the new case described herein (case #9), eight cases have been identified. Of note, the first case of an adult with MDS harboring a *NUP98-NSD1* fusion resulting from a cryptic insertion of *NUP98* into the *NSD1* locus was recently described.[21] In childhood AML the

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NUP98-NSD1 rearrangement may occur with a concurrent del(5q) that appears to be associated with an even poorer prognosis than those cases without the additional deletion.[11] In support of this hypothesis, the new case (#9) presented in this study displayed no obvious deletion at 5q and at present is in complete remission almost four years after diagnosis, whereas the other patient displayed a del(5q) and died 10 months after diagnosis.

The analysis of five patients with an inv(11)(p15q22) detected two new *NUP98-DDX10*-positive cases. Of note, at diagnosis one of these patients (#3) presented with an AML-M3 and showed a t(15;17)(q22;q21) with a *PML-RARA* rearrangement. Five years later he developed a secondary leukemia with an AML-M4 phenotype and the inv(11)(p15q22)*/NUP98-DDX10* fusion confirming the association of *NUP98* aberrations with therapy-related diseases.[22-24] Detailed FISH analysis of the inv(11) cases negative for *NUP98-DDX10* suggested, in at least one case (#1), the existence of another type of inv(11) involving genes that have not been identified yet. Although in two further cases (#4 and 5) the presence of a cytogenetically suspected inv(11) could not be verified with the FISH probes applied, its presence cannot be excluded with certainty.

Patient #2 displaying a *NUP98-DDX10* fusion suffered from aggressive disease and due to treatment failure died 2 months after diagnosis, whereas patient #3 has now been in remission for 6 months. The *NUP98-TOP1* fusion described herein was also found in a patient with secondary AML-M2 with rapid disease progression who died within a few months, again emphasizing the association of *NUP98* aberrations with an adverse outcome. The same held true for the patient with the new *NUP98*/3p24 rearrangement who died of refractory disease 20 months after diagnosis. However, *NUP98* rearrangements are rare and occur in various hematologic diseases, and thus it is difficult to reach any conclusions regarding their definite prognostic significance. Nevertheless, studies of *NUP98* aberrations elucidate the potential mechanisms of leukemic transformation and will eventually identify specific subgroups of leukemia that require particular treatment approaches. So far, 17 *NUP98* fusion partners have been detected,[5] and this evident promiscuity of *NUP98* makes it reasonable to suspect the existence of other yet unknown partners. Screening for *NUP98* aberrations by FISH allows the detection of all rearrangements independently of the partner gene, and seems to be the method of choice for determining the overall incidence of these genetic lesions and for identifying novel fusion partners. In this regard, a further improvement might be achieved by a *NUP98* dual-color split-apart FISH assay that would completely exclude lack of detection of *NUP98* rearrangements associated with 3' deletions.[25] However, unfortunately an adequate probe set for screening studies is not available to date. Interestingly, the low frequency of *NUP98* rearrangements even in selected samples with 11p13-15 alterations suggests the existence of variable chromosomal breakpoints and affected genes in recurrent translocations involving 11p15.[26] These findings emphasize the importance of combining cytogenetics, FISH techniques and molecular analysis in order to determine the specific genes involved in 11p15 rearrangements unequivocally.

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While all authors fulfill the requirements for authorship, more specifically KN, SS, MK and OAH had the major input designing the study and were involved in analyzing and interpreting of data. HHS, DL, WRS, KK, SB and MND were mostly involved in acquisition of available preserved samples and clinical data. KN wrote the first draft of the paper, which was critically revised by SS. Additionally, OAH contributed fruitfully in revision of the paper and the final version was approved by all authors. The authors declare that they have no potential conflicts of interest.

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References

1. Lam DH, Aplan PD. NUP98 gene fusions in hematologic malignancies. Leukemia 2001;1511:1689-95.
2. Powers MA, Forbes DJ, Dahlberg JE, Lund E. The vertebrate GLFG nucleoporin, Nup98, is an essential component of multiple RNA export pathways. J Cell Biol 1997;1362:241-50.
3. Borrow J, Shearman AM, Stanton VP Jr, Becher R, Collins T, Williams AJ, et al. The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. Nat Genet 1996;122:159-67.
4. Nakamura T, Largaespada DA, Lee MP, Johnson LA, Ohyashiki K, Toyama K, et al. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nat Genet 1996;122:154-8.
5. Slape C, Aplan PD. The role of NUP98 gene fusions in hematologic malignancy. Leuk Lymphoma 2004;457:1341-50.
6. Gervais C, Mauvieux L, Perrusson N, Helias C, Struski S, Leymarie V, et al. A new translocation t(9;11)(q34;p15) fuses NUP98 to a novel homeobox partner gene, PRRX2, in a therapy-related acute myeloid leukemia. Leukemia 2005;191:145-8.
7. Arai Y, Kyo T, Miwa H, Arai K, Kamada N, Kita K, et al. Heterogenous fusion transcripts involving the NUP98 gene and *HOXD13* gene activation in a case of acute myeloid leukemia with the t(2;11)(q31;p15) translocation. Leukemia 2000; 149:1621-9.
8. van Oostveen J, Bijl J, Raaphorst F, Walboomers J, Meijer C. The role of homeobox genes in normal hematopoiesis and hematological malignancies. Leukemia 1999; 1311:1675-90.
9. Hussey DJ, Dobrovic A. Recurrent coiled-coil motifs in *NUP98* fusion partners provide a clue to leukemogenesis. Blood 2002;993:1097-8.
10. Lahortiga I, Vizmanos JL, Agirre X, Vazquez I, Cigudosa JC, Larrayoz MJ, et al. NUP98 is fused to adducin 3 in a patient with T-cell acute lymphoblastic leukemia and myeloid markers, with a

new translocation t(10;11) (q25;p15). Cancer Res 2003; 6312: 3079-83.
11. Cerveira N, Correia C, Doria S, Bizarro S, Rocha P, Gomes P, et al. Frequency of NUP98-NSD1 fusion transcript in childhood acute myeloid leukaemia. Leukemia 2003; 1711: 2244-7.
12. Kwong YL, Pang A. Low frequency of rearrangements of the homeobox gene HOXA9/t(7;11) in adult acute myeloid leukemia. Genes Chromosomes Cancer 1999; 251:70-4.
13. Cimino G, Sprovieri T, Rapanotti MC, Foa R, Mecucci C, Mandelli F. Molecular evaluation of the NUP98/RAP1GDS1 gene frequency in adults with T-acute lymphoblastic leukemia. Haematologica 2001; 864:436-7.
14. Strehl S, Konig M, Mann G, Haas OA. Multiplex reverse transcriptase-polymerase chain reaction screening in childhood acute myeloblastic leukemia. Blood 2001; 973:805-8.
15. ISCN. An International System for Human Cytogenetic Nomenclature. F Mitelmann, ed. Basel; S Karger: 1995.
16. Ahuja HG, Felix CA, Aplan PD. Potential role for DNA topoisomerase II poisons in the generation of t(11;20)(p15;q11) translocations. Genes Chromosomes Cancer 2000; 292:96-105.
17. Brown J, Saracoglu K, Uhrig S, Speicher MR, Eils R, Kearney L. Subtelomeric chromosome rearrangements are detected using an innovative 12-color FISH assay (M-TEL). Nat Med 2001; 74:497-501.
18. von Bergh A, Emanuel B, Zelderen-Bhola S, Smetsers T, van Soest R, Stul M, et al. A DNA probe combination for improved detection of MLL/11q23 breakpoints by double-color interphase-FISH in acute leukemias. Genes Chromosomes Cancer 2000; 281:14-22.
19. Konig M, Reichel M, Marschalek R, Haas OA, Strehl S. A highly specific and sensitive fluorescence in situ hybridization assay for the detection of t(4;11)(q21;q23) and concurrent submicroscopic deletions in acute leukaemias. Br J Haematol 2002; 1164: 758-64.
20. Jaju RJ, Fidler C, Haas OA, Strickson AJ, Watkins F, Clark K, et al. A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in de novo childhood acute myeloid leukemia. Blood 2001; 984:1264-7.
21. La Starza R, Gorello P, Rosati R, Riezzo A, Veronese A, Ferrazzi E, et al. Cryptic insertion producing two NUP98/NSD1 chimeric transcripts in adult refractory anemia with an excess of blasts. Genes Chromosomes Cancer 2004; 414:395-9.
22. Nishiyama M, Arai Y, Tsunematsu Y, Kobayashi H, Asami K, Yabe M, et al. 11p15 translocations involving the NUP98 gene in childhood therapy-related acute myeloid leukemia/myelodysplastic syndrome. Genes Chromosomes Cancer 1999; 263:215-20.
23. Arai Y, Hosoda F, Kobayashi H, Arai K, Hayashi Y, Kamada N, et al. The inv(11)(p15q22) chromosome translocation of de novo and therapy- related myeloid malignancies results in fusion of the nucleoporin gene, NUP98, with the putative RNA helicase gene, DDX10. Blood 1997; 8911:3936-44.
24. Block AW, Carroll AJ, Hagemeijer A, Michaux L, van Lom K, Olney HJ, et al. Rare recurring balanced chromosome abnormalities in therapy-related myelodysplastic syndromes and acute leukemia: report from an international workshop. Genes Chromosomes Cancer 2002; 334:401-12.
25. van der Burg M, Poulsen TS, Hunger SP, Beverloo HB, Smit EM, Vang-Nielsen K, et al. Split-signal FISH for detection of chromosome aberrations in acute lymphoblastic leukemia. Leukemia 2004; 185:895-908.
26. Kobzev YN, Martinez-Climent J, Lee S, Chen J, Rowley JD. Analysis of translocations that involve the NUP98 gene in patients with 11p15 chromosomal rearrangements. Genes Chromosomes Cancer 2004; 414:339-52.

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