t(6;9)(p22;q34)/*DEK-NUP214*-rearranged pediatric myeloid leukemia: an international study of 62 patients

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ABSTRACT

Acute myeloid leukemia with t(6;9)(p22;q34) is listed as a distinct entity in the 2008 World Health Organization classification, but little is known about the clinical implications of t(6;9)-positive myeloid leukemia in children. This international multicenter study presents the clinical and genetic characteristics of 62 pediatric patients with t(6;9)/*DEK-NUP214*-rearranged myeloid leukemia; 54 diagnosed as having acute myeloid leukemia, representing <1% of all childhood acute myeloid leukemia, and eight as having myelodysplastic syndrome. The t(6;9)/*DEK-NUP214* was associated with relatively late onset (median age 10.4 years), male predominance (sex ratio 1.7), French-American-British M2 classification (54%), myelodysplasia (100%), and *FLT3*-ITD (42%). Outcome was substantially better than previously reported with a 5-year event-free survival of 32%, 5-year overall survival of 53%, and a 5-year cumulative incidence of relapse of 57%. Hematopoietic stem cell transplantation in first complete remission improved the 5-year event-free survival compared with chemotherapy alone (68% *versus* 18%; $P<0.01$) but not the overall survival (68% *versus* 54%; $P=0.48$). The presence of *FLT3*-ITD had a non-significant negative effect on 5-year overall survival compared with non-mutated cases (22% *versus* 62%; $P=0.13$). Gene expression profiling showed a unique signature characterized by significantly higher expression of *EYA3*, *SESN1*, *PRDM2/RIZ*, and *HIST2H4* genes. In conclusion, t(6;9)/*DEK-NUP214* represents a unique subtype of acute myeloid leukemia with a high risk of relapse, high frequency of *FLT3*-ITD, and a specific gene expression signature.

Introduction

The t(6;9)(p22;q34), frequently reported with a breakpoint in 6p23 but now known to involve the *DEK* gene mapping to 6p22.3, is a rare translocation, estimated to occur in 1-2% of cases of childhood acute myeloid leukemia (AML).[1] The translocation was first identified in 1976, and the first pediatric patient was described in 1982.[2,3] The World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia from 2008 listed the t(6;9)(p22;q34) as a distinct entity.[4] However, our current knowledge of t(6;9)(p22;q34) in AML is drawn from relatively small series of patients, predominantly adults, associating t(6;9) with young age at onset and a poor outcome.[1,5-7] Typically, the t(6;9) presents as *de novo* AML, morphologically associated with French-American-British (FAB) type M2, bone marrow basophilia, Auer rods, and dysplasia.[1,5,7,8] The translocation is primarily the sole cytogenetic abnormality (80%); among the 20% of

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patients with additional secondary changes, gains of chromosomes 8 and 13 are most frequent.[1,8,9] Internal tandem duplication (ITD) in the FMS-related tyrosine kinase 3 (*FLT3*) gene occurs in 20-30% of *de novo* AML in adults and in approximately 10% of childhood AML but in up to 70% of t(6;9)-positive cases.[1,8,10,11] The clinical outcome is poor, with 5-year overall survival rates of 28% reported in children and 9% in adults.[1,5-7] Recent smaller studies, including both adult and pediatric patients, have shown that treatment with early allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission may improve the outcome.[1,12,13]

The t(6;9) results in a fusion of the 5' part of the *DEK* gene at 6p22.3 and the 3' part of the *NUP214* gene, formerly known as *CAN*, located at 9q34, forming the chimeric *DEK-NUP214* gene.[14] The DEK-NUP214 protein has been reported to enhance protein synthesis in cells of the myeloid lineage but is unable to block differentiation.[15,16] The leukemogenic potential of the fusion protein is restricted to a very small subpopulation of hematopoietic stem cells.[17]

The characteristics of t(6;9)/*DEK-NUP214* AML have so far not been separately described in pediatric patients and the prognostic impact in pediatric AML is unclear.[1,12,13,18] The aims of this study were to characterize the clinical, genetic and morphological features of t(6;9)-positive childhood myeloid neoplasms in the largest series so far; to evaluate outcome and to identify genes potentially involved in leukemogenesis of t(6;9) using gene expression profiling.

Methods

Patients

The inclusion criteria comprised age between 0-18 years and a diagnosis of *de novo* AML or myelodysplastic syndrome (MDS) with t(6;9)/*DEK-NUP214* made between 1 January, 1993 and 31 December, 2011.

The study was conducted within the International Berlin-Frankfurt-Munster study group cooperation and 24 study groups and treatment centers participated, providing background data for frequency analysis and submitting clinical and genetic data on 62 patients. In addition, the study groups contributed smears, bone marrow biopsies, and material for gene expression analysis. The nomenclature was reviewed following the International System for Human Cytogenetic Nomenclature 2009[19] by three of the co-authors (JH, BJ, and EF). Available diagnostic smears and bone marrow biopsies were reviewed by co-author GK.

Patients were treated according to national AML trials, and the treatment protocols were approved by local ethical committees in compliance with national regulations.

Gene expression profiling and quantitative real time polymerase chain reaction analysis

Gene expression profiling was performed on 297 pediatric AML patients' samples of which eight were t(6;9)-positive. The gene expression profiling data on the full cohort have already been published but an individual analysis of t(6;9)/*DEK-NUP214* was not performed.[20] Original data are available in the Gene Expression Omnibus repository (*http://www.ncbi.nlm.nih.gov/geo*; accession GSE17855). The gene expression profiles of t(6;9)-positive cases were compared with those of other representative pediatric AML samples.[20] Four top scoring, differentially expressed genes were selected for mRNA expression validation by quantitative real time polymerase chain reaction (RT-qPCR) analysis based on statistical significance, occurrence of multiple probes in the top-list, and log-fold change, combined with potential biological relevance; *EYA3* (eyes absent homolog 3, Drosophila), *SESN1* (sestrin 1), *PRDM2/RIZ* (PR domain containing 2, with ZNF domain), and *HIST2H4* (histone cluster 2, H4). RT-qPCR was performed on t(6;9)/*DEK-NUP214*-positive samples from 17 patients and one cell line (FKH-1) and compared with AML without t(6;9)/*DEK-NUP214* (31 samples from patients and 13 cell lines) using the ABI PRISM 7900HT sequence detector (Applied Biosystems, Foster City, CA, USA). Primer sequences are listed in *Online Supplementary Table S1*.

Statistical analyses

The Kaplan-Meier method was used to estimate the 5-year probabilities of overall survival and event-free survival. The 5-year cumulative incidence of relapse was calculated by the method of Kalbfleisch and Prentice.[21] The median time to HSCT in first complete remission was 150 days, with 98 days as minimum. Patients with an event within 150 days of treatment were excluded from the analysis comparing the effect of HSCT in first complete remission with that of conventional chemotherapy alone. Statistical analyses were conducted using SPSS for Mac, version 20 (SPSS Science, Chicago, IL, USA).

Gene expression profiling data were acquired using Expresso (Bioconductor package Affy). Probe-set intensities were normalized using variance stabilization normalization (Bioconductor package VSN) in the statistical data analysis environment R, version 2.11.[22] An empirical Bayes linear regression model was used to compare the signatures for the t(6;9)-positive cases with those of the other AML cases.[23] Moderated T-statistics *P*-values were corrected for multiple testing using the false discovery rate method, as defined by Benjamini and Hochberg.[24]

Details of the methods and primer sequences are provided in the *Online Supplementary Appendix*.

Results

Of the 24 study groups/centers that participated in the study, 15 provided clinical and cytogenetic data and samples for morphologic and gene expression analyses. The remaining nine study groups reported no patients with t(6;9)/*DEK-NUP214*. Data on 70 children were submitted. Eight patients were excluded; four were diagnosed before 1993 and four had incomplete karyotype with unknown t(6;9) breakpoints and no proof of the *DEK-NUP214* gene fusion. Accordingly 62 patients fulfilled the inclusion criteria. One study group did not report frequency background data and was not included in the frequency analysis, and since this was an AML estimate, EWOG-MDS was not included either. Thus 22 of the 24 study groups/centers reported altogether 7363 childhood AML cases with complete cytogenetic data, 45 of which had t(6;9)/*DEK-NUP214*, corresponding to a frequency of 0.6% of pediatric AML.

Of the 62 t(6;9)/*DEK-NUP214* myeloid malignancies, 54 were diagnosed as *de novo* AML and eight as MDS. The clinical, morphological, and genetic characteristics of the cohort are listed in Table 1. The AML and MDS groups were comparable except for age; children diagnosed with MDS were younger (median age 7.4 years *versus* 11.4 years; $P<0.05$). This notwithstanding, considering all other similarities, we combined t(6;9)/*DEK-NUP214 AML* and MDS cases into one entity.

Morphology

The majority of t(6;9) cases were categorized as FAB type M2 (54%) or FAB type M4 (26%) (Table 1). Peripheral blood and bone marrow smears from 11 and 15 cases, respectively, of which two were MDS cases, were evaluable for review along with 7 bone marrow biopsies. Dysplasia was defined according to EWOG-MDS guidelines.[25] All bone marrow biopsies displayed mild to moderate bilinear dysplasia. Basophils were present in the bone marrow from five (33%) of the reviewed cases, but did not exceed 2% in any case. Data on basophils were available for 16 cases without central review; five had 0.5-2% basophils in the bone marrow while no basophils were reported in the remaining 11 patients. No Auer rods were identified in the material reviewed. Among the cases not centrally reviewed, Auer rods were reported in ten of 28 (36%) cases. The morphological characteristics found by the central review are shown in *Online Supplementary Figure S1.*

Table 1. Characteristics of patients with t(6;9)(p22;q34)/*DEK-NUP214*-rearranged myeloid leukemia.

	AML N =54	MDS N =8	Total N=62
Boys	34 (63%)	5 (63%)	39 (63%)
Median age in years (range)	11.4*(3.4-17.6)	7.4 *(4.1-13.2)	10.4 (3.4-17.6)
0-1 year	0	0	0
2-9 years	21 (39%)	5 (63 %)	26 (42%)
10-18 years	33 (61%)	3 (37%)	36 (58%)
Hematologic parameters median (range)			
WBC (x10⁹/L) (N=58)	16.4 (0.2-191.0)	11.8 (1.9-29.0)	16.0 (0.2-191.0)
Hb (g/dL) (N=56)	8.2 (1.9-12.2)	7.3 (4.0-12.3)	8.0 (1.9-12.3)
Platelets (x10⁹/L) (N=48)	63.0 (6.0-235.0)	76.5 (15.0-92.0)	70.5 (6.0-235.0)
PB blasts (%) (N=50)	36 (0-99)**	9 (0-16)**	31 (0-99)
BM blasts (%) (N=53)	60 (14-95)**	10 (5-19)**	55 (5-95)
FAB			
M0	1 (2%)		1
M1	4 (7%)		4
M2	29 (54%)		29
M4	14 (26%)		14
M5	1 (2%)		1
Unclassifiable	5 (9%)		5
MDS		8	8
Cytogenetics			
t(6;9) sole abnormality	40 (74%)	7 (88%)	47 (81%)
Additional aberrations	10 (19%)	1 (13%)	11 (19%)
FISH or PCR only	4 (7%)	0	4 (6%)
HSCT	32 (59%)	7 (88%)	39 (63%)
First complete remission	18 (55%)	3 (43%)	21 (54%)
Residual disease	0	3 (43%)	3 (8%)
After relapse	14 (45%)	1 (14%)	15 (38%)
Complete remission	50 (93%)	5 (63%)	55 (89%)
No event	20 (37%)	5 (63%)	25 (40%)
Relapse	25 (46%)	3 (38%)	28 (45%)
Survival (SE)			
5-year EFS	32% (7)	55% (20)	35% (7)
5-year OS	53% (7)	86% (13)	57% (7)
5-year CIR	57 % (7)	45% (20)	56% (7)

* $P< 0.05$, ** $P< 0.01$

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; WBC: white blood cell count; Hb: hemoglobin; PB: peripheral blood; BM: bone marrow; FAB: French-American-British subtype; FISH: fluorescence in situ *hybridization, PCR: polymerase chain reaction; HSCT: hematopoietic stem-cell transplantation; EFS: event-free survival; OS: overall survival; CIR: cumulative incidence of relapse*

Cytogenetics

Successful cytogenetic results were available in 58 of the cases; in the remaining four, the *DEK-NUP214* gene fusion was detected by RT-PCR or fluorescence *in situ* hybridization. Among the 58 cytogenetically informative cases, t(6;9)(p22;q34) was the sole cytogenetic abnormality in 47 (81%) while 11 (19%) had additional aberrations, including loss of chromosome Y in three boys and trisomy 8 and trisomy 13 each present in three cases, either alone or combined. The karyotypes with additional aberrations are listed in *Online Supplementary Table S2.*

Outcome and prognostic factors

Outcome data were available for all 62 children. There were no differences in overall and event-free survival between children diagnosed with AML or with MDS; however, due to the heterogeneity of the treatment strategies for AML and MDS, the following survival estimates are based on AML cases only (Table 2). The median follow-up for the survivors was 4.7 years (range, 0.2 – 17.1). Fifty of the children (93%) achieved a complete remission and 25 (46%) experienced relapse. The latest event occurred 41 months after diagnosis. The 5-year event-free survival rate was 32% (±14%), the 5-year overall survival rate was 53% (±14%), and the 5-year cumulative incidence of relapse was 57% (±14%).

Sex, age, and white blood cell count were not prognostic factors for event-free survival, overall survival or cumulative incidence of relapse (Table 2). Patients with FAB M4 had a worse outcome than patients with other FAB subtypes, with a 5-year event-free survival of 9% *versus* 40% (P=0.04) and 5-year cumulative incidence of relapse of 91% *versus* 49% (P<0.01) respectively, but they were over-represented among the *FLT3*-ITD-positive cases (P=0.02).

In total, *FLT3*-ITD status was known in 33 (53%) cases: 14 (42%) were *FLT3*-ITD-positive and 19 (58%) were *FLT3*-ITD-negative (wild-type and one tyrosine kinase domain point mutation). The allelic ratio of *FLT3*-ITD was not available. Among the 29 *FLT3* informative AML cases included in the survival analysis, presence of *FLT3*-ITD had a non-significant negative effect on outcome compared with absence of *FLT3*-ITD (Table 2 and *Online Supplementary Figure S2C,D*).

In the evaluation of HSCT, MDS patients were excluded along with cases with events earlier than 150 days to correct for time to transplantation. Eighteen AML patients were transplanted in first complete remission and 14 after relapse. The characteristics of the HSCT are presented in *Online Supplementary Table S3*. HSCT in first complete remission significantly improved the 5-year event-free survival compared with treatment with chemotherapy alone in first complete remission: 68% *versus* 18% (P<0.01), but did not improve the overall survival rate (68% *versus* 54%; P=0.48; *Online Supplementary Figure S2A,B*). A total of five patients died after HSCT; one from progressive disease following relapse and four from procedure-related toxicity (two infections and two cases of multiorgan failure).

FLT3-ITD status was known for 24 of the 48 cases included in the HSCT analysis. Twelve were *FLT3*-ITD-positive, of which eight received chemotherapy only and four had HSCT in first complete remission. None of the eight patients treated with conventional chemotherapy survived without an event, whereas there were no events among the four ITD-positive patients treated with HSCT in first complete remission. Twelve patients were *FLT3*-

ITD-negative; three were transplanted in first complete remission, of whom one relapsed and nine received chemotherapy alone in first complete remission, of whom six relapsed.

Multivariate analysis was used to assess survival parameters of patients with *FLT3*-ITD treated with HSCT in first complete remission. The limited number of patients and information on *FLT3*-ITD status necessitated a strict selection of variables that could be included in the Cox model. We therefore performed stepwise exclusion and the best possible model was adjusted for HSCT and *FLT3*-ITD. FAB type M4 was overrepresented among *FLT3*-ITD-positive cases and was not an independent prognostic factor; thus, FAB type M4 was excluded from further analysis. Treatment with HSCT in first complete remission was independently associated with higher event-free survival (P=0.03) whereas *FLT3*-ITD mutation was not an independent prognostic factor (*Online Supplementary Table S4*).

Gene expression profiling

The supervised analysis of gene expression levels of *DEK-NUP214*-positive samples *versus* other pediatric AML samples resulted in a top-table of 180 distinctive probe-sets with a false discovery rate-adjusted P value <0.05. The t(6;9)/*DEK-NUP214* cases were characterized by high expression of four genes in particular: *HIST2H4* (log fold-change 2.75; adjusted P value of 8.17×10^{-15}); *PRDM2/RIZ* (log fold-change 1.5, adjusted P value 2.86×10^{-9}), represented by four probe-sets in the top-table, *SESN1* (log fold-change 1.24; adjusted P value 6.30×10^{-5}), and *EYA-3*, represented twice with log fold-changes 0.46 and 0.33 (adjusted P value 1.75×10^{-10}). mRNA expression levels determined by RT-qPCR correlated well with the expression profiles derived from gene expression profiling. The expression of these four genes among cytogenetic subgroups of pediatric AML, determined by both gene expression profiling and RT-qPCR, and their correlations are shown in Figure 1. The unsupervised analysis is presented in *Online Supplementary Figure S3*, revealing that the t(6;9)/*DEK-NUP214* cases did not cluster in an unsupervised manner.

In the top-table, 158 probe sets represented up-regulated genes, including several *HOXA* and *HOXB* genes; (*HOXB2*, *B3*, *B4*, *B5*, *B6*, *B8*, and *B9*). The high expression levels of the *HOXA* and the *HOXB* genes were validated in a previous study.[26]

Table 2. Overall survival, event-free survival, and cumulative incidence of relapse for children with t(6;9)(p22;q34)/*DEK-NUP214*-rearranged AML (N=54).

	5-year OS % (SE)	*P*-log rank	5-year EFS % (SE)	*P*-log rank	5-year CIR % (SE)	*P*-Gray
Year of diagnosis						
1993-1999 (N=18)	44 (12)	0.75	17 (9)	0.72	66 (12)	0.48
2000-2006 (N=18)	61 (12)		44 (12)		52 (12)	
2007-2012 (N=18)	55 (14)		34 (14)		53 (15)	
Sex						
Female (N=20)	51 (13)	0.84	33 (12)	0.90	62 (12)	0.35
Male (N=34)	53 (9)		31 (8)		54 (9)	
Age groups						
2-9 (N=21)	64 (11)	0.29	32 (10)	0.83	50 (11)	0.34
>10 (N=33)	44 (10)		33 (9)		62 (10)	
WBC count						
WBC <20x10⁹/L (N=29)	56 (10)	0.19	35 (10)	0.66	58 (10)	0.51
WBC 20-99x10⁹/L (N=20)	58 (11)		32 (11)		61 (12)	
WBC ≥100x10⁹/L (N=4)	0		0		no relapse	
FAB classification*						
M0 (N=1)	no events	0.47	no events	0.36	no relapse	0.55
M1 (N=4)	67 (27)		50 (25)		25 (22)	
M2 (N=29)	56 (10)		35 (10)		54 (10)	
M4 (N=14)	39 (14)	0.17	9 (8)	0.04	91 (8)	<0.01
M5 (N=1)	no events		no events		no relapse	
Unclassifiable (N=5)	40 (22)		40 (22)		no relapse	
Cytogenetics						
t(6;9)(p22;q34), sole abnormality (N=40)	50 (8)	0.81	28 (8)	0.57	60 (8)	0.32
Additional aberrations (N=10)	55 (17)		37 (19)		48 (21)	
FLT3-ITD**						
Positive (N=14)	22 (14)	0.13	17 (14)	0.29	75 (16)	0.10
Negative (N=15)	62 (13)		31 (14)		46 (16)	
HSCT in first complete remission***						
Yes (N=18)	68 (12)	0.48	68 (12)	<0.01	13 (9)	<0.01
No (N=30)	54 (10)		18 (7)		81 (8)	

The log-rank values are based on a comparison between all FAB subtypes. Only FAB M4 differed from the others, hence the additional analysis of FAB M4 vs. all others. **Four FLT3-ITD-positive patients were treated with HSCT in first complete remission with no events; all eight FLT3–ITD-positive patients not treated with HSCT relapsed. * The analysis of survival is based on patients with EFS >150 days, no patients were censored after HSCT. WBC: white blood cell count; FAB: French-American-British subtype; HSCT: hematopoietic stem-cell transplantation; EFS: event-free survival; OS: overall survival; CIR: cumulative incidence of relapse.*

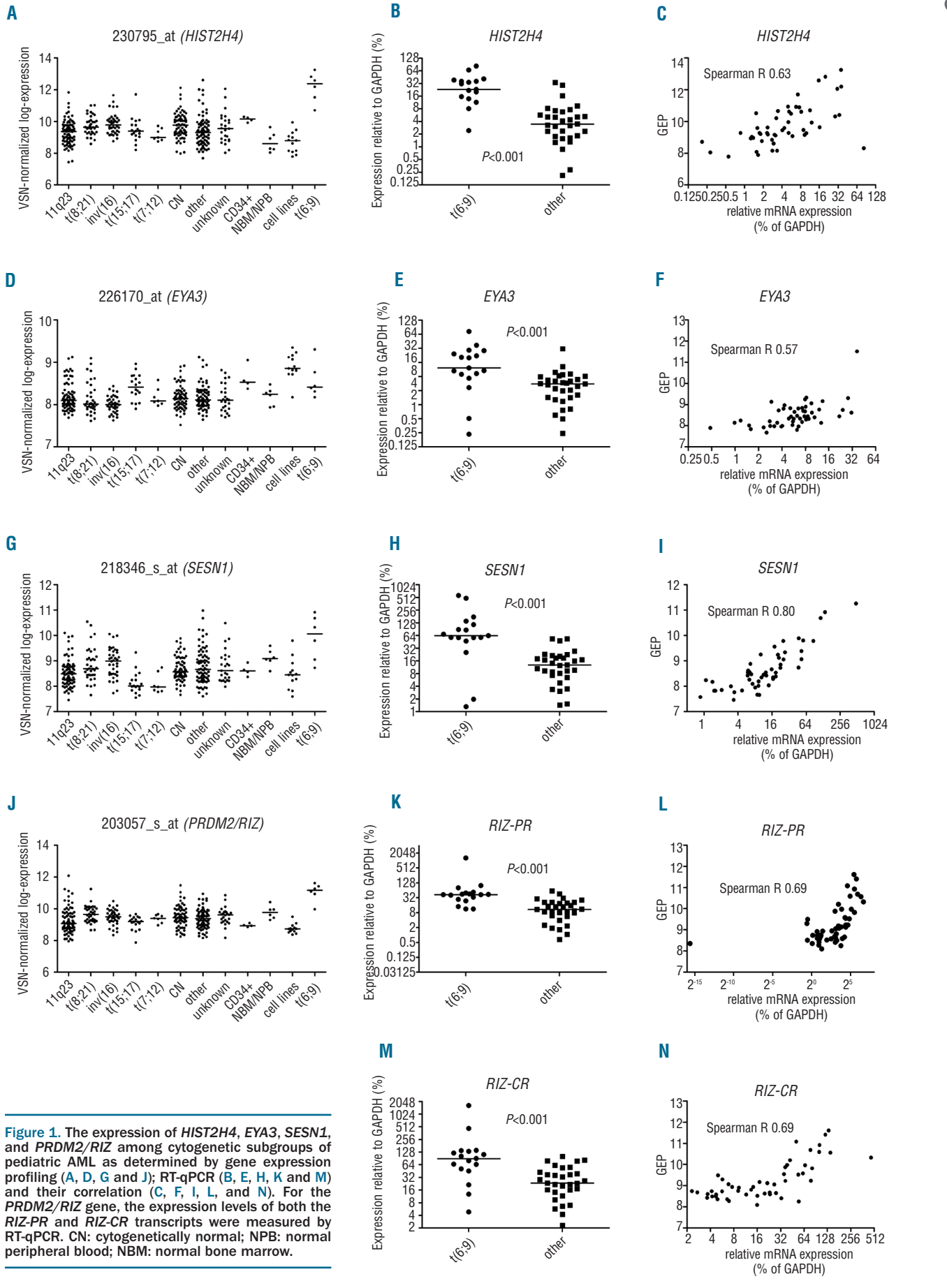

Figure 1. The expression of *HIST2H4*, *EYA3*, *SESN1*, and *PRDM2/RIZ* among cytogenetic subgroups of pediatric AML as determined by gene expression profiling (A, D, G and J); RT-qPCR (B, E, H, K and M) and their correlation (C, F, I, L, and N). For the *PRDM2/RIZ* gene, the expression levels of both the *RIZ-PR* and *RIZ-CR* transcripts were measured by RT-qPCR. CN: cytogenetically normal; NPB: normal peripheral blood; NBM: normal bone marrow.

Discussion

The t(6;9)/*DEK-NUP214*-rearranged cases in this cohort represented less than 1% of childhood AML with available cytogenetics based on the data collected from 22 major study groups/centers - a frequency lower than previously reported.[1,5] The translocation is easily detected by conventional karyotyping and the risk of undetected cases is considered minimal. An explanation for the low frequency could be the inclusion in the denominator of AML children from the study groups who did not report t(6;9) cases in the frequency analysis.

The median age was 10.4 years and no patient was diagnosed before 3 years of age. The age distribution is very different from the general age distribution in AML in which approximately 30% of patients are less than 3 years of age at diagnosis.[27] To our knowledge, no t(6;9)-positive cases have been reported in children below 3 years of age[9], which strengthens our finding. The male dominance in this study has also been observed in adult series of t(6;9).[1,5,7]

As in most previous adult studies on t(6;9) AML, the FAB subtypes FAB M2 and FAB M4 were most common,[1,5,6,28] together constituting 80% of all cases in the present cohort (Table 1). The present study suggests that there are differences in the morphology among pediatric and adult t(6;9) myeloid malignancies. Basophilia seems less common in children than in adults along with a significant but milder degree of dysplasia in childhood myeloid malignancies.[1,7,28] Pseudo-Pelger Huët cells were found in all 15 patients reviewed. This observation is in contrast to that in pediatric MDS, in which such cells are only rarely found (GK, *personal communication 2012*). The similar clinical and morphological characteristics between t(6;9)-positive AML and MDS found in this large pediatric series suggest that t(6;9)/*DEK-NUP214*, like t(8;21)/*RUNX1-RUNX1T1* and inv(16)(p13q22) or t(16;16)(p13;q22)/*CBFB-MYH11*, should be classified as AML regardless of the blast count.

FLT3-ITD mutations were present in 42% of the present cohort, which is less than reported in the literature,[1,6] and had a negative impact on outcome (Table 2), though this did not reach statistical significance. The COG study on t(6;9) AML reported no significant impact of *FLT3*-ITD on outcome (overall survival rates for *FLT3*-ITD-positive and -negative cases were 39% *versus* 20%, respectively). However, the ITD-positive patients in the COG study were allocated to a high-risk treatment protocol including HSCT while ITD-negative cases were assigned to an intermediate-risk protocol without transplantation,[29] which might have influenced the outcome. In the present study, all eight *FLT3*-ITD-positive patients treated without HSCT in first complete remission experienced a relapse and subsequently only two survived (with follow-ups of 12 and 13 months), whereas the four *FLT3*-ITD-positive patients treated with HSCT were alive without disease in first complete remission at follow-up. Six of the nine ITD-negative patients, treated with chemotherapy in first complete remission, relapsed. We found a significant improvement of event-free survival resulting from treatment with HSCT in first complete remission compared with treatment with chemotherapy only, but this did not translate into superior overall survival (Table 2 and *Online Supplementary Table S4*), suggesting a high salvage rate. Only St. Jude reported t(6;9) as a treatment-stratifying aberration in the AML02 and 08 protocols relevant for two patients included in this study and allocated to the high-risk arm including HSCT. It should be emphasized that retrospectively collected data have serious limitations since the factors for allocating patients to HSCT, such as co-morbidity, individual assessment of the treating physician, and availability of donor, remain unknown and this weakness must be taken into account when evaluating the value of HSCT. We have statistically tried to overcome some of the obstacles, such as disease stage, by only evaluating HSCT in first complete remission and corrected for time to transplantation. Parameters important for the outcome of HSCT, such as donor characteristics, and HLA match are given in *Online Supplementary Table S3*. The numbers are small and considering the above-mentioned limitations, our results are in accordance with those of other small series.[12,13] Notably, 14 children were transplanted after relapse and it is possible that a potential beneficial effect of HSCT after relapse is reflected in the high salvage rate. HSCT seems beneficial in patients with t(6;9)/*DEK-NUP214* and in particular for patients with *FLT3*-ITD.

We identified a unique gene expression signature with, among others, high expression of *HIST2H4*, *PRDM2/RIZ*, *EYA3* and *SESN1* in addition to *HOXA* and *HOXB* gene overexpression being characteristic of *DEK-NUP214*-positive cases, as it is for AML with *NPM1*-mutations, *NUP98-NSD1*-rearrangements and partial tandem duplications of the *MLL* gene, suggesting a common pathway of leukemogenesis in these cases. The overexpression of *HOXA* and *HOXB* genes in *DEK-NUP214*-positive cases was previously validated.[26] It is noteworthy that both *DEK-NUP214* and *NUP98-NSD1* are characterized by high *HOXA* and *HOXB* gene expression since these two cytogenetic subgroups share other genetic and clinical characteristics: a high frequency of *FLT3*-ITD (40-70% and >90%, respectively)[1,26] and absence of patients below 2 years of age.[26] Furthermore, the NUP214 and NUP98 oncogenic fusion proteins are similar in many respects. First, both fusions include a nucleoporin-specific FG region (phenylalanine-glycine repeats), which is associated with various histone-modifying complexes.[30,31] Second, both fuse to a nuclear factor, mostly but not uniquely a direct transcription factor.[14,31,32] Third, both fusion products are localized to the nucleus, as opposed to the wild-type nucleoporins, which are mainly present in the nuclear pore complex.[31,33] It is, therefore, likely that these nucleoporin-containing fusion proteins are functionally similar, acting as aberrant transcriptional modulators.

Within this study, we validated the selective up-regulation of four genes in t(6;9)/*DEK-NUP214*–positive pediatric cases: *HIST2H4*, *PRDM2/RIZ*, *EYA3* and *SESN1*. The *HIST2H4*, mapping at 1q21.2, encodes a member of the histone H4 family but the function of *HIST2H4* in leukemogenesis is unknown. The *PRDM2* (1p36.21) gene, also known as *RIZ,* encodes two proteins: RIZ-PR (RIZ1) and RIZ-CR (RIZ2). The proteins are identical except that RIZ-PR has an N-terminal PR domain with methyltransferase activity that is lacking in RIZ-CR. *RIZ*-PR has tumor suppressor activity whereas *RIZ-CR* has been described to acts as an oncogene.[34,35] The probe used in our gene expression profiling analyses did not distinguish between the two transcripts. However, in the RT-qPCR validation of the expression levels, we analyzed the gene expression of each transcript and found that both the tumor-suppressive *RIZ-PR* and the oncogenic *RIZ-CR* were up-regulated. The

EYA3 gene (1p35.3) encodes a member of the 'eyes absent' protein family and is involved in repair and cell survival as a response to DNA damage in organogenesis.[36,37] The fourth gene *SESN1* (6q21) codes for a member of the sestrin family and is known to be a TP53 target.[38,39] Considering their known tumor suppressor function, it is surprising that the *SESN1* gene and the *RIZ-PR* transcript were both up-regulated in t(6;9)-positive cases.

It is striking that several of the genes found to be significantly up-regulated in *DEK-NUP214*–rearranged cases are known to influence the modeling and function of histones. Depletion of both *DEK* and *EYA3* causes phosphorylation of H2Ax (γH2Ax), a subunit of histone H2A,[37,40] DEK protein is able to bind to histones and reduce the levels of histone H3 and H4 acetylation, thus playing an important role in chromatin modification, histone acetylation and transcription.[40,41] In addition, the most significantly up-regulated gene was *HIST2H4* and the tumor suppressor activity of RIZ-PR is related to the histone methyl-transferase activity of the PR domain.[42] We hypothesize that specific histone modifications are key events during leukemogenesis, possibly through modulating the epigenetic state of the cell.

In conclusion, t(6;9)/*DEK-NUP214*-rearranged cases represent less than 1% of all childhood AML and are characterized by a late onset, male predominance, *FLT3*-ITD mutations, and a high risk of relapse. Nevertheless, a large proportion of the patients can be cured and HSCT potentially benefits patients with t(6;9) and especially those with *FLT3*-ITD. In addition, we identified a unique gene expression signature including several up-regulated genes involved in histone modification, and a typical *HOXA/B* profile, which may be a target for future therapy.

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