BCOR and *BCORL1* mutations in pediatric acute myeloid leukemia

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by the presence of different collaborating cytogenetic and molecular aberrations that are associated with treatment response.[1-3] Approximately 20% of pediatric AML patients are classified as cytogenetically normal AML (CN-AML).[4] In the past decade, new prognostic relevant aberrations have been identified in CN-AML, such as *NPM1, WT1, FLT3-ITD* and *CEBPA* double mutations (*CEBPA*dm), which may improve future risk-group stratification.[5-8] In addition to these mutations, we recently described recurrent cryptic *NUP98/NSD1* and *NUP98/KDM5A* translocations in pediatric CN-AML.[9,10] Still, some CN-AML samples have not been fully characterized.

Recently, Grossmann *et al.* discovered a somatic mutation in the *BCL6* co-repressor (*BCOR*) gene in an adult CN-AML patient using whole-exome sequencing.[11] BCOR mutations were subsequently identified in 10 of 262 (approx. 4%) adult CN-AML patients, unselected for cytogenetic abnormalities, and for whom a poor outcome had been predicted. In addition, Li *et al.* discovered mutations in the *BCL6 corepressor-like 1* (*BCORL1*) gene in 2 of 8 patients with secondary adult AML.[12] Further exploration identified somatic *BCORL1* mutations in 10 of 173 (6%) of adult AML patients, of which 5 cases were diagnosed with secondary or treatment-related AML. In 8 of 10 patients these included nonsense, splice site, and frame-shift mutations that were predicted to result in truncation of the protein, suggesting that *BCORL1* is a tumor suppressor gene that may be inactivated by mutations.[13]

In pediatric AML, the role of *BCOR* and *BCORL1* is unknown. Therefore, we explored the frequency and impact of *BCOR* and *BCORL1* mutations in pediatric AML in a molecularly well documented cohort of 230 pediatric AML patients.

Genomic DNA was PCR amplified using the primers described in *Online Supplementary Table S1*. Purified PCR products were directly sequenced. Sequence data were analyzed using CLCWorkbench (CLC Bio, Aarhus, Denmark). *BCOR* variations were determined in comparison to the coding DNA sequence ENST00000342274, and *BCORL1* variations to ENST00000540052. A mutation was defined as a nucleotide change not reported in the dbSNP database. SNPs were defined as a nucleotide change as described in the dbSNP database. Characteristics of the 230 pediatric AML patients included in this mutational screening are listed in Table 1.

The complete coding sequence of *BCOR* and *BCORL1* was screened in a nested cohort of 83 *de novo* pediatric AML patients, and 17 AML cell lines. This cohort represented all relevant cytogenetic subgroups in pediatric AML, with an enrichment for CN-AML (n=48). All CN-AML patients were screened for *NPM1, CEBPAdm, FLT3-ITD, IDH1/2, WT1, cKIT, N/K-RAS, DNMT3A, ASXL1* and *RUNX1* mutations, and *MLL*-PTD, *NUP98/NSD1*, *NUP98/KDM5A*, and MLL-rearrangements (*Online Supplementary Table S2*).

None of the cell lines showed a mutation. In 4 of 83 patients a *BCOR* mutation (detailed in Table 2) was identified; 3 in exon 4 and 1 in exon 12. Three of these cases were CN-AML (3 of 48, 6.3%), and the fourth mutation

Table 1. Clinical characteristics of pediatric acute myeloid leukemia patients included in mutation screening.

	BCOR and BCORL1 Complete coding sequence	BCOR and BCORL1 Exon 4
Number	83	147
Age in years Median (range)	9.8 (0.2-18.0)	8.2 (0.1-18.0)
Sex, n. (%)		
Female	36 (43)	58 (42)
WBC x10⁹/L, median (range)	94.3 (1.2-377.6)	45.2 (2.4-475.0)
Karyotype, n. (%)		
11q23	5 (6)	46 (31)
t(8;21)	5 (6)	13 (9)
inv(16)	6 (7)	28 (19)
t(15;17)	7 (8)	9 (6)
t(7;12)	5 (6)	–
CN	48 (58)	–
Other	7 (8)	40 (27)
Unknown	–	11 (8)
FAB, n. (%)		
M0	8 (10)	6 (4)
M1	17 (21)	10 (7)
M2	19 (23)	19 (13)
M3	9 (11)	9 (6)
M4	22 (27)	49 (33)
M5	4 (5)	42 (29)
M6	–	2 (1)
M7	1 (1)	7 (5)
Unknown	3 (4)	3 (2)
NUP98/NSD1, n. (%)	10 (12)	2 (1)
NPM1, n. (%)	14 (17)	4 (3)
CEBPAdm, n. (%)	7 (8)	3 (2)

WBC: white blood cell count; L: liter; FAB: French-American-British classification; CEBPAdm: CEBPA double mutation.

Table 2. Characteristics of BCOR and BCORL1 aberrations detected in childhood cytogenetically normal-acute myeloid leukemia patients.

UPN	Age (yrs)	Sex	WBC (x10⁹/L)	FAB	Mutated genes	Relapse	Death	Karyotype	BCOR mutation	BCORL1 mutation
1	4	M	354.0	M1	*CEBPAdm, WT1, NRAS*	No	No	46,XY[20]	p.A854T	–
2	11	F	48.2	M4	*FLT3-itd, NPM1*	No	No	46, XX†	p.T60M	–
3	12	F	140.6	M5	*FLT3-itd, WT1*	No	No	46, XX [20]	p.D1302E	–
4	0	F	217.9	M2	*MNX1/ETV6, KIT*	Yes	Yes	47,XX,t(7;12) (q36;p13),+19[32]	p.G86E	–
5	17	M	9.4	M1	*RUNX1*	No	Yes	46,XX[26]	–	p.G158*(Stop)

UPN indicates unique patient number; yrs: years; M: male; F: female; WBC: white blood cell count; L: liter. †number of analyzed metaphases unknown.

was seen in an *MNX1/ETV6* translocated AML. In only one of 83 patients a *BCORL1* mutation was identified which resulted in a stop codon (Table 2). This CN-AML patient had an underlying xeroderma pigmentosum.

An additional 147 pediatric AML patients were screened for mutations in *BCOR* and *BCORL1* exon 4, based on the occurrence of mutations in this exon; 136 cases were *de novo* AML, 11 cases were secondary AML, of which 6 cases were MDS which progressed to AML and 5 were secondary to earlier therapy because of another malignancy. None of the additional cases were CN-AML. No additional mutations were found. In 2 of 230 cases a missense SNP was found in *BCOR* (p.V679I, rs144722432) and in 35 of 230 (15%) a missense SNP in *BCORL1* (p.G209S, rs5932715). Both SNPs are predicted as tolerated by SIFT analysis, and in line with the predicted prevalence in the normal population.[14] In one case missense SNP rs139887979 (p.D94N) was identified in *BCORL1*, predicted as damaging by SIFT analysis.[14] We were unable to confirm this in germline or remission because such material was not available for these patients.

Altogether we identified a *BCOR* mutation in 4 of 230 (1.7%) cases. The frequency of mutations in pediatric CN-AML patients (6.3%; 95%CI: 2.1-16.8) was comparable to that in adult CN-AML (3.8%; 95%CI: 2.1-6.9; *P*=ns). The frequency of *BCORL1* mutations (1 of 83, 1.2%; 95%CI: 0.2-6.5) was significantly lower than in adults (10 of 173, 5.8%; 95%CI: 3.2-10.3; *P*<0.05) in patients of whom we screened the complete exon, and also lower when only analyzing exon 4 of *BCORL1* [1 of 230, 0.4% (0.1-2.4) *vs.* 7 of 173, 4.0% (2.0-8.1); *P*<0.05]. This may be due to the enrichment for secondary AML and treatment-related AML in the adult cohort, 73 of 173 (42%) in the cohort of Li *et al. versus* 12 of 230 (5%) in this cohort.[11,12,15]

Apart from mutation analyses, we studied *BCOR* and *BCORL1* gene expression levels using RT-qPCR in 65 patients of the initial cohort, including all mutated cases. Expression levels were determined using a SYBRgreen (Finnzymes Inc, Woburn, MA, USA) Taqman assay and average cycle threshold (CT) values were compared to the reference gene *GAPDH*, using the comparative cycle time method. Expression levels of 2 BCOR-mutated cases seemed lower than the other pediatric AML cases, but the 4 mutated cases did not significantly deviate from the non-mutated pediatric AML patients (*Online Supplementary Figure S1*). This is in accordance with findings in adult AML patients where both decreased and normal BCOR levels were observed in cases with *BCOR* mutations.[11] In adult AML, *BCORL1* mutations did not affect mRNA levels, similar to the finding of our single case [0.297% expression relative to GAPDH, mean in group (n=65) 0.275%, range 0.049-1.358].[13,15]

In the adult CN-AML cohort, the presence of *BCOR* mutations conferred a poor outcome. In our cohort, the low frequency of mutations did not allow survival analysis to be performed. Clinical outcome of the mutated cases is shown in Table 2. The presence of the non-synonymous SNP in *BCORL1*, rs5932715, did not influence clinical outcome (5-year probability of overall survival 57±9% *vs.* 64±4%, *P*=0.4; 5-year probability of event-free survival 49±9% *vs.* 45±4, *P*=0.9).

In conclusion, in pediatric AML, *BCOR* and *BCORL1* mutations rarely occur. Consequently, the clinical relevance is difficult to determine.

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