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DNMT3A mutations are rare in childhood acute myeloid leukemia

Childhood acute myeloid leukemia (AML) is a complex disease of the hematopoietic stem cell. Overall survival is relatively low with an overall survival rate of 50-70%. Besides cytogenetic changes and response to induction therapy, molecular aberrations are important prognostic markers that can help to risk stratify children with AML. Molecular aberrations include mutations in *FLT3*,[1] *NPM1*,[2] *CEBPA*,[3] *WT1*[4] with the recent addition of *IDH1* and *IDH2*.[5] In a significant number of pediatric and adult AML patients, no known mutation or cytogenetic aberration can be identified. It is, therefore, believed that a large number of gene mutations in AML are still to be identified. Recently, somatic mutations in *DNA methyltransferase 3A* (*DNMT3A*) have been found in adult AML[6,7] but the incidence and prognostic impact in childhood AML is unknown. DNMT3A is involved in epigenetic regulation of genes by enzymatic *de novo* addition of methyl groups to the cytosine residue of CpG dinucleotides. Mutations in *DNMT3A* occur in approximately 20% of adult AML patients. Interestingly, a mutational hotspot in codon R882 located in the catalytic methyltransferase domain has been reported to present approximately 60% of all mutations while the remaining 40% are located throughout the gene with the main focus in the methyltransferase domain.[6,7] Ley *et al.* described an adverse prognostic impact of the mutation for adult AML patients[6] which has been confirmed by our group and others.[7,8] Hence, mutations in *DNMT3A* appear to play an important role as a novel prognostic marker in adult AML. However, so far little is known about the frequency and prognostic impact of *DNMT3A* mutations in childhood AML. The only study to date which looks at *DNMT3A* mutations in a cohort of 180 children with AML did not identify any *DNMT3A* mutations associated with disease.[9] Here we report the frequency, and clinical and molecular characteristics of *DNMT3A* mutations in a well defined cohort of 195 pediatric AML patients. Bone marrow (BM) or peripheral blood (PB) samples from initial diagnosis were obtained from 195 pediatric AML patients. Details regarding the clinical and molecular characteristics of the study cohort are shown in Table 1. All patients were treated within two prospective multicenter trials: the AML-Berlin-Frankfurt-Münster (BFM) 98 or the 2004 (NCT00111345), as previously described.[10-12] The studies were approved by the protocol review committee of the German Cancer Society and by

Table 1. Main clinical and biological features of the study cohort.

Characteristic	Number (n =195)	% 100
Age, years		
median	8.75	
Sex		
male - n. (%)	98	50.3
female - n. (%)	97	49.7
Study		
AML-BFM 98	30	15.4
AML-BFM 04	165	84.6
FAB-subtype		
M0	4	2.1
M1	26	13.4
M2	35	18
M3	9	4.6
M4	57	29.2
M5	42	21.5
M6	2	1
M7	15	7.7
missing data	5	2.6
M. Down		
no	190	97.4
yes	4	2.1
unknown	1	0.5
Cytogenetic standard risk*		
no	121	62.1
yes	48	24.6
unknown	26	13.3
Bone marrow blasts day 15		
≤ 5%	131	67.2
> 5%	40	20.5
missing data	24	12.3
WBC count		
median - (x10^9/L)	31.4	
range - (x10^9/L)	0.8-585	
Platelet count		
median - (x10^9/L)	64	
range - (x10^9/L)	17-376	
FLT3-ITD – n. (%)		
mutated – n. (%)	25	12.8
wild type – n. (%)	123	63.1
missing – n. (%)	47	24.1
NPM1		
mutated – n. (%)	22	11.3
wild type – n. (%)	116	59.5
missing – n. (%)	57	29.2

AML-BFM, multicenter treatment trials AML-Berlin-Frankfurt-Münster (BFM); FAB: French-American-British classification of acute myeloid leukemia; WBC: white blood cell count; FLT3-ITD, internal tandem duplication of the FLT3 *gene; NPM1, nucleophosmin 1 gene. *Cytogenetic standard risk, chromosomal aberrations including t(8;21), inv 16 or t(15;17).*

Table 2. Main clinical and biological features of *DNMT3A* mutated patients.

Patient	*DNMT3A* mutation	Protein change	Age (years)	Sex	FAB	WBC [x10^9/L]	Platelets [x10^9/L]	Karyotype	Other mutations	CR	Relapse	Transplantion	Survival status/ follow up
1	C2645A	R882H	15.6	male	M2	124	51	complex karyotype	*FLT3-ITD*, *NPM1*	yes	yes	MUD	alive (4.67 years)
2	C2644T	R882C	15.3	male	M1	47.5	125	unknown	*FLT3-ITD*	yes	no	MUD	alive (1.1 years)

(R): arginine; (H): histidine F; (C): cysteine; FAB: French-American-British classification of acute myeloid leukemia; ITD: internal tandem duplication; CR: complete remission; MUD: matched unrelated donor transplantation.

the local ethics committees. Written informed consent was obtained from patients, parents or guardians. The median survival time for patients in follow up was 3.53 years (range 0.2-9.5 years). Exon 23 spanning codon R882 was analyzed using primers and PCR conditions as described.[8] Purified PCR fragments were directly sequenced. All mutations were confirmed in a second independent analysis. Two patients had a mutation in codon R882 and one patient had the minor allele of SNP rs61758. A follow-up remission sample was available for one mutated patient; the patient lost the mutation in remission. Although the low frequency of *DNMT3A* mutations in pediatric AML did not allow any formal assessment of clinical and molecular associations or prognostic evaluation to be made, the mutated patients were found to present some interesting characteristics. The 2 children with the mutation were older compared to the median age of the cohort (15.45 *vs.* 8.56 years). In adult AML, *DNMT3A* mutations were associated with older age.[6,8] Our data suggest that the mutation is more likely to occur with advanced age and this, therefore, explains the low mutation rate in childhood AML. Ley *et al.* and our group described an association between mutations in *DNMT3A* with FAB M4/M5 and mutations in *FLT3*, *IDH1* and *NPM1* in adult AML.[6,8] Interestingly, none of the mutated patients belonged to the FAB M4/M5 group. However, our 2 mutated patients were both found to be *FLT3-ITD* positive. While one patient also showed a mutation in *NPM1*, they both had wild-type *IDH1/IDH2* (Table 2). Both patients underwent bone marrow transplantation and are in complete remission (Table 2). The effects of hematopoietic stem cell transplantation in AML patients with mutated *DNMT3A* remain unclear. Our study demonstrates that *DNMT3A* mutations are rare in childhood AML and are associated with older age. However, given that only exon 23 of *DNMT3A* was studied, where the mutational hotspot in adult AML is located, it is conceivable that the mutation rate is underestimated in our study. It may be speculated from this data that embryonic *DNMT3A* mutations may have deleterious effects during development, and that mutations acquired at later stages of life have a weak oncogenic potential resulting in a long latency of leukemic transformation.

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