

## Amplification of *AML1* gene in association with karyotype, age and diagnosis in acute leukemia patients\*

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The *AML1* gene, located in the chromosomal band 21q22, belongs to the runt domain family of genes and encodes the subunit of the core-binding factor (CBF). *AML1* is normally expressed in all hematopoietic lineages and is essential for the transcriptional regulation of a number of hematopoietic specific genes. In acute leukemia three types of abnormality of *AML1* have been observed – chromosomal translocations, point mutation and duplication or amplification of the unrearranged gene. The most common origin of extra copies of the *AML1* gene is polysomy of chromosome 21 or a partial duplication of the long arm of chromosome 21, less frequently ring, isochromosome or the tandem repetition of chromosome 21.

In the study 13 children and 5 adults with ALL and AML or MDS, respectively, have been included. Using standard G-banding and dual color FISH analyses, gain of *AML1* originated in polysomy of chromosome 21 in each group of patients was proved. True high-level amplification was not observed but some uncommon changes in noteworthy association with other chromosome aberrations, age or diagnoses were presented.

*Key words:* *AML1* gene, amplification, ALL, AML

The *AML1* (CBFA2, *RUNX1*) gene located in the chromosomal band 21q22, belongs to a recently identified gene family of transcription factors sharing a highly evolutionarily conserved region of 128 amino acids termed the runt domain (*drosophila* segmentation gene *runt*, human genes *AML1*, *AML2*, *AML3*, mouse homologues *PEBP2αB*, *CBFA3*, *PEPB2αA*) [1].

The *AML1* gene encodes the DNA-binding subunit of the heterodimering transcriptional factor complex, core-binding factor (CBF). *AML1*-CBFβ complex binds a consensus DNA core motif known as the Runt domain binding element (TGTGGT), present in transcriptional regulatory regions of genes essential for myelopoiesis [2, 3].

*AML1* is normally expressed in all hematopoietic lineages and is essential for the transcriptional regulation of a number of hematopoietic specific genes, including those for granulocyte-macrophage colony stimulating factor (GM-CSF),

M-CSF receptor, myeloperoxidase, neutrophil elastase, interleukin 3 (IL-3), and each of the subunits of the T-cell antigen receptor genes. Its key regulatory role in hematopoiesis has been proved on murine model. Mice lacking either *AML1* or *CBFβ* are defective in definitive hematopoiesis of all lineages [2, 3, 4].

In acute leukemia three types of abnormality of *AML1* have been observed – chromosomal translocations with formation of new chimeric oncogene, point mutation of *AML1* and duplication or amplification of the unrearranged gene [5, 6].

More than 40 different patterns of translocations or rearrangements involving the 21q22 region have been described [7]. The *AML1* gene is a target of many common translocations seen in different types of leukemia, such as t(12;21) with *TEL/AML1* fusion in childhood acute lymphoblastic leukemia (ALL), t(8;21) and *AML1/ETO* in acute myeloid leukemia (AML) or recombination of *AML1* with genes *EVII*, *EAP* and *MDS1* on 3q26 in myelodysplasia (MDS) and in the blast phase of chronic myeloid leukemia (CML) [4, 8, 9, 10, 11].

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Mutation within the *AML1* gene has been identified in *de novo* myelodysplasia and acute myeloid leukemia, and in a therapy related to both MDS (t-MDS) and t-AML, respectively, induced by alkylating agents. Congenital mutations in *AML1* caused the rare autosomal dominant disease, familial platelet disorder (FDA) with predisposition to the development of AML [3, 4, 5, 10].

Genomic amplification is a frequent genetic abnormality especially in solid tumors and many oncogenes could be activated in this way. The situation in hematological malignancies appears to be different. Rather than two known forms of amplified genes – extended chromosomes named homogeneously staining regions (HSR) or extra chromosomal elements, called double minute (dmin) chromosomes [4], in acute leukemia the most common origin of extra copies of the *AML1* gene is polysomy of chromosome 21 or a partial duplication of the long arm of chromosome 21, dup(21) [7, 12, 13]. Another, infrequent chromosome aberrations can lead to gain of this gene, such as the amplification caused by ring 21 or by isochromosome, or less frequently by the tandem repetition of chromosome 21 [14]. Albeit polysomy of chromosome 21 or its long arm is usually well demonstrable by conventional cytogenetics (G-banding), a small intrachromosomal amplification can be covered up on cytogenetically unchanged chromosome 21 or by the marker chromosomes occurred in complex karyotype. Therefore, FISH techniques are especially useful for identifying chromosomal aberrations, as well as the identification of marker chromosomes [5, 7, 11, 15].

### Material and methods

**Patients.** Bone marrow samples from 13 children of age ≤15 years, with median age of 5 years (range 2–12) and 5 adults (age >15 years) with a median age of 53 years (range 30–74) were analyzed at the time of diagnosis. None of the 18 patients had Down's syndrome. The leukemia immunophenotype was determined by standard immunofluorescence analysis using a panel of monoclonal antibodies.

**Methods.** Standard chromosomes G-banding analysis was performed on mitotic bone marrow cells after short-term culture. Karyotypes were interpreted according to the International System for Cytogenetic Nomenclature (ISCN, 1995) [16].

Interphase FISH screening was routinely carried out on all children ALL patients for the prognostically significant abnormalities, *TEL/AML1*, *BCR/ABL* and rearrangements of the *MLL* gene. FISH identification of *AML1* aberrations in cases with AML or other diagnoses were realized according to the cytogenetic finding.

FISH analyses were performed on the fixed cell suspensions used for cytogenetic analysis. Either by locus-specific *TEL/AML1* or *AML1/ETO* probe (Vysis), and/or various whole-chromosome and centromere-specific probes were used according to the manufacturer instructions.

### Results

In a group of 13 childhood patients was predominance of boys (8) over girls (5). In 12 cases the diagnosis of common ALL (cALL) and one case of AML was established. Interphase FISH screening analysis by using *TEL/AML1* probe was carried out in all of children. In only 5 cases it was possible to obtain the karyotype (Tab. 1). Four children displayed hyperdiploidy (52–63 chromosomes), but the number of *AML1* signals by FISH was in agreement with the number of chromosomes 21 only in one case (patient 5). The localization of *AML1* extra copies was not known in other three children (2, 3, 6). The sole trisomy of 21 chromosome alike with one additional signal was obtained in patient 12. Only one child patient with AML had one normal but two derived chromosome 21; one small acrocentric chromosome dup(21)(q?) and one as a part of bigger ring chromosome confirmed by FISH could be found (Fig. 1).

In the group of adult patients dominance of 4 AML cases over the one pre-B ALL was observed. Karyotype of all patients was acquired. Three cases (14, 16, 17) presented trisomy of chromosome 21 associated with another numerical or structural changes, confirmed by FISH (Tab. 2). Patient 15 displayed near tetraploidy with four chromosomes 21 but 4–6 *AML1* signals by FISH. The place of extra copies of *AML1* gene was not practicable to identify due to the multiple marker chromosomes. Due to failure in preparing initial karyotype, FISH with panel of probes for childhood ALL was performed in patient 18. Surprisingly, in connection with the age of this patient, *TEL/AML1* double fusion accompanying with loss of normal *TEL* and gain of *AML1* was uncovered (Fig. 2).

### Discussion

This study presents a series of 18 patients with an abnormal number of *AML1* gene in children and adults with differ-

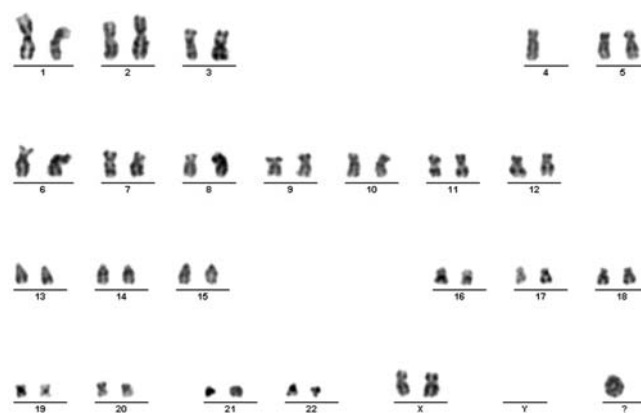


Figure 1. G-banded karyotype of patient 13. 46,XX,der(21)dup(21)(q?), +r. Monosomy of chromosome 4 is not clonal.

**Table 1. Data for childhood patients with extra copies of *AML1* gene**

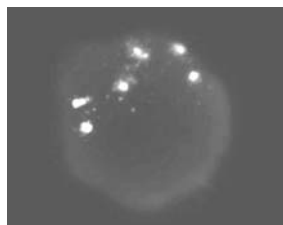
Patient No. (sex, age*)	Phenotype	Karyotype	Number of <i>AML1</i> signals/cell	Other changes
1 (M, 12)	cALL	nk	3–5	/
2 (M, 9)	cALL	±53,XY,+2,+4,+6,+17,+21,+21 1 add(1q), del(12p),+m1,+m2 / 46,XY	3–5	-TEL, +6
3 (M, 4)	cALL	nk	3	+MLL
4 (M, 7)	cALL	54-64,XY,+3,+5,+6,+7,+8,+14,+15,+17,+20,+21 / 46,XY	4	+5,+6,+8
5 (M, 5)	cALL, coexp. CD33	52,XXY,+5,+6,+17,+21,+21 / 53,XXY,idem,+5	4	+5, 2x +5, +6, +17,+X
6 (F, 4)	cALL	50-54,XX / 46,XX	4	/
7 (F, 5)	cALL	nk	4	/
8 (F, 5)	cALL	nk	4	/
9 (M, 4)	cALL, coexp. CD13, CD33	nk	3–5	+TEL
10 (M, 2)	cALL	nk	3	/
11 (M, 7)	cALL	nk	3–4	+ABL
12 (F, 4)	cALL	KD 47,XX,+21/ 46,XX	3	7q-
13 (F, 3)	AML M2, coexp. CD7	KD 47,XX,der(21),+r	4	/

\*age in diagnosis in years, nk – not known, + gain, - loss

**Table 2. Data for adult patients with extra copies of *AML1* gene**

Patient No. (sex, age*)	Phenotype	Karyotype	Number of <i>AML1</i> signals/cell	Other changes
14 (M, 55)	AML	48,XY,+21,+m	3	/
15 (M, 74)	MDS, AML	±90,XY,del(5q),±v1-15, +m1-7,2-18dmin / 46,XY	4–6	5q-, 2x +5p, +TEL, 4-8x c-MYC
16 (F,42)	AML M2	49,XY,+4,+8+,+21 (3) / 46,XX	3	+8
17 (F,55)	AML M1	47,XX,del(11)(q23),+21 / 46,XX	3	MLLso-
18 (F,30)	pre-B ALL	46,XX	6	TEL/AML1, TEL/AML1, -TEL, -TEL

\*age in diagnosis in years, + gain, - loss



**Figure 2. Interphase nuclei FISH of patient 18. Using dual color translocation *TEL/AML1* probe uncovered two yellow fused signals for *TEL/AML1*, 4 extra orange signals for *AML1* gene and loss of normal (green) *TEL* allele.**

ent type of leukemia. In concordance with literature a predominance of both, children patients and ALL diagnose was confirmed.

Though, in both groups of patients with ALL and AML various number of *AML1* extra copies was proved, in no one of them a high-level amplification was demonstrated. Gain of *AML1* gene in all cases originated in polysomy of chromosome 21 (usually trisomy or tetrasomy) but one or more copies localized on marker chromosome were frequently found.

Mostly one copy of *AML1* was included in one marker chromosome with extra material added of unknown origin (patient 2, 6, 15), just in patient 13 a duplication of long arm of chromosome 21 with tandem duplication of *AML1* gene and another extra copy of *AML1* in ring chromosome with unidentified material was shown. Similar cases of tandem repetition of 21(q11q22) with or without *AML1* increase were described by others [10, 14, 17, 18, 19]. Alike intrachromosomal amplification by tandem repeat presence of *AML1* copies in size-variable ring chromosomes have been reported in both types of leukemia even though predominately in myeloid malignancies in adults [11, 17, 19]. In contrast with this, our patient with ring chromosome was, exceptionally, 3-year-old girl with *de novo* AML M2.

ANDERSEN et al [5] classified as amplification of *AML1* gene if, per metaphase cell, two and more signals were located on the same chromosome arm or two and more extra single signals of *AML1* were detected on different chromosome arms. Extra copies of *AML1* solely due to gain of whole extra chromosomes 21 were not considered. In PENTHERS et al [4] study of

149 children and adult patients only two patients presented amplification of the *AML1* gene (6–8 copies), both older children. Similar results were proved even by other investigators [10, 20]. On these observations PENTHER et al [4] considered that *AML1* amplification is closely related to childhood ALL rather than to adult once. Unlike we found four extra copies of *AML1* in 30-year-old patient with pre-B ALL. In spite of relatively low incidence (1–3%) of t(12;21) in adult ALL, JABBAR AL-OBAIDY et al [21] in a study of 159 patients described seven *TEL/AML1* fusion positive cases and three of them together with 3–4 extra signals of *AML1*. Extra copies of chromosome 21 with concordant gains of *AML1* they de-

tected in other 6 patients without fusion. Neither JABBAR AL-OBAIDY nor PENTHER et al [4] registered increase of *AML1* signals more than 4. With a dual-color FISH probe for *AML1* and *TEL* two fusion signals and another 4 orange signals for *AML1* were seen in interphase nuclei of our patient. With normal karyotyp 46,XX and without FISH on metaphase was impossible to define type and localization of amplified *AML1* gene.

The resembling gain of *AML1* was observed also in patient 15, with clone of near tetraploid metaphases and complex karyotypes. Besides the 4–6 copies localized in several markers, FISH revealed *C-MYC* amplification cytogenetically presented like double minute chromosomes (dmin). HILGENFELD et al [22] probed recurring involvement of chromosome 21 and amplification of the *MYC* oncogene in AML M2. Albeit dmin represents an infrequent phenomenon in AML and they are found in only about 1% of cases, in their study of 140 patients two of them displayed *C-MYC* amplification together with trisomy of *AML1* gene. Anyway, our patient 15 was not an extraordinary event, however association between gains of both oncogenes stay unclear yet.

Another specific finding in our study was presented in patient 12, a 4-year-old girl with common ALL phenotype and primary trisomy of chromosome 21; FISH analyses confirmed *AML1* extra copy as well as uncovered partial deletion of long arm of chromosome 7. This combination was already observed [23], however, in connection with myeloid malignancies.

In conclusion, *AML1* gene amplification can occur through polysomy of chromosome 21 or through true high-level amplification due to repetition in tandem fashion and/or presence of *AML1* copies on extrachromosomes. We have confirmed that increase of *AML1* most frequently originated from trisomy or tetrasomy of chromosome 21 in both ALL and AML. HARREWOOD et al [17] suggested that cases of high-level amplification should be distinguished from cases of polysomy of chromosome 21. In case of polysomy, implication of other oncogenes located on chromosome 21 can not be excluded. In case of true high-level amplification, *AML1* seems to be only amplified gene. We have not proved higher incidence of high-level amplification but in other side we have observed some noteworthy and uncommon changes of chromosome 21 with correlation of *AML1* gain and either aberrations or age and diagnoses, respectively. Our observations might contribute to define the exact incidence of *AML1* amplifications and their correlation with specific clinical characteristics.

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## References

- [1] GHOZI MC, BERNSTEIN Y, NEGREANU V, LEVANON D, GRONER Y. Expression of the human acute myeloid leukemia gene *AML1* is regulated by two promoter regions. *Proc Natl Acad Sci* 1996; 93: 1935–1940.
- [2] OKUDA T, TAKEDA K, FUJITA Y, NISHIMURA M, YAGYU S et al. Biological Characteristics of the Leukemia-Associated Transcriptional Factor AML1 Disclosed by Hematopoietic Rescue of *AML1*-Deficient Embryonic Stem Cells by Using a Knock-in Strategy. *Mole Cell Biol* 2000; 20: 319–328.
- [3] PERRY CH, ELDOR A, SOREQ H. Runx1/AML1 in leukemia: disrupted association with diverse protein partners. *Leuk Resch* 2002; 26: 221–228.
- [4] PENTHER D, PREUDHOMME C, TALMANT P, ROUMIER C, GODON A et al. Amplification of *AML1* gene is present in childhood acute lymphoblastic leukemia but not in adult, and is not associated with *AML1* gene mutation. *Leukemia* 2002; 16: 1131–1134.
- [5] ANDERSEN MK, CHRISTIANSEN DH, PEDERSEN-BJERGAARD J. Amplification or duplication of chromosome band 21q22 with multiple copies of the *AML1* gene and mutation of the *TP53* gene in therapy-related MDS and AML. *Leukemia* 2005; 19: 197–200.
- [6] HART SM, FORONI L. Core binding factor genes and human leukemia. *Hematologica* 2002; 87: 1307–1323.
- [7] MARTINEZ-RAMIREZ A, URIOSTE M, CONTRA T, CANTALEJO A, TAVARES A et al. Florescence *in situ* hybridization study of TEL/*AML1* fusion and other abnormalities involving TEL and *AML1* genes. Correlation with cytogenetic findings and prognostic value in children with acute lymphoblastic leukemia. *Hematologica* 2001; 86: 1245–1253.
- [8] DESSEN P, HURET JL. Chromosome 21. *Atlas Genet Cytogenet Onkol Haematol* 2002; URL: <http://www.infobiogen.fr/serices/chromcancer/Genes/AML1.html>
- [9] HURET JL, SENON S. *AML1* (acute myeloid leukemia 1). *Atlas Genet Cytogenet Onkol Haematol* 2003; URL: [http://www.infobiogen.fr/serices/chromcancer/Inex/bychrom/id\\_xa\\_21.html](http://www.infobiogen.fr/serices/chromcancer/Inex/bychrom/id_xa_21.html)
- [10] NIINI T, KANERVA J, VETTENRANTA K, SAARINEN-PIHKALA UM, KNUUTILA S. *AML1* gene amplification: a novel finding in childhood acute lymphoblastic leukemia. *Hematologica* 2000; 85: 362–366.
- [11] STREUBEL B, VALENT P, LECHNER K, FONATSCH C. Amplification of the *AML1* (*CBFA2*) gene on ring chromosomes in a patient with acute myeloid leukemia and a constitutional ring chromosome 21. *Canc Genet Cytogenet* 2001; 124: 42–46.
- [12] ROBINSON HM, BROADFIELD ZJ, CHEUNG KL, HAREWOOD L, HARRIS RL et al. Amplification of *AML1* in acute lymphoblastic leukemia is associated with a poor outcome. *Leukemia* 2003; 17: 2249–2250.
- [13] MOREL F, HERRY A, LE BRIS M-J, DOUET-GUILBERT N, LE CALVEZ G et al. *AML1* amplification in a case of childhood acute lymphoblastic leukemia. *Canc Genet Cytogenet* 2002; 137: 142–145.
- [14] FERRO MT, HERNAEZ R, SORDO MT, GARCIA-SAGREDO JM,

- GARCIA-MIGUEL P et al. Chromosome 21 tandem repetition and *AML1* (*RUNX1*) gene amplification. *Canc Genet Cytogenet* 2004; 149: 11–16.
- [15] MIKHAIL FM, SERRY KA, HATEM N, MOURAD ZI, FARAWELA HM et al. *AML1* gene over-expression in childhood acute lymphoblastic leukemia. *Leukemia* 2002; 16: 658–668.
- [16] MITELMAN F, editor. ISCN. An international system for human cytogenetic nomenclature. Basel: Karger, 1995.
- [17] HAREWOOD L, ROBINSON H, HARRIS R, JABBAR AL-OBAIDI M, JALALI GR et al. Amplification of *AML1* on a duplicated chromosome 21 in acute lymphoblastic leukemia: a study of 20 cases. *Leukemia* 2003; 17: 547–553.
- [18] ROBINSON L, ROBSON L, SHARMA P, WATSON N, HERTZBERG. A novel dicentric deleted chromosome 21 arising from tandem translocation. *Canc genet Cytogenet* 2000; 121: 208–211.
- [19] ROUNIER C, FENAUX P, LAFAGE M, IMBERT M, ECLACHE V et al. New mechanisms of *AML1* gene alternation in hematological malignancies. *Leukemia* 2003; 17: 9–16.
- [20] DAL CIN P, ATKINS L, FORD C, ARIYANAYAGAM S, ARMSTRONG SC et al. Amplification of *AML1* in childhood lymphoblastic leukemias. *Gene Chromosom Cancer* 2001; 30: 407–409.
- [21] JABBAR AL-OBAIDI MS, MARTINEAU M, BENNETT CF, FRANKLIN IM, GOLDSTONE AH et al. *EVT6/AML1* fusion by FISH in adult lymphoblastic leukemia. *Leukemia* 2002; 16: 669–674.
- [22] HILGENFELD E, PADILLA-NASH H, MCNEIL N, KNUTSEN T, MONTAGNAC et al. Spectral karyotyping and fluorescence *in situ* hybridization detect a novel chromosomal aberrations, a recurring involvement of chromosome 21 and amplification of the *MYC* oncogene in acute myeloid leukaemia M2. *Br J Haem* 2001; 113: 305–317.
- [23] CHRISTIANSEN DH, ANDERSEN MK, PEDERSEN-BJERGAARD. Mutations of *AML1* are common in therapy-related myelodysplasia following therapy with alkylating agents and are associated with deletion or loss chromosome arm 7q and with subsequent leukemic transformation. *Blood* 2004; 104: 1474–1481.