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Acute Leukemias

AML1 gene amplification: a novel finding in childhood acute lymphoblastic leukemia

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ABSTRACT

Background and Objectives. **We previously found a high-level amplification in chromosomal region 21q22 in two children with acute lymphoblastic leukemia (ALL) using comparative genomic hybridization. The same region harbors the AML1 gene. The aim of the present study was to investigate whether *AML1* is a target gene in these amplifications.**

Design and Methods. **Bone marrow samples were obtained from 112 childhood ALL patients. The copy number of *AML1* was studied using fluorescent *in situ* hybridization with a dual color DNA probe specific for the *AML1* and TEL genes.**

Results. **Three of the patients had 3-to-8 fold amplification of *AML1* and showed a high-level amplification of 21q22 by comparative genomic hybridization. In two of them the extra copies were shown to be located tandemly in a derivative of chromosome 21. Thirty-seven of the patients (33%) had 1-to-2 extra copies of *AML1*, most probably reflecting the incidence of trisomy 21 and tetrasomy 21. The TEL-*AML1* fusion was less frequent in the patients with extra copies of *AML1* (7/40; 18%) than in the patients with no extra copy (24/72; 33%). None of the three patients with 3-to-8 fold amplification of *AML1* showed the fusion or loss of TEL.**

Interpretation and Conclusions. **Our findings suggest that the *AML1* gene is a target gene in the 21q22 amplicon in childhood ALL. To understand the role, if any, of the *AML1* amplification in leukemogenesis, further studies are needed.**
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Key words: *AML1* gene, chromosome 21, amplification, gain, childhood acute lymphoblastic leukemia.

The *AML1* (CBFA2) gene, located in the chromosomal band 21q22, encodes a transcription factor with an aminoterminal DNA binding domain and a carboxyterminal transactivation domain. The *AML1* protein heterodimerizes with CBFβ, forming a complex called human core binding factor (CBF). CBFβ increases the DNA binding affinity of *AML1*. The *AML1*/CBFβ complex binds an enhancer core sequence, TGTGGT, which has been shown to be critical for tissue-specific expression of several hematopoietic-specific genes. *AML1*/CBFβ may function as a transcriptional organizer that recruits tissue-specific factors to form a nucleoprotein complex that stimulates lineage-restricted transcription. The *AML1* gene is a target of many common translocations seen in different types of leukemia, including t(12;21) in childhood acute lymphoblastic leukemia (ALL), t(8;21) in acute myeloid leukemia (AML), and t(3;21) in myelodysplasia and in the blast phase of chronic myeloid leukemia. CBFβ (16q22) is rearranged in AML-associated inversion of chromosome 16.[1,2]

Translocation (12;21)(p13;q22) is the most frequent molecular genetic aberration in childhood ALL, occurring in about 25% of cases.[3-6] The translocation fuses the *AML1* gene with the *TEL* gene, the latter being a member of the ETS family of transcription factors.[7-9]

Recently, mutations of the *AML1* gene have also been found in leukemias. Osato *et al.*[10] reported point mutations resulting in a defective function of *AML1* protein in 5 of 109 cases of AML and in 1 of 8 cases of chronic myeloid leukemia. Song *et al.*[11] showed that haploinsufficiency of the *AML1* gene causes a form of familial platelet disorder with predisposition to AML. They also studied the *AML1* gene in non-familial leukemias and found insertions with predicted loss-of-function in 1 of 15 patients with pediatric ALL and in 1 of 14 patients with myelodysplastic syndrome.[11]

In our previous studies using comparative genomic hybridization (CGH), the chromosomal region 21q22, to which the *AML1* gene is assigned, was found to be amplified.[12,13] In the present study, we performed fluorescent *in situ* hybridization (FISH) analysis on bone marrow from 112 children with ALL to find out whether *AML1* is a target gene for this CGH amplicon.

Design and Methods

Patients

The bone marrow samples were obtained in the years from 1975 to 1999 from 112 children (57 males and 55 females) with ALL, at the time of diagnosis,

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Table 1. Data for the 40 childhood ALL patients with extra copies of the AML1 gene.

Patient No. (sex/age)*	*Lab code*	*Pheno-type*	*WBC*	*# of AML1 copies*	*TEL-AML1-fusion*	*Deletion of TEL*	*Karyotype*	*DNA copy number changes^*
1 (M, 12.0)	971074	pre B	4.3	10-15	-	-	46, XY, del(18)(p11), der(21)	–18p, +21(**q21-qter**)
2 (F, 5.6)	981693	preB	26.3	6	-	-	48, XX, –20, +der(21), +2mar	+10, +14q21-q31, +15q15-qter, –20, **+21**
3 (F, 2.7)	980611	preB	91.1	4-5	-	-	51-54, inc [4] / 46, XX [2]	+4p15-pter, +6p, +14, +17, +18, **+21**, +X
4 (M, 7.0)	LKL 9	-	8.6	4	-	-	-	-
5 (F, 6.4)	LKL 13	-	3	4	-	-	-	-
6 (F, 4.0)	LKL 16	-	3.1	4	-	-	-	-
7 (F, 9.6)	LKL 18	-	8.2	4	-	-	-	-
8 (M, 3.1)	LKL 20	-	9.2	4	-	-	-	-
9 (F, 5.7)	LKL 25	-	4.6	4	-	-	-	-
10 (F, 11.8)	LKL 35	-	2.7	4	-	-	-	-
11 (M, 4.6)	LKL 74	-	12.9	4	-	-	-	-
12 (F, 1.9)	981372	preB	9.7	4	-	-	56, inc [7] / 46, XX [8]	+4, +6, +8, +10, +14, +17, +18, +21, +X
13 (M, 4.8)	981516	preB	22.9	4	-	-	58, inc [5]	+4, +6, +8, +10, +14, +16, +17, **+18q**, –19, +21, +22
14 (F, 4.2)	990663	preB	1.2	4	-	-	62-66, XX, +X, +2,+3,+4,+5,+6, ?+8, +10, +11, +12, +14, +14, +16, +17, +21, +22, +1-6 mar [cp5] / 46,XX [6]	+3p, +6p+, +10q22-qter, +11q+, +12q, +14, +16, +17, +21, +22
15 (F, 4.3)	LKL 5	-	8.6	3-4	-	-	-	-
16 (F, 6.6)	LKL 7	-	8.2	3-4	+	-	-	-
17 (F, 5.4)	LKL 23	-	4.9	3-4	-	-	-	-
18 (M, 4.9)	LKL 28	-	6.3	3-4	-	-	-	-
19 (M, 7.1)	LKL 54	-	38.2	3-4	-	-	-	-
20 (F, 4.1)	LKL 61	-	10.3	3-4	-	-	-	-
21 (F, 2.0)	LKL 85	-	8.1	3-4	-	-	-	-
22 (F, 2.4)	LKL 87	-	7	3-4	-	-	-	-
23 (M, 13.1)°	990710	preB	3.9	3-4	-	-	55, XY, +X, 1q+, +4, +6, +C, +D, +17, +18, +21, +mar [4] / 55, inc [9] / 46, XX [4]	Normal
24 (F, 3.5)	LKL 1	-	4.9	3	-	-	-	-
25 (F, 5.9)	LKL 11	-	6.7	3	+	+	-	-
26 (F, 4.8)	LKL 24	-	8.8	3	-	-	-	-
27 (F, 2.5)	LKL 32	-	24.5	3	-	+	-	-
28 (M, 3.3)	LKL 46	-	53.5	3	+	+	-	-
29 (F, 2.7)	LKL 53	-	34.8	3	-	-	-	-
30 (M, 3.5)	LKL 75	-	7.9	3	-	-	-	-
31 (F, 10.1)	LKL 82	-	105.9	3	-	-	-	-
32 (F, 7.8)	LKL 91	-	7.3	3	+	-	-	-
33 (M, 4.7)	981395	preB	6.1	3	+	-	46, XY [8]	–9p21-pter, +21q22qter
34 (F, 5.0)	990391	preB	6,1	3	-	-	54, XX, 1q+, del(3p), +6, inc [6] / 46, XX [5]	+1q22-qter, +3q21-qter, +4p, +6, +10, +14q23-qter, +21
35 (M, 10.3)	900100	preB	4.9	3	+	+	45-46, +mar, inc [cp3] / 46,XY [3]	+7p15-pter, –11q21-qter, -12p13-pter, +21
36 (M, 14.3)	981832	preB	2.5	3	-	-	46, XY [8]	-
37 (F, 1.8)	950369	preB	99.2	3	+	+	47-48,+2-4 mar,inc[cp5]/46,XX[7]	+10p12-pter, –12p
38 (M, 5.7)	980667	preB	132	3	-	-	46, XX [3]	-
39 (F, 11.7) DS	900320	preB	5.8	3	-	+	44-46, C, +G, inc [cp4] / 47, XX, +21c [3]	+8q22-qter, –9p, –12p, +21
40 (M, 12.6) DS	981500	preB	41.9	3	-	-	47, XY, –14, +mar +21c [12] / 47, XY, +21c [3]	**+8q**, +21

**age at diagnosis in years; °relapse; DS, Down's syndrome; ^+ = gain; – = loss; high-level amplifications in bold print.*

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except for three samples that were taken at relapse. The median age at diagnosis was 6.2 years (range, 0.2-14.9 years). Two patients had Down's syndrome. The patients were diagnosed and treated at Helsinki University Central Hospital, except four patients who were from Kuopio University Hospital. Table 1 shows some clinical and laboratory characteristics of the patients with ATRA copies of the AML1 gene.

Conventional cytogenetic analysis and comparative genomic hybridization

Standard chromosome banding analysis was performed on mitotic bone marrow cells after short-term culture.[14] CGH was performed as described by El-Rifai *et al.*[15] The karyotype and CGH results were retrieved from the clinical records for the patients for whom these results were available.

Fluorescent in situ hybridization (FISH)

The FISH analyses were performed on 92 smear preparations and 20 fresh preparations. Two fresh and two smear preparations, each from different healthy individuals, were used as controls. We used spectrum green- and spectrum orange-conjugated dual color DNA probes specific for the *TEL* and *AML1* genes. Hybridization and washes were performed according to the supplier's instructions. Smear preparations were pre-treated before hybridization. Briefly, the coverslips were removed using xylene and the slides were dehydrated in alcohol series (70%, 85%, and 100%). Then the slides were fixed in methanol/acetic acid fixative (3:1) at +4°C overnight followed by treatment in 1 M Na-thiocyanate at +65°C for 10 minutes and washing in 2XSSC at room temperature for 5 minutes. Next the preparations were treated in 0.01N HCl at +37°C for 10 minutes and moved into 0.05N HCl with pepsin (0.05 mg/mL) at +37°C for 8 minutes. Finally the slides were washed under cold running tap water for 5 minutes and dehydrated in alcohol series (70%, 85%, and 100%).

Analysis of FISH

The hybridizations were analyzed from images acquired by an Olympus fluorescence microscope and the ISIS digital image analysis system (Metasystems, Altlussheim, Germany) based on an integrated high-sensitivity monochrome charge-coupled device (CCD) camera and automated CGH analysis software. Three-color images were acquired using three filters from Chroma (Chroma Technology Corp, Brattleboro, VT, USA), specific to FITC, Texas-Red, and DAPI. At least 50 interphase cells were analyzed for each patient. From the controls 200 interphase cells with two signals of the *TEL* gene were analyzed. At least 20 metaphases with increased copy number of *AML1* were analyzed from all three patients with more than four copies of *AML1* and from three patients with 3 or 4 copies.

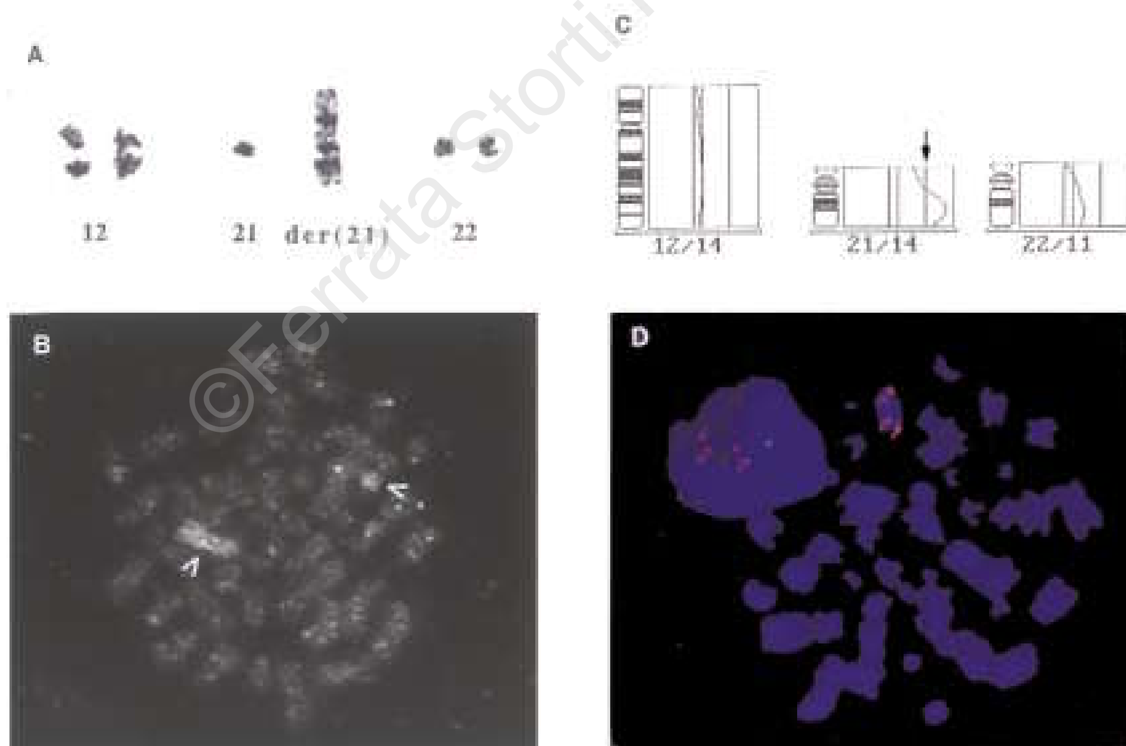

Figure 1. Images depicting the results of different detection methods for patient #1 with 10-15 copies of the AML1 gene. A: G-banding. B: Painting with a chromosome--21 specific probe. In addition to the normal chromosome 21, the whole der(21) is painted. C: Comparative genomic hybridization profiles of chromosomes 12, 21, and 22. The high-level amplification threshold (1.5) is exceeded in chromosome 21 (arrow). D: Double-color FISH with probes specific for AML1 (red) and TEL (green), an interphase cell and a metaphase cell. At least 10 signals of the AML1 gene can be seen in the interphase cell. The metaphase cell shows that the extra copies are situated in the der(21) chromosome.

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Chromosome painting

Chromosome painting using fluorescein isothiocyanate (FITC)-conjugated DNA probes specific to chromosome 21 (Cambio, Cambridge, UK) was performed for three patients with more than four *AML1* copies to detect the chromosomal regions derived from chromosome 21.

Results

Control preparations

In the two fresh control preparations, the 200 analyzed interphase cells with two *TEL* signals gave no extra *AML1* signals. In one control smear preparation, an extra signal of *AML1* was detected in one of the 200 cells.

Patient samples

The number of patients with an extra *AML1* signal in less than 0.5% of the cells analyzed was 72 (64%). These patients were interpreted to be *AML1* amplification negative. Forty of the 112 patients (36%) had at least one extra copy of the *AML1* gene (Table 1). Two of them (patients #39 and 40; 5%) were Down's syndrome patients with one extra copy of *AML1* due to the constitutional 21 trisomy. In five patients (patients #25, 31, 36, 37, and 38) extra copies were seen in less than 50% of the interphase cells (range 14-41%) and in all other patients in 50-100% of the interphase cells. Two patients (patients #1 and 2; 5%) showed a high-level amplification of *AML1* in most cells, with at least 10-15 copies (Figure 1D) and six copies of the gene, respectively. One patient (patient #3; 2.5%) had four copies in most of the cells but at least five copies in 6% of the cells. Twenty of the 40 patients (50%) had four copies of the gene in at least 40% of the aberrant cells. Seventeen patients (43%), including the two patients with Down's syndrome, showed three copies of the gene.

Thirty-one of the 112 patients (28%) showed the *TEL-AML1* fusion. The fusion was found in seven patients (18%)(Table 1) with increased copy number of *AML1* and in 24 of the 72 (33%) with no extra *AML1* copies. None of the three patients with more than four copies of *AML1* (patients 1-3) showed the fusion. Also both of the Down's syndrome patients were fusion-negative. Six of the 31 patients with the fusion had two copies of the fusion gene in 6-92% of the fusion-positive cells. One fusion-positive patient showed no cells with one copy of the fusion gene but 2 to 4 copies were found in all of the cells (2 in 63%; 3 in 28%, and 4 in 8%).

The other *TEL* allele was deleted in 19 of the 31 fusion-positive patients (61%) but only in three of the 81 fusion-negative patients (4%). Two (patients 27 and 39; Table 1) of the three fusion-negative patients with the loss of *TEL* also showed an extra *AML1* gene. One of these two (patient #39) had Down's syndrome. No loss of the *AML1* gene was detected in any of the patients studied.

The *AML1* signals were also studied from metaphases in three of the patients with 3 to 4 *AML1* copies (patients #14, 23, and 34). In all three patients the copies were situated in separate chromosomes, possibly in chromosome 21. In four of the 12 patients with 3 to 4 *AML1* copies and for whom karyotype was available, the G-banding method revealed an extra chromosome 21 (patients #14, 23, 39, and 40) (Table 1). Two of them had Down's syndrome (patients #39 and 40).

In two patients with high-level amplification of *AML1*, the extra gene copies were located in a derivative of chromosome 21 [der(21)]. In the patient with 10 to 15 copies of *AML1* (patient #1), the extra copies were tandemly placed in two sites of a big metacentric chromosome (Figure 1A and 1D). In the patient with six copies (patient #2), the four extra copies were situated as tandem duplicates in two sites of a big acrocentric chromosome. In patient #3 with 4 to 5 *AML1* copies the karyotype was incomplete with 51 to 54 chromosomes. In this patient, both in metaphases with four and five copies, all the copies were seen to be located in different chromosomes. However, the morphology of the metaphases with five copies was too poor to identify the type and size of the chromosomes. Chromosome painting analyses showed the whole der(21) in patient #1 (Figure 1B) and four non-continuous areas of der(21) in patient #2 to originate from chromosome 21. Patient #3 was shown to have at most four copies of chromosome 21, whereas no other region was painted in the 60 metaphases studied.

CGH results were available for 26 of the 112 patients, and 11 of the 26 showed a gain at chromosome 21. These 11 patients with the gain also showed extra copies of the *AML1* gene (Table 1). The limit of high-level amplification was exceeded in three patients, in patient #1 with 10 to 15 copies (Figure 1C), in patient #2 with six copies, and in patient #3 with 4 to 5 copies of the *AML1* gene. In two patients with 1 to 2 extra copies of *AML1* (patients #23 and 37), CGH failed to reveal any gain in chromosome 21, probably because the proportion of the malignant clone in the sample was too low for the method to detect.

Discussion

We report here a novel finding of a high-level amplification of the *AML1* gene in childhood ALL. No gene amplification has been reported in childhood ALL so far.

Three of our patients (patients #1-3) had more than four copies of the *AML1* gene and showed a high-level amplification of 21q22 by CGH. In two of them (patients #1 and 2) with 10 to 15 and six *AML1* signals, respectively, the extra copies were located tandemly in two sites of a derivative chromosome 21. Thus, the increase in copy number of *AML1* in these patients had occurred through intrachromosomal amplification. Because the amplicons were in two different positions, a region with tandem repeats of the gene was probably duplicated after the primary amplification.

Thirty-seven out of the 112 patients (33%) showed one or two extra copies of *AML1*. In these patients, the most evident origin of the gain was polysomy of chromosome 21. The high incidence of trisomy 21 and tetrasomy 21 in the standard cytogenetic studies fits with this suggestion.[16]

No patients with more than four *AML1* copies (patients #1-3) had *TEL-AML1* gene fusion and the frequency of the fusion in the patients with 3-4 *AML1* copies was lower than in those with two copies. This agrees with the results of Raimondi *et al.*[17] who reported that childhood ALL patients with 12p abnormalities and the *TEL-AML1* fusion have a much lower frequency of hyperdiploidy (51+) than the ALL population in general. Our finding is also supported by Lanza *et al.*[18] who studied 11 childhood ALL patients with Down's syndrome and showed the absence of *TEL-AML1* fusion in all of them.

To conclude, our results suggest that the *AML1* gene is a target gene in the 21q CGH amplicon. Before we can understand the role of the amplification of the *AML1* gene in the leukemogenesis of ALL, further studies are needed to investigate whether the amplified *AML1* gene is mutated and whether other co-amplified gene(s) reside in the 21q amplicon.

Contributions and Acknowledgments

SK was responsible for the conception of the study, its design, ethical approval, funding, direct supervision, and recruitment of and contact with the participants. TN performed FISH analysis and wrote the paper. The others took part in the study design. The criteria applied for the order of the authors was the degree of their contribution to the study. The last two authors are senior investigators.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Potential Implications for clinical practice

- AML1 gene amplification is a genetic marker for leukemia follow-up.

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