RUNX1 truncation resulting from a cryptic and novel t(6;21)(q25;q22) chromosome translocation in acute myeloid leukemia: A case report

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Abstract. Fluorescence *in situ* hybridization examination of a pediatric AML patient whose bone marrow cells carried trisomy 4 and FLT3-ITD mutation, demonstrated that part of the *RUNX1* probe had unexpectedly moved to chromosome band 6q25 indicating a cryptic t(6;21)(q25;q22) translocation. RNA sequencing showed fusion of exon 7 of *RUNX1* with an intergenic sequence of 6q25 close to the MIR1202 locus, something that was verified by RT-PCR together with Sanger sequencing. The *RUNX1* fusion transcript encodes a truncated protein containing the Runt homology domain responsible for both heterodimerization with CBFB and DNA binding, but lacking the proline-, serine-, and threonine-rich (PST) region which is the transcription activation domain at the C terminal end. Which genetic event (+4, FLT3-ITD, t(6;21)-RUNX1 truncation or other, undetected acquired changes) was more pathogenetically important in the present case of AML, remains unknown. The case illustrates that submicroscopic chromosomal rearrangements may accompany visible numerical changes and perhaps should be actively looked for whenever a single trisomy is found. An active search for them may provide both pathogenetic and prognostic novel information.

Introduction

Cancer is now accepted to be a genetic disease in the sense that it arises due to acquired genetic abnormalities in susceptible somatic cells (1). Microscopic studies of cancer cells have shown that these aberrations are often visible as balanced chromosomal changes, such as translocations and inversions, as well as unbalanced anomalies, such as deletions, monosomies, duplications, and trisomies (1). Many hematologic malignancies, including acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), are characterized by the presence of acquired chromosome translocations and inversions resulting in chimeric genes of pathogenetic, diagnostic, and prognostic importance (1). Whereas some genes, e.g., *ABL, BCR, RUNX1T1*, and *PML*, have only been reported involved in one or a few translocations, other genes are promiscuous, having numerous fusion partners in various translocations and even in different types of malignancy suggesting that the pathogenetic and phenotypic impact of the chimeras is dependent on both genes participating in the fusion (1).

One such gene is *RUNX1* at 21q22 (2) which codes for the alpha subunit of the heterodimeric transcription factor named core binding factor (CBF) that binds to the core element of many enhancers and promoters. To date, *RUNX1* (previously called *AML1, CBFA2, PEBP2aB*) has been shown in both myeloid and lymphoblastic acute leukemias to fuse with more than 30 different partner genes encoding a heterogeneous group of structurally diverse proteins (1). Recently, *RUNX1* fusions were also found in adenocarcinoma of breast and lung as well as in squamous cell carcinoma of the oral cavity (3). Some of the fusions are common, such as *ETV6-RUNX1* [t(12;21)(p13;q22)] in pre-B-ALL, *RUNX1-RUNX1T1* [t(8;21)(q22;q22)] in AML, and *RUNX1/MECOM* [t(3;21)(q26;q22)] in myelodysplasia (MDS), AML, and chronic myeloid leukemia in blastic phase, whereas others have been reported in single cases, i.e., they have not yet been shown to be recurrent (2,4). The prognostic impact of the common *RUNX1* fusions is well known (5-8). Corresponding knowledge for the infrequent *RUNX1* chimeras is lacking (9).

Acquired point mutations distributed throughout *RUNX1* are also frequently found in both *de novo* and secondary (therapy-related) MDS/AML (10,11). They are not found

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together with *RUNX1* chromosomal translocations or complex abnormal karyotypes, and they are associated with poor outcome in MDS (12-16). The mutation spectrum includes missense, nonsense, frameshift, in-frame insertion/deletion mutations, as well as exon-skipping mutations (15). Nonsense mutations in *RUNX1* account for 11% of the total and generate a repertoire of truncated RUNX1 proteins which to varying degree show lack of the C-terminal region. Most of them affect the transactivation domain (15).

Although less frequent, truncated RUNX1 proteins can also be the result of a chromosomal translocation which generates a premature stop codon in the *RUNX1* open reading frame, leading to expression of C-terminal truncated forms. These chromosome translocations can be divided into two categories: in the first, the translocations produce only out-of-frame fusion transcripts (17-25) whereas, in the second category, they generate both in-frame and out-of-frame fusion transcripts (26-31).

The generation of C-terminally truncated RUNX1 proteins via different mechanisms suggests that their expression is important in leukemogenesis. Truncated RUNX1 protein was shown to reduce the transactivation capacity of CBF on specific myeloid promoters that function as inhibitors of normal RUNX1 (18-20). Recently, the truncated RUNX1 protein resulting from the t(1;21)(p32;q22) chromosomal translocation was shown to impair proliferation and differentiation of human hematopoietic progenitors (25).

Since acute leukemia treatment protocols are in part based on the presence of certain genetic changes, it is of clinical interest to obtain more information also about rare *RUNX1* fusions, even in disease subgroups that so far cannot be treated with medications specifically directed against the leukemogenic defect. It is important to underscore that this may be the case also for infrequent pathogenetic mechanisms where information is gathered by the addition of single case reports, as recently exemplified by the story of the rare *RUNX1-USP42* fusion and 5q deletion in AML (9,32-35).

For this reason, we here present the molecular genetic and clinical features of a case of AML with a cryptic t(6;21)(q25;q22) which resulted in the generation of a truncated RUNX1.

Patient and methods

Ethics statement. The study was approved by the regional ethics committee (Regional komité for medisinsk forskningsetikk Sør-Øst, Norge, http://helseforskning.etikkom.no), and written informed consent was obtained from the patient's parents to publication of the case details. The ethics committee's approval included a review of the consent procedure. All patient information has been de-identified.

Case report. A 7-year-old girl was admitted to the Children's Hospital because of petechiae. Prior to admission she had a one week history of fever, throat and abdominal pain and had been prescribed antibiotics on the suspicion of tonsillitis. On clinical examination, the girl was pale and had petechiae on the extremities and trunk, as well as a few hematomas on the legs. The peripheral blood values were hemoglobin 88 g/l, leukocytes 369.0x10^9/l, platelets 59x10^9/l, lactate dehydrogenase 1886 U/L, and C-reactive protein 71 mg/l. She had continuous epistaxis despite sustained platelet counts of 60x10^9 cells/l, normal international normalized ratio (INR), and activated partial thromboplastin time (APTT). There was no central nervous system involvement. Leucocytes gradually increased to 480.0x10^9 cells/l before start of the treatment.

Morphology and immunophenotypic findings were in keeping with the diagnosis acute myeloid leukemia with minimal differentiation (AML M0). Normal hematopoiesis was completely replaced by large blasts without conspicuous granulation or Auer rods and with lacy chromatin and prominent nucleoli. The blasts were positive for CD34, CD71, CD117, CD123, HLA-DR antigens, and the common myeloid markers CD13, CD33, and CD15. Less than 10% of the blasts were positive for cytoplasmic myeloperoxidase. Of interest, partial expression of Tdt and aberrant expression of CD7 and CD9 were demonstrated. The blasts were negative for B-cell, T/NK-cell as well as for monocytic, erythroid, and megakaryocytic lineage markers.

The bone marrow karyotype was 47,XX,+4[15] (see below). In addition, a FLT3 ITD mutation was detected, but no mutations in the nucleophosmin 1 gene. Upon induction treatment according to the NOPHO-AML 2004 protocol (NOPHO: Nordic Pediatric Hematology and Oncology) (36), morphologic remission (<5% blasts) was obtained. Due to the presence of a FLT3-ITD mutation, the patient became eligible for allogeneic stem cell transplantation (SCT). However, because a suitable donor was not found, consolidation therapy was completed with chemotherapy only. Four months after completed therapy, the patient had a bone marrow relapse. She went into a second remission on a clofarabin-based regimen and was transplanted with stem cells from her 7-month-old matching sibling. Unfortunately, she relapsed again 6 months after SCT and died one month later.

G-banding analysis. Bone marrow cells were cytogenetically investigated by standard methods. Chromosome preparations were made from metaphase cells of a 24-h culture, G-banded using Leishman stain, and karyotyped according to the ISCN 2009 guidelines (37).

Fluorescence in situ hybridization (FISH). As part of our standard cytogenetic diagnosis, initial interphase FISH analyses of bone marrow cells were performed with the Cytocell multiprobe ALL panel (Cytocell, http://www.cytocell.co.uk/) looking for *MYC* rearrangements, *CDKN2A* (P16) deletion, *TCF3 (E2A)* rearrangements, *ETV6-RUNX1* fusion, hyperdiploidy, *MLL* rearrangements, *BCR-ABL1* fusion, and *IGH* rearrangements. On the basis of findings made using the above panel, further FISH was performed on metaphase spreads and interphase nuclei using the Vysis LSI TEL/AML1 ES Dual Color Translocation Probe (Abbott Molecular, http://www.abbottmolecular.com) . This is a mixture of the LSI TEL probe labeled with SpectrumGreen and the LSI AML1 probe labeled with SpectrumOrange. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems, Newcastle, UK).

RNA sequencing. Total RNA (3 μg) extracted from the patient's bone marrow at the time of diagnosis was sent to the Norwegian Sequencing Centre at Ullevål Hospital (http://

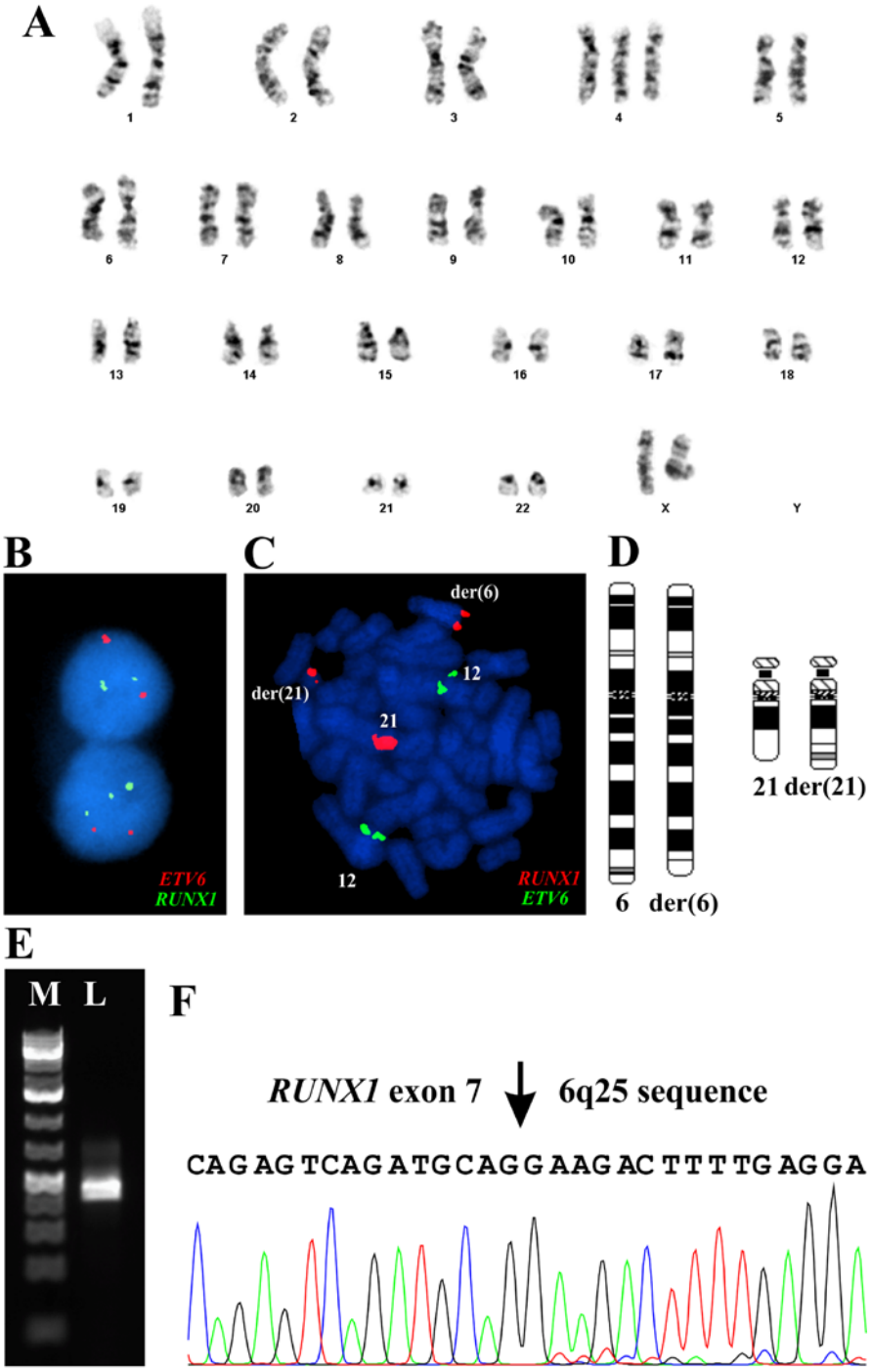

Figure 1. G-banding, FISH and RT-PCR analyses at diagnosis. (A) The G-banding analysis showed trisomy 4 in all 15 cells examined. (B) The *ETV6-RUNX1* probe showed abnormal signals with splitting of the *RUNX1* (green signal) probe in 203 out of 233 interphase nuclei. The red signal is the *ETV6* probe. The results were obtained with the Cytocell multiprobe ALL panel (Cytocell, http://www.cytocell.co.uk/). (C) Metaphase cell in which part of the *RUNX1* probe (red signal) was unexpectedly seen to be located on the distal part of 6q. The green signal is the *ETV6* probe. The results were obtained with the Vysis LSI TEL/AML1 ES Dual Color Translocation Probe. (D) Ideograms showing the der(6)t(6;21)(q25;q22) and the der(21)t(6;21)(q25;q22) together with the corresponding normal chromosome homologs. (E) Amplification of a cDNA fragment using the primers RUNX1-809N-F1 and 6q25-R1 from the bone marrow of the patient (L). M, 1 kb DNA ladder (GeneRuler, ThermoFisher). (F) Partial sequence chromatogram of the cDNA fragment showing the fusion (arrow) of the *RUNX1* gene with a sequence from 6q25.

www.sequencing.uio.no/) for high-throughput paired-end RNA-sequencing. The Illumina software pipeline was used to process image data into raw sequencing data. Only sequence reads marked as 'passed filtering' were used in the downstream data analysis. A total of 103 million reads were obtained. The FASTQC software was used for quality control of the raw sequence data (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The software deFuse was used for the discovery of fusion transcripts (38) (http://compbio.bccrc.ca/software/defuse/).

In addition, the 'grep' command (http://en.wikipedia.org/wiki/Grep) was used to search the fastq files of the sequence

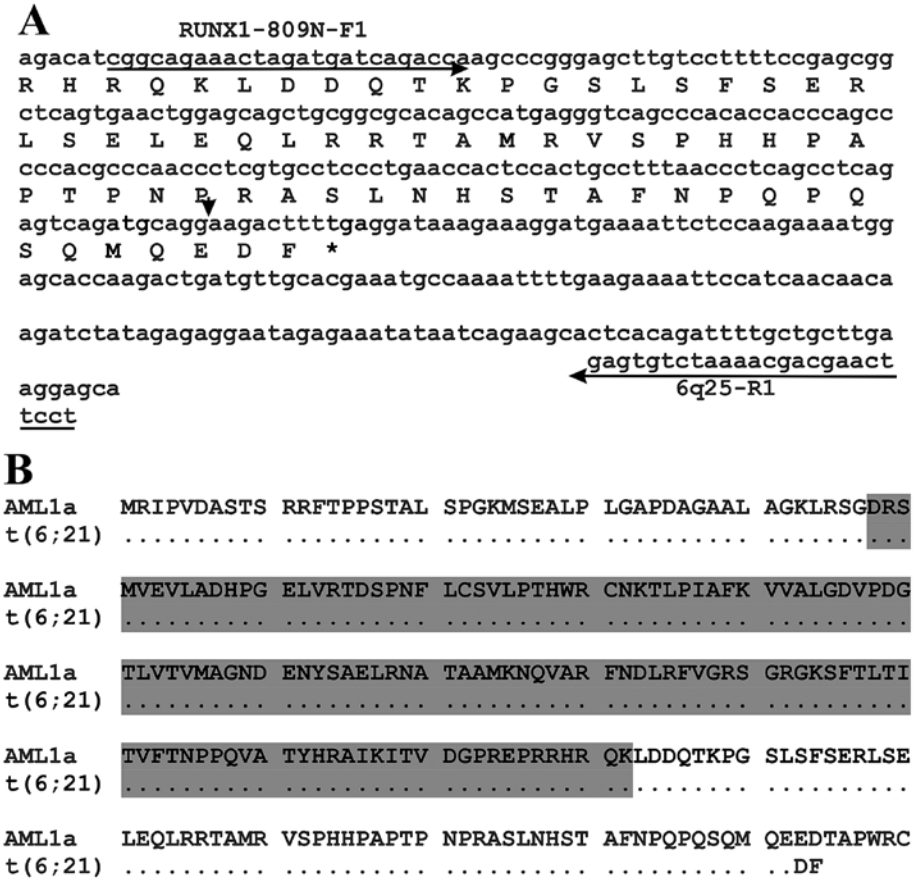

Figure 2. The fusion of *RUNX1* with an intergenic sequence from 6q25 resulting in a putative RUNX1 truncated protein. (A) The sequence of the amplified cDNA fragment from Fig. 1E. The primers are shown by horizontal arrows. Vertical arrow indicates the fusion point. The coding sequence is shown with capital letters. The * corresponds to the stop codon 'tga'. (B) Alignment showing the known runt-related transcription factor 1 isoform AML1a (accession number: NP_001116079 version 1) with the putative RUNX1 protein resulting from the t(6;21)(q25;q22) chromosome aberration in the AML patient. The Runt domain (pfam00853) is the region between 48-182 amino acids. The dots indicate identical amino acids.

data (http://en.wikipedia.org/wiki/FASTQ_format) for *RUNX1* fusion sequences (NM_001754 version 4). To confirm the *RUNX1* fusion identified by the deFuse program (see below), the 'expression' used was 'CAGATGCAGGAAGACTTTTG' which is a sequence of 20 nucleotides (nt) at the fusion point: 10 bases upstream (5'-end of *RUNX1* gene, CAGATGCAGG), and 10 bases downstream from the junction (3'-end of the 6q25 intergenic sequence, AAGACTTTTG). The sequences obtained by 'grep' were blasted against the human genomic plus transcript database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) as well as the reference sequences NM_001754 version 4 *(RUNX1)* and NC_000006.12 (chromosome 6).

PCR analysis. For reverse transcriptase-Polymerase Chain Reaction (RT-PCR), 1 μg of total RNA was reverse-transcribed in a 20 μl reaction volume using iScript Advanced cDNA Synthesis kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad Laboratories, Oslo, Norway). The cDNA was diluted to 50 μl of which 1 μl was used as templates in subsequent PCR assays. The 25 μl PCR volume contained 12.5 μl Premix Ex Taq™ DNA Polymerase Hot Start Version (Takara Bio, AH diagnostics, Oslo, Norway), cDNA, and 0.4 μM of each of the forward and reverse primers. For detection of the *RUNX1* fusion transcript, the forward RUNX1-809N-F1 (CGG CAG AAA CTA GAT GAT CAG ACC A) and reverse 6q25-R1 (TCC TTC AAG CAG CAA AAT CTG TGA G) primers were used. The PCR was run on a C-1000 Thermal cycler (Bio-Rad) with an initial denaturation at 94˚C for 30 sec, followed by 35 cycles of 7 sec at 98˚C, 30 sec at 60˚C, 1 min at 72˚C, and a final extension for 5 min at 72˚C. PCR products (3 μl) were stained with GelRed (Biotium, Hayward, CA, USA), analyzed by electrophoresis through 1.0% agarose gel, and photographed. DNA gel electrophoresis was performed using lithium borate buffer (39). The remaining PCR products were purified using the GeneJET PCR Purification kit (Thermo Fisher Scientific, Oslo, Norway) and sequenced at GATC Biotech (Germany, http://www.gatc-biotech.com/en/home.html). The BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for computer analysis of sequence data.

Results

Cytogenetics. The G-banding analysis at diagnosis showed trisomy 4 in all 15 cells analyzed (Fig. 1A). The *ETV6-RUNX1* probe showed abnormal signals with splitting of the *RUNX1* probe in 203 out of 233 interphase nuclei examined in spite of no cytogenetically visible rearrangement of chromosome arm 21q (Fig. 1B). In the same experiment, 10 metaphase cells were examined in which part of the *RUNX1* probe

Table I. Sequences, obtained with the grep command using the expression '**CAGATGCAGG**AAGACTTTTG', which show the fusion of exon 7 of *RUNX1* (NM_001754.4) with sequence from chromosome band 6q25.

Sequence	NM_001754.4 BP	NC_000006.12
CTTTAACCCTCAGCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCTCCAAGAAAATGGAGCACCAAGACTGATGTTGCAC	996	155903358
CTCGTGCCTCCCTGAACCACTCCACTGCCTTTAACCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCTCCAAGA	996	155903358
CTCGTGCCTCCCTGAACCACTCCACTGCCTTTAACCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCTCCAAGA	**996**	155903358
CAGCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAATTCTCCAAGAAAATGGAGCACCAAGACTGATGTTGCACGAAAATGCCAA	**996**	155903358
CAGCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCTCCAAGAAAATGGAGCACCAAGACTGATGTTGCACAGATCGGAAG	996	155903358
CTCAGCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCTCCAAGAAAATGGAGCACCAAGACTGATGTTGCACGAAATGCA	996	155903358
CCAACCCTCGTGCCTCCCTGAACCACTCCACTGCCTTTAACCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCT	996	155903358
CTCGTGCCTCCCTGAACCACTCCACTGCCTTTAACCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAAAGGATGAAAATTCTCAGATC	996	155903358
CCCAGCCCCCACGCCCAACCCTCGTGCCTCCCTGAACCACTCCACTGCCTTTAACCCTCAGAGTCAGATGCAGGAAGACTTTTGAGGATAAAGAA	996	155903358

RUNX1 sequences are shown in bold.

was unexpectedly seen to be located on the distal part of 6q (Fig. 1C). The data showed a novel cryptic t(6;21)(q25-27;q22) chromosome translocation (Fig. 1D). Other FISH analyses detected no rearrangements of *MYC, TCF3, MLL*, and *IGH*, no *CDKN2A* (P16) deletion, no hyperdiploidy, and none of the fusions *ETV6-RUNX1* and *BCR-ABL1*. Therefore, the whole karyotype was: 47,XX,+4[15].nuc ish(ETV6x2,AML1x3)[209/233].ish t(6;21)(q25-27;q22)(AML1+;AML1+)[10] (Fig. 1A-D).

Analysis of RNA-sequencing with deFuse. Using deFuse on the raw sequencing data, 39 potential fusion transcripts were found (data not shown), among them a fusion between *RUNX1* and a sequence mapping close to the MIR1202 locus which corresponds well to the 6q breakpoint of the t(6;21)(q25-27;q22) suggested by combined G-banding and FISH. In order to verify the fusion obtained with the deFuse software, we used the 'grep' command utility to search for expressions composed of 10 nt of *RUNX1* and 10 nt of 6q25 upstream and downstream of the fusion point (Table I). Using the expression 'CAGATGCAGGAAGACTTTTG', 9 sequences were retrieved which corresponded to the fusion *RUNX1*-transcript found by defuse (Table I).

Molecular confirmation of the RUNX1-fusions. PCR with the RUNX1-809N-F1/6q25-R1 primer combination amplified a 358 bp cDNA fragment (Fig. 1E). Direct sequencing of the amplified fragment verified the presence of the *RUNX1*-fusion transcript. The fusion point was identical to that found with deFuse (Fig. 1F). Therefore, the final karyotype after G-banding, FISH, and molecular examination could be written 47,XX,+4,t(6;21)(q25;q22)[10] (Fig. 1A and D).

Discussion

We present herein a case of childhood AML in which the leukemic cells had trisomy 4, a novel cryptic t(6;21)(q25;q22) chromosome translocation, and FLT3-ITD mutation. The molecular analysis of the translocation showed fusion of the *RUNX1* gene with an intergenic sequence from 6q25 resulting in a putative RUNX1 truncated protein (Fig. 2A and B). The predicted truncated protein would contain the Runt homology domain (RHD) which is responsible for both heterodimerization with CBFB and DNA binding (40). Functionally, the truncated RUNX1 would be similar to the isoform AML1a of the RUNX1 protein (Fig. 2B, protein with accession number NP_001116079) (41-43). The isoform AML1a is a 250 amino acid RUNX1 protein which contains the RHD but lacks the proline-, serine-, and threonine-rich (PST) region which is the transcriptional activation domain at the C terminal end (41-43). AML1a does not itself have any transactivation function, but it inhibits the transcriptional activity of AML1b by competing for the DNA sequence of target genes with higher affinity (43). Overexpression of AML1a was shown to suppress granulocytic differentiation and to stimulate cell proliferation in 32Dcl3 murine myeloid cells treated with granulocyte colony-stimulating factor (43). AML1a was found to inhibit erythroid differentiation induced by sodium butyrate and enhance the megakaryocytic differentiation of K562 leukemia cells (44). AML1a also enhanced hematopoietic lineage commitment

from human embryonic stem cells and inducible pluripotent stem cells (45). AML1a was reported to be highly abundant in the primitive stem/progenitor compartment of human cord blood, and forced expression of AML1a in these cells enhanced maintenance of primitive potential both *in vitro* and *in vivo* (46). Overexpression of AML1a was reported in patients with acute lymphoblastic leukemia and AML-M2 patients (47). In the same study, AML1a was found to repress transcription of promoter of macrophage colony-stimulating factor receptor mediated by AML1b (47). When murine bone marrow mononuclear cells were transduced with AML1a and then transplanted into lethally irradiated mice, the mice developed lymphoblastic leukemia after transplantation (47). Thus, AML1a seems to be an important contributing factor to leukemogenesis.

Truncated RUNX1 proteins generated by chromosomal translocations were shown to have functions similar to those of the AML1a isoform. In a patient with secondary AML carrying a t(19;21)(q13;q22), *RUNX1* was fused out-of-frame to chromosome 19 sequences resulting in a truncated AML protein bearing the DNA binding domain but not the transcriptional activation domain. The fusion AML1 protein functioned as an inhibitor of the normal RUNX1 protein (19). The *RUNX1-RPL22P1* (also known as *AML1-EAP*) fusion gene which is the result of the t(3;21)(q26;q22) chromosome translocation in AML, codes for a truncated RUNX1 protein which acts as an inhibitor of AML1b (17,18). The fusion of *RUNX1* to *CPNE8* in an AML with t(12;21)(q12;q22) also resulted in a truncated inhibitory RUNX1 protein (20). Recently, *in vitro* analysis of transduced human hematopoietic/progenitor stem cells showed that truncated RUNX1 proteins generated by a t(1;21)(p32;q22) chromosomal translocation increased proliferation and self-renewal and disrupted the differentiation program by interfering with AML1b (25). In a mouse model, truncated RUNX1 protein resulting from a point mutation induced pancytopenia with erythroid dysplasia, followed by progression to MDS-RAEB or MDS/AML (48). Dowdy *et al* studied the RUNX1 C-terminus in a mouse model by introducing a premature translational stop codon after amino acid 307 ($Runx1^{Q307X}$) which mimicked RUNX1 mutations found in MDS/AML and CMML patients (49). They found that $Runx1^{Q307X}$ homozygous mice exhibited embryonic lethality at E12.5 due to central nervous system hemorrhage and a complete lack of hematopoietic stem cell function (49). They also showed that while the RUNX1 truncated protein was capable of binding to DNA, it was unable to associate with the nuclear matrix and failed to activate target gene promoters (49).

Taking all the above-mentioned data into consideration, it appears that the truncated RUNX1 protein (or absence from it of the C terminal part which contains subnuclear targeting and transactivation domains) is at least a contributing factor in leukemogenesis.

The patient described here also had, apart from the t(6;21)-*RUNX1* rearrangement, trisomy 4 and FLT3-ITD mutation. The molecular genetic consequences of trisomy 4 are, as for numerical chromosome changes in general, unknown. Possible mechanisms could be global gene expression alterations because of gene dosage effect generated by the trisomy and duplication of any rearranged or mutated genes on chromosome 4. The prognosis for AML-patients with trisomy 4 is unclear, but based on a review of 30 such patients, Gupta *et al* (50) concluded that the outcome is poor compared to that of other cytogenetic subsets within the intermediate risk group. More importantly, a recent international collaborative study on pediatric t(8;21)-AML showed that gain of chromosome 4 in addition to t(8;21) represents a prognostically unfavorable feature (51).

FLT3-ITD mutation has been shown to be a prognostic factor although its impact has to be interpreted against the overall genetic background of the leukemic cells (52). In adult patients with a normal karyotype, FLT3-ITD is associated with poor prognosis (53,54). In core-binding factor (CBF) AML, higher mutant levels of FLT3-ITD were an adverse factor for overall survival (55). However, a recent report on adult patients with CBF AML stated that MRD levels, rather than the FLT3-ITD mutations, were significant prognostic markers for outcome (56). In pediatric patients, FLT3 mutations have been associated with poor prognosis (57,58). Reports on the significance of FLT3 mutations in pediatric CBF AML are lacking.

All in all, we cannot say which genetic event (+4, FLT3-ITD, or t(6;21)-RUNX1 truncation) was more pathogenetically or prognostically important. The case nevertheless illustrates that submicroscopic chromosomal rearrangements may accompany visible numerical changes and perhaps should be actively sought for whenever a single trisomy is found. To what extent and at which frequency such submicroscopic changes target the *RUNX1* gene remains unknown. An active search for them may provide both pathogenetic and prognostic novel information in the future.

Acknowledgements

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References

1. Heim S and Mitelman F: Cancer Cytogenetics: Chromosomal and Molecular Genetic Abberations of Tumor Cells, Forth Edition. Wiley-Blackwell, 2015. http://dx.doi.org/10.1002/9781118795569.
2. De Braekeleer E, Douet-Guilbert N, Morel F, Le Bris MJ, Férec C and De Braekeleer M: RUNX1 translocations and fusion genes in malignant hemopathies. Future Oncol 7: 77-91, 2011.
3. Yoshihara K, Wang Q, Torres-Garcia W, Zheng S, Vegesna R, Kim H and Verhaak RG: The landscape and therapeutic relevance of cancer-associated transcript fusions. Oncogene 34: 4845-4854, 2015.
4. Abe A, Katsumi A, Kobayashi M, Okamoto A, Tokuda M, Kanie T, Yamamoto Y, Naoe T and Emi N: A novel RUNX1-C11orf41 fusion gene in a case of acute myeloid leukemia with a t(11;21)(p14;q22). Cancer Genet 205: 608-611, 2012.
5. Bhojwani D, Pei D, Sandlund JT, Jeha S, Ribeiro RC, Rubnitz JE, Raimondi SC, Shurtleff S, Onciu M, Cheng C, *et al*: ETV6-RUNX1-positive childhood acute lymphoblastic leukemia: Improved outcome with contemporary therapy. Leukemia 26: 265-270, 2012.
6. Cho EK, Bang SM, Ahn JY, Yoo SM, Park PW, Seo YH, Shin DB and Lee JH: Prognostic value of AML 1/ETO fusion transcripts in patients with acute myelogenous leukemia. Korean J Intern Med 18: 13-20, 2003.
7. Gandemer V, Chevret S, Petit A, Vermylen C, Leblanc T, Michel G, Schmitt C, Lejars O, Schneider P, Demeocq F, *et al*; FRALLE Group: Excellent prognosis of late relapses of ETV6/RUNX1-positive childhood acute lymphoblastic leukemia: Lessons from the FRALLE 93 protocol. Haematologica 97: 1743-1750, 2012.

8. Sangle NA and Perkins SL: Core-binding factor acute myeloid leukemia. Arch Pathol Lab Med 135: 1504-1509, 2011.
9. Ji J, Loo E, Pullarkat S, Yang L and Tirado CA: Acute myeloid leukemia with t(7;21)(p22;q22) and 5q deletion: A case report and literature review. Exp Hematol Oncol 3: 8, 2014.
10. Osato M: Point mutations in the RUNX1/AML1 gene: Another actor in RUNX leukemia. Oncogene 23: 4284-4296, 2004.
11. Silva FP, Swagemakers SM, Erpelinck-Verschueren C, Wouters BJ, Delwel R, Vrieling H, van der Spek P, Valk PJ and Giphart-Gassler M: Gene expression profiling of minimally differentiated acute myeloid leukemia: M0 is a distinct entity subdivided by RUNX1 mutation status. Blood 114: 3001-3007, 2009.
12. Dicker F, Haferlach C, Kern W, Haferlach T and Schnittger S: Trisomy 13 is strongly associated with AML1/RUNX1 mutations and increased FLT3 expression in acute myeloid leukemia. Blood 110: 1308-1316, 2007.
13. Harada H, Harada Y, Niimi H, Kyo T, Kimura A and Inaba T: High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. Blood 103: 2316-2324, 2004.
14. Mendler JH, Maharry K, Radmacher MD, Mrózek K, Becker H, Metzeler KH, Schwind S, Whitman SP, Khalife J, Kohlschmidt J, *et al*: RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and MicroRNA expression signatures. J Clin Oncol 30: 3109-3118, 2012.
15. Schnittger S, Dicker F, Kern W, Wendland N, Sundermann J, Alpermann T, Haferlach C and Haferlach T: RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood 117: 2348-2357, 2011.
16. Silva FP, Lind A, Brouwer-Mandema G, Valk PJ and Giphart-Gassler M: Trisomy 13 correlates with RUNX1 mutation and increased FLT3 expression in AML-M0 patients. Haematologica 92: 1123-1126, 2007.
17. Nucifora G, Begy CR, Erickson P, Drabkin HA and Rowley JD: The 3;21 translocation in myelodysplasia results in a fusion transcript between the AML1 gene and the gene for EAP, a highly conserved protein associated with the Epstein-Barr virus small RNA EBER 1. Proc Natl Acad Sci USA 90: 7784-7788, 1993.
18. Zent CS, Mathieu C, Claxton DF, Zhang DE, Tenen DG, Rowley JD and Nucifora G: The chimeric genes AML1/MDS1 and AML1/EAP inhibit AML1B activation at the CSF1R promoter, but only AML1/MDS1 has tumor-promoter properties. Proc Natl Acad Sci USA 93: 1044-1048, 1996.
19. Hromas R, Busse T, Carroll A, Mack D, Shopnick R, Zhang DE, Nakshatri H and Richkind K: Fusion AML1 transcript in a radiation-associated leukemia results in a truncated inhibitory AML1 protein. Blood 97: 2168-2170, 2001.
20. Ramsey H, Zhang DE, Richkind K, Burcoglu-O'Ral A and Hromas R: Fusion of AML1/Runx1 to copine VIII, a novel member of the copine family, in an aggressive acute myelogenous leukemia with t(12;21) translocation. Leukemia 17: 1665-1666, 2003.
21. Mikhail FM, Coignet L, Hatem N, Mourad ZI, Farawela HM, El Kaffash DM, Farahat N and Nucifora G: A novel gene, FGA7, is fused to RUNX1/AML1 in a t(4;21)(q28;q22) in a patient with T-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer 39: 110-118, 2004.
22. Ågerstam H, Lilljebjörn H, Lassen C, Swedin A, Richter J, Vandenberghe P, Johansson B and Fioretos T: Fusion gene-mediated truncation of RUNX1 as a potential mechanism underlying disease progression in the 8p11 myeloproliferative syndrome. Genes Chromosomes Cancer 46: 635-643, 2007.
23. Giguère A and Hébert J: CLCA2, a novel RUNX1 partner gene in a therapy-related leukemia with t(1;21)(p22;q22). Cancer Genet Cytogenet 202: 94-100, 2010.
24. Giguère A and Hébert J: Identification of a novel fusion gene involving RUNX1 and the antisense strand of SV2B in a BCR-ABL1-positive acute leukemia. Genes Chromosomes Cancer 52: 1114-1122, 2013.
25. Rodriguez-Perales S, Torres-Ruiz R, Suela J, Acquadro F, Martin MC, Yebra E, Ramirez JC, Alvarez S and Cigudosa JC: Truncated RUNX1 protein generated by a novel t(1;21)(p32;q22) chromosomal translocation impairs the proliferation and differentiation of human hematopoietic progenitors. Oncogene 35: 125-134, 2016.
26. Chinen Y, Taki T, Nishida K, Shimizu D, Okuda T, Yoshida N, Kobayashi C, Koike K, Tsuchida M, Hayashi Y, *et al*: Identification of the novel AML1 fusion partner gene, LAF4, a fusion partner of MLL, in childhood T-cell acute lymphoblastic leukemia with t(2;21)(q11;q22) by bubble PCR method for cDNA. Oncogene 27: 2249-2256, 2008.
27. Dai HP, Xue YQ, Zhou JW, Li AP, Wu YF, Pan JL, Wang Y and Zhang J: LPXN, a member of the paxillin superfamily, is fused to RUNX1 in an acute myeloid leukemia patient with a t(11;21)(q12;q22) translocation. Genes Chromosomes Cancer 48: 1027-1036, 2009.
28. Hazourli S, Chagnon P, Sauvageau M, Fetni R, Busque L and Hébert J: Overexpression of PRDM16 in the presence and absence of the RUNX1/PRDM16 fusion gene in myeloid leukemias. Genes Chromosomes Cancer 45: 1072-1076, 2006.
29. LaFiura KM, Edwards H, Taub JW, Matherly LH, Fontana JA, Mohamed AN, Ravindranath Y and Ge Y; Children's Oncology Group: Identification and characterization of novel AML1-ETO fusion transcripts in pediatric t(8;21) acute myeloid leukemia: A report from the Children's Oncology Group. Oncogene 27: 4933-4942, 2008.
30. Sakai I, Tamura T, Narumi H, Uchida N, Yakushijin Y, Hato T, Fujita S and Yasukawa M: Novel RUNX1-PRDM16 fusion transcripts in a patient with acute myeloid leukemia showing t(1;21)(p36;q22). Genes Chromosomes Cancer 44: 265-270, 2005.
31. Stevens-Kroef MJ, Schoenmakers EF, van Kraaij M, Huys E, Vermeulen S, van der Reijden B and van Kessel AG: Identification of truncated RUNX1 and RUNX1-PRDM16 fusion transcripts in a case of t(1;21)(p36;q22)-positive therapy-related AML. Leukemia 20: 1187-1189, 2006.
32. Panagopoulos I, Gorunova L, Brandal P, Garnes M, Tierens A and Heim S: Myeloid leukemia with t(7;21)(p22;q22) and 5q deletion. Oncol Rep 30: 1549-1552, 2013.
33. Jeandidier E, Gervais C, Radford-Weiss I, Zink E, Gangneux C, Eischen A, Galoisy AC, Helias C, Dano L, Cammarata O, *et al*: A cytogenetic study of 397 consecutive acute myeloid leukemia cases identified three with a t(7;21) associated with 5q abnormalities and exhibiting similar clinical and biological features, suggesting a new, rare acute myeloid leukemia entity. Cancer Genet 205: 365-372, 2012.
34. Foster N, Paulsson K, Sales M, Cunningham J, Groves M, O'Connor N, Begum S, Stubbs T, McMullan DJ, Griffiths M, *et al*: Molecular characterisation of a recurrent, semi-cryptic RUNX1 translocation t(7;21) in myelodysplastic syndrome and acute myeloid leukaemia. Br J Haematol 148: 938-943, 2010.
35. Paulsson K, Békássy AN, Olofsson T, Mitelman F, Johansson B and Panagopoulos I: A novel and cytogenetically cryptic t(7;21)(p22;q22) in acute myeloid leukemia results in fusion of RUNX1 with the ubiquitin-specific protease gene USP42. Leukemia 20: 224-229, 2006.
36. Hasle H, Abrahamsson J, Forestier E, Ha SY, Heldrup J, Jahnukainen K, Jónsson OG, Lausen B, Palle J and Zeller B; Nordic Society of Paediatric Haematology and Oncology (NOPHO): Gemtuzumab ozogamicin as postconsolidation therapy does not prevent relapse in children with AML: Results from NOPHO-AML 2004. Blood 120: 978-984, 2012.
37. Schaffer LG, Slovak ML and Campbell LJ: ISCN 2009: An International System for Human Cytogenetic Nomenclature. Karger S. (ed). Basel, 2009.
38. McPherson A, Hormozdiari F, Zayed A, Giuliany R, Ha G, Sun MG, Griffith M, Heravi Moussavi A, Senz J, Melnyk N, *et al*: deFuse: An algorithm for gene fusion discovery in tumor RNA-Seq data. PLOS Comput Biol 7: e1001138, 2011.
39. Singhal H, Ren YR and Kern SE: Improved DNA electrophoresis in conditions favoring polyborates and lewis acid complexation. PLoS One 5: e11318, 2010.
40. Ogawa E, Maruyama M, Kagoshima H, Inuzuka M, Lu J, Satake M, Shigesada K and Ito Y: PEBP2/PEA2 represents a family of transcription factors homologous to the products of the Drosophila runt gene and the human AML1 gene. Proc Natl Acad Sci USA 90: 6859-6863, 1993.
41. Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y and Ohki M: t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc Natl Acad Sci USA 88: 10431-10434, 1991.
42. Miyoshi H, Ohira M, Shimizu K, Mitani K, Hirai H, Imai T, Yokoyama K, Soeda E and Ohki M: Alternative splicing and genomic structure of the AML1 gene involved in acute myeloid leukemia. Nucleic Acids Res 23: 2762-2769, 1995.
43. Tanaka T, Tanaka K, Ogawa S, Kurokawa M, Mitani K, Nishida J, Shibata Y, Yazaki Y and Hirai H: An acute myeloid leukemia gene, AML1, regulates hemopoietic myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms. EMBO J 14: 341-350, 1995.

44. Niitsu N, Yamamoto-Yamaguchi Y, Miyoshi H, Shimizu K, Ohki M, Umeda M and Honma Y: AML1a but not AML1b inhibits erythroid differentiation induced by sodium butyrate and enhances the megakaryocytic differentiation of K562 leukemia cells. Cell Growth Differ 8: 319-326, 1997.
45. Ran D, Shia WJ, Lo MC, Fan JB, Knorr DA, Ferrell PI, Ye Z, Yan M, Cheng L, Kaufman DS, *et al*: RUNX1a enhances hematopoietic lineage commitment from human embryonic stem cells and inducible pluripotent stem cells. Blood 121: 2882-2890, 2013.
46. Tsuzuki S, Hong D, Gupta R, Matsuo K, Seto M and Enver T: Isoform-specific potentiation of stem and progenitor cell engraftment by AML1/RUNX1. PLoS Med 4: e172, 2007.
47. Liu X, Zhang Q, Zhang DE, Zhou C, Xing H, Tian Z, Rao Q, Wang M and Wang J: Overexpression of an isoform of AML1 in acute leukemia and its potential role in leukemogenesis. Leukemia 23: 739-745, 2009.
48. Watanabe-Okochi N, Kitaura J, Ono R, Harada H, Harada Y, Komeno Y, Nakajima H, Nosaka T, Inaba T and Kitamura T: AML1 mutations induced MDS and MDS/AML in a mouse BMT model. Blood 111: 4297-4308, 2008.
49. Dowdy CR, Xie R, Frederick D, Hussain S, Zaidi SK, Vradii D, Javed A, Li X, Jones SN, Lian JB, *et al*: Definitive hematopoiesis requires Runx1 C-terminal-mediated subnuclear targeting and transactivation. Hum Mol Genet 19: 1048-1057, 2010.
50. Gupta V, Minden MD, Yi QL, Brandwein J and Chun K: Prognostic significance of trisomy 4 as the sole cytogenetic abnormality in acute myeloid leukemia. Leuk Res 27: 983-991, 2003.
51. Klein K, Kaspers G, Harrison CJ, Beverloo HB, Reedijk A, Bongers M, Cloos J, Pession A, Reinhardt D, Zimmerman M, *et al*: Clinical impact of additional cytogenetic aberrations, cKIT- and RAS mutations and other factors in pediatric t(8;21)-AML: Results from an International Retrospective Study by the International Berlin-Frankfutrt-Munster Study Group. J Clin Oncol 33: 4247-4258, 2015.
52. Levis M: FLT3 mutations in acute myeloid leukemia: What is the best approach in 2013? Hematology Am Soc Hematol Educ Program 2013: 220-226, 2013.
53. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, *et al*; European LeukemiaNet: Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 115: 453-474, 2010.
54. Estey EH: Acute myeloid leukemia: 2014 update on risk-stratification and management. Am J Hematol 89: 1063-1081, 2014.
55. Allen C, Hills RK, Lamb K, Evans C, Tinsley S, Sellar R, O'Brien M, Yin JL, Burnett AK, Linch DC, *et al*: The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3 and CBL mutations in core-binding factor acute myeloid leukemia. Leukemia 27: 1891-1901, 2013.
56. Jourdan E, Boissel N, Chevret S, Delabesse E, Renneville A, Cornillet P, Blanchet O, Cayuela JM, Recher C, Raffoux E, *et al*; French AML Intergroup: Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. Blood 121: 2213-2223, 2013.
57. Meshinchi S, Alonzo TA, Stirewalt DL, Zwaan M, Zimmerman M, Reinhardt D, Kaspers GJ, Heerema NA, Gerbing R, Lange BJ, *et al*: Clinical implications of FLT3 mutations in pediatric AML. Blood 108: 3654-3661, 2006.
58. Zwaan CM, Meshinchi S, Radich JP, Veerman AJ, Huismans DR, Munske L, Podleschny M, Hählen K, Pieters R, Zimmermann M, *et al*: FLT3 internal tandem duplication in 234 children with acute myeloid leukemia: Prognostic significance and relation to cellular drug resistance. Blood 102: 2387-2394, 2003.