Acute Lymphoblastic Leukemia

MLL/GAS7 fusion in a pediatric case of t(11;17)(q23;p13)-positive precursor B-cell acute lymphoblastic leukemia

MLL/GAS7, resulting from t(11;17)(q23;p13), has been reported in one case of treatment-related acute myeloid leukemia (AML). We present a _de novo_ case of t(11;17)-positive pediatric acute lymphoblastic leukemia. Fluorescent _in situ_ hybridization and reverse transcriptase polymerase chain reaction analyses revealed an _MLL/GAS7_ chimera identical to the one previously described in AML. The molecular genetic features of _MLL/GAS7_ and the clinical impact of t(11;17) are discussed.

Haematologica 2006; 91:1287-1288
(*http://www.haematologica.org/journal/2006/09/1287.html*)

To date, the 5' part of MLL has been shown to fuse with the 3' part of approximately 50 different genes in acute myeloid leukemia (AML), chronic myeloid leukemia, myelodysplastic syndromes, acute lymphoblastic leukemia (ALL), and lymphomas.[1] Some of these fusions are quite common, such as *MLL/AFF1(AF4)* [t(4;11)(q21;q23)] in ALL and *MLL/MLLT3(AF9)* [t(9;11)(p21;q23)] in AML, whereas many of them have only been reported in single cases, such as the *MLL/GAS7* [t(11;17)(q23;p13)] in therapy-related AML.[1,2] Although the prognostic impact of the frequent *MLL* fusions is well known,[3,4] the clinical ramifications of the infrequent chimeras are unknown. Considering that most treatment protocols are based on the presence of certain genetic changes in AML and ALL, it is important to describe such rare *MLL* fusions, with the aim that other groups will report additional cases, thus increasing the clinical information available for the medical community, as recently exemplified by the rare *MLL/ARHGAP26(GRAF)* fusion, generated by a t(5;11)(q31;q23), in pediatric AML.[5-7] For this reason, we present here the molecular genetic and clinical features of the first t(11;17)(q23;p13)-positive ALL shown to harbor the *MLL/GAS7* chimera.

A previously healthy 15-month old girl was admitted to hospital in April 2005 because of fever and abnormal blood values (hemoglobin 62 g/L, platelet count 68×10⁹/L, and white blood cell count 3.9×10⁹/L with 28% blasts). The clinical examination revealed hepatomegaly; there was no splenomegaly or mediastinal mass. The bone marrow was

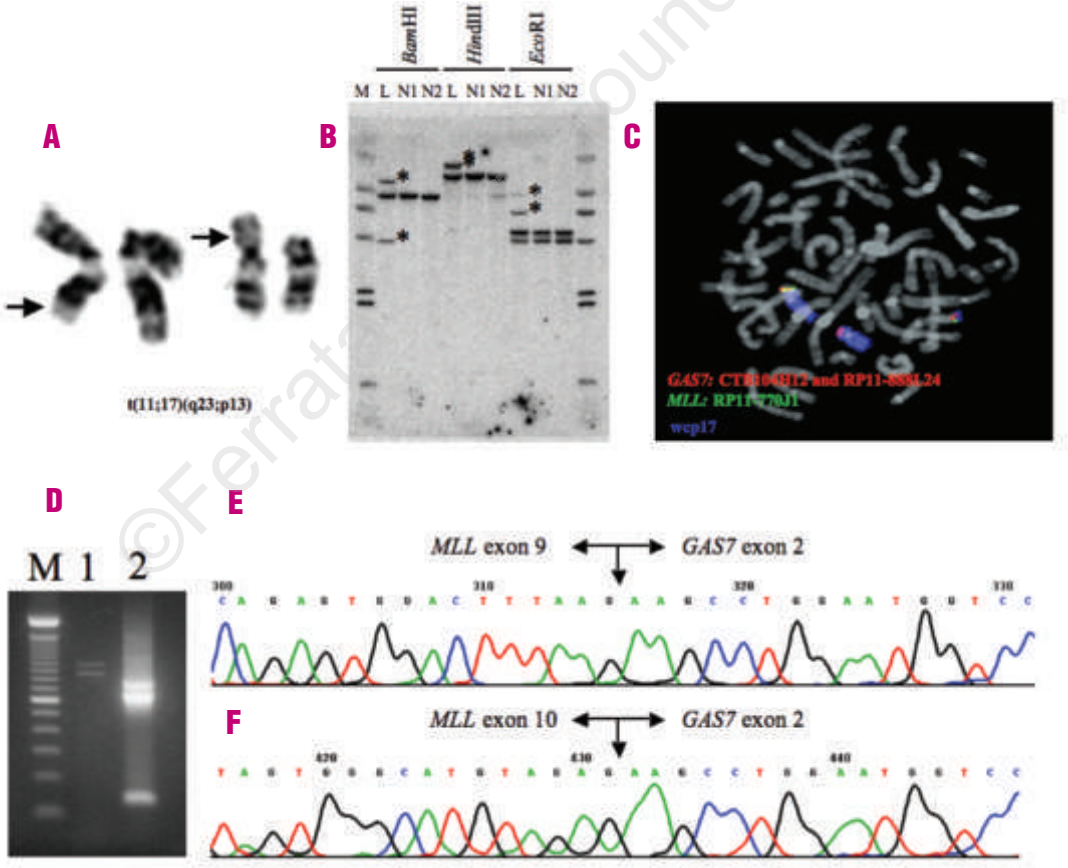

Figure 1. A. Partial karyogram showing the t(11;17)(q23;p13). The derivative chromosomes 11 and 17 and the chromosomal breakpoints are indicated by arrows. B. Southern blot analysis of *Bam*HI-, *Hind*III- and *Eco*RI-digested DNA from the t(11;17)-positive ALL (L) and from two healthy individuals (N1 and N2) using the *MLL* probe B859. Aberrant fragments are indicated by asterisks. M is λ DNA/HindIII fragments. C. FISH using RP11-770J1 for the *MLL* gene (green signal), CTB-104H12 and RP11-888L24 for the *GAS7* gene (red signal), and wcp17 (blue signal). Fusion signals of *MLL* and *GAS7* are seen on both the der(11) and the der(17) of the t(11;17). D. Amplification of a chimeric *MLL/GAS7* transcript. Total RNA was reverse-transcribed, and cDNA was used as a template in PCR amplification using MLL-3735F (CCCATCAGCAAGAGAGGATCCTGC) and GAS7-700R (CATCGTGTCTGG GTGAGGGAACG) primers (Lane 1). Two microliters of the first PCR product were used in a second PCR with MLL-3878F (AGTCAAGCAAGCAGGTCTCCCAGC) and GAS7-634R (CTGCTTTTTGCTTGGCGATGAGG) (Lane 2). M: 100 bp DNA ladder. E and F. Partial sequence chromatogram showing that nt 4241 (NM_005933.2; exon 9) of *MLL* was fused in-frame with nt 345 (NM_201433.1; exon 2) of GAS7 (E) and that nt 4355 (exon 10) of *MLL* was also fused in-frame with nt 345 of GAS7 (F).

Table 1. Clinical, immunophenotypic, and genetic features of t(11;17)(q23;p13)-positive acute leukemias reported in the literature.

Reference	*Diagnosis*	*Sex/age*	*CNS*	*WBC ($\times 10^9$/L)*	*Immunophenotype*	*Cytogenetics*	*Molecular genetics*	*Survival (mo)*
10	AL bi	M/5 mo	Yes	400	HLA-DR$^+$, CD19$^+$, CD33$^+$, CD13$^+$, TdT$^-$, CD10$^-$, CD20$^-$,slg$^-$,CD14$^-$	t(11;17) (Dx) t(11;17),+8 (Rel)	NR	<1
2	t-AML M4	M/14 yrs	NR	NR	NR	t(11;17)	MLL/GAS7	4
9	ALL	M/9 mo	NR	900	pro-B	t(11;17),+22	MLL R	59+
Present case	ALL	F/15 mo	No	3.9	CD34$^+$, CD19$^+$, CD22$^+$, CD24$^+$, CD15$^+$, CD123$^+$, TdT$^+$, cCD79$^+$, CD10$^-$	t(11;17)	MLL/GAS7	14+

AL bi: acute biphenotypic leukemia (FAB type M1/L2); ALL: acute lymphoblastic leukemia; CNS: central nervous system involvement; Dx: diagnosis; F: female; M: male; mo: months; NR: not reported; R: rearrangement; Rel: relapse; t-AML M4: treatment-related acute myeloid leukemia (FAB type M4) occurring after chemotherapy (cyclophosphamide, doxorubicin, vincristine, cisplatin, and etoposide), radiotherapy, and surgery for neuroblastoma; WBC: white blood cell count; yrs: years; +: alive at the time of reporting..

hypercellular, containing mainly blasts. Flow cytometric four-color analysis showed that 95% of the bone marrow cells were positive for CD34, CD19, CD22, CD24, CD15, CD123, TdT, and cCD79a but negative for CD10. A diagnosis of precursor B-cell ALL was made. Genetic analyses of a diagnostic bone marrow sample revealed the karyotype 46,XX,t(11;17)(q23;p13)[25] and an *MLL* rearrangement (Figure 1A and B). Further molecular studies were negative for *BCR/ABL1, ETV6/RUNX1, TCF3/PBX1*, and *FLT3* mutations. A monoclonal IgH rearrangement was identified, characterized and used for evaluating minimal residual disease by real-time polymerase chain reaction (PCR). Based on the finding of an *MLL* rearrangement, the girl continued treatment according to the intensive arm of the Nordic NOPHO-ALL 2000 protocol. Complete cytogenetic, fluorescent *in situ* hybridization, morphologic, flow cytometric, and PCR remission was achieved on day 29. At present, 14 months after diagnosis, she remains in remission. Southern blot analysis, using the *MLL* probe B859[6], yielded two extra bands in the *Bam*HI, *Hind*III, and *Eco*RI digestions (Figure 1B). The t(11;17)(q23;p13) was then further characterized by FISH using wcp11, wcp17, the bacterial artificial chromosome (BAC) RP11-770J1 (covering *MLL*), and BAC CTB-104H12 and RP11-888L24 for the *GAS7* locus. The FISH analysis revealed fusion signals of *MLL* and *GAS7* on both derivative chromosomes 11 and 17 (Figure 1C). Five micrograms of total RNA were reverse-transcribed, PCR-amplified, and sequenced as described previously.[6] The reverse transcription (RT)-PCR with MLL-3735F and GAS7-700R, nested PCR with MLL-3878F and GAS7-634R, and sequence analyses of the two amplified cDNA fragments revealed an in-frame fusion of exon 9 of *MLL* with exon 2 of *GAS7* and, in the smaller fragment, of exon 10 of *MLL* with exon 2 of *GAS7* (Figure 1D-F), both identical to the previously reported *MLL/GAS7* transcripts in AML.[2]

As a consequence, the translocation places the *GAS7* gene under the control of the *MLL* promoter. The putative MLL/GAS fusion protein would retain the *AT-hook* DNA binding domain, the DNA methyl transferase motif, and the transcription repression domain of MLL and all the functional domains of the GAS7 protein.[2] MLL/GAS7 has been shown to transform multipotent hematopoietic progenitors and to induce mixed lineage leukemia in mice.[8] The present case shows that the *MLL/GAS7* fusion may result in ALL as well as in AML, akin to what has been reported for other *MLL* chimeras.[1]

Only four acute leukemias with t(11;17)(q23;p13), two of which with confirmed *MLL/GAS7* chimeras, have been reported to date (Table 1). Thus, it is difficult to draw any firm conclusions as regards its clinical impact. However, all patients have been children and pronounced leukocytosis was reported in two of them. Furthermore, it may be noteworthy that both patients with ALL, i.e., the present case and one previously published,[9] responded well to treatment. On the other hand, the two patients with AML both succumbed to their disease within a few months. Thus, the prognostic impact of *MLL/GAS7* may differ between AML and ALL.

Ioannis Panagopoulos, Henrik Lilljebjörn,* Bodil Strömbeck,* Lars Hjorth,° Tor Olofsson,# Bertil Johansson**

**Departments of Clinical Genetics, °Pediatrics, and #Hematology, University Hospital, Lund, Sweden*

Acknowledgments: this study was supported by the Swedish Children's Cancer Foundation, the Swedish Cancer Society, the Swedish Research Council, the Gunnar Nilsson's Cancer Foundation, and the Siv-Inger and Per Erik Andersson Foundation.

Key words: ALL, translocation, MLL, GAS7, fusion.

Correspondence: Ioannis Panagopoulos, PhD, Department of Clinical Genetics, University Hospital, SE-221 85 Lund, Sweden. Phone: international +46.46.172889. Fax: international +46.46.131061. E-mail: ioannis.panagopoulos@med.lu.se

References

1. Mitelman F, Johansson B, Mertens F. Mitelman Database of Chromosome Aberrations in Cancer. http://cgap.nci.nih.gov/Chromosomes/Mitelman (accessed June, 2006).
2. Megonigal MD, Cheung NK, Rappaport EF, Nowell PC, Wilson RB, Jones DH, et al. Detection of leukemia-associated MLL-GAS7 translocation early during chemotherapy with DNA topoisomerase II inhibitors. Proc Natl Acad Sci USA 2000;97:2814-9.
3. Mrózek K, Heinonen K, Lawrence D, Carroll AJ, Koduru PRK, Rao KW, et al. Adult patients with de novo acute myeloid leukemia and t(9;11)(p22; q23) have a superior outcome to patients with other translocations involving band 11q23: a cancer and leukemia group B study. Blood 1997;90:4532-8.
4. Johansson B, Moorman AV, Haas OA, Watmore AE, Cheung KL, Swanton S, et al. Hematologic malignancies with t(4;11)(q21;q23) – a cytogenetic, morphologic, immunophenotypic and clinical study of 183 cases. Leukemia 1998;12:779-87.
5. Borkhardt A, Bojesen S, Haas OA, Fuchs U, Bartelheimer D, Loncarevic IF, et al. The human GRAF gene is fused to MLL in a unique t(5;11)(q31;q23) and both alleles are disrupted in three cases of myelodysplastic syndrome/acute myeloid leukemia with a deletion 5q. Proc Natl Acad Sci USA 2000;97:9168-73.
6. Panagopoulos I, Kitagawa A, Isaksson M, Morse H, Mitelman F, Johansson B. MLL/GRAF fusion in an infant acute monocytic leukemia (AML M5b) with a cytogenetically cryptic ins(5;11)(q31;q23q23). Genes Chromosomes Cancer 2004;41:400-4.
7. Wilda M, Perez AV, Bruch J, Woessmann W, Metzler M, Fuchs U, et al. Use of MLL/GRAF fusion mRNA for measurement of minimal residual disease during chemotherapy in an infant with acute monoblastic leukemia (AML-M5). Genes Chromosomes Cancer 2005;43:424-6
8. So CW, Karsunky H, Passegué E, Cozzio A, Weissman IL, Cleary ML. MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. Cancer Cell 2003;3:161-71.
9. Chessells JM, Harrison CJ, Kempski H, Webb DK, Wheatley K, Hann IM, et al. Clinical features, cytogenetics and outcome in acute lymphoblastic and myeloid leukaemia of infancy: report from the MRC Childhood Leukaemia working party. Leukemia 2002;16:776-84.
10. Stark B, Vogel R, Cohen IJ, Umiel T, Mammon Z, Rechavi G, et al. Biologic and cytogenetic characteristics of leukemia in infants. Cancer 1989;63:117-25.