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Two new translocations involving the 11q23 region map outside the MLL locus in myeloid leukemias

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Background and Objectives. In acute leukemias, chromosomal translocations involving the 11q23 band are frequently, but not invariably, associated with *MLL* gene rearrangement and their finding is associated with a poor prognosis. We observed two new translocations with a breakpoint in the 11q23 region at standard cytogenetic analysis: a previously undescribed t(3;11)(q21;q23) in a 70-year old woman with a fulminating form of AML-M1 and a new translocation t(6;11)(q15;q23) in a 61-year old man with an atypical chronic myelogenous leukemia. In these two patients, involvement of the *MLL* gene was analyzed by molecular cytogenetic techniques which also allowed a more precise mapping of the breakpoints.

Design and Methods. The *MLL* gene was analyzed by Southern blot and by fluorescent *in situ* hybridization (FISH) with a double-color *MLL* probe. A panel of 11q, 3q and 6q cosmid/YAC probes mapping around the breakpoints was used for breakpoint mapping.

Results. In both patients, FISH analysis and Southern blot showed that the *MLL* gene was not rearranged; in patient 1, *MLL* was retained on the 11q+ derivative, whereas in patient 2 it moved to the 6q- chromosome. In the t(3;11) we localized the chromosome 11 breakpoint at 11q23.3, in a region flanked by CP-939H3 and cos1p3, distal to the *MLL* locus; in the t(6;11) the break occurred at 11q21, in a region flanked by CP-819A5 and CP-829A6, proximal to the *MLL* locus.

Interpretation and Conclusions. Our cases add two new translocations to the list of chromosomal anomalies involving the long arm of chromosome 11, and show that apparent translocation t(11q23) may involve loci and genes other than *MLL*. Characterizing the molecular heterogeneity of 11q23 translocations may identify some prognostic significance.

Key words: *MLL* gene, FISH analysis, atypical-CML, acute myeloid leukemia.

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Rearrangements and/or interstitial deletions involving chromosome band 11q23 are relatively common cytogenetic alterations in acute leukemias.[1] Translocations involving chromosome 11q23 are seen in the majority of cases of infant acute lymphoblastic leukemia (ALL) and topoisomerase-induced acute myelogenous leukemia (AML).[2,3] 11q23 translocations also occur, less frequently, in *de novo* AMLs, adult ALLs, malignant lymphomas and myelodysplastic syndromes (MDS).[4-6]

Chromosome 11q23 breakpoints are characterized by a marked heterogeneity in the number of partner chromosomal bands as more than 80 chromosome loci have been reported as partner sites of reciprocal translocations.[3,7] The majority of 11q23 translocations disrupt the *MLL* gene (also referred to as *ALL-1*, *HRX* and *HTRX1*).[8] This gene is a homolog of *Drosophila trithorax* whose function is required for proper homeotic gene expression and regulation of chromatin structure. It is a complex gene consisting of at least three domains, one causing transcriptional repression, another coding for a zinc finger-type protein and a third with transcriptional activation function.[9]

Acute leukemias carrying *MLL* rearrangements are characterized by a high degree of clinical and immunologic heterogeneity, as demonstrated by the variability in their immunophenotype, consistent with lymphoid or myeloid/monocytic derivation, as well as their occurrence in distinct age groups from infancy to adulthood.[10] At least 40 MLL partner genes have been identified to date.[3,7] However, the presence of 11q23 abnormalities does not always correlate with *MLL* rearrangement.[11,12] Infact, other genes, such as *RCK,* a gene previously identified in a lymphoma cell line, *LARG* encoding a guanine nucleotide exchange factor, *IGSF4*, a novel immunoglobulin superfamily gene, and a gene encoding the α2 subunit of platelet-activating factor acetylhydrolase (*Pafah1a2*) have been described

at the 11q23 region and may have a role in the leukemogenic process.[13-16]

We observed two new translocations with a breakpoint in the 11q23 region at standard cytogenetic analysis. A t(3;11)(q21;q23) translocation was found in a 70-year old woman with a fulminating form of AML-M1; a new t(6;11)(q15;q23) translocation was present in a 61-year old man with a myeloproliferative disorder, who subsequently developed an accelerated phase with an additional 20q- anomaly. We used fluorescence *in situ* hybridization (FISH) in the attempt to map the breakpoints associated with these two chromosome abnormalities using several YAC, PAC and cosmid probes mapping to the regions 11q21-24 and to the partner regions on 3q and 6q, respectively.

Design and Methods

Case reports

Patient #1. The first patient was a 70-year old woman diagnosed in July 1997 as having an acute myeloid leukemia FAB-type M1. At diagnosis, the peripheral blood count showed: hemoglobin (Hb) 8.3 g/dL, white blood cell (WBC) count 128×10^9/L with 40% blast cells, platelet count 104×10^9/L; lactate dehydrogenase (LDH) was 2245 U/L. At immunophenotyping of bone marrow cells, blasts were MPO^+, $CD33^+$, $CD13^+$, $CD34^+$, HLA-Dr^+, CD22, $CD3^-$, and $CD10^-$. Six days after diagnosis, the WBC count increased to 270×10^9/L; the patient started treatment with mitoxantrone, etoposide, and cytosine-arabinoside. On day 28, during bone marrow recovery, a sternal puncture showed persistence of leukemia. The patient refused further chemotherapy and died 45 days after diagnosis because of progressive disease.

Patient #2. A 61-year old man was referred in February 1998 with low-grade fever, weight loss and fatigue lasting for 4 months. Mild hepatosplenomegaly was present. Hb was 7.1 g/dL, WBC 17.9×10^9/L, platelets 220×10^9/L; blood smears showed 54% neutrophils (N), 8% lymphocytes (L), 8% monocytes (M), 2% eosinophils, 2% blasts (BI), 2% promyelocytes, 12% myelocytes (MC), and 12% metamyelocytes (MMC). At bone biopsy, cellularity was 80% with myeloid hyperplasia and dysplasia, conserved megakaryopoiesis and a low percentage of blasts; erythropoiesis was hypoplastic, fibrosis was not present. The patient was diagnosed as having a myeloproliferative disorder. He was treated with red cell transfusions until March 1999, when hydroxyurea was started because of increasing leukocytosis (WBC: 58×10^9/L) and thrombocytopenia (platelets 65×10^9/L). In August 2000, because of the high transfusion requirement and progressive organomegaly, a bone marrow was repeated and demonstrated the increase of the blast percentage to 20% and a cytogenetic evolution in all cells. At immunophenotype, blasts were $CD34^+$, $CD33^+$, and $CD13^+$. In June 2001 splenomegaly was massive, WBC were 149×10^9/L (N 26%, L 6%, M16%, BI 18%, MC 8%, MMC 24%). Treatment with 6-mercaptopurine (6-MP) was added to the hydroxyurea with decrease of WBC, severe anemia and thrombocytopenia. The patient was alive as of July 2002, with a poor clinical condition, still under transfusion support and cytotoxic treatment with hydroxyurea and 6-MP.

Cytogenetics

Two-day cultures without stimulation of bone marrow cells were used for cytogenetics and FISH analysis. G-banding karyotypes were analyzed and classified according to the International System for Human Cytogenetic Nomenclature (*ISCN 1995*).

FISH analysis

Fluorescence *in situ* hybridization was performed on nuclei from the cytogenetic pellet. First, a double color *MLL* probe was used. The *MLL* gene, located at band 11q23, was investigated with a combination of two PAC clones (217A21 and 167K13) from a PAC library (RPCI), which cover the *MLL* gene with a minimal overlap in the breakpoint cluster region.[17] These probes detect the majority of rearrangements at the *MLL* gene locus by showing a double color (red and green) on the normal chromosome 11, a green (5'MLL) signal on the der(11) and a red signal (3'MLL) on the partner of chromosome 11, or no red signal in the case of chromosome 11 deletion.

Subsequently, different YAC probes for 11q loci distal and centromeric to *MLL* were tested. In addition, probes for chromosome 3 and 6, were used in the attempt to map the breakpoints on both chromosome partners. The probes are listed in Tables 1 and 2. These clones were derived from human YAC libraries and constructed at the *Centre d'Etude du Polymorphisme Humain* (CEPH), Paris, France.[18,19] The chromosome 11 cosmid clones were derived from two chromosome 11-specific human cosmid libraries constructed in the vector s-CosI.[20,21] FISH was performed as previously described.[22] Applied DNA probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP using a nick translation kit (Boehringer, Mannheim, Germany). After hybridization, the slides were washed with 50% formamide in 2×SSC (42°C; 15 minutes) and in 2×SSC (37°C; 15 minutes). The biotin dUTP-labeled or digoxigenin-labeled probe was detected with anti-fluorescein-avidin (Vector Laboratories, USA) or anti-digoxigenin

Table 1. Patient 1 with t(3;11): probes applied and results of FISH studies. The probes are listed from centromere to telomere.

Probes #11	*Localization*	*der(3)*	*der(11)*
pac217A21 (5'*MLL*)	11q23.3	–	+
pac167K13 (3'*MLL*)	11q23.3	–	+
pac117 (*RCK*)	11q23.3	–	+
cos19.21	11q23.3	–	+
CP-788B9	11q23.3	–	+
CP-921G9	11q23.3	–	+
CP-939H3	11q23.3	–	+
cos1p3 (*FLI-1*)	11q24	+	–
cos1d1 (*FLI-1*)	11q24	+	–
Probes #3	*Localization*	*der(3)*	*der(11)*
CP-766D8	3q13-21	+	–
CP-790D8	3q21	+	–
CP-785C5	3q22-23	+	–
CP-803F8	3q25	–	+
cos B5-2	3q26	–	+

Table 2. Patient 2 with t(6;11): probes applied and results of FISH studies. The probes are listed from the centromere to telomere.

Probes #11	*Localization*	*der(6)*	*der(11)*
CP-798D6	11q14	–	+
CP-819A5	11q14	–	+
CP-829A6	11q21	+	–
CP-927H11	11q21	+	–
CP-166G16	11q21	+	–
CP-801A3	11q21	+	–
CP-755B11	11q23	+	–
pac217A21 (5'*MLL*)	11q23	+	–
pac167K13 (3'*MLL*)	11q23.3	+	–
Probes #6	*Localization*	*der(6)*	*der(11)*
CP-845B4	6q12	+	–
CP-952B12	6q13	+	–
CP-948B12	6q13	+	–
CP-912H8	6q13	+	–
CP-847H4	6q13	+	–
CP-737B7	6q13	–	+
CP-873A4	6q14	–	+
CP-745G9	6q15	–	+
CP-911A3	6q16	–	+

rhodamine Fab fragments (Boehringer, Mannheim, Germany) producing green or red signals. Fluorescein-labeled and digoxigenin-labeled probes were counterstained with 4'6-diamidine-2'-phenylindole dihydrochloride (DAPI) (Sigma, St Louis, MO, USA).

The FISH analysis was done with a Leitz DMRB fluorescence microscope equipped with a cooled black and white CCD camera run by SmartCapture software (Vysis, Stuttgard, Germany).

Southern blot

Southern blot analysis for the *MLL* gene rearrangement was done only in patient 2 as no DNA was available from patient 1.

DNA was digested with the restriction enzymes BglII and Bam HI, run on 0.8% agarose gel, and subjected to the Southern blotting procedure as previously described.[23] The probe used in this study was a 800 base pair (bp) BamHI cDNA fragment corresponding to the 8.3 Kb *MLL* gene breakpoint cluster spanning exon 8-14.[24]

Reverse transcription polymerase chain reaction (RT-PCR)

RT-PCR was used for the analysis of BCR/ABL transcript in patient 2, in order to rule out a masked Philadelphia chromosome; moreover, both patients were tested for the presence of MLL/AF6, MLL/AF9, MLL/ENL, MLL/ELL transcripts and for the *MLL* internal tandem duplication (MLL/ITD).

Total RNA was obtained from leukemic cells by the Chomczynski and Sacchi method;[25] cDNA was synthesized from total RNA with Moloney murine leukemia virus (MoMLV) reverse transcriptase (Perkin Elmer). The RT-PCR reaction was performed on a 2400 Thermal Cycler (Perkin Elmer, Norwalk, CT, USA); amplification was done with 35 cycles at 92°C for 30 seconds, 62°C for 45 seconds and 72°C for 45 seconds. At the end, 10 mL of amplified product were run on a 2% agarose gel and visualized by ethidium bromide staining. Primers for *MLL* and primers for the identification of fusion genes MLL-AF6, MLL-AF9, MLL-ENL, MLL-ELL were used as described elsewhere.[26] The RT-PCR procedure for BCR/ABL genes was performed according to the protocol established by the European BIOMED 1 Concerted Action.[27] The purified PCR products were sequenced using the Thermo Sequenase Cy 5.5 dye terminator cycle sequencing kit (Amersham Pharmacia Biotech, Uppsala, Sweden).

Results

Patient #1

Cytogenetic data of the cases are represented in Figure 1; our first interpretation on G-banded metaphases was 46,XX,t(3;11)(q21;q23) (Figure 1). At double-color FISH, *MLL* did not split and was retained on the 11q derivative chromosome. RT-PCR for *MLL/AF6*, *MLL/AF9*, *MLL/ENL*, *MLL/ELL* and *MLL/ITD* were negative.

Figure 1. G-banded karyotype of patient 1 at diagnosis: 46,XX,t(3;11)(q21;q23) at standard cytogenetic analysis. After FISH mapping, the translocation was reclassified as t(3;11)(q23-25;q23.3).

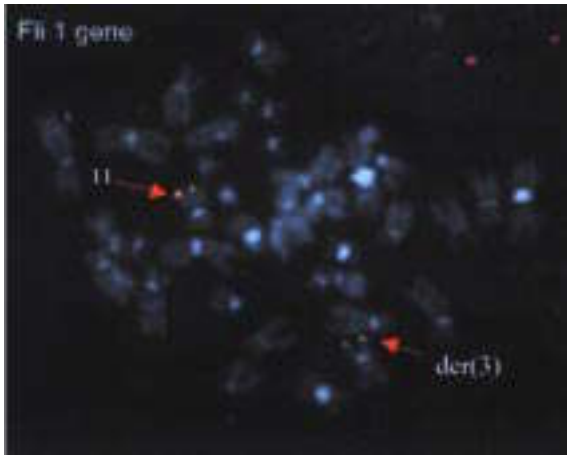

Figure 2. Fluorescence *in situ* hybridization with FLI-1 gene probes (cos1p3-red and cos1d1-green) on metaphase cell from the bone marrow culture of patient 1. Two chromosomes with double colour (red and green) signals are present on the normal chromosome 11 and on der(3), indicating that *FLI-1* gene is not splitted and moves to 3q.

Different probes for 11q loci distal to *MLL* were tested: RCK PAC 117, cos19.21, YACS CP-788B9, CP-921G9 and CP-939H3 (11q23.3) were retained on chromosome 11, whereas *FLI-1* cos1p3 and *FLI-1* cos1d1 (11q24) moved to the 3q derivative (Figure 2). However, a break internal to these loci was not observed. Several YACS for 3q21 (CP-766D8 and CP-790D8) and for 3q22-23 (CP-785C5) were tested, and were retained on chromosome 3, whereas the YAC CP-803F8 (3q25) and the cosB5-2 (3q26) moved to 11q. In conclusion, in this patient the break in chromosome 3 resided in a poorly characterized area between band 3q23 and band 3q25. The break on chromosome 11 did not involve *MLL* or the *RCK* gene and was located in the 11q23.3 band (Table 1). As a result of FISH analysis, the translocation was reclassified as t(3;11)(q23-25;q23.3).

Patient #2

At standard chromosome analysis karyotype at diagnosis was interpreted as 46,XY,t(6;11)(q15;q23) in all cells. At accelerated phase, a 20q- anomaly appeared as an additional anomaly in all metaphases (Figure 3).

FISH for the *MLL* probe demonstrated that the *MLL* gene did not split but 5'*MLL* and 3'*MLL* both translocated on the 6q derivative chromosome (Figure 4). Southern blot analysis did not show bands of rearrangement for *MLL* in addition to the germline fragment. RT-PCR for *BCR/ABL* was negative, and the RT-PCR for *MLL/AF6*, *MLL/AF9*, *MLL/ENL*, *MLL/ELL* and *MLL/ITD* were also negative.

We tested various YAC probes centromeric to *MLL*: the break occurred in the region between YAC CP-829A6 (11q21), which moved on 6q and YAC CP-819A5 (11q14), which remained on 11q. On chromosome 6, the breakpoints were between CP-847H4 (retained on 6q13) and CP-737B7 (6q13) which moved to 11. Refined chromosome breakpoints of the long arm of chromosome 11 and 6 are listed in Table 2.

Figure 3. G-banded karyotype of patient 2 at accelerated phase (August 2000): 46,XX, t(6;11)(q15;q23), del(20)(q11.2). After FISH mapping, the translocation was reclassified as t(6;11)(q13;q21).

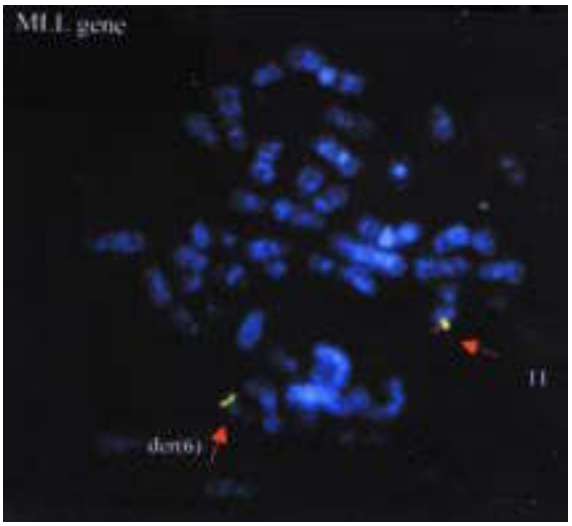

Figure 4. Fluorescence *in situ* hybridization with *MLL* gene probe from patient 2: 5' *MLL* (green) and 3' *MLL* (red). Double colour (red and green) signals are present on normal chromosome 11 and on der(6), showing that the MLL gene translocates on 6q without rearrangement.

Therefore, the breakpoints were more proximal than classified in standard cytogenetics, and the translocation was reclassified as t(6;11)(q13;q21).

Discussion

A variety of recurrent chromosomal abnormalities have been described in association with hematologic malignant neoplasms; one of the most common cytogenetic abnormalities is the 11q23 translocation. The large majority of 11q23 abnormalities in acute leukemias involve the *MLL* gene, and this finding is generally associated with a poor prognosis;[5] however, sporadic cases have been described in which *MLL* is not involved.[12] Cytogenetic analysis of the reported cases showed clonal chromosomal abnormalities in all investigated metaphases. Based on the cytogenetic data, suggesting a break on chromosome 11q23, we expected to find a *MLL* gene rearrangement; however, FISH analysis demonstrated that breakpoints are different in the two patients and *MLL* is not involved.

In both patients, refined FISH analysis has allowed the breakpoints to be mapped more precisely than by standard cytogenetics. Indeed, in the first case the breakpoint was located in 11q23.3, distal to *MLL*, while in the second case the break occurred in the 11q21 region.

The t(3;11)(q23-25;q23.3) has never been described before, even though chromosome 3 has been frequently associated with myeloid hematologic malignancies. In particular, the breakpoints in the 3q21q26 syndrome, including the t(3;3) (q21;q26) and the inv(3)(q21q26), are clustered within a 50kb region of 3q21.[28] The 3q21 region also contains the ribophorin I (*RPN1*) gene and *GR6*. The *RPN1* acquires the function of activating the transcription of the EVI1 gene at 3q26 as an enhancer element through rearrangements involving 3q21 and 3q26.

The *GR6* gene was reported to be expressed in fetal organs and in the AML1-leukemia cell line with a t(3;3) translocation.[29] In our case the region 3q21 was not involved and the breakpoint on 3q was more distal as compared to that in the 3q21q26 syndrome; however, as observed in all described patients with 3q abnormalities, the disease was very aggressive.[30,31] Response to therapy in patients with miscellaneous 3q abnormalities is very poor: it has been shown by the EORTC and GIMEMA leukemia study groups that the survival rate at 3 years is 16.8% among patients with 3q abnormalities versus 41.1% among patients without 3q abnormalities.[32]

In the 11q23.3 region another translocation has been reported, which does not involve the *MLL* gene, namely the t(11;21)(q23.3;q11).[33,34] In the t(11;21) translocation the break was more proximal than observed in our t(3;11), where the break has been mapped between YAC 939H3 and *FLI-1*, whereas in the t(11;21) the breakpoint was between YAC 921G9 and YAC 939H3.[34]

In our patient, the break was at the border between 11q23 and 11q24; the *FLI1* gene, shown to be disrupted by the t(11;22)(q24;q12) in Ewing's sarcoma,[35,36] maps to this region but it too was not involved in our case, as cos1p3 and cos1d1, containing the *FLI1* gene, were both translocated on 3q.

Also in the case of the t(6;11), which was first described by our group, the FISH analysis allowed the *MLL* rearrangements to be excluded and redefined the breakpoints at bands 6q13 and 11q21. This t(6;11)(q13;q21) was associated with a Philadelphia negative, *BRC/ABL* negative myeloproliferative disorder characterized by hyperleukocytosis, presence of immature dysplastic myeloid cells in bone marrow and peripheral blood, low basophil count, monocytosis and spleen enlargement. These features were consistent with a diagnosis of atypical chronic myelogenous leukemia,[37] a rare disease often associated with different chromosome abnormalities, particularly trisomy 8; one case with deletion of 11q (breaks in 11q13 and 11q23) has also been reported.[38] A single case of t(6;8) had a breakpoint on 6p23.[38]

Our cases add two new translocations to the list of chromosomal anomalies involving the long arm of chromosome 11. The marked heterogeneity of rearrangements in this chromosomal region, point-

ing to a high number of relevant genes, has also been demonstrated by a recent series of 32 cases with various hematologic malignancies, including AML and non-Hodgkin's lymphoma, with a clustering of breakpoints at 11q22-23.1 and 11q25, which did not have rearrangement of the *MLL* gene.[39] FISH analysis on 3 cases with AML and new translocations t(11;12), inv(11), and t(11;15) showed that the breakpoints were restricted to 11q22-23.1, a region which is centromeric to *MLL*[39] but telomeric with respect to the 11q21 break in our translocation t(6;11).

These data show that the apparent translocation t(11q23) may involve loci and genes other than *MLL* and suggest that these still unidentified genes could provide important insights into the molecular genetic mechanism involved in the pathogenesis of myeloid malignancies. Characterizing the molecular heterogeneity of 11q23 translocations may identify some prognostic significance. FISH analysis can be used in order to differentiate typical 11q23/*MLL* rearrangements from more unusual chromosomal translocations, apparently involving the same locus at standard cytogenetic analysis.

Contributions and Acknowledgments

EG, GRC, GS and AH: design of the study and writing the paper; EG, IW, PS and BE: cytogenetic and FISH analysis: AS and MS: molecular biology work; AT, MP: clinical data of the patients. EG: primary responsibility for the paper; Table 1 and 2: EG, GRC. Figures 1 and 3: EG, PS: Figures 2 and 4: EG, IW, BE. The authors gratefully thank Dr. E. Schuuring and Prof. Ph. Kluin, Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, for their kind gift of the probes: PAC 217A21 and PAC 167K13.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received May 16, 2002; accepted September 11, 2002.

What is already known on this topic

Chromosome 11q23 breakpoints are characterized by a marked heterogeneity in the number of partner chromosomal bands, and the majority of 11q23 translocations disrupt the *MLL* gene.

What this study adds

This paper adds two new translocations to the list of chromosomal anomalies involving the long arm of chromosome 11, and shows that apparent translocation t(11q23) may involve loci and genes other than *MLL*.

Potential implications for clinical practice

Characterizing the molecular heterogeneity of 11q23 translocations might have prognostic implications.

Mario Cazzola, Editor-in-Chief (Pavia, Italy)