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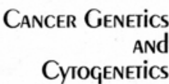

Short communication

# Chromosome 16 inversion-associated translocations in acute myeloid leukemia elucidated using a dual-color *CBFB* DNA probe

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**Abstract**

We describe two cases of acute myelomonocytic leukemia with eosinophilia (AML-M4Eo) that were diagnosed with an inv(16)(p13q22) based on conventional cytogenetics (CC) and fluorescence in situ hybridization (FISH) technique using a chromosome 16p arm specific paint probe. Additional FISH analysis with a dual-color CBFB DNA probe showed that the 3′ portion of the *CBFB* gene was translocated to chromosome 10p13 in the first patient and 1p36 in the other. These two cases indicate that some inv(16)(p13q22) identified by CC and FISH with chromosome arm-specific painting probe may represent cases of inversion-associated translocation. We suggest that all cases with inv(16)(p13q22) should be investigated by FISH with appropriate probes for a possible translocation of 16q22→qter to another chromosome. © 2002 Elsevier Science Inc. All rights reserved.

## 1. Introduction

The inv(16)(p13q22) and t(16;16)(p13;q22) are chromosomal abnormalities typically associated with acute myelomonocytic leukemia with eosinophilia (AML-M4Eo). AML patients with these cytogenetic alterations have a favorable prognosis compared to patients with other chromosome aberrations [1]. The identification of inv(16)/t(16;16), however, can be difficult by conventional cytogenetics (CC), especially on suboptimal G-banded chromosomes and in cases with a masked inv(16) by translocations [2]. The fluorescence in situ hybridization (FISH) technique has been applied using a chromosome 16p-arm painting probe as an adjunt to CC for improving detection of inv(16)/t(16;16) [3,4].

We describe two cases with an AML-M4Eo which were diagnosed as having an inv(16)(p13q22) by CC and FISH using the 16 p-arm paint probe (Oncor, Gaithersburg, MD, USA). However, the application of dual-color LSI *CBFB* DNA probe (Vysis, Downers Grove, IL, USA) demonstrated that both cases were, in fact, inv(16)-associated translocations involving chromosomes 10p13 and 1p36, respectively.

## 2. Material and methods

Patients and cytogenetic methods: Both cases were selected from an AML series of 40 patients in which an inv(16)/t(16;16)(p13;q22) was identified by CC and FISH analysis using the LSI *CBFB* DNA probe (Vysis). Cytogenetic study was performed on 24-h unstimulated bone marrow cells. Chromosomes were G-banded with Wright stain and karyotypes were described according to the ISCN nomenclature [5]. The FISH technique was carried out on destained G-banding metaphases using a commercial chromosome 16 p-arm specific paint probe (Oncor) and a double-color LSI *CBFB* probe (Vysis) which is a mixture of a 5′ *CBFB* probe directly labeled with the SpectrumRed fluorophore and a 3′ *CBFB* probe directly labeled with a SpectrumGreen fluorophore. In case 1, subtelomeric region of chromosomes 10p and 16q were investigated with the TelVysion 10p (green) and TelVysion 16q (red) probes (Vysis), respectively. The hybridization protocol was followed according to the manufacturer's guidelines.

## 3. Results and discussion

Clinical and cytogenetic data of both cases are summarized in Table 1. Diagnosis of inv(16)(p13q22) was based on CC and FISH analysis using a chromosome 16p arm specific paint probe (Oncor) which spans 16p13 breakpoint. The hy-

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Table 1
Clinical and cytogenetic data of both cases with inv(16) associated translocations

| Case | Age/Sex | FAB-type | Karyotype | FISH (*CBFB* DNA probe) | Response to therapy[a] | Survival in months |
|---|---|---|---|---|---|---|
| 1 | 40/M | AML-M4Eo | 46,XY,inv(16)(p13q22)[9]/46,XY[1] | ish der(16)t(10;16)(p13;q22)inv(16)(p13q22) | CR | 41+ |
| 2 | 54/M | AML-M4Eo | 47,XY,inv(16)(p13q22),+22[8] | ish der(16)t(1;16)(p36;q22)inv(16)(p13q22) | CR | 6+ |

*Abbreviations*: AML-M4Eo, acute myelomonocytic leukemia with eosinophilia; CR, complete remission; FAB, French–American–British classification; FISH, fluorescence in situ hybridization; M, male.

[a] Therapy consisted of one course of idarubicin, cytosine arabinoside, etoposide as induction; one course of mitoxantrone, cytosine arabinoside as consolidation; and one course of high-dose cytosine arabinoside as intensification.

bridization pattern for inv(16) is a split hybridization signal on the 16p-arm and 16q-arm (Fig. 1A, Fig. 1C, inset). Since a submicroscopic deletion involving the 3′ portion of the *CBFB* gene in association with inv(16)(p13q22) has been described [6], we performed additional FISH analysis using the LSI dual-color *CBFB* probe (Vysis) which explores the *CBFB* gene. On the inverted 16, the 5′ portion of the *CBFB* gene moves to the 16p13 and the 3′ *CBFB* remains on the 16q22 or is deleted. However, in our two patients, the 3′ *CBFB* probe was unexpectedly translocated to the chromosomes 10p13 and 1p36, respectively, (Fig. 1A, Fig. 1C). Further dual FISH investigations with telomeric 10p and 16q probes showed a reciprocal translocation involving 10pter and 16qter in case 1 (Fig. 1E). There was no pellet available to perform additional FISH experiments in case 2; however, an exhaustive analysis of the G-banded metaphases allowed us to reinterpret the abnormal chromosome 16 as an inv(16)-associated translocation t(1;16)(p36;q22).

The application of FISH studies on so-called variant translocations involving chromosome 16 at 16p13 or 16q22 and other partner chromosomes revealed that such cases might be true inv(16)(p13q22) masked by the translocation [2]. Subsequently, similar cases documented by FISH studies have been reported involving different chromosomes [2,4,7]. To the best of our knowledge, the short arm of chromosomes at 10p13 and 1p36 have never been reported to be involved in the formation of such a subtle inv(16)(p13q22)-associated translocation [8]. Usually, the der(16) looks like a del(16) or an add(16) depending on the size of the translocated material from the partner chromosome; consequently the inv(16)(p13q22) can sometimes be overlooked. In contrast, in our cases the translocated material from 10p13 and 1p36 was similar in size to segment 16q22→qter and the der(16) was identified as an inv(16) without associated translocation. The reverse transcriptase-polymerase chain reaction was positive for *CBFB/MYH11* transcripts (data not shown). Both patients were treated according to the AML-99 CETLAM-protocol which includes high-dose cytarabine as postremission treatment for patients with favorable chromosomal abnormalities, namely, t(8;21) and inv(16)/ t(16;16). They achieved complete remission with one cycle of idarubicin, cytosine arabinoside and etoposide and both are alive 41 and 6 months after diagnosis, respectively. There is no available information from the literature on prognostic differences of patients with an inv(16) as the sole chromosomal abnormality and patients with an inv(16)- associated translocations. Therefore, collection from additional cases is necessary to establish the clinical significance of the variant 3-way translocation of inversion 16.

## Acknowledgments

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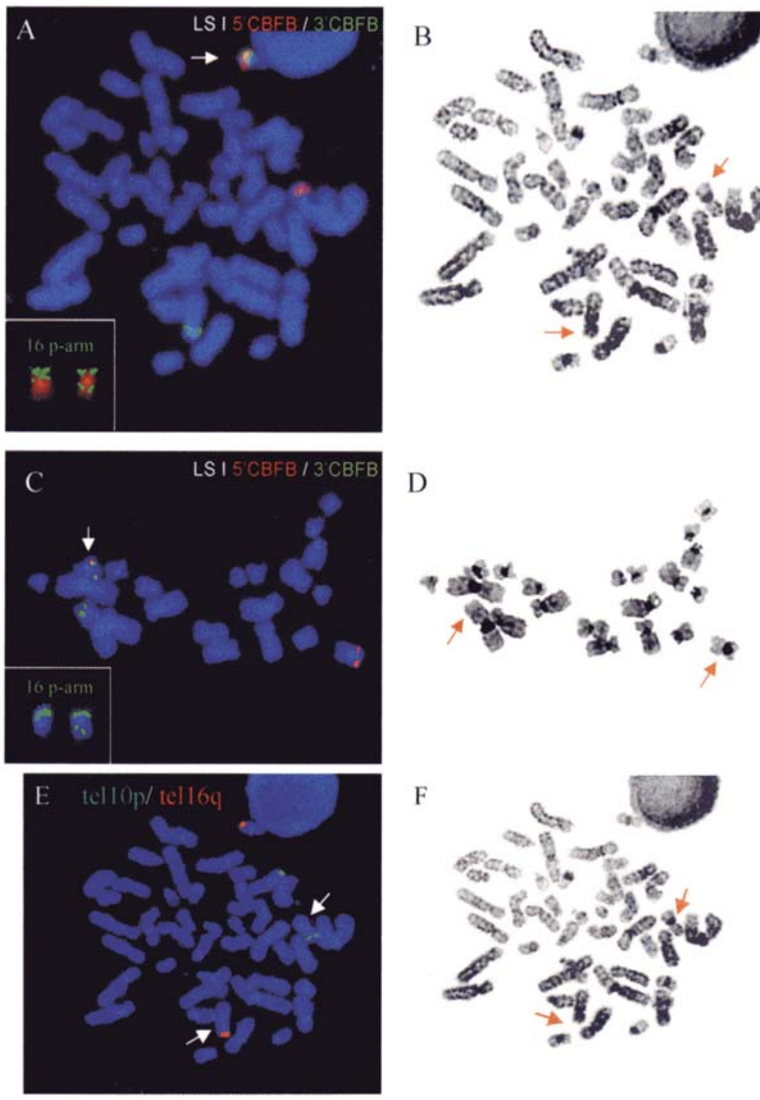


Fig. 1. (A, case 1) (C, case 2) Fluorescence in situ hybridization (FISH) analysis on metaphase spreads using the LSI dual color 5′ CBFB (red) and 3′ CBFB (green) probes (Vysis) showed normal colocalization on the long arm of normal chromosome 16 (arrow) whereas the inverted 16 displayed the 5′ CBFB (red) on the short arm in both cases and the 3′ CBFB (green) on the short arm of chromosome 10p13 and 1p36, respectively. (A, inset)(C, inset) FISH with the use of 16 p13 probe (Oncor) gave two hybridization signals on the inverted 16. (B, case 1) (D, case 2) The same metaphases G-banded showing der(16), der(10) and der(16), der(1) by arrows, respectively. (E, case 1) Metaphase-FISH with telomeric 10p (green) and 16q (red) probes which are translocated on the inverted chromosome 16 and on the der(10), respectively, (arrows). (F, case 1) The same metaphase G-banded.

### *3.2.3 Translocació t(5;11)(q35;p15.5) (III)*

Casas S, Aventin A, Nomdedeu J, Sierra J. Identification of t(5;11)(q35;p15.5) in adult acute myelocytic leukemia with normal karyotype. Cancer Genet and Cytogenet 2003; acceptat, pendent de publicació.

Recentment s'ha identificat la t(5;11)(q35;p15.5) com una nova alteració cromosòmica recurrent associada a casos de LMA pediàtrica (Brown et al., 2002). Aquesta translocació es va detectar mitjançant M-TEL en pacients que presentaven per ACC i M-FISH un cariotip normal. La t(5;11)(q35;p15.5) pot considerar-se una translocació críptica, ja que la grandària del material intercanviat dóna un patró de bandes que pot passar desapercebut per ACC. A més, al tractar-se de regions subtelomèriques tampoc es detecta per M-FISH (Brown et al., 2002).

Es van analitzar per FISH dual, amb sondes TEL-11p i TEL-5q, 40 mostres procedents de pacients adults de LMA que presentaven un cariotip normal diagnosticat prèviament tant per ACC com per M-FISH. En cap cas es va detectar la presència de la translocació t(5;11)(q35;p15.5).

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CANCER GENETICS AND CYTOGENETICS

Letter to the editor

# Cryptic t(5;11)(q35;p15.5) in adult de novo acute myelocytic leukemia with normal karyotype

A new recurrent cryptic translocation t(5;11)(q35;p15.5) has recently been reported as the sole chromosomal abnormality in two young patients with an acute myelocytic leukemia (AML) and an apparently normal karyotype, identified by a multiplex fluorescence in situ hybridization telomere assay [1]. The consequence of this translocation at the molecular level is the fusion of the human homolog of the *Mus musculus* nuclear receptor binding SET-domain protein 1 gene (*NSD1*) on 5q35 and the nucleoporin 98-kDa gene (*NUP98*) on 11p15.5 [2].

To expand upon the findings of Brown et al. [1], we investigated the presence of t(5;11)(q35;p15.5) in a cohort of patients with adult de novo AML with normal cytogenetics using a dual-color fluorescence in situ hybridization analysis (FISH) with chromosome-specific 5q and 11p subtelomeric probes.

A total of 40 de novo AML patients (17–60 years old, 52% male) enrolled in the Spanish CETLAM AML-99 protocol were selected on the basis of normal cytogenetics analyzed either by conventional G-banding or by a multicolor FISH technique (M-FISH). AML cases were diagnosed according to the French–American–British Cooperative Group classification [3] as follows: 10 M1, 9 M2, 4 M4, 16 M5, and 1 M6. Conventional cytogenetics and dual-color FISH using commercial subtelomeric probes for 5q and 11p (Vysis Inc., Downers Grove, IL, USA) were performed as reported previously [4]. M-FISH analysis was carried out using a commercially available set of combinatorially labeled whole chromosome paints with minor modifications (24Xcyte; MetaSystems GmbH, Altslussheim, Germany), following the conditions provided by the manufacturer.

We did not detect the t(5;11)(q35;p15.5) in any of the 40 cases studied. The present results, together with the previously reported study on 39 adult AML [1], suggest that such a rearrangement is a rare event in adult de novo AML. It remains to be investigated whether the formation of the *NSD1/NUP98* fusion gene may be generated by other genomic mechanisms, namely submicroscopic gene insertion.

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## Acknowledgments

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### *3.3 Detecció de guanys i pèrdues de material genètic (IV)*

Refined genetic diagnosis by using comparative genomic hybridization technique in acute myeloid leukemia. Casas S, Aventin A, Fuentes F, Vallespi T, Granada I, Carrio A, Martinez JA, Sole F, Teixidor M, Bernues M, Duarte J, Hernandez JM, Brunet S, Coll MD, Sierra J. Cancer Genet Cytogenet 2003; pendent de publicació.

#### *3.3.1 Anàlisi citogenètica convencional*

Es van analitzar per ACC 121 pacients, dels quals 45 (37 %) van presentar un cariotip normal. En 57 pacients (47 %) es van detectar anomalies cromosòmiques clonals, equilibrades en 42 casos, i desequilibrades en 15 casos. En els 19 pacients restants (16 %) no es van obtenir cèl.lules en metafase.

#### *3.3.2 Hibridació genòmica comparada*

Els guanys i pèrdues de material genètic identificats mitjançant CGH en 128 pacients es representen a la Figura 1. En general es va observar una proporció més elevada de pèrdues en relació a guanys (61 % *versus* 39 %), i una major proporció d'alteracions cromosòmiques parcials en relació a les totals (76 % *versus* 24 %). Així, la lesió cromosòmica més comú en la sèrie analitzada va ser la pèrdua parcial (54 %). Destacar el freqüent guany dels cromosomes 8, 21 i 22, i 3q, així com la pèrdua del cromosoma 7, 7q, 5q, 17p i 16q.

En la sèrie de pacients analitzada, la CGH va mostrar un perfil anormal en 40 casos (31 %). La majoria de desequilibris cromosòmics es van detectar en pacients amb cariotip anormal (32 casos). D'aquests, el resultat de CGH va ser concordant amb el cariotip proposat per ACC en 16 casos (Taula 1, casos 1-16), va aportar més informació en relació a l'ACC en 15 casos (Taula 1, casos 17 a 31), mentre que va ser menys informativa que l'ACC en un casos (Taula 1, cas 39). La resta de guanys i pèrdues es van identificar en 5 pacients on no s'havia pogut obtenir cèl.lules en divisió (Taula 1, casos 32

a 36), en 2 pacients on no es disposava d'ACC (Taula 1, casos 37 i 38) i en un cas amb cariotip normal (Taula 1, cas 40).

En la sèrie de pacients analitzada, la CGH va mostrar un perfil normal en 88 casos (69 %). D'aquests, el cariotip era normal en 44 pacients (resultats no mostrats), 15 casos presentaven una alteració cromosòmica equilibrada (Taula 2, casos 41 a 55), 14 eren pacients on en l'ACC no es van obtenir cèl.lules en divisió (Taula 2, casos 56 a 69), 5 casos corresponien a pacients que no se'ls havia realitzat ACC (Taula 2, casos 70 a 74), i 10 casos presentaven una o més alteracions cromosòmiques desequilibrades. En aquest últim grup, el resultat de CGH va ser menys informatiu en relació a l'ACC, destacar que 6 casos presentaven menys del 50 % de metafases patològiques (Taula 2, casos 75 a 80), mentre que el percentatge de blastes a MO era inferior al 30 % en 2 casos (Taula 2, casos 82 i 83), i en un cas, el nombre de metafases analitzades per ACC era inferior a 10 (Taula 2, cas 81, Estudi IV). En canvi, la discrepància en relació al cas 84 es va interpretar com un resultat de CGH més informatiu respecte al d'ACC.

### *3.3.3 Hibridació in situ fluorescent*

Es va complementar l'estudi mitjançant FISH o M-FISH en 13 pacients, dels quals en 11 casos el resultat de CGH era més informatiu que l'ACC (Taula 1, casos 17 a 19, 21, 23, 25 a 28, 32 i 40), i on en 2 casos el perfil de CGH era normal mentre que per ACC s'havien detectat diferents alteracions cromosòmiques desequilibrades (Taula 2, cas 78 i 84). Excepte en el cas 40 on no es disposava de la sonda LSI adequada, els anàlisis de FISH van confirmar el resultat de CGH.

**Refined genetic diagnosis by using comparative genomic hybridization technique in acute myeloid leukemia**

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Running title: CGH analysis of DNA copy number changes in AML patients

Key words: comparative genomic hybridization; multiplex fluorescent in situ hybridization; DNA copy number changes; acute myeloid leukemia

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## Abstact

A total of 128 adult *de novo* acute myeloid leukemia patients were analyzed by comparative genomic hybridization (CGH) at diagnosis. Abnormal CGH profiles were identified in 40 patients (31 %), in which a greater number of DNA copy number losses (61 %) was observed as compared to gains (39 %), whereas partial chromosome changes (76 %) were more common than whole chromosome changes (24 %). Recurrent losses were detected on chromosome 5q, 7q, 7, 16q and 17p, as well as gains on chromosome 8 and 22. Furthermore, distinct high-level amplifications were identified expanding chromosome regions 21q, 13q12 and 13q21.1. The concordance between CGH results and conventional cytogenetic analysis (CCA) were 62.8 %. In the remaining cases, CGH gave additional information compared to the CCA (17.3 %) and partially failed to identify the alterations previously detected by CCA (9.1 %). The majority of the discrepancies arise from the limitations of the CGH technique, such as the insensitivity to detect unbalanced chromosomal changes that occur at low frequencies. CGH has increased the detection of unbalanced chromosomal alterations and allowed precise defining of partial or uncharacterized cytogenetical abnormalities, and therefore the application of CGH technique is useful as a complementary diagnotic tool to CCA.

## 1. Introduction

Acute myeloid leukemia (AML) is a hematological disease with heterogeneous clinical and biological features. Many chromosomal alterations in *de novo* AML are associated with AML subtypes, as well as characteristic morphologic and immunologic profiles, and represent an important prognostic and therapeutic factor [1,2].

Clonal chromosomal abnormalities are detected by conventional cytogenetic analysis (CCA) in 40-60 % of newly diagnosed patients with AML [1,2,3], but the interpretation of the banding patterns is often complicated by chromosome condensation, imperfect banding and a limited number of metaphases. Molecular cytogenetic techniques have therefore been employed to overcome the limited capacity of CCA and to improve the detection of subtle chromosome aberrations [4]. Comparative genomic hybridization (CGH) has been an important tool for analysis of genetic imbalances at the whole genome level [5]. Unlike the CCA, CGH depends neither to obtain dividing cell nor on chromosome morphology. Genetic analysis by CGH is particularly useful in the diagnosis of unbalanced chromosomal alterations and in the defining of novel genes affected by changes in copy number. Nevertheless, CGH has mainly been applied to solid tumors [6], and only a few studies with short series of cases involving hematological diseases, such as AML, have been published [http://www.helsinki.fi/cmg/cgh_data.html].

The application of this technique complementary to CCA may provide a precise genetic diagnosis of AML. Therefore, the aim of the present study was the application of either CGH or CCA in the genetic diagnosis of a series of 128 AML patients. Fluorescent *in situ* hybridization (FISH) or multiplex-FISH (M-FISH) was used to analyze those cases where CGH results enable a more refined genetic diagnosis subsequent to the initial CCA.

## 2. Patients and Methods

### *2.1. Patients*

This study was performed from 16/09/98 to 12/08/02 on adult *de novo* AML patients consecutively enrolled in the CETLAM AML-99 protocol, in whom blood sample was available to extract DNA. One hundred and twenty-eight patients aged 17-60 years were included in the study. Morphological diagnosis of AML was made according to the FAB classification [7], unless WHO classification was noted [8]. AML typified as M3 were excluded.

### *2.2. Conventional cytogenetic analysis*

Cytogenetic analysis was performed on unstimulated bone marrow cells after short-term culture. Chromosomes were G-banded and classified according to the International System for Human Cytogenetic Nomenclature [9].

### *2.3. CGH and FISH analysis*

DNA was extracted from bone marrow, peripheral blood (cases 4, 9, 26, 48, 51, 55, 83 and 84) and skin (case 34) of selected patients using standard procedures [10]. CGH was performed following conditions provided by the manufacturer (Vysis, Doweners Growe, IL, USA). Briefly, tumoral and normal genomic DNA was labeled by nick translation using FITC and TexasRed®, respectively. Probes were checked in a 1 % agarose gel to obtain fragments between 300 and 3,000 base pairs. A mixture of 300 ng test DNA, 100 ng reference DNA and 10 μg Cot-1 DNA was hybridized with normal metaphase target slides. Thereafter, DAPI II was applied and metaphase images were captured using a fluorescence microscope (DMRB; Leica Mikrosysteme Vertrieb GmbH, Bensheim, Germany) through a charge-coupled device camera (Photometrics SenSys; Roper Scientific Inc., Tucson, USA) and a filter system specific for DAPI, FITC and TexasRed® (Croma Technology Corp, Brat-tleboro, VT, USA). The ratios of the FITC/TexasRed® intensities were calculated along the chromosomes using

the CGH Quips Software (Vysis). Loss and gain thresholds were 0.80 and 1.20, respectively. At least 10 metaphases were analyzed in each case.

Fluorescent in situ hybridization (FISH) analysis was carried out using locus-specific (LSI) (Vysis), subtelomeric (TEL) (Vysis), whole chromosome painting (WCP) (Appligene Oncor-Qbiogene Illkirch, France) and multiplex-FISH (M-FISH) (Vysis) probes according to the manufacturer's guidelines.

## 3. Results

### *3.1. Conventional cytogenetic analysis*

CCA was performed in 121 of 128 patients. Among them, 45 patients (37 %) showed a normal karyotype. Clonal chromosomal abnormalities were detected in 57 patients (47 %) including 42 cases with unbalanced chromosomal aberrations and 15 with balanced chromosomal aberrations. In the remaining 19 patients (16 %) no metaphase cells were obtained after short-term culture.

### *3.2. Overview of DNA sequence copy number changes detected by CGH*

Chromosomal gains and losses identified by CGH technique in 128 de novo AML cases are summarized in figure 1. A greater number of losses was observed as compared to gains (61 % versus 39 %), whereas partial chromosome changes were more common than whole chromosome changes (76 % versus 24 %). Therefore, the most frequent unbalanced chromosomal change was the partial loss (54 %). Indeed, chromosomes 8, 21 and 22 were often whole gained, whereas the most common partial chromosome gain was located at 3q. Additionally, chromosome 7 was the most frequent whole loss, whereas partial losses were mostly observed at 5q, 7q and 16q. High-level amplifications were identified in two cases.

### *3.3. CGH profile and karyotype*

Abnormal CGH profiles were observed in a total of 40 patients (31 %) including 32 patients with abnormal karyotype, 5 cases without mitosis (Table 1, cases 31 to 35), 2 cases in which CCA were not performed (Table 1, cases 36 and 37), and one case with normal karyotype (case 40). Of the group of 32 patients with abnormal karyotype, CGH detected identical chromosome changes in 16 cases, including 2 cases in which chromosomal breakpoints were refined (Table 1, cases 1 to 16). Indeed, CGH gave additional information compared to CCA in a total of 15 cases (Table 1, cases 17 to 31) and partially failed to identify chromosome changes previously observed by CCA (Table 1, case 39).

CGH detected normal profiles in the remaining 88 patients (69 %). Of these, 44 patients showed normal karyotype (data not show), 15 patients had balanced chromosome rearrangement as a sole abnormality (Table 2, cases 41 to 55), 14 cases had no metaphase cells (Table 2, cases 56 to 69), 5 cases were not previously studied by CCA (Table 2, cases 70 to 74), and 10 patients presented unbalanced chromosome alterations (Table 2, cases 75 to 84).

### *3.4. Complementary FISH and M-FISH analysis*

Thirteen patients were examined by FISH or M-FISH to reassess changes detected by CGH or CCA. These included 11 cases in which CGH results identified additional abnormalities compared to the CCA (Table 1, cases 17 to 19, 21, 23, 25 to 28, 32, and 40), and 2 cases in which CGH profiles were normal even though CCA revealed unbalanced alterations (Table 2, cases 78 and 84).

#### 3.4.1. FISH

-Case 16

In case 16, CGH detected loss of 16q13-qter whereas the karyotype was 46,XY,i(1)(q10),del(16)(p11). The CGH result was confirmed by FISH

using WCP-16p probe (Appligene Oncor-Qbiogene). Hybridized metaphases showed two signals, which were located in normal chromosome 16p and in derivative chromosome 16.

In case 18, the chromosomal origin of marker chromosome could not be identified by CCA. CGH found DNA copy number amplification in chromosome 21. FISH with WCP-21 probe (Appligene Oncor-Qbiogene) showed hybridization signals in both chromosome 21 (normal signal) and in each of the marker chromosomes (bigger signal). Cells with 1-4 copies of the marker chromosome were found. The marker chromosome was described as tas(21;21)(q11;q11), concluding that 4-10 copies of chromosome 21 were present in each cell.

-Case 21 and 22

The origin of the additional chromosome materials was detected as 11q22-qter by CGH. In case 21, FISH using WCP-11 (Appligene Oncor-Qbiogene) and LSI-MLL (Vysis) probes confirmed the partial trisomy of chromosome 11 as well as a gain copy of *MLL* gene (case partially reported, Aventín et al., 2003). Unfortunately, no material was available to perform complementary FISH analysis in case 22.

-Case 23

In case 23, CGH partially failed to detect the previously proposed CCA abnormalities. However, FISH analyses with WCP-11 (Appligene Oncor-Qbiogene), TEL-10p (Vysis) and LSI-MLL (Vysis) probes confirmed CGH results and discerned a t(10;11) that was not previously identified by CCA.

-Case 32

CGH detected several copy number changes in a patient with unsuccessful CCA. FISH with LSI-D13S25 (Vysis) was used to investigate the unbalanced region expanding from 13q12 to 13q21.1, as according to the hybridization image of chromosomes 13 nor to the CGH profile, it could include a gain limited by two different amplifications (Fig. 2A). Interphase FISH identified 83 % and 17 % of cells with 2 and 3 copies of the locus 13q14.3, respectively, confirming the presence of two separate

amplifications on 13q (Fig. 2B). No material was available to perform FISH analysis in the remaining informative cases of patients without metaphase cells or CCA. However, certain abnormalities, such as lost chromosome 7, or gained chromosomes 21 and 22 of cases 35, 37 and 36, were detected by CCA in a later study of the disease.

-Case 40

Indeed, in 1 of the 45 AML patients with normal karyotype, CGH detected a gain in 9p23. Metaphase FISH analysis with WCP-9 probe (Appligene Oncor-Qbiogene) showed two normal hybridization signals, which indicated that the 9p23 gain was not located as additional material in the karyotype. Whether this is a case of submicroscopic duplication of 9p23 remains to be investigated by FISH LSI probe.

-Case 78

The i(X)(p10) was confirmed by metaphase FISH with TEL-Xp/Yp probe (Vysis). FISH indicated that the frequency of clonal cells was as low as 4 %.

#### 3.4.2. M-FISH

In cases 25 to 28 and 19, whose karyotypes were complex, M-FISH analyses (Vysis) were performed to confirm CGH results and to reinterpret the final karyotye. The origin of marker chromosomes, unbalanced translocations or additional chromosome materials were corroborated by M-FISH. Unfortunately, there was no material available from cases 20 and 29-31.

Finally, the discrepancy in case 84 was resolved by M-FISH (Vysis), as the unbalanced chromosomal alteration add(9)(q32) observed by CCA was reinterpreted as a subtle balanced t(9;17)(?q;q?). Indeed, it should be mentioned that the M-FISH analysis was further informative, as it identified a t(9;11)(q21~q22;q23) despite of the initial t(9;11)(p12;q23).

## 4. Discussion

### *4.1. Recurrent copy number changes detected by CGH*

#### 4.1.1. Losses

In the present study 128 de novo AML patients were analyzed by CGH at the time of diagnosis. Our results showed that the most frequent unbalanced chromosomal change in AML patients was the partial loss, which agrees with the findings of El-Rifai et al. in a series of 25 refractory AML cases [11]. In detail, the most common loss was detected at the chromosomal region 5q (9 cases), followed by losses of chromosome 7 (9 cases), and deletions of 7q (6 cases) and 17p (5 cases) (Fig. 1). The majority of these copy number changes were not the sole abnormality in the karyotype. These observations correlate with the fact that 5q, 7q and 7 abnormalities have frequently been detected by CGH in AML patients with complex karyotype [12,13]. Indeed, it is well known that losses of 5q, 7q and 17 or monosomy 7 are associated with a poor response to therapy and considered to indicate worse prognosis of the disease [1,2,3,14]. This is in good agreement with the finding that these abnormalities were mostly detected in either refractory or poor prognosis AML cases (87.5 %). Interestingly, the 17p deletions detected in complex karyotypes were mostly associated with 5q deletions.
A finding that should be emphasized is the recurrent losses in 16q (5 cases). In AML, deletions of 16q commonly expanded the 16q22-qter region, and patients with del(16)(q22) tend to have a worse prognosis and are more likely to have complex karyotypes compared to 16q22 alterations, such as inv(16)/t(16;16)(p13;q22) [15,16]. Of the other chromosome 16q abnormalities, which have not been as well described, del(16)(q11) seems to be a recurrent genetic alteration in AML and to be associated with clonal evolution or disease progression [17]. In the present results, although heterogeneous 16q deletions were observed, we confirmed the recurrent loss of 16q11-qter (Fig.1). In 2 cases, 16q deletions were detected as an additional abnormality in the karyotype (Table 1, cases 17 and 23), whereas in 3 cases, 16q deletions appeared with multiple chromosome changes (Table 1, cases 19, 26 and 32). It should be noted that 16q deletions were not associated with M4 subtype.

#### 4.1.2. Gains

Fusion genes as a result of a chromosomal rearrangement are a common event in AML, and could be present in the karyotype in association with secondary abnormalities. These alterations are usually unbalanced chromosomal changes, such as +8, +22 and del(9q) [18]. They indicate clonal evolution of leukemic cells, and consequent disease progression. Thus, it is not surprising that gains of chromosome 8 and 22 were frequently identified in our samples (5 and 6 cases, respectively). Although the gain of chromosome 22 (6 cases) was mostly a secondary abnormality associated with inv(16) (Table 1, cases, 1, 5, 8 and 34), it was also present in complex karyotypes (Table 1, cases 26 and 27), and interestingly, it appeared differently, as a trisomy or as a whole duplication of chromosome 22. Indeed, in 2 of the cases, the gain of chromosome 8 was identified as a sole aberration (Table 1, case 11 and 13).

#### 4.1.3. Multiple gains and amplifications

Although all the recurrent copy number changes mentioned above are common abnormalities in AML, they represent large unbalanced regions, whose molecular equivalents have not yet been found. Besides, whereas gene amplifications are rarely associated with AML [11,19,20], their detection by CGH allows delimitation of a narrow altered region, which could localize an oncogene [21,22]. In the present series, we detected 2 cases with different amplification regions although these were not recurrent (Table 1, case 18 and 32). Of the few reported cases of gene amplifications in *de novo* AML, several were related either to previously anti-neoplasic therapy or exposure to potentially carcinogenic agents [23,24]. Family history of hematological disease, and previous exposure to dyes, occupational or environmental carcinogens or radiation were negative in both patients.

CGH was able to differentiate between a high-level amplification of a small region (case 32) and a multiple gain of a large region (case 18), suggesting that CGH could be used to accurately map amplicon locations, which is in

contrast with considerations in the literature [25]. Furthermore, when the 13q12-q21.1 amplification was examined, two separate regions of high-level amplifications, 13q12 and 13q21.1, were distinguished (Fig. 2A) whereas the CGH profile showed a sole amplification of 13q12-q21.1 bands (Fig. 2B). Separate independent amplifications have previously been described at 17q [25] and 20q [26], indicating the complex origin of some amplifications. Besides our case, 20q amplification was originally thought to consist of a single amplification unit by CGH [27]. If the two narrow amplifications could originate an unreliable gain in the intermediate region 13q13-q14 was excluded by investigating the 13q14.3 locus with interphase FISH (Fig. 2C). Both 13q12 and 13q21.1 are gene rich chromosome band related with few well-known proto-oncogenes. Mutations of *FLT3*, which is located at 13q12, have recently been associated with leukemogenesis of AML [28]. Unfortunately, no material was available to perform complementary molecular analysis to investigate the possible amplification of *FLT3*. Additional similar cases should therefore be investigated to clarify the genetic basis of these amplifications, as novel genes related to the pathogenesis of AML could be located at 13q12 as well as 13q21.1.

The proposed molecular equivalent of chromosome 21 amplification is the activation of *AML1(RUNX1)* oncogene located at 21q22 [29]. Amplification of *AML1* has been identified in either ALL [30,31,32,33] and AML [34], which could be originated through different chromosome 21 abnormalities [35]. In our case, amplification of chromosome 21 seems to be related with copy acquired of the marker chromosome described as tas(21;21)(q22;q22). It should be noted that telomeric associations (tas) have been described as a mechanism to confer chromosomal unstability [36].

### *4.2. Comparison between CGH and CCA results*

It has been well described that when AML cases are analyzed, the concordance between CGH and CCA results is usually high [19,37,38]. In our study, which included 121 patients studied by both techniques, we

observed a concordance of results around 62.8 %. In the remaining cases, CGH gave additional information compared to the CCA (17.3 %) and partially failed to identify the alterations previously detected by CCA (9.1 %).

#### 4.2.1. CGH brought additional information to the CCA

Although improvements are made in CCA, the metaphase analysis has several limitations, and in some cases, chromosomal alterations could remain unidentified or partially characterized. Accordingly, one of the CGH applications was to help in the diagnosis of several cytogenetical alterations (bold font in Table 1). Moreover, CGH detected subtle and cryptic copy number changes hidden in karyotypes (shaded font in Table 1) and could indicate the origin of additional chromosome material (grey font in Table 1). Furthermore, CGH allowed to identify either the origin of marker chromosomes (underlined font in Table 1) and to describe derivative chromosomes (cursive font in Table 1).

As the CGH technique is not dependent on obtaining dividing cells, it provided useful information of copy number changes in CCA cases without mitosis (Table 1, cases 32 to 36). Indeed, another application of CGH was to supply complementary genetic information to the CCA for describing complex karyotypes (Table 1, cases 25 to 31 and 18). However, M-FISH analysis was also needed in most of cases to propose the final karyotype.

#### 4.2.2. Identical results by CGH and CCA

In the present study, a concordance of results between CGH and CCA was observed in patients with an unbalanced karyotype (Table 1, cases 1 to 16). Interestingly, the CGH profile was useful to propose the break point assignation of the unbalanced chromosomal alterations in cases 15 and 16. Moreover, CGH did not identify copy number changes in patients with a balanced chromosome rearrangement as a sole abnormality in the karyotype, and in the majority of cases of the group of patients with normal karyotype.

The low frequency of copy number changes detected by CGH in these groups of patients agrees with previously reports [11,19,20,37,39]. Nevertheless, it should be mentioned that unbalanced abnormalities below to 5~10 Mb or close to telomeric regions remain undetected by CGH [40]. Whether or not these subtle genetic alterations are present in our series of patients should be studied by means of various approaches such us multiplex telomere assay (M-TEL) [41] or CGH array [42].

#### 4.2.3. CGH did not totally identify the abnormalities detected by CCA

The majority of the discrepancies detected in our series of patients stem from the limitations of the CGH technique, such as the insensitivity to detect unbalanced chromosomal changes that occur at low frequencies [42]. In the series analyzed we observed discrepancies when the percentage of blast cells in the sample (Table, cases 82 and 83), or the ratio of blast cells carrying the unbalance chromosomal alteration (Table 2, cases 75 to 80) were below the sensitivity threshold of the technique. However, CGH did not detect the gain of chromosome 8 in case 81, and in case 39, CGH did not totally identify the abnormalities detected by CCA. As there were no available fixed cells to perform complementary FISH analysis, we may hypothesize that the discrepancy between CCA and CGH results on trisomy 8 and 15 in case 81 and 39, respectively, could be related to heterogeneity of the leukemic cell population. However, not all the discrepancies observed between the two techniques were related to CGH limitations, which is reflected in case 84. In this case, the profile observed was normal, whereas CCA proposed an unbalanced chromosomal alteration. In relation, we performed M-FISH analysis, which diagnosed a balanced karyotype. It should thus be noted that in some cases, a discrepancy between CGH and CCA could be informative and, with complementary FISH analysis, it could be used to diagnose subtle chromosomal alterations in the karyotype.

In conclusion, our results show that CGH analysis provides CCA in the genetic diagnosis of AML. The application of CGH complementary to CCA increased the detection of unbalanced chromosomal alterations and allowed precise defining of partial or uncharacterized cytogenetic abnormalities. Identification of unbalanced chromosome regions involved in AML may help to clarify their role in the pathogenesis of AML.

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*Figure 1*

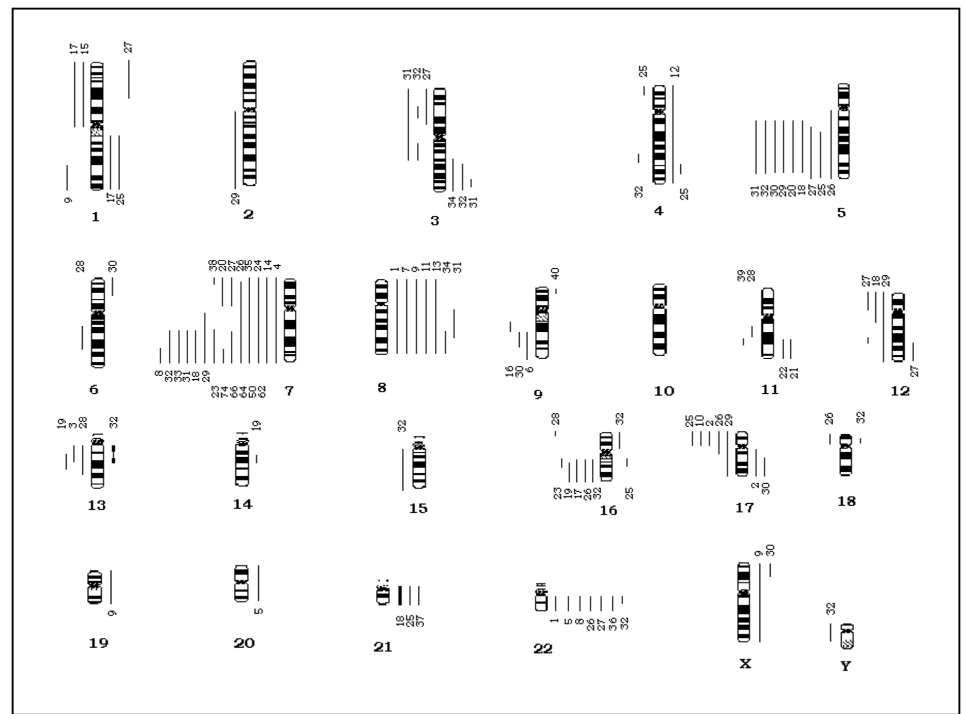


*Figure 2*

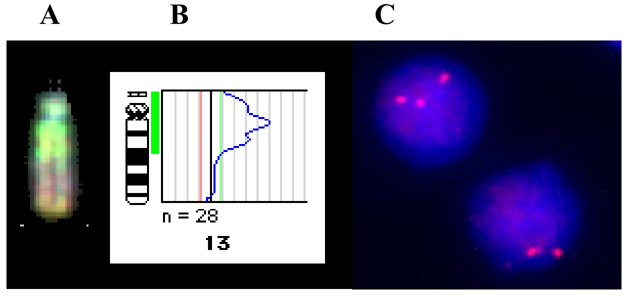

**Figure legends**

**Figure 1.** Distribution of the gains and losses of DNA sequence copy number in 128 adult AML patients analyzed by CGH at diagnosis. Losses are shown on the left side and gains on the right of each chromosome ideogram. Each line represents a genetic alteration seen in one patient, whose case number is indicated. Amplifications are shown as bold lines. Constitutional chromosomal alterations are excluded.

**Figure 2.** CGH and interphase FISH analyses of case 32. Two separate regions of high-level amplifications, 13q12 and 13q21.1, could be distinguished on hybridized 13 chromosomes (A), whereas the CGH profile showed a sole amplification of 13q12-q21.1 bands (B). Interphase FISH with LSI-D13S25 probe identified two or three copies of 13q14.3 locus, which confirmed the presence of a two independent amplification (C).

**Table 1.** AML cases with abnormal CGH profile

| # | Age/ Sex | FAB | Blast in BM (%) | Karyotype | CGH | FISH | Proposed Karyotype |
|---|---|---|---|---|---|---|---|
| *1* | 24 / M | M5 | 97 | 48,XY,+8,+22[12]* | +8<br>+22 | [43] | |
| *2* | 21 / F | M1 | 88 | 46,XX,i(17)(q10)[30] | +17q<br>-17p | - | |
| *3* | 21 / M | M5 | 100 | 46,XY,del(13)(q12q14)[7]/46,XY[7] | -13q12-q14 | - | |
| *4* | 30 / F | M1 | 65 | 45,XX,-7[18] | -7 | - | |
| *5* | 46 / F | M4 | 97 | 48,XX,inv(16)(p13q22),+20,+22[16] | +20<br>+22 | - | |
| *6* | 27 / M | M4 | 70 | 46,XY,del(9)(q22)[12]/46,XY[8]^ | -9q22-qter | - | |
| *7* | 17 / M | M5 | 99 | 47,XY,t(2;10)(p11;q13),+8[18]/46,XY[2] | +8 | - | |
| *8* | 48 / F | M4 | 60 | 47,XX,del(7)(q32),inv(16)(p13q22),+22[25] | +22<br>-7q32 | - | |
| *9* | 33 / F | M2 | 30 | 48,XX,del(1)(q32),+8,inv(12)(p11q14),+19[12]/<br>49,XX,+X,del(1)(q32),+8,inv(12)(p11q14),+19[3] | -1q32-qter<br>+8<br>+X<br>+19 | - | |
| *10* | 22 / F | M4 | 71 | 46,XX,t(10;11)(p13;q21),del(17)(p10)[15]/<br>46,XX[11] | -17p | - | |
| *11* | 59 / F | M5 | 52 | 47,XX,+8[20]^ | +8 | - | |
| *12* | 45 / F | M1 | 27$^{\Omega}$ | 47,XX,+4[20] | +4 | - | |
| *13* | 46 / M | M5 | 58 | 47,XY,+8[14],46,XY[6]^ | +8 | - | |
| *14* | 25 / M | M4 | 87 | 45,XY,-7[17]/46,XY[3] | -7 | - | |
| *15* | 16 / F | M5 | 88 | 46,XX,**del(1)(p?)**[12] | **-1p** | NMA | 46,XX,**del(1)(p11)** |
| *16* | 54 / M | M2 | 20$^{\Omega}$ | 46,XY,t(8;21)(q22;q22),**del(9)(q?)**[8]/46,XY[8] | **-9q13-q21** | - | 46,XY,t(8;21)(q22;q22),**del(9)(q13q21)**/46,XY |
| *17* | 57 / M | M2 | 63 | 46,XY,i(1)(q10),**del(16)(p11)**[9]/ 46,XY[8] | +1q21-qter<br>-1p<br>**-16q12.1-qter** | WCP-16p: 2 signals, on normal cr 16 and on cr der(16) | 46,XY,i(1)(q10),**del(16)(q11)**/ 46,XY |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***18*** | 50 / F | M5 | 46 | 47,XX,del(5)(q13q33),del(7)(q22),−12,der(16) t(12;16)(q13;q24),+2mar[9] | -5q13-q33<br>-12p<br>-12q11-q13<br>-7q22-qter<br>++21 | WCP-21: 4 signals, on each normal crs 21 and on each marker crs | 46−49,XX,del(5)(q13q33),del(7)(q22),−12, der(16)t(12;16)(q14;q24),+tas(21;21) (q22;q22)x1~4 |
| ***19*** | 53 / F | M5 | 70 | 45,XX,t(14;4;13;3)(q12;q25;q14;p13−p14), *der(16)t(16;?)(q13;?)*[16] | *+14q21*<br>-13q14-q21<br>*-16q13-qter* | M-FISH:<br>der(3)t(3;13)(p13−p14;q14)<br>der(4)t(4;14)(q25;q12),<br>der(13) t(4;13)(q12;q13),<br>der(16)t(14;16)(q12;q12) | 45,XX,t(14;4;13;3)(q12;q25;q13;p13−p14), *der(16)t(16;14)(q12;q12)* |
| ***20*** | 31 / M | M5 | 54 | 45,XY,t(4;3;7)(q21;q25;q32),-5[12] | -5q13-q33<br>-7p<br>-7q32-qter | MNA | 45,XY,der(3)t(3;4)(q2?;q12),der(4)t(4;3;7) (q12;q2?;q11),del(5)(q13q33),−7 |
| ***21*** | 42 / F | M1 | 90 | 46,XX,**add(18)(p11.2)**[9]/46,XX[21] | **+11q21-qter** | [44] | 46,XX,**der(18)t(11;18)(q21~q22;p11.2)**/46,XX |
| ***22*** | 42 / F | M4 | 31 | 46,XX,inv(16)(p13q22)[6]/<br>46,XX,add(7)(p22),inv(16)(p13q22)[7] | **+11q21-qter** | - | 46,XX,inv(16)(p13q22)/<br>46,XX,der(7)t(7;11)(p22;q21),inv(16)(p13q22) |
| ***23*** | 30 / M | M1 | 96 | 46,XY,del(11)(q?),**del(16)(q?)**[20]/<br>46,XY,+7,-10[2]^ | **-16q12.1-q21** | WCP-11: 3 signals, on normal cr 11, on cr der(11) and on cr der(10)<br>Tel 10q: 2 signal, on cr der(10) and on normal cr 10<br>LSI MLL: 2 fusions, on normal cr 11 and on cr der(10) | 46,XY,t(10;11)(p15;q22~23),**del(16)(q11q21)**/<br>46,XY,+7,-10 |
| ***24*** | 41 / F | M0 | 80 | 45,XX,-2,add(3)(q?),-7,+mar[13] | -7 | - | 45,XX,t(2;3)(p16;q24),-7 |
| ***25*** | 49 / M | UNC | 58 | 47,XY,+*der(1)t(1;?)(q21;?)*,−4,−5,−16,**add(17) (p13)**, −19,-21,+5mar,frag[10]/<br>47,XY,+*der(1)t(1;?)(q21;?)*,−4,−5,−16,**add(17) (p13)**, −19,-21,+5mar,1dm[2]/46,XY[6] | **-17p**<br>-4p16<br>**-5q15-qter**<br>*+1q21-qter*<br>*+21*<br>+16q12-q13<br>+4q31.3-q32 | M-FISH:<br>der(1)t(1;21)(q21;p10)<br>der(4)t(4;19)(q?;p13)<br>der(5)t(5;17)(?;q10)<br>der(21)t(16;21)(?;p10)<br>der(21)t(19;21)(p13;p10)<br>der(19) | 47,XY,+*der(1)t(1;21)(q21;p10)*,−4,−5,**der(17) t(5;17)(q1?;q10)**,del(19)(?q),+der(19) t(4;19)(q31;p13),der(21)t(16;21)(q12;p10), +der(21)t(19;21)(p13;p10) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***26*** | 53 / M | M5 | 90 | 45,XY,del(5)(q?),−7,**del(16)(q?)**,−17,−18,del(22)(q?),+2mar[16] | -5q<br>-7p11-p15<br>-7q<br>**-16q12.1-qter**<br>-17q11-q12<br>-17p<br>-18p<br>+22 | M-FISH:<br>der(5)t(5;19)<br>der(7)<br>der(12)t(12;18)<br>del(16)<br>-17<br>der(18)t(17;18)<br>der(22) | 45,XY,t(5;19)(q10;p10),der(?)t(?;7)(?;p21),der(12)t(12;18)(p?;q?),−17,**del(16)(q11)**,der(18)t(17;18)(q21;q?),dup(22)(q?) |
| ***27*** | 43 / M | M4 | 67 | 45,XY,del(3)(p?),**add(4)(q?)**,−5,−7,−12,**add(14)(p?)**,**add(22)(q?)**,+2mar[12]/46,XY[2] | **+1p31-pter**<br>-3p14-pter<br>-5q14-qter<br>-7p<br>-7q22-qter<br>-12p<br>-12q21<br>**+12q22-qter**<br>+22 | M-FISH:<br>der(3)t(3;5)(p?;q?),<br>der(12)t(7;12)(q?;?),<br>der(14)t(1;14)(p?;p10),<br>dup(22)(q?q?)<br>ins(4;12)(q?;q?) | 45,XY,der(3)t(3;5)(p13;q13),**ins(4;12)(q12;q22q24?)**,−5,t(7;12)(q11;q11?),**der(14)t(1;14)(p31;p10)**,**dup(22)(q11.2q13)** |
| ***28*** | 39 / M | M0 | 66 | 46,XY,**del(6)(q15)**,**del(11)(p11)**,-13,+1mar[9]/<br>47,XY,**del(6)(q15)**,**del(11)(p11)**,-13,+2mar[2] | **-6q15-q22**<br>**-11q13-q14**<br>-13q12-q22<br>-16p13 | M-FISH:<br>del(6)<br>del(11)<br>del(13)<br>+der(22) | 46,XY,**del(6)(q15q22)**,**del(11)(q13q14)**,del(13)(q12q22),del(16)(p13)/<br>47,XY,**del(6)(q15q22)**,**del(11)(q13q14)**,del(13)(q12q22),del(16)(p13),+del(22)(q?) |
| ***29*** | 58 / F | M1 | 93 | 43,X,-X,*der(2)t(2;?)(q?;?)*,del(5)(q13q33),del(6)(q23),*−7*,−12,der(22)t(?X;22)(?;q12)[20] | *-2q*<br>-5q13-q33<br>*-7q11.2-ter*<br>*-17*<br>-12 | NMA | 43,X,−X,*der(2)t(2;?)(q10;?)*,del(5)(q13q33),del(6)(q23),*−7*,−12,*−17*,der(22)t(?X;22)(?;q12),*+2mar* |
| ***30*** | 46 / F | M0 | 95 | 47,XX,add(1)(p?36.1),**add(4)(q34)**,del(5)(q13q33),+8,?del(8)(q?),**add(10)(p?)**,del(11)(q21q23),**add(12)(p13)**[12]/<br>46,XX,add(1)(p?36.1),**add(4)(q34)**,del(5)(q13q33),+8,?del(8)(q?),**add(10)(p?)**,del(11)(q21q23),**add(12)(p13)**,-13[4]/<br>46,XX,add(1)(p?36.1),der(3)t(3;?)(q29;?),**add(4)(q34)**,del(5)(q13q33),+8,?del(8)(q?),**add(10)(p?)**,del(11)(q21q23),**add(12)(p13)**,-13[3] | -5q13-q33<br>-9q22-q32<br>**+6p21.3-pter**<br>**+17q21-qter**<br>**+Xp22.1-p21** | MNA | 47,XX,add(1)(p?36.1)?,**der(4)t(4;6)(q34;p21)**,del(5)(q13q33),+8?,?del(8)(q?),**der(10)t(10;17)(p15;q21)**,del(11)(q21q23)?,**der(12)t(X;12)(p22;p13)** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***31*** | 40 / F | M4 | 35 | 42~47,XX,–3,del(5)(q13q33),–6,–7,–8,–12,<br>+1~6mar[20] | -5q13-q33<br>-7q22-qter<br>-3q11-q21<br>-3p<br>+3q26.1-q27<br>+8q12-q22 | MNA | 42~47,XX,–3,del(5)(q12q33),–6,del(7)(q22),–8,<br>-12,+1~5mar |
| ***32*** | 48 / M | M1 | 81 | WM | -3p21<br>-3q13.1-3q21<br>-5q13-q33<br>-4q28<br>-7q22-qter<br>-16q12.1-qter<br>-15<br>-Y<br>+3q24-qter<br>+16p<br>+22q11.2<br>+18p11.2<br>++13q12<br>+13q13-q14<br>++13q21.1 | LSI-D13S25: 3 (17 %) and<br>2 (83 %) signals on<br>interphase | |
| ***33*** | 50 / F | M2 | 46 | WM | -7q22-qter | NMA | |
| ***34*** | 53 / M | UNC | 43 | WM | +8q22-qter<br>+3q22-qter | NMA | |
| ***35*** | 44 / M | M4 | 32 | WM | -7 | NMA | |
| ***36*** | 53 / F | M4 | 50 | WM* | +22 | NMA | |
| ***37*** | 50 / F | M4 | 82 | ND | +21 | NMA | |
| ***38*** | 53 / F | M2 | 24$^{\Omega}$ | ND | -7p21-pter<br>-7q22-qter | NMA | |
| ***39*** | 60 / F | M2 | 44 | 48,XX,t(8;21)(q22;q22),del(11)(q21q23),+15,<br>+21c[20] | -11q21q23<br>+21 | MNA | 47,XX,t(8;21)(q22;q22),del(11)(q21q23),+21c/<br>48,XX,t(8;21)(q22;q22),del(11)(q21q23),+15,<br>+21c |
| ***40*** | 54 / F | M2 | 58 | 46,XX[20] | +9p23 | WCP-9: 2 signals, on both<br>crs 9 | |

M, male; F, female; UNC, unclassified; MNA, material not available; WM, without metaphases; cr, chromosome; ND not done; BM, bone marrow; $^{\Omega}$, typified as AML according to WHO classification; *, *CBFB/MYH11* detected by QRT-PCR; ^ negative QRT-PCR for *CBFB/MYH11* and *AML1/ETO*, and negative Southern-blot for *MLL* rearrangement; +, gain; -, loss; ++, multiple gain or amplification; **font**, CGH result was useful to propose the break point related to the cytogenetic alteration; font, CGH result allowed to identify the origin of marker chromosome; **font**, CGH result helped to identify the origin of the additional chromosomal material; *font*, CGH result allowed to describe the derivative chromosome; font, CGH detected a subtle chromosome alteration not previously observed in the karyotype; #, case number; CGH was more informative than ACC; CGH was less informative than ACC

**Table 2.** AML cases with normal CGH profile

| # | Age/ Sex | FAB | Blast in BM (%) | Karyotype | CGH |
|---|---|---|---|---|---|
| ***41*** | 55 / F | M4 | 90 | 46,XX,inv(16)(p13q22)[40] | NAD |
| ***42*** | 22 / M | M4 | 90 | 46,XY,inv(16)(p13q22)[9]/46,XY[2] | NAD |
| ***43*** | 44 / F | M4 | 50 | 46,XX,inv(16)(p13q22)[11]/46,XX[10] | NAD |
| ***44*** | 52 / M | M2 | 67 | 46,XY,inv(16)(p13q22)[15]/46,XY[7] | NAD |
| ***45*** | 52 / M | M4 | 60 | 46,XY,inv(16)(p13q22)[9]/46,XY[2] | NAD |
| ***46*** | 24 / F | M2 | 67 | 46,XX,inv(16)(p13q22)[25] | NAD |
| ***47*** | 43 / M | M5 | 42 | 46,XY,inv(16)(p13q22)[20] | NAD |
| ***48*** | 29 / M | M4 | 90 | 46,XY,t(8;16)(p11;p13)[20] | NAD |
| ***49*** | 42 7 M | M2 | 45 | 46,XY,t(2;14)(q21;q22)[10]/46,XY[10] | NAD |
| ***50*** | 29 / F | M5 | 79 | 46,XX,t(9;11)(p22;q23)[16]/46,XX[3] | NAD |
| ***51*** | 25 / F | M5 | 100 | 46,XX,t(9;11)(p22;q23)[12] | NAD |
| ***52*** | 38 / F | M5 | 90 | 46,XX,t(6;11)(q27;q33)[27]/46,XX[2] | NAD |
| ***53*** | 34 / M | M2 | 49 | 46,XY,t(8;21)(q22;q22)[10] | NAD |
| ***54*** | 30 / M | M4 | 67 | 46,XY,t(5;12)(p14;p14),inv(16)(p13q22)[15]/46,XY[5] | NAD |
| ***55*** | 35 / M | M4 | 77 | 46,XY,inv(14)(q11q32)[18]/46,XY[2] | NAD |
| ***56*** | 58 / M | M5 | 50 | WM | NAD |
| ***57*** | 22 / F | M1 | 90 | WM | NAD |
| ***58*** | 60 / M | M4 | 58 | WM | NAD |
| ***59*** | 49 / M | M4 | 86 | WM | NAD |
| ***60*** | 54 / F | M5 | 32 | WM | NAD |
| ***61*** | 58 / M | M2 | 82 | WM | NAD |
| ***62*** | 25 / M | M0 | 91 | WM | NAD |
| ***63*** | 60 / M | M1 | 90 | WM | NAD |
| ***64*** | 36 / M | M0 | 60 | WM | NAD |
| ***65*** | 19 / F | M2 | 46 | WM | NAD |
| ***66*** | 40 / F | M4 | 70 | WM | NAD |
| ***67*** | 35 / F | UNC | 32 | WM | NAD |
| ***68*** | 45 / F | M5 | 88 | WM | NAD |
| ***69*** | 54 / M | M5 | 80 | WM | NAD |
| ***70*** | 24 / F | M2 | 55 | ND | NAD |
| ***71*** | 34 7 F | M1 | 74 | ND | NAD |
| ***72*** | 48 / M | M1 | 77 | ND | NAD |
| ***73*** | 47 / F | M1 | 84 | ND | NAD |
| ***74*** | 51 / F | M2 | 78 | ND | NAD |
| ***75*** | 53 / F | M1 | 100 | 45,XX,-22[3]/46,XX[25] | NAD |
| ***76*** | 58 / M | M4 | 89 | 46,XY,1dmin[2]/ 46,XY[21] | NAD |
| ***77*** | 26 / M | M1 | 79 | 46,XY,del(10)(q23q24),del(20)(q11q13)[6]/ 46,XY[14] | NAD |
| ***78*** | 54 / F | M2 | 40 | 46,X,i(X)(p10)[6]/46,XX[14] | NAD |
| ***79*** | 29 / F | M0 | 47 | 46,XX,del(13)(q12q14-21)[2]/46,XX[20] | NAD |
| ***80*** | 40 / M | M4 | 78 | 46,XY,del(16)(q22)[4]/46,XY[16] | NAD |
| ***81*** | 36 / M | M5 | 55 | 47,XY,+8[4]^ | NAD |

| 82 | 17 / F | M5 | $24^{\Omega}$ | 48,XX,+3,t(4;15;18)(q12;q21;q21),+7,der(10)t(10;17)(p15;q?),–17[7]/ 49,XXX,+3,t(4;15;18)(q12;q21;q21),+7,der(10)t(10;17)(p15;q?),-17[11] | NAD |
|---|---|---|---|---|---|
| 83 | 32 / M | M1 | 74 | 46,XY,t(3;12)(q26;p12),-7,+mar[22] | NAD |
| 84 | 57 / M | - | 57 | 46,XY,t(9;11)(p12;q23),add(9)(q32)[18][Y] | NAD[Y] |

M, male; F, female; UNC, unclassified; NAD, no abnormality detected; WM, without metaphases; ND not done; BM, bone marrow; $^{\Omega}$, typified as AML according to WHO classification; *, *CBFB/MYH11* detected by QRT-PCR; ^ negative QRT-PCR for *CBFB/MYH11* and *AML1/ETO*, and negative Southern-blot for *MLL* rearrangement; [Y] The discrepancy was further studied by M-FISH, which described the karyotype as 46,XY,t(9;11)(q21~q22;q23),t(9;17)(?q;q?); #, case number; [dark shading] CGH was more informative than ACC; [hatched shading] CGH was less informative than ACC