CLINICAL CASES

Cytogenetic analysis and FISH of terminal deletion of the long arm of chromosome 9 in a patient with acute promyelocytic leukemia

Gonzalo Vásquez-Palacio,1 Olga Botero,1 Margarita Sierra,2 Taknida Tubo,3 Fabiola Quintero-Rivera.4*

1Medical Genetics Unit, School of Medicine, University of Antioquia, Medellín, Colombia.
2Department of Hematology, Children’s Hospital, Hospital Universitario San Vicente de Paúl, Medellín, Colombia.
3Department of Genetics and Development, Columbia University, New York, NY, United States of America (USA).
4The David Geffen School of Medicine at UCLA, Department of Pathology and Lab Medicine, Los Angeles, CA, USA.


Abstract
Deletions of the long arm of chromosome 9, del(9)(q22), are rare aberrations specifically found in acute myeloid leukemia (AML). Yamamoto et al., 1999, reported the first case of acute promyelocytic leukemia (APL) with a terminal 9q deletion as a sole abnormality. Here we describe the second case with the same aberration, the patient, an eleven-years-old girl with APL. Chromosomal analysis by the Giemsa R-banding technique and FISH using BCR/ABL and PML/RARA probe on short-term cell cultures from bone marrow was performed. A deletion of a 9 chromosome, del(9)(q22) was detected. Deletions of 9q have been described in about 3% to 4% of the AML patients, especially in M1 and M2 myeloid leukemia. Sole 9q terminal deletions, are less common than interstitial ones and involve q21~q22 band predominantly. A recent study suggests that 9q deletion, even in the absence of t(15;17), shows a relatively good prognosis. However, our patient died during the treatment.

Análisis citogenético y FISH de la deleción terminal del brazo largo del cromosoma 9 en un paciente con leucemia promielocítica aguda

Resumen
Las delecciones del brazo largo del cromosoma 9, del(9)(q22), son raras, y se observan especificamente en la Leucemia Mieloide Aguda (LMA). Yamamoto et al., 1999, publicó el primer caso de leucemia promielocítica aguda (LPA) con una deleción terminal 9q, como la única aberración cromosómica presente. Aquí nosotros describimos el segundo caso con la misma aberración. El paciente, una mujer de 11 años de edad con LPA. El análisis cromosómico fue realizado en cultivos celulares de médula ósea usando la técnica de bandas R con Giesma y FISH con sondas para detectar las fusiones BCR/ABL y PML/RARA. Fue identificada la deleción de una copia del cromosoma 9, del(9)(q22).

*Corresponding author:* Fabiola Quintero-Rivera, M.D., F.A.C.M.G. 1000 Veteran Avenue, 22-26, Los Angeles, CA 90024 Telephone: 310-794-1287, Fax: 310-794-5099 E mail: fquintero@mednet.ucla.edu <mailto:fquintero@mednet.ucla.edu>

1665-5796 © 2009 Revista Medicina Universitaria. Facultad de Medicina UANL. Publicado por Elsevier México. Todos los derechos reservados.
Introduction
According to FAB classification (French - American - British cooperative group), acute promyelocytic leukemia (APL) is defined as an acute myeloid leukemia (AML) subtype M3.1 APL is distinguished from other acute myeloid leukemias (AMLs) by cytogenetic, clinical, and also biological characteristics,2-6 Although the basis of the diagnosis remains in the morphological characteristics, additional studies including immunophenotyping, cytogenetic evaluation, and molecular genetic analysis have become critical, and in some specific cases mandatory and complementary tools.4

APL typically carries a specific reciprocal chromosome translocation t(15;17) in the 95% of cases, leading to the expression of a leukemic-generating fusion protein, PML-RARα,2,5,6 The APL molecular defect was found to be the disruption of the alpha receptor of retinoid acid (RARα) and its reciprocal, in frame fusion, with one of five partner genes: PML, located in chromosome 15q22, PLZF in 11q23, NuMA in 11q13, NPM in 5q32 and STAT5b in 17q11.2.5 The cytogenetic abnormality in rare cases involves reciprocal translocations of chromosomes 5, 6, 7, 8, 11, 13, 19 and deletion (9q-).5

Terminal deletions of the long arm of chromosome 9, del (9q22-ter), are rare structural aberrations specifically found in AML.7 Yamamoto et al, reported the first case of APL with a terminal 9q deletion as sole abnormality.8 Here we describe the second case with the same structural aberration.

Case report
An eleven years old girl was admitted to the Pablo Tobón Uribe Hospital, Medellín, Colombia, in February 2000 with severe fatigue, and fever. Physical examination showed fever, tachycardia, pallor, weight loss, hepatomegaly and splenomegaly. Blood analysis revealed a hemoglobin level of 8.8g/dL, platelet count 12x10⁹/L, and white blood cells count of 55.2 x 10⁹/ul with 12% neutrophils, 15% lymphocytes, and 71% blasts, diagnostic of acute leukemia. Examination of the bone marrow showed myeloid blasts (40%)

Figure 1. R-banded karyotype of the patient: 46,XX,del(9)(q22). The arrowhead indicates del(9)(q22)
Materials and methods

Chromosome analysis
Chromosomal analysis was performed on unstimulated 24-hours in vitro culture of a bone marrow specimen by the Giemsa R banding technique. Thirty metaphases and 300 nuclei were analyzed. Karyotype was described according to the International System for Human Cytogenetic Nomenclature.9

Fluorescence in situ hybridization (FISH) analysis
FISH to interphase nuclei and metaphase chromosomes was performed at the cytogenetics laboratories of Columbia University and UCLA, using a Locus Specific Identifier BCR/ABL Dual Color-Dual Fusion and PML/RARA Translocation Probe (Vysis, Inc.).

Results

Chromosome analysis
Chromosome analysis of bone marrow cells showed 46,XX,del(9)(q22.3) in all 30 metaphases (figure 1).

Fluorescence in situ hybridization (FISH) analysis
FISH analysis to interphase nuclei and metaphase chromosomes revealed del (9)(q22) as shown by the absence of one signal on chromosome 9 (figure 2).

Discussion

In this study, R-banding and chromosome painting analyses showed both normal chromosome 9, and del(9)(q22). Deletions of the long arm of chromosome 9, del(9)(q22), are rare structural aberrations specifically found in acute myeloid leukemia (AML) as a sole chromosomal abnormality or as a secondary change, particularly together with translocations as t(8;21)(q22;q22).10 Terminal deletions of 9q are less common than interstitial ones and involve 9q21~9q22 as observed in this case.10 The most common breakpoints proximal and distal present in the interstitial deletions are 9q21 and 9q22 respectively.11 Deletions 9q—occurs predominantly in APL subtype M1 and M2, but only seven cases of APL with 9q—deletions have been reported to date.7 Yamamoto et al., reported the first case of APL with a terminal deletion of 9q— as sole chromosomal abnormality.4 Our study is the second case with the same abnormality.

Chromosomal rearrangements in addition to t(15;17) have been reported in 25%~40% of APL.2,12-19 The most frequent additional change was trisomy 8. Other abnormalities were far less frequent and usually involved chromosome 9, 17, 7, 21, 16, 6 and 12. The prognostic value of those additional cytogenetic abnormalities in APL patients remains controversial: Hiorns et al, found them to be associated with a poorer prognosis, Schoch et al, and Grimwalde et al., found them to be associated with a similar prognosis and Slack et al, with a slightly better prognosis.10,14,16,19
As shown in Table 1, nine cases of APL with 9q- have been reported. All but the last two cases had t(15;17) and five of them had terminal deletions. No specific morphological feature has been reported in APL with 9q_. Survival in five of these cases seems to be very short, although four cases were treated without ATRA. FISH performed in our patient with a DNA probe flanking the breakpoints of t(15;17) did not show the retinoic acid receptor alpha (RARA*)/PML fusion signal usually gene-rated on the der(17)t(15;17) (Figure 3). This result does not exclude the diagnosis of APL. It is possible that this patient had a cryptic chromosome rearrangement undetectable by karyotype and FISH analysis. This is known to occur in approximately 1% of APL cases. In such cases, reverse-transcriptase PCR, RT-PCR, is the best test to perform with a detection sensitivity of ~1 in 10^5 cells compare to the 1% sensitivity of FISH. Unfortunately, RT-PCR analysis was not performed in our patient. At the time of diagnosis this test was not available for clinical diagnosis. Nowadays, it is widely available, not only to identify a PML-RARA fusion due to a cryptic rearrangement in patients with flow cytometric and bone marrow morphology features characteristic of APL but also to follow up patients for minimal residual disease. A fresh sample for RNA extraction is needed to perform the RT-PCR, however, this is not available in our case.

The present patient did not respond to chemotherapy with cytarabine and mercaptanopurine and died in the course of the treatment four months after the diagnosis of APL. Therefore, our report suggests that 9q- may also be an adverse prognostic factor in APL subtype AML, M3. More cases need to be investigated to elucidate the precise role of 9q- in the pathogenesis of APL and its clinical significance.

### Table 1. Reported cases of APL with 9q deletion

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Karyotype</th>
<th>Survival (weeks)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>38/M</td>
<td>46,XY,del(7)(q22),del(9)(q22),t(15q+;17q-)</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>22/M</td>
<td>46,XY,del(9)(q22?),t(15q+;17q-)</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>50/F</td>
<td>46,XX,del(9)(q22?),t(15q+;17q-)</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>30/F</td>
<td>46,XY,del(9)(q21q33?),t(15q+;17q-)</td>
<td>9a</td>
<td>12</td>
</tr>
<tr>
<td>NA</td>
<td>46,X?,del(9)(q?),t(15;17)</td>
<td>NA</td>
<td>21</td>
</tr>
<tr>
<td>59/M</td>
<td>46,XY,del(9)(q11q23),t(15;17)(q22;q21)</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>38/M</td>
<td>46,XY,del(9)(q1?q22),t(15;17)(q22;q21)</td>
<td>NA</td>
<td>22</td>
</tr>
<tr>
<td>25/F</td>
<td>46,XX,del(9)(q22)</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>11/F</td>
<td>46,XX,del(9)(q22)[30]</td>
<td>16</td>
<td>Present case</td>
</tr>
</tbody>
</table>

NA: not available
*aSurvival from chromosome analysis at relapse

### References