Table 1. Baseline demographic characteristics and mean values for the three hypercoagulative markers in the patients and in the control subjects.

Variable	*Hip*	*Knee*	*Controls*
Number of cases	53	26	33
Age (years)			
Mean	64	67	68
Percentiles 25-75%	60-73	64-70	62-73
Sex			
Male	27	3	17
Female	26	23	16
Indication for surgery *(more than one diagnosis could be present in the same patient)			
Osteoarthrosis	39	23	–
Necrosis	10	1	–
Rheumatoid arthritis	7	2	–
Miscellaneous	2	2	–
Markers			
Mean			
D-D (ng/mL)	1,135.5	847.4	727.5
TAT (µg/L)	5.3	9.0	2.8
F1+2 (nmol/L)	1.7	2.6	1.5
Percentiles 25-75%			
D-D (ng/mL)	665.14-1,339.43	403.42-992.27	365.03-812.4
TAT (µg/L)	2.1-7.6	2.05-9.8	2-2.9
F1+2 (nmol/L)	1.4-2.1	1.3-3.0	1.2-1.7

Keys words

Hypercoagulability, hip and knee arthroplasty, venous thromboembolic disease

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References

1. Francis CW, Marder VJ, McCollister E, Yaukoolbodi S. Two-step warfarin therapy. JAMA 1983; 249: 374-8.
2. Stulberg BN, Insall JN, Williams GW, Ghelman B. Deep-vein thrombosis following total knee replacement: an analysis of six hundred and thirty-eight arthroplasties. J Bone Joint Surg 1984; 66-A:194-201.
3. Whitehouse S, Wrawick D. Clinical thromboembolism after knee replacement. XVIth Congress of the International Society on Thrombosis and Haemostasis. Florence, Italy, June 1997 [abstract]. Thromb Haemostas Suppl. June 1997. p. 718.
4. Hursting MJ, Stead AG, Crout FV, Horvath BZ, Moore BM. Effects of age, race, sexe and smoking on prothrombin fragment 1,2 in healthy population. Clin Chem 1993; 39:683-6.
5. Bauer KA, Weiss LM, Sparrow D, Vokonas PS, Rosenberg RD. Aging-associated changes in indices of thrombin generation and protein C activation in humans. Normative aging study. J Clin Invest 1987; 80:1527-34.
6. Mari D, Mannucci PM, Coppola R, Bottaso B, Bauer KA, Rosenberg RD. Hypercoagulability in centenarians: the paradox of succesful aging. Blood 1995; 85: 3144-9.
7. Sue-Ling HM, Johnston D, McMahon MJ, Philips PR, Davies JA. Pre-operative identification of patients at high risk of deep venous thrombosis after elective major abdominal surgery. Lancet 1986; i:1173-6.
8. Vogel G, Dempfle C-E, Spannagl M, Leskopf W. The value of quantitative fibrin monomer determination in the early diagnosis of postoperative deep vein thrombosis. Thromb Res 1996; 81:241-51.
9. Rocha E, Alfaro MJ, Páramo JA, Cañadell JM. Preoperative identification of patients at high risk of deep venous thrombosis despite prophylaxis in total hip replacement. Thromb Haemostas 1988; 59:93-5.
10. Jorgesen LN, Lind B, Hauch O, Leffers A, Albrecht-Beste E, Konradsen LAG. Thrombin-antithrombin III-complex and fibrin degradation products in plasma: surgery and postoperative deep venous thrombosis. Thromb Res 1990; 59:69-76.

Cryptic insertion (15;17) in a case of acute promyelocytic leukemia detected by fluorescence *in situ* hybridization

Sir,

We report the case of a patient with acute promyelocytic leukemia (APL) with no detectable cytogenetic abnormalities. Fluorescence *in situ* hybridization (FISH) studies demonstrated an insertion of the RARα gene into one copy of chromosome 15. RT-PCR studies showed a PML/RARα transcript. The patient achieved complete remission with chemotherapy and ATRA, but relapsed during maintenance therapy with ATRA.

Acute promyelocytic leukemia (APL) is characteristically associated with the reciprocal chromosomal translocation t(15;17)(q22;q21) which is identified in up to 90% of cases by conventional cytogenetics. However, a few cases with submicroscopic rearrangements of RARα gene have been described.[1]

A 27-year-old man was admitted to our hospital because of a one-week history of weakness and fever. Blood cell count showed: Hb 79 g/L; WBC 45×10^9/L with 79% hypergranular blast cells and platelets 39×10^9/L. The bone marrow findings were consistent with classical APL (AML-M3) according to the FAB criteria. The immunophenotype showed: $CD13^+$, $CD33^+$, $HLA\text{-}DR^-$ and $CD34^-$. He was treated according to the European APL/93 protocol (ATRA in combination with cytosine arabinoside and daunorubicin) and achieved a complete remission on day 30 of treatment. The patient relapsed, 20 months after diagnosis, during maintenance therapy with ATRA. A second remission was obtained with Ara-C, mitoxantrone and etoposide. Afterwards, he received an allogeneic peripheral blood stem cell transplantation (PBSCT) from his HLA-identical sister. The patient developed a veno-occlusive disease and acute graft-versus-host dis-

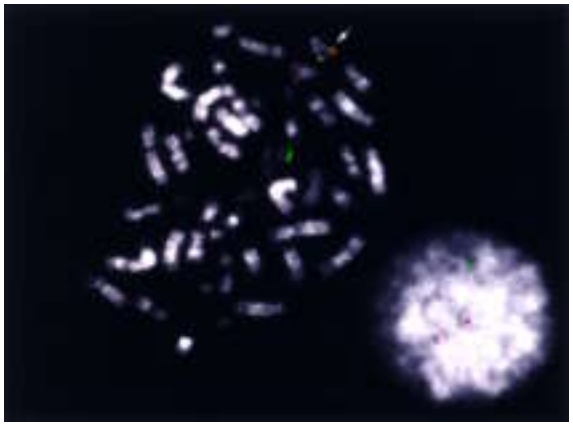

Figure 1. FISH study with specific PML (red)-RARα (green) probe. The PML-RARα fusion is the result of the interstitial insertion of RARα gene into PML gene on chromosome 15 (arrow).

ease and died on day 40 after PBSCT.

Cytogenetics: at the time of diagnosis and relapse, bone marrow samples were cultured for 48 hours according to standard procedures. A normal karyotype was observed in the 20 metaphases examined.

FISH: two-color FISH was performed using painting probes for whole chromosomes 15 (Cambio, Cambridge, UK) and 17 (Oncor, Gaithersburg, MD, USA) and revealed two intact copies of both chromosomes in the 35 metaphases analyzed. An APL t(15;17) translocation probe (Vysis, Stuttgart, Germany) demonstrated the presence of the PML/RARα fusion gene on one copy of chromosome 15 (Figure 1).

RT-PCR: in vitro reverse transcription (RT) of 1 μg of total RNA to cDNA and RT-PCR amplification of PML/RARα and RARα/PML fusion transcripts were performed using standard methods (GeneAmp RNA PCR kit; Perkins Elmer-Cetus, Norwalk, CT, VSA). A PML/RARα transcript of the bcr-1 type (DNA fragment of 326 bp) was observed, however the reciprocal RARα/PML transcript failed to be amplified.

This report describes an interstitial insertion of RARα gene from chromosome 17 into the PML gene on chromosome 15 in an APL patient with an apparently normal karyotype. The cryptic PML/RARα rearrangement was detected by FISH with an APL t(15;17) probe and was confirmed by RT-PCR, which showed the presence of a hybrid PML/RARα transcript but not of the reciprocal RARα/PML transcript. A number of variant translocations associated with APL including submicroscopic translocations have been described.[2-7] However, the characterization by FISH of cases with cryptic PML/RARα rearrangements in apparently normal chromosomes 15 and 17 is unusual.[6-8] The presence of the PML/RARα fusion gene determines the sensitivity to ATRA treatment, while the cytogenetic variants of APL not leading to a PML/RARα fusion, for instance t(11;17) and t(5;17), fail to respond to ATRA.[9,10] Although the molecular consequences of this interstitial insertion are apparently identical to those observed in the classic RARα rearrangement, the molecular mechanisms should be different since another chromosome break distal to RARα has been produced to allow the insertion. Whether or not this different molecular mechanism implies a different clinical course and an unfavorable prognostic factor which could be related to the relapse of the patient during maintenance therapy with ATRA needs to be clarified.

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Funding

Partially supported by a grant from Fundación Ramón Areces, Spain.

Key words

Acute promyelocytic leukemia, insertion (15;17), cytogenetics, FISH.

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References

1. Grignani F, Fagioli M, Alcalay M, et al. Acute promyelocytic leukemia: from genetics to treatment. Blood 1994; 83:10-25.
2. Borrow J, Shipley J, Howe K, et al. Molecular analysis of simple variant translocations in acute promyelocytic leukemia. Genes Chromosom Cancer 1994; 9: 234-43.
3. McKinney CD, Golden WL, Gemma NW, Swerdlow SH, Williams ME. RARα and PML gene rearrangements in acute promyelocytic leukemia with complex translocations and atypical features. Genes Chromosom Cancer 1994; 9:49-56.
4. Eclache V, Benzacken B, Le Roux G, Casassus P, Chomienne C. PML/RARα rearrangement in acute promyelocytic leukaemia with t(1;17) elucidated using fluorescence in situ hybridization. Br J Haematol 1997; 98:440-3.
5. Gogineni SK, Shah HO, Chester M, et al. Variant complex translocations involving chromosomes 1, 9, 9, 15, and 17 in acute promyelocytic leukemia without RARα/PML gene fusion rearrangement. Leukemia 1997; 11:514-8.
6. Hiorns LR, Min T, Swansbury GJ, Zelent A, Dyer MJS, Catovsky D. Interstitial insertion of retinoic acid receptor-α gene in acute promyelocytic leukemia with normal chromosomes 15 and 17. Blood 1994; 83:2946-51.
7. Lafage-Pochitaloff M, Alcalay M, Brunel V, et al. Acute promyelocytic leukemia cases with nonreciprocal PML/RARα or RARα/PML fusion genes. Blood 1995;

85:1169-74.
8. Grimwade D, Gorman P, Duprez E, et al. Characterization of cryptic rearrangements and variant translocations in acute promyelocytic leukemia. Blood 1997; 90:4876-85.
9. Licht JD, Chomienne C, Goy A, et al.Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). Blood 1995; 85:1083-94.
10. Brunel V, Sainty D, Carbuccia N, et al. Unbalanced translocation t(5;17) in an atypical acute promyelocytic leukemia. Genes Chromosom Cancer 1995; 14:307-12.

C→T mutation at -158 $^{G}\gamma$ HPFH associated with 4 bp deletion (–225–222) in the promoter region of the $^{A}\gamma$ gene in homozygous β^0 39 nonsense thalassemia

Sir,

Two Caucasian brothers from Central Spain were found to have homozygous β^0 thalassemia with mild anemia and mild physical stigmata of thalassemia. Molecular studies revealed that both subjects were homozygotes for the nonsense mutation of codon 39 (C→T), and heterozygotes for the C→T mutation at position –158 to the $^{G}\gamma$ gene [*Xmn* I-γ (+)] and for the 4 bp deletion (–225–222) in the promoter of the $^{A}\gamma$ gene.

β-thalassemias are a heterogenous group of genetic alterations characterized by a deficient synthesis (β+) or an absence (β^0) of β globin chains. The clinical expression of this disease can range from asymptomatic cases in most heterozygote forms of β-thalassemia (thalassemia minor) to severe forms of the disease (thalassemia major) in which the patients, usually homozygotes or double heterozygotes, are transfusion dependent. However, between these two extreme clinical forms there are a wide range of clinical phenotypes.[1]

We have studied two Caucasian brothers, 26 (II_1) and 31 (II_2) years old, from Central Spain. Physical examination revealed normal body structure with a splenomegaly of 5 cm in II_1 and 6 cm in II_2, and mild signs of thalassemic facies and conjunctival jaundice in both. Their father (I_1) and mother (I_2) were not related but both had thalassemia minor. The subjects had a more severe phenotypic expression than their parents with mild anemia (Table 1).

Both subjects were homozygotes for the nonsense mutation of codon 39 (C→T) and their parents were heterozygotes for this mutation (Figure 1). This mutation produces a lack of expression of the β gene (β^0) and has been reported to be responsible for thalassemia major.[2] The existence of α-thalassemia, which would have produced a less pronounced phenotypic expression of the disease,[3] was ruled out by Southern blot analysis with *Bam* HI, *Bgl* II, *Hph* I, *Nco* I and *Eco* RI restriction enzymes and α and ζ probes.

In the last decade some forms of non HPHF-deletion, which can "improve" the expression of the disease, have been described. These forms are due to point mutations of one base upstream of the $^{G}\gamma$ or $^{A}\gamma$ gene. Most of these mutations are associated with levels of HbF from 5 to 25% in heterozygotes and levels of HbF greater than 5% when associated with heterozygote β-thalassemia.[4] In the two cases reported here the parents are carriers of heterozygous β-thalassemia and the levels of HbF are lower than 3% in both (Table 1). On the other hand, the substitution C→T at position –158 of the $^{G}\gamma$ gene [*Xmn* I-γ (+)] is associated with increases in HbF in situations of severe anemia and stress erythropoiesis (homozygote SS, homozygote or double heterozygote β-thalassemia) which would result in a decrease in the clinical severity of these situations.[5-7] However, these *Xmn* I-γ (+) are not associated with a significative increase in HbF in normal individuals or heterozygote β-thalassemias.[7] The molecular studies revealed that the mother and the two brothers had the C→T mutation at position –158 to the $^{G}\gamma$ gene [*Xmn* I-γ (+)] in the heterozygote form (Figure 1). This finding could explain the clinical picture of the disease, with a mild anemia of 10.5 to 11.5 g/dL of HbF and a $^{G}\gamma/^{A}\gamma$ ratio of 2:1, higher than the expected 2:3, in the brothers, and a HbF level less than 3% in the mother who has heterozygote β-thalassemia. Other forms of non HPHF-deletion are associated with levels of HbF greater than 5% when associated with heterozygote β-thalassemia.[4] In this context, the presence of another form of non HPHF-deletion associated in this family is not probable.

At the level of the promoter of the gene $^{A}\gamma$ both the brothers and the parents had a 4 bp deletion (–225–222) (Figure 1). This deletion of 4 base pairs is

Table 1. Hematologic values and biochemical studies.

Measurement	*I_1 (father)*	*I_2 (mother)*	*II_1*	*II_2*
RBC × 10^{12}	6.9	6.3	4.5	4.6
PCV (L/L)	41.6	38.1	33.7	31.4
Hb (g/dL)	13.6	12.5	11.3	10.6
MCV (fL)	59.2	61	75	67.6
MCH (pg)	19.6	20	25.5	22.8
MCHC (g/L)	33.1	32.8	33.6	33.8
RDW (%)	15.5	14.9	26.1	26
Reticulocytes (‰)	6.2	5.9	7.4	8.3
IMR: (MFR+HFR)×100/LFR	3.5	6.9	13.4	10.7
Hb A_2 (%)	5	5.2	2.5	3.1
Hb F (%)	2.9	2.3	97.5	96.9
$^{G}\gamma/^{A}\gamma$	-	-	2/1	2/1
LDH (U/L)	-	-	226	186
Total bilirubin (mg/dL)	-	-	4.8	3.40
Serum iron (g/dL)	-	-	171	108
TIBC (g/dL)	-	-	193	184
Ferritin (ng/mL)	-	-	368.2	454.8