To the editor:

Intron 1 factor VIII gene inversion in a population of Italian hemophilia A patients

Bagnall and collegues[1] report a technique to investigate an inversion that disrupts the factor VIII (*F8*) gene and that represents a frequent cause of severe hemophilia A. This large genomic rearrangement, identified for the first time by Brinke et al[2] in 2 haemophilic monozygotic twins, affects intron 1 of the *F8* gene. Bagnall et al[1] demonstrated that this inversion of intron 1 derives from a homologous recombination between 2 nearly identical 1041–base pair (bp) sequences, *int1h-1* and *int1h-2*, in opposite orientation, positioned respectively in intron 1 of the gene and in a more telomeric region, 140 kilobases (kb) downstream, between the *C6.1A* and *VBPI* genes. This rearrangement results in the production of 2 chimeric mRNAs; the first, possibly under the control of the F8 gene promoter, contains the first exon of the *F8* gene, followed by some exons of *VBP1*. The other chimeric mRNA, under control of the C6.1A gene promoter, contains almost all of the coding region of the *C6.1A* gene followed by part of intron 1 and exon 2 to 26 of the *F8* gene.

We have examined 28 patients with severe to moderate hemophilia A (21 with severe, 2 with moderately severe, and 5 with moderate hemophilia A phenotype). All of the patients were investigated for the inversion of intron 22[3-4]; 8 of them are characterized by the presence of this common mutation. The other affected males that were negative for the inversion of intron 22 were screened for the inversion of intron 1; this mutation occurs repeatedly and seems to have a prevalence of 5% in patients with severe disease in the United Kingdom.[1]

We have performed an analysis directly on genomic DNA extracted with a QIAamp DNA blood mini-kit (QIAGEN, Hilden, Germany) under polymerase chain reaction (PCR) conditions suggested by Bagnall et al.[1] We performed 2 amplifications. One reaction contained 3 different primers: 9F and 9cR, specific for the *int1h-1* region, and int1h-2F, which is specific for the region flanking *int1h-2*. The other reaction contained 2 primers, int1h-2F and int1h-2R, which are specific for the amplification of the *int1h-2* region, and a third primer, 9F, which flanks the telomeric side of the *int1h-1* region. These PCRs are able to differentiate wild type from the patients with inversion and the patients with inversion from carriers. The amplified segments were readily separated on a 1.5% agarose gel. One patient out of 20 with severe disease tested positive for intron 1 inversion (Figure 1).

These observations demonstrate, in accordance with Bagnall et al, that this approach is a rapid and efficient method for the individuation of intron 1 inversion and it should be routinely performed in patients with severe hemophilia A.

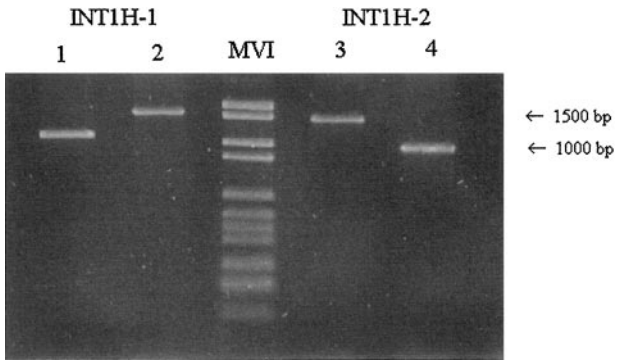

Figure 1. Amplification of int1h-1 and int1h-2 regions. Regions were amplified using 9F, 9cR, and Int1h-2F primers (lanes 1,2), and int1-2F, int1h-2R, and 9F primers (lanes 3,4). Lanes 1 and 3 show a male with inversion; lanes 2 and 4 show a wild-type male. MVI indicates DNA molecular weight marker VI (Boehringer Mannheim, Mannheim, Germany). The dimensions of the amplified fragments are approximately 1500 bp and 1000 bp.

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References

1. Bagnall D, Waseem N, Green PM, Giannelli F. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe haemophilia A. Blood. 2002;99:168-174.
2. Brinke A, Tagliavacca L, Naylor J, Green P, Giangrande P, Giannelli F. Two chimeric transcription units result from an inversion breaking intron 1 of the factor VIII gene and a region reportedly affected by reciprocal translocations in T-cell leukaemia. Hum Mol Gen. 1996;5:1945-1951.
3. Lakich D, Kazazian HH Jr, Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nat Genet. 1993;5:236-241.
4. Liu Q, Nozari G, Sommer SS. Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A [letter]. Blood. 1998;92:1458-1459.

To the editor:

Lack of *p21^{CIP1}* DNA methylation in acute lymphocytic leukemia

Recently, Roman-Gomez et al[1] reported an incidence of *p21^{CIP1}* methylation of 41% in 124 patients with acute lymphocytic leukemia (ALL). Most importantly, they observed that *p21^{CIP1}* methylation was an independent predictor of poor prognosis both in adults and children with this disease.

To follow these observations, we have analyzed the methylation status of *p21^{CIP1}* in a cohort of patients with ALL who were previously studied for methylation of multiple genes.[2,3] We studied a total of 31 patients (19 male; median age, 39 years [range, 7-77 years]; 6 Philadelphia (Ph) chromosome positive). For methylation analysis, we used widely accepted methods based on bisulfite modification of DNA because these assays are sensitive and have a low rate of false positivity. We used 2 different bisulfite polymerase chain reaction (PCR) methods to assess methylation of 3 different