formally contraindicated in most cases of clinical DIC. In conclusion, enhanced fibrinolysis plays an important role in preventing the development of organ failure in a model ofTF-induced DIC.

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Key words: DIC, tissue factor, fibrinolysis, tranexamic acid.

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References

1. Asakura H, Suga Y, Aoshima K, Ontachi Y, Mizutani T, Kato M, et al. Marked difference in pathophysiology between tissue factor- and lipopolysaccharide-induced disseminated intravascular coagulation models in rats. Crit Care Med 2002;30:161-4.
2. Asakura H, Suga Y, Yoshida T, Ontachi Y, Mizutani T, Kato M, et al. Pathophysiology of disseminated intravascular coagulation (DIC) progresses at a different rate in tissue factor-induced and lipopolysaccharide-induced DIC models in rats. Blood Coagul Fibrinol 2003;14:221-8.
3. Mannucci PM. Hemostatic drugs. N Engl J Med 1998;339:245-53.
4. Pelzer H, Schwarz A, Heimburger N. Determination of human thrombin-antithrombin III complex in plasma with an enzyme-linked immunosorbent assay. Thromb Haemost 1988;59:101-6.

Disorders of Hemostasis

Duplication of exon 13 causes one third of the cases of mild hemophilia A in northern Italy

A rearrangement of exon 13 in the factor VIII gene has been identified as the causative mutation in 32% of Northern Italian patients with mild hemophilia A. We have demonstrated that all share a common haplotype, thus suggesting that the mutation likely occurred in a single ancestor. To date, no predominant mutation has been identified in mild hemophilia A, therefore it would be extremely useful to carry out more extensive studies to ascertain whether the mutation is confined to northern Italy.

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Hemophilia A (HA) is an X-linked recessive disease, caused by wide-spread mutations in the factor VIII (FVIII) gene (*http://europium.csc.mrc.ac.uk*). Direct high performance liquid chromatography (DHPLC) screening detected a causative mutation in 9 of the 18 mild (FVIIIC≥5%) HA patients among a cohort of 31 patients referred to us from northern Italy. Since direct sequencing of the whole FVIII gene of 3/9 uncharacterized patients revealed no nucleotide alterations, and since these patients had high residual FVIII:C (>8%) activity, we hypothesized that there could be a mutational mechanism which: 1) skips the routine screening techniques; 2) is not as disruptive as the intron 1[1] and intron 22 inversions;[2] 3) does not cause severe damage to the FVIII protein. An unusual duplication of exon 13[3-4] was previously characterized in a patient treated at our Center. The duplication is the result of an unequal crossing-over between two non-homologous regions of misaligned X-chromosomes. Figure 1A shows that the break points occur on the two X chromosomes within FVIII gene intron 13 and intron 12. In the recombined chromosome the two normally oriented exons 13 are separated by an AT rich region of 19 tandemly duplicated nucleotides (Figure 1B). We hypothesized that the mRNA generated could be elongated or produced in two forms by alternative splicing.

To our knowledge, this patient is still an isolated case. We, therefore, studied the possibility that this rearrangement may also have been present in some of the uncharacterized patients with mild HA. Based on the FVIII genomic DNA sequence (*http://genome.ucsc.edu*; X-chromosome positions 151650711-151791079, which we assumed as nucleotide 1 and nucleotide 140369, respectively) we designed a pair of oligonucleotides encompassing the breakpoint region of the recombined X-chromosome: PAL13F 5'- TCAGTTTGGAAGTATTTTTC -3', extending from nt 77539 to 77558 of intron 13 and PAL13R 5'-TGTGTAC-TAAAGTATTGAGA-3', extending from nt 74331 to 74350 of intron 12. The polymerase chain reaction (PCR) was generated in standard conditions, and carried out for 30 cycles (94°C for 1 min, 55°C for 20 sec, and 72°C for 2 min), resulting in the amplification of a 337 bp fragment only in the patients with the mutation. Intra-test control consisted in the simultaneous amplification of exon 8 (*data not shown*).

We examined the 9 uncharacterized patients by PCR and found that 6 of them, including the 3 in whom direct gene sequencing had been negative, were in fact positive for this rearrangement. These findings prompted us to study our whole series of patients with mild HA, using the exon 13 duplication as first screening in the patients who had not yet been investigated. The overall frequency of this mutation was 32% (10/31 patients).

As seen from the pedigree analysis, all patients were apparently unrelated. HA was familial in 5 cases and sporadic in the remaining ones. In 3/5 sporadic cases, the two available generations consisted of mothers, all of whom proved to be carriers, and their sons. The recurrence of the same breakpoint (*data not shown*), as established by direct sequencing of all fragments, coupled with the fact that 7/10 positive patients were born in our region (Liguria), led us to hypothesize that this duplication could have spread from a single, ancestral event. As a matter of fact, data from bi-allelic and multi-allelic FVIII intragenic polymorphisms showed that all patients with mutations had the same haplotype (BclI/IVS18, + allele; XbaI/IVS22, - allele; VNTR IVS 13, 20 CA repeats).

Therefore, the mutation may have arisen as a single event and was then disseminated throughout the northern Italian population by a founder effect. The lack of kinship among the families as well as the fact that about 50% of the cases were apparently sporadic, observed from the reported family history, are two features that seemingly contrast with these results. However, we must remember that most positive patients had a FVIII:C>8%, that they had been diagnosed in adulthood following trauma and/or surgical procedures, and that they could have had undiagnosed ancestors. Moreover, for the same reasons we were only able to examine the members of two generations. The body of information we obtained allows us to make some remarks. Firstly, conventional mutation screen-

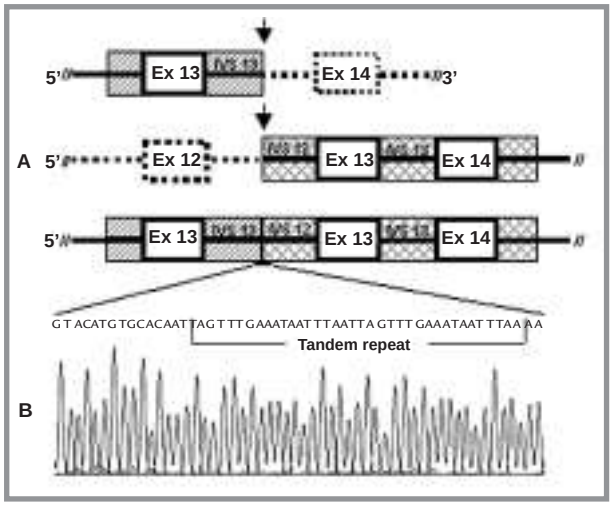

Figure 1. Schematic representation of exon 13 duplication. (A) The location of exon 13 in two misaligned X-chromosomes is shown. The crossing over (// = break points) between non-homologous regions creates a recombined chromosome with two exons 13 duplicated in frame. (B) The new sequence originating from the rearrangement, which includes the breakpoint, can be seen. The AT rich sequence, which is normally present in intron 12, appears to be tandemly repeated, in patients with the mutation.

ing using PCR-sequencing of the essential region of the FVIII gene on genomic DNA leaves some gene alterations undetected. Secondly, it is possible that the high frequency of this mutation is the result of a founder effect although it will be essential to study a broader population of mild HA patients from central and southern Italy in order to unravel this matter. Since 3/10 patients were born outside our region, we believe that the first mutation we should look for in Italian patients with mild HA is duplication of exon 13.

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Key words: hemophilia A, mutation, exon 13.

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References

1. Bagnall RD, Waseem N, Green PM, Gianelli F. Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A. Blood 2002;99:168-74.
2. Lakich, D, Kazazian HH Jr, Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. J Nat Genet 1993;5:236-41.
3. Casula L, Murru S, Pecorara M, Ristaldi MS, Restagno G, Mancuso G, et al. Recurrent mutations and three novel rearrangements in the factor VIII gene of hemophilia A patients of Italian descent. Blood 1990;75:662-70.
4. Murru S, Casula L, Pecorara M, Mori PG, Cao A, Pirastu M. Illegitimate recombination produced a duplication within the FVIII gene in a patient with mild hemophilia A. Genomics 1990;7:115-8.

Disorders of Hemostasis

Treatment of acquired factor VIII inihibitor with recombinant activated factor VIIa: data from the Italian registry of acquired hemophilia

We report on the data collected in the Italian Registry of acquired haemophilia (AH) in 2001. Recombinant activated factor VII (rFVIIa) was selected as first-line therapy in 19 bleeding episodes because of their severity and as salvage in one case. Bleeding was controlled in 90% of the episodes, indicating the efficacy of rFVIIa in HA.

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Acquired hemophilia (AH) is a rare syndrome caused by inhibitors to factor VIII (rarely to factor IX), characterized by the sudden onset of bleeding, usually severe, in patients with no family or personal history of bleeding diatheses.[1,2] It is a medical emergency, requires prompt treatment and is clinically and financially demanding. Various therapies can be used: human or porcine FVIII, DDAVP, immunoglobulins, activated prothrombin concentrate, immunoadsorption and recombinant activated factor VII (rFVIIa). The available data are from anecdotal case reports and retrospective studies with a limited number of patients; none of the available agents is effective in all the patients. rFVIIa, a systemic hemostatic agent with topical action, is beneficial in a variety of bleeding conditions: hemophiliacs with inhibitor, elective surgery (prostatectomy, hepatectomy, liver transplantation), hereditary platelet disorders, and severe bleeding secondary to oral anticoagulation and trauma.[3,4]

The multicenter study by Hay *et al.* on the use of the rFVIIa is the largest so far reported.[5] Our study is a retrospective analysis of the patients reported to the Italian registry in 2001 (*www.emonet.org*) who were treated with rFVIIa. The following information was requested: primary condition, cause of bleeding or its spontaneous occurrence, site, severity evaluated by the hemoglobin level and by transfusion requirements, inhibitor titer, therapy, selection criteria and results. The clinical response was evaluated by the patient's physician at 6, 12, 24 and >24 hours and was scored as very effective (complete cessation of bleeding), effective (residual minor bleeding), partially effective (reduced but still significant bleeding); ineffective. Any adverse event occurring within two months of the end of treatment was recorded. Twenty-eight new patients were registered, 15 of whom were treated with rFVIIa, 11 with other modalities, and 2 who did not require treatment (Table 1). rFVIIa was selected as first-line treatment in 19 bleeding episodes because of the severity of the episode and as salvage treatment in case 1 (Table 2). The median total dose to control the bleeding (primary end-point) in groups treated with bolus or continuous infusion was 309 and 474.5 μg/kg, respectively, with a wide range. One patient concomitantly received tranexamic acid. Treatment was very effective or effective in 13/15 patients (86.6%) and in 18/20 bleeding episodes (90.0%), within 24 (14 episodes) and 48 hours (4 episodes), including the recurrences. There were no differences between the groups treated with intermittent or continuous infusion (*data not shown*). Treatment was scored par-