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INVERSION MUTATION AS A MAJOR CAUSE OF SEVERE HEMOPHILIA A IN ITALIAN PATIENTS

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ABSTRACT

We investigated the presence of a recombinant event between the F8A gene located in intron 22 of the factor VIII gene and the two additional copies of F8A lying 500 Kb upstream of FVIII in severe hemophilic patients. The genomic DNA of 146 unrelated Italian patients with severe hemophilia A (HA) was hybridized with an F8A gene probe to detect the abnormal band patterns. A recombinant event was found in 71/146 patients, confirming the high incidence of this mutation in the Italian hemophilic population also. We conclude that the high frequency of the mutation in HA subjects allows us to make a direct and safe diagnosis in about 50% of our families without resorting to RFLP analysis.

Key words: hemophilia A, carrier detection, prenatal diagnosis

Hemophilia A (HA) is a bleeding disorder that affects approximately 1/5000 males. It is caused by a deficiency in the function or in the production of blood coagulation factor VIII (FVIII). It is considered a heterogeneous disease not only at the clinical[1] and biochemical levels but also at the molecular level because of the great variety of mutations described (point mutations, deletions, insertions). Recently, a recombinant event followed by factor VIII disruption was identified as a cause of the disease in about 45% of severe HA patients (FVIII < 1%).[2] The factor VIII gene contains 2 other genes, one of which, F8A intronless, is wholly located in intron 22 and transcribed in the opposite direction as FVIII and two or more F8A copies lying 500 Kb upstream from the FVIII gene. Recombination occurs between the homologous sequences of F8A on the same X-chromosome, resulting in a large inversion that disrupts the FVIII gene.

Molecular analysis was performed in hemophilic patients coming from different Italian regions. The aim of our study was to evaluate the percentage of Italian hemophiliacs carrying this mutation and the impact of this new recombination assay on prenatal diagnosis and carrier detection as compared to the RFLP test.

Materials and Methods

In all, 146 unrelated patients with severe hemophilia A (factor VIII < 1%) coming from different Italian regions were studied. Genomic DNA was isolated from peripheral blood leukocytes by standard procedures. In order to screen the inversion mutation, 5 g samples of DNA were digested with BclI restriction enzyme. The resulting fragments were electrophoresed on 0.6% agarose gel and then transferred to nylon membranes (Gene Screens Plus, Dupont) and hybridized to a 0.9 Kb EcoRI/SstI fragment of plasmid p482.6[2] at 65° C overnight. The filters were exposed to X-AR autoradiographies (Kodak) with intensifying screen.

Results

Digestion of DNA with the BclI enzyme and hybridation with the F8A probe detected a normal pattern of bands of 21.5 Kb, 16 Kb and 14 Kb, corresponding to the F8A gene contained in intron 22 and the two additional copies located upstream of the FVIII gene.

To investigate the presence of this mutation, we tested 146 severely affected Italian HA patients. Recombination was found in 71/146 (49%) cases; in 58 of these the rearrangement involved a recombination between the intron 22 copy of the F8A gene and the most distal upstream F8A gene (type 1). Only 13 subjects showed a pattern compatible with a recombination between the intron 22 copy of gene F8A and the more proximal upstream F8A gene (type 2). Four of the rearranged patients showed inhibitors against the factor VIII gene. Moreover, there was no association between inversion and RFLP haplotype.

We did not find any rare patterns resulting from an inversion in an individual carrying more than two extragenic copies of the F8A gene (type 3). In one family, which was uninformative at RFLP analysis and in which the affected male was dead, the presence of the inversion in a pregnant female was used to carry out a prenatal diagnosis: the fetus, having inherited the defective gene (20 Kb and 17.5 Kb bands) was affected (Figure 1).

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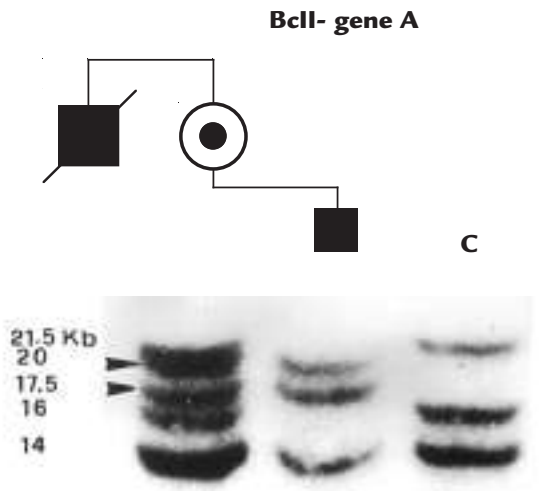

Figure 1. In a sporadic family uninformative for all RFLPs, in which the affected male was dead, the presence of the inversion in the pregnant woman was used to perform a prenatal diagnosis: the fetus who inherited the defective gene (20 Kb and 17.5 Kb bands) was affected. C = normal control.

Comment

As far as the incidence of this new FVIII gene mutation in the Italian population is concerned, our results are in agreement with those found in other countries.[3]

The most important achievement obtained with the F8A rearrangement assay is in the field of carrier detection and prenatal diagnosis. In fact, at present RFLPs have certain limits, such as: i) the percentage of informativity due to the mother's homozygosity; ii) the risk of a recombinant event (5%) due to the use of extragenic markers; iii) the need to study a great number of family members, including non affected males, in order to check RFLP inheritance and verify the reliability of the markers; iv) the inability to detect, in sporadic cases, at which level the mutation took place and therefore whether the hemophiliac's mother is a carrier or not; v) when the hemophiliac is not available, carrier detection may be impossible in some families.

The use of intragenic markers (BclI and XbaI) with an informativity of about 60% in the Italian population,[4,5] combined with detection of the F8A gene inversion, allowed us both to reach high diagnostic accuracy in a great number of HA families and to find the generation and the germ line in which the mutation occurred. Pedigree analysis identified 14 certainly sporadic families: in 10 the mutation was found in the germ cells of the maternal grandfather of unaffected hemophiliacs according to Rossiter *et al.*;[6] three mutations came from the hemophiliac's maternal grandmother, and in 1 case the recombination originated de novo in the hemophiliac's maternal meiosis.

As far as homozygosity and unavailability of the hemophiliac are concerned, Figure 1 describes how it was possible to carry out a prenatal diagnosis in a family in which the hemophiliac was dead and his mother was homozygous with all the RFLPs.

In conclusion, detection of the F8A rearrangement is the test of choice for HA carrier identification and prenatal diagnosis. Moreover, as analysis with intragenic markers becomes more frequent today, diagnosis is getting more and more accurate.

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