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
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.

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, 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.; 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.

References