Letters to the Editor

Inversion of intron 1 of the factor VIII gene for direct molecular diagnosis of hemophilia A

An inversion of the factor VIII gene has been recently described as a consequence of an intrachromosomal recombination involving a 1041bp specific duplionic inside and outside the gene. We investigated the inversion in a cohort of 201 Spanish hemophilia A (HA) families. The inversion was detected in 4 families with severely affected cases of HA and no inhibitor history. The frequency of the inversion among cases of severe HA cases was 5% (4/79), confirming that this inversion is a recurrent mutational event.

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The most frequent mutation in severe hemophilia A patients is an inversion of intron 22 of the factor VIII gene, described 8 years after the cloning of the gene.1,2 In 1996 an inversion breaking intron 1 was detected in two hemophilic monozygotic twins.2 This was originally regarded as a rare event, but 6 years later, the same group in the United Kingdom reported that this inversion was a recurrent event in patients with hemophilia A (HA).3 A 1041-bp pair sequence (int1h–1) of the intron 1 was found to be duplicated (int1h–2) and orientated in the opposite direction 140 kb outside the gene between the C6.1A and VBP1 genes. This inversion arises from a recombination event between the two homologous sequences int1h–1 and int1h–2 (Figure 1).

One hundred and eighty-five unrelated HA patients and 16 mothers of deceased hemophiliacs, in whom inversion of intron 22 had been excluded, were investigated for the presence of inversion of intron 1. Out of 201 cases, 79 had severe disease, 53 had moderate disease and the remaining 69 had a mild phenotype. For inversion analysis, two polymerase chain reactions (PCR) were performed as previously described5 with slight modifications. In the first reaction, primers specific for int1h–1 (9F, 9cR) plus the primer int1h–2F were used in an amplification reaction that yielded a 1908 bp product from normal DNA and a 1776 bp product in inversion DNA and a 1323 bp product if the inversion was present.

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References

the sporadic patient, the mother was a carrier. None of the patients reported inhibitors. All the remaining cases tested had the expected size of the normal band according to the first or second PCR reaction. The at-risk haplotype defined by DNA markers was consistent in all families with no detected recombination. There was no specific association with the different alleles, although the 6.2 kb allele of the intron 22 XbaI polymorphism was detected in the four chromosomes with the inversion. This allele is present at a frequency of 0.34 in our population (Fisher’s exact test p=0.016). The frequency of intron 1 inversion in Spanish HA patients agrees with that reported by Bagnall et al., who found 10 cases of inversion among a total of 209 patients (4.8%) with severe HA previously screened for intron 22 inversion. Because of the description of intron 22 inversion in a moderately affected patient the screening of every hemophilic patient for this inversion, regardless of the severity of the disease, could be justified until more multicenter data are available. None of the patients of our series developed inhibitors whereas one of the ten patients reported by Bagnall et al. did. Duplicons in the human genome facilitate rearrangements within large intervening sequences of genes potentially causing dis-

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Figure 1. Schematic representation of the locations of int1h1 and int1h2 within the Xq28 region. The upper part illustrates the normal situation and the lower part the inversion case. The int1h1 is a 1041 bp sequence located inside intron 1 (22.9 kb) and the int1h2 is an inverted duplicon located 140kb upstream of the factor VIII gene towards the telomeric side. The homologous intrachromosomal recombination of both sequences yields a partition of factor VIII gene with exon 1 and 2 separated by approximately 150 kb and in opposite orientations. PCR fragments: A= 1191 bp fragment amplified by primers int1h2F and int2h2R, which corresponds to the int1h2 wild type sequence. B= 1908 bp fragment amplified by primers 9F and 9cR, corresponding to the int1h1 wild type sequence. C= a 1776 bp fragment amplified by primers 9F and int1h2R, which corresponds to the exon 1 recombinant int1h sequence. D= a 1323 bp fragment amplified by primers 9F and int1h2R, corresponding to the exon 2 recombinant int1h sequence. Data modified from Bagnall et al. assuming reciprocal exchange of material.

Figure 2. PCR test for intron 1 inversion. Lanes 1-3 illustrate the result of PCR 1 (primers 9F, 9cR plus int1h –2F) and lanes 4-6 the results of PCR 2 (primers int1h –2F, int1h –2R plus 9F). Lanes 1 and 4 represent a patient with intron 1 inversion, lanes 2 and 5 his carrier mother and lanes 3 and 6 a case without the inversion. See Figure 1 for interpretation of the size of the PCR products. The reactions were performed in 25µL volume with 300µM dNTPs, 2mM MgCl2, 5% DMSO and 0.7 and 0.4 units of Taq polymerase. The PCR products were visualized on ethidium bromide 1 % agarose gel after electrophoresis. M = 1kb ladder marker.
ease. In the factor VIII gene, the largest of these sequences is intron 22, spanning almost 40 kb, followed by intron 1, 14 and 25 with approximately 22 kb and intron 6 and 13 with around 15 kb. In addition to the reported intron 22 and 1 duplicons, it is reasonable to hypothesize that the remaining large introns harbor sequences that could be repeated outside the gene. These sequences would be prone to rearrangements by recombination, causing a molecular pathology that is unrecognized by mutation detection methods based on the analysis of the entire coding sequence. Thus, the presence of inversions involving different intron rearrangements could be a common mechanism in HA cases in whom analysis of the entire coding region is negative for mutations. Our results confirm the earlier observation that intron 1 inversion is a recurrent event, albeit 10 times less frequent than intron 22 inversion. It is readily detectable by PCR and is effective in diagnosing carriers at risk. The implementation of intron 1 inversion testing is a useful addition to that for the previously reported intron 22 inversion. Despite its low frequency, data collection from various Centers should help to define the prevalence of this inversion in different populations, its mutation rate, its paternal origin, its relationship with inhibitors and its linkage disequilibrium with DNA markers.

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