Improved strategy for rapid genetic analysis of hemophilia A.

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Background

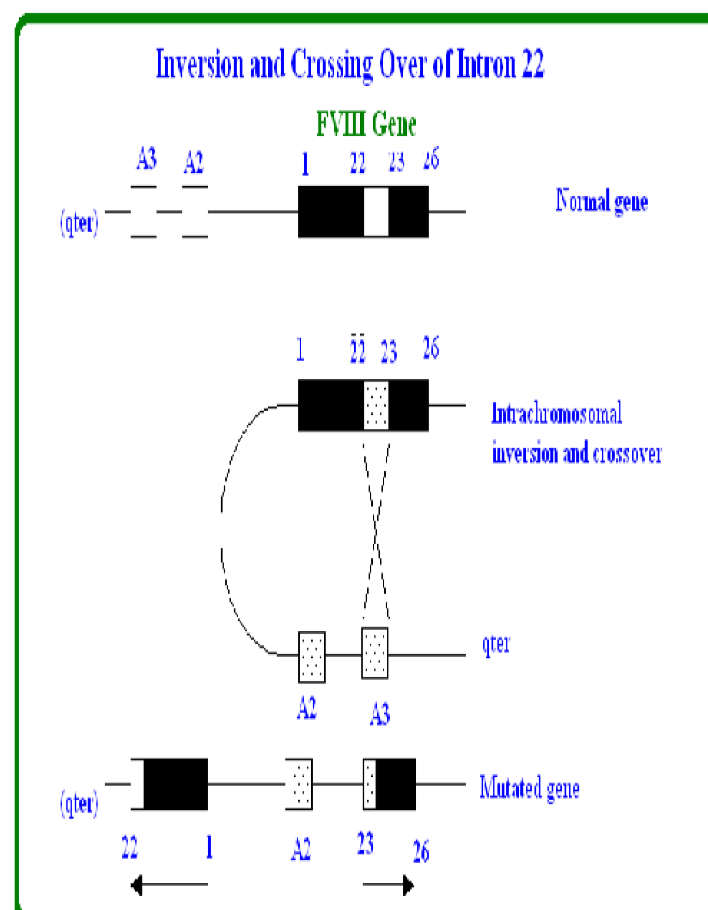
Hemophilia A is a bleeding disorder caused by deficiency or impaired function of factor VIII (FVIII). FVIII is encoded by a large and complex gene (F8) comprised of 26 exons spanning 186 kb, located at the most distal region of the long arm of the X chromosome (Xq28). About 50% of severe hemophilia cases are caused by intron 22 inversions in the F8 gene. The remaining 50% include mainly missense mutations spread over the gene, with about 1500 unique mutations causing hemophilia A reported up to date. In view of this mutation heterogeneity, examination of potential hemophilia A carriers for definite determination of their carrier status and further prenatal diagnosis offered to the carriers are commonly based on linkage analysis using short tandem repeat (STR) markers. Accurate genetic analysis of hemophilia A using STR markers should be based on at least 2 markers: one intragenic and one extragenic marker, or preferably, two extragenic markers located on different sides (at the 3' and 5' ends) of the F8 gene. The aim of this approach is to exclude recombination between the marker used for analysis and the hemophilia-causing mutation.

Aims

1. To improve the sensitivity of Inverse-shifting PCR (IS-PCR) assay for analysis of intron 22 inversions in the FVIII gene.
2. To identify additional extragenic STR markers proximal to the 5' region of the FVIII gene, which is very close to the chromosome X telomere, since to date, the majority of extragenic STR markers useful for analysis of hemophilia A are located at the 3' region of the F8 gene.

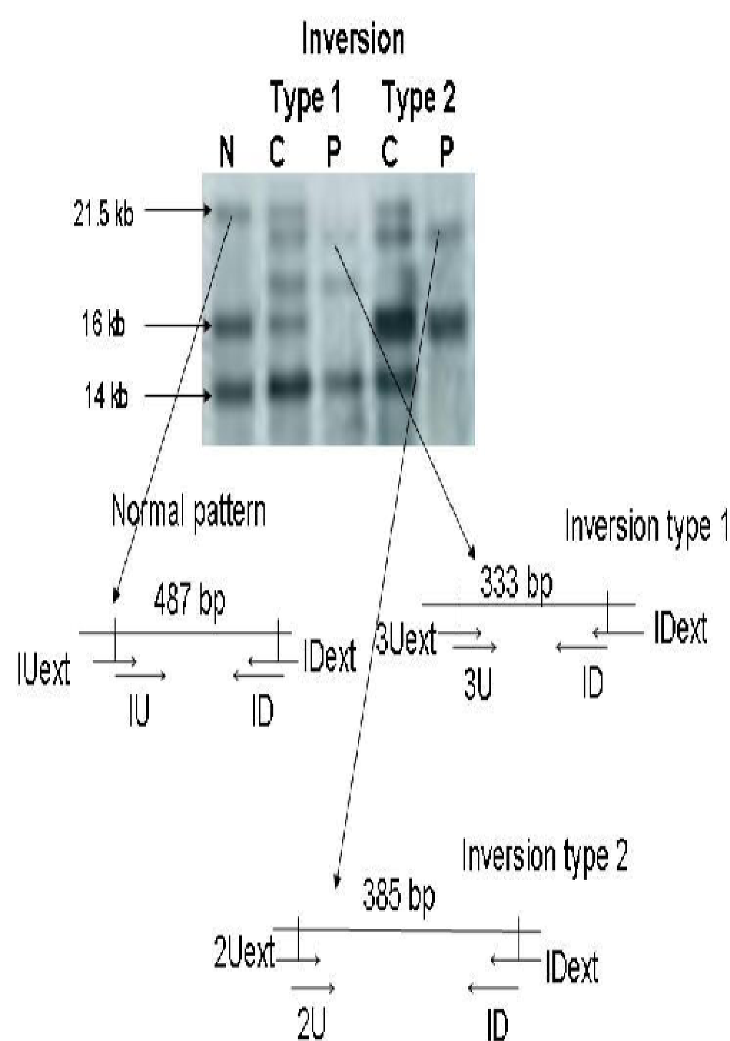
Methods

Genomic DNA samples were prepared for IS-PCR as described elsewhere (Fujita et al., J. Thromb. Haemost. 2012, 10:2099-2107).
PCR fragments specific for the normal, inversion type I and inversion type II patterns were amplified separately, using two sets of primers for each ("nested" PCR system).
Sequences of the internal primers were as described by Rossetti et al (J. Thromb. Haemost. 2008, 6: 830-836).
Potential new STR markers were identified by searching the telomeric region of the X chromosome for dinucleotide repeats (UCSC website, http://genome.ucsc.edu/). The polymorphic nature of the potentially polymorphic regions was either confirmed or excluded by fluorescent PCR analysis of 20 chromosomes.

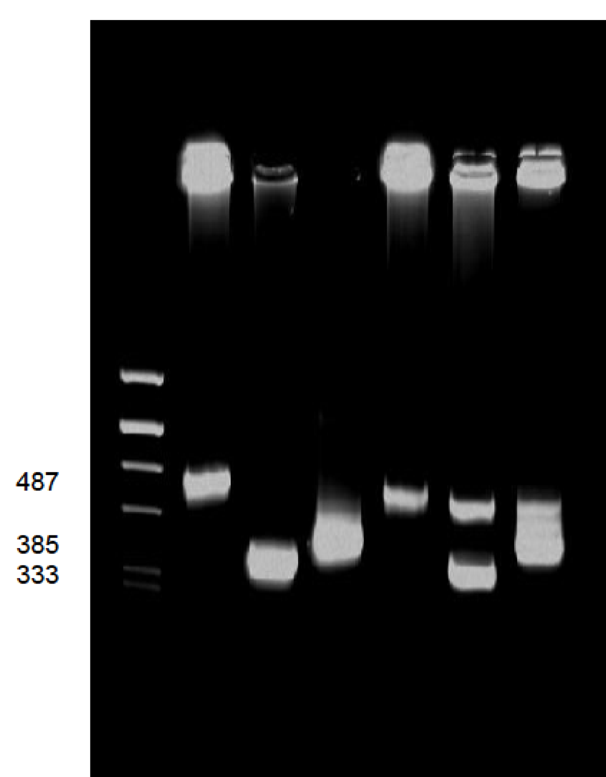


http://www.intechopen.com/books/targets-in-gene-therapy/using-factor-vii-in-hemophilia-gene-therapy

Correlation between traditional Bcl I fragments observed on Southern blots and PCR fragments obtained by inverse shift PCR following self- ligation

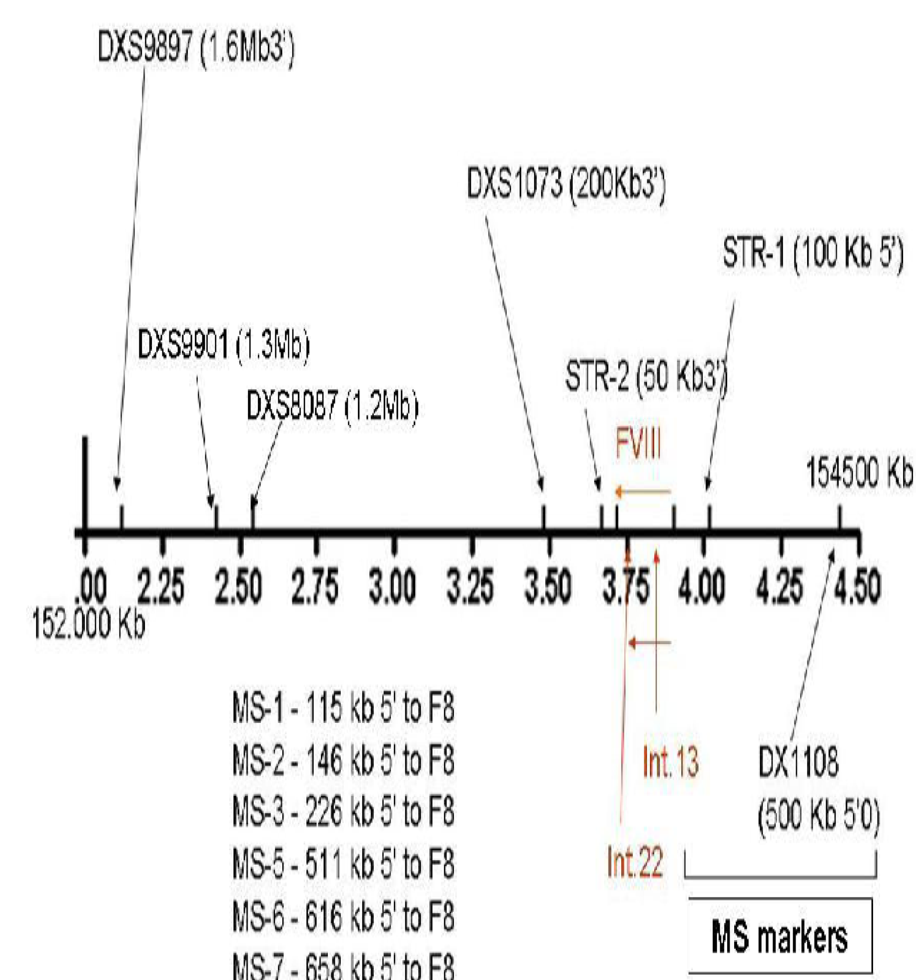


Nested IS-PCR products



N – Normal
P1 – Inversion type 1 patient
P2 – Inversion type 2 patient
C1 - Inversion type 1 carrier
C2 - Inversion type 2 carrier

Intragenic and extragenic STRs in and around the FVIII gene



Results

1. Using the "nested" PCR system, we significantly improved the sensitivity of IS-PCR assay for detection/ exclusion of intron 22 inversions in the FVIII gene.
2. Using the approach of screening potentially polymorphic dinucleotide repeats, we identified 6 new STR markers at the distance of 100 – 700 Kb from the 5' end of the F8 gene, in addition to only 2 STR markers previously identified in this region.

Summary

We suggest an improved strategy for rapid genetic analysis of hemophilia A, based on a "nested" PCR system for detection/exclusion of intron 22 inversions, followed by fluorescent PCR analysis of 15 highly polymorphic STR markers (2 intragenic, 8 and 5 at the 5' and 3' ends of the F8 gene, respectively). During the period of 2009 - 2012, this approach enabled us to perform accurate genetic diagnosis of hemophilia A, complying with the requirement of at least 2 polymorphic markers, thus ensuring lack of recombination between the marker and the hemophilia-causing mutation, in 239 potential carriers and 36 male fetuses of established carriers.