MOLECULAR ANALYSIS OF CONGENITAL FACTOR XI DEFICIENCY BY HIGH-RESOLUTION MELTING

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INTRODUCTION

Coagulation factor XI (FXI) plays an essential role in the intrinsic blood-coagulation pathway through the activation of factor IX. Factor XI deficiency is a rare bleeding autosomic disorder (estimated prevalence of severe deficiency is ~1: 1,000,000) resulting in a wide range of bleeding manifestations, from asymptomatic to injury-related bleedings, irrespective of genotype and circulating FXI levels. The FXI gene (F11) maps on chromosome 4 (4q35.2) and contains 15 exons spread over ~ 25 Kb of genomic DNA.

AIMS

This study analysed the F11 gene in 13 patients, related to Haemophilia Centres of Emilia-Romagna Region (Parma and Bologna), identified by pre-surgical or routine laboratory screening or by personal history of bleeding symptoms. The aim of the present study was to identify FXI gene mutations with HRM.

METHODS

High-Resolution Melting (HRM) analysis (fig. 1) is a useful technique for genotyping, mutation scanning and sequence matching. It is a refinement of the melting curve analysis that uses a saturating DNA-binding dye. After PCR amplification and subsequent melting under HR conditions, LightCycler480 software (Roche) processes the raw melting curve data to form a difference plot. This method can reveal variations between samples (mutations or polymorphisms) and display those variations on a graph that is easy to interpret. Patients samples with revealing difference plots are further analysed with direct sequencing. Promoter regions and all coding exons were screened by designing 23 amplicons (182-298 bp).

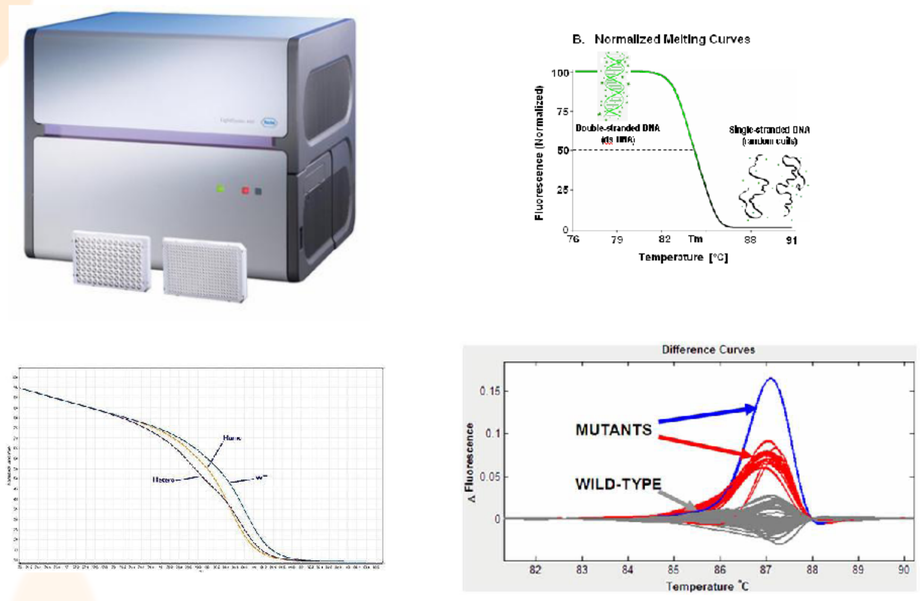

fig.1 HRM instrument and melting curves

RESULTS AND CONCLUSIONS

Pathological mutations were identified (1253G>T, Gly418Val in homozygosis and 403G>T, Glu135X in heterozygosis) in one severe patient (FXI:C=2%).
Three missense mutations were identified in other 3 mild patients (268A>C, Asn90Thr and 1171G>A Gly391Arg in heterozygosis; 1786G>T Gly596Cys in homozygosis).
In two patients (30%-32% of FXI:C) a variation was detected (-445G>C) in the promoter region that could have an impact on the transcription. We characterised also some genetic known polymorphisms. In our study, molecular screening of FXI confirmed a high level of allelic heterogeneity.

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