Identification of a third rearrangement at Xq28 that causes severe hemophilia A due to homologous recombination between inverted repeats

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Introduction

Hemophilia A (HA) is a common, X-linked, recessive disorder caused by deficiency of coagulant factor VIII (FVIII). The gene encoding this factor is large and complex [186 kb and 26 exons] and is situated at about 1 Mb from the telomere at the Xq28[1]. The Xq28 locus appears to be prone to several types of rearrangements. The presence of several homologous repeats accounts for the high frequency at which these rearrangements occur. Two such rearrangements have been previously described that disrupt *F8* and lead to severe hemophilia A. Here we describe a third rearrangement causing a severe phenotype in an index patient without mutations in the *F8* coding sequence.

Material and Methods

In this study we investigated the molecular mechanisms causing a severe HA phenotype (FVIII:C <1 IU dl^{-1}) in a patient where no mutation was found in the coding sequence (cDNA) of *F8*. We have already reported absence of mRNA in the patient as well as his mother and sister[2]. For mutation screening direct sequencing of all exonic sequences and exon/intron boundaries was done. These results were verified using a comparative genome hybridization (CGH) array. A Long Range PCR (LR-PCR) approach was performed to investigate the integrity of *F8* locus, followed by southern blot analysis and an inverse PCR approach for verification of the rearrangement.

Results

The index patient was negative for intron 1 and 22 inversions. Complete cDNA sequencing revealed no deleterious mutations. LR-PCR was performed to determine the integrity of *F8* locus by amplifying 28 overlapping fragments. Inability to amplify a 5.25 kb fragment from *F8* intron 1 from the index patient indicated the presence of a genomic breakpoint in this region (Fig. 1A,B). Southern blotting verified the presence of the rearrangement in the patient and his mother (Fig. 1C). Analysis of the intronic sequences flanking the breakpoint revealed the presence of a 687 bp sequence with 98.9% homology to a sequence at Xq28, but in inverted orientation, Int1R-1 and Int1R-2 respectively (Fig. 2A,B). This approach indicated a possible homologous recombination between the identified repeat in *F8* intron 1 and the highly homologous inverted repeat in intron 2 of *IKBKG*. Analysis was followed by performing an inverse PCR to identify the breakpoint junctions. Here we were able to localize the junction of breakpoint within the repeat. Due to the fact that mutations disrupting the *IKBKG* gene are lethal in males[3], we excluded disruption of *IKBKG* by LR-PCR. We postulated the presence of a third copy of the repeat and screened the patient for duplications using a CGH array (Fig. 3A,B). Our analysis revealed the presence of a large duplication (≈ 94 kb) which extends from Int1R-2 in *IKBKG* to *IKBKGP* (pseudo gene of *IKBKG*)(Fig 3.C). A multiplex PCR was designed to detect the rearrangement in patients and carriers (Fig. 1D,E).

Conclusion

We identified a third rearrangement causing hemophilia A due to homologous recombination between inverted repeats and developed a PCR-based diagnostic method that can be used to screen for this genetic rearrangement in cases of severe hemophilia A for which mutations cannot be identified[4].

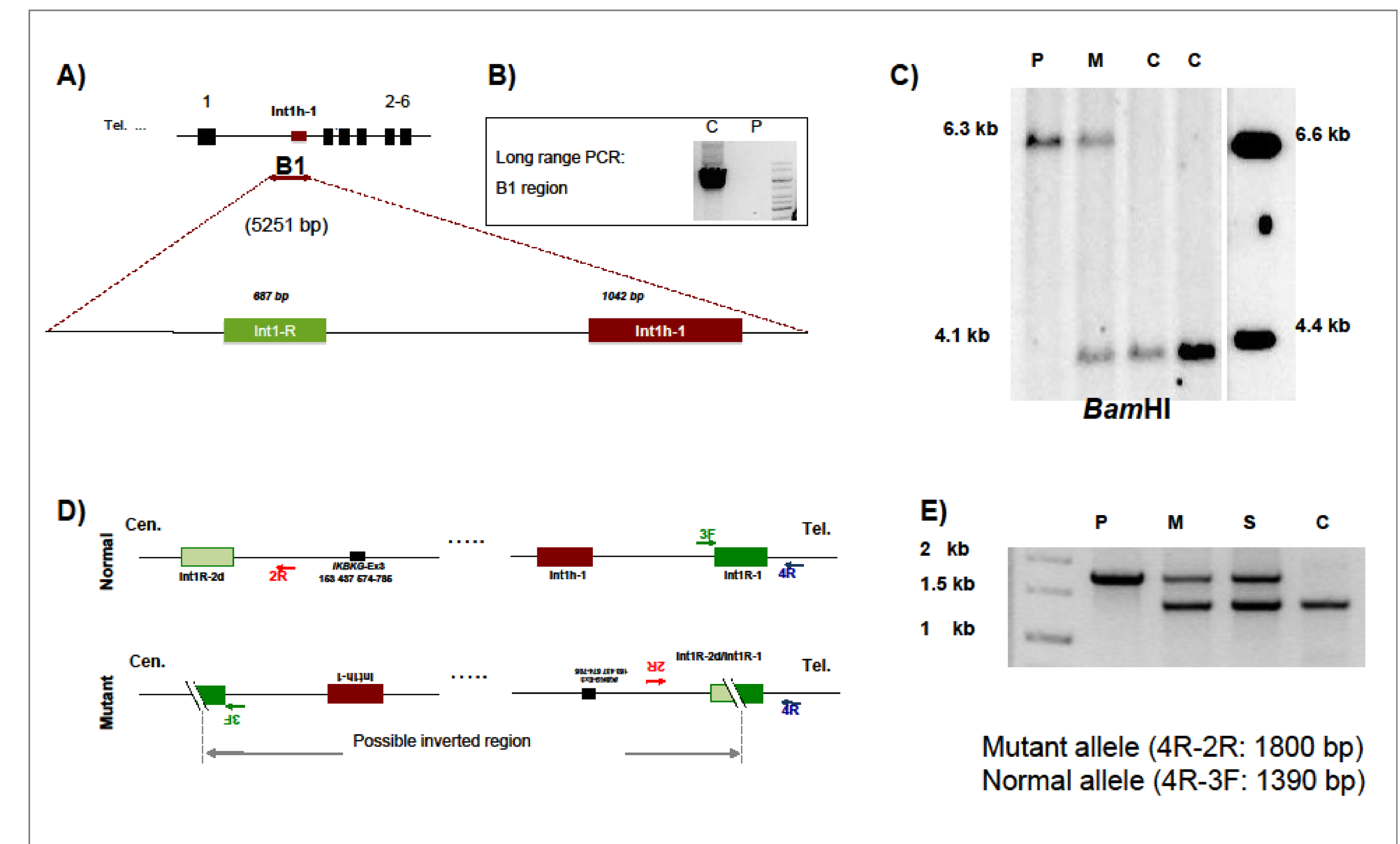

Figure 1. Identification and characterization of a genomic rearrangement between inverted repeats. A) Schematic representation of the location of the breakpoint (B1) in intron 1 of *F8*. B) LR-PCR amplification of B1 Region. C) Southern blot analysis using *BamHI*, with a probe upstream the repeat in Int1-R1 (P: Patient, M: Mother, C: Control). D) A schematic diagram showing the genomic rearrangement found in the patient. E) Multiplex PCR to detect the rearrangement in the patient and carriers (P: Patient, M: Mother, S: Sister, C: Control). Red arrows indicate the position of primers.

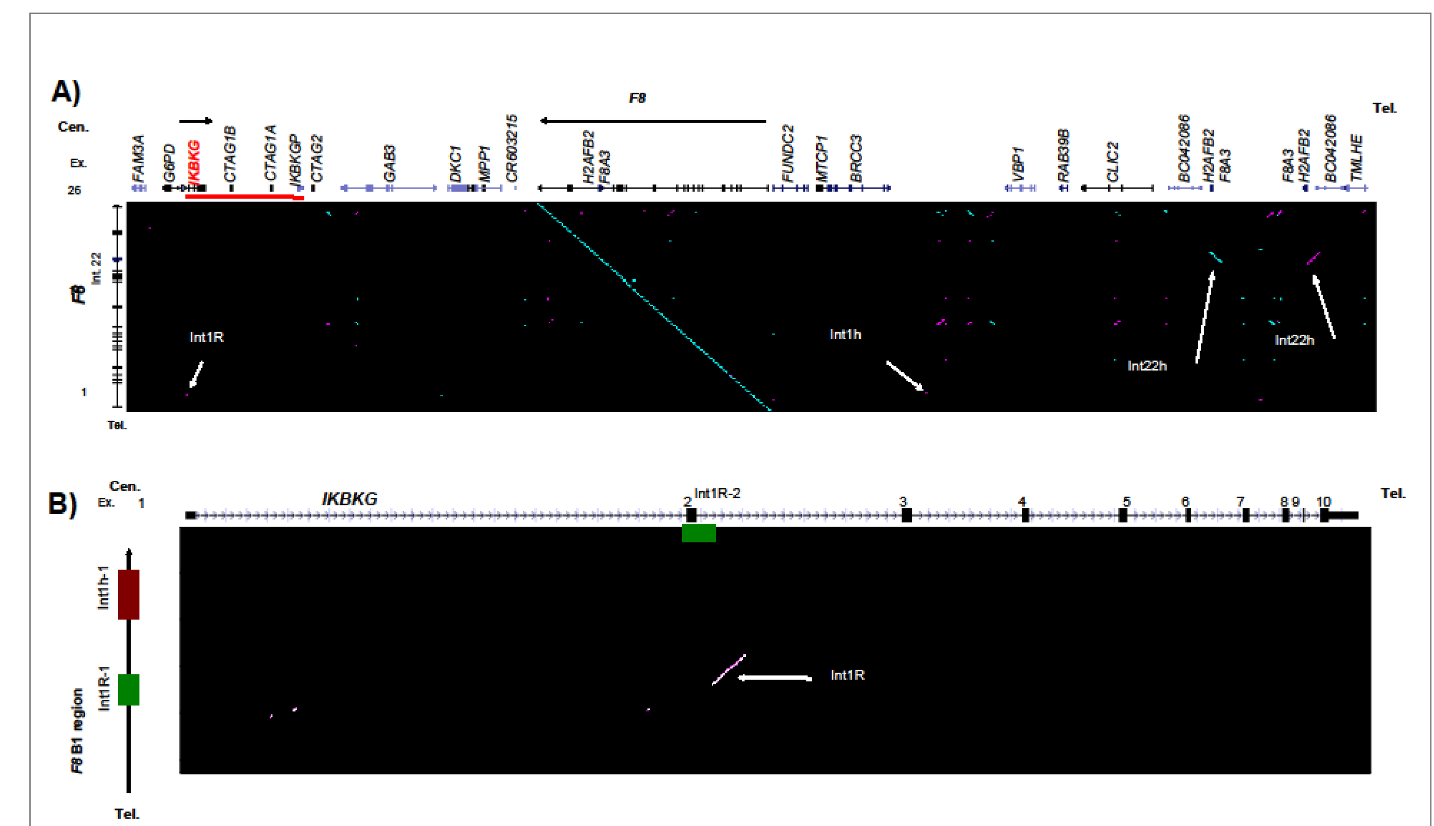

Figure 2: Homology analysis to identify identical repeats within 1Mb; 500 kb upstream and 500 kb downstream of the breakpoint. A) Dot plot of 1 Mb region containing the B1 region at the center vs. *F8* locus. The identical repeats, Int22h and Int1h, known to be involved in inversions in *F8* and the newly identified Int1R, are labeled. The horizontal red bar at the top left of the figure represents the duplicated 94.4 kb region in the index patient. B) Dot plot of *IKBKG* vs. the *F8* B1 region; The Int1h-1 and Int1R repeats are labeled in red and green, respectively. Inverted repeats in dot plot are colored purple and direct repeats are blue. The word size and the tile size used for the dot plot are 50 and 2000, respectively; Geneious software was used for the analysis.

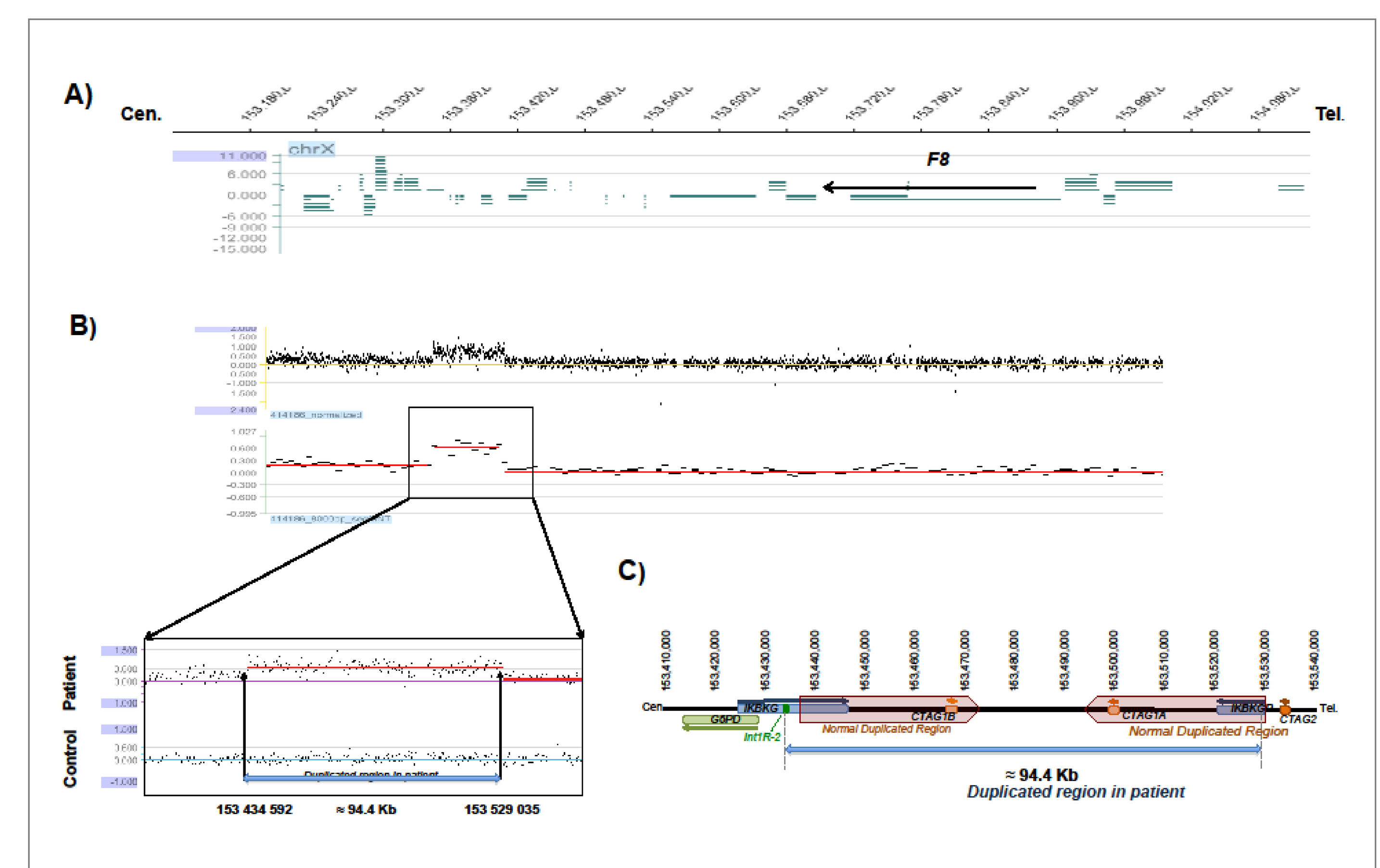

Figure 3. Genomic region harboring alterations at Xq28. A) The upper panel shows the position of *F8* according on X chromosome (hg:18, NCBI build 36). Arrow indicates the direction of *F8* transcription. B) Extent of the duplication in patient C) Map of the involved region; *G6PD*, *IKBKG*, *CTAG1B*, *CTAG1A* and *IKBKGP* are shown: the thin arrows above genes indicate direction of transcription; the thick pink/transparent horizontal arrows indicate the naturally occurring duplicated region; the extent of the duplication in the index patient is indicated by the blue line with arrows below the map (Cen: Centromere, Tel: Telomere).

References

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