An int22h-related deletion combined with unbalanced X-chromosome results in severe haemophilia A in a female

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Introduction

Haemophilia A (HA)– a common hereditary bleeding disorder with an incidence of one in 5,000–10,000 male newborns – is caused by a wide spectrum of different mutations in the *factor 8* (*F8*) gene. Being an X-linked recessive disorder, females are generally not affected, although they can be carriers of the disorder. In carriers, the disease phenotype mainly depends on the mutation type in *F8* and the degree of X chromosome inactivation. Here, we report the first case of severe HA in a female due to intron 22-related deletions and skewed lyonization.

Patient and Methods

The index patient is a one-year old girl with FVIII:C<1 IU/dl born of a consanguineous marriage with no family history of HA. *F8* was analyzed by the conventional routine technique including: intron 22 (Inv22) and intron 1 inversion PCRS as well as direct sequencing of all 26 exons. Moreover, Multiplex ligation-dependent probe amplification (MLPA) for *F8* was done according to manufacturers instructions. X chromosome inactivation pattern was determined using the *HUMARA* assay[1]. The extended intron 22 genetic analysis was performed using the new generation of inverse-Shift (IS-)PCR test[2].

Results

The patient was negative for both intron 22 (Figure 1) and intron 1 inversion PCRs. Direct sequencing of exons of *F8* and its adjacent intronic regions revealed no mutation. Furthermore, MLPA analysis showed neither duplications nor deletions involving *F8* and the adjacent control regions, which excludes a Turner syndrome as well. As after performing the routine assays no genetic defect was found, we investigated the possibility of the hypothesized of int22h-related deletions by performing the new IS-PCR diagnostic test. This IS-PCR test is mainly used for discrimination of type 1 and type 2 Inv22 pattern. But more importantly the test allows the detection of the int22h-related deletions (Del22). The new diagnostic IS-PCR test revealed a heterozygous Del22 type 1 pattern, resulted from the deletion of int22h-3 (Figure 2). The conventional Inv22 IS-PCR does not detect this deletion and the patients would be misdiagnosed as non-carrier. Furthermore, analysis of the patient's X-inactivation pattern clearly showed preferential inactivation of the unaffected X chromosome (80:20) (data not shown).

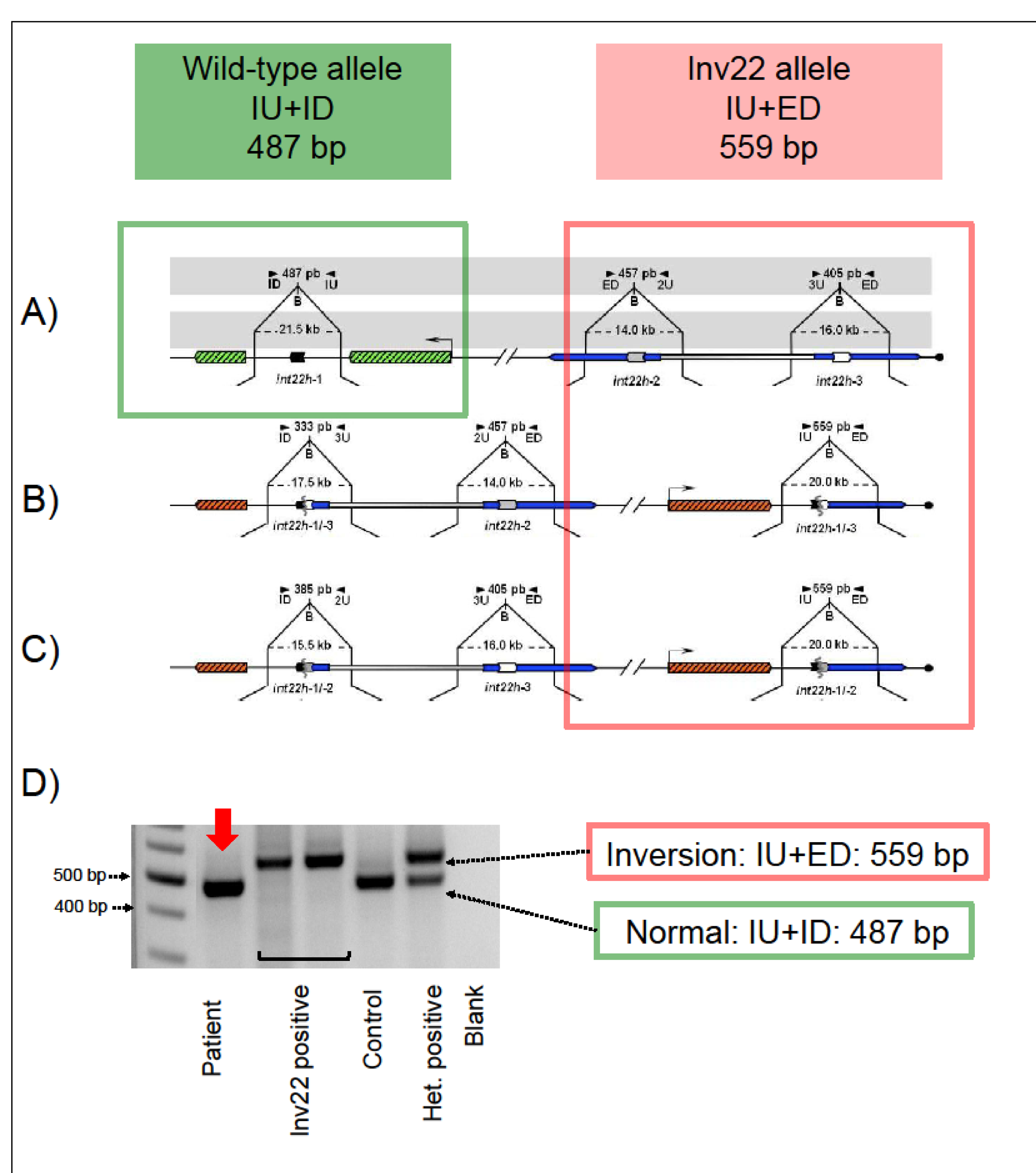
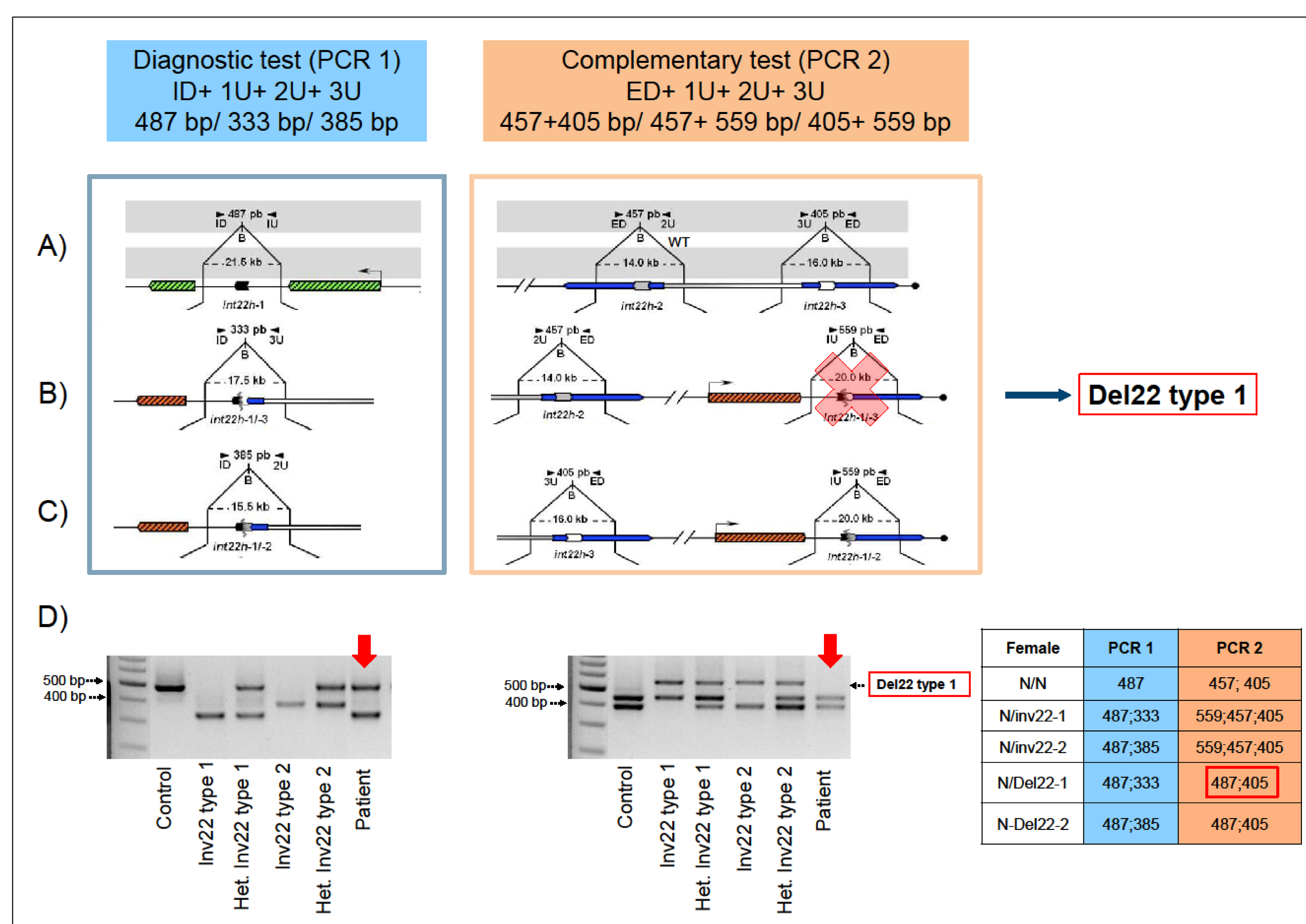


Figure 1: Conventional IS-PCR. Inv22 analysis is achieved using three different primers that yields a 487 bp amplicon (ID/IU) for wild-type intragenic allele and a 559 bp amplicon (ED/IU) for Inv22 allele. **(A)** The Normal *F8* variant; **(B)** and **(C)** the Inv22 type 1 and type 2 variants; **(D)** The index patient is misdiagnosed as non HA carrier.



Female	PCR 1	PCR 2
N/N	487	457; 405
N/inv22-1	487;333	559;457;405
N/inv22-2	487;385	559;457;405
N/Del22-1	487;333	487;405
N-Del22-2	487;385	487;405

Figure 2: IS-PCR for diagnostic and complementary test of Inv22 for full characterization of possible int22h-related rearrangements. (A) The normal *F8* wild-type variant h123; **(B)** HA-associated Inv22 type 1 originating from recombination between *h1* and *h3* and **(C)** HA-associated Inv22 type 2 originating from recombination between *h1* and *h2*; **(D)** IS-PCR analysis reveals heterozygous Inv22 type 1 inversion using the diagnostic test but the wild-type pattern after the complementary test indicating the presence of the Del22-related deletion (absence of the 559 bp band).

Conclusion

Here, we present a case of HA caused by a heterozygous Del22 type 1 mutation in combination with non-random X chromosome inactivation that leads to the clinical manifestation of severe HA in a female. The data here shows an example of a rare mechanism leading to HA in females. Moreover, the results presented here underline the limitations of genotyping by Long Distance-PCR approaches[3,4] and the conventional IS-PCR[5] test. Diagnostic based on these conventional but widely used tests would lead to misdiagnosis of two group of patients: **first** females with a Dup22 will be diagnosed as an Inv22 carrier and **second** females carrying the Del22 inversion as non-carriers.

References
[1]Tost et al Nucleic Acids Res. 2007; [2]Rossetti et al. JTH. 2008, [3]Bagnall et al. JTH. 2006, [4]Liu Et al. Blood. 1998, [5]Rossetti et al. ClinChem. 2005

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