Genotyping of a cohort of 12 HA families from Suriname.

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Introduction and Objectives

Hemophilia A (HA) is a recessive X-linked bleeding disorder caused by a deficiency or abnormality in the procoagulant activity of factor VIII (FVIII). The prevalence is 1:5,000 male births. HA can be divided in three different subclasses: severe (FVIII activity < 1%), moderate (1-5%) and mild (6-30%). The objectives are genotyping a cohort of 12 families with 52 family members from Suriname, suspected to be affected by HA. All family members were screened for mutations in the *F8* gene. This information is important for proper genetic counseling. Moreover, analysis of *F8* gene variations is important to understand the patient's clinical phenotype.

Materials and Methods

DNA of patients suffering from HA was analyzed by Sanger sequencing. All coding regions including the intron-exon boundaries of the *F8* gene were sequenced. Intron 22 inversions were analyzed using inverse-PCR described by Rossetti et al. (*Clinical Chemistry, 2005*) . The *F8* intron 1 breaking inversion was analyzed using a PCR method as described by Bagnall et al. (*Blood, 2002*).

Results

Up to April 2014, 42 family members were analyzed. 21 family members had a genetic abnormality (table 1, figure 1). So far, 2 nonsense mutations (n=4 patients), 2 missense mutations (n=2), 1 small deletion (n=3), 2 splice site mutations (n=8) and 2 polymorphisms (n=12) were identified. No mutations were found in 21 family members. Of all mutations detected 6 mutations were reported previously (n=14) and 1 deletion was novel (n=3). The novel deletion (c.180delC, p.Asn41fs) induces a frameshift leading to a premature stop codon (figure 2, 3, 4). Polymorphism D1241E was found in 8 family members and polymorphism M2238V was found in 4 family members. Both polymorphisms were reported previously.

Gene	Missense mutation	Nonsense mutation	Splice site mutation	Deletion	Insertion
F8	c.1421 G>A p.Gly455Glu (homo) c.491 G>A p.Gly145Asp (homo)	c.2440 C>T p.Arg795X (het) c.2440 C>T p.Arg795X (homo) c.1063 C>T p.Arg336X (homo)	c.787+3 A>G (het) c.787+3 A>G (homo) c.5219+1 G>A (het) c.5219+1 G>A (homo)	c.180 delc p.Asn41fs (het) c.180 delc p.Asn41fs (homo)	-

Table 1: Overview of the mutations found in HA families from Suriname.

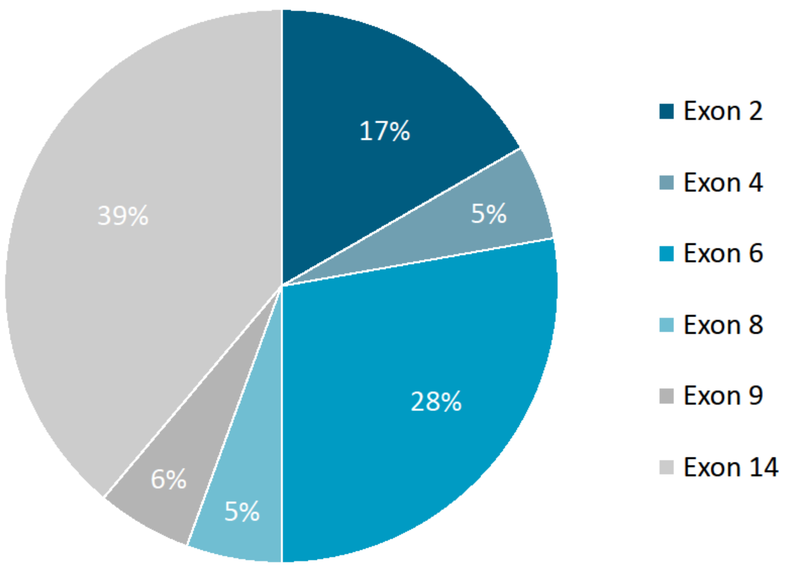

Figure 1: Distribution of the mutations found in the F8 gene.

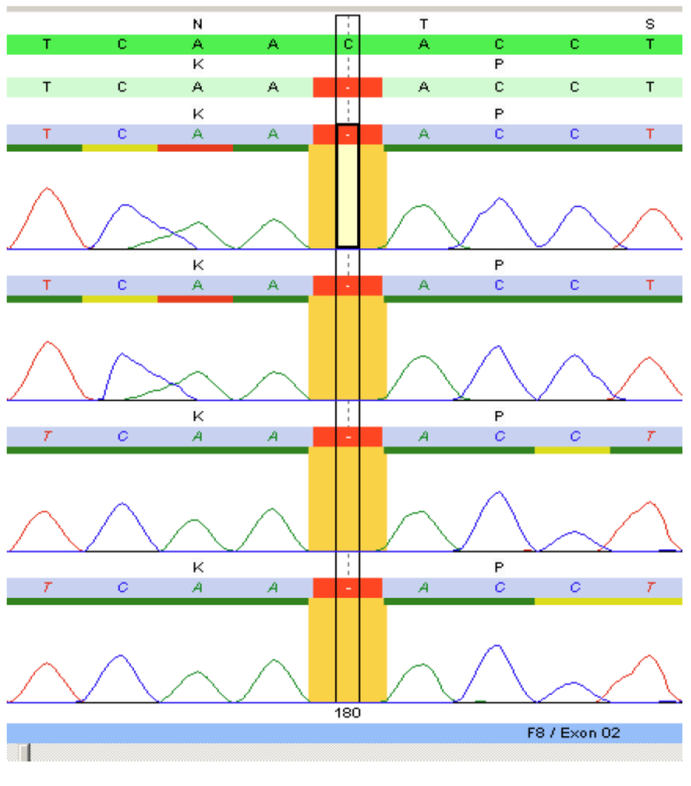

Figure 2: Sequence Pilot plot of the novel deletion c.180 delc p.Asn41fs (homo) found in the F8 gene.

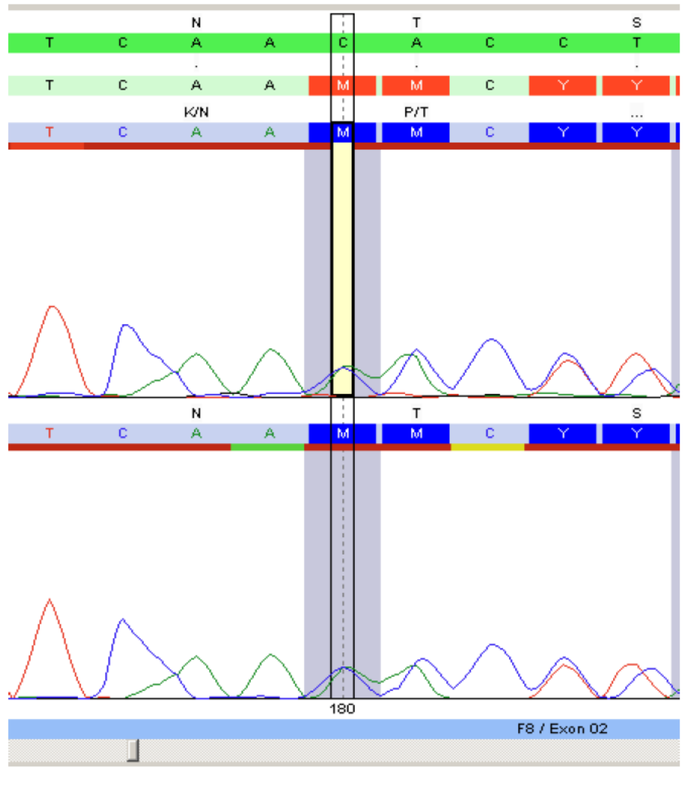

Figure 3: Sequence Pilot plot of the novel deletion c.180 delc p.Asn41fs (het) found in the F8 gene.

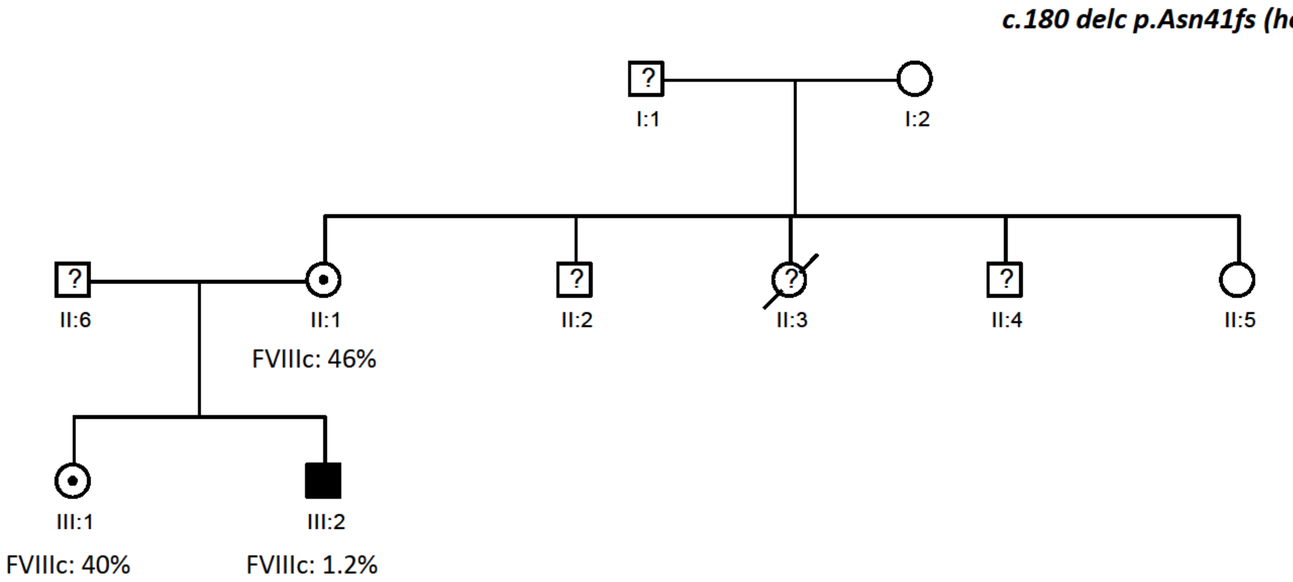

Figure 4: Pedigree of the novel deletion c.180 delc p.Asn41fs found in the F8 gene.

Conclusion

Our genotypic approach confirmed our phenotypic suspicion, based on FVIII levels, of HA in 12 families from Suriname origin. So far, 11 HA carriers were identified. In one family a novel deletion (c.180delC, p.Asn41fs (n=3)) was found. This deletion leads to severe HA. Finally, the D1241E polymorphism leads to a mild decrease in FVIII activity as reported previously (*Viel e.a., Blood, 2007*) which may have clinical impact.

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