Characterization of Rare Hemostatic Disorders by Sanger Sequencing.

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Introduction and Objectives

Rare Hemostatic Disorders (RHDs), including Rare Bleeding Disorders (RBDs), are rare diseases. Haemophilia A and B, together with von Willebrand disease, include 95% to 97% of all inherited coagulation deficiencies. The other 3-5% are represented by less common inherited disorders. This includes deficiencies of fibrinogen (Fg), prothrombin (factor II), factors V, VII, X, XI and XIII. Moreover, rare thrombotic disorders also occur. All these disorders are autosomal recessive (except for factor XI) and their prevalence is approximately 1:500,000 or less in western countries. Clinical presentations of patients affected by RBDs vary from mild or moderate bleeding tendencies, to potentially serious or life-threatening hemorrhages. The clinical presentation of rare thrombotic disorders is more severe. The objective of our centre is to focus on RBDs. Therefore we started to screen for genotypic abnormalities by genotyping the genes that are suspected to cause RHDs, including RBDs. RHDs are usually due to mutations in the genes that encode the corresponding coagulation factors. A cohort of 33 patients has been screened. Characterization of mutations in the *"RBD"* genes is important to understand the patient's clinical phenotype. FXII was analyzed to determine the gain of function mutation causing hereditary angioedema (HAE) type III.

Materials and Methods

DNA of patients suffering from RHDs was analyzed using Sanger sequencing. All coding regions including intron-exon boundaries of the genes were sequenced.

Results

Up to April 2014, 33 patients were analyzed for RBD (table 1). 10 genes (*F2, F7, F11, F12, F13A1, F13B, FGα, FGβ, FGγ and PROC*) were sequenced. 15 patients had a genetic abnormality; 2 nonsense mutations (*F11*;n=2, *PROC*;n=4), 7 missense mutations (*F2;n=2,* 3**F7*;n=1, *F12*;n=1, *F13A1*;n=1, *FGγ;n=1*), 2 splice site mutations (*FGα*;n=1) and 1 duplication (*F7*;n=1). 11 mutations were reported previously (n=15) and 1 mutation was novel (n=1). The novel missense mutation (c.1328 A>G, p.Tyr443Cys) was found in the *F7* gene (figure 4). With respect to congenital thrombotic disorder, one patient was identified with a homozygous nonsense mutation (c.1042C>T, p.Arg348X) in the *PROC* gene, inducing a premature stop codon (figure 1, 2, 3).

Gene	N screened	N abnormal	Missense mutation	Nonsense mutation	Splice site mutation	Deletion	Insertion	Known in HGMD?
F2	2	2	c.260A>G p.Tyr87Cys (het) c.1256 C>T p.Thr419Met (het) c.1328 A>G p.Tyr443Cys (het)	-	-	-	-	Y Y N
F11	4	2	-	c.682C>T p.Arg228X (het)	-	-	-	Y
F12 (HAE, type III)	13	1	c.983C>A p.Thr328Lys (het)	-	-	-	-	Y
F13A1	1	1	c.233G>A p.Arg78His (homo)	-	-	-	-	Y
F13B	1	0	-	-	-	-	-	-
FGα	4	2	-	-	c.510+1G>T (homo) c.180+1G>C (het)	-	-	Y Y
FGβ	1	0	-	-	-	-	-	-
FGγ	1	1	c.323C>G p.Ala108Gly (het)	-	-	-	-	Y
PROC	4	4	-	c.1042C>T p.Arg348X (het) c.1042C>T p.Arg348X (homo)	-	-	-	Y Y

Table 1: Overview of the mutations found in RHDs

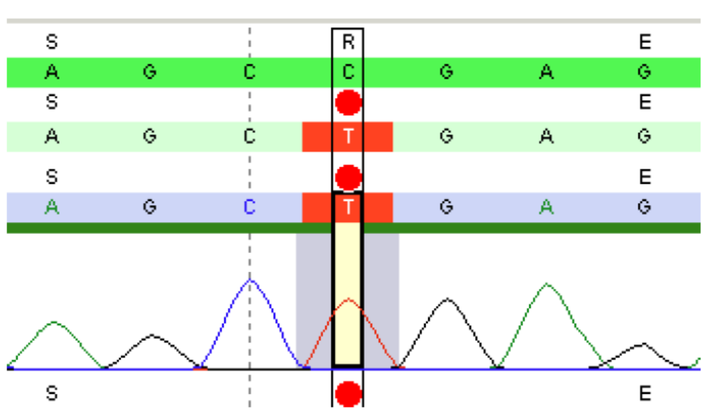

Figure 2: Sequence Pilot plot of nonsense mutation c.1042C>T p.Arg348X (homo) in the PROC gene

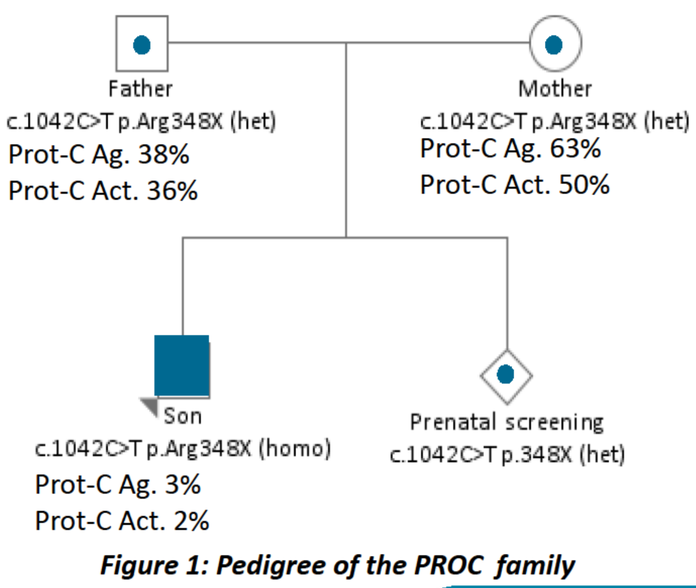

Figure 1: Pedigree of the PROC family

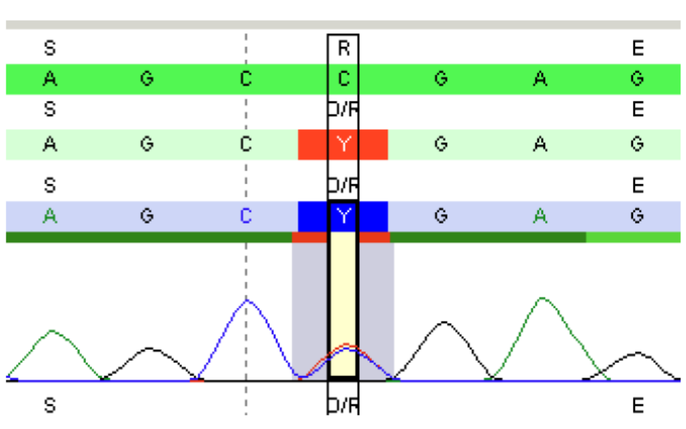

Figure 3: Sequence Pilot plot of nonsense mutation c.1042C>T p.Arg348X (het) in the PROC gene

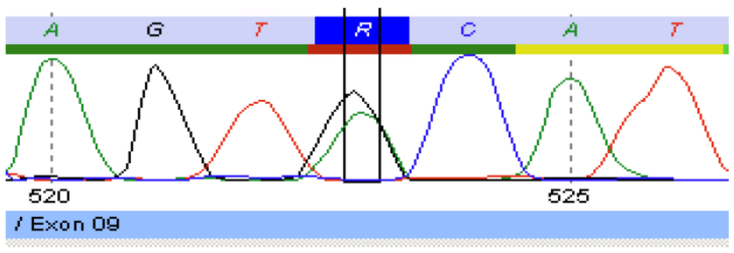

Figure 4: Sequence Pilot plot of the novel missense mutation c.1328A>G p.Tyr443Cys (het) found in the F7 gene

Conclusion

Our study indicates the presence of genetic abnormalities in patients suspected to be affected by RHDs, including RBDs. Our genotypic approach confirmed our phenotypic suspicion, based on FII, FVII, FXI, FXII, FXIII, Fg and Protein-C levels. The homozygous protein-C patient had a severe clinical phenotype, due to thrombosis, whereas the other heterozygous family members had a mild clinical phenotype.

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