Disorders of Hemostasis

Prothrombin Suresnes: a case of homozygous F299V mutation responsible for hypodysprothrombinemia

A patient with a severe prothrombin deficiency and a hemorrhagic diathesis was found to have positive cross-reactive material in plasma and a homozygous F299V mutation (F7V in the A chain). This mutation reinforces the previous conclusion that the A chain affects the geometry of the catalytic triad. Marked prolongation of the Taipan venom and Russell venom clotting times also demonstrated a defective activation mechanism and a defective interaction with factor Xa.

haematologica 2006; 91:431-432

(*http://www.haematologica.org/journal/2006/03/431.html*)

Prothrombin is synthesized as a polypeptide of 622 amino acids. Several Glu residues in the amino terminal part are gamma-carboxylated, the propeptide is glycosylated, and a 43-amino acid fragment is cleaved before prothrombin is secreted as a single chain of 579 amino acids (MW 71.6 kDa). Proteolytic cleavage of prothrombin by the prothrombinase complex yields α-thrombin, which is a serine protease consisting of two polypeptide chains (A and B) linked by a disulfide bridge. Thrombin amplifies the coagulation pathway by cleaving various elements of the coagulation cascade. The gene for human prothrombin contains 14 exons, is 21 kb long, and maps to 11p11-q12.[1] Hereditary prothrombin deficiency is a rare bleeding disorder with a slightly enhanced prevalence in the Latino-American population.[2] To date, a few defects in the prothrombin gene have been identified in patients with dysprothrombinemia or hypoprothrombinemia. Among these natural dysprothrombins, amino acid residues of the thrombin A-chain were found to be rarely involved.[3] We report on a patient bearing a novel missense mutation in the A chain.

The patient is a 20- year old female originating from Algeria. She was born from a consanguineous marriage. The parents and siblings were unavailable for testing. The prothrombin deficiency was diagnosed when the girl was 4 years old, following a substantial hemorrhage after tooth extraction. She suffers prolonged bleeding and important hematomas after minor trauma. She also suffered menorrhagia requiring contraceptive treatment. She was operated on for onychocryptosis under Kaksadil prophylactic infusion, and was given another postoperative infusion. No abnormal bleeding occurred.

Coagulation tests showed the data presented in Table 1. Chronometric evaluation of FII indicated a severe prothrombin deficiency (<5%), but an antigen assay showed a significant amount of FII (23%). The presence of positive cross-reacting material in her plasma indicated that the mutant protein is at least partially secreted. The discrepancy between the antigen and chronometric values indicates that the circulating molecule is not active and that the patient has a hypodysprothrombinemia. It is remarkable that FVIII, FIX, FXI and FXII levels were elevated in the patient, while FVII and FX were rather on the low side. Some modifications could result from a combination of the effects of gender, pill, and blood group, all conditions previously shown to affect coagulation factor levels.[4,5] All 14 exons and exon-intron boundaries of the prothrombin gene were amplified using gene-specific primers designed for this study. Sequencing of the amplified fragments was performed using the ET-terminator kit and a Megabace sequence analyzer (Amersham-Biosciences, Piscataway, NJ, USA). Primers used for sequencing were the same as those used for the polymerase chain reaction amplification. The G20210A mutation was searched for as described by Raoul *et al.*[6] and found to be absent.

Table 1. Coagulation test results for the propositus.

	Patient	*Control or normal range*
Prothrombin time	26.5 sec	12 sec
Factor V	111%	65-150%
Factor II	<5%	70-150%
Factor II (Ag)	23%	70-150%
Taipan venom time	119 sec	35-38 sec (normal plasma)
		50 sec (plasma diluted to 24%)
		47 sec, Antivitamin K-treated patient, FII=25%
Russell venom time	63 sec	34-40 sec (normal plasma)
		44 sec (plasma diluted to 25%)
Factor VII	60%	70-150%
Factor X	82%	70-150%
APTT ratio	1.42	0.80-1.20
Fibrinogen	4.28 g/L	2.00-4.00 g/L
Factor VIII	163%	50-150%
Factor IX	142%	50-100%
Factor XI	122%	50-100%
Factor XII	168%	50-100%
platelets	318 G/L	150-400 G/L

Coagulation test methods: routine coagulation tests were performed with Thromborel S Reagent (Dade-Behring, Liederbach Germany). Factor II antigen (Ag) was assayed by ELISA with an anti human FII (Cedarlane Laboratories, Hornby, Ontario, Canada). The control values for the Taipan venom time (Diagnostic Reagents Ltd, Oxfordshire UK) and the Russell venom time were obtained by diluting normal plasma (FII 100%) in deficient plasma. Calibration curves were determined and the clotting times ranged from 59 sec (FII: 5%) to 36 sec (FII: 100%) for Taipan venom, and from 48 sec (FII: 10%) to 34 sec (FII: 100%) for Russell venom.

A homozygous T14053G transversion (base numbering refers to the sequence M17262.1) was found in exon 9, predicting a homozygous F299V substitution. A homozygous deletion was also found five bases upstream of the exon 2 splicing site (IVS1 -5Tdel). This deletion is a polymorphism, since it was found on every tested chromosome of 30 control individuals (*not shown*).

The thrombin A-chain is organized mainly in a multiple-turn and partly helical conformation. The A-chain has a boomerang-like shape, giving the B-chain part a smooth contour, opposite to the active site pocket.[7] V299 is located in the rigid part of the A chain of the α-thrombin catalytic domain (Figure 1), in the vicinity of the Xa-catalyzed cleavage sites (R320-I321 then R271-T272) and of the autoactivation cleavage site (R284-T285). Prolonged Russell venom time indicated that the Xa-prothrombin interaction is affected. Thrombin ΔK301 is a naturally occurring mutant[8] with similar consequences (severe deficiency with hemorrhagic diathesis and positive cross-reacting material). A simulation of the molecular dynamics of ΔK301 thrombin provided support to the role of the A-chain in affecting conformation and catalytic properties of the B-chain, as well as in the geometry of the catalytic triad residues.[3] However, the vicinity of the cleavage sites also suggests that the activation can be deficient. Using Taipan venom, which has been shown to activate the prothrombin directly,[9] indicated that the clotting time

©Ferrata Storti Foundation

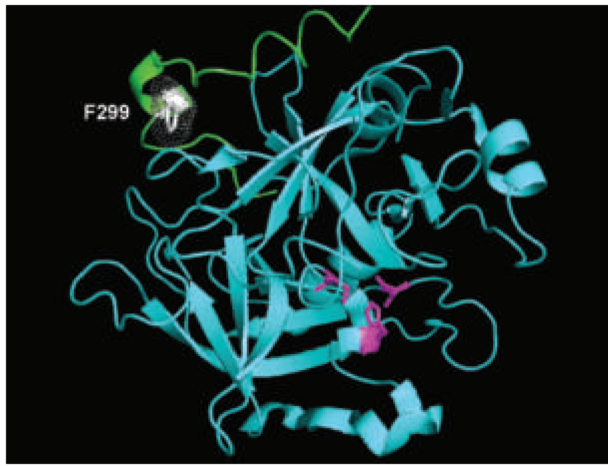

Figure 1. Crystal structure of the human α-thrombin (PDB access 1WAY). The B chain is in blue, the A-chain is in green, and the F299 residue is in white, in a dot and stick representation. The catalytic triad is in purple, represented by sticks. F299 refers to the circulating prothrombin. This residue is F342 when referring to the translation initiation codon, and F7 when referring to the A-chain numbering. The figure was generated with PyMol.

was significantly longer than the clotting time of the patients's prothrombin of an equivalent amount of normal prothrombin (dilutions in deficient plasma).

The case reported here is consistent with previous conclusions that the residues in the rigid part of the A-chain are crucial to the catalytic properties of the B-chain.[3,7] It also shows that F299 not only contributes to the three-dimensional organization of the A-chain, but that this mutation results in a defective activation mechanism, and affects the Xa-prothrombin interaction.

Dominique François, Caroline Chevreaud,° Dominique Vignon,* Philippe de Mazancourt°#*

**Laboratoire de Biologie Clinique, Hôpital Foch, Suresnes; °Laboratoire de Biochimie et Biologie Moléculaire, Hôpital Poincaré, Garches; #Faculté de Médecine Paris Ile de France-Ouest, UVSQ EA 2493, Garches, France*

Key words: prothrombin deficiency, hypodysprothrombinemia, mutation.

Prepublished online on February 17, 2006. PII: 03906078_8727.

Correspondence: Philippe de Mazancourt, Laboratoire de Biochimie et de Biologie Moléculaire, Hôpital Raymond Poincaré, 92380 Garches, France. Fax: international +33.1.47107923. E-mail: philippe.de-mazancourt@rpc.ap-hop-paris.fr

References

1. Degen SJF, Davie EW. Nucleotide sequence of the gene for human prothrombin. Biochemistry 1987;26:6165-77.
2. Acharya SS, Coughlin A, Dimichele DM. North American rare bleeding disorder study group. Rare bleeding disorder registry: deficiencies of factors II, V, VII, X, XIII, fibrinogen and dysfibrinogenemias. J Thromb Haemost 2004;2:248-56.
3. De Cristofaro R, Akhavan S, Altomare C, Carotti A, Peyvandi F, Mannucci PM. A natural prothrombin mutant reveals an unexpected influence of A-chain structure on the activity of human alpha-thrombin. J Biol Chem 2004;279:13035-43.
4. Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L. Cross-laboratory audit of normal reference ranges and assessment of ABO blood group, gender and age on detected levels of plasma coagulation factors. Blood Coagul Fibrinol 2005; 16:597-605.
5. Norris LA, Bonnar J. Haemostatic changes and the oral contraceptive pill. Baillieres Clin Obstet Gynaecol 1997;11:545-64.
6. Raoul M, Mathonnet F, Peltier JY, Collet C, Boucly C, Van Amerongen G, et al. An improved method for the detection of the G20210A transition in the prothrombin gene. Thromb Res 1997;88:441-3.
7. Bode W, Turk D, Karshikov A. The refined 1.9Å crystal structure of D-Phe-Pro-Arg chloromethylketone-inhibited human α-thrombin: structure analysis, overall structure, electrostatic properties, detailed active site geometry, and structure-function relationships. Protein Sci 1992;1:426-71.
8. Akhavan S, Mannucci PM, Lak M, Mancuso G, Mazzucconi MG, Rocino A, et al. Identification and three-dimensional structural analysis of nine novel mutations in patients with prothrombin deficiency. Thromb Haemost 2000;84:989-97.
9. Chen L, Rezaie AR. Proexosite-1-dependent recognition and activation of prothrombin by Taipan venom. J Biol Chem 2004; 279:17869-74.

©Ferrata Storti Foundation