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A Haut-Doubs FVII variant depending on species-derived-thromboplastin reagent (*F7*:p.Arg337His)

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Congenital factor VII (FVII) deficiency is a rare bleeding disorder characterized by a wide molecular heterogeneity and a poor relationship between FVII coagulant activity (FVII:C) and bleeding diathesis severity. It is inherited in an autosomal recessive manner. Severe haemorrhagic manifestations tend to occur mainly in homozygous or compound heterozygous individuals. To date, up to 200 mutations scattered throughout the *F7* gene and numerous polymorphisms have been reported (FVII mutation database, http://www.umd.be/F7/W_F7/index.html). Among these, some FVII variants p.Arg139Gln, p.Arg364Gln (FVII Padua) and p.Gly391Asp are known to be associated with variable FVII coagulant activities (FVII:C) depending on the species-derived thromboplastin reagent [1–3]. Recombinant human tissue factor preparations and highly sensitive clotting assays are recommended for the correct assignment of FVII:C levels in these cases [4]. In this study, we reported on a thromboplastin-dependant FVII variant: p.Arg337His highlighting the need to test FVII-deficient plasma with reagent of human origin.

Each patient consented to participate in the study, providing a written informed consent in accordance with the French law. Clinical features including both severity and frequency of the haemorrhagic symptoms were recorded by the physicians in charge of each patient. FVII coagulant activity was determined by a one stage-clotting assay using a rabbit thromboplastin (Neoplastin™; STA automated clotting analyser; Diagnostica Stago, Asnières-sur-Seine, France) and a human recombinant thromboplastin (Recombiplastin2G™; Instrumentation-Laboratory, Bedford, MA, USA). Factor VII antigen (FVII:Ag) was performed using the AsserachromVII:Ag Kit (Diagnostica Stago, Asnières-sur-Seine, France) according to the manufacturer's instructions. Direct sequencing of the whole coding regions and the 5′ flanking region containing the *F7* promoter was performed as previously described [5]. Primers and polymerase chain reaction conditions used are available upon request.

The proband was an asymptomatic 15 years old girl, incidentally diagnosed for FVII deficiency prior to surgery. The FVII:C level was initially measured with thromboplastin of rabbit origin. The result was validated again with another batch of rabbit thromboplastin, confirming the FVII:C level at 4%. When recombinant human thromboplastin was used, our study showed that the FVII:C level reached normal values of 80%. FVII:Ag levels revealed a cross-reactive-positive deficiency with a value of 81%. Direct sequencing of the *F7* gene identified two missense mutations: p.Arg139Gln and p.Arg337His at the heterozygote state. The p.Arg337His mutation had been already characterized in six unrelated French patients in our registry. Then, four of them were further analysed using a recombinant human thromboplastin. All of them displayed a discrepancy between the FVII:C values obtained from reagents of human or rabbit origins. Patients 1 and 2 were asymptomatic, whereas patients 3 and 4 had a mild bleeding phenotype (Table 1).

The p.Arg337His (previously named R277H) was reported once at the heterozygote state in an asymptomatic Yemenite-Jewish patient displaying FVII:C levels of 25% using a thromboplastin prepared from rabbit brain. No control using thromboplastin of human origin had been performed at that time [6]. In our series, we present two pairs of unrelated p.Arg337His carriers with the same *F7* genotype: [p.Arg139Gln/p.Arg337His] and [p.Arg337Cys/Arg337His] for patients (CC-2) and patients (1–3) respectively. Both pairs displayed concordant biological phenotypes assessing the reproducibility of these findings among similar patients.

In the (CC-2) first pair, the Arg139Gln variant was already known to be associated with higher FVII:C levels of 40% using human thromboplastin [1]. Nevertheless, heterozygosity for p.Arg139Gln could not, on

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Table 1. Genotypic and phenotypic characteristics of the patients harboring the p.Arg337His variant.

N	Age/gender	*F7* genotype mutation	FVII:C (%) Rabbit TF	FVII:C (%) Human TF	FVII:Ag (%)	Clinical features	Geographical distribution in France
CC	15/F	p.Arg139Gln/p.Arg337His	4	80	81	Asymptomatic	Haut-Doubs
1	38/F	p.Arg337Cys/p.Arg337His	<5	39	28	Asymptomatic	Western
2	33/F	p.Arg139Gln/p.Arg337His	7.5–8	105	85	Asymptomatic	Northern
3	53/M	p.Arg337Cys/p.Arg337His	4	41	32	Provoked bleeds (professionally exposed to haemostatic challenges)	Haut-Doubs
4	42/F	p.Pro311Leu/p.Arg337His	12	49	42	Bruising	Haut-Doubs

The p.Pro311Leu is reported here for the first time.

its own, explain normal range in FVII:C values. This result led us to hypothesize that the p.Arg337His variant was also associated with thromboplastin-dependent FVII:C values. The (1–3) second pair harboured the p.Arg337Cys mutation in compound heterozygous state with the p.Arg337His mutation. The p.Arg337Cys mutation has already been described in two homozygous patients with severe cross-reacting material negative FVII deficiency meaning that FVII:C levels using recombinant human thromboplastin and FVII:Ag levels were below 1% for the first case [7] and that FVII:C with unmentioned thromboplastin and FVII:Ag levels were of 6% and 12%, respectively, for the second case [8]. Therefore, the *F7* allele carrying the p.Arg337Cys mutation could only contribute up to 3% to the high FVII:C levels observed in these patients. Furthermore, the increase in FVII:C levels observed using reagent of human origin could be mostly attributed to the p.Arg337His variant.

In the crystal structure of the activated FVII: Tissue factor (FVIIa:TF) complex [9], Arg337 binds to the loop 48–54 of tissue factor (TF) and does not appear in close contact with the FIX or FX substrate [10]. Arg337 is partially buried as demonstrated by its depth location and makes three inter-residue hydrogen bonds with TF and thus position 337 is likely important for the stability of the FVIIa:TF complex (Fig 1). Consequently, a mutation in FVII at position 337 that reduced the total number of hydrogen bonds ought to destabilize the FVIIa:TF interaction. Conversely, a modification of the conformation in loop 48–54 of tissue factor, due to a mutation for instance, may also destabilize the FVIIa:TF interaction.

It turns out that in the rabbit TF, there are two residues radically different from the human TF in the loop 48–54. One is the change of Ser51->Leu and the other is Gly52->Glu. The former one appears the most different as Ser51 in human is fully solvent-exposed; thus, a replacement by a Leu, a hydrophobic residue, is expected to modify the loop structure in the rabbit TF. The latter one presents a serious modification of the protein backbone flexibility by replacing a Gly with a Glu. Two structural interpretations are possible: first, due to the two amino acid changes in rabbit TF, we may hypothesize that the loop conformation is different in rabbit TF compared with that in human

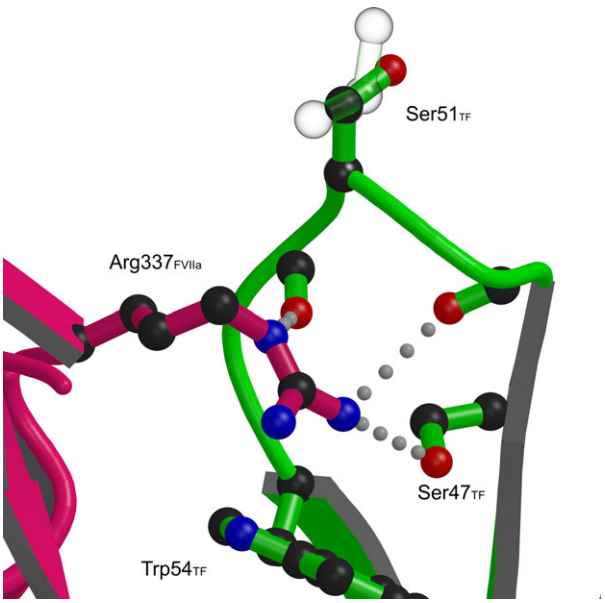

Fig. 1. Focus on the position Arg337 of factor VII (FVII) a bound to human TF loop 48–54. Coagulation FVIIa is drawn in pink, whereas the tissue factor is drawn in green. Protein side chains are displayed using balls-and-sticks with classical colour assignment (grey for carbon, blue for nitrogen and red for oxygen). Hydrogen bonds are shown as grey dotted lines. On top the TF loop is drawn the residue Ser51 which is fully solvent exposed. Replacement of Ser51 by Leu is also drawn in transparent balls-and-sticks highlighting one of the major differences between the human (plain colour) and rabbit (transparent) TF in the loop 48–54.

TF; second, if the TF loop conformation is similar in both orthologs then the stability of that loop ought to be decreased in rabbit versus human (solvation and entropic penalties). Thus, with both hypotheses the results is an expected reduction in binding strength with human FVIIa with rabbit TF. The reduction can be even emphasized with specific mutations occurring at position 337 in human FVIIa.

In our series, the FVII:C values obtained using human thromboplastin were better correlated to the clinical pauci-symptomatic phenotypes than the values obtained using reagent from rabbit origin. Structural bioinformatics analysis revealed that testing the activity of FVIIa mutants with TF obtained from different species may lead to divergent conclusions in which the cause of the observation is not the one due to mutations in FVIIa but rather due to the change in local structure of TF. These findings confirm the reliability

of recombinant human tissue factor preparations for the correct assignment of FVII:C levels in FVII-deficient patients.

Author contributions

GM, CZ, MT, MAB had the patients in charge and enrolled them in the study. GT collected and analysed the data. JLP performed the structural analysis and wrote the paper. JFS critically reviewed the manuscript. MGB designed the study and wrote the manuscript.

Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict of bias.

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FXI concentrate use and risk of thrombosis

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In contrast to haemophilia A and B, the management of surgery in patients with factor XI deficiency is not straightforward due to the poor relationship between factor XI level in the plasma and the bleeding risk [1,2]. Emerging evidence suggests that thrombin generation assays may correlate better with bleeding history [3], but a reliable assay is not readily available and some other studies did not show this [1,4]. While most FXI deficient individuals do not suffer from either spontaneous or surgery-related bleeding complications, treatment may sometimes be required particularly for surgery in areas of high fibrinolytic activity.

Two factor XI concentrates are available for treatment from BioProducts Laboratory, UK (BPL) and LFB Biomedicaments, France, Hemoleven (LFB) but both have been associated with venous and/or arterial thrombotic complications [1,5,6] particularly in elderly patients with pre-existing thrombotic risk factors. Guidelines and Hemoleven SmPC recommended a dose of not more than 30 IU kg^{-1}, aiming at a target level of 30–40 IU dL^{-1} according to Hemoleven SmPC and peak level of not more than 70 IU dL^{-1} according to the guidelines [7] which also recommend that antifibrinolytic agents should not be used at the same time.

From 2002, 12 thromboembolic events have been reported to LFB (11 since 2007) five of which were

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