Homozygous FVII deficiencies with different reactivity towards tissue thromboplastins of different origin

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The reagents most frequently used for FVII activity assay are obtained by rabbit brain or human placenta. In recent years, human recombinant thromboplastins have received great attention. FVII activity in FVII deficiency is usually low, regardless of the thromboplastin used. There are a few exceptions to this rule. These are represented by FVII Padua (Arg304Gln), FVII Nagoya (Arg304Trp), and FVII (Arg79Gln). In these three instances, clear discrepancies were noted in the FVII activity depending on the thromboplastin used. This indicates that at least two areas of FVII are involved in tissue binding, namely an epidermal growth factor domain of the light chain (Arg79Gln) and the catalytic domain (Arg304), controlled by exons 4 and 8, respectively. Since these three variants are cross reactive material positive, namely they are Type 2 defects, all other variants with normal antigen should be investigated by a panel of at least three tissue thromboplastins (rabbit brain, human tissue or human recombinant, and ox brain derived) in order to obtain a satisfactory classification.

Keywords: Tissue thromboplastins, FVII activity discrepancies, FVII deficiency

Introduction

The prothrombin time (PT) is the most widely used clotting test. The tissue factors commonly used in this test are obtained from rabbit brain, from human placenta, or human recombinant preparations. Less frequently an ox brain preparation is used either alone or in combination with absorbed normal plasma (thrombotest). The main use of PT is the follow-up of anticoagulated patients.

PT is also the key test for the diagnosis of FVII deficiency, which is the most frequently encountered condition among the rare coagulation disorders.[1] PT is always prolonged in FVII deficiency while partial thromboplastin time and thrombin time are always normal.

In the late 1970s and early 1980s it was demonstrated that a Factor VII variant, namely FVII Padua, had a different reactivity towards tissue thromboplastins of different origin.[2,3] It was subsequently shown that the molecular biology alteration at the basis of the defect was the substitution of Arginine 304 with a Glutamine (Arg304Gln) in exon 8.[4–6]

In this variant, FVII activity was 6–8% of normal using rabbit brain thromboplastins whereas it is normal using ox brain thromboplastins. Intermediate values around 30–40% of normal were obtained using human placenta or human recombinant thromboplastin. Several confirmatory papers were subsequently published.[7–10]

By now, the laboratory picture is well established and the clotting pattern is the simplest way to formulate a tentative diagnosis of this FVII variant.[11,12] Recently, at least two other mutations of FVII have been associated with similar discrepancies towards different tissue thromboplastins, namely FVII Nagoya (Arg304Trp) and the Arg79Gln mutation.[9,13,14]

The purpose of the present paper is to evaluate similarities and discrepancies among these three peculiar FVII variants.

Patients, materials, and methods

All papers dealing with congenital FVII deficiencies which showed different levels of FVII activity, with at least two thromboplastins of different origin, were selected. The availability of FVII antigen, a compatible autosomal hereditary pattern, and mutation responsible for the defect, were also sine qua non-inclusion criteria. Compound heterozygotes and heterozygotes were excluded in order to deal with a homogeneous homozygous population. This allowed

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the elimination of interferences from another mutation (compound heterozygotes) or from the normal allele (heterozygotes). Patients showing the same mutation but lacking even one of the other inclusion criteria were also excluded.

The FVII activity level difference between at least two thromboplastins had to be at least about eight times as high as the lowest level obtained. Patients with smaller differences were disregarded as probably due to the range of the methods used. FVII activities obtained with all different thromboplastins were reported for each patient. The thromboplastins selected were rabbit brain, human placenta, human recombinant, simian brain, and ox brain. Different types of rabbit brain were used, on the contrary only one type of human placenta was used (Thromborel, Dade-Behring, Marburg, Germany). Human recombinant thromboplastins was that supplied by Lilly or by Dade-Behring laboratories.

Finally, the sources of the ox brain and simian brain thromboplastins were only seldom described. Usually they were made in-house or kindly prepared by Stago laboratories or Nyegard laboratories after a specific request by the individual investigators.

Factor VII antigen, regardless of the method used, electroimmunoassay or Elisa, were also gathered on individual basis. The bleeding tendency was recorded as stated by the authors of the single papers. The presence of associated risk factors or disease was also recorded. For calculation purposes, values of FVII activity of less than 1% were entered as 1% and values of activity and of antigen given as normal were entered as 100% of normal.

Results

The number of homozygous patients with FVII deficiency due to the three mutations involved is still limited. We have collected 16 cases with the Arg304Gln mutation (Factor VII Padua). This number includes six personal cases. Then, there is one case with an Arg304Trp mutation (FVII Nagoya) and four cases with the Arg79Gln mutation. Only in a few cases all thromboplastins were used. In the majority of cases only rabbit brain and human placenta or human recombinant thromboplastins were used. Ox brain thromboplastin was used in about 50% of cases (Table 1).

In a few cases a simian brain preparation was used. In FVII Padua (Arg304Gln) FVII activity using rabbit brain varied between 1 and 14% (mean 5.5). The values varied instead between 21 and 57% of normal (mean 33.8), between 17 and 57% (mean 32.1), and between 70 and 130% (mean 101.2) for human placenta, human recombinant, or ox brain thromboplastin, respectively. The mean value for simian brain was 30.0 (range 26–35). The single case with Arg304Trp mutation showed values of 5, 16, and 60% of normal for rabbit brain, Human recombinant, and ox brain, respectively.

The FVII activity mean value for the Arg79Gln mutation were 6.8% of normal (range 35–11); 62.3% (range 30–100); 47.7% (range 38–57), and 109% (range 68–150) for rabbit brain, human placenta, human recombinant, and ox brain, respectively. The activity value using simian brain was 30% (range 25–35).

Factor VII antigen as obtained by rabbit raised polyclonal antibody was reported in all cases. It varied between 95 and 241 (mean 130) for the Arg304Gln mutation, 100% of normal for the Arg304Trp mutation, and varied between 65 and 125 (mean 93.3) for the Arg79Gln mutation.

Discussion

FVII is a single-chain glycoprotein synthesized in the liver with a molecular weight of about 50 000 Da. It is activated to FVIIa by thrombin, IXa, Xa, or XIIa.[15] In the presence of calcium and TF, FVIIa by its turn activates both FIX and FX.

The transformation of single-chain FVII to activated FVII involves the cleavage of an Arg153Ile bond with consequent formation of a heavy chain (254aa) and a light chain (152aa) joined by a disulphide bond at Cys135 and Cys262. The heavy chain contains the catalytic domain while the light chain contains the Gla domain, the amphipathic helix, the growth factor domain, and the connecting peptide. The two forms of FVII, zymogen and activated form, circulate in plasma approximately in a 100 to 1 proportion.[1,15] At least two areas of FVII seem to be involved in tissue factor binding.[16–19] The Arg304Gln or the Arg304Trp mutations belong to the catalytic area of the heavy chain controlled by exon 8. On the contrary, the Arg79Gln mutation belongs to the first epidermal growth factor (EGF)-like domain controlled by exon 4.[15]

The area around the Arg304 residue is surely involved as demonstrated by the relatively large series of patients with FVII Padua (Arg304Gln) described.[2,3,8,10,20] The observation that the mutation of Arg304 to Trp instead of Gln (FVII Nagoya) suggest that this Arg plays a pivotal role. Regardless of its change to Gln or to Trp, the result is the same, namely low activity with rabbit brain, normal with ox brain, and intermediate with human placenta or human recombinant.[21]

It is more difficult to explain the picture in the case of Arg79Gln, located in the first EGF-like domain of the light chain. Many authors have proposed this area as fundamental for the binding to tissue factor.[16–19] However, in this case the pattern of reactivity to different thromboplastins seems different from that seen in FVII Padua or FVII Nagoya (Table 1).

Table 1 Cases of homozygous with Arg304Trp, Arg304Gln (FVII Padua), or Arg79Gln FVII deficiency studied by at least two thromboplastins of different origin as reported in the literature

Author (years)	Eponym	FVII Act. (% of normal) Rabbit brain	Human placenta	Human recombinant	Ox brain	Simian brain	FVII Ant. (% of normal)	Bleeding tendency	Mutation (exon)	Comments
Girolami *et al.* (1978–1993)										
Case 1		8	36	30	105	29	95	Mild	Arg304Gln[8]	Daughter of case 1
Case 2		9	32	28	110	23	110	Mild		Different kindred
Case 3		13	36	32	100	36	105	None		Different kindred
Case 4		10	34	29	105	30	110	Mild		Different kindred
Case 5		8	33	30	105	32	120	Mild		So far unpublished. (Different kindred)
Case 6		7	29	27	95	34	95	Mild		
O'Brien *et al.* (1991)		1	30	n.r.	n.r.	n.r.	100	None	Arg304Gln[8]	Studies carried out on purified material
Shurafa *et al.* (1993)		14	21	n.r.	124	n.r.	n.r.	None	Arg304Gln[8]	Afro-American
Peyvandi *et al.* (1997–2000)										
Case 1		1	n.r.	37	Normal	n.r.	240	None		
Case 2		1	n.r.	27	Normal	n.r.	224	Mild	Arg304Gln[8]	Levels obtained with ox-brain reagents unspecified
Case 3		1	n.r.	24	Normal	n.r.	156	None		Cases 1–4 belong to same kindred
Case 4		1	n.r.	17	Normal	n.r.	106	None		
Case 5		1	n.r.	22	Normal	n.r.	130	None		
Horellou *et al.* (2007)										
Case 1		2–5	25–89	25–89	n.r.	n.r.	Normal	None	Arg304Gln[8]	Values supplied as a range
Case 2		2–5	25–89	25–89	n.r.	n.r.	Normal	None	Arg304Gln[8]	
Kirkel *et al.* (2010)		7	n.r.	77	n.r.	n.r.	121	None	Arg304Gln[8]	
Matsushita T *et al.* (1994)	Nagoya	5	n.r.	16	60	n.r.	100	None	Arg304Trp[8]	
Takamiya *et al.* (1995)	Shinjo	11	100	n.r.	150	25	115	None	Arg79Gln[4]	One brother similarly affected
Takamiya *et al.* (1998)	Tondabayshi	6	36	38	68	35	65	None	Arg79Gln[4]	
Horellou MH *et al.* (2007)	n.r.	2–5	25–89	n.r.	n.r.	n.r.	Normal	None	Arg79Gln[4]	Values entered as range

For calculation purposes, clotting levels of less than 1% of normal are entered as 1%. Similarly, antigen values supplied as normal have been entered as 100%.

These three variants have in common the Type 2 pattern, namely are cross reactive material (CRM) positive and are asymptomatic or only mildly symptomatic. Furthermore and most importantly, an Arg to Gln or Trp change is involved in all cases, regardless of the site.

Unfortunately, there are other Type 2 defects which have not been fully investigated by this approach.[20,22–24]

These defects should also be investigated by the use of all different tissue thromboplastins in order to obtain more information on the reaction between TF and FVII. Very little is known, for example, for the Gly331Asp mutation. There are two heterozygotes for this mutation, which have been shown to yield similar FVII activity levels with different tissue thromboplastins together with normal antigen.[25,26] Unfortunately, no homozygote for this mutation has ever been studied by this approach and therefore no final conclusions can be drawn. On the basis of the results obtained in the two reports dealing with

heterozygous patients, it could be surmised that homozygotes for the CRM-positive Gly331Asp mutation have an inert protein.

Unless all Type 2 variants are investigated by the use of thromboplastins of different origin, it will be difficult to correlate laboratory data and clinical features. The results obtained with a given thromboplastin may not represent the real haemostatic condition. This is probably at least one of the causes for the discrepancies observed between FVII level and bleeding tendency, as reported in some series of patients.[27]

For example, FVII Padua (Arg304Gln) could appear a moderately severe condition if evaluated only on the basis of rabbit brain thromboplastin (1–10% of normal). It would appear a mild defect if judged by human tissue preparations (FVII activity around 30% of normal), and completely normal if assayed with ox brain thromboplastins.[2] No case of true FVII deficiency (Type 1) has ever been described as having this different reactivity towards different thromboplastins. This is not surprising since it is plausible that the discrepancies depend on the presence of the abnormal FVII.

The double homozygosity (Arg152Gln + Arg79Gln) described by Chaing *et al.*[17] is also of interest. The abnormality was characterized by different reactivity towards different thromboplastin. However, it was demonstrated by means of expression studies that the variable reactivity towards tissue thromboplastin was due to the Arg79Gln mutation and not to the Arg152Gln which was inert. These studies confirm the other findings about the role of residue Arg79 in the binding to tissue factor.[16,18]

The different prevalence of the three mutations may indicate that the Arg304 residue is a hot-spot subject to frequent mutations.[6] The homozygous patients with the Arg304Gln defect in exon 8 are at least five times more frequent than the other two variants considered together. As a matter of fact, for the Arg304Trp mutation, only two homozygotes have been described so far,[17,21] but only one (FVII Nagoya) has been studied using thromboplastins of different origin.[21] The levels of factor VII activity found in FVII Padua patients with human placenta are fairly uniform and similar to those observed with human recombinant.[2,3,28]

The pattern seen in the two mutations (Arg304Gln and Arg304Trp) seems similar and well established (low level with rabbit brain, normal level with ox brain). On the contrary, the discrepancies observed for the Arg79Gln mutation are less clear cut. In this case it seems that the difference in activity between human tissue and ox brain is less evident. However, FVII activity levels obtained with rabbit brain preparations are always the lowest values observed. The limited number of cases so far observed may, at least in part, explain the still unclear pattern.

In conclusion, it seems that at least three mutations of FVII, belonging to areas controlled by exon 4 (Arg79) and by exon 8 (Arg304), are associated with a sure discrepant sensitivity towards thromboplastins of different origin. The exact mechanism underlying the sequential involvement of these two areas is not completely clarified yet. Available data suggest that the EGF area (exon 4) mainly binds the protease to its receptor whereas interaction with one or more than one protease domain residues (exon 8) are needed for the development of full catalytic activity of the bound protease.[16]

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