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Variability of clinical manifestation of factor VII-deficiency in homozygous and heterozygous subjects of the European *F7* gene mutation A294V

Inherited factor VII deficiency (FVIID) is a rare autosomal bleeding disorder. Subjects with reduced FVII activity and identified *F7* gene mutation are registered in the International Greifswald registry of FVIID.[1] The mutation A294V is the most frequent among 717 FVIID patients in Central and North-Eastern Europe. The high prevalence of FVIID homozygous and heterozygous subjects, who share the same mutation (A294V), offers the unique possibility to study the clinical variability in a relatively large number of individuals with the identical defect in the *F7* gene. In the reported study we have analyzed the clinical variability of 14 homozygous and 99 heterozygous subjects with *F7* gene mutation A294V. Subjects with combinations with other bleeding disorders such as von Willebrand Disease, deficiency of FIX, FII etc., were excluded.

We collected data on factor VII clotting (FVII:C) activity, FVII antigen (FVII:Ag), date of initial onset of bleeding and bleeding symptoms.[1,2]

FVII:C was assayed locally with standard one-stage methods using thromboplastin (mostly recombinant and human-derived, Thromborel S® Dade Behring). FVII:Ag was measured at some of the centers using an immunoenzymatic method (Asserachrom, Diagnostica Stago, Asnieres, France). The following bleeding symptoms were evaluated according to published criteria:[1-4] intracranial hemorrhage (ICH), gastrointestinal (GI) hemorrhage, hemarthrosis, subcutaneous hematoma as well as epistaxis, oral bleeding, and menorrhagia in accordance with Peyvandi and Mannucci.[5] Endogenous thrombin potential (ETP) was measured in platelet poor plasma from controls and subjects homozygous for A294V in the presence of activated protein C.[6] Citrated plasma samples from 8 control subjects without FV_{Leiden} or FVHR2 (factor V wild type; FV_{wt}) and without A294V mutation were used as a reference. We used the mutation H1299R as marker for FVHR2.[7] The FV_{Leiden} mutation was determined according to Bertina *et al.*[8]

The 14 homozygous subjects for the F7 gene mutation A294V are characterized by mean levels of FVII:C of 10±6% and of FVII:Ag of 52±11%. Nine of the homozygous patients (64%) had spontaneous bleeding symptoms (classified as symptomatic) and 5 (36%) did not (classified as asymptomatic). There was no significant difference in FVII level between symptomatic and asymptomatic homozygous A294V subjects.

The spontaneous bleeding profile of the 9 symptomatic subjects was characterized by GI (22%) bleeds, hemarthrosis (22%), epistaxis (33%), easy bruising (11%), gingival bleeding (22%), subcutaneous hematoma (11%),

Table 1. Endogenous thrombin potential, bleeding symptoms and co-inheritance of FVHR2 in nine patients homozygous for the mutation A294V with available plasma samples.

Patient	Age	FVII:C [%]	FV HR2	GI Bleed	Hemarthrosis	Easy Bruising	Epistaxis	Gum Bleed	Hematuria	Menorrhagia	Hematoma	Severity*	Endogenous thrombin potential compared to the references (reference (100%))	
G-9125	6	8	hz									asympt.	203%	
G-9297	18	25	ho									asympt.	146%	
G-9130	15	4	wt						x			mild	93%	
G-9623	32	8	hz								x	mild	82%	
G-13255	10	7	wt					x				mild	59%	
G-9773	59	6	wt				x	x		x		moderate	56%	
G-13325	74	11	wt		x							severe	65%	
G-12990	44	9	wt	x								severe	57%	
G-10833	57	3	wt		x		x		x			severe	47%	

hematuria (22%), and menorrhagia (33%) but no ICH.

The thrombotic risk factor FV_{Leiden} has already been reported to have a moderating effect on bleeding symptoms in patients with severe deficiency of factors VIII (hemophilia A),[9] factor IX (hemophilia B)[10] or with the mutation FVII g.9726+5G>A ($FVII_{Lazio}$).[11] We analyzed the presence of the thrombophilia risk factors FV_{Leiden}, FV_{HR2} and FIIG20210A mutation in 14 patients homozygous for A294V. In 3 out of 5 asymptomatic cases (60%) we found a co-inheritance for FV_{Leiden} (heterozygous) or FV_{HR2} (heterozygous and homozygous). Among 9 symptomatic homozygous patients 8 had neither FV_{Leiden} nor FV_{HR2} or FIIG20210A and only one case with mild bleeding characterized by *occasional hematomas* was heterozygous for the FV_{HR2} (p=0.09, Fisher exact test). These data suggest that the presence of FV_{Leiden} or FV_{HR2} could attenuate the bleeding severity in homozygous A294V patients; however, due to the small size of patient population investigated, further studies are needed to clarify this. With a FV_{HR2} allele frequency of 7.4% in the German population, the frequency of 5.5% FV_{HR2} alleles among the symptomatic A294V patients matches the expected value, but the frequency of 30% FV_{HR2} alleles among the asymptomatic cases is above the expected value for a random selection. The high frequency of FV_{HR2} alleles suggests a moderating effect.

Recently we have shown an increased ETP in plasma samples from carriers of the mutations FV_{Leiden} or *FVHR2*.[6] On the basis of these findings we studied the ETP in available plasma samples from 9 FVII deficient patients, homozygous for A294V (Table 1). The ETP values of all seven plasma samples of symptomatic patients were significantly (p=0.002, Student's t-test) lower than the reference value. Increased ETP values were only observed in the two samples of asymptomatic individuals. The ETP study supports a possible modulating influence of FV_{HR2} on differential clinical manifestations in homozygous A294V subjects. Further studies are required to confirm this trend.

This present study included 99 individuals heterozygous for A294V (52 males, mean age 33.4 years; 47 females, mean age 33.6 years). Their mean FVII level was 44±15%. Twenty-three (23%) and 76 (77%) heterozygous A294V carriers demonstrated or lacked spontaneous bleeding symptoms respectively. Nine of the 23 symptomatic heterozygous patients were sporadic cases; the others belonged to families (parents, siblings or children) of a FVII deficient index patient.

The FVII level did not differ significantly between symptomatic and asymptomatic heterozygous A294V subjects. The prevalence of FV_{Leiden} or FV_{HR2} was similar among the symptomatic and asymptomatic heterozygous cases. The bleeding pattern of the 23 heterozygous patients consisted of epistaxis (65%), gum bleeds (17%), easy bruising (30%), hematoma (3%), and menorrhagia (56% in women older than 14 years), but hemarthrosis, ICH or GI bleeding were not observed. Three patients (*Online Supplementary Table S2*) were treated as needed with pdFVII (patients f.10 and 9) or tranexamic acid (pt. 4).

In previously published papers, heterozygous probands were usually characterized as clinically asymptomatic and only single cases of heterozygous patients with clinical manifestations have been described.[2,4] For some mutations (Arg304Gln) heterozygous subjects have moderate bleeding symptoms;[4,12] but systematic studies of heterozygous subjects for *F7* gene mutations do not exist. Our large-scale analysis of A294V heterozygous subjects shows that the proportion of symptomatic carriers of FVIID is relatively high.

*Falko H. Herrmann,[1] Karin Wulff ,[1] Rüdiger Strey,[1] Annelie Siegemund,[2] Jan Astermark,[3] and Sam Schulman[4] for the International Greifswald Registry of FVII deficiency**

[1]Institute of Human Genetics, Greifswald, Germany; [2]Clinical Haemostaseology, University of Leipzig, Leipzig, Germany; [3]Department for Coagulation Disorders, Malmö, University Hospital, Malmö, Sweden; [4]Thrombosis Service, McMaster Clinic, HHS-General Hospital, Hamilton, Canada

**Members of the International Greifswald Registry of FVII deficiency, see appendix.*

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Key words: factor VII deficiency, mutation A294V, clinical variability, endogenous thrombin potential.

Correspondence: Falko H. Herrmann, University Greifswald, Medical Faculty, Institute of Human Genetics, Fleischmannstr. 42-44, D-17487 Greifswald, Germany. Phone: international +49.3834865371. Fax: international +49.3834865393.
E-mail: herrmafh@uni-greifswald.de

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The online version of this article contains a supplemental appendix.

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Endothelial-coagulative activation during chronic obstructive pulmonary disease exacerbations

Patients with chronic obstructive pulmonary disease (COPD) are prone to clinical exacerbations of their disease and this is known to be associated with increased airway inflammation.[1] A prothrombotic condition resulting from the inflammatory activation of the endothelium may well occur during COPD exacerbations and could be a significant causative factor for pulmonary thromboembolism (PTE).[2] We tested the hypothesis that acute inflammatory responses of COPD exacerbation are associated with activation of the endothelial-coagulative system.

Surrogate markers of inflammation (IL-6), endothelium activation (von Willebrand Factor antigen – vWF:Ag), fibrinolytic activation (D-Dimer - D-D) and clotting stimulation (prothrombin fragment 1+2 - F1+2) were prospectively assayed in the blood of patients with COPD by commercial ELISA kits (IL-6, R&D Systems, Oxon, UK; Asserachrom vWF, Diagnostica Stago, Asnieres, France; Zymutest D-Dimer, Hyphen Biomed, Neuville-Sur-Oise, France; Enzygnost F1+2 Micro, Dade Behring, Marburg, Germany) at the time of hospital admission for acute exacerbation of their disease (visit 1), and after clinical resolution (visit 2). The diagnosis of COPD and the presence of disease exacerbation were documented during hospital admission based on current criteria. All patients were prescribed a standardized treatment regimen consisting of oral antibiotics, IV corticosteroids, nebulized bronchodilators and oxygen. Patients with commonly acquired thrombotic risk factors (e.g. hypertension, diabetes mellitus, malignancy, etc) were excluded. No patient received heparin, anticoagulants, statins or antihypertensive drugs during the study. All other treatment (including inhaled corticosteroids) remained constant throughout the study. The data obtained from the COPD patients were compared with that from control patients (with no diagnosis of COPD) who were hospitalized in the same institution for other respiratory conditions.

Complete datasets were available for 30 COPD patients and for 12 non-COPD controls for final analyses (*see summary data in Tables 1 and 2 in Supplementary Appendix*). At the time of exacerbation (visit 1), IL-6, vWF:Ag, D-D and F1+2 levels (mean±SEM) were elevated and decreased significantly when patients were clinically stable (visit 2) with IL-6 declining from 6.7±0.8 to 3.9±0.4 pg/mL ($p<0.001$; paired Student's *t*-test) (Figure 1A; left panel); vWF:Ag from 186.3±14.1 to 124.7±7.9% ($p<0.001$; paired Student's t-test) (Figure 1A; right panel); D-D from 232.1±30.0 to 129.5±7.4 ng/mL ($p<0.002$; paired Student's t-test)(Figure 1B; right panel); and F1+2 from 1.36±0.61 to 0.72±0.22 nmol/L ($p<0.001$; paired Student's t-test) (Figure 1B; left panel). A significant reduction in CRP levels from 32.5±6.0 to 2.3±0.4 mg/L ($p<0.001$; paired Student's *t*-test) was also reported. In the control group the levels of all the parameters studied were also elevated at admission (visit 1), but significant reductions were not observed at follow-up (visit 2) with the exception of D-D (*data not shown*).

This study shows that blood levels of vWF:Ag, D-D and F1+2 together with IL-6 are elevated in COPD patients during acute exacerbation of their disease, thus suggesting a relationship between acute inflammation, endothelial activation and clotting initiation. The present findings are in agreement with those of others. Earlier work by Wedzicha *et al*.[3] postulated an enhanced platelet activity in COPD by showing lower platelet aggregate ratio in hypoxemic COPD patients compared to controls with a trend to lesser aggregate ratios in the more hypoxemic patients. Further support for the view that a prothrombotic condition is present in COPD was provided by Alessandri *et al*.,[4] who demonstrated that about two-thirds of patients with stable COPD have elevated plasma levels of the thrombin generation marker prothrombin F1+2 fragment. This study also underlines the importance of systemic inflammation in COPD and it is in agreement with previous work showing that COPD patients have increased blood levels of fibrinogen and IL-6 during exacerbations of their disease.[5] Clinically, elevated vWF:Ag, D-D and F1+2 concentrations in blood have been reported to increase the likelihood of throboembolic events,[6] which are noted during acute exacerbations of COPD.

COPD exacerbations may be caused by severe pneumonia and this *per se* is likely to explain the underlying activation of the endothelial-coagulative system.[7,8]

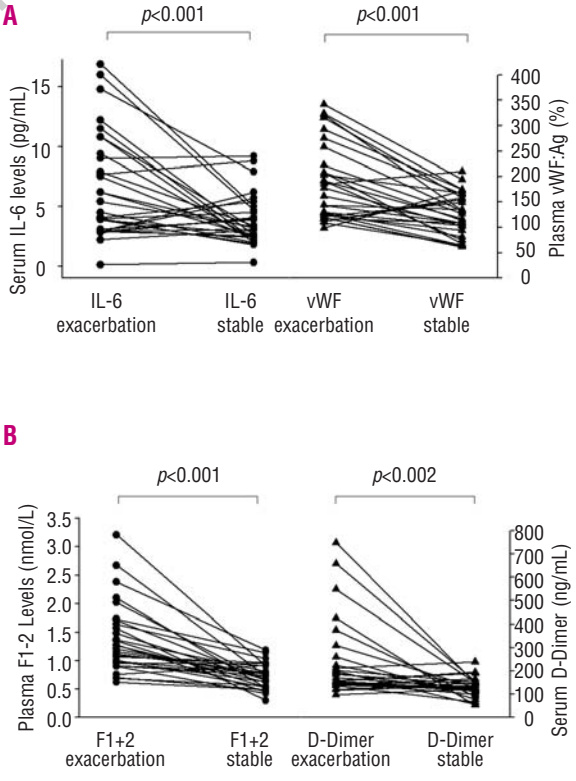

Figure 1. (A) Serum levels of IL-6 (pg/mL) and plasma vWF:Ag (%) in COPD subjects during exacerbation and in stable state. (B) Plasma levels of F1+F2 (nmol/L) and serum D-Dimer (ng/mL) in COPD subjects during exacerbation and in stable state.