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著者	Takahashi Ryoichi, Ono Kenjiro, Ikeda Tokuhei, Akagi Akio, Noto Daisuke, Nozaki Ichiro, Sakai Kenji, Asakura Hidesaku, Iwasa Kazuo, Yamada Masahito
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Coagulation and fibrinolysis abnormalities in familial amyloid polyneuropathy

Ryoichi Takahashi[1], Kenjiro Ono[1], Tokuhei Ikeda[1], Akio Akagi[1], Daisuke Noto[1], Ichiro Nozaki[1], Kenji Sakai[1], Hidesaku Asakura[2], Kazuo Iwasa[1], Masahito Yamada[1]

[1]Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, 13-1 Takara-machi, Kanazawa 920-8640, Japan.

[2]Department of Internal Medicine (III), Kanazawa University Graduate School of Medical Science, 13-1 Takara-machi, Kanazawa 920-8640, Japan.

Correspondence: Masahito Yamada, MD, PhD, Professor & Chairman, Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, 13-1 Takara-machi, Kanazawa 920-8640, Japan; Tel: +81-76-265-2290; Fax:

+81-76-234-4253; E-mail: m-yamada@med.kanazawa-u.ac.jp

Running Head: Coagulation and fibrinolysis abnormalities in FAP

Abstract

Objective. Familial amyloid polyneuropathy (FAP) is an autosomal dominant form of hereditary amyloidosis. Several studies reported coagulation factor X deficiency and excessive fibrinolysis in immunoglobulin light chain amyloidosis. However, few have investigated coagulation and fibrinolysis in FAP. The objective of this study was to determine abnormalities in plasma biomarkers of coagulation and fibrinolysis in FAP.

Methods. We prospectively recruited eight FAP patients with transthyretin mutations and ten age-matched control patients with other neuropathies in our university. We examined plasma biomarkers of coagulation and fibrinolysis including prothrombin time, activated partial thromboplastin time, fibrinogen, fibrin/fibrinogen degradation products, D-dimer, α_2-antiplasmin, antithrombin, plasminogen, thrombin-antithrombin complex, plasmin-α_2-antiplasmin complex, prothrombin fragment 1+2, and coagulation factor X. The Mann-Whitney U test was performed for statistical comparisons between FAP and control groups.

Results. FAP patients exhibited significantly decreased levels of coagulation factor X, plasminogen and α_2-antiplasmin, and significantly increased levels of prothrombin fragment 1+2 compared to control patients.

Conclusion. Our results indicate abnormalities of coagulation and fibrinolysis in FAP patients.

Keywords: amyloidosis, coagulation factor, prothrombin fragment, α_2-antiplasmin, plasminogen

Abbreviations: α_2-AP = α_2-antiplasmin; APTT = activated partial thromboplastin time; AL amyloidosis = immunoglobulin light chain amyloidosis; AT = antithrombin; CIDP = chronic inflammatory demyelinating neuropathy; F1+2 = plasma prothrombin fragment 1 + 2; FAP = familial amyloid polyneuropathy; Fbg = fibrinogen; FDPs = fibrin/Fbg degradation products; FVIIa = activated coagulation factor VII; FX = coagulation factor X;

PAP = plasmin-α_2-antiplasmin complex; PT = prothrombin time; TAT = thrombin-antithrombin complex; TTR = transthyretin

Introduction

Familial amyloid polyneuropathy (FAP) is an autosomal dominant form of hereditary amyloidosis. Amyloidogenic mutated transthyretin (TTR) is the most common FAP-related protein worldwide [1]. Several studies reported coagulation factor X (FX) deficiency and excessive fibrinolysis in immunoglobulin light chain amyloidosis (AL amyloidosis) patients [2-4]. However, few have investigated coagulation and fibrinolysis in FAP. Most researchers did not observe coagulation abnormalities in FAP patients because of low incidence of hemorrhagic events [5]. Here we examined coagulation and fibrinolysis in FAP and observed decreased levels of FX, plasminogen, and α_2-antiplasmin (α_2-AP) and elevated levels of plasma prothrombin fragment 1 + 2 (F1+2).

Patients and methods

FAP patients

We investigated coagulation and fibrinolysis in eight consecutive FAP patients admitted to our hospital from 2004 to 2011. In our district, FAP with a TTR Val30Met

mutation was endemic [6], and a FAP pedigree with rare TTR Leu58Arg mutation was present [7]. Diagnoses were confirmed in all patients by positive family history, clinical and neurophysiological examinations, tissue biopsies of the sural nerve and gastrointestinal tract demonstrating amyloid deposits, and gene analysis indicating the TTR Val30Met (n = 7) or Leu58Arg mutations (n = 1). All patients were from separate kinships. Patient age ranged from 34 to 77 years (median age: 66 years); six of the eight patients were males and two were females. Three patients experienced hemorrhagic events: one had intestinal hemorrhage, one had hematuria and a hemorrhagic kidney cyst on abdominal computed tomography, and one had seminal vesicle hemorrhage; no patient experienced serious hemorrhagic events requiring blood transfusion or resulting in death. All patients had no genetic abnormalities of coagulation and fibrinolysis. All patients had no liver dysfunction and showed normal levels of transaminase, albumin, and choline esterase (data not shown).

Control patients

We prospectively recruited control patients with peripheral neuropathy at our hospital. Each peripheral neuropathy was diagnosed by clinical, laboratory, and neurophysiological

examinations, and nerve biopsy. We excluded patients being administered anticoagulation drugs and those having severe hepatic injury or innate coagulopathy. The age of ten control patients with peripheral neuropathies ranged from 57 to 67 years (median age: 61 years; not significantly different from that of FAP patients). Diagnoses included alcoholic neuropathy (n = 3), diabetic neuropathy (n = 2), vasculitic neuropathy (n = 1), Crow-Fukase syndrome (n = 1), neuropathy with anti-myelin-associated glycoprotein antibody (n = 1), chronic inflammatory demyelinating neuropathy (CIDP) (n = 1), and Charcot-Marie-Tooth disease (n = 1).

Coagulation and fibrinolysis tests

This study was approved by the medical ethical committee of our university. After obtaining written informed consent, blood samples were obtained from all FAP and control patients. Plasma samples were analyzed for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), fibrin/Fbg degradation products (FDPs), D-dimer, α_2-AP, antithrombin (AT), plasminogen, thrombin-antithrombin complex (TAT), plasmin-α_2-antiplasmin complex (PAP), F1+2, and FX. PT and APTT were measured by

light scattering spectroscopy using diagnostic kits (PT: Thromborel® S, Siemens Healthcare Diagnostics, Inc. APTT: Actin FSL, Siemens Healthcare Diagnostics, Inc.). Fbg and FX were measured by the coagulation time method using reagents (Fbg: Fibrinogen determination; Siemens Healthcare Diagnostics, Inc. FX: HemosIL Factor X deficient plasma and HemosIL Recombiplastin; Mitsubishi Chemical Medience Co., Ltd., Japan). FDP was measured by latex photometric immunoassay using a diagnostic kit (Nanopia® P-FDP; Sekisui Medical Co., Ltd., Japan). AT, α_2-AP, and plasminogen were measured using synthetic chromogenic peptide substrate (AT: Test Team® S AT III, Sekisui Medical Co., Ltd., Japan. α_2-AP: Test Team® S APL; Sekisui Medical Co., Ltd., Japan. Plasminogen: test team® S PLG; Sekisui Medical Co., Ltd., Japan). TAT, PAP, and F1+2 were measured by enzyme immunoassay , latex agglutination turbidimetric immunoassay, and enzyme-linked immunosorbent assay using diagnostic kits (TAT: TAT S; TFB Co., Ltd., Japan. PAP: LPIA-S PPI II; Mitsubishi Chemical Medience Co., Ltd., Japan. F1+2: Enzygnost®F1+2 Monoclonal; Siemens Healthcare Diagnostics, Inc., Japan), respectively.

The two patient groups were statistically compared using the Mann-Whitney U test.

Statistical analyses were performed using software package StatView version 5.0. *P* values <0.05 were considered significant.

Results

The table 1 shows the coagulation and fibrinolysis test results of FAP and control patients. The levels of α_2-AP, plasminogen, F1+2, and FX were abnormal in the FAP group but not in the control group. FAP patients exhibited significantly decreased levels of α_2-AP ($p = 0.039$), plasminogen ($p = 0.0037$), and FX ($p = 0.0010$), and significantly elevated levels of F1+2 ($p = 0.019$) compared with control patients.

Some FAP patients also presented with prolonged PT (n = 2), elevated TAT level (n = 1), and mildly elevated PAP levels (n = 4). No abnormalities were observed in levels of Fbg, FDP, or D-dimer in FAP patients.

Some control patients exhibited prolonged PT (n = 1), elevated TAT levels (n = 2), and elevated levels of F1+2 (n = 4). However, no abnormalities were observed in levels of α_2-AP, Plg, Fbg, FDP, D-dimer, or FX.

Discussion

Our results indicate coagulation and fibrinolysis abnormalities in FAP patients.

The figure 1 shows the coagulation and fibrinolysis cascades and the abnormalities observed in FAP patients. For coagulation markers, we observed significant elevation of F1+2 and decrease of FX in FAP patients. F1+2 is an activation peptide generated during prothrombin conversion to thrombin, and the elevation of F1+2 indicates *in vivo* thrombin generation, which means the activation of coagulation. Among fibrinolysis markers, we observed significantly decreased levels of α_2-AP and plasminogen. PAP was elevated in FAP patients compared to normal value, but this elevation of PAP was not significant. The decreased levels of α_2-AP and plasminogen indicate the activation of fibrinolysis. Our FAP patients had no liver dysfunction, congenital blood coagulation disorders, or thrombotic disorders, such as atherosclerosis, thromboembolism, inflammation, cancer, sepsis, and atrial fibrillation [8].

As for the mechanisms underlying the activation of coagulation and fibrinolysis in

FAP, we speculate that the injury of vascular endothelial cells by amyloid deposits might lead to expression of tissue factor and initiate the extrinsic pathway of the clotting cascades, activating coagulation. The activation of coagulation by amyloid deposits might trigger secondary fibrinolysis. However, as discussed below, further investigations are necessary to understand the mechanism by which activation of coagulation and fibrinolysis occurs in FAP patients.

F1+2 and TAT are biomarkers of thrombosis, and, commonly, F1+2 plasma concentration is correlated with that of TAT in thrombosis [9]. In our study, TAT levels were not elevated despite thrombin neutralization by antithrombin-mediated TAT generation. The reason why TAT was not elevated in FAP patients might be explained by the shorter half-life of TAT (5 min versus 90 min for F1+2) [10-11]. The decrease of α_2-AP and plasminogen could indicate the activation of fibrinolysis; however, PAP was not significantly elevated. It was reported that the levels of PAP were significantly elevated in the patients of AL and AA amyloidoses, which would be related to pathological bleeding, but the PAP levels were not elevated in FAP [2], compatible with our results; this would be

consistent with less severe bleeding tendency in FAP compared with AL and AA amyloidoses [3]. In our study, we found the mild activation of coagulation and fibrinolysis in FAP patients. The half-life (0.5 days) of PAP is shorter compared with that of plasminogen (2.2 days) and α_2-AP (2.6 days) [12-13] during the activation of fibrinolysis. The shorter half-life of PAP as well as mild activation of fibrinolysis might be related to insignificant elevation of PAP in FAP. Further, it remains to be determined why the FDP levels were not elevated in spite of activation of fibrinolysis in FAP patients, though we speculate that the FDP levels were unchanged because the activation of fibrinolysis was very mild.

Decreased FX was also observed in FAP patients. Decrease of FX may be explained by FX consumption due to excessive coagulation. On the other hand, deficiencies of coagulation factors such as FX have been reported in AL amyloidosis [3]. FX deficiency has been demonstrated to be due to *in vivo* binding of FX to the amyloid fibrils [14]. FX binding to amyloid fibrils may occur in FAP as well as AL amyloidosis. However, there is no immunohistochemical proof of FX binding to FAP amyloid deposits.

Thus, we first demonstrated that the coagulation and fibrinolysis cascades were activated in FAP. Our study has certain limitations. The sample size was relatively small. Patients with AL or other amyloidoses were not examined. A prospective study with a large sample size should be conducted to confirm coagulation and fibrinolysis abnormalities in FAP.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Table 1. Tests for coagulation and fibrinolysis in FAP and control patients.

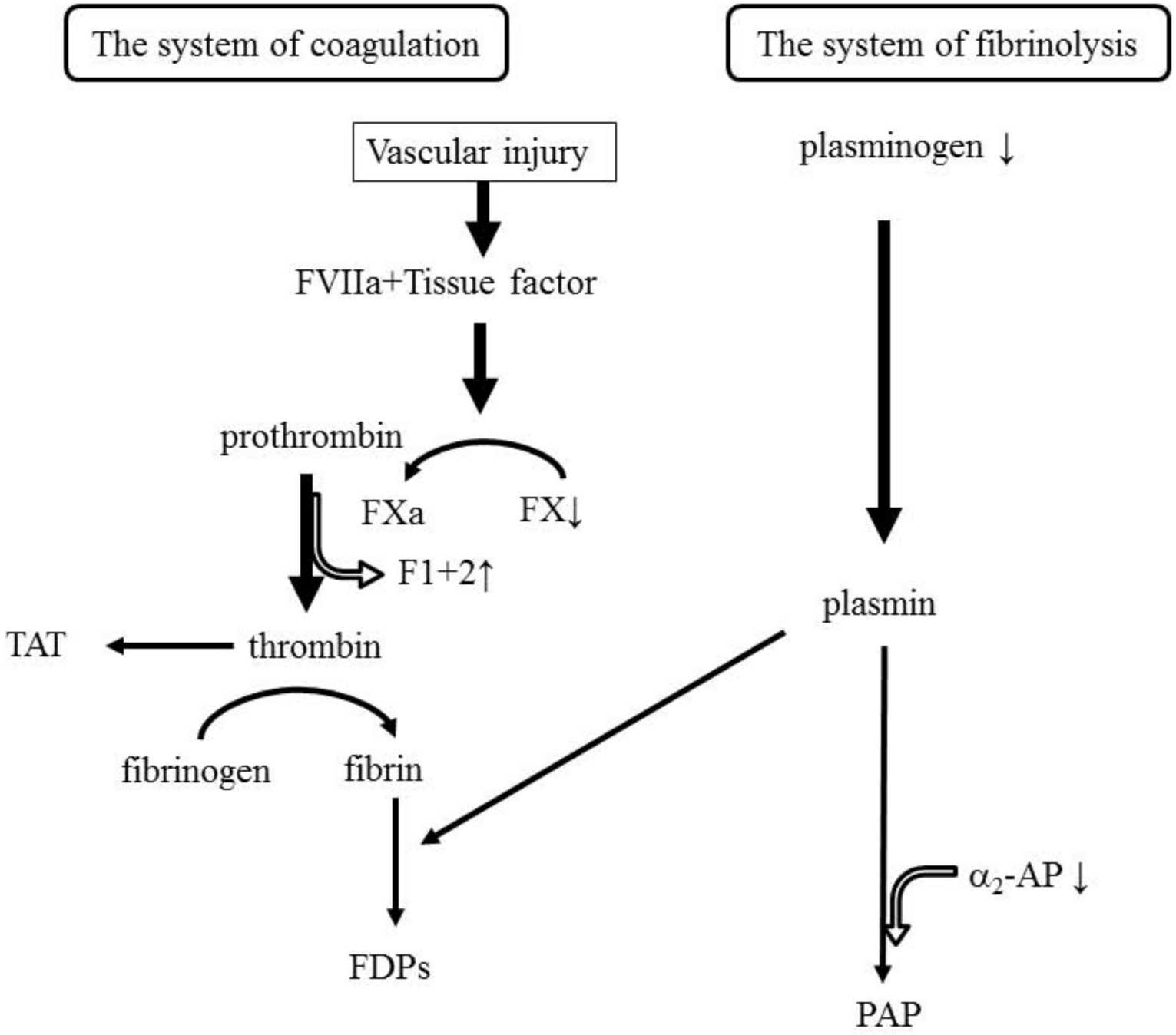
	Normal range		FAP		Other neuropathies		*p*-value
Male/Female			6/2		6/4		
Age			66.5 ± 5.0	(n = 8)	61.5 ± 2.88	(n = 10)	0.267
PT (s)	10.5-13.5		12.3 ± 0.59	(n = 8)	11.7 ± 0.43	(n = 10)	0.11
APTT (s)	24.3-36.0		28.3 ± 1.81	(n = 8)	25.8 ± 2.26	(n = 10)	0.13
Fbg (mg/dL)	150-400		265.5 ± 23.3	(n = 8)	309.0 ± 38.9	(n = 10)	0.17
FDPs (μg/mL)	<4		1.6 ± 0.58	(n = 8)	1.1 ±0.78	(n = 10)	0.35
α_2-AP (%)	85-115	↓	80.0 ± 5.13	(n = 6)	108.5 ± 14.3	(n = 8)	0.039*
AT (%)	70-130		92.0 ± 6.75	(n = 7)	105.0 ±2.0	(n = 5)	0.09
Plg (%)	75-125	↓	76.0 ± 6.75	(n = 6)	99.0 ± 8.63	(n = 9)	0.0037**
TAT (ng/mL)	<3.0		1.3 ± 0.3	(n = 8)	1.8 ± 0.61	(n = 10)	0.16
PAP (μg/mL)	<0.8		1.0 ± 0.16	(n = 8)	0.8 ±0.23	(n = 10)	0.29
F1+2 (pmol/L)	69-229	↑	281.0 ± 51.4	(n = 8)	197.0 ±36.0	(n = 10)	0.019*
FX (%)	70-130	↓	62.0 ± 3.5	(n = 8)	92.5 ± 9.0	(n = 10)	0.0010**

Values are median values ± quartile deviation. * $p < 0.05$, ** $p < 0.01$.

Figure legend:

Figure 1. Coagulation and fibrinolysis cascades.

FAP patients showed elevated F1+2 and decreased FX, plasminogen and α_2-AP levels, indicating activation of coagulation and fibrinolysis.


The system of coagulation
The system of fibrinolysis
Vascular injury
plasminogen ↓
FVIIa+Tissue factor
prothrombin
FXa
FX↓
F1+2↑
plasmin
TAT
thrombin
fibrinogen
fibrin
α2-AP ↓
FDPs
PAP