Acquired dysfibrinogenemia: monoclonal λ-type IgA binding to fibrinogen caused lower functional plasma fibrinogen level and abnormal clot formation

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Abstract

Background: We reported a case of acquired dysfibrinogenemia with monoclonal gammopathy of undetermined significance presenting λ-type IgA M protein. The patient showed lower functional (0.38 g/dL) and normal immunological fibrinogen (3.24 g/dL). To examine the cause of the false lower value of fibrinogen, we performed experiments using the patient's purified fibrinogen and IgA.

Methods: Fibrinogen was purified from the patient's plasma, and IgA was purified from patient's plasma or serum by immunoaffinity chromatography. We performed thrombin-catalyzed fibrin polymerization, scanning electron microscopy (SEM), immunoblotting analysis, and enzyme-linked immunosorbent assays (ELISAs).

Results: Fibrin polymerization in the patient's plasma was markedly reduced, and SEM showed no fiber bundles or sponge-like structures. Purified IgA did not influence polymerization, whereas immunoprecipitated plasma with an anti-IgA (α-chain) antibody indicated normalization of polymerization and clot structure. Western blotting analysis revealed the presence of monoclonal λ-type IgA-bound fibrinogen, and the proportion was significantly higher than normal control plasma using ELISA.

Conclusion: Our results suggested that IgA M protein-bound fibrinogen is not normally converted into fibrin, and instead finally forms an aberrantly structured fragile clot. The

patient’s reduced plasma fibrinogen level was caused by the presence of IgA M protein-bound fibrinogen and not by IgA M protein alone.

Keywords:

Acquired dysfibrinogenemia, false lower fibrinogen value, M protein, fibrin structure

Introduction

Fibrinogen is a 340 kDa hexameric plasma glycoprotein that is synthesized by hepatocytes. Fibrinogen consists of a double set of three polypeptide chains, Aα, Bβ, and γ, which are encoded by the genes *FGA*, *FGB*, and *FGG*, respectively [1]. Circulating fibrinogen concentrations range between 1.8 and 3.5 g/L, and congenital fibrinogen disorders are characterized by functional and antigenic fibrinogen concentrations; patients with lower fibrinogen levels or who are under the detection limit of functional and antigenic fibrinogen are fibrinogen-deficient (type I; afibrinogenemia as a homozygote and hypofibrinogenemia as a heterozygote). Patients with lower levels of functional fibrinogen and a normal level of antigenic fibrinogen have dysfunctional fibrinogenemia (type II; a homozygote and heterozygote) [2].

Most cases of dysfunctional fibrinogenemia are caused by genetic mutations of fibrinogen genes, namely congenital dysfibrinogenemia [3, 4]. However, dysfunctions of fibrinogen conversion into fibrin due to the presence of acquired factors or plasma factors – namely acquired dysfibrinogenemia – have been reported previously. Acquired dysfibrinogenemia is a rare abnormality and is observed in patients with some liver diseases (including cirrhosis [5], chronic active hepatitis [6], alcoholic liver disease, post necrotic cirrhosis [7], and obstructive jaundice [8]), plasma cell disorders [9, 10],

paraneoplastic syndrome [11], and autoimmune disorders [12]. Furthermore, it may also be induced by anti-fibrinogen autoantibodies [13-15] and drug usage [16]. Laboratory tests of acquired dysfibrinogenemia show prolongation of either thrombin time (TT), reptilase time, prothrombin time (PT), or activated partial thromboplastin time (APTT); on the other hand, functional and antigenic fibrinogen levels are within normal ranges in some cases. Acquired dysfibrinogenemia can be distinguished from congenital dysfibrinogenemia by the absence of similar abnormalities in family members or mutations in the fibrinogen genes.

Acquired dysfibrinogenemia associated with plasma cell disorders and/or M proteins have been reported, such as AL amyloidosis [10], multiple myeloma [17-19], and monoclonal gammopathy of undetermined significance (MGUS) [20]. Most of these cases reported elevated M protein levels and prolonged results of TT and reptilase time, but their fibrinogen levels were normal. Only two cases of acquired dysfibrinogenemia that showed lower functional fibrinogen level have been reported for amyloidosis [10] and MGUS [20], both of which were patients with λ-type Bence Jones proteins (BJP).

We have already reported a case of λ-type IgA M protein, in which the level of functional fibrinogen was lower and immunological fibrinogen was normal, and the phenomenon was counteracted by immunoprecipitated plasma with anti-IgA (α-chain)

antibody [21]. This report is the first case of lower functional fibrinogen level caused by whole immunoglobulin molecules. In this study, to clarify the cause of the false lower value of fibrinogen, we performed experiments using the patient's purified fibrinogen and IgA.

Materials and methods

This study was approved (#383) by the Ethics Review Board of Shinshu University School of Medicine. After informed consent had been obtained from the patients, blood samples were collected for biochemical and genetic analyses.

Patient history

The patient was a 40-year-old Japanese man, diagnosed as anaphylactoid purpura at another hospital. The chief complaint was ulceration of the lower leg and purpura, and there was no additional medical history. At the age of 42, during prednisolone treatment and follow-up observation at our hospital, monoclonal λ-type IgA was determined in serum by immunofixation electrophoresis. Because he had no end-organ damage such as hypercalcaemia, renal insufficiency, anemia and bone lesions (CRAB), with no increase in plasma cells by bone marrow examination, he was diagnosed with MGUS

[22]. Routine coagulation tests showed that his plasma fibrinogen level decreased gradually, but serum liver enzymes were within reference ranges (**Table 1**).

Coagulation tests

Blood was drawn into evacuated anticoagulant tubes with a blood to 0.109 mol/L sodium citrate ratio of 9：1. Plasma was separated by centrifugation at 2,000 ×*g* for 10 minutes at 20°C. The PT and APTT tests were performed on an automated analyzer, Coapresta 2000 (Sekisui Medical Co., Tokyo, Japan) using Thromborel S (Sysmex, Kobe, Japan) and APTT-SLA (Sysmex). The functional fibrinogen level determined using the Clauss method were measured with Coapresta 2000 (Clauss-automatic analyzer) and the manual clotting method (Clauss-manual method) using Thrombocheck Fib-L (Sysmex). The immunological fibrinogen level was determined using anti-fibrinogen antibody-coated latex particles (Q-May Co., Ohita, Japan). The thrombin clotting time for plasma was also measured as described previously [23].

DNA sequence analysis

Genomic DNA purification, PCR, and DNA sequencing were performed as described previously [24].

Immunoprecipitation

Immunoprecipitation was performed by mixing anti-human IgA (α-chain) antibody (Medical and Biological Laboratories Ltd, Nagoya, Japan) and plasma at a ratio of 3:1 and standing overnight at 4°C. After centrifugation at 9,500 ×*g* for 10 min at 4°C, supernatant was collected as immunoprecipitated plasma.

Purification of fibrinogen and IgA

Fibrinogen was purified from citrated plasma obtained from the patient and a normal control subject by immunoaffinity chromatography using an IF-1 monoclonal antibody (LSI Medience Co., Tokyo, Japan), and concentrations were determined as described previously [25, 26]. IgA was purified from the patient's citrated plasma or serum by immunoaffinity chromatography using an anti-human IgA (α-chain) antibody (Medical and Biological Laboratories Ltd), and concentrations were measured using an N-assay TIA IgA-SH (Nittobo Medical Co., Tokyo, Japan) with an automated biochemical analyzer JCA-BM6050 (JOEL Ltd., Tokyo, Japan).

Thrombin-catalyzed fibrin polymerization

Polymerization was followed by measuring changes in turbidity at 350 nm at ambient temperatures using a UVmini-1240 spectrophotometer (Shimadzu, Kyoto, Japan). Reactions were performed as described previously [27] using 20 mM N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES), pH 7.4, 0.12 M NaCl (HBS buffer) and human α-thrombin (Enzyme Research Laboratories, South Bend, IN, USA).

Scanning electron microscopy

SEM preparation was performed as described previously [28]. Thrombin (10 μL, 0.5 U/mL) was added to diluted plasma (40 μL, fibrinogen; 0.4 mg/mL) and mixed by repeated pipetting. Polymerization proceeded overnight at 37°C, and clots were then fixed in 2.5% glutaraldehyde overnight, stained with 1% osmium tetroxide, mounted, osmium plasma-coated at 5 nm thickness in Neo-AN (Meiwafosis Co. Ltd, Tokyo, Japan), and viewed on a JSM-6000F (Japan Electron Optics Laboratory Co. Ltd, Tokyo, Japan).

Immunoblotting analysis

The characterization of proteins was performed by sodium dodecyl sulfate (SDS)-

polyacrylamide gel electrophoresis (PAGE) in reducing conditions (10% polyacrylamide gel) and non-reducing conditions (7% polyacrylamide gel). These gels were treated by Coomassie Brilliant Blue R-250 staining or western blotting analysis developed using rabbit anti-human fibrinogen (DAKO, Carpinteria, CA, USA), anti-human IgA (α-chain), anti-human kappa light chain (Medical and Biological Laboratories Ltd), and anti-human lambda light chain (Medical and Biological Laboratories Ltd) antibodies, and horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Medical and Biological Laboratories Ltd). The blots were visualized using 3,3'-diaminobenzidine (DAB) and hydrogen peroxide.

Enzyme-linked immunosorbent assays and statistical analysis

We performed ELISAs in order to determine the presence or otherwise of anti-fibrinogen IgA antibody and the IgA-bound fibrinogen in the patient's plasma. Normal plasma with an equivalent IgA concentration to the patient's plasma was used as the control. For the detection of anti-fibrinogen IgA antibody, recombinant fibrinogen (1.2 μg/well) was coated as the solid phase, and diluted plasmas were reacted. After washing, rabbit anti-human IgA (α-chain) antibody was reacted as a capture antibody and followed horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Medical

and Biological Laboratories Ltd) as the second antibody. For the detection of IgA-bound fibrinogen, goat anti-human fibrinogen antibody (Cosmobio, Co., Ltd, Tokyo, Japan) was coated as a solid phase, and diluted plasma with fibrinogen equivalent to 500 ng/mL was reacted. After washing, the IgA-bound fibrinogen was reacted as described above. Finally, a TMB Microwell Peroxidase Substrate System (KPL, Gaithersburg, MD) was used as a substrate, and the absorbance at 450 nm was measured using an ELISA reader (BioRad, Tokyo, Japan). The reactions were performed in triplicate, and the statistical significances of differences among groups were determined using a one-way ANOVA with Dunnett post-hoc test. A difference was considered significant when the p value was lower than 0.05.

Results

Coagulation screening tests and DNA sequence analysis

The results of routine laboratory tests are presented in **Table 1**. The patient's fibrinogen level had decreased during the follow-up period, although he had no history of thrombosis or liver disorder. At the age of 42, the IgA level had increased to 5.23 g/L and M protein of λ-type IgA was detected by immunofixation electrophoresis (data not shown). His fibrinogen concentration has decreased to 0.38 g/L (Clauss-automatic

analyzer), but his PT, APTT, and enzyme levels derived from the liver were all within normal ranges. However, the patient's plasma fibrinogen concentration using an immunological method was 3.24 g/L (normal range: 1.80–3.50 g/L). We have already reported that lower functional fibrinogen levels were markedly improved by the elimination of plasma IgA with immunoprecipitation using an anti-IgA (α-chain) antibody, and we demonstrated the presence in small quantities of IgA-bound fibrinogen in patient's purified plasma [21].

At the age of 42 and six months, the functional and immunological fibrinogen concentrations were 0.30 g/L (Clauss-automatic analyzer) and 2.93 g/L, respectively; whereas the fibrinogen concentration, determined by the Clauss-manual method and thrombin clotting time, were 2.50 g/L (normal range: 1.80–3.50 g/L) and 9.9 sec (normal range: 10.0–14.0 sec), respectively. However, the patient's fibrin clot configuration using the manual method was smaller and more fragile than for normal clots [21]. Fibrinogen levels for immunoprecipitated plasma with an anti-IgA (α-chain) antibody were 2.88 g/dL and within normal range.

To ascertain the congenital dysfibrinogenemia, DNA sequence analysis was performed, with none of the mutations confirmed in any of the three fibrinogen genes (*FGA*, *FGB*, and *FGG*).

Characterization of purified fibrinogen and IgA

We purified plasma fibrinogen and serum IgA as described in the *Materials and methods* section, and both were analyzed using SDS-PAGE and subsequent immunoblotting analysis. In non-reducing or reducing conditions, Coomassie staining showed that the normal control and the patient's fibrinogen indicated the usual patterns corresponding to fibrinogen, or the Aα, Bβ, and γ-chain (**Fig. 1a, b**). However, the patient's fibrinogen showed the presence of a small amount of an additional band (**Fig. 1b ▸ and ▹**). The patient's serum IgA showed the usual pattern of α chain and light chain (**Fig. 1b**). An immunoblotting analysis for purified fibrinogen (**Fig. 1c**) also demonstrated that the band reacted with α chain and λ light chain antibody but not with κ light chain antibody, and both bands corresponded to the α chain and light chain in Coomassie staining. Namely, a small amount of λ-type IgA M protein was co-purified with the patient's fibrinogen.

Furthermore, we performed immunoblotting analysis for the patient's IgA purified from plasma or serum. In non-reducing conditions, Coomassie staining showed a usual pattern of IgA (about 350 kDa) and two extra bands (**Fig. 2a ▸ and ▹**) were present in IgA purified from patient's plasma or serum (**Fig. 2a**). Subsequent immunoblotting

analysis demonstrated that fibrinogen was present only in patient's plasma IgA but not in serum IgA (**Fig. 2b Anti-Fbg**), and two extra bands in the patient's serum and plasma were reacted with α chain and λ light chain antibody but not with κ light chain antibody (**Fig 2b**). Namely, both were suggested as degradation products of λ-type IgA M protein.

Thrombin-catalyzed fibrin polymerization

Thrombin-catalyzed fibrin polymerization was performed using the procedure described in the Materials and methods section. In conditions with 0.18 mg/mL fibrinogen in the presence of 1.0 mM $CaCl_2$ and 0.05 U/mL thrombin, the fibrin polymerization for patient plasma was markedly reduced (**Fig. 3a**). To determine whether the patient's plasma inhibited or participated in fibrin clot formation of normal fibrinogen, a mixing experiment with the patient's plasma and normal plasma was performed. The turbidity observed for an equimolar mixture (0.1 mg/mL fibrinogen each) was markedly decreased (**Fig. 3a**). On the other hand, for the immunoprecipitated plasma with an anti-IgA (α-chain) antibody, normalization of fibrin polymerization was observed (**Fig. 3b**).

Polymerization of the purified patient's fibrinogen (0.18 mg/mL) showed similar

turbidity changes to that of normal purified fibrinogen (**Fig. 3c**). Moreover, polymerization of patient's purified fibrinogen (0.18 mg/mL) was performed in the presence of IgA (0.15 mg/mL) purified from the patient's or normal control plasma. As a consequence, the patient's IgA did not influence the polymerization of his fibrinogen (**Fig. 3d**).

Scanning electron microscopy

To analyze differences in the ultrastructures of fibrin clots formed from normal plasma, the patient's plasma, or both IgA-depleted plasmas, we observed fibrin clots using SEM. A clot prepared from the patient's plasma displayed a lack of fiber bundles and an unusual sponge-like structure with large pores (**Fig. 4b**). On the other hand, the clot structure of the patient's IgA-depleted plasma (**Fig. 4d**) was not significantly different from that of IgA-depleted normal plasma (**Fig. 4c**).

Confirmation of presence of fibrinogen-reacted IgA and IgA-bound fibrinogen in plasma

The presence of anti-fibrinogen IgA antibody in the patient's plasma was confirmed using an ELISA coated with recombinant fibrinogen as a solid phase and anti-human

IgA α-chain antibody as a capture antibody. The samples were examined at various dilutions, but the absorbances of normal control and the patient's plasma showed no significant difference (**Fig. 5a**).

The presence of IgA-bound fibrinogen in the patient's plasma was confirmed by ELISA coated with anti-human fibrinogen antibody as a solid phase antibody and anti-human IgA α-chain antibody as a capture antibody. The absorbance of the patient's plasma was 0.325 ± 0.021 (mean ± SD, n = 3), which was about 2–3 times higher than that of normal control plasma: 0.099 ± 0.006, 0.101 ± 0.011, and 0.158 ± 0.007 (**Fig. 5b**). Thus, the IgA-bound fibrinogen in the patient's plasma was significantly higher than that of normal control plasma ($p < 0.01$).

Discussion

We described a patient with MGUS, λ-type IgA M protein, and lower functional and normal immunological fibrinogen levels determined using an automated analyzer. However, no mutations were found in fibrinogen genes. Moreover, functional fibrinogen levels determined using the Clauss-manual method and thrombin clotting time were normal.

Subsequently, immunoprecipitated plasma using an anti IgA α-chain antibody showed

a normal fibrinogen value. These results suggested that the patient had an acquired dysfibrinogenemia associated with M protein [21]. In this study, we purified the patient's fibrinogen and IgA to examine the detailed mechanism of the effect of the patient's IgA on fibrin polymerization.

Eight cases of acquired dysfibrinogenemia caused by the presence of M-protein have been reported, and among them were three of κ-type IgG, two of λ-type IgG, one of κ-type IgA, and two of λ-type BJP. Mechanisms of the inhibition of fibrin formation were speculated as follows: (1) higher M-protein levels in plasma may increase plasma viscosity, resulting in an alteration in fibrin polymerization; (2) M-protein may bind to fibrinogen through nonspecific reactions; or (3) M-protein reacts with fibrinogen as an autoantibody. Only two cases, both of which were patients with λ-type BJPs [10, 20], showed lower functional fibrinogen levels, as found in congenital dysfibrinogenemia. Our report is the first case of a lower functional fibrinogen level caused by whole immunoglobulin molecules.

In this report, the inhibition of fibrin polymerization was not reproduced in a mixed experiment with the patient's purified fibrinogen and IgA derived from plasma. On the other hand, SDS-PAGE and western blotting analyses demonstrated the presence of an IgA-fibrinogen complex in purified IgA from the patient's plasma, and λ-type IgA-

bound fibrinogen in the patient's purified fibrinogen. Furthermore, ELISA, which used fibrinogen as a solid phase, showed no significant reactivity with the patient's plasma IgA compared with the normal control. Polymerization of the patient's purified fibrinogen was not affected in the presence of purified IgA from the patient's plasma; however, there is a possibility that the concentration of free IgA that might be reactive to fibrinogen was low, because the patient's M protein concentration was relatively low. On the other hand, focusing on the patient's fibrinogen, western blotting, and ELISA (which used an anti-fibrinogen antibody as a solid phase) revealed that IgA-bound fibrinogen was present in the patient's plasma. However, polymerization of the patient's purified fibrinogen showed a normal pattern. Therefore, we presumed that IgA-bound fibrinogen can be purified with a lower recovery rate than intact fibrinogen, because IgA-bound fibrinogen may not react with the anti-IF-1 monoclonal antibody due to steric inhibition.

Furthermore, the patient's plasma formed abnormal fibrin clots, but the patient's IgA-depleted plasma did not. These results suggest that the patient's IgA-bound fibrinogen may be causing an abnormal fibrin structure, not the IgA M protein alone. Kotlin et al. [19] reported a case of a κ-type IgG multiple myeloma patient, whose serum was reactive to the fibrinogen γ-chain, which altered protofibril formation and finally

resulted in the formation of a clot with an amorphous structure and no fibers. Fibrin clot formation is a multi-step process: 1) fibrinopeptide release; 2) protofibril formation; and 3) lateral association of protofibrils and fiber formation [29]. In our case, we presumed that protofibril formation was normal, but lateral aggregation did not proceed normally, and finally formed an abnormal structured clot, as observed by SEM. As reported previously [21], we also observed that the configuration of fibrin clots using the Clauss-manual method was smaller and more fragile than that of the normal control, and scattered light intensity was lower than that of the normal control. These results supported our hypothesis that IgA-bound fibrinogen interacts with and alters protofibril formation and the resultant fibrin network structure, causing an abnormal measurement of fibrinogen as calculated from scattered light intensity in an automated analyzer. Because lower molecular weight IgA bands were observed in the patient's purified IgA but not his purified fibrinogen, we speculated that the fragile clot (by megascopic observation) and aberrant structure (by microscopic observation) might be associated with the presence of lower molecular weight λ-type IgA M protein observed only in the patient's purified IgA fraction.

This study raises some questions: 1) are the interactions between λ-type IgA M protein and fibrinogen specific or nonspecific? 2) Where is the fibrinogen epitope in λ-

type IgA M protein? 3) How does IgA-bound fibrinogen function in the patient's blood and body? 4) Are the fragile fibrin clots formed in the patient associated with onset of anaphylactoid purpura? Anaphylactoid purpura in our patient was highly resistant to steroid therapy. Focusing on wound healing, one of the functions of fibrinogen, we considered that the existence of IgA-bound fibrinogen may be associated with the resistance of the therapy.

In conclusion, we report a case of MGUS, which indicated a lower functional fibrinogen level caused by the presence of λ-type IgA M protein. The mechanism speculated to be present was that IgA M protein-bound fibrinogen does not convert into fibrin in the normal way, and finally forms an aberrantly structured fragile clot. However, we did not clarify whether the interactions between IgA M protein and fibrinogen are specific or nonspecific.

Author contributions

All authors contributed to the study conception and design. Material preparation, date collection and analysis were performed by S Arai, T Kamijo, Y Takezawa and N Okumura. The first draft of the manuscript was written by S Arai and N Okumura, and all authors commented on previous versions of the manuscript. Critical revision of the

manuscript was performed by S Arai, M Sugano, R Yanagisawa, T Uehara, T Honda and N Okumura. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure legends

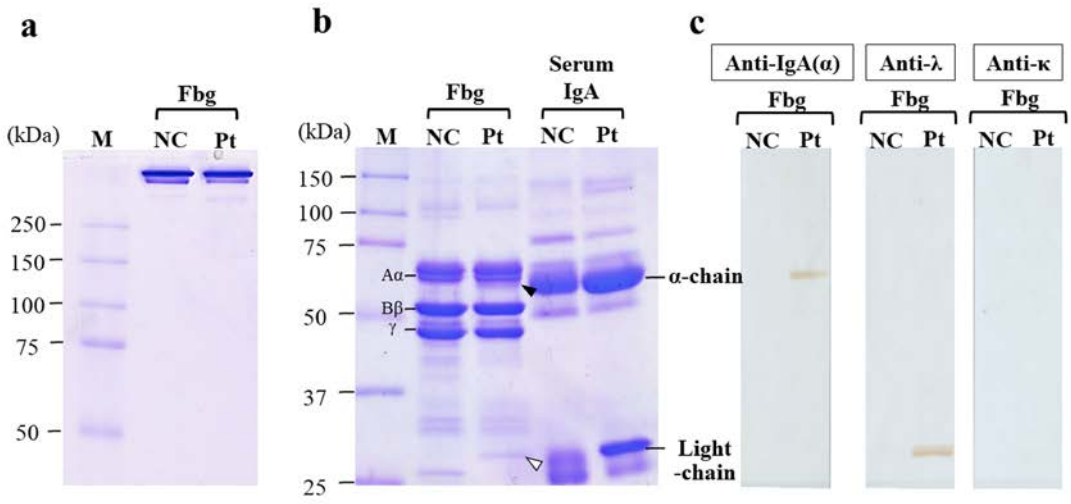

Figure 1 SDS-PAGE and western blotting analysis of patient's purified fibrinogen.

Purified plasma fibrinogen was resolved on 7% SDS-PAGE gels in non-reducing conditions and stained with Coomassie Brilliant Blue R-250 (a). Purified plasma fibrinogen or purified serum IgA was resolved on 10% SDS-PAGE gels in reducing conditions and stained with Coomassie Brilliant Blue R-250 (b). Resolved fibrinogens were electrically transferred to nitrocellulose sheets and reacted with an anti-human IgA (α-chain) antibody, anti-human λ-light chain antibody, or anti-human κ-light chain antibody (c) as described in Materials and methods. M: molecular marker, NC: normal control, and Pt: patient. The extra bands observed for patient purified fibrinogen are designated as ▶ and ▷.

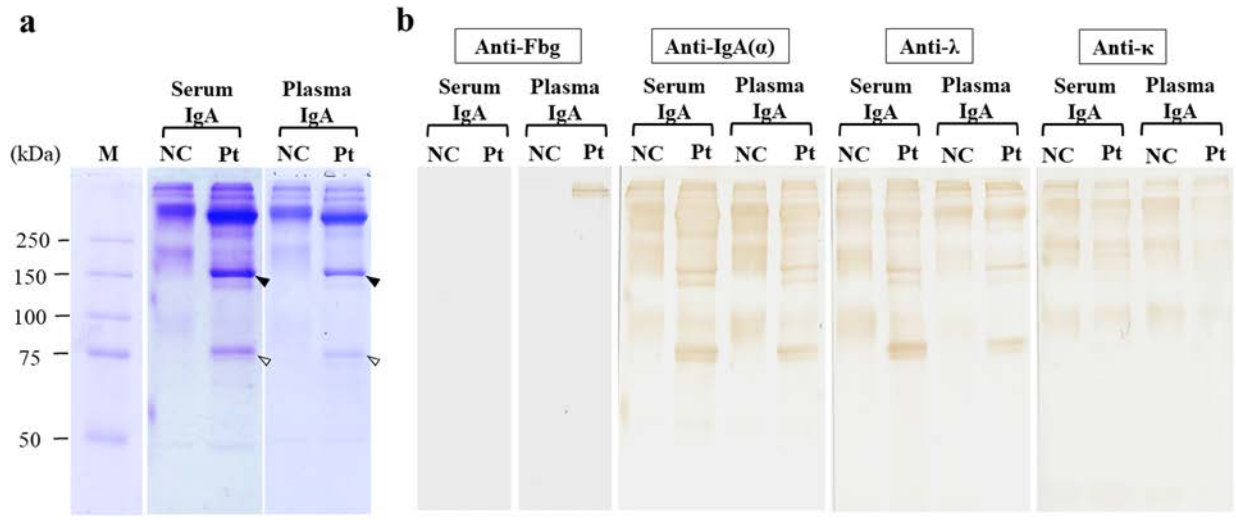

Figure 2 SDS-PAGE and western blotting analysis of patient's purified IgA.

Purified serum or plasma IgA were resolved on 7% SDS-PAGE gels in non-reducing conditions and stained with Coomassie Brilliant Blue R-250 (a). The extra bands observed for the patient's purified IgA are designated as ▶ and ▷, respectively. Resolved IgA from plasma or serum was electrically transferred to nitrocellulose sheets and reacted with an anti-human fibrinogen antibody, anti-human IgA (α-chain) antibody, anti-human λ-light chain antibody, or anti-human κ-light chain antibody (b) as described in the Materials and methods section. M: molecular marker, NC: normal control, and Pt: patient.

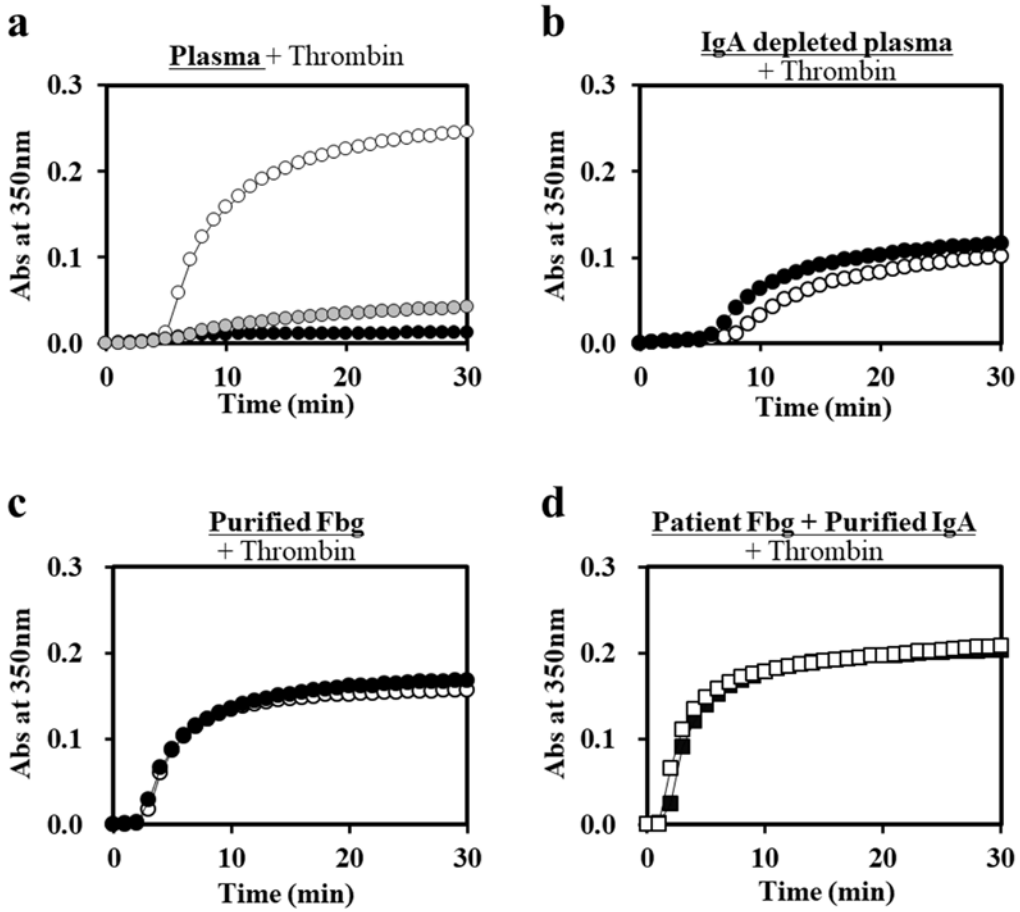

Figure 3 Thrombin-catalyzed fibrin polymerization.

Polymerization of plasma (a), immunoprecipitated plasma with an anti-IgA (α-chain) antibody (b), purified fibrinogen (c), and purified patient's fibrinogen mixed with purified IgA (0.15 mg/mL) from the patient's plasma or normal control plasma (d) was initiated with fibrinogen (0.18 mg/mL) and thrombin (0.05 U/mL), and the change in turbidity at 350 nm was followed over time. Representative polymerization curves for normal control fibrinogen (open circle), patient's fibrinogen (closed circle), equimolar mixture of normal control and patient's fibrinogen (hatched circle), purified patient's

fibrinogen mixed with purified IgA from normal control (open square), and patient's plasma (closed square) are shown. Fbg: fibrinogen.

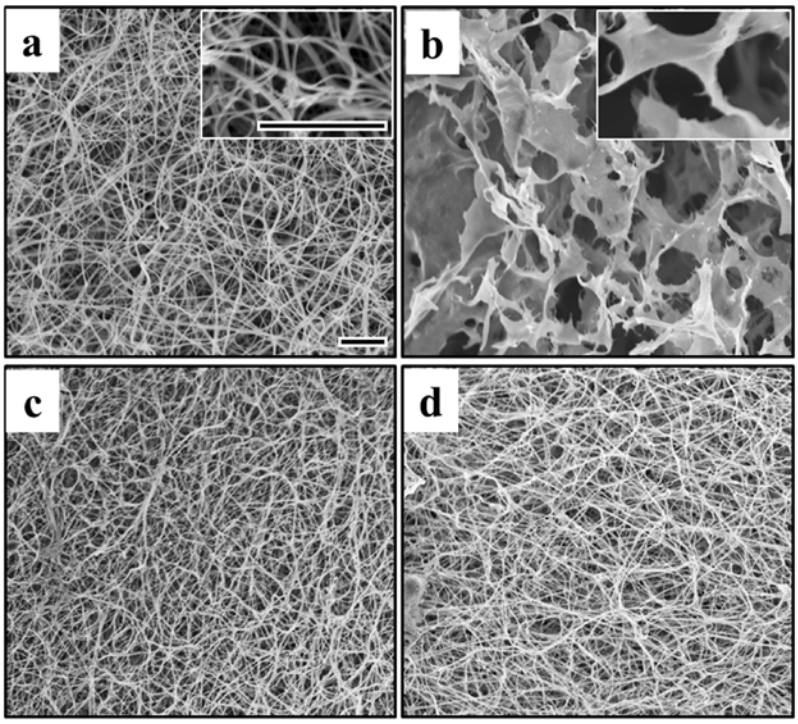

Figure 4 Scanning electron microscopy of fibrin clots.

The samples were prepared and observed as described in the Materials and methods section. Micrographs of fibrin clots for plasma (a, b) and immunoprecipitated plasma with an anti-IgA (α-chain) antibody (c, d) were taken at 3000×. The right upper micrographs in panels (a) and (b) were taken at 20,000×. Panels (a) and (c) show

normal control clots, and panels (b) and (d) show clots from the patient. The black scale bar represents 3 μm.

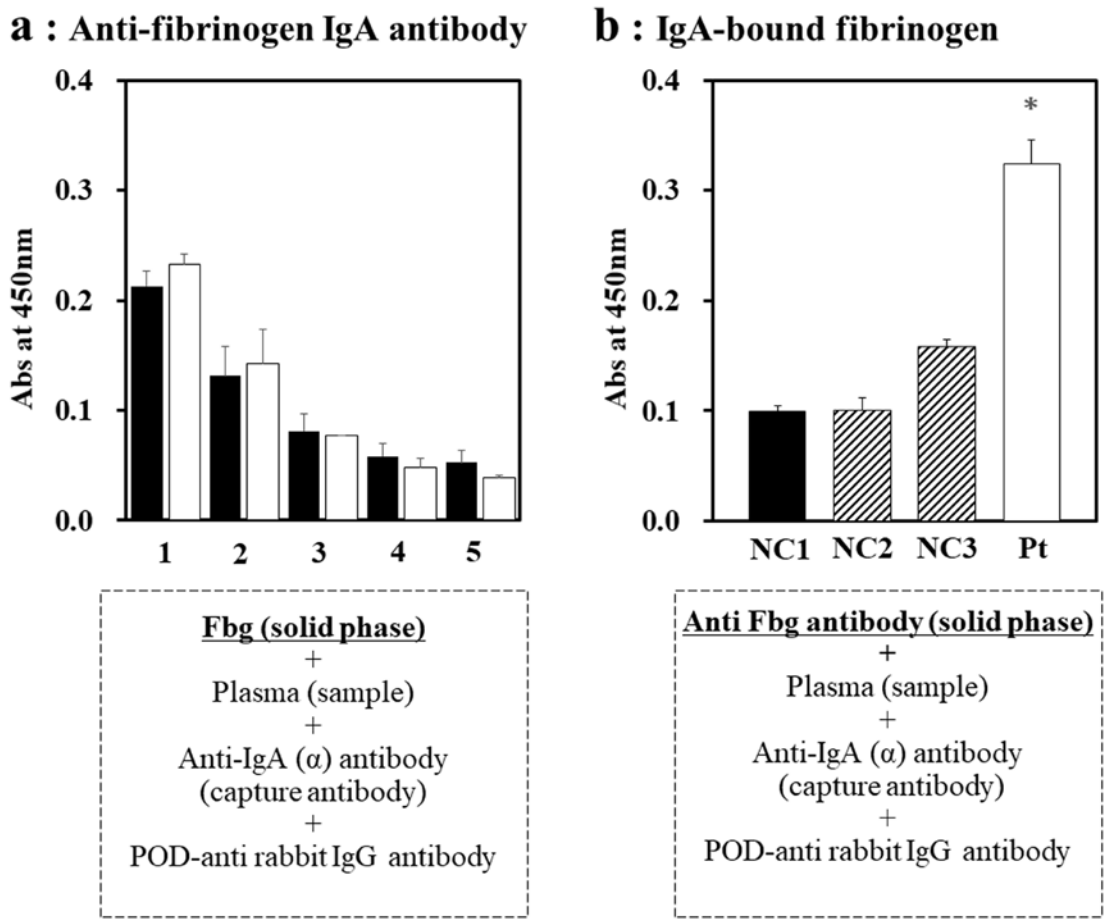

Figure 5 ELISA for the anti-fibrinogen IgA antibody or IgA-bound fibrinogen.

The presence of fibrinogen-reacted IgA and IgA-bound fibrinogen in plasma were measured using an ELISA, as described in Materials and methods. Coated with recombinant fibrinogen (a) or anti-human fibrinogen antibody (b) as a solid phase, and

using anti-human IgA α-chain antibody as capture antibody. The mean values are presented with standard deviations indicated by error bars. Sample 1: plasma diluted 200-, 2: 400-, 3: 800-. 4: 1600- and 5: 3200-fold (a). Fbg: fibrinogen, NC1–3: normal control plasmas (1–3), and Pt: patient plasma (b). Significantly different from NC (** $p < 0.01$). Closed column: normal control plasma 1; hatched column: normal control plasma 2 or 3; open column: patient's plasma.

Table 1 Laboratory test results

Assay	Patient (years)			reference range
	Age 40	Age 42	Age 42.5	
Prothrombin time (s)	10.6	10.2	10.8	10.0-13.0
INR	0.96	0.88	0.93	0.85-1.15
Activated partial thromboplastin tin	28.0	25.6	27.7	23.0-38.0
Fibrinogen activity (g/L)	1.09	0.38	0.30	1.80-3.50
Fibrinogen antigen (g/L)	n.d.	3.24	2.93	1.80-3.50
D-dimer (μg/mL)	3.0	3.8	10.1	0.0-1.0
Factor XIII (%)	133	n.d.	100	70-140
Hb (g/dL)	13.8	14.1	12.3	12.9-17.4
Platelet ($\times 10^9$/L)	319	325	366	143-333
Total protein (g/L)	7.2	7.6	7.3	6.5-8.0
Albumin (g/L)	4.1	4.7	4.3	4.0-5.0
Serum creatinine (mg/dL)	0.76	0.89	0.75	0.63-1.05
Aspartate aminotransferase (U/L)	21	13	17	11-28
Alanine aminotransferase (U/L)	26	14	16	9-36
γ-Glutamyltransferase (U/L)	56	31	32	13-70
Alkaline phosphatase (U/L)	191	220	222	115-330
Lactate dehydrogenase (U/L)	149	167	146	120-230
C-reactive protein (mg/L)	0.16	0.13	0.26	0.00-0.10
IgG (g/L)	12.92	10.19	n.d.	8.70-17.00
IgA (g/L)	3.76	5.23	5.61	1.10-4.10
IgM (g/L)	0.75	0.60	n.d.	0.35-2.20
Free κ (mg/L)	n.d.	n.d.	10.30	2.42-18.92
Free λ (mg/L)	n.d.	n.d.	51.70	4.44-26.18
κ/λ ratio	n.d.	n.d.	0.199	0.248-1.804

n.d., *not determined*