Congenital Deficiency of α_2-Plasmin Inhibitor Associated with Severe Hemorrhagic Tendency*

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ABSTRACT α_2-Plasmin inhibitor (α_2PI) is a recently characterized, fast-reacting plasmin inhibitor in human plasma that appears to play an important role in regulation of in vivo fibrinolysis. We report here a case of complete deficiency of α_2PI in man. The patient, a 25-yr-old Japanese man, had a life-long severe bleeding tendency (hemarthrosis and excessive bleeding after trauma). The following tests were within normal limits: platelet count, bleeding time, thrombin time, prothrombin time, partial thromboplastin time, titers of known clotting factors, platelet glass bead retention, Factor VIII-related antigen, platelet aggregation by ADP, collagen and ristocetin, and clot retraction. Routine liver function tests were also normal. The only abnormal finding was that whole blood clot lysis was extemely rapid and was complete in 4–8 h. The concentration of plasma protease inhibitors, including α_2-macroglobulin, antithrombin III, α_1-antitrypsin, and C$\bar{1}$INH, were all normal. The concentration of α_2-PI in the patient's plasma, assayed by immunological methods, was <0.1 mg/100 ml (normal concentration, 6.1±0.88 mg/100 ml [mean±SE]) and functional assays showed a complete deficiency of α_2PI. Addition of purified α_2PI to the patient's whole blood completely corrected the accelerated fibrinolysis. The patient's parents, four siblings, and four other members of this family were asymptomatic, but the titers of α_2PI in their plasmas were ≅50% of normal pooled plasma. There were three consanguineous marriages in this family, and the α_2PI deficiency appears to have been inherited as an autosomal recessive trait. We speculate that α_2PI deficiency in this patient has led to uninhibited in vivo fibrinolysis that probably causes the severe hemorrhagic tendency. Thus, this study indicates the important role of α_2PI in hemostasis.

INTRODUCTION

A plasma proteinase inhibitor termed α_2-proteinase inhibitor or α_2-plasmin inhibitor (α_2PI)[1] is a glycoprotein of estimated molecular weight 67,000 (1) that functions as a primary and fast-acting inhibitor of plasmin (1–6). The inhibitor has been also called primary plasmin inhibitor (3) or antiplasmin (4). Its average concentration in the plasma of healthy individuals is 6.1 mg/100 ml (0.9 μM) (7) and it inhibits roughly 35% of the plasmin generated when all the plasminogen in plasma (2.5 μM on the average) is fully activated to plasmin. α_2PI may be produced in the liver as the concentration in plasma decreases in liver diseases (7). The concentration also decreases as a result of its consumption in fibrinolytic states, such as accompany disseminated intravascular coagulation or thrombolytic therapy, the decrease preceding that of α_2-macroglobulin (2, 8). α_2PI inhibits plasmin activity instantaneously by forming a stable complex with the enzyme even in the presence of substrates of the enzyme (1, 9–11); α_2PI is unique in this respect among plasmin inhibitors in plasma (9–11). In addition, α_2PI interferes with adsorption of plasminogen to fibrin, thereby inhibiting efficiently the plasminogen activator-induced lysis of fibrin clot (9, 12). These

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[1] *Abbreviations used in this paper*: α_2PI, α_2-plasmin inhibitor; C$\bar{1}$INH, C$\bar{1}$ esterase inhibitor; FRA, fibrinogen/fibrin-related antigen.

results suggest that α_2PI plays an important role in the regulation of fibrinolysis (13).

In the present study, a patient with congenital deficiency of α_2PI is described. The patient has had a hemorrhagic tendency since his early childhood. The α_2PI deficiency, which resulted in increased fibrinolytic activity, was the only abnormality found in the study of the patient's coagulation and fibrinolytic system. These observations imply that α_2PI has an important biological role in hemostasis. The defect appeared to have been inherited as an autosomal recessive trait.

METHODS

Plasma. Blood was collected from antecubital veins into 0.1 vol of 3.8% sodium citrate and was centrifuged at 2,000 *g* for 20 min to prepare plasma. In some experiments, citrated plasma from normal individuals and patients with congenital clotting factor deficiencies was prepared as described (14). To prepare platelet-rich plasma, citrated blood was centrifuged at 650 *g* for 4 min. Plastic syringes, centrifuge tubes, and storage containers were used. Venipuncture in patients and in normal subjects was performed after informed consent for the procedure was obtained.

Tests of platelets. The bleeding time was measured by the method of Duke (15). Platelet retention by glass bead columns was estimated by the method of Salzman (16). Platelet aggregation studies were performed by the turbidimetric method of Born (17) using an aggregometer (model 169, Evans Co., England); the final concentrations of aggregating agents, ADP (Sigma Chemical Co., St. Louis, Mo.), collagen (Hormon-Chemie, Munchen, West Germany), and ristocetin (H. Lundbeck & Co. A/S, Copenhagen-Valby, Denmark) used were 5 μM, 2 μg/ml, and 1.5 mg/ml, respectively. Von Willebrand factor activity in plasma was assayed by a published method (18).

Tests of blood coagulation. The whole blood clotting time, thrombin time, prothrombin time, and activated partial thromboplastin time were measured by standard methods (19). Plasma fibrinogen was measured as described earlier (20). Levels of prothrombin, Factors V, VII, and X were assayed by a modification of one-stage prothrombin time (21) using artificially depleted or congenitally deficient plasmas as substrates. Titers of Factors VIII, IX, XI, XII, prekallikrein (Fletcher factor), and high molecular weight kininogen (Fitzgerald factor) were measured by a modification of kaolin-activated partial thromboplastin time (22) using congenitally deficient plasmas as substrates. Fitzgerald trait plasma was obtained through the courtesy of Dr. R. Waldmann, Henry Ford Hospital, Detroit, Mich., and Fletcher trait plasma was purchased from George King BioMedical, Inc., Salem, N. H. Factor VIII-related antigen in plasma was estimated by an electroimmunoassay using a monospecific antiserum against Factor VIII (23). Assays for Factor XIII (24) and antithrombin III (25) were performed as reported previously. The results of these tests were expressed as percent relative to normal pooled plasma obtained from 24 male subjects (100%).

Tests of fibrinolysis. The euglobulin clot lysis time was performed by the method of von Kaulla and Schultz (26). Plasma plasminogen was assayed by the caseinolytic method described previously (25) and by an amidolytic method (27) using the chromogenic substrate H-D-Val-Leu-Lys-*p*-nitroanilide (S-2251) (Kabi Diagnostica, Stockholm, Sweden). Concentrations of fibrinogen/fibrin-related antigens (FRA) in serum were estimated by hemagglutination inhibition test (28). Care was taken to avoid in vitro generation of FRA by adding 0.01 vol of an aprotinin-tranexamic acid mixture (aprotinin, 5,000 KIU/ml and tranexamic acid, 10 wt/vol%) to blood immediately after drawing the blood from veins. Aprotinin and tranexamic acid were purchased from Mochida Pharmaceutical Co., Tokyo, and Daiichi Pharmaceutical Co., Tokyo, respectively. The concentrations of proteinase inhibitors in plasma such as α_2-macroglobulin, α_1-antitrypsin, and α_2PI were determined by single radial immunodiffusion technique (29). Partigen plates (Behring-Werke AG, Marburg/Lahn, West Germany) were used for α_2-macroglobulin and α_1-antitrypsin. The assay for α_2PI has been described in detail (7). The α_2PI antigen concentration in the patient's plasma was also determined by a radioimmunoassay by a double-antibody technique. ^{125}I-labeled α_2PI was prepared by enzymatic iodination of purified α_2PI with Na^{125}I, using lactoperoxidase (30). The radioimmunoassay mixture consisted of 0.5 ml of bovine crystalline serum albumin (1 mg/ml in barbital-saline buffer, pH 7.4), 0.02 ml of ^{125}I-α_2PI (usually 20,000–30,000 cpm), 0.1 ml test sample diluted appropriately in barbital-saline buffer, and 0.1 ml of a 1:2,000 dilution of rabbit anti-α_2PI serum, in 10 × 75-mm polystyrene tubes. After incubation at 4°C for 3 d, 0.1 ml of a 1:25 dilution of normal rabbit serum and 0.1 ml of a 1:10 dilution of goat anti-rabbit immunoglobulin (Ig)G serum (Antibodies, Inc., Paris, Calif.) were added to the mixture. Normal rabbit serum was used as a carrier to increase the volume of the precipitates formed. After incubation overnight at 4°C, the tubes were centrifuged at 1,800 *g* for 10 min at 2°C, the supernatant fluids were aspirated, and the radioactivity of the precipitates was measured in a γ-counter (model 1085, Nuclear Chicago Corp., Des Plaines, Ill.). The values obtained were expressed as percent of bound (precipitated) radioactivity relative to that of total radioactivity added to the tube initially. Nonspecific binding (precipitation) of ^{125}I-α_2PI, as determined by substituting normal rabbit serum for anti-α_2PI serum, was consistently <5%. The activity of C1INH was kindly assayed by Dr. Jack Pensky, Veterans Administration Hospital, Cleveland, Ohio (31). α_2PI activity in plasma was determined by a fibrinolytic assay (2) and by an amidolytic assay (11). In the fibrinolytic assay, the test plasma was filtered through a column of lysine-Sepharose (Pharmacia, Inc., Uppsala, Sweden) to remove plasminogen before being tested for its inhibitory activity on urokinase-induced clot lysis. The test was performed as follows: 0.1 ml of plasminogen-depleted and five times-diluted test plasma was mixed with urokinase, Cohn Fraction I, and thrombin. The lysis time of the clot formed was compared with that of the control without test plasma. The prolongation of lysis time was proportionately related to the activity of α_2PI present in the test plasma. In the amidolytic assay, the inhibitory effect of the test plasma on the initial rate of plasmin-catalyzed hydrolysis of the chromogenic substrate, S-2251, was determined. The decrease of initial rate of hydrolysis was proportional to the amount of the α_2PI in the test plasma. The result obtained with the test plasma was compared with the result of normal control plasma and expressed as percent of the control.

Purified α_2PI. α_2PI was purified from human plasma by a method previously described (1). β-Mercaptoethanol, which had been used as a stabilizer, was found to be unnecessary and was not used in the present study.

RESULT

Case history. The patient is a 25-yr-old male who has had a hemorrhagic diathesis since his early childhood. He had no abnormal umbilical bleeding at birth.

The most frequently encountered types of bleeding were prolonged hemorrhage from cuts and subcutaneous hemorrhage. The first noticeable episode of hemorrhage occurred at the age of six when his sole was cut by a small piece of broken glass. It took almost 2 mo to stop the bleeding in spite of local pressure continually applied with gauzes. He did not receive blood transfusions. At the age of eight, he had luxation of his left hip joint. When the joint was repositioned, he had bleeding into the joint and its surrounding tissues. Because of this hemorrhage, he was forced to lie in bed for 3 mo. At the age of nine, he oozed for a few weeks after cutting his finger. In his early teens, he began to have hemorrhage into both knee and ankle joints and their surrounding tissues. Bleeding into joints occurred most frequently after a trauma. "Spontaneous" joint bleeding occurred with less frequency and tended to occur when the season changed; from winter to spring and from summer to autumn. At the age of 16, he had submucosal bleeding into the gingiva and his jaw was extensively swollen. At the age of 18, he hit his right knee joint violently against an edge of a desk and developed severe bleeding into the joint. Since then he has been unable to bend his right knee more than 90 degrees. Since the age of 20, he has been given a blood transfusion whenever severe hemarthrosis occurred. The patient felt that the transfusions were effective. At the age of 20, he had bleeding into the muscles of his back after being hit on his back. Swelling and pain of the muscle persisted for months. At the age of 24, he had right hemothorax for which he had a surgical drainage and received transfusions of a total of 3.2 liters of blood. A few months before the present investigation, he stumbled on the stairs and had bleeding into his right ankle joint and his thigh muscles. Swelling and pains in these regions persisted for more than a month. x-ray examinations revealed that his right knee joint had narrowing of the joint space associated with subcondylar sclerosis indicative of degenerative change. Other joints were radiologically unremarkable. Routine liver function tests were all normal.

Laboratory studies. The patient was evaluated for hemostatic abnormalities at a time when he was asymptomatic. Abnormalities found by routine laboratory examinations were unusually rapid lysis of whole blood clots and a shortened euglobulin lysis time (Table I). There were no abnormalities in platelet count or function or von Willebrand factor activity (Tables I and II). Clotting time, prothrombin time, partial thromboplastin time, and thrombin time were all within normal limits, suggesting that there was no gross abnormality in blood coagulation. This was further substantiated by the assay of each coagulation factor activity (Table II). However, we constantly observed during the patient's study the late development of small subcutaneous bleeding at the sites of incisions made for bleeding time tests and also at the sites of venipuncture in spite of a local pressure carefully applied for >5 min after the punctures. The sizes of these subcutaneous bleedings were small and the diameter of the purpuric spot was usually <10 mm.

TABLE I
Routine Hemostatic Studies in the Propositus

Studies	Propositus	Normal or control
Bleeding time, *min*	2–4	1–4
Clotting time, *min*	8	5–13
Clot retraction, %	60	40–60
Platelet count, *per μl*	19×10^4	$15–40 \times 10^4$
Prothrombin time, *s*	13	12–13
Kaolin partial thromboplastin time, *s*	56	47–57
Thrombin time, *s*	20	19–24
Antithrombin III activity, %	98	90–110
Euglobulin lysis time, *min*	65	90–240
Whole blood clot lysis, *h*	4–8	Negative at 24 h

The most remarkable laboratory finding in this patient was the clot lysis. Blood drawn by venipuncture from the patient was immediately placed in a glass test tube and incubated at 37°C. The clot was formed

TABLE II
Detailed Hemostatic Studies in the Propositus

Studies	Propositus	Normal or control
Platelet retention by glass beads, %	77	20–75
Platelet aggregation, %		
ADP	45	43–80
Collagen	73	67–93
Ristocetin	42	44–68
VIII VWF, %	98	70–120
VIII RAG, %	100	57–137
Prothrombin, %	88	80–130
Factor V, %	88	80
Factor VII, %	110	65–135
Factor VIII, %	95	60–170
Factor IX, %	140	70–180
Factor X, %	100	45–155
Factor XI, %	172	50–156*
Factor XII, %	66	35–183*
Plasma prekallikren	59	56–148*
High molecular weight kininogen	47	46–132*
Factor XIII, %	80	70–120
Antithrombin III antigen, *mg/100 ml*	29.3	32.8±4.2‡

VIII VWF, von Willebrand Factor activity in plasma; VIII RAG, Factor VIII-related antigen.
* Normal range, mean±2 SD of 40 normal adults.
‡ Mean±SD from 50 normal adults.

and retracted normally, but the clot thus formed underwent lysis. After several hours incubation (from 4 to 8 h on 4 different times of examinations) the clot was hardly visible, and only a small strand of fibrin, if any, was found when examined by pouring the blood over a gauze. The blood clot formed in a plastic or silicon-coated glass tube was also similarly lysed.

Evaluation of fibrinolysis. The results of the analysis of the patient's fibrinolytic system are presented in Table III. Although the concentrations of α_2-macroglobulin, α_1-antitrypsin, and CĪINH were normal in the patient's plasma, essentially no α_2PI was detectable by either immunological or functional assays. A single radial immunodiffusion study showed no precipitate with undiluted patient's plasma (Fig. 1). In a radioimmunoassay of α_2PI, the addition of the patient's plasma did not displace the binding of radioactive α_2PI at all, whereas that of either purified α_2PI or normal plasma did (Fig. 2). Thus, the failure to detect α_2PI by a radial immunodiffusion was not a result of slow diffusion of the patient's α_2PI in agar. No activity of α_2PI was detected by fibrinolytic assay, and only 3% activity of the normal control was detected by amidolytic assay. Plasminogen was present in normal titer, as assayed by its activity and its antigen concentration. The fibrinogen concentration was within normal limits. The concentration of FRA was not elevated.

Several samples of the patient's plasmas obtained and frozen during the past 2 yr also lacked α_2PI, although those of normal plasmas similarly stored were found to have normal concentrations of α_2PI. These results suggest the long-standing nature of α_2PI deficiency in the patient.

TABLE III
Fibrinolysis Studies in the Propositus

Studies	Propositus	Normal or control
Fibrinogen, *mg/100 ml*	325	150–400
Plasminogen, *mg/100 ml*	17.5	17.2±2.6*
Plasminogen, activity		
Caseinolytic, *U*	1.9	1.7±0.14*
Amidolytic, %	120	100
α_2-Plasmin inhibitor, *mg/100 ml*	< 0.1	6.13±0.88*
α_2-Plasmin inhibitor activity		
Fibrinolytic, *U*	0	1,461±298*
Amidolytic, %	3	100
α_2-Macroglobulin, *mg/100 ml*	270	211±57*
α_1-Antitrypsin, *mg/100 ml*	245	215±42*
CĪINH, *U/ml*	3.9	4.2‡
Serum FRA, *μg/ml*	0–5	0–5

* The means±SD were obtained from 28 normal adults for plasminogen, and from 50 for the inhibitors.
‡ Pool of normal adult plasmas.

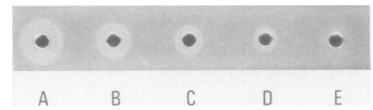

FIGURE 1 Single radial immunodiffusion study of the patient's plasma (conc, concentration). (A) Undiluted normal pooled plasma, (B) 1:2 dilution of normal pooled plasma, (C) 1:4 dilution of normal pooled plasma, (D) 1:8 dilution of normal pooled plasma, and (E) undiluted patient's plasma.

The patient's increased fibrinolytic activity was completely corrected in vitro by the addition of purified α_2PI. 5 ml of blood drawn from the patient was immediately mixed with 0.1 ml of purified α_2PI (198 mg/100 ml), providing a normal concentration of α_2PI, and whole blood clot lysis test and euglobulin lysis time were measured. The whole blood clot did not lyse within 24 h. The euglobulin lysis time was prolonged from 55 min observed without the addition of α_2PI to 100 min, a normal value.

Various amounts of α_2PI were added to the patient's plasma and the effect on fibrinolysis was determined by fibrinolytic as well as amidolytic assays. 30 μl of purified α_2PI (198 mg/100 ml) was added to 1 ml of plasma, and the plasma was tested for its inhibitory activity on urokinase-induced clot lysis (α_2PI fibrinolytic assay; see Methods). The inhibitory activity of the patient's plasma was completely restored to normal, as anticipated from the amount of α_2PI added (Fig. 3). The inhibitory activity of the patient's plasma measured

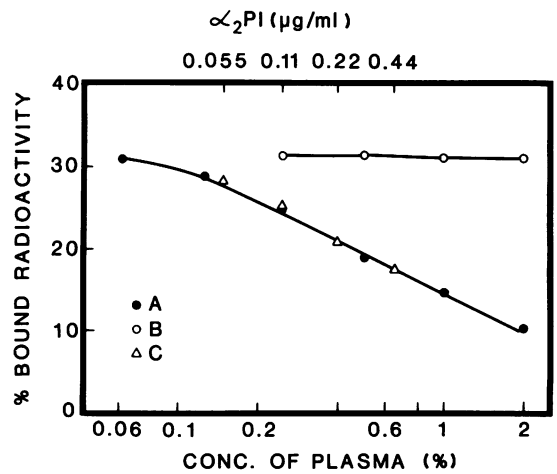

FIGURE 2 Radioimmunoassay of α_2PI. Effect of the addition of purified α_2PI, normal pooled plasma, or the patient's plasma upon the bound (precipitated) radioactivity. 0.1 ml of serial dilutions (shown on the abscissa) of either purified α_2PI, normal pooled plasma, or the patient's plasma was tested in the assay system described in Methods. The results were expressed as percent bound radioactivity relative to that added to tubes. (A) Normal pooled plasma, (B) the patient's plasma, and (C) purified α_2PI.

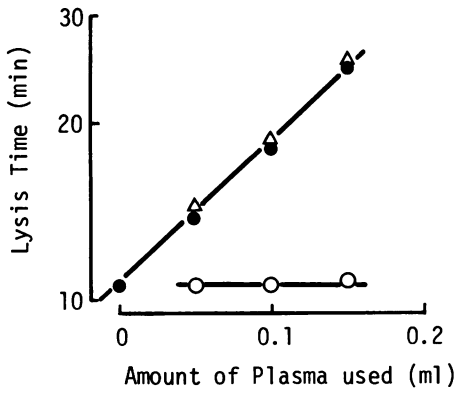

FIGURE 3 Restoration to normal of inhibitory activity of the patient's plasma for urokinase-induced clot lysis by the addition of purified α_2PI. The amount of plasma, expressed on the abscissa, is that of plasminogen-depleted and five times-diluted plasma. The clot lysis time is plotted on a logarithmic scale on the ordinate. ○, The patient's plasma; △, the patient's plasma supplemented with 59.4 μg/ml purified α_2PI, ●, normal control plasma (α_2PI 58 μg/ml).

by the amidolytic method increased in proportion to the amount of α_2PI added (Fig. 4). When α_2PI concentration was 5.9 mg/100 ml, the activity was 104% of normal control (α_2PI, 5.8 mg/100 ml), indicating that there was an excellent recovery of activity.

Family studies. The patient (subject VI 3; Fig. 5) belongs to a kindred that has lived for generations on the island Okinawa, southwest of Japan. The pedigree that was constructed from the official census registration and information obtained from the living members of the family is shown in Fig. 5. All the family members descended from common ancestors, and there were three consanguineous marriages. The patient (VI 3) was born of a consanguineous union, and his parents were also offsprings of consanguineous marriages.

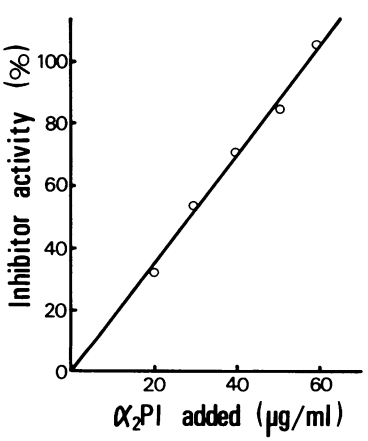

FIGURE 4 The linear increment of α_2PI inhibitor activity, measured by amidolytic assay, in the patient's plasma upon addition of purified α_2PI. The amount of purified α_2PI added to 1 ml of the patient's plasma is expressed on the abscissa.

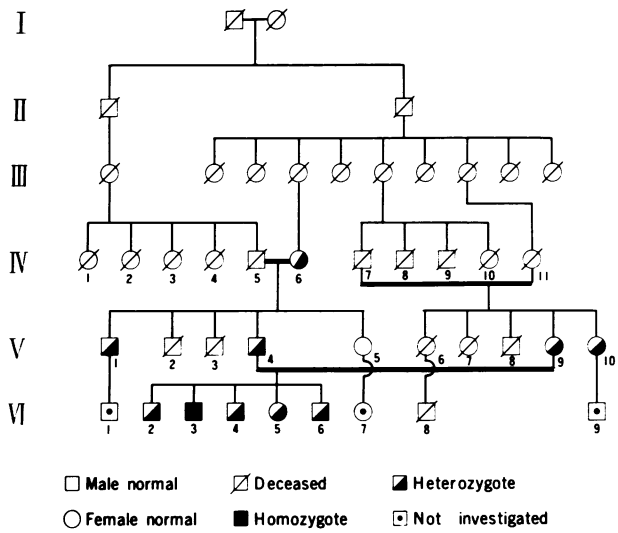

FIGURE 5 The pedigree of family M with α_2PI deficiency. Each subject may be located in the pedigree by a Roman numeral indicating generation and an Arabic numeral indicating position within the generation. Subjects possessing ≅2–4 mg/100 ml α_2PI in plasma were believed to be heterozygotes. The patient (VI 3) was believed to be homozygous because of the absence of α_2PI in his plasma.

None of the family members except the patient reported any episode suggestive of hemorrhagic diathesis. None of the deceased family members were said to have had a hemorrhagic diathesis, and the causes of their death, when known, did not appear to be related to excessive hemorrhage.

The living members of the family were examined for blood coagulation and fibrinolysis abnormalities except for three cousins (VI 1, -7, -9), none of whom were available. Coagulation data of all the family members examined were within normal limits. In analysis of fibrinolysis, the characteristic finding in these family members were the decreased levels of α_2PI concentration in plasma (Table IV).

α_2PI concentrations measured by a single radial immunodiffusion in plasma of all the members studied except for V 5 were below the normal mean minus 3SE (3.5 mg/100 ml).

Among the family members tested, V 5 was the only member who had normal concentration of α_2PI in plasma. α_2PI concentration in plasma of the abnormal family members except for the propositus was calculated to be 2.92±0.43 mg/100 ml (mean±SE, n = 9). This mean value is approximately half of that of the normal control group (6.1±0.88 mg/100 ml, n = 50). The difference between the means of the normal control group and the abnormal members was highly significant ($P < 0.001$). The activity of α_2PI as measured by the amidolytic method, in the plasma of all the abnormal members was also low; the mean activity was calculated

TABLE IV
The Concentration of α_2-Plasmin Inhibitor in Plasmas of Family Members

Pedigree position*		Activity‡	
		Amidolytic	Fibrinolytic
	mg/100 ml	%	
IV 6	3.6	55	—
V 1	2.6	43	—
4	2.5	39	42
5	7.0	117	120
9	2.5	44	46
10	3.4	51	—
VI 2	2.5	44	40
3§	<0.1	3	0
4	2.9	43	—
5	3.2	50	52
6	2.9	48	—

* Pedigree position refers to the pedigree in Fig. 5.
‡ Standard normal plasma (α_2 PI, 5.8 mg/100 ml) was used as a reference and the results are expressed as percent of the standard.
§ The patient.

to be 46±5% (mean±SE, n = 9) of a normal pooled plasma. The results of fibrinolytic assays of α_2PI activity performed on some of the members are in good agreement with the results of amidolytic assays (Table IV). The plasmas of the abnormal members were subjected to crossed immunoelectrophoresis for α_2PI and no abnormal component was found. Plasma concentrations of the other proteinase inhibitors, including α_2-macroglobulin, α_1-antitrypsin, and antithrombin III, were all within normal limits. The plasminogen antigen concentration and activity in plasma of the family members were 16.6±1.9 mg/100 ml (n = 10) and 1.7±0.18 U/ml (n = 6), respectively. These values are not significantly different from the normal values; 17.2±2.6 mg/100 ml (n = 28) for antigen concentration and 1.68±0.14 U/ml (n = 28) for caseinolysis.

DISCUSSION

Normal human plasma contains many inhibitors of fibrinolysis that may maintain a state of dynamic equilibrium with the activating components of fibrinolysis. An increased tendency for thromboembolism has been attributed to high levels of naturally occurring inhibitors (32). To our knowledge, however, the reverse situation of a bleeding tendency because of low levels of inhibitors has not yet been reported. The case described in this paper indicates that a severe deficiency of α_2PI in plasma is associated with increased fibrinolytic activity and a hemorrhagic tendency. Thus, this case illustrated the important role of α_2PI in hemostasis as well as fibrinolysis.

The patient has had a prolonged bleeding after minor trauma since his early childhood. After adolescence, the patient developed hemarthrosis and the clinical features similar to those of hemophilia. However, bleeding occurred most frequently after trauma, while spontaneous hemorrhage was rare, in contrast to severe hemophilia.

The striking abnormality found in this patient was spontaneous lysis of the blood clot formed in vitro. Although α_2PI is also known to inhibit some activated clotting factors (XIa or thrombin) in purified systems (33, 34), there was no significant abnormality in blood coagulation tests in this patient's plasma. The abnormally increased fibrinolytic activity was solely ascribed to the severe deficiency of α_2PI, because the addition of purified α_2PI to the patient's blood completely corrected its abnormality.

α_2PI added to the patient's plasma exerted its full activity without inhibition, indicating that there is no mechanism operating to hinder the action of the inhibitor in the patient's blood. Whether the deficiency of α_2PI in plasma was because of absent synthesis of this protein in the liver or because of the hypercatabolism of α_2PI is not known at present time.

When blood clots, vascular activator, and plasminogen are bound to fibrin and plasminogen activation will take place on fibrin molecules (35–37). Thus, fibrinolysis caused by a vascular or blood activator may be dependent on the binding of plasminogen to fibrin (35, 38). α_2PI decreases the binding of plasminogen to fibrin (9, 12), thus efficiently inhibiting fibrin clot lysis. When blood lacks α_2PI, as seen in the case of this patient, plasminogen activation on fibrin molecules will take place freely without any hindrance because α_2-macroglobulin and probably other plasmin inhibitors do not inhibit binding of plasminogen to fibrin (9).

In this patient, any fibrin formed in vivo, including a hemostatic plug formation, might readily lyse because of the deficiency of α_2PI. The fibrin strands are major constituents of the hemostatic plug, and lysis of these strands might make the hemostatic plug very friable. Because laboratory evaluation of blood coagulation and platelet functions in this patient were all within normal limits, the patient's hemorrhagic diathesis may be totally attributable to the deficiency of α_2PI and the resultant dissolution of hemostatic plugs once formed when the blood vessels are injured. The late development of small subcutaneous bleeding at the site of venipuncture or incision for bleeding time test might be a result of the friable nature of the hemostatic plugs formed.

In spite of the strikingly increased fibrinolytic activity in this patient, the fibrinogen concentration in plasma was within normal range, and only small

amounts of FRA (0–5 μg/ml) were detected when plasmin inhibitors (aprotinin and tranexamic acid, or α_2PI) were added to the blood immediately after drawing the blood. This suggests that abnormal fibrinogenolysis does not occur in vivo in this patient. Perhaps α_2-macroglobulin, which is present in a normal concentration in this patient, prevents fibrinogenolysis by inhibiting plasmin that may form in vivo, but α_2-macroglobulin may not be able to inhibit fibrinolysis induced by a vascular activator (9).

The euglobulin lysis time was moderately shortened. The euglobulin lysis time has been generally considered to reflect plasminogen activator activity and be relatively unsensitive to the inhibitor activity of plasma (26). The shortened euglobulin lysis time of this patient, however, was normalized when α_2PI was added to blood before preparing euglobulin fraction. This suggests that α_2PI may be partially co-precipitated in euglobulin fraction, thereby inhibiting the euglobulin clot lysis to some extent, and the shortened euglobulin lysis time observed in this patient is not because of increased plasminogen activator activity but rather because of the absence of α_2PI in his blood.

α_2PI deficiency is apparently transmitted by autosomal genes (Fig. 5). Those family members with approximately half the normal concentrations of α_2PI were believed to be heterozygotes. The concentration of both α_2PI antigen and activity averaged ≅50% of normal in heterozygotes. It is striking that 9 out of 10 family members tested were heterozygotes. This high frequency of heterozygosity may be derived from an unusually high incidence of consanguinity.

None of these heterozygotes reported any episode suggestive of hemorrhagic tendency. This suggests that half of normal concentration of α_2PI is sufficient to maintain normal hemostasis in usual daily life. It is not known, however, whether or not they would have any trouble from excess bleeding after major surgery because none of the heterozygotes has undergone major surgical operations.

Intravenous injection or oral intake of the antifibrinolytic agent, tranexamic acid, seemed to correct the patient's abnormal fibrinolysis, and the studies on this subject as well as on the long-term effect of the drug on the patient's clinical course is now in progress.

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