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A Congenitally Abnormal Fibrinogen (Vlissingen) with a 6-Base Deletion in the γ-Chain Gene, Causing Defective Calcium Binding and Impaired Fibrin Polymerization*

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Jaap Koopman‡§, Frits Haverkate‡, Ernest Briët§, and Susan T. Lord¶

From the ‡Gaubius Institute TNO and the §University Hospital, Leiden, The Netherlands and the ¶Department of Pathology, University of North Carolina, Chapel Hill, North Carolina 27514

A congenitally abnormal fibrinogen (Vlissingen) was isolated from the blood of a young woman suffering from massive pulmonary embolism. Fibrinogen Vlissingen showed an abnormal clotting time with both thrombin and Reptilase™. The release of the fibrinopeptides A and B by thrombin was normal, but fibrin polymerization was impaired both in the presence and absence of Ca^{2+} ions. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis performed according to Laemmli the γ-chain of fibrinogen Vlissingen showed two bands, one normal and one having an apparently lower molecular mass of about 1500 daltons. The previously described protective effect of Ca^{2+} ions on plasmin degradation of the carboxyl terminus of the γ-chain of normal fibrinogen was only partially detectable in fibrinogen Vlissingen. In addition the binding of Ca^{2+} ions was decreased. Fibrinogen Vlissingen bound 2.4 Ca^{2+} ions per fibrinogen molecule at pH 7.4, whereas normal fibrinogen bound 3.1 Ca^{2+} ions. At pH 5.8 fibrinogen Vlissingen bound 1.1 Ca^{2+} ions, whereas normal fibrinogen bound 2.0 Ca^{2+} ions per molecule fibrinogen in the D-domains, again indicating a structural change in the carboxyl terminus of fibrinogen. The structural defect was determined by sequence analysis of DNA amplified by use of the polymerase chain reaction. Exons VIII, IX, and X of the γ-chain gene were amplified and the DNA sequence of the amplified fragments was determined. A 6-base deletion was found in 50% of the fragments corresponding to exon VIII, indicating that the patient was heterozygous for the mutation. This deletion codes for amino acids Asn-319 and Asp-320 in the normal fibrinogen γ-chain. The data indicate that Asn-319 and Asp-320 are crucial for maintaining the integrity of the carboxyl-terminal polymerization sites, the protective effect of Ca^{2+} ions on plasmin degradation of the carboxyl terminus of the γ-chain, and the calcium binding domain at the carboxyl terminus of fibrinogen.

Fibrinogen, a soluble plasma protein, is a dimer of three polypeptide chains, called Aα, Bβ, and γ. The amino termini of all six chains form a central E domain, while the carboxyl termini of each fibrinogen molecule form two outer D domains. During coagulation fibrinogen is cleaved by thrombin to generate fibrin monomers which then polymerize to form an insoluble fibrin matrix. Fibrin monomer polymerization arises from the interactions of polymerization sites on different molecules. Sites located in the amino terminal part of the molecule are exposed by thrombin cleavage of fibrinopeptides from the Aα and Bβ chains; these sites are designated "A" and "B," respectively. Complementary polymerization sites, designated "a" and "b," are located in the D domains of fibrinogen which include the carboxyl terminus of the γ-chain (1–3).

Human fibrinogen binds three Ca^{2+} ions per molecule with high affinity at pH 7.5. Each D-domain contains one high affinity Ca^{2+} binding site (4–6). Plasmin degradation of the carboxyl terminus of the γ-chain results in loss of Ca^{2+} binding (7). On the other hand, binding of Ca^{2+} ions at this site protects the carboxyl terminus of the γ-chain from plasmin degradation (8). Plasmin degradation of normal fibrinogen in the presence of Ca^{2+} ions results in fragment D_1, with the carboxyl terminus of the γ-chain intact (M_r 38,000), in the absence of Ca^{2+} ions fragment D_3 is formed, with the carboxyl terminus of the γ-chain degraded (M_r 26,000) (8).

More than 200 cases of inherited dysfibrinogenemia have been reported (9), and most of these show prolonged clotting times with both plasma and purified fibrinogen due to a defect in the thrombin-catalyzed release of fibrinopeptides or a defect in polymerization of fibrin monomers or both. At least 12 congenitally abnormal fibrinogens have abnormalities in the carboxyl terminus of the γ-chain (10–22) and correspondingly defective fibrin polymerization. In fibrinogen Haifa (γ 275 Arg → His) the protective effect of Ca^{2+} ions on plasmin degradation of the γ-chain was impaired (23), but this was not evident in fibrinogen Saga I (γ 275 Arg → His) (24). Up to now the structural defects in abnormal fibrinogens have been elucidated by amino acid sequence analysis of the purified protein (25). Since the complete cDNA sequences (26–28) and part of the genomic DNA sequences (28, 29) of the Aα, Bβ, and γ-chain genes are known, the polymerase chain reaction (30) can be employed to determine structural defects in abnormal fibrinogens (31, 32). This report describes the functional and structural defects of a congenitally abnormal fibrinogen called fibrinogen Vlissingen. We found a 6-base deletion in the γ-chain gene as determined by DNA sequence analysis of polymerase chain reaction-amplified DNA.

This mutation results in a deletion of two amino acids in the protein, affecting fibrin polymerization, Ca^{2+} binding, and the protective effect of Ca^{2+} on plasmin degradation.

EXPERIMENTAL PROCEDURES

Materials

A reversed-phase C_{18} column, 250 × 4.6 mm, was obtained from Chrompack (Middelburg, The Netherlands). The HPLC[1] device was

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[1] The abbreviations used are: HPLC, high performance liquid chro-

manufactured by LKB Biotechnology Inc. Bovine thrombin was purchased from Leo (Ballerup, Denmark); Reptilase™ from Boehringer (Mannheim); $^{45}CaCl_2$ (3.92 Ci/mmol; 59 μg/ml) from Amersham; water for inorganic trace analysis, with a Ca^{2+} concentration below 3×10^{-7} M, from Fluka. Plasmin was prepared as described (33). Taq DNA polymerase was obtained from New England Biolabs. Oligonucleotides were synthesized on an Applied Biosystems 380A DNA synthesizer. Puc 13/*Sma*I cut/Bap, T7 sequence kit, dATP, dCTP, dGTP, and dTTP were purchased from Pharmacia. DNA-grade agarose and Chelex-100 were from Bio-Rad, aprotinin from Bayer, and x-ray film from Kodak.

Methods

Fibrinogen Purification—Fibrinogen was purified from plasma of the propositus and two normal controls as described by Van Ruijven-Vermeer *et al.* (34). Venous blood was collected in 0.01% (w/v) EDTA and 40 KIU/ml aprotinin. Plasma was prepared by centrifugation of the blood for 20 min at 3000 × *g* and 4 °C. The fibrinogen obtained was concentrated by precipitation in 50% saturated $(NH_4)_2SO_4$ solution, centrifuged, dissolved in 0.15 M NaCl, and dialyzed against this solution for 24 h at 4 °C. The purified fibrinogen solution was stored at −20 °C.

Determination of Various Parameters in Plasma—Venous blood was collected in one-tenth volume of 0.11 M sodium citrate. Platelet-poor plasma was prepared by centrifugation at 2300 × *g* for 30 min at 4 °C. Thrombin and Reptilase™ clotting times were obtained as described (35). The concentration of plasma fibrinogen was measured by radial immunodiffusion (36) and by the functional assay according to Clauss (37). Fibrin(ogen) degradation products were measured in plasma using a monoclonal antibody-based immunoassay (38). Antithrombin III was measured by a chromogenic assay (39), and protein C (40) and protein S (41) were measured by an immunoassay.

Coagulation of Purified Fibrinogen—Thrombin and Reptilase™ clotting times of purified fibrinogen were determined as described (35). Release of FpA and FpB was determined essentially according to Kehl *et al.* (42) and modified as described previously (35). Fibrin polymerization was determined by measuring the increase in absorbance at 350 nm as a function of time using a spectrophotometer (Pye Unicam SP 1700). Fibrinogen solutions used had been dialyzed against 0.1 M Tris-HCl, pH 7.5, containing 1 mM $CaCl_2$ or 1 mM EDTA. The fibrinogen concentration was determined by measuring the absorbance at 280 nm ($A^{1\%}_{1cm}$ = 15.0) and adjusted to 0.25 mg/ml. To initiate polymerization, 10 μl of thrombin (10 NIH units/ml) or undiluted Reptilase™ solution was added to 0.7-ml aliquots of these fibrinogen solutions.

SDS-PAGE—SDS-PAGE was performed according to Laemmli (43) or Weber and Osborn (44).

Plasmin Degradation of Fibrinogen—Plasmin degradation of fibrinogen in the presence of Ca^{2+} or EDTA was performed as described (8). Purified fibrinogen was dialyzed against 0.04 M sodium 5,5-diethylbarbiturate, pH 7.8, containing 0.1 M NaCl, and the fibrinogen concentration was adjusted to 0.5 mg/ml. To 0.5 ml of fibrinogen solution was added 10 μl of 0.1 M $CaCl_2$ or 0.1 M EDTA. After mixing and adding 10 μl of a plasmin solution containing 0.13 mg/ml, the samples were incubated for 120 min at 37 °C. The reaction was stopped by adding an equal volume of incubation buffer containing 0.1 M Tris-HCl, pH 6.8, 2% (w/v) SDS, 7 M urea, and 5% (v/v) 2-mercaptoethanol, the samples were boiled for 10 min, and 25 μl of each sample was loaded on a 10% SDS-PAGE gel.

Calcium Binding of Fibrinogen—Ca^{2+} binding of fibrinogen was performed essentially as described by Marguerie *et al.* (45). Fibrinogen solutions were treated with EGTA, and buffer solutions were prepared in water with a Ca^{2+} concentration below 3×10^{-7} M and treated with Chelex-100 to remove traces of Ca^{2+} ions (45). Ca^{2+} binding was measured at 15 different Ca^{2+} concentrations, *i.e.* 1×10^{-4} M; 8, 6, 4, 3, 2, 1.5, and 1×10^{-5} M; and 8, 6, 4, 3, 2, 1.5, and 1×10^{-6} M in 5 mM Tris-HCl, pH 7.5, or 5 mM sodium acetate, pH 5.8, buffer, both containing 0.5 M NaCl.

A 0.25-ml volume of fibrinogen solution (3.0 mg/ml) was dialyzed against 100 ml of the Ca^{2+}-free buffers containing one of the above-mentioned Ca^{2+} concentrations and 10 μl of $^{45}CaCl_2$ stock solution. After 48 h of dialysis at 25 °C, 0.1 ml of inner and outer fluid was added to 10 ml of scintillation fluid and counted on a liquid scintillation counter (1900CA Tricarb, Packard). The data were quantified according to Scatchard (46).

Isolation of Genomic DNA—Genomic DNA was isolated from the blood cells which remained after plasma had been collected for fibrinogen purification. The cells were stored at −70 °C until use. The isolation of genomic DNA was performed as described by Gustafson *et al.* (47).

DNA Amplification, Cloning, and Sequencing—Primer pair 4a (5′ GAGGAGGGTCAGCATGTGATGGTTGAATTCCCTTC 3′) and 4b (5′ AACTTGGAATCTAAGAAAGGAAAACATACC 3′) and primer pair 5a (5′ ATGTACATCTACGACTTGTTTTAG 3′) and 5b (5′ AAAAAAGGAATTCTCTTTTGAAACGGTC 3′) were used for amplification of a 348- and a 780-bp fragment of the fibrinogen γ-chain gene including exon VIII (4a/4b) or exon IX, intron I and exon X (5a/5b), respectively. Amplification was performed in a 100-μl reaction volume containing 1 μg of genomic DNA, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, and 0.3 μM each of primer a and b in 1 × reaction buffer (10 mM Tris-HCl, pH 8.3, at 25 °C, 50 mM KCl, 3.0 mM $MgCl_2$, and 0.01% (w/v) gelatin). The DNA was denatured at 95 °C for 8 min after which 1.0 unit of Taq DNA polymerase was added. Each cycle consisted of a 1-min 95 °C, 0.5-min 60 °C, and 3-min 72 °C incubation. After 30 cycles, 10 μl of a sample was run on a 1.5% (w/v) agarose gel, from which the band with the expected size was isolated and cloned into Puc 13/*Sma*I/Bap. Individual clones were sequenced using the T7 sequence kit.

RESULTS

Clinical and Laboratory Data—A 23-year-old woman was hospitalized suffering from massive pulmonary embolism as diagnosed from x-ray and perfusion scan. The laboratory studies revealed that the functional fibrinogen values were lower than the gravimetric and immunological values, and that both the thrombin and Reptilase™ clotting time were prolonged (Table I). The fibrin(ogen) degradation product and protein C concentrations were slightly increased, while antithrombin III activity and protein S antigen were within the normal range (Table I). The father as well as the daughter of the propositus showed low functional fibrinogen values and prolonged clotting times by thrombin and Reptilase™ but no clinical symptoms.

SDS-PAGE Analysis of Purified Fibrinogen—Purified fibrinogen Vlissingen analyzed by SDS-PAGE in the Laemmli system (43) showed two types of γ-chains: the normal γ-chain with an apparent M_r of 48,000 and the γ-chain variant γ-Vlissingen with an apparent M_r of 46,500 (Fig. 1). Higher concentrations of 2-mercaptoethanol and longer reduction times had no effect on the subsequent electrophoretic migration rate of γ-Vlissingen, indicating that the cleavage of intrachain disulfide bonds was complete. In contrast, SDS-PAGE performed according to Weber and Osborn (44) showed no difference in electrophoretic migration between normal γ-chain and the γ-variant. Similar observations have been reported for a number of other abnormal fibrinogens, all having a single amino acid substitution in the carboxyl terminus of the γ-chain (11, 13, 14, 18, 20–22).

TABLE I

Parameters of normal plasma and the patient's plasma (Vlissingen)

	Normal	Vlissingen
Thrombin time (s)	18.6	57.5
Reptilase® time (s)	20.1	41.3
Fibrinogen concentration (mg/ml)		
Clauss	2 to 4	0.4
Gravimetric	2 to 4	1.8
Immunologic	2 to 4	3.1
FDP[a] (μg/ml)	<0.5	0.8
Antithrombin III (% of normal)	75–120	83
Protein C (% of normal)	75–135	153
Protein S (% of normal)	67–125	91

[a] FDP, fibrin(ogen) degradation products.

matography; KIU, kallikrein inactivating unit; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; FpA, fibrinopeptide A; FpB, fibrinopeptide B; Bap, bacterial alkaline phosphatase; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

Fibrinopeptide Release and Fibrin Polymerization—The HPLC profiles of the FpA and FpB released by thrombin or Reptilase™ from fibrinogen Vlissingen were normal (data not shown). As shown in Fig. 2, the rate of thrombin-catalyzed fibrinopeptide release from fibrinogen Vlissingen was equivalent to that of normal fibrinogen. In contrast, polymerization of fibrinogen Vlissingen induced by thrombin or Reptilase™ was impaired. When thrombin was used to induce polymerization, the impairment was more pronounced in the presence of EDTA (Fig. 3*B*) than in the presence of Ca^{2+} (Fig. 3*A*). When Reptilase™ was used in the presence of EDTA (Fig. 3*D*) no polymerization was detected; the addition of calcium ions only partially restored polymerization (Fig. 3C).

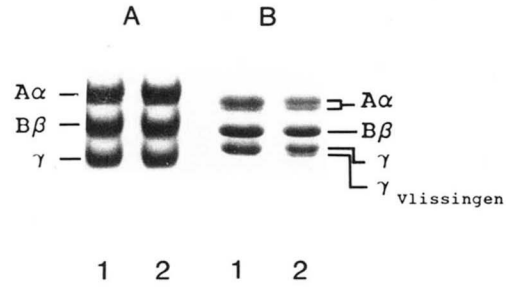

FIG. 1. **SDS-PAGE of purified fibrinogen after reduction with 2-mercaptoethanol.** *A*, 8% SDS-PAGE gel according to Weber and Osborn; *B*, 10% SDS-PAGE gel according to Laemmli. *Lane 1*, normal fibrinogen; *lane 2*, fibrinogen Vlissingen.

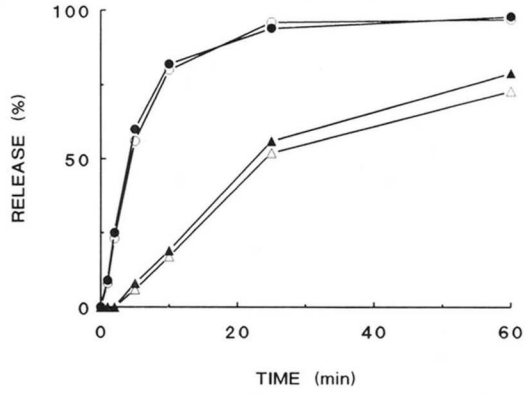

FIG. 2. **Percentage of FpA and FpB release from purified fibrinogen by thrombin as determined by HPLC.** Normal FpA (●), normal FpB (▲), Vlissingen FpA (○), and Vlissingen FpB (△).

Plasmic Digestion of Fibrinogen in the Presence of Calcium or EDTA—The above results prompted us to study the γ-chain more closely. Since the carboxyl terminus of the γ-chain of normal fibrinogen is protected against plasmin degradation by Ca^{2+} ions (8), we analyzed reduced plasmin digests of fibrinogen Vlissingen formed in the presence of Ca^{2+} or EDTA. As shown by SDS-PAGE in Fig. 4, the plasmin digest of fibrinogen Vlissingen formed in the presence of Ca^{2+} ions, differed from that of normal fibrinogen. With fibrinogen Vlissingen a band with the electrophoretic mobility of the γ-chain of normal fragment D_3 was formed in the presence of Ca^{2+} ions; a band equivalent to the γ-chain of normal fragment D_1 was also present in this plasmin digest. The plasmin degradation products formed in the presence of EDTA from normal fibrinogen and fibrinogen Vlissingen had the same electrophoretic mobility. This result indicates that fibrinogen Vlissingen is heterozygous for the defect, which is consistent with the two γ-chains demonstrated in Fig. 1*B*. As the protective effect of Ca^{2+} ions against plasmin degradation of the carboxyl terminus of the γ-chain, is related to the Ca^{2+} binding to this part of the molecule, we determined the Ca^{2+} binding to fibrinogen Vlissingen.

Calcium Binding to Fibrinogen—Using equilibrium dialysis, we quantitively compared Ca^{2+} binding of fibrinogen Vlissingen with that of normal fibrinogen. We measured binding at pH 7.5, where 3 mol of Ca^{2+} bind per mol of fibrinogen (5, 6) and at pH 5.8, where fibrinogen (bovine) binds only 2 mol of Ca^{2+} per mol of fibrinogen (45). Scatchard analysis of the calcium binding data (Fig. 5*A*) show at pH 7.5 approximately 3 Ca^{2+} binding sites with a K_d of 2.6×10^{-5} M in normal fibrinogen, which is in agreement with previously reported data (5, 6). In contrast, human fibrinogen Vlissingen contains 2.4 binding sites with a K_d of 2.8×10^{-5} M (Fig. 5*A*). At pH 5.8, normal fibrinogen showed 2.0 Ca^{2+} binding sites with a K_d of 2.4×10^{-5} M, and fibrinogen Vlissingen showed only 1.1 sites with a K_d of 1.8×10^{-5} M (Fig. 5*B*). The observed decrease in the number of binding sites from 3 at pH 7.5 to 2

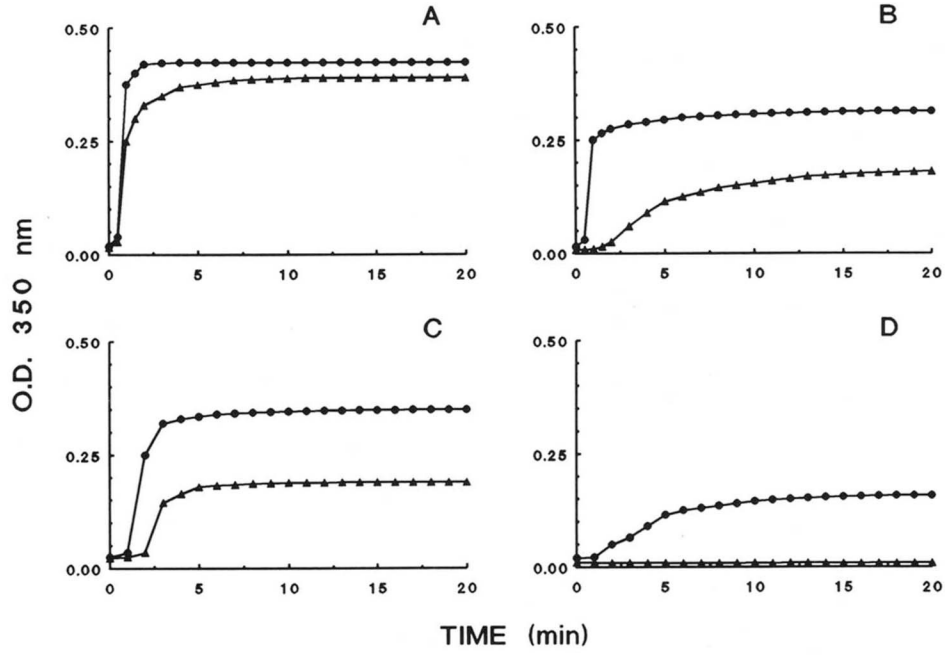

FIG. 3. **Coagulation profiles of purified fibrinogen.** Rate and degree of coagulation of normal (●) and Vlissingen (▲) fibrinogen induced by thrombin in the presence of (*A*) Ca^{2+} (1 mM) or (*B*) EDTA (1 mM) and by Reptilase™ in the presence of (*C*) Ca^{2+} (1 mM) or (*D*) EDTA (1 mM).

at pH 5.8 is the same as that reported for bovine fibrinogen (45). In contrast to the results with bovine fibrinogen, our results obtained with human fibrinogen show that the K_d at pH 7.5 does not differ significantly from that at pH 5.8.

Amplification and DNA Sequence Analysis of Fibrinogen γ-Chain Gene Exon VIII, IX, and X—Based on evidence implicating the γ-chain, we determined the DNA sequence of polymerase chain reaction-amplified genomic DNA coding for the carboxyl terminus of the γ-chain. After 30 cycles of amplification, the polymerase chain reaction samples contained DNA fragments of the size predicted from the genomic DNA sequence (29). These fragments were cloned and individual isolates were sequenced. All the clones containing the γ-chain exon IX and X fragment showed the normal γ-chain gene sequence. We sequenced 12 clones containing the exon VIII fragment. Five clones had the normal sequence, but seven clones had a 6-base deletion (Fig. 6) corresponding to amino acids Asn-319 and Asp-320. Since about half of the clones containing the exon VIII fragment showed this deletion, we conclude that the patient is heterozygous for this mutation.

DISCUSSION

Using the polymerase chain reaction, we have identified a 6-base deletion in the gene coding for the γ-chain of the abnormal fibrinogen designated fibrinogen Vlissingen. SDS-PAGE analysis of plasmic digests of fibrinogen Vlissingen in the presence of Ca^{2+} ions or EDTA show that the protective effect of Ca^{2+} ions is only partially present in fibrinogen Vlissingen. This protective effect is associated with the calcium binding site located in the carboxyl terminus of the γ-chain. We, therefore, designed oligonucleotide primers which spanned the γ-chain carboxyl-terminal exons, amplified these genomic segments, and cloned and sequenced the amplified products. Sequence analysis demonstrates that the patient was heterozygous for a deletion where the codons for Asn-319 and Asp-320 of the γ-chain were missing. The size of this deletion probably arises from its location within a sequence repeat where the repeat unit is 6 bases. Deletion of a repeat unit, by either unequal crossing-over during meiosis or strand slippage during DNA replication, is a commonly noted mutation.

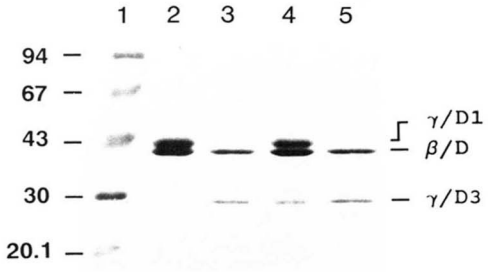

FIG. 4. **Plasmic degradation of the fibrinogen γ-chain analyzed by SDS-PAGE according to Laemmli after reduction with 2-mercaptoethanol.** *Lane 1*, low molecular weight standard (Pharmacia); *lanes 2* and *3*, plasmic digest of normal fibrinogen in the presence of Ca^{2+} and EDTA, respectively; *lanes 4* and *5*, plasmic digest of fibrinogen Vlissingen in the presence of Ca^{2+} and EDTA, respectively.

On SDS-PAGE using the method of Laemmli, γ-Vlissingen showed an apparent M_r approximately 1500 smaller then the normal γ-chain. The two-amino acid deletion, however, justified a decrease of only 265 Da. Such unexpected changes in mobility on Laemmli gels have been observed previously with mutant chains, even when only a single amino acid substitution was found. These changes have been ascribed to changes in hydrophobicity or local conformation, which then alters the mobility of the protein-SDS complexes (48, 49).

The interaction of Ca^{2+} ions with fibrinogen Vlissingen was measured by equilibrium dialysis. Scatchard analysis of the data showed a decrease in the number of high affinity binding sites for Ca^{2+} ions in fibrinogen Vlissingen as compared with normal fibrinogen. As has been described for bovine fibrinogen (45), we found that the number of high affinity binding sites in human fibrinogen depends on the pH. At both pH values 5.8 and 7.5, the number of sites was decreased in fibrinogen Vlissingen. The difference in the number of Ca^{2+} binding sites between normal fibrinogen and fibrinogen Vlissingen is the clearest at pH 5.8, because under these conditions two identical high affinity binding sites remain in normal fibrinogen, being those in the carboxyl terminus of the two D domains (45). We therefore conclude that the deleted amino acids are essential for the calcium binding associated with the D domain of fibrinogen. Previously, this binding site was localized in the region γ 303–336 (50). On the basis of amino acid sequence analogy with known calcium binding sites in a number of proteins and the results obtained with different spectrophotometric methods, it has been suggested that the amino acids involved in calcium binding are γ Asp-316, Asp-318, Asp-320, Phe-322, Gly-324, and Glu-328 (51). Since amino acids Asn-319 and Asp-320 are missing in fibrinogen Vlissingen, our results are consistent with this hypothesis.

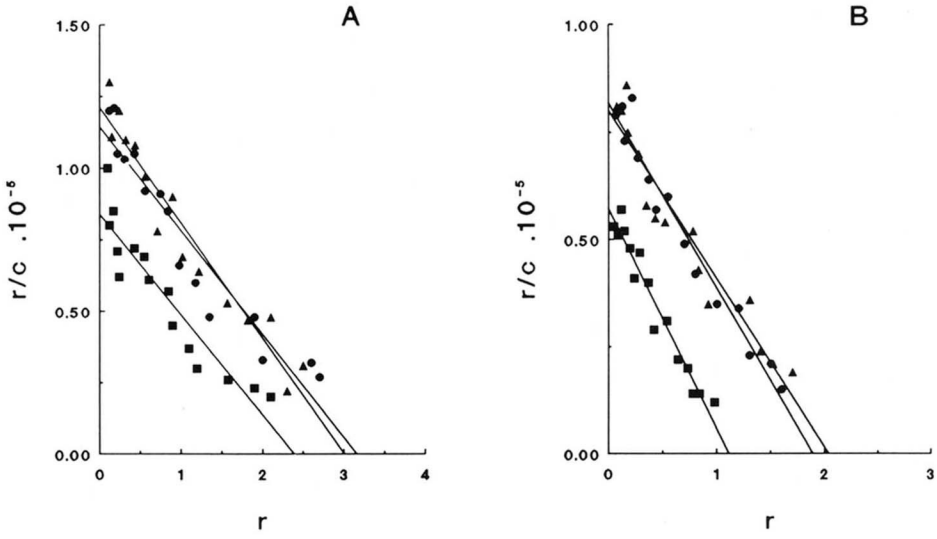

FIG. 5. **Scatchard plot of Ca^{2+} binding to purified fibrinogen.** Measured at pH 7.5 (*A*) and 5.8 (*B*) for two normal samples of fibrinogen (●, ▲) and fibrinogen Vlissingen (■). *r*, number of Ca^{2+} ions bound per molecule of fibrinogen; *c*, equilibrium concentration (M). The Ca^{2+} concentration was varied between 10^{-4} and 10^{-6} M.

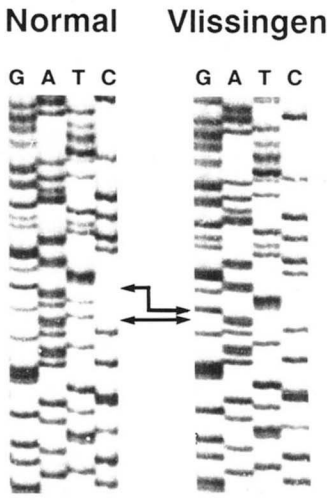

FIG. 6. **DNA sequence analysis of individual clones containing the amplified γ-chain gene exon VIII fragment, coding for normal fibrinogen, and fibrinogen Vlissingen (*arrows* indicate the deletion).**

As the thrombin-catalyzed release of FpA and FpB was normal and the defect is localized in the carboxyl terminus of the γ-chain, we conclude that the prolonged clotting time of fibrinogen Vlissingen is due to defective fibrin polymerization. Since the presence of Ca^{2+} ions is known to increase the rate of fibrin polymerization (52) by a number of mechanisms (53, 54), the loss of the high affinity calcium binding site in the D domain may fully account for the altered polymerization. However, this is unlikely because the results with fibrinogen Vlissingen using Reptilase™ (Fig. 3*D*) or thrombin (Fig. 3*B*) show that polymerization in the absence of calcium is also altered.

The polymerization defect in fibrinogen Vlissingen with Reptilase™ in the absence of Ca^{2+} indicates that the a polymerization site is defective. This is in conjunction with the data of Laudano and Doolittle (53), who demonstrate that the presence of Ca^{2+} ions does not significantly alter the interaction between the carboxyl-terminal "a" polymerization site and a peptide analog of the amino-terminal "A" polymerization site. Whether there is an additional influence of the defective calcium binding on polymerization of fibrinogen Vlissingen is difficult to determine because our measurement of polymerization requires formation of aggregates through multiple interactions, and because fibrinogen Vlissingen is heterozygous. Thus it is not possible to interpret our results more specifically. However, our data clearly demonstrate an association of this small γ-chain deletion with altered fibrin polymerization.

In summary, we have used the polymerase chain reaction to determine the structural defect in an abnormal fibrinogen, and have found a 6-base deletion encoding Asn-319 and Asp-320 of the γ-chain. Studies on the patient's plasma and purified fibrinogen indicate that the mutation is associated with reduced calcium binding and impaired fibrin polymerization. This study demonstrates the effectiveness of studies on dysfibrinogenemias where 1) the primary abnormality of this large complex protein can readily be identified by DNA sequence analysis, and 2) the altered protein can be functionally characterized *in vitro*.

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