Fibrinogen Brescia

Hepatic Endoplasmic Reticulum Storage and Hypofibrinogenemia Because of a $\gamma 284$ Gly→Arg Mutation

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The proposita suffered from liver cirrhosis and biopsy showed type 1 membrane-bound fibroglass inclusions. The hepatic inclusion bodies were weakly periodic acid-Schiff diastase-positive, and on immunoperoxidase staining reacted specifically with anti-fibrinogen antisera. Coagulation investigations revealed low functional and antigenic fibrinogen together with a prolonged thrombin time of 37 seconds (normal, 17 to 22 seconds) suggestive of a hypodysfibrinogenemia. DNA sequencing of all three fibrinogen genes showed a single heterozygous mutation of GGG (Gly)→CGG (Arg) at codon 284 of the γ -chain gene. However, examination of purified fibrinogen chains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, reverse-phase high-performance liquid chromatography, ion-exchange high-performance liquid chromatography, and isoelectric focusing, failed to show any evidence of the mutant γ^{Br} chain in plasma fibrinogen. This finding was substantiated by electrospray ionization mass spectrometry, which showed only a normal γ (and B β) chain mass, but a large increase in the portion of their disialo isoforms. We speculate that misfolding of the variant protein causes hepatic retention and the subsequent hypofibrinogenemia, and that the functional defect (dysfibrinogenemia) results from hypersialylation of otherwise normal B β and γ chains consequent to the liver cirrhosis. These conclusions were supported by studies on six other family members with hypofibrinogenemia, and essentially normal clotting times, who were heterozygous for the $\gamma 284$ Gly→Arg mutation. (*Am J Pathol* 2000, 157:189–196)

respectively.^{1,2} The symmetrical molecule consists of a central E domain joined to two peripheral D domains by triple-helix spacers. Single biantennary oligosaccharide side chains, of sequence, -NAcGlc₂-Man-(Man-NAcGlc-Gal-NAcNeu)₂, are attached to the B β and γ chains at Asn 364 and 52, respectively.³

After activation by thrombin, the fibrin monomer spontaneously polymerizes through D:E interactions to form a half-staggered bimolecular array that is stabilized by noncovalent end-to-end D:D interactions between adjacent monomer units.^{1,2} Covalent cross-links are subsequently inserted to buttress this interaction and form an insoluble clot. The C-terminal of the γ chain is intimately involved in each of these interactions; however, the finely balanced process of clot formation can be disrupted by both genetic and acquired perturbations of molecular structure. The acquired dysfibrinogenemias result from alterations in the pattern of posttranslational modification and are associated with chronic liver disease.⁴ Their impact on function or thrombin clotting time (TCT) is through elevation of the level of sialylation of the biantennary oligosaccharide.⁵

Specific genetic mutations in fibrinogen have long been associated with bleeding and thrombosis and more recently with extracellular amyloid deposits that can result in fatal renal disease.^{6,7} Putative undefined mutations have also been suspected of causing hepatic endoplasmic reticulum (ER) storage disease and consequent hypofibrinogenemia. In 1987 we described a family from Brescia, Italy, who suffered from hereditary hypofibrinogenemia with intrahepatic fibrinogen storage.⁸ A similar clinical and pathological picture had been reported earlier in two German families.^{9,10} Subsequent investigations of other families with cryptogenic chronic hepatitis/cirrhosis established that fibrinogen can be stored in the liver as four different distinguishable morphological types.¹¹

Z-antitrypsin deficiency is perhaps the most well-recognized and characterized ER storage disease, and blockage of secretion is because of a single 342 Glu→Lys mutation in the 55-kd inhibitor.¹² Accumulation

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Fibrinogen is synthesized in the liver as a 340-kd glycoprotein. The molecule is a dimer with each half consisting of three disulfide-linked polypeptide chains (A α , B β , and γ) with nominal molecular weights of 66, 55, and 48 kd,

and subsequent liver disease results from polymerization of the Z protein within the ER. This polymerization and fibril formation is initiated by insertion of the mobile reactive site loop of one molecule into a five-stranded (A) β sheet of a neighbor.¹³

By analogy it seemed that the different morphological fibrinogen inclusions might result from a series of mutations in one or another of the three fibrinogen genes. Here we investigate the molecular basis of the type I inclusions in fibrinogen Brescia and identify a novel γ 284 Gly $\rightarrow$ Arg mutation in the five-strand β -sheet structure of the γ -D domain.

Materials and Methods

Morphological Study

Liver specimens were fixed in Holland's fluid and embedded in paraffin. Serial 4- μ m sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) with and without previous amylase digestion, Masson's trichrome, and with a conventional immunoperoxidase technique using commercial polyclonal antibodies against fibrinogen, α -1-antitrypsin, α -1-antichymotrypsin, albumin, complement C3/C4, haptoglobin, α -2-macroglobulin, and IgG (DAKO, Copenhagen, Denmark). Both standard and immunoelectron microscopy were carried out on dewaxed material as reported previously.¹⁴

Coagulation Studies

Standard clinical coagulation tests, including thrombin and reptilase clotting times and antigenic fibrinogen levels, were performed on citrated plasma. Functional fibrinogen levels were determined by the Clauss method and gravimetric concentrations by fibrinopeptide quantitation.¹⁵

Fibrinogen Purification and Protein Analysis

Fibrinogen was purified by precipitation with 22% saturated ammonium sulfate. The pellet was washed three times with 25% saturated ammonium sulfate and redissolved at 5 mg/ml in distilled water.¹⁵

Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7.5% reducing and 4% nonreducing) was essentially as described by Laemmli.¹⁶ For isoelectric focusing fibrinogen was first reduced in 0.1 mol/L dithiothreitol, 8 mol/L urea, 5 mmol/L Tris/HCl, pH 8.0, and the chains focused over a pH range of 4 to 8 in 7% acrylamide gels in 8 mol/L urea, 0.5% Triton X-100.¹⁷

Ion-exchange high-performance liquid chromatography (HPLC) was performed on a 10-cm CX-300 column (Brownlee, Santa Clara, CA) in 8 mol/L urea, 10 mmol/L phosphate buffer, pH 6.5, and 0.1% mercaptoethanol. Gradient elution was with this same buffer made 0.2 mol/L with respect to NaCl. The flow rate was 0.75 ml/min and the absorbance was monitored at 280

nm. Fifty μ l of fibrinogen (5 mg/ml) in 8 mol/L urea, 10 mmol/L phosphate buffer, pH 6.5, and 10% mercaptoethanol was acidified with 0.25 μ l H_3PO_4 immediately before injection.¹⁸

For reverse-phase separations, fibrinogen (5 mg/ml) was dissociated by reduction (4 hours at 37°C) in 8 mol/L urea, 0.1 mol/L Tris/HCl, pH 8.0, 15 mmol/L dithiothreitol, and individual chains were resolved by HPLC on a Phenomenex (Torrance, CA) C-4 column (25 $\times$ 0.45 cm). The column was monitored at 215 nm and developed with a 0.05% trifluoroacetic acid/acetonitrile gradient system at a flow rate of 0.75 ml/min.¹⁹

Electrospray ionization mass spectrometry (ESI MS) was performed on a VG Platform II quadrupole analyzer (Micromass, Manchester, UK) as previously outlined.^{19,20} Peak crests from reverse-phase purified chains were collected and 20 μ l of each was directly injected into the ion source at a flow rate of 5 μ l/minute. The probe was charged at + 3500 volts and the source maintained at 60°C. The mass range 700 to 1500 m/z was scanned every 2 seconds and a cone voltage ramp of 30 to 60 volts was applied over this range. Up to 120 scans were averaged in acquiring the raw data. Calibration was made over this same m/z range using the charge series generated by human α globin. Data were acquired and processed using Mass-Lynx software and transformed on to a true molecular mass scale using maximum entropy (Max-Ent) software.^{19,20}

DNA Amplification and Sequence Analysis

Genomic DNA was extracted from whole blood²¹ and the coding regions and the intron/exon boundaries of all three fibrinogen chain genes were amplified by polymerase chain reaction²² and sequenced. Exon 8 of the γ gene was amplified for 30 cycles using the oligonucleotides g5515 (5' TAT CTA TTG CCT CTT GCC A) and g6126 (5' ACT TGG TAT TAT CCA CTT CC) with cycling parameters of denaturation at 94°C for 20 seconds, annealing at 56°C for 20 seconds, and extension at 72°C for 20 seconds. The polymerase chain reaction products were purified before sequencing using a High Pure kit (Boehringer Mannheim, Mannheim, Germany). Cycle sequencing of γ exon 8 was performed with the internal primer g5607 (5' CCT ACG AAG AGG GAA CTT C), using ³³P-radiolabeled terminators and Thermosequase (Amersham, Arlington Heights, IL) according to the manufacturer's instructions.

Screening for the γ G284R mutation used a mutagenic polymerase chain reaction/*Bbr*PI digestion procedure. The upstream mutagenic primer γ 5725*Bbr*PI (5' G TAC CGC CTA ACA TAT GCC TAC TTC GCA CGT; mismatches underlined) and γ 5837 (5' CTG CAT GCC ATT ATG GGA TG) were used to amplify a 114-bp fragment using the above conditions. On digestion with *Bbr*PI (Roche Diagnostics, Indianapolis, IN) the product of the normal allele is specifically hydrolyzed to 85- and 29-bp fragments, whereas the variant product remains uncut.

Results

Clinical History

In 1983 the *proposita*, at age 64, underwent an abdominal operation consisting of a cholecystectomy for gallbladder stones and a duodenal polypectomy. A wedge liver biopsy was performed because of the unexpected finding of liver cirrhosis. The postoperative course was uneventful; in particular there were no problems with hemostasis or wound healing. At the time of the operation there was a mild transaminase elevation, a Clauss fibrinogen of 0.2 mg/ml (normal, 1.0 to 3.0), an immunoreactive fibrinogen of 1.0 mg/ml, a prolonged TCT of 47 seconds, and HBsAg was negative. She had esophageal bleeding because of varices in 1977. Plasma fibrinogen levels have remained consistently low throughout the last 15 years (0.2 to 0.6 mg/ml) and presently the patient shows signs of decompensated cirrhosis (aspartate aminotransferase 59; alanine aminotransferase 65); her fibrinogen is currently 0.5 mg/ml, TCT 37 seconds, and she is hepatitis C virus antibody-negative.

Morphological Studies

Histological examination of the liver biopsy revealed features of early cirrhosis with thin connective tissue septa linking portal tracts and surrounding parenchymal nodules. Portal tracts contained inflammatory cells with evidence of piecemeal necrosis. The vast majority of hepatocytes contained cytoplasmic inclusions (arrow in Figure 1) appearing either as round or polygonal globules with an irregular outline, often surrounded by a clear halo, at times centered by small lipid droplets, or as acicular structures (asterisk). The latter filled the entire cytoplasm and imparted a fibreglass appearance to the cell. The inclusions were weakly stained on H&E preparations (Figure 1b) and faintly positive on PAS staining (Figure 1c). Under electron microscopy the globular inclusions corresponded to dilated rough endoplasmic reticulum (RER) cisternae filled with densely packed tubular structures arranged in curved bundles resulting in a fingerprint-like appearance (Figure 1, f and g). The acicular fibreglass inclusions corresponded to longitudinally cut cisternae of the RER containing tubular structures arranged in a parallel fashion. The electron microscopy observations confirmed the presence of lipid droplets within the large intracisternal inclusions. Neither the smooth endoplasmic reticulum, the Golgi, or the secretory vesicles, showed signs of dilation or endoluminal precipitates and the remaining organelles (mitochondria, lysosomes, and peroxisomes) were normal in size and number. The cytosol contained free ribosomes and a variable number of glycogen particles.

The intracisternal material reacted selectively and exclusively with an anti-fibrinogen antibody at both the light (Figure 1, d and e) and electron microscopy level (not shown). No immunoreaction for fibrinogen was observed outside the RER and the tubular intracisternal material was negative on immunostaining for all other proteins tested.

Family Studies

Plasma and DNA were obtained from fifteen other family members and the coagulation results and genotypes are summarized in Figure 2. All but one direct descendant of the *proposita* (II-2) had normal plasma fibrinogen concentrations and normal TCTs; none of these individuals had inherited the γ 284 mutation (see below). The exception (III-6) had both hypofibrinogenemia and liver cirrhosis, however he died in 1985 and archival material was not available for genetic analysis. The sister (II-1) and all but one of her descendants had hypofibrinogenemia together with a very slightly extended TCT and these were later found to be heterozygous for the fibrinogen Brescia mutation. To exclude the possibility of coincidental segregation of the γ 284 mutation with the hypofibrinogenemia we also examined 20 randomly selected Italians using mutagenic primer amplification followed by a *Bbr*PI digestion; none of the 40 alleles examined had the Brescia mutation.

Protein and DNA Analysis

Examination of purified plasma fibrinogen showed the expected 340- and 305-kD protein bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and after reduction, a normal pattern of $A\alpha$, $B\beta$, and γ chains was observed. Reverse-phase HPLC separations also showed a normal elution profile of $A\alpha$, $B\beta$, and γ chains.

Further examination of the HPLC-purified chains from the *proposita* by ESI MS indicated a single normal $A\alpha$ peak of mass 66,228 d (control, 66,230 d) and as expected the $B\beta$ and γ chains were heterogeneous, each displaying both mono- and disialo-isoforms. Again the masses of these chains did not differ significantly from the controls; 54,510 d for the disialo $B\beta$ chain *versus* a control of 54,504 d, and 48,404 d for the monosialo γ chain *versus* two independent control values of 48,404 d and 48,408 d (Figure 3). What was surprising however was the preponderance of the disialo isoforms of the Brescia $B\beta$ (not shown) and γ chains. The Brescia $B\beta$ chains were 77% fully sialylated (not shown) and the γ chains 46% (Figure 3) compared to normal control values of 45% and 24%, respectively.

Because this protein analysis failed to indicate which polypeptide chain contained the putative mutation, DNA encoding each of the three genes was amplified and sequenced. Only one mutation was identified, the *proposita* was found to be heterozygous for a single GGG (Gly)→CGG (Arg) mutation at codon 284 of the γ chain gene (Figure 4).

This finding was rather enigmatic because the mass difference between glycine and arginine is 99 d and an increase of this magnitude should have been detected in the ESI analysis of the isolated γ chain. Although the analysis would not have been expected to resolve the two forms, in a 50/50 heterozygous mixture of mutant γ^{Br} and normal γ^A chains the average chain mass would have been expected to increase by 99/2 d. Indeed we have previously measured the predicted 60/2 d change associated with heterozygosity for a γ 280 Tyr→Cys mutation

Figure 1. Immunohistochemistry of liver sections. **a** and **b**: H&E stained section showing cytoplasmic inclusions (**arrow**) within the hepatocytes. There are a few vacuolated nuclei and steatosis vacuoles present (**a**) (original magnification, $\times 175$); inclusions appear in the form of round or polygonal (**arrow**) or elongated bodies (**asterisk**) (**b**). A clear halo surrounds the largest inclusions, a few of which are often centered by small lipid droplets. The inclusions are weakly eosinophilic (original magnification, $\times 325$). **c**: PAS after diastase treatment; the inclusions are weakly positive after staining (original magnification, $\times 325$). **d** and **e**: Immunoperoxidase staining with fibrinogen antisera; both globular and elongated (acicular) inclusions stain positively (**d**) (original magnification, $\times 175$); acicular fibrinogen inclusions within periportal hepatocytes (**e**) (original magnification, $\times 325$). **f**: Electron microphotograph showing cytoplasmic fingerprint inclusion surrounded by electrondense remnants of the RER membrane (original magnification, $\times 18,400$). Glycogen particles are present in the cytosol. Neither peroxisomes, mitochondria, or multivesicular bodies contained tubular or fibrillar material. **g**: Electron microphotograph showing elongated RER cisternae containing fibrillar-tubular structures (original magnification, $\times 48,000$). The peripheral electrondense material represents remnants of the RER membranes. Cytosol, lysosomes, damaged mitochondria, lipid inclusions, and multivesicular bodies do not contain fibrillar material.

Figure 2. Family tree showing the probanda (arrow) and other carriers of the γ G284R mutation (filled semicircle). The fibrinogen concentrations (mg/ml, Claus) are shown below the TCT (s). nt, not tested.

at precisely 31 d.²³ The implication from this is that the mutant Brescia γ chain is not expressed to a significant extent in plasma fibrinogen.

This possibility was confirmed by a more detailed analysis of plasma fibrinogen chains by ion exchange chromatography and isoelectric focusing in the presence of 8 mol/L urea. Because the Gly $\rightarrow$ Arg mutation introduces a charge increase of +1, these procedures would also be expected to detect γ^{Br} chains if present. Positive controls were included in these experiments and heterozygotes for a β 14 Arg $\rightarrow$ Cys mutation showed two clear 50/50 peaks on CX-300 chromatography and a γ 279 Ala $\rightarrow$ Asp heterozygote was clearly identified by a -1 anodal shift of the γ components on isoelectric focusing (Figure 5). However, in the Brescia case only a single normal γ peak was observed on CX-300 chromatography (not shown) and a normal distribution of γ isoforms on isoelectric focusing. Specifically there was no new cathodal band that would be expected if the γ^{Br} chain, with its new Arg,

Figure 3. Transformed ESI MS of purified γ chains from patient (center) and two normal controls. The paired peaks correspond to mono- and disialo isoform of the chain as depicted in the schematic. There is no evidence for the presence of the abnormal γ^{Br} chain, but there is increased sialylation of otherwise normal γ chains.

Figure 4. Radiograph showing DNA sequence from exon 8 of the fibrinogen γ chain gene. N, normal sequence; B, sequence from probanda with fibrinogen Brescia. The heterozygous mutation (asterisk) of GGG $\rightarrow$ CGG results in a Gly $\rightarrow$ Arg substitution at codon γ 284.

was actually present in circulating fibrinogen molecules (Figure 5).

Further family members were screened for the codon 284 GGG $\rightarrow$ CGG mutation using mutagenic primer amplification followed by a *Bbr*PI digestion and six carriers were identified. Two additional affected family members (II-1 and III-1) and two normals (IV-2 and III-3) were further analyzed by ESI MS. The average monosialo γ chain mass of the two affected individuals was 48,389 d

Figure 5. Isoelectric focusing of purified plasma fibrinogen showing separation of γ chain isomers. 1, Normal family member (III-3); 2 and 3, heterozygous carriers of Brescia mutation (III-1 and III-2); 4, heterozygous control with γ 279 Ala $\rightarrow$ Asp mutation showing increased anodal migration of the variant γ isoforms; 5, normal control. Note absence of additional cathodal band (in bracketed region) that would signify presence of γ^{Br} chains in the γ 284 Gly $\rightarrow$ Arg heterozygotes (anode at top).

and that of the normals was 48, 391 d and the amount of disialo isoform averaged 29% and 23% for the affected and normals, respectively. The level of B β chain sialylation averaged 46% and 35%, respectively. These results suggest that there was no significant plasma expression of the γ^{Br} chain in these heterozygotes and no significant increase in sialylation of the γ^{A} chain. This was substantiated by isoelectric focusing of purified fibrinogen from the heterozygotes III-1 and III-2.

Discussion

Here we found hereditary hypofibrinogenemia and hepatic accumulation of fibrinogen associated with a novel γ chain mutation. The γ 284 Gly $\rightarrow$ Arg substitution was inexorably linked to the hypofibrinogenemia because the seven proven carriers had an average plasma fibrinogen of 0.7 mg/ml and the nine normal family members had average concentrations of 2.4 mg/ml (Figure 2). Although the hepatic storage seems to predispose to the development of cryptogenic chronic liver disease, the link between the mutation and storage is not as strong as that with hypofibrinogenemia. There was however, no other discernible cause of the chronic liver disease in the *proposita*, and it seemed to segregate with the fibrinogen mutation because her only direct descendant with hypofibrinogenemia (III-6) died from decompensated cirrhosis. Whereas none of the other surviving carriers of the γ 284 mutation has yet developed any symptoms, this is similar to Z-antitrypsin deficiency where only a minority of individuals develop significant liver injury.^{12,24}

In individuals with the MZ-antitrypsin phenotype the Z allele contributes 15% of the plasma antitrypsin, however no γ^{Br} chains were present in circulatory fibrinogen from Brescia heterozygotes. Because of the dimeric nature of fibrinogen, random incorporation of the γ^{Br} chain into nascent dimers in the ER would mean that 75% of molecules would contain at least one variant chain and potentially contribute to inclusions. With this scenario we would expect the mutation to induce a more severe hypofibrinogenemia than would result from the simple nontranslation of a gene; this was indeed the case because carriers had one-third the fibrinogen concentration of their nonaffected relatives.

The pathology associated with the γ 284 Gly $\rightarrow$ Arg mutation seems to be of liver cirrhosis rather than of bleeding or thrombosis. For the latter, aberrant molecules would first have to be present in plasma and the evidence from ion-exchange HPLC, isoelectric focusing, and ESI MS is that they are not. How then do we explain the functional defect implied by the prolonged TCT (37 s) if the *proposita* has no significant plasma expression of the variant? The polymerization defect can best be explained by the hypersialylation of the B β and γ chains because Martinez et al⁴ have shown that the acquired dysfibrinogenemia associated with chronic liver disease is because of excess sialic acid on these chains. They showed that whereas normal fibrinogen contained six residues of sialic acid, their five subjects with liver disease had between 1.4 and 3.4 residues more per mole-

Table 1. Percentage of Full Sialylation and Number of Sialic Acid Residues Per Molecule Calculated from ESI Spectra

Chain(s)	Maximum number	Normal controls		Brescia <i>proposita</i>	
		% full	No. residues	% full	No. residues
A α	0	NA	0	NA	0
B β	2	45	1.45	77	1.77
γ	2	24	1.24	46	1.46
A α B β γ	4		2.69		3.23
(A α B β γ) ₂	8		5.38		6.46

Maximum number of sialic acid residues is based on presence of four biantennary oligosaccharides per 340-kd molecule. NA, not applicable.

cule and this extended their thrombin times by between 12 and 22 seconds. Here ESI MS shows B β and γ chains of normal mass but with an increased proportion of disialo isoforms. Indeed if we assume a maximum of eight sialic acids per (A α ₂ B β ₂ γ)₂ molecule, the data shows 5.4 residues/mole for normal controls and 6.5 for the patient (Table 1). This might be expected to increase the TCT by approximately 12 seconds, which is reasonably consistent with the observed increase of 15 seconds.

The Gly at γ 284 is absolutely conserved, not only in all known γ -chain sequences, but also in all B β and extended α E chains.²⁵ This conservation between homologous chains suggests a structural rather than functional importance for the site, because the C-terminals of the B β and α E chains do not have the critical role in polymerization that the C-terminal of the γ chain does, but all three chains have similar three-dimensional structures.²⁶

Although located in the functionally important γ D domain, X-ray structures²⁵ indicate that residue γ 284 is situated away from regions directly involved in D:E or D:D polymerization sites and therefore the mutation would not be expected to have a direct effect on function. The Gly residue itself lies at the end of a β strand²⁷ that starts at γ 280 Tyr and extends away from the D:D interface to Ala 286. Tyr 280 is however intimately involved in D:D polymerization and forms the closest contact across this surface.^{23,25} The strand containing Gly 284 is the most distal of five that make up an extended β -sheet structure (Figure 6).^{25,27} It seems that this strand might be quite mobile because in the double D structure of Spraggon et al²⁶ residues 280 to 284 appear as a random coil. This sheet structure is reminiscent of the A sheet in antitrypsin that undergoes a transition from a metastable five-stranded structure to stable six-stranded structure on cleavage within its reactive center loop.²⁸ The Z mutation at position 342 is at the hinge point of this loop-sheet insertion and the Glu $\rightarrow$ Lys substitution facilitates intermolecular loop to sheet insertion leading to antitrypsin Z-fibril formation and the blockage of hepatic secretion.¹³ The two other rare antitrypsin mutations that result in ER storage, 52 Phe deleted and 53 Ser $\rightarrow$ Phe, also seem to perturb the movement of strands in the A sheet by destabilizing its supporting structure.²⁹

Like antitrypsin, the fibrinogen molecule is in a metastable state because its D:D polymerization sites pre-

Figure 6. Molecular model of the C-terminal D domain of the fibrinogen γ chain showing the position of $\gamma 284$ Gly. The conserved Gly residue is at the end of the last strand of an extended β sheet structure reminiscent of the A-sheet in antitrypsin. The position is away from functional D:E or D:D interaction sites. As shown, the β strand containing $\gamma 284$ runs away from Tyr 280, which forms a close contact across the DD interface. The figure was prepared using pcRIBBONS³⁰ from the co-ordinate file 3fib.pdb.

exist and do not require thrombin activation. The $\gamma 284$ Gly $\rightarrow$ Arg mutation may perturb the β -sheet array and allow intramolecular strand insertions leading to fibrinogen fibril formation and the blockage of its secretion. Certainly the electron microscopy images of fibrinogen Brescia inclusions show a degree of order similar to Z-antitrypsin fibrils.

This is the first fibrinogen mutation identified that results in hypofibrinogenemia and it seems that defective secretion may account for the hepatic storage and plasma deficiency. Although this new form of ER storage disease maybe an uncommon cause of cirrhosis, fibrinogen inclusions have been detected in eight out of 700 biopsies on patients with hepatitis.¹¹ This suggests that it is an unrecognized entity. Fibrinogen storage should be excluded in appropriate cases and, when immunoperoxidase stains are positive, genetic analysis undertaken. The recognition of four distinct morphological forms also suggests that several different mutations may be involved.

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