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A novel missense mutation in the *FGB* g. 3354 T>A (p. Y41N), Fibrinogen Caracas VIII

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Summary

A novel dysfibrinogenemia with a replacement of Tyr by Asn at B β 41 has been discovered (fibrinogen Caracas VIII). An asymptomatic 39 year-old male was diagnosed as having dysfibrinogenemia due to a mildly prolonged thrombin time (+ 5.8 sec); his fibrinogen concentration was in the low normal range, both by Clauss and gravimetric determination, 1.9 g/l and 2.1 g/l, respectively. The plasma polymerization process was slightly impaired, characterized by a mildly prolonged lag time and a slightly increased final turbidity. Permeation through the patients' clots was dramatically increased, with the Darcy constant around 4 times greater than that of the control ($22 \pm 2 \times 10^{-9} \text{ cm}^2$ compared to $6 \pm 0.5 \times 10^{-9} \text{ cm}^2$ in controls). The plasma fibrin structure of the patient, by scanning electron microscopy, featured a mesh composed of thick fibers ($148 \pm 50 \text{ nm}$ vs. $120 \pm 31 \text{ nm}$ in controls, $p < 0.05$) and larger pores than those of the control fibrin clot. The viscoelastic properties of the clot from the patient were also altered, as the storage modulus (G' , 310 ± 30) was much lower than in the control (831 ± 111) ($p = 0.005$). The interaction of the fibrin clot with a monolayer of human microvascular endothelial cells, by confocal laser microscopy, revealed that the patients' fibrin network had less interaction with the cells. These results demonstrate the significance of the amino terminal end of the β chain of fibrin in the polymerization process and its consequences on the clot organization on the surface of endothelial cells.

Keywords

mutation; abnormal fibrinogen; clots; permeation; viscoelastic properties; scanning electron microscopy; confocal microscopy

Introduction

Fibrinogen is a major plasma protein (normal concentration 1.5–4.0 g/l), which is synthesized in hepatocytes (1). It comprises three pairs of non-identical polypeptide chains, (A α B β γ)₂, held together by 29 disulphide bonds. Thrombin removes short sequences from the N-termini of A α and B β chains (FpA and FpB denote the fibrinopeptides A and B) (2–4). The removal of FpA (A α 1-16) exposes the polymerization site termed knob 'A', comprising residues α 17 to 20 (GPRV) (5), and at a slower rate occurs the removal of FpB, exposing an independent polymerization site termed knob 'B', beginning with β 15-18 (GHRP) (6). These exposed sequences in the α and β chains interact noncovalently with ever-present specific amino acid sequences forming 'holes' near the C-termini of the γ and β chains on neighbor molecules, to form oligomers termed protofibrils. The protofibrils aggregate laterally to form fibers, which then branch to yield a three-dimensional network (the fibrin clot). As polymerization progresses, thrombin-activated factor XIII incorporates covalent cross-links, initially between the carboxy terminal segments of γ chains, and later (at a much slower rate) between the carboxy terminal segments of α chains, and to a minor extent between α and γ chains (7), stabilizing the clot against mechanical, chemical, and proteolytic insults (4).

The formation of fibrin at the site of vascular injury provides a temporary matrix for wound healing and revascularization (8, 9). Endothelial cells express integrin-type receptors responsible for adhesion to the proteins of the extracellular matrix. Fibrinogen contains multiple recognition sites for integrins, some of which appear cryptic and become exposed upon its conversion to fibrin (10). Of the many endothelial integrin receptors, only the $\alpha_v\beta_3$ receptor is capable of recognizing all the provisional matrix proteins including fibrin(ogen) and fibronectin (11, 12). The integrin $\alpha_v\beta_3$ recognizes a single RGD-containing sequence near the C-terminus of the fibrinogen α -chain (α residues 572–574) (11), and a non RGD motif in the fibrinogen γ chain 148–226 region (13). Furthermore, there are at least two more endothelial cell non-integrin receptors with which fibrin can interact with: VE-cadherin (through the residues β 15-42) (14), and the intercellular adhesion molecule-1 (ICAM) (15).

Fibrinogen is a heterogeneous protein with both inherited and non-inherited variations that can influence fibrinogen function and its levels in the normal population (16). Dysfibrinogenemia is defined as the presence of an abnormal fibrinogen variant in plasma, usually associated with normal antigenic levels (17).

Dysfibrinogenemic abnormalities are usually reflected by changes to one or more phases of the fibrinogen-fibrin conversion and fibrin assembly processes, including impaired release of fibrinopeptides, defects in fibrin polymerization, and faulty factor XIIIa-mediated cross-linking (18).

In the present work, we report some functional and structural abnormalities found in clots formed from a novel fibrinogen variant, fibrinogen Caracas VIII, with a missense mutation in the *FGB* g. 3354 T>A (p. Y41N), and we explore the interaction of the variant fibrin with human dermal microvascular endothelial cells (HMEC-1), which is important for clotting and thrombosis *in vivo*.

Materials and Methods

All chemicals were of analytical Grade, most of them from Sigma Chemical Company (St. Louis, MO, USA). The human microvascular endothelial cells (HMEC-1) were kindly donated by Dr. Edwin Ades and Mr. Francisco J. Candal of the Centers for Disease Control and Prevention (CDC) (Atlanta, USA) and Dr. Thomas Lawley of Emory University

(Atlanta, USA). Human α -thrombin was purchased from American Diagnostica (Greenwich, CT, USA). Alexa Fluor® 488, di-8-anepps (aminonaphthylethylenpyridinium), epidermal growth factor, and the LabTek glass chamber slides were obtained from Invitrogen (Nalge Nunc International, Rochester, NY, USA). The MCDB 131 medium, penicillin, streptomycin, fungizone, L-glutamine and fetal bovine serum were from GIBCO (Grand Island, NY, USA).

Blood Collection

Blood was collected in citrate (1 volume of 0.13 mol/l trisodium citrate and 9 volumes of blood) and immediately centrifuged at 2500 g for 20 min. Plasma was supplemented with benzamidine 10 mM (final concentration), aliquoted and kept frozen until use. The fibrinogen concentration was determined by means of the gravimetric method (19).

Mutation Analysis

Genomic DNA was isolated using the Invisorb Spin Blood Mini Kit (Invitex GmbH, Berlin, Germany) according to the manufacturer's protocol. Sequences comprising all exons and exon-intron boundaries from the three fibrinogen genes: *FGA*, *FGB*, and *FGG* were amplified by the polymerase chain reaction (PCR) according to standard protocols. After purification of the PCR products using the Invisorb Spin PCRapid Kit® (Invitex, Berlin, FRG), direct DNA cycle sequencing was performed, applying the Big Dye kit from Applied Biosystems (Foster City, CA, USA), according to the manufacturer's recommendations.

Fibrin polymerization

The polymerization process was examined in the patients' plasma and in the plasma of two healthy male donors, as control, and in purified fibrinogen (purified from these same samples) at room temperature. The plasma was diluted 1:10 with Tris- buffered saline (TBS) (50 mM Tris, 0.15 M NaCl, at pH 7.4) and clotted with a solution of 0.6 units/ml of thrombin and 20 mM CaCl_2 (prepared in TBS, final concentrations). Purified fibrinogen (obtained by β -alanine precipitation (20)) at 1 mg/ml in TBS was incubated for 1 min with 10 mM of CaCl_2 (final concentration), then clotted with 2 units/ml of thrombin (final concentration). Changes in absorbance were followed for 13 and 5 min, plasma and purified fibrinogen, respectively, at 350 nm using a Genesys 2 spectrophotometer (Spectronic Instruments, Rochester, NY, USA). Duplicate samples were analyzed each time. The lag time, slope and maximum turbidity were recorded for each curve and averaged.

Permeation and scanning electron microscopy

Permeation experiments were performed as described elsewhere (21). Briefly, 100 μl of undiluted plasma were mixed with a solution of 1 unit/ml of thrombin and 20 mM CaCl_2 (final concentrations). The mixture was immediately transferred to a plastic column (internal diameter: 0.2 cm). The columns were left in a moist environment at room temperature for two hours, and then the top part of the column was filled with TBS, so that the head pressure applied on the upper surface of the clot was 4018 dyne/cm². The clot was perfused with buffer to wash out plasma proteins that had not been incorporated into the formed clots, before starting the flux measurements. The flux was calculated from the weight of the drops that percolated in a given time, which is equivalent to the volume of a drop with a density of 1 g/ml (approximately the same as that of water at room temperature). Six recordings for each clot (three replicates of each sample) were taken and the Darcy constant was calculated (22).

After the permeation experiments, the clots were processed for scanning- electron microscopy evaluation as described elsewhere (23). The clot networks were observed using

a Philips XL20 microscope (FEI, Hillsboro, OR, USA). Digital images were saved and the thicknesses of 300 fibers each were measured using the NIH ImageJ 1.42 program. Triplicate clots of each sample were made, and several fields of each clot were examined randomly to obtain fields that were characteristic of the entire clot.

Viscoelastic properties

The rheological properties of clots formed from plasma samples were measured using a torsion pendulum (24). Undiluted plasma was clotted with 1 unit/ml of thrombin and 20 mM CaCl_2 (final concentrations). The clot was left 1 h in static conditions before measurements, keeping it in a moist environment. The dynamic storage modulus, G' (dyne/cm²), the loss modulus, G'' (dyne/cm²), and the loss tangent ($\tan \delta$), the ratio between G''/G' (the ratio of energy lost to energy stored in a cyclic deformation), were calculated from free oscillations of three records from each clot (in triplicate) by the application of a momentary, standardized impulse to the arm of the pendulum at room temperature.

Fibrin interaction with human dermal microvascular endothelial cells (HMEC-1)

HMEC-1 were cultured in MCDB 131 medium supplemented with 10 % fetal bovine serum, epidermal growth factor (10 ng/ml), penicillin (100 U/ml), streptomycin (100 µg/ml), fungizone 0.25 µg/ml and L-glutamine (2 mM). Cells (120.000) were seeded in LabTek glass chamber slides and maintained at 37 °C in a humid atmosphere with 95 % air and 5 % CO_2 and grown to >80% confluence. The culture medium was removed and cells were labeled with 4 µM di-8-anepps for 15 min. The cells were then washed with saline phosphate buffer three times. Separately, a solution of plasma from the patient or a healthy male as control was mixed with Alexa Fluor 488-labeled fibrinogen (19 µg/206 µl sample volume), and a solution of 0.3 units/ml of thrombin and 20 mM CaCl_2 (final concentrations) was added. The mixture was quickly transferred on to the top of the cells. Another clot was prepared under the same conditions, but it was loaded directly in the glass chamber, in order to compare the influence of HMEC-1 on clot organization. Clot formation was allowed to progress for 2 h in a tissue culture incubator at 37 °C. Afterwards, the top of the clot was loaded with 200 µl of MCDB 131 medium with no supplement. The clot structure on the surface of HMEC-1 was visualized using a Nikon Eclipse TE 2000-U laser microscope with a 488 nm Argon or a 543 nm HeNe laser. The objective used was Plan Apo VC 60X in water immersion with a work distance of 0.27. The acquisition pinhole was set to 60 µm. For analysis, a Z- stack was imaged from the bottom of the dish (0 µm) up to 30 µm, with step sizes of 0.5 µm. Duplicates of each sample were made, and several fields of each clot were examined randomly to obtain fields that were characteristic of the entire sample, then five areas of 212 × 212 µm (x,y) were selected at random from each and digitized.

Statistical analysis

The data from the measurements of fibrin fiber diameters was analyzed, first comparing the variances by applying an F-test and if the variances were similar, the t-test for similar variance was applied; otherwise, the Welch approximation was used. A probability (p) of less than 0.05 was regarded as statistically significant. The viscoelastic fibrin properties, the storage modulus and loss tangents, were analyzed by non- parametric statistics using the Mann-Whitney U test. A probability (p) of less than 0.05 was regarded as statistically significant.

Results

Case Report

A dysfibrinogenemia was suspected in an asymptomatic 39 year old male, due to his prolonged thrombin time (+ 5.8 sec). His fibrinogen concentration was in the low normal

range, as determined by Clauss (STA Compact®, Stago, France) and gravimetric assays, and which gave 1.9 g/l and 2.1 g/l, respectively. This abnormal fibrinogen was discovered because his son was first studied for a severe FVII deficiency (2.5 U/dl), homozygous for the mutation FVII G283S, while his father had 39 U/dl FVII, heterozygous for this mutation (25). DNA sequencing of the three fibrinogen genes of the proband and his son revealed a heterozygous missense mutation in the *FGB* gene g.3354 T>A (p.Y41N), with numbering starting at residue 1 of the predicted mature polypeptide. We name this fibrinogen variant as fibrinogen Caracas VIII. All the studies were performed with the plasma of the proband (case 1). The blood coagulation screening tests were performed at the Metropolitan Blood Bank of Caracas and results are summarized in Table 1.

Healthy males from the staff of the Instituto Venezolano de Investigaciones Científicas, without taking any medication at the moment of blood withdrawal, not age matched, were chosen and compared to the patient in all the experiments performed.

Fibrin polymerization

Plasma fibrin polymerization showed that the final turbidity of the sample from the patient was slightly higher than controls. The two controls used contained 1.1 and 1.4 g/l more plasma fibrinogen than the plasma from the patient, in which that concentration remained at 2.1 g/l. The patient's lag time was 30 sec longer than in the controls but their slopes were similar. This is opposite to what would be expected, whereby as fibrinogen concentration increases there is an increase in the final turbidity (26). Fibrin polymerization curves using purified fibrinogen revealed that the patient showed a final turbidity 1.5 times higher and a 19 sec longer lag time than those of the two controls, whereas as in the case of plasma, all the slopes were identical (Fig. 1). Since the site of this mutation is near a plasmin cleavage site, we assessed potential changes in lysis by measuring the rate of decrease of turbidity after polymerization of plasma by thrombin and subsequent lysis due to the presence of tPA, but observed no differences from control plasma (results not shown).

Permeation, scanning electron microscopy and viscoelastic properties

Since the Darcy constant is dependent on fibrinogen concentration, a control with a plasma fibrinogen concentration close to that of the patient was chosen (2.9 g/l vs. 2.1 g/l, respectively). The permeation was drastically increased in the patient's clots, to almost 4 times higher than in the control, with Darcy constants of $(22 \pm 2) \times 10^{-9} \text{ cm}^2$ and $(6 \pm 0.5) \times 10^{-9} \text{ cm}^2$, respectively.

The architecture of the patient's plasma fibrin clot was visualized by scanning electron microscopy (Fig. 2). The patient's fibrin network was formed of thicker fibers than in the control, with wider pores, congruent with permeation and turbidity results. The mean fibrin fiber diameter from the patient was $148 \pm 50 \text{ nm}$ as compared to $120 \pm 31 \text{ nm}$ in the control, and the difference was statistically significant ($p < 0.05$).

The viscoelastic measurements revealed that the patients' clots were less rigid, characterized by a storage modulus (G') of $310 \pm 30 \text{ dyne/cm}^2$ and a loss tangent ($\tan \delta = G''/G'$) of 0.100 ± 0.007 compared to $831 \pm 111 \text{ dyne/cm}^2$ and 0.092 ± 0.006 for the control. These differences were statistically significant (at $p = 0.005$ and $p = 0.048$, respectively), according to the Mann-Whitney U test. These results indicate that the patient's clots undergo more deformation when subjected to mechanical stress (Table 2).

Fibrin interaction with human dermal microvasculature endothelial cells (HMEC-1)

We analyzed the fibrin structure of the clots formed on the cultured cells at various distances from the cells' surface, starting from the bottom of the dish, distance 0 μm , where almost no

fibrin fibers were seen, up to 30 μm . It was noted that the structure of the fibrin network changed with the distance from the cell surface. Close to the monolayer of cells, the control showed a great density of thin fibers adhered to the cells' surface (Fig. 3A, at 7.5 μm). In contrast, in the sample from the patient, the quantity of fibers associated with the cells was much less (Fig. 3B). Furthermore, the structure of the patients' fibrin network was very different than that of the control. It was composed of thicker and less branched fibers, with big pores, in agreement with what had been observed by scanning electron microscopy of clots without cells.

At greater distances, a different fiber arrangement was seen. The control clot fibers were interwoven very tightly around some cells (Fig. 3C, 10 μm), while those structures were less tight and more lax in the patient's samples (Fig. 3D). From 15 to 30 μm , the fibrin fibers were more randomly distributed. In Figures 3E and F, the control and the samples from the patient are shown at 30 μm .

In order to visualize the fibrin structure in three dimensions, we carried out Z-projections and deconvolved the images. For the first 10 μm closest to the cell surface, in the control samples very dense areas were observed (indicated by arrows) surrounded by looser ones (Fig. 4A), whereas in the samples from the patient those dense areas were much less frequent (Fig. 4B). The difference in fibrin pore sizes between control and patient's clots were remarkable, with the latter showing larger pores near the cell surfaces. When Z-projections were generated at greater distances, from 20 to 30 μm , the fibrin networks were homogeneous, both for control and patient (Figs. 4C and D, respectively), and the fibrin structure of the patient resembled that observed between 0–10 μm .

The fibrin structure observed farther away from the cells' surface was similar to that of clots formed without cells, both for normal control and patient, Fig. 4E and 4F, respectively.

Discussion

In the present work we report a new fibrinogen variant with a missense mutation in the *FGB* at g.3354 T>A, which at the protein level causes the replacement of the amino acid tyrosine for asparagine at B β 41. This amino acid is located in the sequence B β 15–42, for which different roles in fibrin polymerization (27), endothelial cell (EC) spreading (28), and VE-cadherin receptor binding (14) have been documented. One limitation of our findings is that the expression and quantification of the abnormal B β chains was not assessed. However, there was strong evidence from the fibrin polymerization and biophysical studies that the mutated B β chains were expressed.

During fibrin polymerization the newly exposed sequence, knob "A" and "B" interact with the hole "a" and "b", respectively, forming the well known fibrin interactions A:a and B:b (29). Unfortunately, knob A and B has not been visualized at atomic level, as hole "a" and "b", because these regions appear to be flexible and do not show up in the crystal structure (29). The crystal structures of the end regions of fibrinogens, called fragment D with various GPR or GHR containing peptides fit into holes "a" while GHR peptides fit into holes "b". However, we do not know at present if the interactions of these peptides represent the entire binding sites or if these could be more extensive regions involved, as suggested by some studies (27, 30).

The plasma polymerization process of fibrinogen Caracas VIII was slightly impaired, as a mildly prolongation of the lag phase and a final turbidity increase was detected. However, when purified fibrinogen was used, a drastic change in the final turbidity, roughly twice that of the control, was observed. It seems likely that the replacement of the bulky aromatic side chain of tyrosine by the small polar uncharged side chain of asparagine probably altered the

conformation of knob “B”, and consequently the B:b interactions. It is possible that this mutation decreases the rate of protofibril initiation, as can be inferred from Figures 1c and 3 in Weisel and Nagaswami (31).

The network stiffness is strongly dependent on fiber thickness and branchpoint concentration (32). Fiber lengths and diameters decreased with increased branching (32, 33), contributing to increased clot rigidity. Fibrin is a viscoelastic polymer, which means that it has both elastic and viscous properties (34). Thus, the mechanical properties of fibrin may be typified by the stiffness or storage modulus (representing its elastic properties) and creep compliance or loss modulus/loss tangent (representing its inelastic properties) (4). The variant fibrinogen B β Y41N examined by us showed a diminished storage modulus and a slightly increased inelastic component, which means that its clot will deform more with applied mechanical stress than one with higher storage modulus and loss tangent. The elastic and inelastic properties of a clot are very sensitive to small changes that affect polymerization and clot structure. Clots ligated with Factor XIIIa usually show higher stiffness and a lower inelastic component of deformation (4). We disregarded the effect of fibrin ligation by Factor XIIIa, as its value was normal (results not shown). The protein mutation largely affected the viscoelastic properties of the clot, highlighting the importance of this region in the clot mechanical properties. Recently, it has been demonstrated that the stiffness of clots formed from fibrinogen 325 (fibrinogen lacking the B β 1-42, after digestion with *Crotalus atrox* protease III), decreased by about 8-fold (35). Although the clots formed with this degraded form of fibrinogen are not equivalent to those from the variant B β Y41N, the former results confirmed the role of B β 1-42 in oligomer formation and on the elastic properties of the fibrin clot.

We also wished to explore the structure of the patients' fibrin clot formed on ECs, especially since the fibrinogen mutation presently reported was located in the fibrin binding site to VE-cadherin (14), and this non-integrin receptor is likewise located on the adherens junction (zonula adherens) which allows calcium-dependent homophilic recognition between ECs (36) that controls vascular permeability (37).

It has been reported elsewhere that fibrin near the EC surface is more organized and occurs in tighter bundles than fibrin 50 μ m away from the surface (38). When antibodies against α_V or β_3 integrin subunits or the ligand-mimetic peptide d-RGDW were used (38), the fibrin organization on the cells' surface was lost, indicating that the endothelial receptors responsible for the fibrin arrangement were associated or formed part of the integrin $\alpha_V\beta_3$. In our attempt to explore what the *in vivo* clot organization of fibrinogen Caracas VIII subjects would be like, it was found that the patient's fibrin structure near the EC surface was much less tight than that of the control, closely resembling that observed farther away from the cells' surface. It appears that this mutation upsets the normal association of fibrin with the integrin $\alpha_V\beta_3$ EC receptor. Some researchers do not agree with the results of Jerome et al. (38) on the effects of ECs on fibrin lysis; others have found that the fibrin structure near the cell surface depends on the thrombin generation, and that the presence of the ligand-mimetic peptide did not alter the fibrin structure in this zone, concluding that fibrin structure on the surface of ECs was not related to fibrin binding to the integrin $\alpha_V\beta_3$ (39, 40). Nonetheless, our results are in agreement with those of Jerome et al. (38), and the addition of the mimetic peptide abrogated the tighter structure near the cell surface in the case of the control (results not shown). Evidently more research has to be carried out to discern these apparent discrepancies.

In conclusion, the amino acid exchange B β Y41N in the fibrinogen variant herein described has profound effects on clot structure, leading to increased fibrin fiber diameters and pore size, which impair the fibrin mechanical properties, increase dramatically the permeation

through the clots and decrease the interaction between the fibrin and the surface of HMEC-1 cells. Furthermore, although this was not examined in the present work, it is to be expected that the patient's fibrin interaction with the VE-cadherin receptor should be impaired. Further studies ought to address these issues and their interactions, in order to clarify the functional consequences on the barrier function of ECs.

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What is known about this topic?	What does this paper add?
<ul style="list-style-type: none">* The fibrinogen Bβ 15-42 sequence has a role in the fibrin polymerization process.* VE-cadherin interacts with fibrin through residues β 15-42.	<ul style="list-style-type: none">* The missense mutation in the <i>FGB</i> g.3354 T>A, is a novel mutation* The effect of this mutation on fibrin structure, viscoelastic properties, permeation, and ECs-fibrin interaction.

Figure 1.

Fibrin polymerization from plasma and purified fibrinogen. A) Plasma samples, diluted 1:10, were clotted with 0.6 units of human thrombin and 20 mM CaCl_2 (final concentrations). The changes in the OD at 350 nm were recorded during the first 13 min. Two controls with different plasma fibrinogen concentrations were used, $\blacklozenge$ control 1 (3.5 g/l), $\blacksquare$ control 2 (3.2 g/l), $\blacktriangle$ patient (2.1 g/l). B) Purified fibrinogen. A fibrinogen solution of 1 mg/ml in Tris-buffered saline was clotted with 2 units/ml of human thrombin and 10 mM of CaCl_2 (final concentrations). The changes in the OD at 350 nm were recorded over the first 5 min. $\blacklozenge$ control 1, $\blacksquare$ control 2, and $\blacktriangle$ patient.

Figure 2.

Scanning electron micrographs of plasma clots. One hundred μl of undiluted plasma was mixed with a solution of 1 unit/ml of human thrombin and 20 mM CaCl_2 (final concentrations). After 2 h of incubation, the clots were perfused with Tris-saline buffer to measure the permeation rate, or Darcy constant. Thereafter the clots were intensively washed for preparation for scanning electron microscopy. A) Control plasma clots. B) Patient plasma clots. The magnification bar represents 10 μm .

Figure 3.

Confocal microscopy images of plasma clots formed on HMEC-1 monolayer. Fibrin is labeled with Alexa 488 and shown in green in these images, while the cells were labeled with di-8-anepss and are shown in red in these images. A) Control sample and B) Patient samples; the planes shown correspond to a height of 7.5 μm from the bottom of the dish. C) Control and D) Patient; the planes shown correspond to a height of 10 μm . E) Control and F) Patient; the planes shown correspond to a height of 30 μm . The magnification bar represents 30 μm .

Figure 4. Z-projections of plasma clots visualized by confocal microscopy in the presence and absence of HMEC-1. Z projections 0–10 μm from the cells' surface: A) Control sample and B) Patient. Z projections 20–30 μm from the cells' surface: C) Control sample and D) Patient. Z projections of clots obtained without cells: E) Control sample and F) Patient. The magnification bar represents 30 μm .

Table 1

Summary of the blood coagulation screening tests performed on plasma samples of the propositus and his father.

Coagulation test	Control	Case 1 (Father)	Case 2 (Son)
Prothrombin time (sec)	12.8 (12–15)	16	54
Thrombin time (sec)	18 (16–19)	23.8	28
aPTT (sec)	31 (27–37)	34	31
Fibrinogen (g/l)			
Clauss	1.5–3.5	1.90	2.69
Gravimetric	2.0–4.0	2.30	2.40

aPPT activated thromboplastin partial time

Table 2

Viscoelastic properties of plasma fibrin clots measured using the torsion pendulum. One hundred μl of undiluted plasma was clotted with 1 unit/ml of human thrombin and 20 mM CaCl_2 (final concentrations) and left for 1 h in static conditions before measurements. The dynamic storage modulus, G' (dyne/cm²), the loss modulus, G'' (dyne/cm²) and the loss tangent, the ratio between G''/G' were calculated for three clots (three records each) and averaged. The results are expressed as the mean $\pm$ SD.

Viscoelastic properties	Control	Case 1
Fg (g/l)	2.4	2.1
G' (dyne/cm ²)	831 $\pm$ 111	310 $\pm$ 30 *
G'' (dyne/cm ²)	77 $\pm$ 15	32 $\pm$ 5
$\tan \delta$ (G''/G')	0.092 $\pm$ 0.006	0.100 $\pm$ 0.007 *

* Statistically significant at $p < 0.05$ (Mann-Whitney U test)