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Fibrin clot structure in patients with congenital dysfibrinogenaemia

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ABSTRACT

The clinical phenotype of patients with congenital dysfibrinogenaemia is highly heterogeneous, from absence of symptoms to mild bleeding, or thrombosis. A few mutations are associated with a specific phenotype, but generally the clinical course is not predictable. We investigated whether fibrin clot properties are correlated with the patient's phenotype and/or genotype. Ex vivo plasma fibrin clot characteristics, including turbidity, fibrinolysis, clot permeability and fibrin fibre density assessed by laser scanner confocal microscopy were investigated in 24 genotyped patients with congenital dysfibrinogenaemia compared to normal pool plasma. Compared to normal pool plasma, the patients were characterised by slower fibrin polymerisation (lag time, 345.10 ± 22.98 vs. 166.00 s), thinner fibrin fibres (maximum absorbance, 0.15 ± 0.01 vs. 0.31), prolonged clot lysis time (23.72 ± 0.97 vs. 20.32 min) and larger clot pore size (21.5 × 10−9 ± 4.48 × 10−9 vs. 7.96 × 10−9 cm2). Laser scanning confocal microscopy images confirmed disorganised fibrin networks in all patients. Patients with tendency to bleed showed an increased permeability compared to asymptomatic patients (p=0.01) and to patients with a thrombotic history (p=0.02) while patients with thrombotic history had a tendency to have a prolonged clot lysis time. Fibrin clot properties were similar among hotspot mutations. Further studies including a larger number of patients are needed to evaluate whether analysis of permeability and clot lysis time may help to distinguish the clinical phenotype in these patients and to assess differences according to the genotype.

1. INTRODUCTION

Congenital fibrinogen disorders include quantitative (afibrinogenaemia and hypofibrinogenaemia) and qualitative (dysfibrinogenaemia and hypodysfibrinogenaemia) diseases [1]. Congenital dysfibrinogenaemia is characterised by a discrepancy between the functional and the antigen levels of circulating fibrinogen, reflecting the altered functional properties of the molecule due to structural defects [2].More than 100 causative mutations have been identified in congenital dysfibrinogenaemia, mainly heterozygous missense mutations in the amino-terminal portion of the Aα chain or in the carboxyl terminal region of the γ chain [3]. Almost all these molecular anomalies lead to ineffective polymerisation of the fibrin clot and may affect several other functions of the fibrinogen/fibrin [4].

The clinical phenotype of dysfibrinogenaemic patients is highly heterogeneous, from absence of symptoms to major bleeding or thrombotic events, including pulmonary hypertension and renal amyloidosis [3]. During the natural course of the disease, even asymptomatic patients at the time of the diagnosis are at risk of developing adverse outcomes [5]. However, neither standard haemostasis assays nor specific genotypes, with exception of some thrombotic-related mutations [6], are able to predict the clinical phenotype.

Recently, abnormal fibrin structure has been correlated with several thrombotic and cardiovascular diseases [7,8]. Fibrin clots with high fibre density and increased resistance to fibrinolysis have been consistently associated with an increased risk of thrombosis [9] whereas abnormal clots with lower fibrin network density and increased porosity have been associated with a bleeding phenotype [10]. Many studies on dysfibrinogenaemic families have described how fibrinogen variants can affect the fibrin clot in both purified fibrinogen and plasma conditions [11–14]. Sugo et al. provided a classification of the fibrin network structures formed from congenital dysfibrinogenaemia, suggesting that specific clot architecture could be correlated with a given phenotype [15]. In the present study, we aimed to determine the structural properties of fibrin clots generated from dysfibrinogenaemic plasma and to assess whether they are predictive of clinical outcomes. In addition, we assessed whether the genotype is associated with distinct clot properties.

2. MATERIALS AND METHODS

2.1. Patients

This study was performed with institutional review board approval and with written informed consent from all patients, in accordance with the Declaration of Helsinki. Known dysfibrinogenaemic patients were recruited in French (Lille, Besançon, Montpellier) and Swiss (Geneva, Bern, Sion) Hospitals. Patient plasma was obtained after collection of venous blood in 0.1mol/L sodium citrate (9 parts blood per 1 part citrate). Within 1 h after the collection, the samples were centrifuged at 3000 g for 12min at room temperature and stored at−80 °C until analysis. Normal pool plasma was obtained after collection of venous blood from the antecubital vein of 14 healthy volunteers aged between 20 and 35 in 0.1 mol/L sodium citrate (9 parts blood per 1 part citrate).Within 1 h after collection, the samples were centrifuged at 2400 g for 20min at room temperature to obtain platelet-poor plasma. Plasma samples were pooled and frozen in aliquots in liquid nitrogen, and stored at −40 °C until analysis.

Ethical approval was obtained from the local Leeds NHS Trust research ethics committee (Ref: 03/142). Plasma levels of functional fibrinogen were measured by the Clauss method (Multifibren* U, Siemens, Germany). The PT-derived fibrinogen assay is not recommended in the case of dysfibrinogenaemia as it can overestimate the plasmatic level of fibrinogen [16]. Levels of total fibrinogen antigen were measured by a latex immunoassay (Liaphen Fibrinogen, Hyphen BioMed, France) on a BCS® XP coagulometer (Siemens, Germany). The patient genotype was determined as previously reported [5]. Causative fibrinogen mutations are described with amino acid residues and substitutions numbered from the initiator methionine. Patients with a bleeding phenotype, defined by an ISTH/SCC bleeding assessment tool [17] greater than 2, were included in the bleeders group. The thrombotic group comprised patients with objectively documented venous or arterial thrombotic events.

2.2. Turbidity and lysis

For the fibrin polymerisation assessment [18], plasma samples were diluted 1/6 with TBS (50mMTris, 100mMNaCl, pH 7.4) and incubated with 0.1 U/ml human thrombin (Calbiochem; Nottingham, UK) and 5mMCaCl2 (final concentrations) in a final volume of 150 µl in polystyrene 96-well plates (Greiner Bio-one International, Stonehouse, UK). Absorbance was monitored at 340 nm, every 12 s for 60 min, using a BioTek PowerWave HT microplate reader (BioTek; Swindon, UK). For the fibrinolysis measurement, plasma samples were diluted 1/6 with TBS and incubated with 85 ng/ml tissue plasminogen activator (tPA; TechnoClone GmbH; Vienna, Austria), 0.5 U/ml human thrombin (Calbiochem; Nottingham, UK) and 22.5 mM CaCl2 (final concentrations) in a final volume of 150 µl in polystyrene 96-well plates. Absorbance was monitored at 340 nm, every 12 s for 180 min, using the BioTek PowerWave HT microplate reader (BioTek; Swindon, UK). All measurements were made in triplicate and analysed with the Gen5™ software (BioTek; Swindon, UK). Lag time and maximum absorbance were calculated from the turbidity curves as previously described [19]. The clot lysis time was defined as the time from the midpoint in the transition from the initial baseline to maximum turbidity to the midpoint in the transition from maximum turbidity to the final baseline turbidity.

2.3. Permeation

The permeability assay was performed with slight modifications as previously described [20]. Briefly, plasma samples (100 µl) were incubated with 1 U/ml human thrombin (Calbiochem; Nottingham, UK) and 16 mM CaCl2 (final concentrations), in a final volume of 110 µl, into a clotting tip and placed for 2 h in a humidity chamber at room temperature. The clotting tips were connected via plastic tubing to a reservoir containing TBS with a constant pressure drop of 4 cm. After washing the clots for 2 h, flow rates of buffer through the fibrin gels were measured every 30 min for 2 h for each tube. The permeation coefficient (Ks, Darcy constant) was calculated as described [20]. All samples were analysed in triplicate.

2.4. Laser scanner confocal microscopy

Laser scanning confocal microscopy was performed as previously described [21,22]. In brief, plasma samples were diluted 1/6 with TBS and incubated with 0.6 U/ml of human thrombin (Calbiochem; Nottingham, UK), 50 µg/ml AlexaFluor 488 fibrinogen (Invitrogen; Paisley, UK) and 5mMCaCl2 (final concentration), in a final volume of 60 µl. The reaction mixture was transferred into the channel of an uncoated Ibidi slide (Ibidi GmbH, München, Germany) and left in a humidity chamber for 4 h at room

temperature. Imaging was performed using an upright Zeiss LSM700 microscope (Oberkochen, Germany) with a 63× oil immersion objective lens. The fibrin density was determined by counting the number of fibres crossing an arbitrary line of 100 μm drawn through a single optical section using an in-house macro for Image J (Fiji, National Institute of Health, Bethesda, Maryland, USA). Each fibrin clot was prepared in duplicate and 20 density measurements were performed on each sample.

2.5. Statistical analysis

Data are expressed as means (with standard deviation, SD) or medians (with Interquartile ranges, IQR) as appropriate. Means of normal pool plasma (normal distribution) were compared to patients by chi-square test. Means of groups were compared by an unpaired Student t-test with Welch's correction. Pearson correlation (r) was used to test for correlation between functional and antigen fibrinogen levels and
fibrin clot properties. Two-sided p-values b 0.05were considered statistically significant. Statistical analyses were performed using STATA® version 11.2 (StataCorp, College Station, TX, USA) and graphs were prepared using GraphPad Prism® version 6.0 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. Demographic data

A total of 24 patients (12 probands and 12 relatives) with congenital dysfibrinogenaemia were included in this study. Demographic, clinical and biological data of proband and relatives are summarised in Table 1. A total of 5 (21%) patients reported a bleeding phenotype, including cutaneous bleeding, menorrhagia or post-partum haemorrhage (one requiring treatment with a fibrinogen concentrate). The median ISTH bleeding assessment tool was 4 (range 2–5). A total of 8 (33%) patients experienced 12 thrombotic events, 11 venous (6 provoked) and 1 arterial. Three patients suffered from recurrent venous thrombosis. Patients with history of thrombosis neither carried a Leiden mutation of factor V nor a G20210A mutation of factor II. All patients presented a discrepancy between normal antigenic and decreased functional fibrinogen levels, with a mean ratio activity/antigenic of 0.23 (SD 0.1) compatible with the dysfibrinogenaemia diagnosis. As expected, most patients carried a hotspot mutation (n = 20, 83.3%) either at residue Arg301 of exon 8 in FGG (n = 13), or at Arg35 of exon 2 of FGA (n = 7). All other patients harboured a heterozygous missense mutation in FGA (Arg38Gly, n=2; Cys184Arg, n=1; Arg573Cys, n=1). The latter mutation is known to be the fibrinogen Paris V (also named Chapel Hill III), which is strongly associated with a thrombotic phenotype. Clinical phenotype according to genotype is resumed in Table 1. No patient was receiving antithrombotic treatment at the time of inclusion.

3.2. Turbidity

All patients demonstrated an abnormal polymerisation profile, as indicated by a significantly prolonged lag time (345.10± 22.98 vs. 166 s; p b 0.01) and a decreased maximal absorbance compared to normal pool plasma (0.15 ± 0.01 vs. 0.31; p = b0.01) (Fig. 1A–B). The maximum absorbance was increased in the bleeding group compared to the asymptomatic group (0.20 ± 0.01 vs. 0.13 ± 0.01; p = 0.01) (Fig. 1B).

Hotspot mutations were associated with a prolonged lag time (368.30 ± 23.28 vs. 229.50 ± 42.31 s; p = 0.03) compared to other mutations (Fig. 1D). In addition, both the FGG Arg301Cys and FGG Arg301His mutations showed a prolonged lag time compared to the FGA Arg35Hismutation (476±41.76 and 367±38.18, respectively vs. 323.32 ± 19.08) (Fig. 1D). The maximum absorbance was similar among all the mutations (Fig. 1E).

Clot lysis time was prolonged in all patients compared to normal pool plasma (23.72 ± 0.97 vs. 20.32 min; p = b0.01) (Fig. 1C). The thrombotic group presented a tendency to longer lysis time compared to bleeders (25.53 ± 1.99 vs. 21.46 ± 0.38; p = 0.08) (Fig. 1C). The hotspot mutations demonstrated similar clot lysis time compared to other mutations (28.30 ± 5.39 vs. 22.86 ± 0.55 min; p = 0.41) (Fig. 1F). One patient with a thrombotic phenotype had a very long clot lysis time (39 min). This patient carried the Arg573Cys (Paris V fibrinogen) known to result in a hypofibrinolysis. Clot lysis time was similar among the three hotspot mutations: 22.62 ± 0.94, 24.60 ± 1.31, and 22.33 ± 0.77 for FGA Arg35His, FGG Arg301Cys and FGG Arg301His, respectively (Fig. 1F).

Overall turbidity results were similar between proband and their asymptomatic relatives (Supplementary Table 1). The maximum absorbance was significantly correlated with functional but not with antigen fibrinogen levels (r = 0.40; p = 0.04 and r = −0.10; p = 0.68, respectively). The lag time and the clot lysis time were not correlated (Fig. 2 A-C).

3.3. Clot permeability

Patients had an increased clot pore size compared to normal pool plasma (21.5 × 10−9 ± 4.48 × 10−9 vs. 7.96 × 10−9 cm2; p = b0.01) (Fig. 3A). The permeability was particularly increased in the bleeders group (47.1 × 10−9 ± 15.6 × 10−9 cm2) compared to the thrombotic (11.8 × 10−9 ± 4.07 × 10−9 cm2; p = 0.02) and asymptomatic (16.9 × 10−9 ± 3.04 × 10−9 cm2; p = 0.01) patients, as well as in probands compared to their asymptomatic or thrombotic relatives (Supplementary Table 1). In addition, there was no difference among normal pool plasma and thrombotic/asymptomatic patients. The Ks coefficient was increased in hotspot mutations (Fig. 3C) compared to other mutations (24.5 × 10−9 ± 5.13 × 10−9 vs. 6.57 × 10−9 ± 2.01 × 10−9 cm2; p=b0.01), but similar among the three hotspot mutations.

Neither the functional nor the antigen fibrinogen levels were correlated with permeability (r = −0.27; p = 0.18 and r = 0.30; p = 0.15, respectively) (Fig. 2D).

3.4. Fibrin fibre density

The fibrin fibre density was similar in patients and normal pool (10.57 ± 0.70 vs. 11.57 fibre/100 μm; p = 0.40), between each phenotype group (Fig. 3B) and proband and their asymptomatic relatives (Supplementary Table 1). However, visual inspection of the confocal micrographs of most dysfibrinogenaemic clots revealed a markedly disorganised fibrin network compared to normal pool plasma (Fig. 4 B-F), with less straight fibres and some denser clumps of fibres. The patient with Paris V fibrinogen showed a highly branched, tight and dense fibrin network compared to normal pool plasma (37.33 vs. 11.57 fibre/100 μm, p = b0.01) (Fig. 4C). The fibrin fibre density was decreased in hotspot mutations compared to other mutations (9.64 ± 0.53 vs. 19.21±6.14 fibre/100 μm; p=b0.01) but similar among the three mutations (Fig. 3B). The functional fibrinogen but not antigen levels were significantly correlated with fibrin fibre density (r = 0.47; p = 0.02and r = 0.1688; p=0.43, respectively) (Fig. 2E).

4. DISCUSSION

The management of patients with congenital dysfibrinogenaemia is particularly challenging. Except for a few fibrinogen mutants such as Naples, Ijmuiden, Nijmegen, New York I, Caracas V, Paris V, and Melun clearly associated with a thrombotic phenotype [23], the only valuable predictive information helping in the clinical care of dysfibrinogenaemic patients are the patient's personal and familial history [24]. In this study we investigated correlations between fibrin clot structure and the clinical phenotype in a series of well characterised patients with congenital dysfibrinogenaemia.

Almost all mutations leading to congenital dysfibrinogenaemia are associated with an abnormal polymerisation profile resulting mainly from defective fibrinopeptide cleavage and/or impaired thrombin binding, such as for the FGA Arg35 mutation, or altered end-to-end alignment of fibrin such as for the FGG Arg301 mutation [15]. The prolonged lag time and the decreased maximum absorbance observed in all our dysfibrinogenaemic patients are consistent with results from number of isolated family studies and series [12,25–27]. Similar to other reports showing decreased fibrin polymerisation [28,29], we did not find a statistically significant difference between the different groups of patients. Therefore, even though the relatively small number of patients do not allow formal conclusions, the fibrin polymerisation assessment does not seem to properly predict the clinical outcome of dysfibrinogenaemic patient but its utilisation could be rather useful to describe the functional abnormalities in fibrin formation, i.e. by comparing the thrombin and reptilase-induced polymerisation profiles to identify the mechanisms of the defective conversion of fibrinogen to fibrin [30].

The clot lysis time has been widely used to assess the plasma fibrinolytic potential in a broad range of diseases [31–35]. In the setting of congenital dysfibrinogenaemia, fibrinogen variants with a well-identified thrombotic phenotype have been associated with hypofibrinolysis [36]. Several mechanisms, often overlapping, account for the hypofibrinolysis in these mutations, including impaired binding of tPA and plasmin to the abnormal fibrinogen as well as abnormal fibrin clot structure with an increased stiffness [3]. In this study, the FGA Arg573Cysmutation showed an abnormal fibrin network with thin fibres and a very dense clot, resulting in greater resistance to fibrinolysis. Several studies have shown reduced plasminogen binding for this fibrinogen variant, impaired tPA-induced fibrinolysis [11,37] and increased γXL self-association and γ-chain cross-linking [38]. However, for most other cases of thrombosis-related dysfibrinogenaemia, the molecular anomaly is probably not directly responsible for the vascular event but rather a contributing factor to the overall haemostatic balance. Thus, testing the clot lysis time may improve the identification of dysfibrinogenaemic patients with an increased thrombotic risk. In our study, even though patients with a thrombotic phenotype showed a tendency to a prolonged clot lysis time compared to bleeders and asymptomatic patients, we lack the statistical power to distinguish between these phenotypes.

The fibrin clot permeability measurement is a standardised technique to determine the fibrin structure. The pore size between fibrin fibres is evaluated by the permeation constant, which may be decreased in subjects with thrombotic disease and increased in patients with bleeding phenotype [20]. The clot permeability has generally been correlated with the fibrin fibre density and the fibrinogen level [39]. A dysfunctional fibrinogen may present with a wider range of fibre width resulting from the heterozygosity of the molecule (depending on the proportion of hetero-homodimers and normal molecules in circulation) [15]. A few single-case dysfibrinogenaemic

reports have suggested increased clot permeability in dysfibrinogenaemic families with a bleeding phenotype [40,41]. In our study, the patients with a bleeding phenotype showed significantly increased clot permeability compared to patients with a thrombotic phenotype or those without symptoms. It is likely that a more permeable fibrin structure is more susceptible to tPA-induced fibrinolysis. Indeed, permeable clots have larger pores and thicker fibres, which promote the interaction between fibrin and plasmin leading to an increased fibrinolysis while fibrin networks composed of thin, highly branched fibres usually are less permeable, stiffer and less prone to lysis [42]. This assay is relatively time-consuming and requires expert laboratory handling; however, it has recently been standardised [20] and may provide useful and clinically relevant information to better assess and predict the patient phenotype. How to integrate this in the management of dysfibrinogenaemic patients may be the subject of further studies.

Although this study indicates that fibrin clot structure and function analysis may indeed be useful for the assessment of the bleeding or thrombotic phenotype in dysfibrinogenaemic patients, there are several limitations. First, a relatively small number of patients were included, with few different causative mutations. Larges series of specific mutations are needed to highlight how the different molecular anomalies may affect the clinical course of patients. Second, many genetic and environmental factors known to modify the fibrin structure such as genetic polymorphisms and comorbidities were not investigated. However, it is likely that in the setting of congenital dysfibrinogenaemia the dysfunctional fibrinogen is the major determinant of the fibrin clot properties. Note that in this study cardiovascular risk factors were not correlated with fibrin clot structure measurements (data not shown). Third, controls were not matched for gender and age, which are known to potentially have a moderate effect on the fibrin properties, with the patients. Fourth, the retrospective design: asymptomatic patients at the time of the inclusion may in fact experience adverse outcomes in the future but due to the low prevalence of the congenital dysfibrinogenaemia and the low incidence of its complications, a prospective study would be difficult to perform.

In conclusion, this is the largest study correlating the clinical outcomes of a well-defined population of dysfibrinogenaemic patients to the fibrin clot properties. Further studies including a larger number of patients are needed to further evaluate whether analysis of permeability and clot lysis time may help to distinguish the clinical phenotype in these patients.

AUTHOR CONTRIBUTIONS

A. Casini designed and performed the research, analysed the data and wrote the paper. C. Duval designed the research and wrote the paper. X. Pan helped with experiments. C. Biron-Andreani and V.Tintillier provided clinical data. R. Ariëns designed the research and provided overall supervision of the project.

CONFLICT OF INTERESTS

None declared.

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Table 1

Patients' clinical and biological characteristics.

Patient	Birth year	Fibrinogen levels*		Genotype	Bleeding, ISTH BAT	Thrombosis	Cardiovascular risk factors
		Functional, g/L	Antigenic, g/L				
P1	1931	0.8	2.6	FGA Arg35His	None, 0	None	Hypertension, smoking
P1.1	1958	0.7	3.1	FGA Arg35His	Cutaneous, menorrhagia, 3	None	None
P1.2	1962	0.8	3.1	FGA Arg35His	Cutaneous, menorrhagia, 3	None	Obesity
P1.3	1989	0.6	2.7	FGA Arg35His	None, 0	None	Smoking
P2	1962	0.6	2.5	FGA Arg35His	None, 0	Splanchnic	Obesity, diabetes, hypertension
P2.1	1986	0.7	3.1	FGA Arg35His	None, 0	None	None
P2.2	1989	0.5	1.8	FGA Arg35His	None, 0	None	None
P3	1929	0.7	2.7	FGG Arg301Cys	None, 0	None	None
P4	1973	0.6	2.4	FGA Arg38Gly	None, 0	None	Smoking
P5	1950	0.9	3.7	FGA Arg38Gly	None, 0	Retinal	Smoking, diabetes, hypertension
P6	1982	0.7	2.1	FGG Arg301His	Menorrhagia, 4	None	Smoking
P6.1	1986	0.9	3.8	FGG Arg301His	None, 0	None	None
P6.2	1956	0.8	3.1	FGG Arg301His	None, 0	Deep venous thrombosis	Obesity, hypertension
P7	1994	1.3	2.5	FGA Arg573Cys	None, 0	Cerebral	None
P8	1958	0.6	3	FGG Arg301Cys	Post-partum, menorrhagia, 5	None	Hypertension
P8.1	1988	0.4	3	FGG Arg301Cys	None, 0	Deep venous thrombosis	None
P9	1960	0.3	3.7	FGG Arg301His	None, 0	None	None
P9.1	1991	0.3	2.9	FGG Arg301His	None, 0	None	None
P10	1956	0.7	1.5	FGA Cys184Arg	None, 0	Stroke	Hypertension, diabetes
P11	1972	0.4	1.5	FGG Arg301His	None, 0	Pulmonary embolism	Smoking
P11.1	2000	0.3	1.5	FGG Arg301His	None, 0	None	None
P12	1956	0.6	3.2	FGG Arg301His	Cutaneous, post-partum, 2	None	None
P12.1	1996	0.7	3.1	FGG Arg301His	None, 0	None	Smoking
P12.2	1983	0.7	3.1	FGG Arg301His	None, 0	Deep venous thrombosis	Smoking

* Functional: Clauss method; antigenic: latex immunoassay; ISTH BAT: International Society on Thrombosis and Haemostasis Bleeding Assessment Toll.

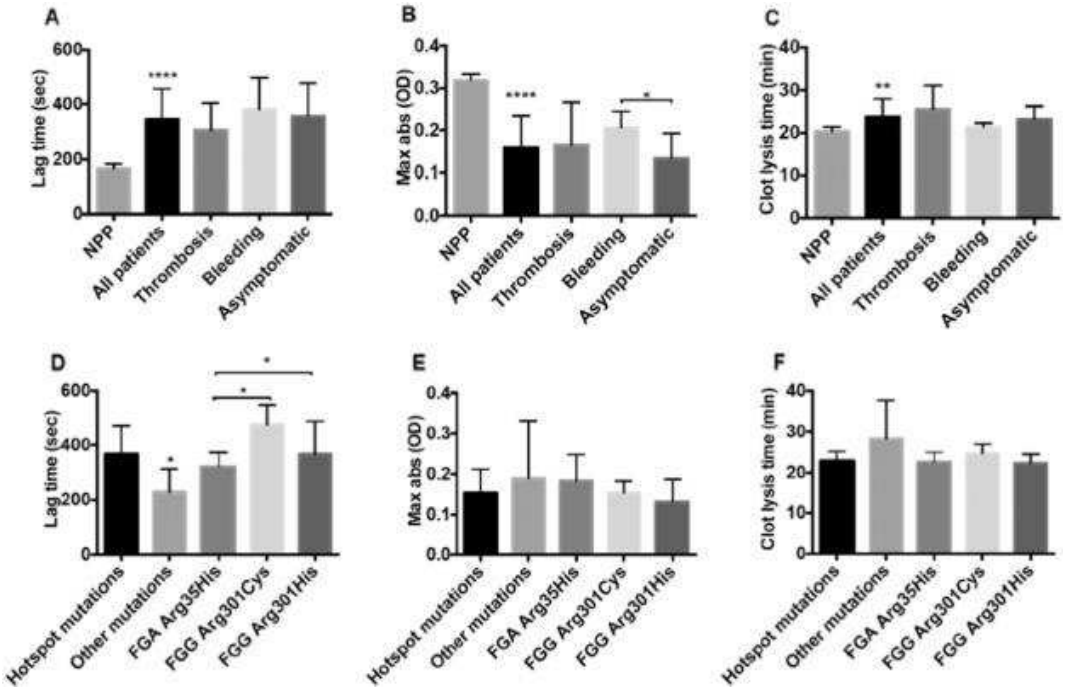

Fig. 1. Turbidity assays among normal pool plasma (NPP) and patients according to the phenotype (A–C) and the causative mutations (D-F). Hotspot mutations: FGA Arg35His, FGG Arg301His, FGG ARg301Cys; other mutations: FGA Arg38Gly, FGA Cys184Arg, and FGA Arg573Cys. Error bars represent median with interquartile range. *p b 0.05; **p b 0.01; and ****p b 0.0001.

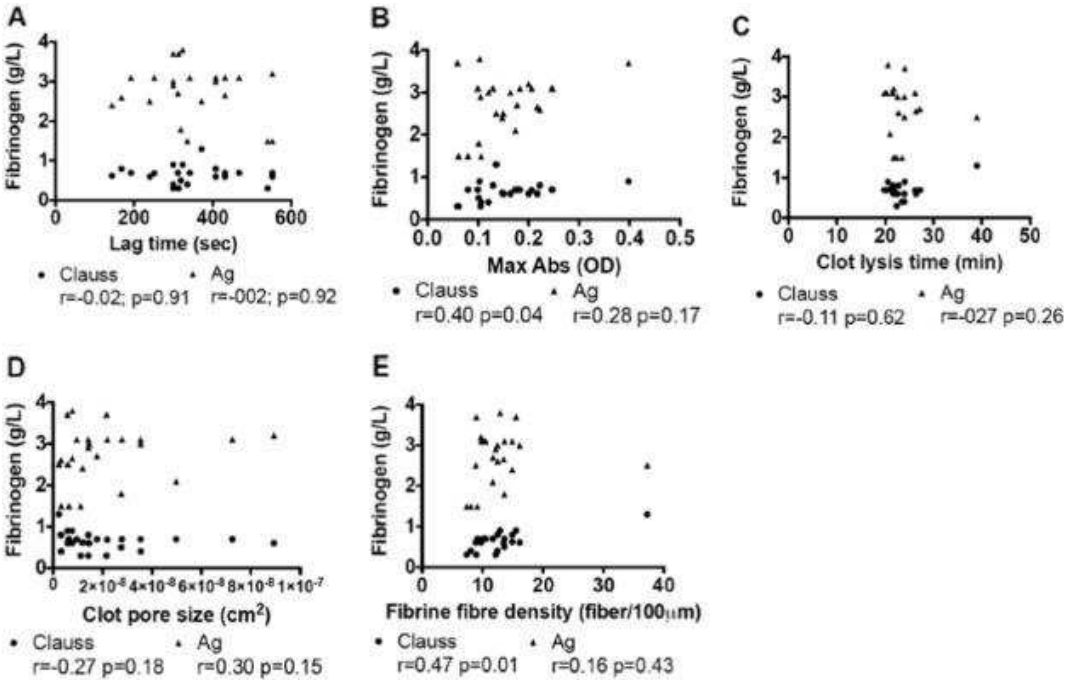

Fig. 2. Spearman's correlation of functional (Clauss) and antigen fibrinogen levels with parameters from the turbidity assay (A–C), permeation assay (D) and fibrin fibre density (E).

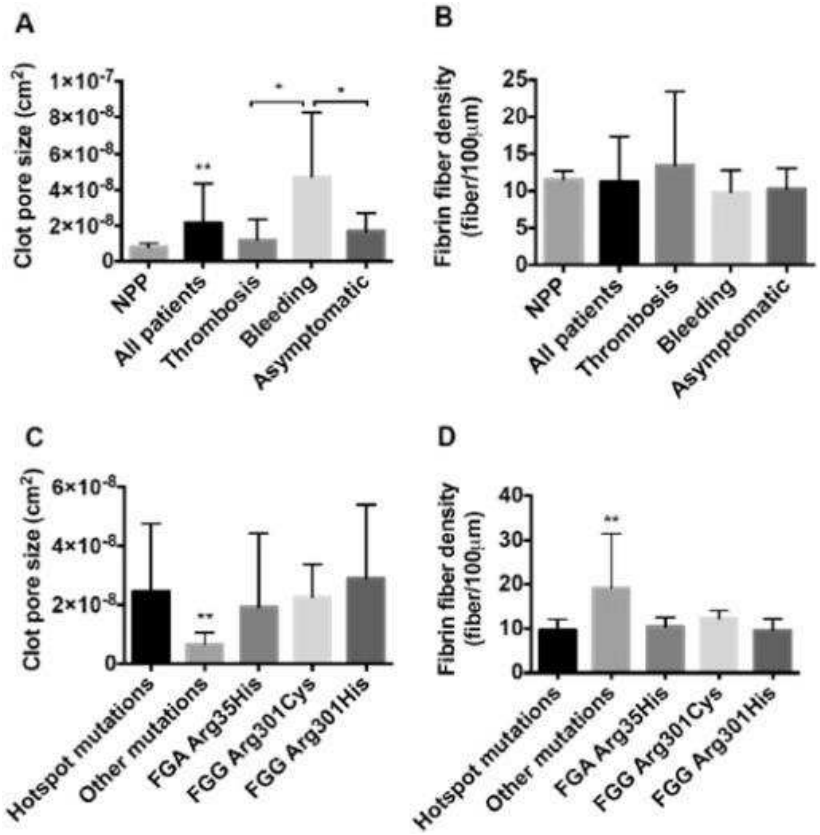

Fig. 3. Permeation assay (A, C) and fibrin fibre density (B, D) among normal pool plasma (NPP) and patients according to the phenotype and the causative mutations. Hotspot mutations: FGA Arg35His, FGG Arg301His, and FGG ARg301Cys; other mutations: FGA Arg38Gly, FGA Cys184Arg, and FGA Arg573Cys. Error bars represent median with interquartile range. *p b 0.05 and **p b 0.01.

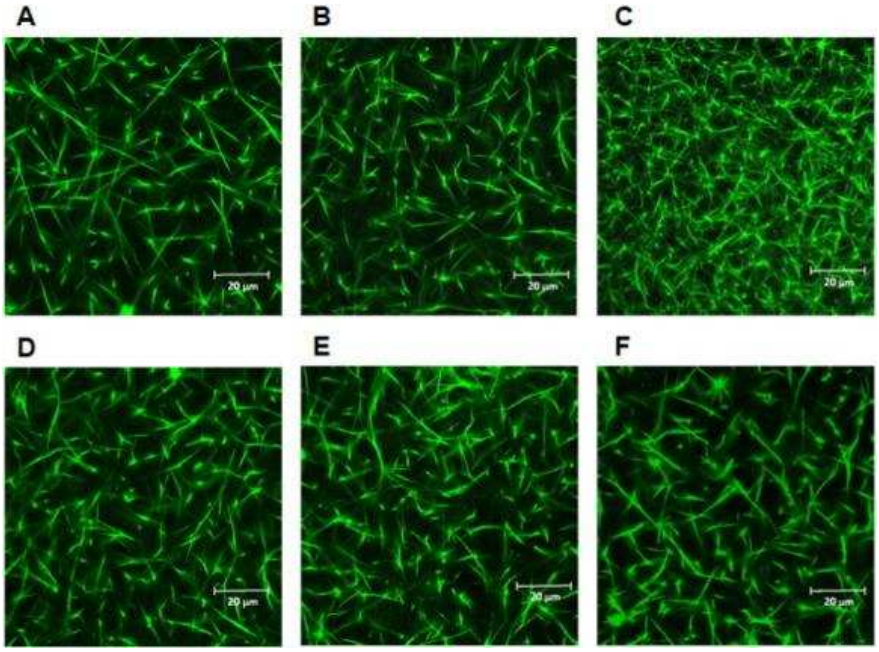

Fig. 4. Laser scanning confocal microscopy images of fibrin clots from normal pool plasma (A), asymptomatic patient with FGG Arg301His (B), thrombotic patient with FGA Arg573Cys (C), bleeder patient with FGG Arg301His (D), asymptomatic patient with FGA Arg38Gly (E) and thrombotic patient with FGA Cys184Arg (F). The scale bars indicate 20 μm.

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