Blood Cells, Molecules and Diseases 55 (2015) 308–315

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

Clinical features and molecular basis of 102 Chinese patients with congenital dysfibrinogenemia

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ARTICLE INFO

Article history:
Submitted 4 March 2015
Revised 8 May 2015
Accepted 10 June 2015
Available online 14 June 2015

Editor: Mohandas Narla

Keywords:
Congenital
Dysfibrinogenemia
Hemorrhage
Mutation
Thromboelastography

ABSTRACT

Introduction: Congenital dysfibrinogenemia (CD) is a rare qualitative disorder of fibrinogen (Fg) with heterogeneous clinical manifestations. We aimed to analyze clinical phenotype and molecular basis of 102 Chinese CD patients and to evaluate the application of thromboelastography (TEG).
Materials and methods: Clinical manifestations were recorded and quantified using the consensus ISTH bleeding assessment tool. Kaolin activated TEG and functional Fg TEG were applied in 30 patients. Genetic analysis of Fg genes were performed by direct sequencing.
Results: 27.5% patients experienced bleeding, 3.9% had thrombosis and 68.6% were asymptomatic. Females were more prone to experience bleeding ($P = 0.01$). Significant difference ($P < 0.05$) in TEG results were found between patients with hot-spot mutations at AαArg35(16) and γArg301(275), but were not identified between patients with and without bleeding. Normal TEG results were found in patients with mutations at AαArg35(16), AαPro37(18) or AαArg38(19). Six novel mutations were identified, including AαGly33(14)del, AαAsp57(38)_Trp60(41)delIVS2 + 1_ + 2GTdel, AαPhe742(723)Tyr, γAsn334(308)Thr, γGly335(309)Cys and γTrp395(369)Leu.
Conclusions: CD patients have similar clinical manifestations and hot-spot mutations worldwide with no ethnic difference. TEG results could not indicate the bleeding risk in patients, but priority of mutation screening at thrombin cleavage site or polymerization site on Aa chain may be given if TEG results are normal.

1. Introduction

Fibrinogen (Fg) is a 340 kDa glycoprotein playing key roles in fibrin clot formation, non-substrate thrombin binding, platelet aggregation and fibrinolysis [1]. It is composed of three pairs of non-identical chains $(A\alpha, B\beta, \gamma)_2$, which are encoded by *FGA*, *FGB* and *FGG* genes, respectively, clustered in a region of 50 kb on 4q31. Upon activation of the coagulation cascade, fibrin is produced by proteolytic cleavage of the fibrinogen Aα and Bβ chains by thrombin, thus releasing fibrinopeptides A and B and allowing polymerization to occur [1]. Activated factor XIII (FXIIIa) mediated cross-linking stabilizes this network into a covalently-linked fibrin clot [2].

Congenital fibrinogen disorders are rare and affect either quantity (afibrinogenemia and hypofibrinogenemia) or function (dysfibrinogenemia) of circulating fibrinogen, or both (hypodysfibrinogenemia). To date, more than 400 cases of congenital dysfibrinogenemia (CD) have been reported [3]. The clinical manifestations of dysfibrinogenemia are heterogeneous. Nearly 55% of them were asymptomatic, 30% of them experienced bleeding and 15% of them had thrombosis [4,5]. The majority of dysfibrinogenemia defects, inherited as a dominant trait, are caused by heterozygous missense mutations in one of the three fibrinogen genes, which may impair conversion to fibrin monomer or affect critical interactions between fibrin and FXIIIa, fibrinolysis mediators or cell-surface integrins [6]. Only a few of dysfibrinogenemias are caused by homozygous mutations and most of patients in this kind are symptomatic [3].

Abbreviations: ACA, anticardiolipin antibody; anti-β2GP1, anti-β2 glycoprotein 1; APTT, activated partial thromboplastin time; BAT, bleeding assessment tool; CD, congenital dysfibrinogenemia; Fg, fibrinogen; Fg:Ag, antigen level of fibrinogen; Fg:C, activity level of fibrinogen measured by Clauss assay; Hcy, total homocysteine; TEG, thromboelastography; FF-TEG, functional fibrinogen TEG; LA, lupus anticoagulant; MA, maximum amplitude; MA-CFF, maximum amplitude measured by FF-TEG; PT, prothrombin time; TT, thrombin time

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http://dx.doi.org/10.1016/j.bcmd.2015.06.002
1079-9796/

Global assays, such as thromboelastography (TEG), have been shown to have potential providing a better evaluation of haemostatic state in individuals. Common TEG applications include assessment and decision making for fibrinolytic and anticoagulant therapies and for transfusion for acute blood loss [7]. Functional fibrinogen TEG (FF-TEG), which eliminates the contribution of platelets to clot strength, is special to evaluate all aspects of Fg function reflected by maximum amplitude (MA-CFF). Moderate correlations between fibrinogen level measured by Clauss assay (Fg:C) and MA measured by kaolin activated TEG or MA-CFF measured by FF-TEG have been identified both in healthy volunteers and in patients operated for ischemic heart disease without known coagulopathy or hepatopathy [8–10]. Mutant fibrinogen purified from the plasma of dysfibrinogenemia patients were shown to affect the signal generation of TEG [11]. However, the evaluation of the whole blood sample from dysfibrinogenemia patient using TEG has not been reported.

In this study, we analyzed clinical phenotype and molecular basis of 102 Chinese patients with CD. In addition, the potential application value of TEG in CD patients was also evaluated.

2. Materials and methods

2.1. Study population

A total of 102 patients enrolled between 2005 and 2014 in Shanghai Ruijin Hospital were studied on informed consent. CD was diagnosed by low Fg:C with normal antigen level of Fg (Fg:Ag), combined with molecular defect identified in one of the three fibrinogen genes. Clinical manifestations were recorded in a standard questionnaire and were quantified using the consensus ISTH BAT. These were compared to bleeding scores determined from an age- and sex-matched group of health volunteers (n = 50; 25 female, median age 35, Fig. 1) using the Mann–Whitney *U* test. We documented all objectively confirmed venous or arterial thromboses.

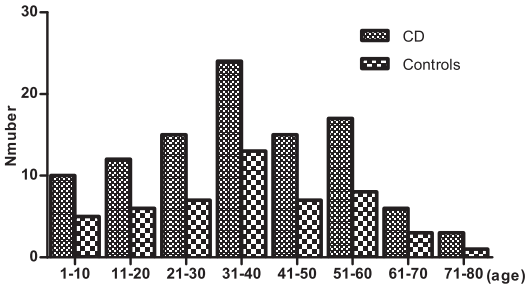
2.2. Hemostatic assays

Venous blood samples were taken using a 21-gauge needle with minimal suction and 2.7 ml of venous blood was collected in a BD Vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, USA) containing 0.3 ml of 0.109 mol/L (M) sodium citrate. Routine coagulation screening assays including activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), Fg:C measured by Clauss assay and Fg:Ag measured by immunoturbidimetry were performed as previously reported [12]. Reptilase time was detected with the method of magnetic sand on a STAGO semi-automatic blood coagulation analyzer (Diagnostica Stago, Asniéres sur Seine, France). Thrombophilia screening tests, including the activities of protein C (PC:A) and antithrombin (AT:A), antigen of the free PS (FPS:Ag), lupus anticoagulant (LA), anticardiolipin antibody (ACA), anti-β2 glycoprotein 1 (anti-β2GP1) and total homocysteine (Hcy), were assayed as described previously in all subjects with thrombosis [13].

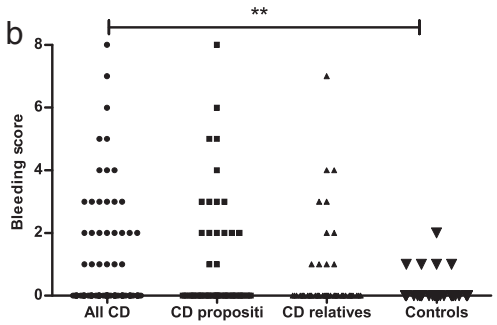
2.3. Thromboelastography assay

A total of 30 CD patients (16 females) who were available for this assay, including 22 propositi and 8 affected relatives, and 20 age- and sex-matched healthy volunteers (10 females) as normal controls were involved in this study. The kaolin activated and functional fibrinogen thromboelastography (TEG) assays were provided by Haemonetics (Heamoscope Corporation, Niles, USA) and performed on a TEG 5000 device according to the manufacturer's instructions. Briefly, for the kaolin activated TEG assay, 1 mL of citrated blood was added to the designated kaolin vial and mixed gently. To perform the FF-TEG assay, 0.5 mL of citrated blood was added to the designated FF vial including a mixture of tissue factor and a monoclonal glycoprotein IIb/IIIa receptor antagonist and mixed gently. For both assays, a 340 μl aliquot was transferred from either the kaolin vial or the FF vial to a 37 °C TEG cup preloaded with 20 μl 0.2 mol/L $CaCl_2$.

a



b



c

Incidental clotting screen	**44/54 (81.5%) propositi**
Investigation of bleeding or thrombosis	**10/54 (18.5%) propositi**
Asymptomatic	**70/102 (68.6%)**
Any bleeding symptom (bleeding score ⩾1)	**28/102 (27.5%)**
epistaxis	5/102 (4.9%)
easy bruising	8/102 (7.8%)
bleeding from minor wound	5/102 (4.9%)
oral cavity bleeding	4/102 (3.9%)
tooth extraction bleeding	4/102 (3.9%)
bleeding after surgery or major trauma	6/102 (5.9%)
gastrointestinal bleeding (GI)	1/102(1.0%)
menorrhagia	3/49 (6.1% of women)
obstetric and gynecology hemorrhage	6/49 (12.2% of women)
miscarriage	3/49 (6.1% of women)
Thrombosis at any site	**4/102 (3.9%)**
lower extremity venous thrombosis	2/102 (2.0%)
pulmonary embolism	1/102 (1.0%)
portal vein thrombosis	1/102 (1.0%)
dorsal vein of foot thrombosis	1/102 (1.0%)

Fig. 1. Clinical characteristics of 102 study subjects with CD. a) Age distributions in both CD patients and normal controls. b) Bleeding scores in subjects with CD were determined using the ISTH bleeding assessment tool and were compared to scores from an age- and sex-matched control group (n = 50). Data are presented as bleeding scores from the entire CD group, from CD propositi, from relatives with CD and from normal controls. c) Historical symptoms of bleeding were determined by patients' interview and inspection of clinical records. Bleeding symptoms were recorded as abnormal if the bleeding scores ≥1. All objectively confirmed venous thromboses were recorded. CD, congenital dysfibrinogenemia.