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## Hypodysfibrinogenemia causing mild bleeding and thrombotic complications in a compound heterozygote of A $\alpha$ IVS4+1G>T mutation and A $\alpha$ 4841delC truncation (A $\alpha$ <sup>Perth</sup>)

Asier Jayo<sup>1</sup>, Erin Arnold<sup>2</sup>, Consuelo González-Manchón<sup>1</sup>, David Green<sup>3</sup>, and Susan T. Lord<sup>2</sup>

<sup>1</sup> Department of Cellular and Molecular Physiopathology. Centro de Investigaciones Biológicas (CIB-CSIC), Madrid, Spain

<sup>2</sup> Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina, USA

<sup>3</sup> Division of Hematology & Oncology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

Fibrinogen, a 340 KDa plasma glycoprotein, is essential for fibrin clot formation and platelet aggregation. Fibrinogen is composed of three pairs of polypeptide chains called A $\alpha$ , B $\beta$  and  $\gamma$ . The N-termini of all six chains are associated in the central E domain, while the peripheral D domains are composed predominantly of the C-terminal regions of B $\beta$  and  $\gamma$ . The C-termini of the A $\alpha$  chains, known as  $\alpha$ C-domains, fold back from D and interact with each other and with the central E domain (1). Familial dysfibrinogenemia, caused by qualitative changes in circulating fibrinogen, shows a high phenotypic heterogeneity; nearly half the cases are clinically silent, a quarter show a bleeding tendency, and the remaining experience thrombosis with or without haemorrhage (2). A prolonged thrombin time that is corrected by the addition of normal plasma and discrepancy between functional and antigenic levels of circulating fibrinogen are usually diagnostic.

We report a new case of hypodysfibrinogenemia in a Caucasian woman with a life-long mild bleeding disorder characterised by menorrhagia and postpartum bleeding. At age 60 she developed cholecystitis. Laboratory studies showed prolonged thromboplastin, prothrombin, thrombin and reptilase times that were corrected in mixing studies. Assays of factors II, V, VII, X, and XIII were normal. Clottable fibrinogen was 59 mg/dl and fibrinogen antigen 184 mg/dl (reference values: 200–400 mg/dl). The cholecystitis resolved with medical therapy, and she was diagnosed with congenital dysfibrinogenemia. Three years later she suffered a crushing injury to her left fingers; after 1–2 weeks, the initial bruising became cyanotic and the third finger gangrenous. She was treated with cryoprecipitate infusions, raising clottable fibrinogen levels from <60 to 218 mg/dl. A magnetic resonance examination the next day revealed occlusion of the left carotid from its origin at the aorta, and high-grade stenosis of the right brachiocephalic artery. She did not receive additional cryoprecipitate and the left third finger was amputated without complications. A computerised tomographic angiogram performed one month later confirmed occlusion of the left subclavian artery with vertebral artery “steal syndrome”. A large plaque at the origin of the right subclavian artery and aortic atheromas were also noted.

Correspondence to: Susan T. Lord, Department of Pathology and Laboratory Medicine, University of North Carolina. Chapel Hill, NC, USA, Tel.: +1 919 966 3548, Fax: +1 919 966 6718, [stl@med.unc.edu](mailto:stl@med.unc.edu).

Fibrin polymerisation studies with the patient's plasma showed a near absent response in turbidity (Fig. 1A). The patient's daughter and granddaughter, clinically asymptomatic, had clottable fibrinogen levels of 131 and 177 mg/dl (3), respectively. Their plasmas showed a severe polymerisation defect with a prolonged lag phase and markedly diminished final turbidity (Fig. 1A). Immunoblot analysis with a polyclonal antibody (Dako, Carpinteria, CA, USA) showed no normal A $\alpha$  chain in the patient (data not shown). Subsequent studies with A $\alpha$  chain-specific antibodies (Y18, Gaubius Institute, TNO, Leiden, The Netherlands) showed a unique truncated circulating A $\alpha$  chain of ~56 KDa in the patient (Fig. 1B). This truncated chain was not detected with antibodies against the  $\alpha$ C residues 603–610 (F48, kindly provided by Gary Matsueda, Bristol-Meyers Squibb, New Brunswick, NJ, USA). The densitometry showed that patient's relatives expressed approximately equal levels of both normal and truncated circulating A $\alpha$  chains.

We isolated DNA from the patient and performed genomic DNA sequence analysis of PCR-amplified products corresponding to the exonic, intronic flanking regions and promoter of the *FGA* gene. We identified the IVS4+1G>T mutation in heterozygosity. This transversion is the most prevalent mutation causing afibrinogenemia in homozygous individuals (4). The consensus splice donor site in intron 4 is lost, leading to alternative splicing and degradation of the transcript (5). This mutation has been linked to different haplotypes suggesting that it is a mutational hot spot in the *FGA* gene (4).

In the other allele, we found the 4841delC mutation, reported in the A $\alpha$ <sup>Perth</sup> variant (6). This deletion induces a frameshift in the  $\alpha$ C domain causing truncation of the A $\alpha$  chain at residue 517 including incorporation of 23 new residues. Therefore, the circulating protein lacks the terminal RGDS sites, the Lys<sup>508</sup> residue that participates in FXIII-mediated crosslinking, and a tPA binding domain (1). Analysis of cloned PCR products showed the A $\alpha$ <sup>Perth</sup> 4841delC was linked to the Ala312 polymorphism. This polymorphism increases FXIII-mediated crosslinking activity and resistance to fibrinolysis (7). It has been reported that carriers are more likely to suffer venous thromboembolism (8).

At least 25 other familial mutations have been reported in the  $\alpha$ C domain (<http://www.geht.org/>) and many of these are associated with reduced expression of the mutant chain. No circulating A $\alpha$ <sup>Otago</sup> chain was found in heterozygotes for Otago truncation (9), 0.2:1 ratio in A $\alpha$ <sup>Lincoln</sup> (10), Wilmington (11) and the Perth case (6), and less than 10% of the circulating A $\alpha$  chains in fibrinogen Marburg (12), suggesting the  $\alpha$ C domain is involved in fibrinogen assembly and/or secretion from the hepatocyte (6,13). However, the hemizygous patient, expressing only the variant chain, and her heterozygous relatives, showed near normal circulating levels of truncated A $\alpha$  chains, indicating the absence of a severe defect in mature protein folding in this case.

Interestingly, in contrast to heterozygous carriers in the first reported familial case, our patient's relatives had no obvious bleeding tendency. Thus, our patient's bleeding phenotype apparently reflects her compound mutation, hypofibrinogenemia resulting mainly from the IVS4+1G>T and the polymerisation defect due to the  $\alpha$ C domain truncation. The etiology of the thrombotic complications associated with her hand injury is likely complex. Her atheromatous disease is likely causal, but the cryoprecipitate infusions and her fibrinogen genotype, both the A $\alpha$ <sup>Perth</sup> and the 312Ala polymorphism, probably exacerbated development of the large vessel occlusions.

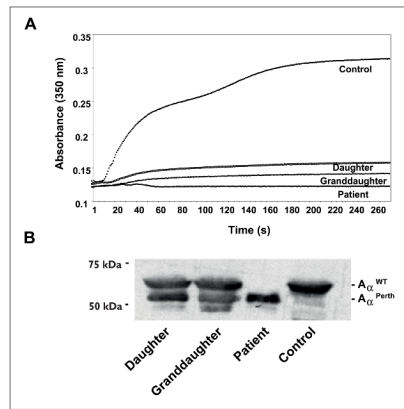
In summary, our data show dysfibrinogenemia associated with the Perth mutation has a heterogeneous phenotype and suggest factors other than the mutation impact on plasma fibrinogen levels.

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**Figure 1. Fibrin polymerisation and A $\alpha$  chain characterisation**

A) Representative fibrin polymerisation curves. Thrombin (0.1 U/ml) was added to plasma 1:10 diluted in HBS (CaCl<sub>2</sub> 10mM) and the polymerisation was monitored in triplicate, in a microplate reader at room temperature. Turbidity increase was abolished in the patient's plasma, while the daughter and granddaughter's revealed a longer lag-phase and a severe reduction in final turbidity. Control plasma was from a healthy non-related donor. B) Immunoblot analysis of reduced plasma samples run on 7.5% SDS-PAGE, developed with an A $\alpha$  chain specific antibody. While no normal A $\alpha$  chain could be seen in the patient, the daughter and granddaughter showed both normal and variant A $\alpha$  chains at similar levels. Loading was normalised to total plasma protein, and verified by Ponceau stain after SDS-PAGE (data not shown).