

Fibrinogen Martin: A Novel Mutation in *FGB* (Gln180Stop) Causing Congenital Afibrinogenemia

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Semin Thromb Hemost 2016;42:455–458.

We read with great interest the topical article by de Moerloose et al¹ in *Seminars in Thrombosis and Hemostasis* regarding congenital afibrinogenemia. Accordingly, we recently corresponded a novel mutation in an afibrinogenemic patient with a proposed new pathological molecular mechanism underlying afibrinogenemia.² We have named this novel mutation “fibrinogen Martin”, and now wish to expand our initial report to also describe findings with the hypofibrinogenemic kindreds of the index patient.

Fibrinogen is a 340 kDa glycoprotein comprising pairs of three polypeptide chains termed A α , B β , and γ . Fibrinogen has a trinodular structure with a central nodule (E-domain) that contains the N-terminus of each chain and two lateral globular domains (D-domains) that contain the C-terminus of B β - and γ -chains. The E-domain is linked to the two D-domains by a coiled-coil triple helix structure.^{3,4} The three genes encoding fibrinogen B β (*FGB*), A α (*FGA*), and γ (*FGG*), ordered from

centromere to telomere, are clustered in a region of 50 kb on human chromosome 4q28.^{1,4}

Congenital afibrinogenemia is an autosomal recessive bleeding disorder, referring to the total absence of plasma fibrinogen as measured by an antigenic assay, and is caused by variations in these genes, and associated with homozygous or compound heterozygous mutations, while hypofibrinogenemia is usually linked with heterozygous mutations.⁵ Mutations of *FGB* are less common and of particular interest since the B β -chain is considered the rate-limiting factor in the hepatic production of the fibrinogen hexamer.⁶ The estimated prevalence of congenital afibrinogenemia is 1 in 1,000,000,¹ and according to our present knowledge, in Slovakia is 1 in 5,000,000.⁷

We recently performed genetic analysis of *FGA*, *FGB*, and *FGG* as well as coagulation tests in a patient with congenital afibrinogenemia plus his immediate family (i.e., his parents and two older sisters). The patient with congenital

Table 1 Phenotype of the proband and selected family members

Member of family, age and control	PT (s)	APTT (s)	TT (s)	RT (s)	Fbg (Clauss method, g/L)	Fbg (Laurell method, g/L)
Proband (26)	II-3 > 300	> 300	> 300	> 150	Not detected	0.0
Older sister (32)	II-2 12.4	31	15	20	1.6	1.4
The oldest sister (36)	II-1 16.2	27	15	19	1.8	1.5
Father (62)	I-1 12.3	28	16	19	1.3	1.3
Mother (60)	I-2 11.8	27	16	18	1.5	1.5
Normal control	10.4–12.6	22–32	15–22	16–22	2.0–4.0	2.0–4.0

Abbreviations: APTT, activated partial thromboplastin time; Fbg, fibrinogen; PT, prothrombin time; RT, reptilase time; TT, thrombin time.

published online
May 5, 2016

Issue Theme The Role of Fibrinogen and Factor XIII in Hemostasis, and the Identification and Treatment of Associated Disorders; Guest Editors: Ton Lisman, PhD, and Philippe de Moerloose, MD.

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Tel: +1(212) 584-4662.

DOI <http://dx.doi.org/10.1055/s-0036-1581104>.
ISSN 0094-6176.

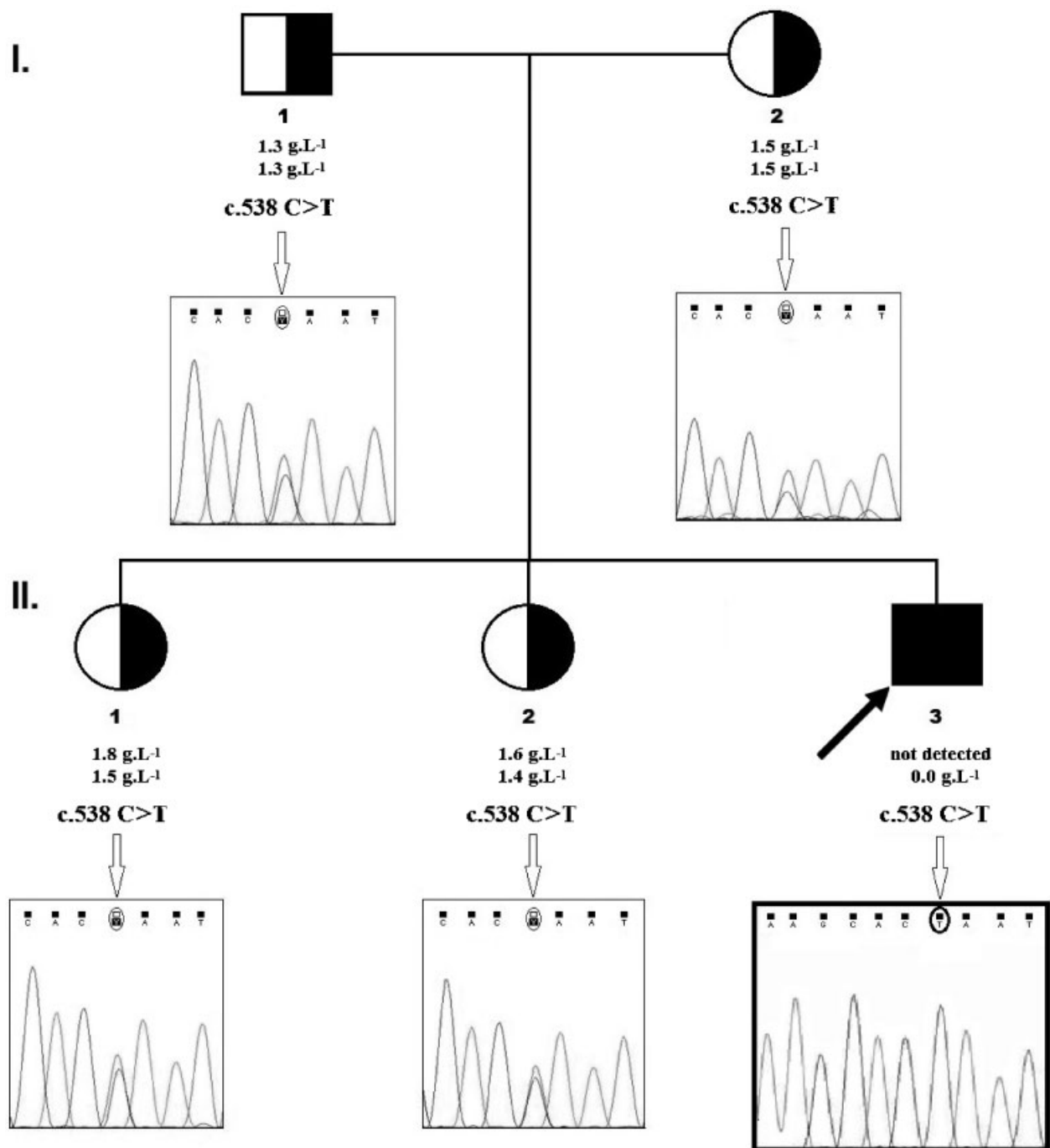


Fig. 1 Family pedigree of the patient with afibrinogenemia and electropherogram of the novel mutation fibrinogen martin. Functional and immunoreactive plasma fibrinogen levels, as well as the identified 538C > T mutations in *FGB* are depicted. The black filled-out symbol (black square) with arrow represents the homozygous patient. The half-filled-out, symbols with right half black represent the genotypically heterozygous kindreds.

afibrinogenemia reported in Slovakia is a 26-year-old man, and umbilical cord bleeding and development of epidural hematoma and hygroma in the occipital region were the first signs of bleeding. In the patient's history there were many other bleeding episodes, including repeated hemorrhage into the muscles, joints, soft tissues, and mucocutaneous bleeding. Examined members of the patient's family (mother, father, and two older sisters) instead have hypofibrinogenemia without any clinical manifestation. The patient and members of the family have not developed

any thrombotic episodes. Screening tests for thrombophilic mutations in the family were also negative. The patient's parents are unrelated, but they both come from the same Orava region in northern Slovakia. Results of coagulation assays are depicted in **Table 1**. All routine coagulation tests, that is, activated partial thromboplastin time, prothrombin time, and thrombin time of the index patient were infinitely prolonged (i.e., > 300 seconds), and fibrinogen plasma level measured according to the Laurell and Clauss method was undetectable. Family members instead

had reduced levels of fibrinogen below the normal range. Plasma fibrinogen according to the Clauss method was 1.3 g/L in father and 1.5 g/L in mother, respectively.

We identified a novel mutation in the index patient with afibrinogenemia. The mutation was localized in exon 4, nucleotide position 9661 of *FGB* and caused by the transversion C > T, leading to the switch of amino acid glutamine to stop codon, as was recently proposed and preliminary briefly reported by our research group.² The novel *FGB* mutation was subsequently also confirmed in the hypofibrinogenemic kindreds of the afibrinogenemic patient by direct sequence analysis of the three fibrinogen genes *FGA*, *FGB*, and *FGG*. The kindreds (i.e., the patient's parents and two sisters), were identified as heterozygous for the novel mutation which was later named "fibrinogen Martin" after the town of its discovery (► **Fig. 1**). However, hypofibrinogenemia secondary to the novel mutation was present with only the mild decrease of the fibrinogen level (activity and antigen between 1.0 and 2.0 g/L) in kindreds. Based on the results of the coagulation and functional tests of fibrinogen, as well as the results of genetic analysis of the patient kindreds, we have proposed the new pathological molecular mechanism underlying afibrinogenemia. The presence of the stop codon, caused by the substitution C9661T in exon 4 of *FGB* eliminates the aberrant mRNA encoding incomplete B β polypeptide by the process of nonsense-mediated mRNA decay.⁸ We presume that formation of an A α B β γ half-molecule is not possible. Thus, the fibrinogen cannot be formed by the dimerization of two hexamers A α B β γ . The homozygous mutation in the patient with afibrinogenemia correlates well with the results of the coagulation assays where a total absence of fibrinogen was found.

The identification of the precise genetic defect of congenital afibrinogenemia is of value, to permit early testing of other at-risk individuals, to understand the correlation between genotype and clinical phenotype, to assist in therapeutic choices, and as an essential prerequisite for the development of new specific treatments, such as gene therapy.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This work has a general support by departmental chairs. We would like to thank the support of projects Vega 1/0168/16, Grant Comenius University (UK/334/2015), Martin Center of Biomedicine (BioMed Martin, ITMS 26220220187), and Agency for the Support of Research and Development (APVV) 0222-11.

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