

# A novel frameshift mutation in *FGA* (c.1846 del A) leading to congenital afibrinogenemia in a consanguineous Syrian family

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Congenital afibrinogenemia is a rare autosomal recessive coagulation disorder characterized essentially by bleeding symptoms, but miscarriages and, paradoxically, thromboembolic events can also occur. Most reported mutations leading to congenital afibrinogenemia are located in *FGA* encoding the fibrinogen A  $\alpha$ -chain. In this study, we analysed 12 individuals from a consanguineous Syrian family with reduced or absent fibrinogen levels: those with fibrinogen levels around 1 g/l ( $n = 7$ ) were found to be heterozygous for a novel frameshift mutation in *FGA* exon 5 (c.1846 del A) and those with undetectable fibrinogen levels ( $n = 5$ ) were homozygous for the same mutation. This novel frameshift mutation is the most C-terminal causative *FGA* mutation identified to date in afibrinogenemic patients. The resulting aberrant A $\alpha$ -chain (p.Thr616HisfsX32) is most likely synthesized, but is less efficiently assembled and/or secreted into the circulation given the phenotype of asymptomatic hypofibrinogenemia

## Introduction

Fibrinogen is a 340 kDa glycoprotein predominantly synthesized in the liver and composed of two sets of three homologous polypeptide chains known as A $\alpha$ -chain, B $\beta$ -chain and  $\gamma$ -chain that are encoded by distinct genes (*FGA*, *FGB* and *FGG*, respectively) clustered in a region of 50 kb on chromosome 4q31 [1]. Since the first case of afibrinogenemia described in 1920 [2], and the first causative mutation published in 1999 [3], more than 80 mutations resulting in quantitative hereditary fibrinogen disorders have been reported to date, the majority situated in *FGA* [4,5].

The estimated prevalence of afibrinogenemia is one in 1 000 000, but in populations in which consanguineous marriages are common, the prevalence of afibrinogenemia is increased. Bleeding is usually the main clinical manifestation and affects the umbilical cord at birth, skin, gastrointestinal tract, genitourinary tract, spleen and the central nervous system, the latter the major cause of death. Hemarthrosis and muscle hematomas are, however, less frequent and result in less disability than in hemophilia patients. As fibrinogen is important to maintain a pregnancy until term, first-trimester abortion is common. Paradoxically, both arterial and venous thromboembolic complications are observed, either spontaneously or therapy related [4,5]. In this study, we

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performed fibrinogen gene mutation analysis of 13 members of a consanguineous Syrian family, five members with afibrinogenemia, seven with hypofibrinogenemia and one with normal fibrinogen levels.

## Patients and methods

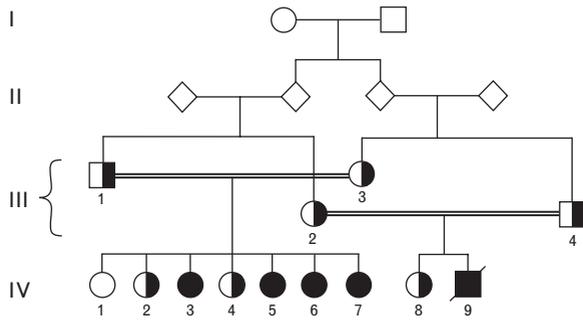
### Patients

Thirteen members of a consanguineous family (Fig. 1) living in the south of Syria were enrolled in this study. Five patients presented congenital bleeding diathesis with undetectable fibrinogen levels and infinitely prolonged prothrombin time, partial thromboplastin time and thrombin time. Of these five patients, one (IV-9) died recently at 28 years old from intracranial hemorrhage and two (IV-6 and IV-7) suffered from thromboembolic events and first-trimester miscarriages (three miscarriages for IV-6 and five for IV-7). The most frequent bleeding symptoms were severe menorrhagias in all homozygous women. No case of hemarthrosis was reported. The other eight family members were asymptomatic, with the exception of IV-4 who presented three first-trimester miscarriages but who ultimately successfully maintained three other pregnancies until term.

### Fibrinogen level measurement

Fibrinogen levels (activity and antigen) were determined for all 13 samples (Table 1). Clottable fibrinogen was

Fig. 1



Pedigree of the consanguineous family from Syria.

measured by a modified Clauss method (Multifibren\*U with a BCS analyzer, Siemens, Marburg, Germany). Fibrinogen antigen was measured by immunoturbidimetry (Turbitimer, Siemens, Marburg, Germany). In the five patients with undetectable fibrinogen by immunoturbidimetry, fibrinogen was also measured by ELISA as previously described [6].

### Genetic analysis

Our mutation screening strategy for afibrinogenemia is based on a flowchart described elsewhere [5]. For amplification and sequencing of *FGA* exon 5, the primers used were forward *FGA*x5N1L: 5'CTACCAGGAACCTCAA TAGACG3' and reverse *FGA*3'R: 5'ATGGCTCTGT ACTGTTAGGC3'. The PCR program consisted of denaturation at 94°C (5 min) followed by 10 touchdown cycles: 94°C (30 s), 60–50°C (30 s, –1°C per cycle), 72°C (1 min); and 25 cycles: 94°C (30 s), 50°C (30 s), 72°C (1 min). A final elongation was performed at 72°C for 5 min. Sequencing was performed using a capillary genetic analyzer system from Applied Biosystems (ABI 3130xl; Foster City, California, USA). This protocol has been approved by the Ethics Committee of the Faculty of Medicine of Geneva. Mutation description is according to standardized nomenclature as in

Table 1 Fibrinogen levels

Individual	Fibrinogen (g/l) measured by modified Clauss method	Fibrinogen (g/l) measured by immunoturbidimetry
III-1	1.2	1.2
III-2	1.3	1.6
III-3	1.2	1.7
III-4	1.2	1.2
IV-1	2.1	2.0
IV-2	1.2	1.5
IV-3	0	0
IV-4	1.0	1.0
IV-5	0	0
IV-6	0	0
IV-7	0	0
IV-8	1.3	1.4
IV-9	0	0

our Mutation Update for fibrinogen gene mutations [4,5].

### Results and discussion

Twelve individuals from a consanguineous Syrian family with reduced or absent fibrinogen levels were screened for causative mutations in fibrinogen genes: those with low fibrinogen levels (approximately 1 g/l,  $n = 7$ ) were found to be heterozygous for a novel single base-pair deletion in *FGA* exon 5 (c.1846 del A), those with undetectable fibrinogen levels ( $n = 5$ ) were homozygous for the same mutation. This mutation causes a frameshift in the coding sequence and subsequent read-through of the normal stop codon (TAG) of the common  $\text{A}\alpha$ -chain. The resulting putative protein (p.Thr616HisfsX32) is slightly longer than the wild-type  $\text{A}\alpha$ -chain with a stretch of 32 aberrant amino acids with the sequence HIAPREAMLNAL SEVSTLLLWGS LPPRLS (instead of the 29 amino acid sequence THSTKRGHAKSRPVRGIHTSPLGKPS LSP), with an enriched leucine content. This aberrant protein is probably not able to be efficiently assembled and/or secreted and leads to a phenotype of afibrinogenemia. However, because this novel frameshift mutation is the most C-terminal *FGA* causative mutation identified to date in afibrinogenemic patients, we wished to determine whether traces of fibrinogen, undetectable with routine laboratory assays, were present in the plasma of the homozygous individuals. Using an antifibrinogen ELISA [6], we were indeed able to detect traces of fibrinogen in three of the five afibrinogenemic patients (up to 5% of the level quantified in control plasma), whereas heterozygous family members ( $n = 4$ ) had an average level of 60% compared with control. These antigenic levels are probably insufficient to qualify the homozygous individuals as hypodysfibrinogenemic, but may explain the relatively moderate bleeding episodes in most members of the family. However, with the development of more sensitive assays allowing the detection of very low levels of fibrinogen, the definition of afibrinogenemia (traditionally defined as undetectable fibrinogen plasma levels) may need to be respecified in the future.

To date, 18 frameshift mutations leading to afibrinogenemia have been reported, the majority (nine) in *FGA* exon 5 [5]. Interestingly, seven single base-pair deletions in *FGA* exon 5 result in usage of the same new reading frame. All seven mutations are predicted to encode a long stretch of aberrant amino acids before terminating at the same premature stop codon 69–158 codons downstream. The aberrant amino acid sequence may lead to abnormal folding of the  $\text{A}\alpha$ -chain, thus, affecting fibrinogen chain assembly or secretion. Computer-assisted analysis of two of these putative C-terminal sequences (p.Ser312A-lafsX109 and p.Gly316GlufsX104) predicted the presence of several  $\alpha$ -helices in the mutant domains due to enrichment in leucine and valine residues [7]. These motifs that are absent in the wild-type fibrinogen

A $\alpha$ -chain may perturb the assembly and/or secretion of fibrinogen hexamer molecule, the longer the aberrant polypeptide, the likelier the impact on fibrinogen synthesis. In the novel c.1846 del A mutation described here, resulting in the longest putative aberrant protein to date (p.Thr616HisfsX32) but with only 32 aberrant amino acids, enrichment in the basic amino acid leucine in the C-terminus of the A $\alpha$ -chain could also explain the disturbed assembly and/or secretion of the fibrinogen. However, in this study, the aberrant A $\alpha$ -chain is not severely truncated and its size is similar to the wild-type chain. This probably explains in part why the aberrant protein is still synthesized and why traces of fibrinogen are detected, with a very sensitive ELISA, in the plasma of our homozygous patients.

The *FGA* c.1846 del A mutation is the first reported fibrinogen mutation in a Syrian family at this time. Moreover, this novel frameshift mutation is the most C-terminal *FGA* causative mutation identified to date in afibrinogenemic patients. Although afibrinogenemia is a rare disorder, its incidence is increasing in countries with migratory flows from Muslim countries or from some parts of southern India in which consanguineous marriages are traditionally frequent [8].

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