Clinical, biological, and genetic features in an afibrinogenemia patient series in Algeria

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Abstract

Introduction: The incidence of afibrinogenemia had not been previously reported in Algeria. Afibrinogenemia patients are prone to both haemorrhagic and thrombotic complications. Predictive markers of thrombosis in afibrinogenemia patients are not existent.

Aims and methods: Clinical and biological data from 46 afibrinogenemia patients are reported. Biological investigations included routine tests, genetics analysis and thrombin generation.

Results: FGA mutations (four novel and four previously described) and FGB mutations (seven mutations; five novels) were homozygous in all but one family as a result of 28 consanguineous marriages out of 30 discrete families. Incidence of afibrinogenemia in Algeria is at least 3 per million births. Umbilical bleeding was reported in 39/46 cases and was the main discovery circumstance. We also report post trauma or post-surgery (3/46) bleeding and spontaneous deep vein thrombosis (DVT) in adulthood (1/46), as discovery circumstances. The median age (10.5-year-old) of the population reported here explains why there are few hemarthrosis and obstetrical or gynaecological complications in this series. Thrombotic events were reported in seven patients (four spontaneous). Endogenous Thrombin Potential was significantly increased in thrombosis-prone patients compared to afibrinogenemic patients with and without personal or familial history (1118 vs. 744 and 817 nM IIa × min, respectively).

Conclusion: The incidence of afibrinogenemia in Algeria is the consequence of consanguineous marriage in families carrying private mutations. The thrombin generation test (TGT) could identify, among afibrinogenemic patients, those presenting a thrombotic risk.

Key words
afibrinogenemia, bleeding complications, fibrinogen mutations, thrombin generation, thrombosis

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INTRODUCTION

The final stage in a series of blood coagulating reactions is the fibrinogen to fibrin conversion catalysed by thrombin. Fibrinogen is circulating in the blood as a disulfide-linked 340 kDa glycoprotein composed of two identical heterotrimers, each consisting of three different chains (\(\alpha\), \(\beta\), and \(\gamma\)). The three chains are encoded by the FGA, FGB, and FGG genes, respectively, clustered in a 50 kb region on chromosome 4q31.3-4q32.1. Congenital afibrinogenemia (MIM 202400, PMID: 30076675) is a rare autosomal recessive disorder characterized by absence of both functional and antigen fibrinogen levels in patient’s plasma. The prevalence of the disorder is around 1 in 1,000,000. However, the prevalence of afibrinogenemia is found to be increased in populations in which consanguineous marriages are common. Over 200 families of patients with afibrinogenemia have been described so far in the GEHT database [https://site.geht.org/base-fibrinogene/] or in publications including a large series of 55 patients from Iran and two reports of 204 afibrinogenemia and 100 hypofibrinogenemia cases. The majority (>65%) of the mutations is found in FGA and consist of null, that is, large deletion, frameshift, early-truncating nonsense or splice-site mutations. Because it is a rare disease that results from private mutations, <20% are due to compound heterozygosity, and the 80% homozygous cases result mainly from consanguineous marriage. Affected patients encounter a lifelong bleeding diathesis, either spontaneous or following trauma or surgery, and paradoxical thrombosis is not uncommon, even in the absence of fibrinogen infusion. However, reports on the frequency of some events are rather conflicting, possibly because of the size of most of the published series. We report on the clinical features, thrombin generation and molecular biology characterization of the fourth largest series of afibrinogenemia patients, all originating from Algeria.

MATERIAL AND METHODS

2.1 Patients

We included 46 afibrinogenemia patients from 30 discrete families and 85 healthy or heterozygous related individuals. All patients gave informed written consent.

Blood was collected on EDTA for DNA extraction and on .109 M trisodium citrate for fibrinogen assay and thrombin generation test (TGT). The platelet-poor plasma (PPP) was obtained by centrifugation at 2000 g for 20 min and was aliquoted and stored at -80°C until use.

2.2 Fibrinogen determination

Functional fibrinogen concentration was assessed according to Clauss method, using thrombin 100 U NIH/ml (Dade Behring) and Owren-Koller buffer (Diagnostica Stago, Asnières, France) in a coagulation analyzer (STA Diagnostic Stago).

Dideoxy sequencing was performed with the BigDye® Terminator sequencing kit (Applied Biosystems; Austin, TX). Dideoxy sequencing products were loaded onto an ABI3130 (Applied Biosystems) fluorescent analyzer. The sequence numbering refers to the GenBank access numbers NM_00508, NM_005141 and NM_000509 for the FGA, FGB, and FGG genes.

2.3 DNA purification, amplification, and sequencing

DNA was available from the index cases and also from 81 of the relatives, either heterozygous or unmutated, whose genotypes were used for segregation studies and ACMG classification, when necessary. DNA was extracted using the QIAamp DNA blood purification kit (QIAGEN, Courtaboeuf, France). DNA was used for amplification by polymerase chain reaction (PCR) of exons and intron-exon boundaries of the FGA, FGB, and FGG genes.

2.4 Sequencing of FGA, FGB, and FGG genes

The reptilase was from Siemens–Behring, Marburg, Germany. Reptilase treatment causes defibrination without activation or depletion of other coagulation factors.

2.5 Defibrination of PPP

TGT was measured in the presence of 5 pM tissue factor (FT) and 4 \(\mu\)M phospholipids (PL) (PPP-reagent HIGH, Diagnostica Stago) with fluorogenic substrate and CaCl\(_2\) (FluCa-Kit, Diagnostica Stago), using calibrated automated thrombogram (CAT) assay (Diagnostica Stago, Asnières, France).

First, thrombin generation was evaluated in normal citrated PPP and reptilase-treated normal PPP (fibrinogen-depleted plasma). Second, thrombin generation was evaluated in PPP from afibrinogenemic patients at basal state (\(n = 36\)) and in reptilase-treated PPP from healthy related subjects (\(n = 85\)); in these two groups fibrinogen was <.1 g/L.

The molar amount of thrombin present in clotting plasma was calculated using the Thrombinscope software. All experiments were carried out in triplicate and the mean value was reported.

From the thrombin-time curve, four parameters were determined: endogenous thrombin potential (ETP, i.e., area under the curve [AUC]), peak height, time to peak, and lag time for thrombin detection.
TABLE 1  Main clinical features of the 46 patients of this series and comparison with the literature

| Clinical features                              | ALGERIA(This study)| International(Peyvandi et al.\textsuperscript{7})| IRAN(Lak et al.\textsuperscript{4})| International(Casini et al.\textsuperscript{8}) |
|------------------------------------------------|-------------------|----------------------------------|--------------------------------|----------------------------------|
|                                                 | N = 46            | N = 72                           | N = 55                         | N = 204                          |
| Umbilical cord bleeding                         | 85\% (39/46)      | NA                               | 85\%                          | 42\%\textsuperscript{b}          |
| Post-trauma or post-surgery bleeding            | 43\% (20/46)      | NA                               | 40\%                          | 40\%                             |
| Post-trauma muscle hematomas                    | 41\% (19/46)      | 17\%                             | 72\%                          | 38\%                             |
| Oral cavity bleeding                            | 22\% (10/46)      | NA                               | 72\%                          | NA                               |
| Epistaxis                                       | 15\% (7/46)       | 10\%                             | 72\%                          | NA                               |
| Thrombosis events                               | 15\% (7/46)       | 3\%                              | 10\%                          | 18\% (6\% in children)           |
| (four spontaneous thrombotic events; three post fibrinogen or post FFP infusion thrombosis) | | | | |
| Spontaneous CNS bleeding                        | 9\% (4/46)        | 4\%                              | 10\%                          | 23\%                             |
| Hemarthrosis                                    | 6\% (3/46)        | 25\%                             | 54\%                          | 38\%                             |
| Gastro-intestinal haemorrhage                   | 0-                | 17\%                             | 0                             | NA                               |
| Menorrhagia                                     | 75\%\textsuperscript{a} (3/4) | 7\%                             | 70\%\textsuperscript{b} (14/20) | 74\%                             |
| Miscarriage                                     | irrelevant        | 13\%\textsuperscript{c} (4/30)   | NA                            | 85\%                             |

\textsuperscript{a}Percentage refers to the relevant cases.

\textsuperscript{b}Frequency of umbilical stump bleeding with other items such as cephalohematoma or cheek hematomata.

2.7 Statistical analysis

Parameters of thrombin generation were first analyzed by Kruskal-Wallis. Post-hoc analysis was made using non-parametric Mann-Whitney test with Bonferroni correction. All analyses were performed using SPSS statistical software.

3 RESULTS

In this report, 30 index cases and 16 additional related cases of afibrinogenemia from 30 discrete families were investigated (median age 10.5-year-old; range 25 days-40-year-old; sex ratio 20F/26 M). Clinical features of the afibrinogenemia patients are summarized in Table 1. The most characteristic clinical feature of afibrinogenemia was bleeding episodes. First events usually occurred in early childhood and varied from mild to severe. Premature death occurred in 6/46 patients (13\%); ischemic stroke two cases, postperfusion ischemic thrombosis one case; spontaneous and traumatic intracranial haemorrhage one case each, umbilical bleeding one case).

Umbilical bleeding at birth was the most commonly reported event, affecting 39 out of 46 (85\%) patients. The next more frequent reported events were bleeding at venipuncture sites (30/46; 65\%), post traumatic muscular hematomas (19/46; 41\%) and postoperative bleeding (20/46; 43\%). Spontaneous central nervous system (CNS) bleeding occurred in 9\% (4/46; one fatal) patients. A fifth patient deceased of post-traumatic intracranial bleeding. Joint bleeding occurred in three individuals only. In the present series, six fatal complications are reported including two spontaneous ischemic strokes, one spontaneous intracranial bleeding, one umbilical bleeding, one traumatic intracerebral bleeding and one mesenteric thrombosis following fibrinogen infusion. The ischemic origin of the fatal cases was documented by appropriate imaging. All patients but one (98\%) reported bruising or ecchymosis, 6/46 (13\%) reported epistaxis. We have no reliable information about wound healing or bone cysts in this series.

Four spontaneous thrombotic events were also observed in the present series, including two fatal cases of ischemic stroke, one deep vein thrombosis (DVT) of the leg, and another DVT complicated with pulmonary embolism in a heterozygous FV Leiden patient. The presence of the FV Leiden was documented in two individuals of a sole family in the present report; one did not experience thrombotic events. The other risk factors sought for (FII G20210A, antithrombin, protein C and protein S deficiency) were all absent. The explanation for these thrombotic events relies on the absence of the antithrombin effect of fibrinogen, allowing platelet aggregation. Additionally, three patients presented thrombotic events following fresh frozen plasma (one cutaneous necrosis, one venous mesenteric thrombosis) or fibrinogen concentrate (DVT) infusions.

To attempt to identify the afibrinogenemia patients with a thrombotic risk, we studied the thrombin generation in the 36 available samples from afibrinogenemia patients and 85 healthy related individuals as controls. As fibrinogen interferes with the thrombin generation, we performed a defibrination of normal plasma with reptilase. As shown in Table 2, the defibrination resulted in a decreased rate of thrombin generation in the residual fibrinogen-depleted plasma.

The results of ETP, peak height, time to peak and lag time for the 36 available afibrinogenemia patients and 85 healthy related individuals after defibrination are shown in Table 3.

The comparison of thrombin generation parameters showed that ETP and time to peak from plasma of afibrinogenemia patients were
**TABLE 2** Parameters of thrombin generation test in normal plasma with fibrinogen 2.5 g/L before and after reptilase treatment

| Fg     | Lag time(min) | ETP(nM IIa x min) | Time to peak(min) | Peak height(nM IIa) | n  |
|--------|---------------|-------------------|-------------------|---------------------|----|
| 2.5 g/L | 1.2 ± 0.9     | 1645 ± 550        | 3.3 ± .9          | 347 ± 105           | 73 |
| <.1 g/L | 1.2 ± 0.9     | 910 ± 116         | 3.8 ± .7          | 212 ± 60            | 30 |

Note: The results were expressed as mean ± 1.96 SD. 
Abbreviations: ETP: endogenous thrombin potential; n: number of experiments.

**TABLE 3** Results of thrombin generation parameters

|                          | Healthy related subjects (n = 85) | Afibrinogenemia (n = 36) | Patient without personal or familial thrombotic history (n = 27) | Patient with personal thrombotic history (n = 4) | Patients with familial thrombotic history (n = 5) |
|--------------------------|-----------------------------------|--------------------------|---------------------------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| ETP (nM IIa x min)       | 988 (263; 640–1840)               | 776 (192; 574–1258)      | 744 (176; 574–943)                                           | 1118 (308; 869–1258)                          | 817 (122; 804–1113)                          |
| Peak height (nM)         | 251 (73; 144–464)                 | 253 (82; 118–382)        | 251 (86; 118–382)                                           | 194 (175; 129–333)                            | 297 (67; 241–329)                            |
| Time to peak (min)       | 4.0 (1.4; 2.1–6.7)                | 3.4 (8; 1.8–6.7)         | 3.4 (9; 1.8–5.6)                                            | 5.4 (2.6; 3.5–6.7)                            | 3.2 (8; 2.6–3.7)                             |
| Lag time (min)           | 1.4 (7; 2–3.2)                    | 1.3 (7; .5–3.0)          | 1.2 (.6; .5–2.1)                                            | 2.4 (.9; 2.0–3.0)                             | 1.0 (.5; .8–1.6)                             |

Note: Data are expressed as median [interquartile range; min - max]. 
Abbreviations: ETP, endogenous thrombin potential; VT: venous thrombosis. *p < .05.

significantly less than from defibrinated plasma from healthy related subjects (p < .0001 and 004, respectively) while lag time and peak thrombin were no different (Table 3).

Comparison of parameters of thrombin generation according to the personal or familial thrombotic history of afibrinogenemia patients is shown in Table 3. Out of the 36 available patients for thrombin-generation studies, twenty-seven had no personal or familial history of thrombosis, five had familial history thrombosis, and four had personal thrombotic history (one spontaneous DVT, two after fibrinogen infusion, and one fatal spontaneous ischemic stroke).

ETP from patients without personal or familial thrombotic history was significantly less than ETP from patients with personal thrombotic history and from patients with familial thrombotic history (p < .009 and .048, respectively). No difference was found between ETP from patients with personal thrombotic history and with familial thrombotic history.

The lag time from patients without thrombotic history was significantly less than the lag time from patients with personal thrombotic history (p = .006) but not significantly different from patients with familial thrombotic history. Lag time from patients with personal thrombotic history was significantly different from patients with familial thrombotic history (p = .042).

The time to peak from patients without thrombotic history was significantly higher than the lag time from patients with personal thrombotic history (p = .042) but not significantly different from patients with familial thrombotic history. No difference was found between ETP from patients with personal thrombotic history and with familial thrombotic history. The peak height was not different between the three groups.

Using a receiver operator characteristic curve (ROC curve) we explore the optimal cutoff threshold to identify thrombotic risk among afibrinogenemic patients. Figure 1 depicts the ROC analysis with an AUC of .945 (95% CI 853–1.000). Using a cut-off of 869 nM thrombin x min of ETP, there was 100% of sensitivity and 81% of specificity.

Consanguinity was acknowledged in all but two families. Mutation findings are summarized in supplemental material table 4. Mutation studies evidenced four novel homozygous mutations in the FGA gene: FGA c.261delG predicting p.(Lys87Asnfs*19); two occurrences in two discrete families); c.546delGinsTATTAAGATCC predicting p.(Ser183Ilefs*7), one occurrence in one family and four occurrences in another family, two of them inferred from clinical history; these two families originate from the same area; c.1771delA (p.Arg591Gufs*84); one occurrence in one family; and c.1798delA predicting p.(Ser600Alafs*75), genotype of the deceased
| Individual | Relationship to the propositus | Year of birth (or age at death) | Gender (M or F) | Thrombotic events | Gene | Exon | cDNA | Status | Protein nomenclature | Plasma protein |
|-----------|-------------------------------|-------------------------------|----------------|------------------|------|------|------|--------|---------------------|---------------|
| Family 1  | 1                             | 2002                          | F              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| 2          | F                             | deceased 35-yo ischemic stroke | M              | Spontaneous ischemic stroke | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 2  | 3                             | 2002                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 3  | 4                             | 1975                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| 5          | B                             | deceased 6-yo post perfusion ischemic thrombosis | M | Mesenteric thrombosis post FFP infusion | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 4  | 6                             | 2000                          | F              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| 7          | B                             | 2004                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 5  | 8                             | 1994                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| 9          | S                             | 2000                          | F              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| 10         | B                             | 2003                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| 11         | B                             | 2006                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 6  | 12                            | 2009                          | F              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 7  | 13                            | 2002                          | F              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 8  | 14                            | 2010                          | M              |                  | FGA  | Exon 2| c.117delT| homozygous | p.(Val40Trpfs*31) | Val21Trpfs*31 |
| Family 9  | 15                            | 1998                          | M              |                  | FGA  | Exon 3| c.261delG| homozygous | p.(Lys87Asnfs*19) | Lys68Asnfs*19 |
| Family 10 | 16                            | 2006                          | F              |                  | FGA  | Exon 3| c.261delG| homozygous | p.(Lys87Asnfs*19) | Lys68Asnfs*19 |
| Family 11 | 17                            | 2002                          | F              |                  | FGA  | Intron 3| c.364+4_364+7delAGTA| homozygous | ? | ? |
| Family 12 | 18                            | 1996                          | F              |                  | FGA  | Exon 5| c.546delIGins TATTAAGATCC fc.546delIGins TATTAAGATCC f | homozygous | p.(Ser183Ilefs*7) | Ser164Ilefs*7 |

(Continues)
| Individual | Relationship to the propositus | Year of birth (or age at death) | Gender (M or F) | Thrombotic events | Gene | Exon | cDNA | Status | Protein nomenclature | Plasma protein |
|------------|--------------------------------|-------------------------------|----------------|-----------------|------|------|------|--------|---------------------|----------------|
| Family 13  | 19d                            | 1976                          | F              | DVT post        | FGA  | Exon 5 | c.546delGinsTATTAGATCC Homozygous | p.(Ser183Ilefs*7) | Ser164Ilefs*7 |
| Family 14  | 20a                            | 1976                          | F              | DVT post        | FGA  | Exon 5 | c.885G > A Homozygous | p.(Trp295*) | Trp276* |
| Family 15  | 21a                            | 1997                          | M              | Spontaneous     | FGA  | Exon 5 | c.1771delA fc.1771delAf Homozygous | p.(Arg600Alafs*75) | Ser581Alafs*75 |
| Family 16  | 22a                            | 1984                          | M              | Spontaneous     | FGA  | Exon 5 | c.833G > A Homozygous | p.(Glu285Ser) | Gly258Ser |
| Family 17  | 23a                            | 2007                          | F              | Spontaneous     | FGA  | Exon 6 | c.895T > C Homozygous | p.(Tyr299His) | Tyr269His |
| Family 18  | 24a                            | 1997                          | F              | Spontaneous     | FGA  | Exon 6 | c.862G > A Homozygous | p.(Glu285Ser) | Gly258Ser |
| Family 19  | 25a                            | 2001                          | M              | Spontaneous     | FGB  | Exon 5 | c.823G > Tfc.823G > Tf Homozygous | p.(Glu285Ser) | Gly258Ser |
| Family 20  | 26a                            | 2006                          | M              | Spontaneous     | FGB  | Exon 6 | c.833G > Afc.833G > Aa Homozygous | p.(Glu285Ser) | Gly258Ser |
| Family 21  | 27a                            | 2009                          | M              | Spontaneous     | FGB  | Exon 6 | c.895T > C Homozygous | p.(Tyr299His) | Tyr269His |

(Continues)
| Individual | Relationship to the propositus<sup>1</sup> | Year of birth (or age at death) | Gender (M or F) | Thrombotic events | Gene | Exon | cDNA | Status | Protein nomenclature | Plasma protein |
|------------|----------------------------------|-------------------------------|----------------|-----------------|------|------|------|--------|---------------------|---------------|
| Family 23  | 35                               | 1991                          | M              |                | FGB  | Exon 6 | c.905G > A | homozygous | p.(Gly302Glu)       | Gly272Glu     |
|            | 36                               | 1992                          | F              |                |      |        |      |         |                     |               |
|            | 37                               | 1998                          | M              |                |      |        |      |         |                     |               |
| Family 24  | 38                               | 2005                          | M              |                | FGB  | Exon 6 | c.905G > A | homozygous | p.(Gly302Glu)       | Gly272Glu     |
| Family 25  | 39                               | 2009                          | F              |                | FGB  | Exon 6 | c.905G > A | homozygous | p.(Gly302Glu)       | Gly272Glu     |
|            | 40<sup>a</sup>                   |                               |                |                |      |        |      |         |                     |               |
| Family 26  | 41<sup>d</sup>                   | 1971                          | M              | Spontaneous DVT| FGB  | Exon 8 | c.1299G > T | homozygous | p.(Trp433Cys)       | Trp403Cys     |
| Family 27  | 42                               | 1987                          | M              |                | FGB  | Exon 8 | c.1427C > A | homozygous | p.(Ser476*)         | Ser446*       |
| Family 28  | 43                               | 2001                          | F              |                | FGB  | Exon 8 | c.1427C > A | homozygous | p.(Ser476*)         | Ser446*       |
| Family 29  | 44                               | 1997                          | M              |                | FGB  | Exon 8 | c.1427C > A | homozygous | p.(Ser476*)         | Ser446*       |
| Family 30  | 45                               | 2001                          | M              |                | FGB  | Exon 8 | c.1427C > A | homozygous | p.(Ser476*)         | Ser446*       |
|            | 46                               | 1997                          | F              |                |      |        |      |         |                     |               |

Note: There are three occurrences of the FGA c.117delT mutation in the GFHT database, two in Italy and one in California, all of them as compound heterozygote ones. There are three records of the FGA c.364+4,364+7delAGTA mutation in the GFHT database (originating from the USA, Turkey, and Iraq), all of them homozygous. FGA exon 5 c.885G > A; p.(Trp295*) was already reported in an Algerian girl in 2007.<sup>35</sup> We could not confirm whether this report was describing individual 26 of this series or an unrelated case, and other occurrences of this mutation are recorded in the GFHT database (three in Turkey, one in Italy, although the latter was described as a hypodysfibrinogenemia). For FGB c.895T > A predicting p.(Tyr299His), the GFHT record states that the patient originates from Algeria. It is thus possible that the present report is a second description of the case.

Abbreviations: DVT, deep vein thrombosis; FFP, Fresh Frozen Plasma.
<sup>1</sup>Genotype inferred from fibrinogen level and relative's genetic status.
<sup>2</sup>P: propositus; B: propositus’ brother; S: propositus’ sister; F: propositus’ father.
<sup>3</sup>Heterozygous FV Leiden carrier.
<sup>4</sup>Thrombotic events.
<sup>5</sup>Non consanguineous marriage.
<sup>6</sup>Novel mutation.
<sup>?:</sup> unknown effect on transcription and/or translation.
Novel occurrences of four previously reported FGA mutations were also found. The FGA homozygous c.117delT in exon 2 (predicting p.(Val40Trpfs*31) or Val21Trpfs*31 in the mature protein) has been found in 13 afibrinogenemic patients from seven discrete families. Consanguineous marriage is acknowledged in six of these seven families. This mutation was also observed in the second nonconsanguineous marriage family in association with an intron three deletion c.364+4_364+7delAGTA. We also report the occurrence of this homozygous deletion in one family. Exon prediction software consistently predict a suppression of the donor site. Interestingly, fibrinogen was detectable in this case (.1 g/l) reflecting that some transcripts still undergo normal splicing as described for other splicing mutations. A homozygous FGA exon 5 c.885G > A; p.(Trp295*) mutation was found in two unrelated families. The index case in one of these families was an 18-year-old girl. Her sister died at age 23 of ischemic cerebral infarction. A homozygous c.945delT mutation in exon 5 was found in one family predicting p.(Gly316Glufs*105).

In the FGB gene we found seven mutations, all homozygous, five of them being novel, in 15 afibrinogenemic patients from 12 families. The five novel mutations are the following: c.823G > T in exon 5; p.(Glu245*), one occurrence in one family; c.833G > A in exon 6; p.(Gly278Glu), the genotype of the deceased index case is inferred from the parents, both heterozygous for the mutation; c.905G > A in exon 6 (p.(Gly302Glu); six cases in three unrelated families. Another mutation at this residue p.(Gly302Arg) is recorded in the GFHT database. We also report four cases in one family of c.1299G > T in exon 8 predicting p.(Trp433Cys); the mutation status is inferred for three of the four siblings as DNA was unavailable. Another mutation at this residue (p.(Trp433Leu)) is recorded in the GFHT database. We also report six cases in four unrelated families from the same area of the c.1427C > A mutation in exon 8 predicting p.(Ser476†). In addition, two previously reported mutations were found: c.862G > A predicting p.(Gly288Ser) and c.895T > A predicting p.(Tyr299His).

4 Discussion

4.1 Haemorrhagic complications

Table 1 compares the clinical features in this series with the three main reported series. When treated, all patients received fresh frozen plasma as no fibrinogen concentrate was available in Algeria. The two exceptions are patients 5 and 19, treated abroad.

As in previous reports, first haemorrhagic events occurred in early childhood and varied from mild to severe. Most features of the present data are comparable to previous series: in the literature over 40% of patients with afibrinogenemia or hypofibrinogenemia had a history of spontaneous major bleeding including hematomas, hemorrhosis, CNS, gastrointestinal, and umbilical cord bleeding; 3%–5% had only spontaneous minor bleeding, such as bruising, ecchymosis, oral cavity bleeding, epistaxis, and menorrhagia and 19% had experienced bleeding that occurred after trauma or drug injection. Sixty percent of afibrinogenemia or severe hypofibrinogenemia patients had more than one bleeding event per year.

The prevalence of umbilical bleeding at birth was also identical to values reported elsewhere but departs from the 32% reported in a third series. Occurrence of bleeding at venipuncture sites (65%), post traumatic muscular hematomas (41%) and postoperative bleeding (43%) were identical to values in an international study. In contrast, a higher incidence (72%) of muscle hematomas was reported in the Iranian series of 55 afibrinogenemia patients. Spontaneous CNS bleeding occurred in 10% of patients in the Iranian series, similar to 9% of spontaneous bleeding (one fatal) patients in this report. The incidence was twice higher (23%) in the international study.

However, the present report points to clear differences with other studies: median age was 20-year-old, with 60% of women being of child bearing age. In our series, median age is 10.5-year-old, with 30% of patients older than 18-year-old and striking differences are related to the age of the cases related here. Most of the cases are paediatric cases, possibly contributing to the lowest incidence of hemarthrosis (6% in this series vs. 25%, 54%, and 38% in the literature). Underreporting or unrecognized events such as bone cysts due to restricted access to healthcare in some areas might also contribute to the difference.

Age difference in the present series applies to gyanaecological and obstetrical issues. The general opinion is that women with afibrinogenemia have high rates of recurrent, spontaneous abortion (at around 5–8 weeks) or decreased fertility. In one series of 13 pregnancies in six
women with afibrinogenemia, seven (54%) ended in spontaneous abortion at 6–7 weeks gestation supporting requirement of fibrinogen for embryo implantation. Studies in KO mice models have demonstrated that fibrinogen plays a critical role in maintaining pregnancy, both by supporting normal development of foetal vascular communication and in stabilizing embryo implantation. The striking difference with the literature is the apparent absence of spontaneous miscarriage in this series. However, of the 20 affected females, only four were old enough to bear child and none actually tried. One died at age 23, the three others are unmarried. Thus, there is a clear bias in this series explaining the absence of obstetrical complications. Most women with afibrinogenemia were reported to suffer severe menorrhagia. In the present series, menorrhagia is reported in 3 out of the 4 (75%) cases of living female individuals older than 14-year-old.

### 4.2 Thrombotic events

Paradoxically, both arterial and venous thromboembolic complications have been reported in afibrinogenenic patients, including thrombosis in peripheral arteries and in cerebral and hepatic veins. Thrombosis events occurred in 6% of the paediatric population of the Casini’s series. Besides the genetic background that could explain for the thrombosis in two relatives (patients 19 and 21 in Table 4) or pulmonary embolism in FV Leiden carrying patient 34, several factors may contribute to thrombosis in patients with afibrinogenemia, such as increased thrombin activity, platelet activation, increased antifibrinolytic effect, unstable clot lacking fibrin. This leads to increased levels of other procoagulants, and could patient prothrombotic tendency.25

### 4.3 Thrombin generation

The TGT was an interesting approach to evaluate the balance between procoagulant and anticoagulant potential of patients. The defibrinogenation resulted in a decreased rate of thrombin generation in the residual fibrinogen-depleted plasma. These results are in agreement with those of Duchemin et al. and Szanto et al., showing a decrease of ETP in the absence of fibrinogen and that ETP increased with increasing fibrinogen levels.26,27 Our study shows that the decrease of thrombin generation (ETP) is less important in patients with personal thrombotic history than in patients without personal or familial thrombosis history. Using a ROC curve, we determined a cut-off for ETP to select all afibrinogenemia patients with personal history of thrombosis, but five other patients (two patients with familial thrombotic history and three without thrombotic history) have an ETP above the cut-off. The limitation of this result is the number of patients, and a larger cohort study is needed to validate this TGT as a predictor for thrombotic risk in afibrinogenenic patients. This is difficult in regard to the low prevalence of the disease. The clinical data are controversial for fibrinogen replacement therapy. It has been suggested that treatment with fibrinogen concentrates may be a risk factor for thrombosis. However, it should be noted that there is no clear evidence of a direct link between fibrinogen concentrate administration and the development of thrombosis.28 Rather, the TGT could identify the afibrinogenemic patients most likely to develop thrombosis, particularly during supplementation with FFP or fibrinogen.

### 4.4 Demographic characteristics and genetics

We observed a high prevalence of the FGA exon 2 c.117delT (in 7 [25%] unrelated families). We also observed multiple occurrences of FGB c.1427C > A and c.905G > A mutations. Otherwise, we evidenced private mutations. As a rule, FGB and FGG heterozygous mutations result in hypofibrinogenemia as a consequence of haploinsufficiency, whereas FGA mutations behave as recessive ones, that is, A-alpha chain gene mutations require two affected alleles either at the homozygous or compound heterozygous state to cause afibrinogenemia: because of A-alpha chain synthesis in excess relative to B-beta and gamma chains, heterozygous carriers of FGA mutations do not bear hypofibrinogenemia. Very few exceptions of dominant-negative FGA mutations are described.29–31 This study reports a fourth exception: in the relatives’ population, two heterozygous carriers of the A-alpha chain Ser581Ala fsX+48 truncation had fibrinogen levels of 1.25 and 1.75 g.L−1. The explanation relies on preferential association of Ser581Ala fsX+48 A-alpha chain with its normal counterpart during fibrinogen assembly and alteration of secretion of any mutant-containing molecule. Five false sense mutations in the FGB gene were evidenced (p.(Gly288Ser); p.(Gly278Glu), p.(Tyr299His), p.(Gly302Glu) and p.(Trp433Cys)). Taking into account either the occurrence in unrelated family, the monoclonal disease, or the description of other cases or substitution at the same residues reported in the GFHT database, the 5 variants were classified as “likely pathogen” or “pathogen” according to the ACMG criteria’s population.

During the period of survey (2000–2012) most diagnosed case in Algeria would have been referred to us. Some afibrinogenemia may have been missed because undiagnosed. However, assuming that the series reported here is close to exhaustive in the Algerian population, the overall prevalence is in the 1/1,000,000 range (the Algerian population estimate was 3.65 million as of 2012). However, because affected patients have a reduced life expectancy, we focused on the 23 cases under 13-year-old. Based on the 23 patients born between 2000 and 2012 and the 7,564,557 birth in Algeria in the same period (http://www.indexmundi.com/algeria/population.html accessed on 2nd May 2013), the calculated incidence of afibrinogenemia turns out to be 3 per million births. This high incidence likely results from Algeria’s high consanguineous marriage rate, in the 22%–30% range32,33; http://www.consang.net/index.php/Global_prevalence. However, it is lower compared to Iran (38% in a 74 million population34). Consanguinity was acknowledged in all but two families in the present series.

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designed the research study. Tristan Mirault performed the statistical analysis. Kahina Guenounou contributed to the biological analysis. Dominique Helley wrote the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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