Pseudohomozygous dysfibrinogenemia

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Abstract
Hypodysfibrinogenemia (HD) is a heterogeneous disorder in which plasma fibrinogen antigen and function are both reduced but discordant. This report addresses the key clinical question of whether genetic analysis enables clinically useful subclassification of patients with HD. We report a new case and identify a further eight previously documented cases that have the laboratory features of HD but biallelic inheritance of quantitative and qualitative fibrinogen gene variants. The cases displayed both bleeding and thrombosis and sometimes had undetectable fibrinogen activity. In all cases, the predicted effect of the coinherited variants is reduced levels of circulating fibrinogen that is all dysfunctional. We propose the term pseudohomozygous dysfibrinogenemia for this subtype of recessively inherited HD that is distinct from the more commonly recognized monoallelic HD caused by a single fibrinogen gene variant.

KEYWORDS
afibrinogenemia, bleeding, fibrinogen deficiency, hypodysfibrinogenemia

Heritable fibrinogen disorders have variable and overlapping clinical manifestations and are by convention subclassified according to the results of quantitative and qualitative fibrinogen testing of plasma. According to this classification, a concordant reduction of plasma fibrinogen antigen and function indicates the disorders afibrinogenemia and hypofibrinogenemia (Online Mendelian Inheritance in Man [OMIM] No. 202400) in which there are reduced circulating levels of fibrinogen. These disorders are usually associated with variants in the fibrinogen genes FGA, FGB, or FGG that predict failure of expression or secretion of the fibrinogen polypeptide and which are usually biallelic in afibrinogenemia and monoallelic in hypofibrinogenemia. In dysfibrinogenemia (OMIM No. 616004) plasma fibrinogen antigen is normal but function is reduced, usually because of an underlying monoallelic missense variant disrupting a functionally critical region of the fibrinogen polypeptide. A further disorder termed hypodysfibrinogenemia (OMIM No. 616004) is characterized by reduced fibrinogen function but also a less marked reduction of plasma fibrinogen antigen that may arise from abnormal assembly, secretion, or increased clearance of the fibrinogen chains. Here, we report a new case with a severe heritable fibrinogen disorder and review the literature to highlight that hypodysfibrinogenemia is genetically heterogeneous and includes a distinct subset of cases better classified as pseudohomozygous dysfibrinogenemia.
CASE DESCRIPTION

The index case is a 5-year-old boy (case 9; Figure 1A) with noncon-sanguinous, Caucasian parents born after an uncomplicated term pregnancy. He was asymptomatic until 17 months old when there was prolonged bleeding after removal of a 5-mm facial skin tag. His subsequent clinical course consisted of only mildly prolonged bleeding after minor trauma that responded to oral tranexamic acid and never required hospital treatment. Bleeding has never been severe enough to require fibrinogen replacement treatment. Plasma clotting times and Clauss fibrinogen activity (FGN-ACT) could not be evaluated because of failure to reach a fibrin clot formation end point (Figure 1B). Rotational thromboelastometry with the EXTEM reagent (thromboplastin-initiated clot formation) or the FIBTEM

| Case | PT  9.5-12.0 s | APTT 20-25 s | TT 15-22 s | RT 16-23 s | FIB-ACT 2.0-5.0 g/L | FIB-ATG 2.0-5.0 g/L | FGA genotype |
|------|--------------|-------------|-----------|-----------|-------------------|-------------------|---------------|
| 3    | 10.4         | 23.0        | NR        | NR        | 2.2               | 2.2               | p.Arg178*     |
| 4    | 13.7         | 24.8        | NR        | 32.6      | 0.5               | 3.63              | p.Arg35Ser    |
| 5    | 14.0         | 22.6        | 27.2      | 30.8      | 0.4               | 3.99              | p.Arg35Ser    |
| 6    | 13.7         | 23.4        | 26.8      | 31.5      | 0.4               | 3.85              | p.Arg35Ser    |
| 7    | 9.7          | 22.6        | 14.9      | 21.3      | 6.2               | 5.73              | normal        |
| 8    | 10.4         | 25.2        | 14.1      | 21.6      | 4.8               | 4.73              | normal        |
| 9    | >120         | >200        | >120      | >120      | <0.5              | 2.1               | p.[Arg35Ser + Arg178*] |

FIGURE 1  Characteristics of the study pedigree. A, The family relationships of the index case (9; arrowed) with gray and black symbols indicating the laboratory features of dysfibrinogenemia and hypodysfibrinogenemia, respectively. Below each symbol are indicated the Clauss fibrinogen activity (g/L) and the FGA genotype (+= normal sequence; H= p.Arg178* and D= p.Arg35Ser). B, Results of coagulation and fibrinogen tests. Plasma samples anticoagulated with 0.105 M trisodium citrate were analyzed using a Sysmex CA7000 coagulometer and Dade-Behring reagents according to manufacturer's instructions unless otherwise stated. C, Viscoelastometric analysis of plasma samples from case 9 and a healthy control performed using a ROTEM delta thromboelastometer (Tem International GmbH, Munich, Germany) with EXTEM and FIBTEM reagents. D, Light transmission aggregation of platelet-rich plasma from a healthy control and case 9 after stimulation with the stated reagents (thrombin receptor activating peptide, HART Biologicals, Hartlepool, UK; collagen, Takeda Pharmaceutical Company, Tokyo, Japan) using a PAP8 aggregometer (Alpha Laboratories, Hampshire, UK). APTT, activated partial thromboplastin time (Actin FS); FIB-ACT, Clauss fibrinogen activity (thrombin reagent; 33 μg/mL bovine thrombin); FGN-ANT, FIB-ATG fibrinogen antigen (Liaphen immunoturbidometric assay, Hyphen Biomed, Neuville-sur-Oise, France); NR, not recorded; PT, prothrombin time (Innovin); RT, reptilase time (4.8 BU batroxobin); TT, thrombin clotting time (Thromboclotin reagent; 1.25 U/mL bovine thrombin)
# Table 1: Characteristics of the pseudohomozygous dysfibrinogenemia cases

| ID | Qualitative variant | Quantitative variant | Clinical Phenotype | FGN-ACT 2.0–5.0 g/L | FGN-ATG 2.0–5.0 g/L | TT 15–19 s | RT 15–19% | Ref |
|----|---------------------|----------------------|--------------------|----------------------|----------------------|-------------|-----------|-----|
| 1. | FGA p.Arg35Cys      | Disrupts fibrinopeptide A cleavage site. | FGA p.Trp52* Aα chain truncation | 4 F | <0.10 | 0.31 | >120 | >120 | 15 |
| 2. | FGA p.Arg35Cys      | Disrupts fibrinopeptide A cleavage site | FGB p.Arg47* Aα chain truncation | 54 M | <0.16 | 1.6 | 65 | 240 | 16 |
| 3. | FGA p. Arg35His     | Disrupts fibrinopeptide A cleavage site. | FGA 11kB deletion (intron 1 to 3’UTR) Aα chain truncation | 23 F | <0.01 | 0.6 | 52.4 | >600 | 17 |
| 4. | FGA p.Gln347*       | AαC domain truncation; abnormal lateral protofibril aggregation. | FGA p.JVS4+1 G>T Aberrant splicing, frameshift, Aα chain truncation | 53 F | 0.3 | – | 46 | – | 18 |
| 5. | FGA p.Gln347*       | AαC domain truncation. Abnormal lateral protofibril aggregation. | FGA p.JVS4+1 G>T Aberrant splicing, frameshift, Aα chain truncation | 49 M | 0.3 | 0.5 | 52 | – | 18 |
| 6. | FGA p. His494fsTer23| AαC domain truncation; abnormal lateral protofibril aggregation. | FGA p.JVS4+1 G>T Aberrant splicing, frameshift, Aα chain truncation | 60 F | 0.59 | 1.84 | – | – | 19 |
| 7. | FGA p. Glu481LysfsTer3 | AαC domain truncation. | FGA p.JVS4+1G>T Aberrant splicing, frameshift, Aα chain truncation | 40F | Bleeding after dental extraction Postpartum bleeding | limited laboratory data reported |            |       |     |
| 8. | FGB p. Asp170Lys    | Abnormal thrombin-catalysed polymerisation | FGB c.1245-17_1262del or -16_1263del Aberrant splicing, frameshift, Bβ chain truncation | 31F | Postpartum bleeding | 1.05 | 1.24 | – | – | 11 |

Note: FGA, FGB, and FGG variants were identified from HGMD Pro (https://my.qiagendigitalinsights.com/bbp/view/hgmd/pro/start.php) and Clinvar (https://clinvarminer.genetics.utah.edu/) and through PubMed (https://pubmed.ncbi.nlm.nih.gov/; all URLs accessed April 26, 2021) using the search terms [hypodysfibrinogenaemia or hypodysfibrinogenemia]. Variants are annotated to the ENSEMBL transcripts ENST00000403106.8 and ENST00000302068.9.

*European Network of Rare Bleeding Disorders classification.

Abbreviations: FIB-ACT, Clauss functional fibrinogen activity; FIB-ATG, fibrinogen antigen; RT, reptilase clotting time; TT, thrombin clotting time.
reagent (thromboplastin initiate clot formation with cytochalasin D to eliminate the platelet component of clot strength) both showed no detectible clot formation, confirming absent fibrin formation (Figure 1C). Although these data initially suggested afibrinogenemia, the plasma fibrinogen antigen (FGN-ATG) was 2.1 g/L. Platelet-rich plasma from case 9 stimulated with 10 μM of thrombin receptor activating peptide or 10 μg/mL of collagen showed normal platelet aggregation consistent with the presence of fibrinogen in plasma (Figure 1D).

Analysis of the coding regions of all three fibrinogen genes from case 9 showed two different missense variants in FGA. The first variant was FGA c.103C>A, predicting a p.Arg35Ser substitution in the fibrinogen Aα chain and reported previously in multiple pedigrees with dysfibrinogenemia. FGA p.Arg35Ser was present as a monoallelic trait in the asymptomatic mother of the index case (case 4) and two of her siblings (cases 5 and 6) and segregated with prolonged prothrombin, thrombin, and reptilase times and with normal FGN-ATG, indicating dysfibrinogenemia (Figure 1A and B). The second variant in case 9 was FGA c.532C>T predicting the stop gain codon substitution p.Arg178* that predicts nonsense-mediated decay of the variant FGA mRNA. Homzygosity for p.Arg178* has previously been associated with afibrinogenemia, confirming that this variant prevents expression of the fibrinogen Aα chain. Within the study pedigree, monoallelic FGA p.Arg178* was also detected in the father of the index case (case 3), who had concordant FGN-ACT (2.2 g/L) and FGN-ANT (2.2 g/L) (Figure 1 plates A, B). Since the laboratory 95% reference intervals for FGN-ACT and FGN-ANT are 2.0–5.0 g/L in a healthy population, the predicted range in carriers of a fibrinogen null allele is approximately half of this (1.0–2.5 g/L). Thus, these measurements in case 9 are consistent with genotype.

2 | SURVEY OF PREVIOUSLY REPORTED CASES

To better understand the characteristics of cases with coinherited quantitative and qualitative fibrinogen gene variants, we surveyed the primary reports of fibrinogen gene variants deposited on Clinvar or the Human Gene Mutation Database (432 unique variants) and additional variants identified by searching PubMed and MEDLINE for cases with hypodysfibrinogenemia laboratory features (117 case reports or series). Case reports were evaluated further only if they harbored both quantitative and qualitative fibrinogen gene variants classified as pathogenic or likely pathogenic according to American College of Medical Genetics criteria and were confirmed to be monoallelic through testing of other pedigree members.

Eight cases from seven pedigrees were identified in the literature search (Table 1) harboring a total of 11 different fibrinogen gene variants. Five of the six qualitative variants were in FGA. Two of these (cases 1-3) predicted disruption of the fibrinopeptide A cleavage site, thereby preventing generation of fibrin monomers. Three qualitative FGA variants (cases 4-8), predicted partial truncation of the αC domain of the fibrinogen Aα chain thereby disrupting lateral aggregation of fibrin protofibrils during polymerisation. The remaining qualitative variant (case 8) was a FGB missense variant shown experimentally to alter fibrin polymerization but through an unknown mechanism. The co-inherited quantitative variants were either stop gains (cases 1 and 2) or were predicted to disrupt transcript splicing (cases 3-8) and in all but two cases have also been reported as homozygous traits in cases with afibrinogenemia. The eight cases had a median age of 45 years and comprised six women. Clinical features included surgical, traumatic, postpartum and heavy menstrual bleeding that was typically moderate or mild. Arterial thrombosis was reported in three cases, although in one case it was associated with multiple acquired atherothrombotic risk factors (Table 1). Venous thrombosis was reported in two cases after surgery or postpartum. In the seven cases for which laboratory data were reported, the FGN-ATG was below or near the lower end of laboratory reference intervals but in all the cases there was a greater reduction in FGN-ACT, indicating hypodysfibrinogenemia (Table 1). In cases 1 through 3, FGN-ACT was below the lower limit of assay detection, and clotting times were unrecordable, which was similar to the new case identified in our clinic.

3 | CONCLUSION

The data presented in this report are consistent with a biallelic disease model in which there is absent expression of one fibrinogen allele but preserved expression of abnormally functioning fibrinogen from the other allele. Although this results in hypodysfibrinogenemia laboratory features, this represents a disease mechanism that is distinct from most previously reported cases of autosomal dominant hypodysfibrinogenemia in which there is a single underlying fibrinogen gene variant resulting in diminished secretion and function of the product from that allele.

Since the circulating fibrinogen polypeptide is a heterohexamer comprising three pairs of fibrinogen Aα, Bβ, and γ chains, the biallelic genotype of the cases in this series predicts that the total level of circulating fibrinogen is partially reduced but also that all of the residual circulating fibrinogen polypeptide will be dysfunctional because only the variant allele is expressed. The consequence of this is best illustrated by the new case from our clinic and cases 1 through 3 in the literature series in which the qualitative variants at codon 35 of the fibrinogen Aα chain prevent fibrin polymerization by disrupting thrombin-mediated cleavage of fibrinopeptide A. In the relatives with these qualitative variants in monoallelic form, circulating fibrinogen is expected to be a mixture of both substituted and normal hetero-hexamers, enabling partial fibrin polymerization and resulting in a mild defect in fibrinogen function. However, in the cases with a co-inherited quantitative variant, the remaining circulating fibrinogen was highly dysfunctional and unable to form fibrin in any of the functional assays, although it did enable platelet aggregation, which is
mediated by fibrinogen monomer as well as fibrinogen and fibrin. These laboratory features are reminiscent of the ultra-rare disorder homozygous dysfibrinogenemia in which all circulating fibrinogen is dysfunctional because of biallelic qualitative variants but with additional reduction in total circulating fibrinogen. In contrast to patients with heterozygous dysfibrinogenemia, we further predict that in settings such as pregnancy or inflammation, patients with pseudohomozygous dysfibrinogenemia will not display increased levels of functional fibrinogen because of the presence of a null allele, thereby remaining at risk of bleeding or thrombotic manifestations.

In view of this distinctive molecular pathogenesis, we propose the term pseudohomozygous dysfibrinogenemia for the disorder described in this report and suggest that this be considered as a subgroup of hypodysfibrinogenemia in the proposed phenotype-driven classification of fibrinogen disorders proposed by the ISTH. It is noteworthy that the described cases have a wide range of clinical disease severity but include examples of bleeding and arterial and venous thrombosis similar to some rare cases with afibrinogenemia, dysfibrinogenemia, or monoallelic hypodysfibrinogenemia. The key features that distinguish pseudohomozygous dysfibrinogenemia from other heritable fibrinogen disorders remain autosomal recessive inheritance and the laboratory features of discordant reductions in quantitative and qualitative fibrinogen tests.

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RP contributed to the study design, initial search and data acquisition, data interpretation, and drafting of the manuscript. LF contributed to the initial search and data acquisition and drafting of the manuscript. CR-S and CD performed coagulation testing. EP contributed to study design and data acquisition. AM contributed to the study design, initial search and data acquisition, data interpretation, and drafting of the manuscript. All authors approved the final manuscript.

RELATIONSHIP DISCLOSURE
The authors declare no conflicts of interest.

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