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Hypofibrinogenaemia resulting from novel single nucleotide deletion at codon Bbeta58 (3404del A) associated with Thrombotic Stroke in infancy

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Case Report

Hypofibrinogenemia resulting from novel single nucleotide deletion at codon B β 58 (3404del A) associated with thrombotic stroke in infancy

Classical afibrinogenemia is an autosomal recessive disorder characterized by the complete absence of circulating fibrinogen and is caused by homozygous or compound heterozygous mutation of one or more of the three fibrinogen genes. The condition is usually detected at birth by uncontrolled bleeding from the umbilical cord and, while spontaneous intracerebral hemorrhage and splenic rupture can occur at any time, it is not unusual for individuals to present with thrombosis or transient cerebral ischemia (1, 2). Recurrent spontaneous abortion (3) is another common feature of the condition, and heterozygotic family members usually display hypofibrinogenemia.

Hypofibrinogenemia is characterized by low levels of fibrinogen, and both bleeding and thromboembolic disease occur in this condition, although generally to a milder extent than in afibrinogenemia (4, 5). Most cases result from heterozygous null (large deletions, frameshift, nonsense, or splice-site) or missense mutations, each causing a total lack or major decrease of the corresponding chain in plasma fibrinogen molecules (5–7). In this report, we describe a family with a unique mutation in the fibrinogen β gene resulting in hypofibrinogenemia in affected members of the kindred. The proposita developed a thrombotic stroke in her infancy, but other family members have been asymptomatic.

Case report

The proposita, a twenty-year-old female, was referred for diagnostic workup at the age of twelve because of a history of recent bruising over her lower extremities. At the age of fourteen months she had experienced sudden onset of seizure activity concomitant with a thrombotic stroke that was diagnosed on the basis of multiple imaging studies, and characterized by a moderately dense left hemiparesis that resolved slowly but completely. Except for occasional seizure activity beginning at age eight, she had been well and had experienced no unusual bleeding or bruising prior to this episode. Hematological workup at the time revealed a low functional and antigenic fibrinogen (Table 1) to-

gether with a slight prolongation of the prothrombin time (13.3 s), the partial thromboplastin time (38.5 s) and the thrombin time (25.8 s). Assessments of antithrombin III, protein C, protein S, the factor V Leiden mutation, including screening for von Willebrand's disease were either negative or normal. The proposita's father and brother also had low fibrinogen levels (1.57 and 1.50 g/l, respectively) and both were found to carry the genetic defect (Table 1). However, they have been asymptomatic to date. Her mother had a normal fibrinogen level of 2.88 g/l, does not carry the fibrinogen gene mutation, but had been previously diagnosed with von Willebrand's disease. She has experienced excessive bleeding during elective facial surgery. The maternal grandmother suffered a thrombotic stroke at age 73, and a paternal grandmother suffered a cerebrovascular event at age 63, however no blood was available for further gene or coagulation studies.

Examination of purified plasma fibrinogen by both reducing and non-reducing SDS PAGE showed only a normal pattern of bands. However direct sequence analysis of the three fibrinogen genes established that the proposita was heterozygous for two novel mutations; a single nucleotide (a) deletion at codon 58 of the B β chain (3404del A) and a four nucleotide deletion of aata in intron eight of the γ gene (7769–7772del aata). Both of these mutations segregated with hypofibrinogenemia in the father and brother and were absent from normal family members and controls. The three carriers of these deletions were also heterozygous for the A α 312 Ala polymorphism while normal family members were homozygous for the more common A α 312 Thr variant indicating that the B β and γ deletions occur on an allele encoding Ala at position A α 312 (Table 1).

Since the B β 3404del A and γ 7769–7772del aata deletions occur on the same allele, the question arises as to which one actually causes the disease. The former is fully capable of doing so as the deletion and consequent frame shift occurs at codon B β 58 and result in a unique non-fibrinogen sequence and premature chain termination forty-one residues later (Fig. 1). This prevents formation of the disulphide rings that are critical for the

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Table 1: Fibrinogen level and genotype of family members.

	Proposita	Father	Mother	Sister	Brother
Clauss fibrinogen (g/l) (normal 1.50–3.50)	1.13	1.57	2.55	2.88	1.50
Immunologic fibrinogen (g/l) (normal 1.50–3.50)	1.25				
β mutation, 3404delA	+	+	-	-	+
γ mutation 7769–72del aata	+	+	-	-	+
A α 312 polymorphism	Ala/Thr	Ala/Thr	Thr/Thr	Thr/Thr	Ala/Thr

formation of the coils that link the central E region to the peripheral D regions; hence no viable molecules can be assembled from this translation product. Although the γ intron eight aata deletion is well separated from the intron boundaries, it could still impact on mRNA processing by creating a new 5' splice site with in the intron. Whilst a new ag sequence is created by deletion of the aata sequence (aaaaaataaagtagata), the resulting acceptor lacks the required 5' polypyrimidine track and was not recognised by splice-site prediction programs as a potential site (8).

Whilst it is clear that the B β gene codon 58 frame shift caused the hypofibrinogenemia, the basis for the thrombotic stroke in infancy is less obvious. Other than hypofibrinogenemia, the proposita was free of known risk factors, and it seems therefore probable that hypofibrinogenemia contributed to the stroke, perhaps as a consequence of a reduced thrombin binding potential by fibrin (antithrombin I) (4). An additional predisposing factor might be that the mutation is inherited together with the rarer A α 312Ala polymorphism which has been associated with altered clot structure (stiffer clots with increased A α chain cross-linking) (9) and increased risk of embolism in both the arterial (10) and venous systems (11). However, other unknown genetic or environmental factors may also be involved since neither the af-

	58
normal	E R K A P D A G G C L H A D P D L G V L C P T
base seq:	tagaa agt a agg ccc ctg atg ctg gag gct gtc ttc acg ctg acc cag acc tgg ggg tgt gtc cta cag
variant	E R K P L M L E A V F T I T Q T W G C C V L Q
normal	G C Q L Q E A L L Q Q E R P I R N S V D E L
base seq:	gat gtc agt tgc aag agg ctt tgc tac aac agg aaa ggc caa tca gaa ata gfg tfg atg agt taa
variant	D V S C K R L C Y N R K G Q S E I V L M S STOP

Figure 1: Consequences of single adenosine deletion in exon two of the β gene. The deletion of one of the four adenosines (boxed) between nucleotides 3404 and 3407 results in a frame shift at codon 58 and the transcription of 41 irrelevant residues before an in-frame taa stop codon is encountered.

ected father or brother have experienced thromboembolic events.

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