Fibrinogen is an essential component of the coagulation cascade and the acute phase response. The native 340 kDa molecule has a symmetrical trinodular structure composed of a central E-domain connected to outer D-domains by triple helical coiled-coils. Several mutations known to cause hypofibrinogenemia occur within the C-terminal gammaD-domain and have helped to elucidate the structurally and functionally important areas of this domain.

Here we report the identification of a novel point mutation gammaG200V (fibrinogen Columbus) causing hypofibrinogenemia and cosegregating with three genetic thrombophilia risk factors.

The proband is a two year old boy (III.3; Figure 1). He and his deceased twin (III.4; Figure 1) were both found to be hypofibrinogenemic with a Clauss fibrinogen of 0.3 g.L-1 at birth. Twin III.4 was noted, post-partum, to have seizure-like activity. An MRI revealed subdural and subarachnoid hemorrhaging as well as diffuse areas of hypoxic ischemia. Both III.3 and III.4 showed a prolonged thrombin clotting time (TCT), positive D-dimer reactivity and heterozygosity for the factor V Leiden mutation. III.4 was developmentally challenged and his neurological function continually deteriorated, resulting in death at 7 months of age due to intracranial thrombotic and hemorrhagic events. III.3 was asymptomatic post-partum and has been developmentally normal to date. The proband’s female sibling (III.1; Figure 1) is also heterozygous for the factor V Leiden mutation and has raised levels of circulating D-dimers.

The father (II.3; Figure 1) was genotypically and phenotypically normal. The mother (II.4; Figure 1) has a history of deep vein thrombosis (DVT) and heparin was administered prophylactically for her first pregnancy (III.1) and for the birth of the twins (III.3 and III.4). She displays borderline hypofibrinogenemia with a prolonged TCT (32 seconds; control 15 seconds), heterozygosity for the factor V Leiden and MTHFR C677T polymorphic mutations. The maternal grandmother (I.2; Figure 1) has a history of DVT, increased bleeding during surgery, and is heterozygous for the factor V Leiden mutation and has raised levels of circulating D-dimers.

DNA sequencing revealed a heterozygous GCC→GTC mutation in exon 7 (c. G677T) of the fibrinogen γ gene (FGG). This novel γ200Gly→Val mutation was found in both II.4 and III.3 but was absent in II.3. Further screening for the three haplotype-tagging single nucleotide polymorphisms (htSNPs) of the FGG-H2 haplotype revealed homozygosity (H2H2) in II.3, heterozygosity (H2Hx) in III.3 and a wild type genotype (HxHx) in II.4 (Figure 1).

Normal patterns of fibrinogen chains were seen under reducing and non-reducing SDS-PAGE and chain separation by reverse-phase HPLC showed a normal pattern of peaks. Further analysis of the separated γ chains by electrospray ionisation mass spectrometry (ESI-MS) indicated a normal mass (48,374 Da) of the γ chain compared to a control value of 48,375 Da. This indicates no expression of the variant chain in plasma fibrinogen. The γ200Gly→Val mutation appears to be the direct cause of hypofibrinogenemias. It segregates with low functional and physical fibrinogen levels in the affected individuals (II.4 and III.3). This substitution has not been reported as a polymorphism and was not detected by us in 50 normal subjects. Sequence alignment of all fibrinogen chains and fibrinogen related proteins shows that the affected γ200 glycine residue is totally conserved. Absolute conservation between functionally unrelated proteins and chains suggests that this residue is structurally, rather than functionally
tionally, important. Residue 200 is situated just after the first strand of the five stranded β-sheet of the γD-domain in an external bend, 3.74 Å from the side chain of tyrosine 348 (Figure 2). Mutation to valine may result in a conformational change in the area due to bulk or hydrophobicity, in turn compromising molecular integrity.

The factor V Leiden mutation (and to a lesser extent MTHFR C677T) is commonly associated with thrombophilia. Thrombosis was evident with I.2 and II.4, who both experienced DVT. Subject III.4 had an intracranial sinus thrombosis and diffuse hypoxic ischemia. The FGG-H2 haplotype has recently been reported as an additional risk factor for thrombosis and involves three SNPs located in intron 8 (7874 g→a), intron 9 (9615 c→t) and downstream of the polyadenylation site for exon 10 (10034 c→t) of FGG. The 10034 c→t htSNP is likely to result in decreased production of the alternately spliced γ prime chain, which has been reported to increase the risk of deep venous thrombosis.7 The γ prime chain itself is a major component of antithrombin I (fibrin) which is an important inhibitor of thrombin generation in clotting blood.8,9

In conclusion, the γG200V mutation found in two of the three tested individuals is the cause of hypofibrinogenemia and may contribute to the cumulative effect of other (factor V Leiden, MTHFR C677T and FGG-H2) thrombophilic mutations in this kindred.

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