Fibrinogen Columbus: a novel gamma Gly200Val mutation causing hypofibrinogenemia in a family with associated thrombophilia

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.[1] 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.[2–5] Here we report the identification of a novel point mutation gammaG200V (fibrinogen Columbus) causing hypofibrinogenemia and co-segregating with three genetic thrombophilia risk factors.

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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 postpartum 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 MTHFR C677T mutation.

DNA sequencing revealed a heterozygous GGC→GTC mutation in exon 7 (c. G677T) of the fibrinogen γgene (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)[6] 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

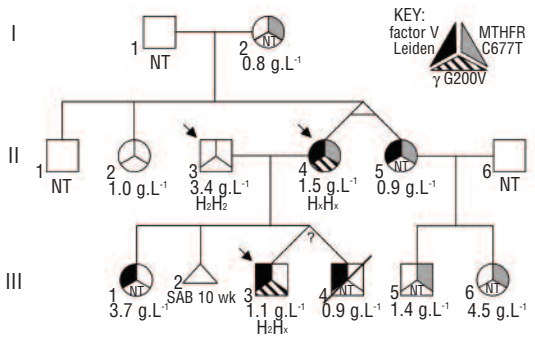

Figure 1. Genetic pedigree showing heterozygosity for two thrombophilic mutations (factor V Leiden and MTHFR C677T) and the novel fibrinogen mutation (γ Gly200Val). (→) Denotes individuals for whom DNA and plasma were available for fibrinogen gene analysis. Values below individuals are Clauss fibrinogen concentration (g.L^{-1}) and FGG-H$_2$ haplotypes for tested individuals (H$_2$H$_2$: homozygous; H$_2$H$_x$: heterozygous; H$_x$H$_x$: wild type). NT: not tested.

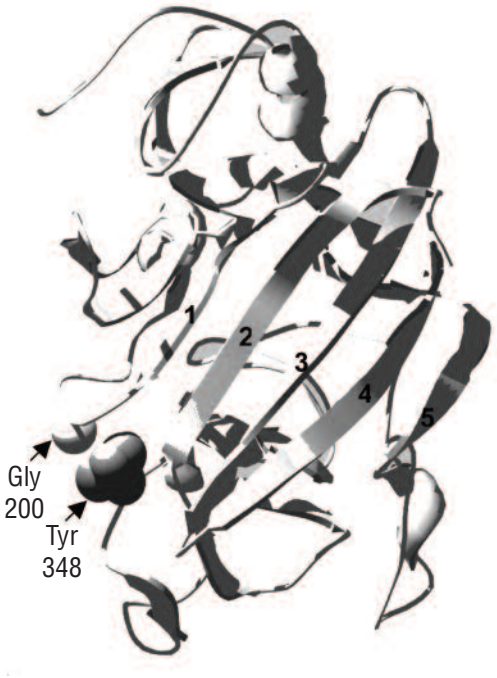

Figure 2. Crystal structure of the γD domain showing Gly200 just after the first strand of the five stranded β sheet (1) and in close association with Tyr348. The side chain is solvent exposed, so the hydrophobic valine is likely to be poorly tolerated at this position. Furthermore, the close proximity of the bulky valine side chain to the side chain of tyrosine 348 is likely to destabilise the tertiary structure.

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 func-

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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References

1. Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. Annals of the New York Academy of Sciences 2001;936:11-30.
2. Everse SJ, Spraggon G, Doolittle RF. A three-dimensional consideration of variant human fibrinogens. Thromb Haemost 1998;80:1-9.
3. Brennan SO, Fellowes AP, George PM. Molecular mechanisms of hypo- and afibrinogenemia. Ann NY Acad Sci 2001;936:91-100.
4. Matsuda M, Sugo T. Hereditary disorders of fibrinogen. Ann NY Acad Sci 2001;936:65-88.
5. Maghzal GJ, Brennan SO, Homer VM, George PM. The molecular mechanisms of congenital hypofibrinogenaemia. Cell Mol Life Sci 2004;61:1427-38.
6. Brennan SO. Electrospray ionisation analysis of human fibrinogen. Thromb Haemost 1997;78:1055-8.
7. Uitte de Willige S, de Visser MC, Houwing-Duistermaat JJ, Rosendaal FR, Vos HL, Bertina RM. Genetic variation in the fibrinogen γ gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen γ levels. Blood 2005;106:4176-83.
8. Mosesson MW. Antithrombin I. Inhibition of thrombin generation in plasma by fibrin formation. Thromb Haemost 2003;89:9-12.
9. Siebenlist KR, Mosesson MW, Hernandez I, Bush LA, Di Cera E, Shainoff JR, et al. Studies on the basis for the properties of fibrin produced from fibrinogen-containing γ chains. Blood 2005;106:2730-6.