A homozygous duplication of the *FGG* exon 8-intron 8 junction causes congenital afibrinogenemia. Lessons learned from the study of a large consanguineous Turkish family

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Supplementary Methods for Guipponi et al.

Fibrinogen measurements:

After local measurements, platelet poor plasmas were sent to the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center Milan, Italy, for confirmation of diagnosis. Fibrinogen coagulant activity (Fg:C) was measured by a functional assay using Fibrinogen C (Instrumentation Laboratory) as a reagent on a coagulation analyzer ACL-Top according to Miesbach et al. (1). An in-house developed enzyme-linked immunosorbent assay (ELISA) with a sensitivity of 0.05 mg/dL was used to measure the antigen fibrinogen levels (Fg:Ag). The normal range for both assays was 160–400 mg/dl (2).

Primer sequences for identification of the duplicated sequence:

A forward primer located in *FGG* intron 7: FGGi7F2: 5'AGTGCTATAGTCAGTAGACCT3'and a reverse primer located in the 3'UTR portion of *FGG* exon 10: FGG3'R 5'GCTTTGCAAGTCCATTGTCC3' were used.

RT, PCR amplification of cDNAs for identification of splicing variants.

cDNAs, normal and mutant, were obtained from transfected cell total RNA extracts (1 microgram) using M-MLV Reverse Transcriptase and Oligo (dT)15 primer from Promega according to the manufacturer's protocols. PCRs were performed on cDNAs using the previously described oligonucleotides FGGi7F2 and FGG3'R, to amplify products with exon 10 corresponding to the major γ transcript, and FGGi7F2 and FGGstopR 5'CAAATCATCCTCAGGGTAAAG3', in the exon 9 sequence specific to γ' transcripts. PCR products were visualised on agarose gels and the main products were purified and sequenced using a primer located closer to FGG exon 8: FGGx8L1 5'TTCCAAGGAAGCATCCTACGA3'. Since additional minor bands were visible for each PCR, products were cloned using the Dual Promoter pcRII TOPO TA cloning kit (Invitrogen) and clones individually sequenced.

References:

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