

Why dysfibrinogenae-mias still matter

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Con-genital abnormalities of fibrinogen that result in functional defects have been important in discoveries of structure-function relations for this protein and correlations with thrombosis and bleeding, and more than 450 such so-called dysfibrinogenae-mias have been identified, although most have only been characterised very superficially. Much is now known about fibrinogen, including the atomic level structure of large parts, but not all, of the molecule and the functions of some domains (1–3). Some basic molecular mechanisms of fibrin polymerisation have been discovered, although little is known about lateral aggregation and branching of fibers, for example. So, with all of this information, why should clinicians or basic scientists care about newly identified dysfibrinogenae-mias? A paper by Kotlín et al. published in this issue of *Thrombosis and Haemostasis* provides one answer (4).

Hereditary dysfibrinogenae-mias are caused by a variety of mutations in the three fibrinogen genes that code for its polypeptide chains and may be uncovered in patients who present with thrombosis or bleeding or may be discovered by routine coagulation testing (5, 6). Mutations can result in quantitative defects, such as afibrinogenae-mia or hypofibrinogenae-mia or qualitative defects resulting in functional deficiencies in the protein's activities or a combination of both. Studies of afibrinogenae-mias have given us data about the synthesis, assembly, and secretion of fibrinogen (7–9), while investigation of qualitative dysfibrinogenae-mias has provided a great deal of information on fibrin polymerisation, fibrinolysis, platelet aggregation, and other functions (2, 10, 11). Dysfibrinogenae-mias can also give us clues about relationships between molecular structure/function and clinical consequences (5, 6).

Unfortunately, these conclusions do not usually come easily. Most individuals are heterozygous for the mutation and hence have some normal fibrinogen chains, and effects of the mutations can be confused by accompanying altered concentrations of fibrinogen in the blood. Furthermore, there are dysfibrinogenae-mias for which several subjects are known with the identical mutation and yet very different clinical outcome, such as bleeding versus thrombosis. Most likely, the observed clinical consequences are not the direct result of this mutation at all but arise

from some other genetic and/or environmental causes. In other cases, it could be that the mutation does have a contributing effect but the overall consequences arise as a result of other aspects of the coagulation system, such as the well known factor V Leiden or prothrombin G20210A mutations, or several single nucleotide polymorphisms with additive effects, together with lifestyle factors (12).

The paper by Kotlín et al. (4) presents two new dysfibrinogenae-mias, fibrinogen Praha III with a γ Y363N mutation and fibrinogen Plzen with an A α N106D mutation. Both patients presented with thrombotic problems, and both mutations have major effects on fibrin polymerisation, fibrinolysis, and platelet aggregation. It seems likely that the tendency toward thrombosis of these patients may arise from the clot structure consisting of very thin fibers and the resulting delayed fibrinolysis. The γ 363 tyrosine is an important part of hole 'a', which is the binding pocket into which fits knob 'A' exposed as a result of thrombin cleavage of fibrinopeptide A. The A α 106 asparagine is in the coiled-coil region near the plasmin-sensitive hinge region and part of one of the RGD sequences.

Quite a lot is known about the structure and chemistry of hole 'a', yet Praha III is the first dysfibrinogenae-mia identified with a mutation of tyrosine 363, which is highly conserved and has been shown to be important in binding of the GPRP peptide that mimics knob 'A', so it is useful to learn the functional effects of this mutation. The effects of this mutation were characterised and the structural changes in the binding pocket are described using an atomic level model of GPRP interactions. The replacement of the bulky aromatic side chain of tyrosine by the small polar uncharged side chain of asparagine could possibly alter the conformation of the binding pocket. Their results of fibrin polymerisation with thrombin and reptilase together with what is known of hole 'a' indicate that the thin fibers they observed are likely not a defect of lateral aggregation of protofibrils but an effect of defective oligomer formation. Questions remain. For example, how could such a mutation affect platelet aggregation? Similarly, why is the extent of phosphorylation of A α serine 3 affected by this mutation?

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The fibrinogen Plzen mutation in the coiled-coil region appears to have two functional effects, defective fibrin polymerization and platelet aggregation. This mutation likely disrupts the coiled-coil conformation because of the substitution of an uncharged residue with an acidic one. It has been proposed that the middle of the coiled-coil acts as a hinge and may be important in polymerisation (3, 13). The results presented here seem to point to the importance of the hinge in oligomer formation. Since this substitution is near one of the major cleavage sites by plasmin, its effect on fibrinolysis observed in these studies is understandable. Also, this mutation is near an RGD sequence, which in most adhesive proteins is involved in binding to integrins. In the early 1990s, evidence from two sources indicated that the γ chain dodecapeptide of fibrinogen, and not either of the RGD sequences, was necessary and sufficient for platelet aggregation via binding of fibrinogen to the integrin α IIb β 3. Structural studies showed that α IIb β 3 bound to the ends of fibrinogen, where the γ chain dodecapeptide is located, and not the parts of fibrinogen containing either RGD site (14). In addition, both RGD sequences were mutated with no effect on platelet aggregation, while mutation of the C-terminal γ chain dodecapeptide of fibrinogen completely abrogated platelet aggregation (15). Therefore, the findings of this new paper are significant in that they could imply that this

RGD sequence may in fact have some as yet unknown role in platelet aggregation.

More research is needed to understand the functional consequences of these mutations and address some unresolved issues. Experiments with heterozygous mutations are often difficult to interpret, because there is commonly a mixture of normal and abnormal fibrinogen chains. With some dysfibrinogenemias, recombinant homodimers have been made, to avoid the difficulties of interpreting experiments with heterodimers or mixtures of two forms of homodimers that occur with heterozygous mutants. Such experiments with homogeneous recombinant fibrinogens have yielded very interesting results that can be more easily turned into clear structure-function relations (11, 16). Importantly, X-ray crystal structures of some of these mutants have been obtained, demonstrating that the mutation did not affect the molecular structure and that the observed functional effects can be attributed to the amino acid substitution itself. Yet, with all of the limitations of these initial experiments with naturally occurring dysfibrinogens from patients, this new paper demonstrates that dysfibrinogenemias can still be interesting and provide clues to structure-function relations and significant correlations with clinical consequences.

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