A new method to detect causative mutations in fibrinogen.

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Fibrinogen plays a central role in coagulation and therefore has been studied in great detail since its discovery as coagulation’s factor I. Structure-function studies have deciphered minute details on how this molecule works, and the extend of the studies is not surprising, as fibrinogen contains all the “knobs”, “holes”, loops and binding sites to interact with the machinery that activates the formation of the clot mesh, while also the ability to eliminate it by controlled proteolysis (1). The result of this research provides a picture of one of the most astonishing molecular machines in biology: a soluble protein that is capable of forming an enormous polymeric network that confers the capacity to stop bleeding. Fibrinogen does this task with efficacy and also with elegance by rapidly forming dimers and then oligomers that pack in protofibrils and fibrils to then branch and form the network that nests platelets and erythrocytes, bringing consistency and flexibility to the clot and avoiding the loss of blood from an injured vessel, which could otherwise be lethal in a short time. Fibrinogen is so admirably fitted for its function that structure/function studies are especially rewarding in terms of how they help us to understand the way this molecule works.

A field of research that has been more fruitful in this regard is the study of naturally occurring mutations that are detected in the three genes responsible of the synthesis of fibrinogen. In Thrombosis and Haemostasis, several recent studies are
good examples of this research. For a long time it has been known that some common genetic variants in are correlated with fibrinogen levels (2). These variants could define the concentration of plasma fibrinogen, and they correlate with hematological parameters. Still, a full span of effects on fibrinogen could be observed, from functional defects without affecting the total amount of fibrinogen to the complete or almost complete absence of fibrinogen in plasma in cases of afibrinogenaemia (3). Because most mutations are found in patients and/or families suffering from diseases of haemostasis, they allow us to study the relationship of the detected genetic variants with their manifestation in a given phenotype. Although most mutations in fibrinogen manifest as an increased bleeding tendency, some mutations are associated with thrombophilia, i.e. an increased risk of thrombus formation. This is due to the subtle relation between structure and function that occurs in the fibrinogen molecule, where a mutation in specific residues could affect not only its synthesis but also the formation of the polymer, its stability, or the capacity of the fibrinolytic system to degrade it. For instance, fibrinogen Perth causes a partial deletion in the αC domain and codes for a free cysteine that is associated with tighter and thinner clots and impaired fibrinolysis, resulting in familial thrombophilia (4). Similar results are observed in mutations affecting the tPA binding site associated with thrombosis, (5), but also in mutations where the main effect seems to be structural rather than regulatory (6). An interesting addition to the array of effects studied is provided by a mutation in the coiled-coil module of Aα chain, that was shown to affect platelet aggregation (7). The calcium binding site affected in the fibrinogen Tolaga Bay mutation showed an effect on the stabilization of the clot through delaying the factor XIIIa-catalysed γ and α chain cross-linking (8). Interestingly, Park et al show that the direct mutation of one of the factor XIIIa crosslinking acceptor sites (fibrinogen Seoul II) affected polymerization but alternative sites could substitute for factor XIIIa crosslinking (9). Finally, the study of Marchi et al shows that some fibrinopathies could manifest with bleeding and thrombotic episodes (10).
Although advances in DNA sequencing technology have facilitated the identification of numerous mutations in hereditary dysfibrinogenaemia, one of the persisting problems is to determine the genetic changes that are really the molecular basis of a disease is not mutation detection, but deciding whether or not an identified sequence variation is responsible for the disease phenotype. In the present issue of Thrombosis and Haemostasis, the study of Brenner et al describes a simple method to analyze mutations in fibrinogen with extreme simplicity. The method uses a small amount of blood processed through HPLC and time of flight (TOF) mass spectrometry. To test the methodology, they analyzed a patient with hypofibrinogenaemia where they could detect three mutations associated with three mass changes in the Bβ and γ chains. They could determine the relative amount of protein derived from each allele, and, together with modelling information and sequence preservation, determine which is the mutation causative of hypofibrinogenaemia in the patient.

New technologies as the one described here will facilitate the faster determination of mutations and its correlation with protein data, providing new insights on how the different parts of fibrinogen function. Also this research will allow us to continue admiring the sheer beauty of this superb molecule.

References


