Editorial Focus

Genetic variation in the fibrinogen gene cluster

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Elevated plasma levels of fibrinogen have been identified as a cardiovascular risk factor in a number of epidemiological studies with a range of endpoints, such as acute myocardial infarction and ischemic stroke (1). The underlying mechanism for this association is still unclear. One explanation for the association can be found in the acute phase character of fibrinogen. Inflammation is an important mechanism in cardiovascular disease and plasma fibrinogen levels will reflect the level of vascular inflammation. However, the fibrinogen and fibrin that are present in the atherosclerotic plaque can directly contribute locally to the progression of atherosclerosis by increasing the chemotaxis of smooth muscle cells (2), and affecting the structure and stability of the plaque matrix (3).

When we accept that fibrinogen and fibrin causally contribute to the progression of cardiovascular disease, it is important to know the mechanisms that determine the plasma levels of fibrinogen. A large number of environmental and life-style factors have been identified that are associated with the plasma fibrinogen concentration, such as age, gender and diet (4). In addition, twin studies and family studies have shown that a substantial part of the fibrinogen concentration is determined by genetic factors, with estimates varying from 27% to 51% (5, 6). These genetic factors include polymorphisms in the fibrinogen genes, but also polymorphisms in genes that regulate the fibrinogen synthesis may contribute.

Of primary interest are polymorphisms in the coding region that result in an amino acid substitution because these may alter the function of the protein. Indeed, the Thr312Ala (rs#6050) polymorphism in the Aα-chain and the Lys478Arg (rs#4220) in the Bβ-chain have been identified and the Thr312Ala polymorphism has been associated with the fibrinogen clotting characteristics and the structure of the fibrin clot (7, 8). The Aα-chain Thr312Ala polymorphism is also associated with risk of venous thrombosis (9).

To study an association between polymorphisms and levels of fibrinogen, the obvious first region of interest is the promoter region of the fibrinogen β-gene that was associated with plasma fibrinogen levels and with the biological variation of the fibrinogen levels. This was the BclI (6638ins28) polymorphism in the 3′ region of the gene. It soon became clear that this polymorphism was in almost complete linkage disequilibrium with the -455G/A polymorphism, first known as the HaeIII polymorphism (rs#1800790), and subsequent research focused on the promoter polymorphism. A large number of studies have since then studied this polymorphism and the relation with fibrinogen levels and many, but not all, studies confirmed the presence of this relationship (6). Some studies were also performed with other polymorphisms in the promoter region of the fibrinogen β-gene (-148C/T [rs#1800787], –249C/T [rs#1800788], –854G/A [rs#1800791], –993C/T [rs#2227389], –1420G/A [rs#1800789]), but since these polymorphisms are in almost complete linkage disequilibrium with the –455G/A polymorphism in Caucasians, the results were similar (6).

After the first studies suggested that the synthesis of the Bβ-chain was rate-limiting for fibrinogen synthesis, other studies have shown that also the synthesis of the other fibrinogen chains affects the fibrinogen levels (12). However, polymorphisms in the promoter of the α and γ genes are hardly studied.

Although a substantial number of polymorphisms have been identified in the fibrinogen genes, and although a number of them have been associated with fibrinogen levels, they explain only a minor part of the total variance of the fibrinogen levels. A genome-wide search for genes affecting fibrinogen levels in the Framingham Heart Study did not identify the 4q28 region that harbors the genes encoding the Aα, Bβ and γ chains of fibrinogen (13). In this study, the highest LOD scores were seen on chromosomes 2 and 10, but no genes that are known to regulate fibrinogen have been identified on those chromosomal segments.

Many studies find an association between polymorphisms in the fibrinogen genes and plasma fibrinogen levels, but in most studies there was no association between fibrinogen β-gene polymorphisms and risk of cardiovascular disease (14, 15). However, the studies on the relationship between polymorphisms in the fibrinogen genes and cardiovascular disease are hindered by the fact that until now, only a few polymorphisms in...
the region that spans the 3 fibrinogen genes have been studied, and that it is unclear whether the analysed polymorphisms have functional effects. The polymorphisms that were studied may be functional or they may be linked to truly functional polymorphisms elsewhere in the fibrinogen genes, or in nearby gene(s), which can then explain the associations observed. Thus, to perform a comprehensive study on the contribution of variation in the fibrinogen genes to plasma levels of fibrinogen and to cardiovascular risk, one first has to document the haplotype structure of the fibrinogen locus, and then to determine the relationship between the haplotype-tagging polymorphisms with relevant phenotypic endpoints such as plasma levels and risk of disease.

In this issue of Thrombosis and Haemostasis, Maria Nastase Mannila and colleagues (see pp. 570-7)(16) have performed a study where genetic variation in the total fibrinogen-spanning region is being considered. They have used an approach that first identified the haplotype-structure of the gene cluster, then they selected the haplotype-tagging polymorphisms and finally they studied the association between these haplotypes with plasma fibrinogen levels and with the risk of acute myocardial infarction. This approach is a clear improvement to the previous studies where only a limited number of polymorphisms, often only one, were considered. In the study of Mannila and colleagues, the genetic variation in the fibrinogen locus, reflected by the haplotype comprised of 8 single nucleotide polymorphisms (SNPs) in the fibrinogen α, β and γ genes, is associated with the risk of acute myocardial infarction. This observation indicates that the haplotype approach is stronger than the analysis of single polymorphisms. The results also make one wonder what the underlying mechanism is of the observed relationship with risk. It could be an effect of a functional polymorphism on fibrin clot structure, as has been described for the fibrinogen αThr312Ala polymorphism (8), or it could be a polymorphism in the promoter region that gives a hyperresponse of fibrinogen to an inflammatory trigger. Furthermore, the present study included polymorphisms in the region spanning the three fibrinogen genes, but regulatory elements much further away from the gene may also be relevant and these may also be part of the haplotype. Another option could be that it is not a polymorphism in the fibrinogen gene, but one in the flanking region that affects the cardiovascular risk. Further studies will have to evaluate these possible underlying mechanisms.

Before genetic variation can be used in clinical routine, it is necessary to identify the functional polymorphism in the haplotype that is associated with the clinical endpoint and to understand the underlying mechanism. One of the techniques is to compare the association between genetic variation and phenotype in different races. It is known for many polymorphisms, including the −455G/A and the 6638ins28 (BclI) in the fibrinogen β gene, that their frequency greatly varies among races (17, 19). Functionality of polymorphisms can also be studied by various other techniques depending on the location of the polymorphism: for polymorphisms in the promoter region luciferase expression studies comparing different haplotypes will indicate the functional variants, for polymorphisms that give amino acid changes the production and characterization of recombinant variants containing the different haplotypes will provide information on the functionality of the variants.

The study of Mannila and colleagues (16) is the first study that combines information on genetic variation in the three fibrinogen genes, and further studies to explain the relationship between genetic variation and cardiovascular risk will help to understand more of the role of fibrinogen in this disease.

References