Quest for Genes Regulating Plasma Fibrinogen Concentration
Still a Long Way to Go
Anders Hamsten, Maria Nastase Mannila, Angela Silveira

Identification of the genes and genetic variants that confer proneness to coronary heart disease (CHD) remains an outstanding challenge to the biomedical research community. A host of genes are likely to contribute, yet only a small fraction have so far been unequivocally identified. This is not least because of the limited increase in risk contributed by each susceptibility gene. Thus, in most instances, more than one gene aberration will be required (heritability is polygenic), and clinical disease results from multifaceted interactions between susceptibility genes and environmental factors. Heritability is also heterogeneous; ie, different constellations of susceptibility genes are involved in different individuals. Adding to the complexity is epistasis or gene–gene interactions, where interactions among loci result in the genetic effects at one locus differing in magnitude or direction as a consequence of the genotype at another locus.1

One of the preferred molecular genetic approaches is to focus on the dissection of measurable quantitative traits that are associated with disease susceptibility in epidemiological studies. This is generally considered to be a more practicable strategy than to target affection status itself, the latter end point being even more complex, further distant from gene function, and statistically less informative. One example of an intermediate trait that translates variation at the gene level into variation in the risk of CHD is the measure of plasma fibrinogen concentration. The plasma fibrinogen concentration is an established risk factor for CHD,2 and several pathophysiological mechanisms have been identified, linking plasma fibrinogen to the development and progression of atherothrombotic disease.3

The fibrinogen molecule comprises 2 symmetrically arranged halves, each consisting of 3 polypeptides, Aα, Bβ, and γ, encoded by 3 genes located across a 51-kb region on chromosome 4q28 in the order fibrinogen gamma (FGG), alpha (FGA), and beta (FGB).4 A complex interplay between environmental and genetic factors accounts for the regulation of plasma fibrinogen concentration. However, whereas the genetic heritability of the plasma fibrinogen trait is substantial, estimates based on family and twin studies range from 20% to 51%.5−7 A multitude of genotype–phenotype association studies strongly indicate that only a limited proportion of the genetic determination is accounted for by single nucleotide polymorphisms (SNPs) in the fibrinogen genes themselves.8 This implies that other, presently unidentified genes that influence fibrinogen synthesis play major roles in the regulation of plasma fibrinogen concentration, and the question arises of how to best identify them. Because the function is known for only a small percentage of the genes catalogued in the Human Genome project, the a priori chances of guessing the right gene(s) are low using the candidate gene approach. Consequently, unbiased genome-wide strategies are preferable.

Against this background, Soria et al conducted a genome-wide screen in 21 three- to-five-generation Spanish families, the results of which are reported in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology.9 Of the 21 extended families investigated using a standard selection of microsatellite DNA markers, 12 were selected through a proband with idiopathic thrombophilia whereas 9 were recruited without consideration of phenotype. In all, 398 individuals were examined, all of whom participated in the Genetic Analysis of Idiopathic Thrombophilia (GAIT) project.10 The subsequent linkage analysis, which used standard multipoint variance component linkage methods (the SOLAR software) revealed 1 quantitative trait locus (QTL, a chromosomal region contributing to the variability in plasma fibrinogen concentration) with genome-wide significance located on chromosome 14 (LOD score of 3.1) and suggestive evidence of a second QTL on chromosome 12 (LOD score of 2.1). In addition, weaker linkage signals of potential interest were picked up on chromosomes 1 and 17. Interestingly, no gene with a known effect on plasma fibrinogen concentration is contained within the QTL on chromosome 14, which means that this region may be harboring a novel hemostasis-related candidate gene, potentially implicated in cardiovascular disease. The QTL on chromosome 12, on the other hand, contains the gene (designated TCF1) encoding hepatocyte nuclear factor 1, which may be implicated in the transcriptional regulation of the FGA and FGB genes. Based on associations between three SNPs in the TCF1 gene and the plasma fibrinogen concentration, together accounting for ≈5% of its variation, the authors advocate that the plasma fibrinogen level is genetically influenced by the TCF1 locus. However, it is apparent from the statistical analyses that the functional sites/variants remain to be identified. Also, and more importantly, other

See page 1287

© 2005 American Heart Association, Inc.
Arterioscler Thromb Vasc Biol is available at http://www.atvbaha.org
DOI: 10.1161/01.ATV.0000167519.47426.83

From the Atherosclerosis Research Unit, King Gustaf V Research Institute, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.
Correspondence to Professor Anders Hamsten, MD, PhD, FRCP, King Gustaf V Research Institute, Building M1, Karolinska University Hospital, Solna, S-171 76 Stockholm, Sweden. E-mail anders.hamsten@medks.ki.se

potential candidates exist in this region, functional variants of which may be in strong linkage disequilibrium (LD) with the TCF1 variants.

It is notable that the region on chromosome 4 that contains the three fibrinogen genes failed to show evidence of linkage (LOD scores <1.0). As pointed out by the authors, the fibrinogen genes may exert too small an effect to allow detection in a study with a quite limited power (10% to detect a single QTL accounting for 5% of the phenotypic variation). This conclusion is in accordance with the results of previous SNP-based association studies and does not rule out the possibility that the fibrinogen genes are implicated in CHD through mechanisms other than an influence on plasma fibrinogen concentration. Alternative mechanisms include effects on fibrin cross-linking resulting in altered fibrin gel structure and resistance to fibrinolysis. In fact, haplotypes across the fibrinogen gene cluster were recently reported to contribute to variation in risk of myocardial infarction, independently of the plasma fibrinogen concentration. It should also be emphasized in this context that trans-acting loci (such as genes encoding transcription factors) in general appear to be quantitatively more important for the regulation of gene expression than cis-acting variation in the flanking DNA sequence of the gene itself.

The present study is not the first genome-wide effort targeting the plasma fibrinogen concentration. Recently, the Framingham Heart Study reported the results of a genome-wide scan conducted in 330 extended families. No linkage with genome-wide significance was detected, but suggestive evidence of linkage was found on chromosome 10. Again, no linkage peak corresponding to the fibrinogen gene cluster on chromosome 4 was detected.

How should we interpret the discrepant findings of the two genome-wide searches? In the first place, both studies suffered from a low power to detect a QTL with a modest impact on variation in plasma fibrinogen concentration. Accordingly, some of the QTLs may represent false-positive observations (type-I errors), and/or some true QTLs may have gone undetected. Secondly, differences in pedigree size might contribute. In this respect, the GAIT study, which was based on considerably larger families, had greater power per study subject. Thirdly, families were drawn from different populations. Each of these factors may be in strong linkage disequilibrium (LD) with the TCF1 variants.

In the linkage peaks, using appropriate selections among the several hundreds of thousands of SNPs spanning the genome, with subsequent association analyses in relation to the plasma fibrinogen trait. This is now a realistic option after recent advances in genotyping technologies and SNP mapping, but costs remain quite high. Also, the TCF1 gene needs to be explored in much greater detail with the emphasis being placed on detection and characterization of functional polymorphisms.

In all, the findings reported by Soria et al represent a step forward, but the ultimate goal of identifying the gene(s) conferring the strong genetic heritability on plasma fibrinogen concentration and the related molecular etiology is still out of sight. However, pedigree studies of QTLs, such as this one, are a starting point that should invoke cautious optimism.

References
Quest for Genes Regulating Plasma Fibrinogen Concentration: Still a Long Way to Go
Anders Hamsten, Maria Nastase Mannila and Angela Silveira

Arterioscler Thromb Vasc Biol. 2005;25:1100-1101
doi: 10.1161/01.ATV.0000167519.47426.83
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/25/6/1100

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/