To lyse or not to lyse: is it genetic?

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The development of cardiovascular disease is influenced by complex interactions between environmental and genetic factors. Despite a flurry of activity in the past decade to identify common genetic variants that predispose to cardiovascular disease the results have, on the whole, been inconclusive. In the present review we summarise the available information regarding genetic variants of fibrinolytic factors and discuss the issues pertinent to understanding the inconsistencies in their reported relationship to cardiovascular disease. Finally, we suggest that work in this area should continue, but with the emphasis firmly directed towards the use of genetic markers as tools for investigating and understanding the complexities of cardiovascular disease.

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Introduction

Atherothrombotic disorders are characterised by the development of atherosclerotic plaques on the arterial vessel wall, followed by plaque instability, rupture and superimposed thrombus formation, which culminate in an acute thrombotic event. The complexity of this phenotype with the myriad risk factors for each component of the disease has contributed to the difficulty in elucidating genetic markers and an understanding of disease at the scientific level. This is in marked contrast to the clinical situation where a series of environmental markers have been identified (smoking, dyslipidaemia, dysglycaemia, obesity, lack of exercise) which clearly influence the course of disease. Clustering within families of these risk markers and of cardiovascular disease (CVD) itself has indicated the importance of genetic factors in the pathogenesis of coronary thrombosis. However, due to the heterogeneity of CVD and the contribution of complex interactions between environmental and genetic factors, the genetic variants contributing significantly to the pathogenesis of CVD have remained frustratingly elusive.

Clinical studies of circulating fibrinolysis and vascular disease

Studies of circulating components of fibrinolysis in relation to vascular disorders have produced results that are difficult to interpret. The first Northwick Park Heart study demonstrated an association of decreased global fibrinolytic activity (as assessed by the euglobulin clot lysis time) with future risk of ischaemic heart disease in middle-aged men[1]. This result was entirely plausible and appeared to confirm the potential importance of the fibrinolytic system in the maintenance of vascular patency. Since then a number of large prospective and case-control studies have investigated the potential influence of components of the fibrinolytic system, in particular tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1), on the development of cardiovascular disease[2-6] and it is in this area that there is some controversy.

t-PA is an endothelial cell-derived activator of the fibrinolytic system which is considered to be protective against thrombotic disorders, a view supported by the clinical use of recombinant t-PA following acute myocardial infarction. In contrast, however, results from a number of clinical studies have demonstrated an association of elevated plasma levels of t-PA with CVD[2-4]. The reason for this apparently paradoxical observation is unclear but may reflect complexing of t-PA with its plasma inhibitor, PAI-1. PAI-1 is a serine protease inhibitor (SERPIN) and exists in the circulation in molar excess over t-PA thereby preventing the development of systemic fibrin(ogen)olysis. Elevated PAI-1 has been consistently associated with CVD[5,6] and high PAI-1 levels are found in atherosclerotic plaques[7]. Clinically, PAI-1 clusters with a number of features of the insulin

resistance syndrome[8,9], which is itself strongly associated with cardiovascular events, and the combination of elevated t-PA and PAI-1 with overall suppression of fibrinolysis in association with this condition may explain the relation between t-PA and vascular disease.

Heritability of fibrinolysis

There has been a great deal of effort expended in attempting to determine the extent to which genetic and environmental factors contribute to the interindividual variation in plasma levels of haemostatic factors. Heritability estimates for t-PA and PAI-1 from a study of 397 individuals in 21 extended pedigrees in Spain were 0.27 ± 0.07 and 0.30 ± 0.08 respectively[10]. For PAI-1 there was an additional household estimate of 0.14 ± 0.06 with the remaining variance of PAI-1 and t-PA attributable to environmental influences and random error. These estimates varied from results from the St. Thomas' twins study in which genetic factors were estimated to contribute to 0.60 (95% confidence interval 0.51-0.68) and 0.62 (95% confidence interval 0.53-0.69) of the variation of PAI-1 and t-PA, respectively, with the remainder attributable to environmental influences[11]. The reason for the difference in estimates is likely to reflect stronger similarities in both genes and environmental factors between twins compared with those observed in extended families. However, these studies highlight the contribution of genetic factors to the variance in plasma levels of these factors and further unpublished studies suggest a significant degree of pleitropy between PAI-1 and features of insulin resistance which indicate that common genetic and environmental determinants underpin this vascular syndrome.

Genetics of fibrinolysis

The genes encoding these proteins have been intensively studied, a number of common polymorphisms have been identified and their association with CVD has been investigated. Several polymorphisms in the t-PA gene exist including a common Alu insertion/deletion in intron 8[12]. There is no evidence of association of this polymorphism with arterial or venous t-PA levels; however an association with local basal t-PA release rate determined by a modified perfused forearm model has been described[13]. Higher release rates were observed in subjects homozygous for the insertion allele compared with those possessing the deletion allele. The insertion allele of this polymorphism has also been associated with myocardial infarction in the Rotterdam study[14], although this association has not been confirmed in other studies[15].

Several common polymorphisms of the PAI-1 gene have been identified, including a 4G/5G insertion/deletion at -675, a CA dinucleotide repeat within intron 3 and a 3' *Hind* III RFLP. The only consistent associations with plasma PAI-1 have been observed for the -675 4G/5G polymorphism, with approximately 25% higher levels in those homozygous for the 4G compared to the 5G allele[16-18]. *In vitro* studies have confirmed higher rates of transcription associated with the 4G allele and differences in binding of nuclear proteins, with the 4G allele apparently binding an enhancer whereas the 5G allele binds both an enhancer and a repressor resulting in decreased transcription of the 5G allele relative to the 4G allele[19]. Furthermore, the 4G/5G promoter region has been shown to exhibit genotype specific responses to triglycerides with highest levels of PAI-1 in 4G/4G individuals with elevated triglycerides[20,21]. These findings have been supported by laboratory studies in which a triglyceride responsive region has been identified adjacent to the 4G/5G polymorphic site[22]. An interaction between 4G/5G and an exercise-induced decrease in PAI-1 has also been reported, with a significant reduction (36%) in PAI-1 observed only in those of 4G/4G genotype[23]. This was suggested to be related to an exercise-induced decrease in triglycerides, which would be reflected in a more profound effect on PAI-1 in the triglyceride responsive 4G/4G genotype. A number of studies have demonstrated an increased prevalence of the 4G allele in subjects with myocardial infarction. However, as with many gene-disease association studies, there have been an equal number of studies refuting these associations[15].

Genetics: the answer or just another question?

Why are the results of the above gene-disease association studies so inconsistent? The answer to this question lies at many levels and ultimately may point to the futility of our current approach to the genetics of complex disorders. 1) It is clear that genes do not commonly operate in isolation from the environment in the development of complex multifactorial disorders. An exception to this would be familial hypercholesterolaemia occurring in ~1 in 500 individuals, however in the general population all migratory epidemiological studies highlight the importance of environment. This being so it is possible that all genetic markers are either neutral or relatively protective in the absence of an environmental influence and studies clearly need to take this into account. 2) The phenotype of coronary artery disease and myocardial infarction is incredibly complex with perhaps up to 200 candidate genes with variable environmental interactions. On this basis it is possible to imagine that the potential haplotypes and environmental interactions would require a study greater than the size of the human race to answer all the potential genetic issues. This might be scientifically valuable but it would be clinically useless and impractical to even consider. 3) As an adjunct to 2) the candidate gene approach has at its heart the philosophy that some genes are more impor-

tant than others. This view is difficult to sustain and even in coagulation and fibrinolysis alone we may need to study dozens of genes and hundreds of polymorphisms. 4) For the majority of haemostatic factors there is a wide interindividual variation in levels, with the greatest interindividual variation observed for PAI-1, and despite convincing evidence of a significant heritable component to t-PA and PAI-1 levels the currently identified polymorphisms account for a very limited proportion (up to 5%) of this variation. It is, therefore, highly unlikely that a polymorphic variant accounting for such a relatively small variation in levels would be related to disease. Additionally, any polymorphisms identified within the genes encoding these proteins may interact to either augment or attenuate the influence of another thereby confounding the difficulty in interpretation of the results of gene-disease associations. One clear solution to this is to account for the total genetic variance in the gene of interest by evaluation of all polymorphic variants and analyse the association of common haplotypes with plasma levels of e.g. PAI-1 and presence of disease. 5) An additional important factor to consider is that we have tended to focus on the proteins which contribute to fibrinolysis and forget that there are many that modulate resistance to fibrinolysis *in vivo*. The most obvious omission to the majority of clinical studies is plasminogen, the precursor of plasmin, for which there is little data regarding plasma levels and CVD and no data regarding polymorphic variants and disease. Additional factors include PAI-2 and urokinase-type plasminogen activator (and its receptor), which may play an important role in the pathogenesis of cardiovascular disease because of their potential influence on fibrinolysis and also their role in extravascular proteolysis and cellular migration. Factor XIII-dependent cross-linking is a major determinant of both endogenous and exogenous fibrinolysis as indicated in animal studies[24,25] and the level of fibrinogen and generated thrombin (the latter perhaps an indicator of the overall activity of the intrinsic and extrinsic coagulation pathways) determine the structure and lytic properties of cross-linked fibrin. Finally thrombin activatable fibrinolysis inhibitor is a recently identified fibrinolysis inhibitor which has been shown to determine the efficacy of exogenous fibrinolysis in animals[26]. Therefore genetic variation in all of the genes encoding these proteins and the proteins involved in their regulation may influence an individual's fibrinolytic potential. In this context, the inability to identify a significant and consistent genetic contribution of one genotype in particular reflects the underlying genetic complexity of CVD.

Conclusions

What are the implications for those of us who have devoted many years to identifying polymorphisms in candidate genes which we hoped would contribute significantly to the pathogenesis of CVD? All is not lost. CVD is a clearly modifiable disease and although a single polymorphism or cluster of polymorphisms within a single candidate gene will not be particularly informative in determining overall risk for CVD, analysis of the functional effects of polymorphisms will provide invaluable information regarding protein function and regulation. This information is likely to be fundamental in identification of novel targets for the development of new antithrombotic and pro-fibrinolytic agents. In addition, knowledge of an individual's genetic profile may help in defining individual drug regimens to elicit maximum therapeutic benefits. Finally we should begin to turn our attention towards the transcriptional regulation of coagulation and fibrinolysis in the search for factors which regulate the whole system rather than merely individual proteins. An example of the success of this approach has been the identification of the PPAR-γ transcription factor which regulates multiple genes related to insulin resistance and the development of agonists which have now been introduced into clinical practice as effective therapeutic agents in the management of type 2 diabetes mellitus.

We should continue our work in this area with optimism, but employ the use of genetic markers as tools for investigation rather than as the answer in itself. We must develop better skills in post genomic functional analysis of polymorphisms to inform us as to how we can better understand the systems we study and to develop novel therapeutics and markers for therapeutic responses. Finally we should approach molecular epidemiology in this area with caution, understanding the many pitfalls that lie in the path of attempting to nail a single polymorphism to this complex disorder[15].

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