Molecular genetics of coronary artery disease: measuring the phenotype

Conventional risk factors explain only about half the risk of coronary artery disease (CAD) [1]. The strong familial predisposition to CAD [2], combined with advances in DNA analysis, has led to a proliferation of studies in recent years attempting to identify genetic factors that influence risk. The approach taken by most studies has been to examine the association of naturally occurring genetic variation (polymorphisms) in candidate genes with risk of or severity of CAD. Given the complex pathophysiology of coronary atherosclerosis and the processes that lead to the clinical syndromes [3], there is no lack of candidate genes worthy of study.

In this issue of Clinical Science, two studies examine the role of variation in the gene for transforming growth factor-β1 (TGF-β1). TGF-β1 is a cytokine with several functions which could both inhibit and advance the development and progression of atherosclerosis. However, in 655 patients with CAD confirmed angiographically and 244 angiographically normal individuals, Syrris et al. [4] found no association of five different polymorphisms in the gene with CAD. Haplotypes constructed from linkage of individual polymorphisms also did not associate with CAD. Wang et al. [5], on the other hand, examined whether a polymorphism (C-509T) in the promoter region of the gene, one of those also studied by Syrris et al. [4], influenced the severity of coronary disease, as judged by the number of significantly diseased vessels at angiography in 371 patients. Once again, no association was found of the polymorphism with this phenotype, or indeed with a prior history of CAD. In the other 655 patients with CAD, Wang et al. [5] found no association of five different polymorphisms in the gene with CAD. Haplotypes constructed from linkage of individual polymorphisms also did not associate with CAD. Wang et al. [5], on the other hand, examined whether a polymorphism (C-509T) in the promoter region of the gene, one of those also studied by Syrris et al. [4], influenced the severity of coronary disease, as judged by the number of significantly diseased vessels at angiography in 371 patients.

Association studies examine the role of individual polymorphisms and, by inference, any variations that are in significant linkage disequilibrium. However, when negative as in the studies of Syrris et al. and Wang et al., they cannot exclude an effect of a gene locus. Construction of haplotypes as done by Syrris et al. brings association studies closer to the type of locus exclusion permitted by linkage analysis, although in this case the study was probably underpowered to do this adequately. Thus it remains possible that other variations at the TGF-β1 locus still play a role in the pathogenesis of CAD. However, the two studies usefully serve to highlight a more general dilemma facing researchers in this area, namely which CAD phenotype to look at and how best to measure it.

Because of both clinical relevance and patient access, two CAD phenotypes have been most commonly studied – myocardial infarction and angiographically documented coronary disease. Myocardial infarction has the great advantage of having well-defined criteria for diagnosis. An important consequence of this is that it is also relatively easy to identify controls who have not suffered an event, although as the condition can occur without any prior symptoms, and is an age- and gender-dependent process, it is important that controls are well matched for these criteria, in addition to others such as ethnicity which can impact on allele frequencies. The other major advantage of myocardial infarction as a phenotype is that in most cases it can be fairly accurately timed. This means that, in addition to any overall association, one can also examine whether an allele or genotype is associated more strongly with myocardial infarction in younger subjects or with a younger mean age at time of myocardial infarction, features one may expect of a risk factor. The main disadvantage of studying myocardial infarction is the unavoidable loss of subjects through fatality when subjects are recruited after the incident event, even if this is done in admission wards or coronary care units, as more than half the acute deaths occur before subjects even reach hospitals. This could become of paramount importance if a genetic factor, unknowingly, also influences survival after myocardial infarction. This is of course the major reason why findings from prospective studies carry extra weight.

Use of the presence of coronary disease documented angiographically as a phenotype poses different problems. First, it is a chronic process and the onset is difficult to define. Secondly, although angiography is useful in identifying the presence of haemodynamically significant coronary stenoses (often defined as stenoses > 50%), it is an insensitive technique for quantifying the extent of atheroma. Patterns vary from focal lesions to diffuse involvement of large segments of vessels. Indeed, an interesting but unresolved question is whether different biological processes influence the pattern of coronary atheroma deposition. Furthermore, recent studies using intravascular coronary ultrasound [8,9] have shown that considerable atheroma may already exist in even angiographically 'normal' coronary segments due to extrinsically directed remodelling [10]. Therefore, classification of severity of coronary disease simply on the basis of the
The number of vessels with any segment stenosed by more than 50%, as used in the study by Wang et al. although of prognostic relevance, provides only a poor estimate of the underlying biological process and is thus of limited use as a phenotype. Better tools to quantify the extent of coronary atheroma are urgently required.

The insidious development of coronary atheroma and the unpredictable timing of its clinical presentation adds a further complexity to its use as a phenotype, namely identifying appropriate controls. One cannot assume that subjects are free of the disease simply because they are symptom free. Thus, allowing for the limitation of angiography in excluding atheroma discussed above, an impressive feature of the study by Syrris et al. is the use of only subjects shown to be completely free of angiographically visible coronary disease as controls. Since normal coronary arteries are more common in younger than older subjects, it is remarkable that they achieved a very reasonable age match. However, these advantages are counterbalanced by the fact that their control subjects were still patients (with other disorders requiring angiography or studied for chest pain) and not randomly selected individuals from the general population; thus selection bias remains possible. Furthermore, since angiographically normal coronary arteries are more common in women being investigated for chest pain than men, there was a not unexpected inversion in the sex ratio with 80% of cases being male but only 30% of controls. This is relevant as CAD occurs later in women than in men. The study therefore illustrates the inevitable trade-offs necessary in selecting a control population for this phenotype.

Finally, it is worth emphasizing that although the two phenotypes (myocardial infarction and presence/severity of coronary atheroma) are related, the association is less than perfect. It is well recognized that an acute coronary event is as likely to occur from disruption of a minor (and angiographically insignificant plaque) as from obstruction of a severe stenosis [11,12]. Thus, depending on its precise role in the pathophysiology of coronary disease, a genetic factor may be associated more strongly, or indeed only, with one of the two phenotypes. This may, at least partly, explain the inconsistent findings reported with regard to some polymorphisms.

The limitations related to measuring the coronary phenotypes are neither new nor specific to genetic studies. However, in the excitement created by DNA analysis they can be forgotten. Therefore, a reminder of some of the issues that the reader needs to consider when interpreting the burgeoning and often conflicting literature on the genetics of CAD may be helpful.

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REFERENCES