Genome-wide association studies of coronary artery disease: have the results of replication studies been replicable?

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This editorial refers to ‘Replication study of 10 genetic polymorphisms associated with coronary heart disease in a specific high-risk population with familial hypercholesterolemia,’ by J.B. van der Net et al., on page 2195.

The recent wave of publications describing genome-wide association (GWA) studies of coronary artery disease (CAD) is beginning to wane. These GWA studies have identified a host of genetic variants that are associated with CAD. As expected, within a span of a few months this initial flood of GWA studies has inspired a matching surge of replication studies. At this juncture it is perhaps pertinent to ask the question: Have the results generated from these studies, thus far, been reproducible?

Findings from the first GWA study for CAD were reported by the Wellcome Trust Case Control Consortium.1 A day later, two independent studies2,3 simultaneously reported that two variants in the chromosome 9p21 region are significantly associated with two closely related disease phenotypes, CAD and myocardial infarction (MI), respectively. An association with chromosome 9p21 had not been reported in previous studies of CAD or MI. This region contains the sequences of genes coding for two cyclin-dependent kinase inhibitors, CDKN2A (encoding p16INK4a) and CDKN2B (p15INK4b). In less than 2 months, a replication study carried out using the GWA approach confirmed the original findings for 9p21 and also identified four additional CAD-associated regions on other chromosomes.4

While the first four GWA studies were carried out with Caucasian populations, a recent replication study conducted in Korean and Japanese populations demonstrated that the same CAD-associated genetic variants on chromosome 9p21 are consistently observed in East Asians.5 This was followed by another replication study involving a huge sample of nearly 10,000 subjects of European ancestry from seven individual case–control studies.5 The authors of this study also carried out a meta-analysis of all previously published data, totalling 12,004 cases and 28,949 controls. These studies provided increased support for the autosomal additive model of the CAD association of chromosome 9p21. In van der Net et al.’s replication study,7 10 genetic polymorphisms were investigated for an association with CAD in a high-risk familial hypercholesterolaemic cohort. Of these, however, only four significant associations were replicated, including one variant at chromosome 9p21.

As was recently reviewed by McCarthy et al.,8 a major problem confronting the investigators of association studies has been a failure to fully replicate findings from published reports. Unlike the previous GWA studies, all subjects in the longitudinal study of van der Net et al.7 had familial hypercholesterolaemia, which meant that the control group differed from the experimental cases only by not having CAD at the endpoint. Consequently, a positive association with CAD or MI would be detected only if a genetic variant contributed to the susceptibility of MI or CAD through non-hypercholesterolaemia-related risk factors, since both control and case subjects were hypercholesterolaemic. McCarthy et al.8 remark that the failure to replicate a significant association in an otherwise well-performed and well-powered study is usually interpreted as indicating that the initial finding was spurious. However, they also point out that the failure to replicate previous results can also result from substantive differences between the discovery and replication studies in certain parameters, such as sample ascertainment. Therefore, the failure of van der Net et al.7 to replicate 6 of the 10 polymorphisms might be another good example of the ‘informative heterogeneity’ that McCarthy et al. refer to.

The replication studies cited in this editorial were conducted using the best practices in that they had moderate to large sample sizes, included systematic appraisal of potential sources of error and bias, and used independent replication samples. In addition, the investigators used distinct genotyping assays, which
is necessary to stamp out technical artefacts. The various genotyping platforms that have been employed include Affymetrix GeneChip Human Mapping 500K, the Illumina Hap300 chip, the TaqMan SNP genotyping assay (Applied Biosystems), and custom oligonucleotide arrays.

There are many possible reasons why the results of an association study can fail to reproduce previously published results. The most common reasons include genetic heterogeneity, population stratification, insufficient statistical power, differences in the ethnic background of the study subjects, and variation in study design. Despite years of research and ample funding, the precise genetic factors underlying complex diseases remain obscure today. This has led sceptics to liken the efforts of complex disease investigators to the alchemists’ futile quest for the philosopher’s stone. Judging from the results of a series of replication studies, we can now proudly proclaim that chromosome 9p21 is reproducibly a locus of significant association with CAD. This, in my opinion, constitutes progress; for, as long as an observation is shown to be reliable, we have moved a step closer to the truth. Failure to replicate some other significant associations from previous studies is to be expected. After all, one of the main reasons for conducting a replication study is to weed out spurious associations and ascertain true ones.

Taking these well-conducted replication studies together, we can see that they have not failed in fulfilling the objective of confirming genuine associations of reported genetic variants with disease phenotypes and dismissing false-positive discoveries. The unanimous finding across many studies that alleles on chromosome 9p21 are associated with CAD attests to the usefulness of GWA studies. Through replication studies and meta-analysis, we can be confident that concerted worldwide efforts in this direction will continue to be fruitful. In this regard, we are without doubt making commendable progress in the identification of susceptibility genes for complex diseases.

References

1. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447:661–678.