The success of genome-wide association studies (GWAS) has breathed new life into our genetic understanding of common cardiovascular diseases such as heart disease, stroke, and hypertension. These combined diseases account for the largest mortality rates in the West, surpassing cancer and infectious diseases. The strategies deployed in dissecting out their genetic etiology have ranged from candidate gene–, family-based linkage studies to GWAS. Now that whole genome approaches are beginning to bear fruit, should we consign the oft-used candidate gene–based association study to history? Certainly it appears that, at least for stroke, our etiologic understanding of these conditions becomes increasingly tested, GWAS, not being hampered by such limitations and requiring no a priori hypothesis, lends itself open to identifying novel gene loci and therapeutic targets.

However, the article by Ding et al in this issue of Circulation Research serves to remind us that an intelligent genetic approach to common diseases still has mileage. Synthesis of the vasodilator NO is suppressed by the amino acid asymmetrical dimethylarginine (ADMA), which displaces l-arginine, the natural substrate for NO synthesis. Consequently, factors that elevate plasma ADMA levels will secondarily reduce NO synthesis. Given that NO exerts significant protection against a wide variety of vascular disorders, ranging from stroke, atherosclerosis, hypertension, hyperlipidemia, diabetes, and renal failure, genetic variants that modify ADMA levels would themselves be predicted to act as risk factors for cardiovascular disease. One such candidate, tested by the authors here, is the gene coding for the enzyme dimethylarginine dimethylaminohydrolase (DDAH), which hydrolyzes ADMA to L-citrulline and dimethylamines. Two isoforms of DDAH (1 and 2) are known to exist, with DDAH1 being the dominant isoform. Hence, polymorphisms associated with reduced DDAH functioning might be reasonably expected to lead to ADMA accumulation and reduction in NO signaling.

The authors speculated that because NO has been shown to influence many vascular disorders (an observation not lost on the Nobel prize committee), its regulator DDAH1 should be a good candidate gene for disease involvement. They set about investigating the DDAH1 gene.

The authors first sequenced the entire DDAH1 gene in 48 randomly selected unrelated controls from the Han Chinese populations. This resulted in 9 polymorphisms being identified: 1 in the promoter region, 1 in exon 4; 5 in intronic regions, and; 2 in the 3’ untranslated region. The variant that resulted in a synonymous change (exon 4) was understandably discounted, although choosing to ignore the 3’ untranslated region may be short-sighted because such variations have been shown to influence disease pathways. Notwithstanding such an omission, they decided to concentrate on the novel 4-nucleotide deletion–insertion polymorphism (−396 4N del/ins) polymorphism in the promoter region. Several functional analyses of this variant confirmed disruption of a metal-regulatory transcription factor (MTF)1 binding site, thereby reducing gene transcriptional activity. Consistently, mean ADMA plasma concentrations increased in a codominant fashion in individuals with −396 4N ins alleles.

The authors then proceeded to undertake a case–control association study using this ins/del allele in 1388 patients with imaging-confirmed ischemic strokes and 1027 controls, as well as 576 coronary heart disease cases and 557 controls. The −396 4N ins allele was significantly associated with increased risk of ischemic stroke (odds ratio, 1.33) in 716 patients of the ischemic stroke group, and this was replicated in an independent dataset, although combining all subtypes (lacunae and hemorrhage) did not replicate the results from the discovery dataset. A positive association was found, however, in both the discovery (odds ratio, 1.5) and replication sets with coronary heart disease. Although this ins/del allele was associated in both the discovery and replication population with coronary heart disease, this was only the case for the ischemic subtype of stroke. This is not surprising because stroke is a heterogeneous condition encompassing many subtypes with likely different etiology, with some subtypes may having a greater genetic liability.

Like other molecular variants that have been positively associated with vascular disease, the results of this work will need to be confirmed in other populations. The investigators studied individuals from Han Chinese ancestry. Whether this variant is associated with ischemic stroke or coronary heart
disease in other ethnic populations with similar attributable risk ratios remains to be seen. Certainly risk variations are known to occur in different ethnic populations in stroke.13

The relationship described here between −396 4N polymorphism and ADMA levels informs the debate over whether ADMA serves as an independent risk factor for cardiovascular disease. If the level of disease risk associated with a polymorphism squares with the level of risk expected from the influence of that polymorphism on a biochemical intermediate X, then this supports the case for X being causative rather an effect of the disease. This is because the genetic effects on X are found in healthy individuals, i.e., before disease has arisen. One can derive the expected risk for a genetically determined biochemical modification using a mendelian randomization approach (see Figure). For example, the increment in plasma homocysteine secondary to the MTHFR polymorphism C677T is associated with a level of cardiovascular risk quantitatively similar to that found by the polymorphisms itself using a mendelian randomization strategy.14 Conversely, genetic determinants of C-reactive protein level are not associated with cardiovascular risk, in spite of heart disease and stroke being consistently associated with elevated C-reactive protein levels,15 suggesting reverse causation.

When we apply a similar mendelian randomization based logic to the present data from Ding et al, and in conjunction with cardiovascular disease risk data from a 24-year-old healthy cohort in whom ADMA levels were measured at baseline,16 it is apparent that the increase in ADMA level secondary to −396 4N approximates to a level of stroke and heart disease risk similar to that observed for the polymorphism itself. Particularly noteworthy is that the ∼10% greater risk of heart disease relative to stroke seen for the −396 4N polymorphism is paralleled by differences in expected risk for the 2 diseases derived from ADMA level data. However, data relating ADMA levels and risk are far from being consistent. For example, an 11-year cohort of 2956 individuals from the Framingham Offspring Study17 found a nonsignificant decrease in risk with unit increase in ADMA. This was reconciled by Leong et al,16 who noted that in the latter study, cardiovascular risk among individuals with high-baseline ADMA levels (>0.71 μmol/L), only became apparent after 12 years. Conversely, risk estimates for ischemic events associated with ADMA levels appear to be even stronger than those of Leong et al16 when based in populations with preexisting ischemic disease, e.g., peripheral vascular disease18 or coronary disease,19 suggesting risk factor synergy. However, smoking appears to reduce the risk associated with ADMA, possibly because of induction of ADMA catabolism by tobacco smoke.20

The question of whether the variant described by Ding et al would have been identified by a genome-wide approach needs to be asked. Given a large enough sample size a GWAS approach may well have stepped up to the mark. However, the logistics of recruiting huge patient databases and the expense of the gene chips used cannot be underestimated. Furthermore, a broad locus of interest would have emerged with no guarantee of isolation of the actual gene or an understanding of its underlying mechanism or an assessment for a causal relationship.

So, candidate gene based association studies when intelligently performed still have value. The candidate gene needs to be thoughtfully chosen from our understanding of disease processes, physiological studies need to demonstrate a functional role for identified molecular variants, and a causal relationship could be shown using strategies such as mendelian randomization.

Proponents of whole genome approaches should swagger lightly.

Sources of Funding
P.S. and P.B. hold senior fellowships from the Department of Health (United Kingdom).

Disclosures
P.S. has received honoraria for lecturing in industry-sponsored meetings, has received industry funding for attending national and international meetings, has received research grants from pharmaceutical companies, has been a paid consultant to the biotech industry, and is a member of industry advisory boards. P.B. declares no conflict of interest.
References


Down but Not Out: Candidate Gene-Based Studies Still Have Value in a World Dominated by Whole Genome Approaches
Pankaj Sharma and Paul Bentley

Circ Res. 2010;106:1019-1021
doi: 10.1161/CIRCRESAHA.110.217992

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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