

A call for accurate phenotype definition in the study of complex disorders

To the editor:

Gretarsdottir *et al.* recently reported on the association between risk of ischemic stroke and the gene encoding phosphodiesterase 4D (*PDE4D*)¹. There was no significant association with ischemic stroke as a whole¹, and so the authors focused on various etiological subgroups of stroke. They found that different haplotypes were associated with different risk of combined carotid and cardiogenic strokes. Their work warrants serious criticism. There is an important methodological problem relating to combining individuals with cardiogenic and carotid stroke into one subgroup for genetic analysis. Combined carotid and cardiogenic strokes represented 40–45% of all ischemic strokes in the authors' sample^{1,2}, which means that they excluded 55–60% of individuals with stroke from the genetic association study. Excluding that many individuals (and losing that much genetic information) is appropriate only if it refines the stroke phenotype in the remaining sample. Unfortunately, this was not the case in the study of Gretarsdottir *et al.*

The authors claim that cardiogenic and carotid strokes are most clearly related to atherosclerosis. This is true for carotid strokes (14–16% of all ischemic strokes in their population^{1,2}) but not for cardiogenic strokes (24–29% of total). Data from the literature show that only 25% of cardiogenic strokes directly result from complications of coronary atherosclerosis³, whereas ~65% are due to valvular heart disease or atrial fibrillation (with less than 20% of all atrial fibrillations being attributable to coronary heart disease⁴). The combined subgroup resulting from the admixture of carotid and cardiogenic strokes is, in fact, highly heterogeneous and, in our opinion, unsuitable for genetic analysis. Readers should receive with caution the authors' assumptions regarding the putative role of *PDE4D* in atherogenesis and subsequent risk

of stroke. In agreement with this caveat, we note that *PDE4D* genotypes or haplotypes do not influence the risk of peripheral artery occlusive disease or coronary artery disease (see Supplementary Table 7 from ref. 1), although atherosclerosis is the cause of both disorders in more than 90% of cases.

The genetic analysis of the whole group of individuals with ischemic stroke showed a possible though nonsignificant association of anonymous markers at the *PDE4D* locus with stroke. In this context, the additional finding of downregulation of several *PDE4D* mRNA isoforms in individuals with ischemic stroke further suggests that *PDE4D* is a very good candidate for genetic modulation of ischemic stroke risk. Gretarsdottir *et al.* only considered the possible involvement of *PDE4D* in atherogenesis, which is supported by few experimental data¹. By contrast, phosphodiesterase 4 has repeatedly been reported to be involved in susceptibility to ischemic brain damage in animal models^{5–8}. Such a mechanism directly related to cerebral ischemia could explain why the *PDE4D* locus was found to be in linkage with stroke regardless of cause in the Icelandic population².

It seems that the *PDE4D* functional variant that influences the risk of ischemic stroke has yet to be identified, as statistically significant associations were found only in a pathophysiologically inadequate subgroup of individuals in the present study. Genotyping studies are becoming more comprehensive and including growing numbers of polymorphisms in each study. Accurate phenotype definition seems more than ever to be a necessary prerequisite to establish reliable genotype-phenotype relationships in the study of complex disorders.

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In reply

Definitions of clinical phenotypes are often arrived at through consensus criteria that may have little to do with the underlying biology. Stroke is an entity with subtypes that lack clear biological correlates.

Funalog *et al.* argue that there is a methodological problem in our genetic association study, namely, our combined analyses of the carotid and cardiogenic stroke subtypes. They do not agree that atherosclerosis is the primary pathological process for both carotid and cardiogenic stroke and claim that only 25% of cardiogenic strokes result directly from coronary atherosclerosis and 65% are due to valvular heart disease or atrial fibrillation (<20% of those being attributable to coronary atherosclerosis).

This is a different pattern from that seen in our Icelandic stroke cohort, in which at least 47% of individuals with cardiogenic stroke have coronary artery disease. Atrial fibrillation is observed in 79% of individuals with cardiogenic stroke, and 44% of those have documented coronary artery disease. Recent studies, especially in Scandinavian populations, of atrial fibrillation show that

46% of affected individuals have ischemic heart disease, 20% have congestive heart failure and only 4.5% have valvular heart disease or cardiomyopathy¹. Because over 60% of congestive heart failure is a long-term consequence of ischemic heart disease, at least 60% of atrial fibrillation is due to ischemic heart disease^{1,2}. Therefore, as much as 65% of cardiogenic strokes may be attributed directly or indirectly to atherosclerosis of the coronary arteries. In addition, many individuals with cardiogenic stroke have emboli from atherosclerotic plaque of their cerebrovasculature and thoracic aorta and therefore should be more appropriately diagnosed with carotid artery or large vessel occlusive disease. Moreover, the current population at the highest risk for stroke had a lower rate of acute rheumatic fever in their earlier years, owing to use of antibiotics during the previous 50 years. Therefore, the prevalence of stroke due to rheumatic valvular dysfunction has markedly declined and the relative effects of atherosclerosis have increased. Therefore, our ischemic stroke cohort of individuals with carotid and cardiogenic stroke may not be as heterogeneous as Funalot *et al.* suggest.

In our association analyses of each of the stroke subcategories, we saw that individuals who carried the disease-associated variants had equal risk of developing carotid and cardiogenic strokes.

We felt that one link between cardiogenic and carotid stroke was underlying atherosclerosis either in the coronary arteries or in the cerebrovasculature. Given the known effects of cAMP on vascular smooth muscle cell proliferation and migration, we proposed this as a mechanism of action of PDE4D underlying stroke risk. Further support for a vascular role for PDE4D in stroke comes from preliminary experiments showing that a PDE4 inhibitor reduces lesion formation in a rat carotid balloon injury model (J. Fingerle *et al.*, personal communication).

As mentioned in our paper, however, cAMP is a key signaling molecule regulating myriad cellular functions. Consequently, there are numerous ways in which it could be connected to the pathogenesis of stroke, for example, in inflammation-mediated plaque instability, thrombogenicity, vascular permeability, NO-production and oxiradical stress³⁻⁷. Funalot and colleagues rightly point out that PDE4D has been postulated to protect against the effects of ischemia in rodent models. It is an interesting hypothesis that wild-type variants of PDE4D may protect individuals from an infarct (permanent brain damage) resulting from decreased blood flow. This is certainly possible, but space limitations prevented us from enumerating all the possible roles that PDE4D could have in increasing risk of

stroke. In our opinion, however, this hypothesis is less likely, because our PDE4D variants show similar risk ratios for transient ischemic attacks as for the infarcts, which would not be expected if the predominant role of PDE4D is prevention of permanent damage to the central nervous system from initial ischemia. Further work is needed to confirm our initial genotype-phenotype correlations in other populations and to show that inhibition of PDE4D can directly affect carotid atherosclerosis and stroke in animal models.

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