The Gly389Arg beta-1 adrenoceptor polymorphism and cardiovascular disease: time for a rethink in the funding of genetic studies?

In recent years, there have been an expanding number of publications relating human genetic variation to cardiovascular risk. Following the recent sequencing of the human genome, we might anticipate a great deal more. Most will use a similar strategy: a system (or system component) will be suggested to play a role in the pathogenesis of cardiovascular disease. Its gene sequence will be determined, and common minor variations (polymorphisms) in this candidate gene identified. These can then be used in candidate gene-association studies, which themselves appear in one of two principle forms. Firstly, the frequency of the polymorphic variants amongst cases and controls are compared. Differences in allele frequency suggest a causative role for the candidate gene in the aetiology of the disease process in question. Secondly, the magnitude of a given phenotype (or its change with environmental stimulus) is compared amongst those of different genotype — again inferring a role for the candidate gene in the regulation of the phenotype itself. Such studies might be applied, for instance, to left ventricular mass or growth responses, or to cholesterol levels or profile. When appropriately applied, such genetic approaches can be very powerful indeed, and might in the future serve two strategic aims: they will contribute to our understanding of the pathophysiology of complex traits, and may (later) have a role in risk stratification or therapeutic targeting of specific treatments to specific patients.

In this issue White and colleagues report a candidate gene-association study in which the frequency of a polymorphic variant of the beta-1 receptor (BAR-1) gene is compared amongst cases of myocardial infarction, and controls. The authors report no difference in allele frequency between the two groups, and infer that this polymorphic variant is unlikely to be involved in mediating cardiovascular risk. How dependable are such conclusions? It is perhaps instructive to compare this study to a (perhaps unattainable) ‘perfect’ template:

The polymorphic variant should be functional

This generally means that different amounts of the protein are made, or that the ‘functional power’ of the protein is different. Several strategies may be used to demonstrate functionality. One may go ‘direct to humans’ — ‘stimulating’ (for instance) the individual appropriately and measuring response differences (for instance interleukin-6 levels) amongst those of different (IL-6) genotype.

Alternatively, the two gene variants may be expressed in cell lines, and the function of their product assessed in vitro. In this case, a polymorphic variant is found which substitutes a Glycine (Gly) for Arginine (Arg) at position 389 in the BAR-1 molecule. This is near the intracytoplasmic tail of the receptor, in a region potentially involved in receptor signalling. The two versions of the gene were expressed in a hamster fibroblast cell line (CHW-1102), and both basal and stimulated adenylyl cyclase activity were found to be lower in Gly389 than Arg389 receptors. One might infer, therefore, that the ‘Gly389’ receptor behaves as if ‘endogenously beta-blocked’: indeed, this is the premise upon which the current study is based. However, as the authors eloquently discuss, the function of a polymorphism may strongly depend upon the wider genetic context in which it is found — both in terms of other variants within the same gene (different haplotypes) and the presence of nearby control sequences. The lack of this genetic context — and of the physiological control systems which may be founding in vivo in the cell, tissue and body — make in vitro expression studies hard to rely upon as evidence of in vivo effect.
expression of human receptors in vitro in animal cell lines may thus not easily extrapolate to what is happening in vivo in a human.

**The polymorphism should be functional in vivo**

Thus, we should seek proof of an in vivo functionality of the polymorphism. Even if functional differences seen in vitro are also present in vivo, there is currently no way of inferring how the magnitude of the two will correlate. In this case, the Gly389 polymorphism is used as a marker of long-term ‘endogenous beta-blockade’. However, resting heart rates and haemodynamic variables were unrelated, suggesting that (at least basally) such an effect was not large. As in the in vitro studies, the functionality of the polymorphism may only become evident during sympathetic activation. However, even when exercising (when the sympathetic nervous system is activated) there seems no association of the polymorphism with haemodynamic response. Neither does the polymorphism seem to influence response to antagonists in humans. Currently, the sole data suggesting a functional role for the polymorphism in humans is the finding of an association of the Arg389 variant with the presence of hypertension. Further human physiological data (such as genotype-association with heart rate/blood pressure responses to infusion of a beta-1 agonist) would go a long way to help address such issues.

**The study should be appropriate powered**

The absence of a known physiological effect of a polymorphism in vivo makes candidate gene-association studies such as this very hard to power appropriately. Thus, on the basis of resting haemodynamics alone, pharmacological beta-blockade seems to be far greater than the ‘endogenous’ beta-blockade related to the Gly389 allele. If such pharmacological beta-blockade is associated with (say) a 20% reduction in secondary risk of myocardial infarction over 5 years, the much smaller ‘endogenous’ blockade associated with the Gly389 allele is unlikely, therefore, to be associated with anywhere near the reduction in relative risk associated with drug therapy and may need huge numbers of cases and controls in order to be identified. Further, atherosclerosis and myocardial infarction are multifactorial and polygenic traits, in which any one polymorphic variant generally only confers a relative risk of 1.0–1.3. A study of 580 events in a case-control study is thus, as the authors point out, unlikely to detect such an alteration in risk associated with an allele (Arg389) with a frequency of >70%.

**The phenotype should be very tightly defined**

We now recognize that pathogenesis of atherosclerosis is multifactorial. It involves endothelial dysfunction, altered lipid profile and levels, glucose handling, oxidative stress, exposure to mitogens, as well as inflammation and its response. Each of these may have a thousand provocateurs, and may be signalled in a thousand interrelated ways. We are also increasingly understanding that the unstable coronary syndromes (including myocardial infarction) may be similarly triggered by a diverse range of processes, which themselves differ from those involved in the pathogenesis of stable atheromatous disease. Here, the inclusion of cases of non-fatal and fatal myocardial infarction together with those needing angioplasty or surgical coronary artery bypass grafting produces a mixed cardiovascular phenotype. Whilst the beta-adrenoceptor might play a role in diverse aspects of atherogenesis and in the triggering of MI, such roles will vary in strength. Thus, a very powerful role for the BAR-1 polymorphism in causing plaque instability in inflammation or during blood pressure surges in exercise, or in causing atherogenesis itself in non-diabetic non-smokers (for instance) would be missed.

**Use of confounding agents should be excluded**

The angiotensin-converting enzyme (ACE) gene demonstrates a common polymorphic variant in which the insertion (‘I’) allele is associated with lower ACE activity than the deletion (‘D’) allele. In recent years, many studies have addressed the association of this polymorphism with cardiovascular disease, on the understanding that higher ACE activity (marked by the ‘D’ allele) may contribute to cardiovascular disease pathogenesis. Many such studies are flawed by the inclusion of patients taking ACE-inhibitors: the role of genetically-lower ACE levels cannot be studied in a mixed group which included some with pharmacologically-lowered ACE levels. In the present study, the assessment of the role of ‘endogenous beta-blockade’ by the Gly389 allele is hampered by the fact that nearly 12% of cases are pharmacologically beta-blocked. In addition, the ratio of B1-to
B2-adrenoceptors in normal human myocardium is 2:1[11], and it seems that cross-talk exists between them[12]. The use of beta-blockade may thus amplify functional effects of myocardial beta-2 receptors.

Other agents may also have unexpected confounding roles. Here, 41% of cases were on pravastatin, and both beta-blockade and statin use may have effects on the inflammatory response[8,9] and hence on cardiovascular risk[10]. Sympathetic nervous system activity may alter coagulability and hence vascular risk[13], and 6% of patients (and 2-9% of controls) were taking aspirin. Pharmacogenomic interactions may thus have occurred, weakening the effect of the beta-adrenoceptor polymorphism on cardiovascular risk.

Other difficulties also exist. The authors (rightly) excluded the hypertensive population from heart rate or blood pressure analysis. However, given past reports of an influence of the polymorphism on risk of hypertension[7], it may be that such exclusion selectively removes those subjects in which the effect of the polymorphism is most strong.

Further studies

There is a strong scientific rationale to support a role for the beta-1 adrenoceptor in the pathogenesis of cardiovascular disease. Further, pharmacological trials have conclusively demonstrated such a role. It has yet to be demonstrated whether intrinsic modulation in BAR-1 receptor activity or signalling may modulate such risk. The role of the Gly389Arg polymorphism also requires elucidation in further studies. Firstly, in vivo functionality must be demonstrated. Secondly, the association of the polymorphism with tightly-defined cardiovascular phenotypes must be examined. Thirdly, the roles of other adrenoceptor genotypes in modulating cardiovascular risk must be examined. To date four beta adrenergic receptors (Beta 1–4) have been identified, which seem to share a similar structure (an extracellular amino terminus, seven transmembrane helical domains and an intracellular carboxy tail). It has been shown that functional genetic polymorphisms exist in the beta 1, 2 and 3 receptors. The gene for beta 1 adrenergic receptor, comprising of a single exon, is located on chromosome 10q24-26 and has been shown to exhibit a myriad of single nucleotide polymorphisms, some of which result in amino acid changes. It is likely that the other receptors may exhibit similar diversity.

The way forward?

The work of White and colleagues represents the largest association study of this polymorphism with cardiovascular risk yet reported. It is helpful in suggesting the relative potency of the Gly389Arg polymorphism in causing cardiovascular disease within (as the authors correctly state) ‘the practical constraints of this type of research’. However, these constraints apply to us all, and are major limitations to the success of such work. Perhaps it is thus time to engage new genetic strategies of research. In physiological investigation applied to case-control studies, patients should be of the same race and sex, with as close to one aetiology of atheromatous disease as possible (e.g. all similar diet, non-smokers, similar occupation), and with one phenotype measured (e.g. Q-wave infarction). Ideally, studies should be both large-scale and prospective. To obtain adequate numbers for such studies, huge collaborative efforts will be required both nationally and internationally. Research funding and strategies would have to change. Should small studies of one polymorphism in one place still be funded? Or should grant-giving bodies now consider investing heavily in the collection of massive quantities of complete phenotypic data without questioning, a priori, which polymorphisms will be studied?

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References

Acute coronary syndrome: the struggle for the best in risk stratification and therapy

See doi:10.1053/euhj.2001.3043 for the article to which this Editorial refers

It is a common dilemma in clinical trials dealing with acute coronary syndromes without persistent ST elevation that there are no hard criteria identifying the patient with coronary artery disease at risk. Up to 15% of patients with typical symptoms will have normal vessels or insignificant disease in the coronary angiograms. This dilutes any patient cohort at risk and thereby reduces the likelihood of showing significant treatment benefits. Accordingly, study designs have to balance between limiting the inclusion criteria to patients not representative of the 'real world' or to enormously increasing the trial size. Alternatively, it is a common practice to analyse pre-defined high-risk subgroups after study completion. In acute coronary syndrome trials these are commonly the cohorts with ST segment deviations or elevated cardiac biomarkers, particularly troponins. Both may be understood as markers of an ongoing thrombotic process. Elevated troponins indicate minor but irreversible cell injury caused by repetitive thrombus embolization downstream of the culprit lesion. Similarly, depression of the ST segment in the ECG of more than 1 mm reflects ischaemia resulting from active thrombus formation at the site of a ruptured atherosclerotic plaque.

There is ample evidence that unfractionated heparin as well as low molecular weight heparins compared with placebo improve the short-term outcome in patients with acute coronary syndromes[1]. Enoxaparin was shown in two trials to be more effective than unfractionated heparin[2,3]. A similar benefit was shown for glycoprotein IIb/IIIa inhibitors when added to unfractionated heparin and aspirin[4]. Post-hoc analyses revealed consistently that the benefit was mainly accomplished in patients with elevated troponin[5–9]. Furthermore, patients with elevated troponins seem to have the best outcome with early invasive management (<48 h) after upstream treatment with the glycoprotein IIb/IIIa inhibitor tirofiban[10]. Accordingly, the concept for hospitals with direct access to invasive facilities seems to be well delineated. For other places, however, two questions remain open: which patient must be sent on to coronary angiography, and what is the best treatment for the time in between.

The FRISC II study is a landmark trial, because it established the important role of coronary interventions for the outcome of patients with unstable angina as opposed to a very conservative strategy[11]. In fact, it is the first study to establish that percutaneous coronary interventions reduce the risk for death and myocardial infarction. The effect of extended anticoagulant treatment with dalteparin on the outcome in the other arm of this study was less convincing. Only marginal benefit was shown in patients with elevated troponin T[12]. In this issue, the results of continuous 12-lead ECG or vectorcardiography over 24 hours as prognostic marker are presented[13]. Patients with ischaemic episodes at study entry had a significant 34% reduction of the triple end-point death, myocardial infarction, or revascularization when treatment with dalteparin was continued for 3 months after 5 days of open label treatment. Although the study population of 629