Genetic determinants of coronary vasomotor tone in humans

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APART FROM AND IN ADDITION to all established mechanisms and mediators in the regulation of coronary blood flow under normal and pathological circumstances (1), there remains substantial interindividual variability in coronary vasomotor responses, both in the experimental animal and in humans. Such interindividual variability has, among other factors such as age, diet, and environmental factors, a genetic background. The human genome contains about 1.4 million (12) to 2.1 million (32) single nucleotide polymorphisms that may or may not encode proteins with a function that is more or less different from that encoded by the wild-type genome.

There is currently a large number of association studies that link a certain polymorphism with a certain clinical disease; notably there are also a number of such association studies that link the respective polymorphism with coronary artery disease, e.g., an angiotensin-converting enzyme gene insertion/deletion polymorphism (6), and polymorphisms in the angiotensinogen gene (35) that may both affect circulating angiotensin II levels or an insertion/deletion polymorphism in the α2B-adrenoceptor gene (29). There exists an extensive list of other polymorphisms potentially associated with coronary artery disease (for review, see Ref. 30). However, what is lacking in most studies is the analysis of a functional phenotype that links the observed polymorphism causally with the observed clinical entity. In this medical editorial we focus on two typical examples where such a functional phenotype is established: one with exaggerated coronary vasoconstriction and one with attenuated coronary vasodilation.

Better insight in such genetic background will in the future have considerable impact, because identification of such polymorphisms will help identify individuals at increased risk for coronary events already in childhood and, eventually, enable a targeted therapy that eliminates the functional phenotype that causally links the polymorphism with the coronary event.

G PROTEIN β3 SUBUNIT GENE C825T POLYMORPHISM AND CORONARY VASOCONSTRICTOR RESPONSIVENESS

Because most receptors located in the coronary circulation mediate their action via heterotrimeric G proteins, functionally significant mutations in G protein subunits are expected to exert a major impact on coronary vasomotor tone.

Studies on skin fibroblasts and immortalized B lymphoblasts from patients with essential hypertension had yielded strong evidence for a genetically fixed enhanced intracellular signal transduction in selected patients with essential hypertension (21, 27). This enhanced signal transduction was observed on stimulation with agonists coupling to receptors that predominantly activate pertussis toxin-sensitive G proteins (lysophosphatidic acid, thrombin), whereas signals produced by agonists such as bradykinin, which predominantly activate pertussis toxin-insensitive G proteins, were not increased. Moreover, such cell lines with increased signal transduction showed an enhanced proliferation pattern on stimulation with platelet-derived growth factor or serum. Interestingly, this increased signal transduction was completely abrogated in cells treated with pertussis toxin which by ADP ribosylation of a COOH-terminal cystein in Gαi subunits, prevents receptor activation of G proteins (21, 27). Together, these findings led to the hypothesis of a genetically fixed increased G protein activation. Whereas mutations in the genes encoding Gαi2, Gαi3, Gβ1, and Gβ2 were ruled out, systematic sequencing yielded a C825T polymorphism in the gene G protein β3 subunit (GNB3) encoding the ubiquitously expressed β3-subunit of heterotrimeric G proteins (28). Whereas the C825T polymorphism is silent in terms of

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amino acid composition of Gβ3, the 825T allele located in exon 10 of GNB3 is associated with alternative splicing of exon 9. The underlying mechanism remains to be fully understood. It appears that this nucleotide exchange and potential cooperative effects of other nucleotide exchanges in linkage disequilibrium with 825T, e.g., the 1429T allele in the 3' untranslated region, may change the secondary structure of the pre-mRNA, thereby favoring the additional use of a cryptic splice site located in exon 9 of GNB3 (22, 28). Carriers of at least one 825T allele express two Gβ3 proteins, the “wild-type” variant and, additionally, a deletion variant, which is 41 amino acids smaller. This deletion variant is, nevertheless, functionally active in G protein heterotrimers and, on expression in COS7 cells, reconstitutes the phenotype of increased cell activation as seen from enhanced agonist-evoked chemotaxis (33). Thus the 825T allele is associated with a "loss of structure, gain of function" variant.

Multiple genetic association studies (10, 24) as well as case-control studies (4, 5, 7, 28) were able to show an association between the 825T allele and hypertension or its sequelae, e.g., stroke (15). The exact mechanism, however, through which enhanced signal transduction may be a risk factor for hypertension is not completely clear. It appears relatively unlikely that increased blood pressure results from increased vasoconstriction in 825T allele carriers because hypertension apparently develops slowly over many years. One mechanism may involve an increased tendency of 825T allele carriers for obesity (8, 9, 26).

There exists functional evidence, however, that coronary vasomotion is strongly influenced by the C825T allele status. Baumgart et al. (2) performed intracoronary bolus injection of α-adrenoceptor agonists in Caucasian individuals undergoing coronary angiography due to chest pain of unknown cause (2). They used both methoxamine, which predominantly activates α1-adrenoceptors, and BHT-933, which predominantly activates α2-adrenoceptors. In humans with normal coronary vessels, predominantly α2-adrenoceptor activation reduces coronary blood flow, mainly through microvascular constriction. In the presence of atherosclerosis, both α1- and α2-adrenergic epicardial and microvascular constrictions of human coronary vessels are augmented and can then induce myocardial ischemia. In line with the concept that α1-adrenoceptors couple predominantly to pertussis toxin-insensitive Gq/11 proteins, we found no association between coronary blood flow reduction on methoxamine injection and GNB3 C825T allele status, and the magnitude of methoxamine-induced coronary vasoconstriction was almost exclusively determined by the presence or absence of coronary artery disease in these individuals. In contrast, α2-adrenoceptors are known to activate G protein heterotrimers comprising Gβ3. Interestingly, the coronary blood flow reduction induced by BHT-933 was significantly larger in 825T allele carriers than in homozygous C825 allele carriers (58% vs. 28%, P < 0.001), with only minimal overlap between genotype groups. Moreover, clinical signs of ischemia in patients receiving BHT-933 (like chest pain or ST-segment depression) were almost exclusively observed in 825T allele carriers (3).

Meirhaeghe et al. (14) examined the response of angiographically normal human coronary arteries following intravenous injection of methylergonovine maleate, a vasoconstrictor that presumably activates α-adrenoceptors as well as serotonin receptors, followed by injection of isosorbide dinitrate, a vasodilator, according to GNB3 genotypes. Subjects carrying at least one 825T allele had greater vasoconstrictor responsiveness to methylergonovine maleate than CC subjects. In contrast, vasodilation in response to isosorbide dinitrate did not differ among the different genotypes.

It thus appears, that genotyping for the GNB3 C825T polymorphism is highly predictive for coronary vasoconstriction in response to different agonists. The increased responsiveness of coronary vessels in 825T allele carriers might also explain why these individuals are at increased risk for unstable angina pectoris. Beside these observations, an increased activation of platelets (17), neutrophils (34), and lymphocytes (13) has been observed in 825T allele carriers, which in concert with obesity, may contribute to an increased propensity for coronary artery disease in these individuals. Enhanced coronary vasoconstriction together with enhanced platelet aggregation may contribute to the reported increased propensity for myocardial infarction in 825T allele carriers (18).

ENDOTHELIAL NO SYNTHASE POLYMORPHISM AND CORONARY SPASM

Given the delicate interplay between vasoconstrictor and vasodilator mechanisms, it is immediately evident that gene alterations that may reduce vasodilator mechanisms will exert an impact on coronary vasomotion. Using DNA samples from patients with coronary spasm, Nakayama et al. (19) searched the gene encoding endothelial nitric oxide (eNOS) located on chromosome 7q35–36 for potential mutations. They described a genetic T(−786)C polymorphism in the eNOS gene, with the −786C allele being significantly associated with coronary spasm in their Japanese patient sample. Moreover, using transfection studies, they could show that promoter activity was significantly reduced by ~50% with the mutant allele. This would imply a reduced synthesis of eNOS in vivo being responsible for an increased vasomotor tone in coronary arteries. Apart from these statistical associations, no study has so far investigated whether or not endothelium-dependent vasodilation is actually impaired in (−786)C allele carriers.

In addition to this genetic promoter polymorphism, there exists another frequent G894T polymorphism in the eNOS gene, which results in a glutamate or aspartate, respectively, at position 298 in the eNOS protein (36). Because glutamate and aspartate are conservative substitutions, it has been postulated that this polymorphism might be less important and one allele in linkage disequilibrium with a truly important gene alteration elsewhere in the gene (31). However, it could...
be demonstrated through transfection studies that the eNOS gene with polymorphisms at nucleotide 894 generates protein products with different susceptibility to cleavage (31). Thus in contrast to earlier predictions, the 894T allele may indeed have a functional effect on the eNOS protein, and the subsequent nitric oxide synthesis might be attenuated in individuals carrying an aspartate at position 298. In fact, the 298Asp variant was found associated with coronary spasm (36) and myocardial infarction (25) in Japanese individuals and with coronary artery disease in a patient sample from the United Kingdom (11). Functionally, the 894T allele carriers have increased baseline coronary vascular resistance, suggesting endothelial dysfunction, but no attenuation of adenosine-recruitable coronary reserve (16). The 894T allele also predicted an increased systemic blood pressure rise following infusion of the α1-adrenoceptor agonist phenylephrine in patients undergoing cardiac surgery with cardiopulmonary bypass (20). Schneider et al. (23), however, found no effect of the Glu298Asp polymorphism on endothelium-dependent vasodilation in healthy subjects following intrartrial infusion of acetylcholine in the forearm circulation. The reason for this discrepancy is unclear; it is quite possible that endothelium-dependent dilation is not mediated by NO, but by endothelium-derived hyperpolarizing factor, and that NO, on the other hand, antagonizes α1-adrenergic vasoconstriction better than endothelium-derived hyperpolarizing factor. In any event, these discrepant findings refer to the peripheral circulation and may not be easily extrapolated to the coronary circulation.

PERSPECTIVES

More and more polymorphisms that are associated with cardiovascular disease in general and coronary artery disease more specifically are being detected. Causal conclusions cannot be drawn from linkage analyses. It is definitely important to identify the functional phenotype, e.g., enhanced coronary vasoconstriction or attenuated coronary vasodilation, which is causally responsible for the observed clinical entity. This is an area for truly translational research, where an initial linkage analysis in humans prompts physiological studies in transgenic animals, which obviously need to be confirmed again in humans.

REFERENCES


