Impairment of endothelium-derived vasodilation has been observed in hypercholesterolaemia, atherosclerosis, diabetes and hypertension, and there is increasing evidence that oxidative stress is one of the causes [1–3]. It has been proposed that increased superoxide anion production in these conditions leads to a diminished bioavailability of nitric oxide (NO) and this, in turn, leads to vascular damage and the loss of dilation [4,5]. In support of this hypothesis, antioxidant therapy has been demonstrated to improve endothelial function [6–8].

The NAD(P)H oxidase system is the most important source of superoxide production in vascular cells [9]. It is a multisubunit protein complex consisting of membrane-bound and cytosolic subunits. The p22phox subunit is membrane bound, it is expressed in phagocytic, endothelial and vascular smooth muscle cells, and antisense studies have shown that the p22phox subunit is a critical component of the NAD(P)H oxidase system [10].

Several polymorphisms of the p22phox gene (CYBA) have now been reported, and two have been studied for association with coronary artery disease (CAD). The C242T polymorphism is located in the potential haem-binding site and codes for the substitution of histidine by tyrosine, the A640G polymorphism is located in the 3′- untranslated region of the gene. Inoue and co-workers [11] found the T/T genotype of the C242T polymorphism to be associated with a lower risk of developing CAD in a Japanese cohort. Other studies, however, found no association or the opposite, that is the C/T and T/T genotype to be associated with CAD and disease progression [12,13]. In contrast, Gardemann and co-workers [14] found no association with the C242T polymorphism, but association between the A640G polymorphism (A/A genotype) and CAD. There are no genetic studies assessing the role of CYBA gene polymorphisms with hypertension; however, a recent small study found no association with the C242T polymorphism and pre-eclampsia [15].

The case-control studies investigating CYBA gene polymorphisms are not consistent in their findings, however, there is an ‘in vitro’ study that has found a direct association between the C242T polymorphism and vascular superoxide levels [16]. Guzik and co-workers [16] found the p22phox gene was expressed at both the mRNA and protein level in human blood vessels and that the 242T genotype was independently associated with decreased basal and NADH-stimulated superoxide production in saphenous veins and mammary arteries taken from patients undergoing a routine coronary bypass. This interesting observation suggested ‘functionality’ of the polymorphism.

In this issue of Clinical Science, Schneider and co-workers [17] have reported one of the first ‘in vivo’ studies investigating whether the C242T polymorphism is associated with endothelium-dependent or NO-mediated vasodilation. Previous work by Li and co-workers [18] has shown no association between this polymorphism and endothelial-dependent vasodilation; however, they did not investigate NO-mediated vasodilation [18].

In the study by Schneider et al. [17], hypercholesterolaemic patients were genotyped for the C242T polymorphism and plethysmography was used to measure forearm blood flow after intra-arterial infusion of acetylcholine, nitroprusside and NOS-monomethyl-l-arginine (l-NMMA). No difference in forearm blood flow was observed between the three genotype groups after infusion of each agent. The authors [17] concluded that the C242T polymorphism is not a major determinant of acetylcholine-dependent vasodilation or basal NO-mediated vascular tone in hypercholesterolaemic subjects.

Schneider et al. [17] enrolled 90 hypercholesterolaemic patients consecutively and used stringent exclusion criteria to ensure this was the primary phenotype under investigation. In their power calculations, 90 patients would provide 80 % power to detect a difference due to genotype of a 170 % change of forearm blood flow in response to infusion of 48 µg/min acetylcholine. In Tables 3 and 4, we can clearly see that there are no differences between the genotype groups for most measurements, although with 48 µg/min acetylcholine there is a trend for increased forearm blood flow in the T/T genotype group. In this study, there were only 11 individuals with the T/T genotype; this is a small group to demonstrate any statistically significant difference if indeed true. Although no association was found with the C242T polymorphism, further studies are required with larger numbers of patients in each genotype group. The genotype frequencies also need to be taken into account in the power calculation, this is mandatory before this gene can be excluded as a major player in endothelium-dependent or NO-mediated vasodilation. It would also be interesting to study the same hypothesis in patients with other disorders in which oxidative stress is present, as it is possible that this gene polymorphism does play a major role.
Schneider and co-workers [17] as well as others have assumed the C242T variant of the CYBA gene is a major determinant of NAD(P)H activity in vascular cells. This may not be true as there are now seven polymorphisms described in this gene. Thus further studies assessing the functionality of each or of the haplotypes should be assessed. Other subunits of the complex are also good candidates, as some are also expressed in endothelial cells [9,19]. Ultimately, ‘in vivo’ functional studies, as described by Schneider et al. [17], are key to teasing out the role of genetic polymorphisms and disease; however, of equal importance is that there is good statistical evidence of disease association with a genetic polymorphism and replication of results. In the literature, most of the case-control studies reported are on relatively small numbers of patients and, although some associations are convincing [20], reported are on relatively small numbers of patients. In the literature, most of the case-control studies involving much larger numbers are required. Efforts to expedite such analyses are now being addressed and very large cohort collections and studies are being planned. The UK Biobank project (http://www.ukbiobank.ac.uk/) is such an example. This will be the world’s biggest study (comprising 500,000 participants) and will assess the role of genes and environmental influences in health and disease.

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REFERENCES


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