SCIENTIFIC LETTER

Haplotype analysis of the endothelial nitric oxide synthase gene in relation to acute myocardial infarction

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In addition to directly affecting the blood pressure, endothelium derived nitric oxide has been suggested to play an important part in the development of atherosclerosis. Several genetic variations in the gene encoding endothelial nitric oxide synthase (eNOS) have been reported to affect the expression of eNOS, which synthesises nitric oxide. Among them, polymorphisms in the promoter (−786T>C) and exon 7 (+894G>T) were shown to be associated with reduced vascular nitric oxide production or increased proteolytic cleavage of eNOS.[1] In view of the important role of eNOS in atherogenesis these variants have been hypothesised to influence the susceptibility to atherogenesis. However, findings from different studies are inconsistent and the exact molecular effects of these polymorphisms on eNOS enzyme function and activity are still debated. We conducted a case–control study to elucidate whether polymorphisms of −786T>C and +894G>T in the eNOS gene are associated with acute myocardial infarction (AMI) in the Chinese population.

METHODS

The enrolment criteria of AMI cases and controls for the present study have been reported in detail previously.[2] Briefly, 506 patients with a definite history of AMI from among hospitalised patients of the Fuwai Heart Hospital and Cardiovascular Institute, Beijing, were recruited between October 1997 and September 2001. A group of 506 normal controls were also randomly recruited from participants in a community based survey of cardiovascular risk factors in Beijing.

A structured questionnaire was used to characterise all participants. This included details of medical history, family history of cardiovascular disease, and the other traditional risk factors of AMI such as drug use, cigarette smoking, and alcoholic consumption. Blood pressure, weight, height, waistline, and hip circumference were also recorded. Body mass index and waist to hip ratio were calculated. All participants gave written informed consent. Venous blood was drawn from all participants after an overnight fast. Serum total cholesterol, triglycerides, high density lipoprotein cholesterol, and glucose were measured. We selected −786T>C and +894G>T for genotyping. Polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism. Visualisation was achieved with 3% agarose gel and ethidium bromide staining.

The Hardy-Weinberg equilibrium was assessed by the χ^2 test. Pairwise linkage disequilibrium coefficients were calculated from estimated haplotype frequencies with the 2LD program. Frequencies of categorical variables were compared by the χ^2 test or Fisher's exact test. Haplotype based hypothesis tests of generalised linear models were conducted by use of the haplo.stats software package,[3] in which the AMI status was the dependent variable and haplotypes and the other risk factors were independent and covariate variables. Associations between haplotypes and AMI risk were assessed by odds ratios and 95% confidence intervals. The significance level (two tailed) was taken as $p \leqslant 0.05$.

RESULTS

The traditional risk factors of AMI such as hypertension, smoking, and diabetes were more prevalent in the case group than in the control group. The case group also had significantly higher triglyceride concentrations, body mass index, waist to hip ratio, fasting glucose, and low density lipoprotein cholesterol, and significantly lower high density lipoprotein cholesterol concentrations than the control group. However, no significant differences were found in total cholesterol concentration and systolic blood pressure between the case and the control groups. Similarly, diastolic blood pressure was significantly lower in the cases than in the controls, which may be caused by medication after the patients were informed of their disease.

Table 1 Results of haplo.stats analysis of the 506 patients with acute myocardial infarction and 506 controls according to the haplotypes derived from T-786C and G894T*

Variables	Frequency†	Coefficient	OR	95% CI	p Value
Hypertension (yes/no)	NA	1.33	3.78	2.78 to 5.13	0.000
Diabetes (yes/no)	NA	1.42	4.16	2.63 to 6.55	0.000
Alcohol consumption (yes/no)	NA	−0.79	1.78	1.26 to 2.51	0.000
Smoking (yes/no)	NA	0.58	0.45	0.32 to 0.64	0.001
BMI (kg/m^2)	NA	0.06	1.06	1.02 to 1.09	0.001
HDL-C (mmol/l)	NA	−0.30	0.74	0.68 to 0.81	0.000
T-T (−786T, +894T)	0.102	−0.36	0.70	0.49 to 0.99	0.025
T-G (−786T, +894G)	0.801	NA	1.0	NA	NA

*Dependent variable: presence or absence of acute myocardial infarction; independent variables: diabetes, hypertension, alcohol consumption, smoking, body mass index (BMI), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol, total cholesterol, triglycerides, and three haplotypes. The T-G haplotype was the reference group. Variables with p>0.05 are not listed.
†Haplotype frequency estimated by expectation-maximisation algorithm; frequencies <0.01 are not listed.
CI, confidence interval; NA, not applicable; OR, odds ratio.

Both polymorphisms of −786T>C (p = 0.99) and +894G>T (p = 0.95) did not deviate significantly from the Hardy-Weinberg equilibrium. Table 1 displays the results of haplotype analysis for −786T>C and +894G>T combinations after adjustment of conventional risk factors. For the four haplotypes, the T-G haplotype (−786T and +894G, estimated frequency 0.801) was the reference group. The C-G haplotype had a frequency less than 0.01 (not shown in table 1). Compared with the T-G haplotype, the T-T haplotype had a significant independent protective effect against the risk of AMI (p = 0.025). Patients with the C-T haplotype also had a lower risk of AMI than did those not carrying this haplotype (odds ratio 0.95), though its effect did not reach significance (p = 0.387).

DISCUSSION

In contrast to previous studies that obtained positive results,[4] this study found no sign of associations between polymorphisms of −786T>C and +894G>T and AMI in the Chinese population. One large scale association study genotyping 112 polymorphisms of 71 candidate genes in 2819 unrelated Japanese patients with AMI and 2242 unrelated Japanese controls also found no relation between both polymorphisms and AMI status.[5] This discrepancy may be caused by the genetic heterogeneity across ethnic groups. This is consistent with previous studies that found two groups of greatly different allele frequencies in white and Japanese populations for −786T>C and +894G>T polymorphisms, respectively.[1 4 5] Our result indicates that allele frequencies of both polymorphisms in the Chinese population were similar to those in the Japanese.

Additionally, the present study observed a weak but significant linkage disequilibrium between −786T>C and +894G>T (D' = 0.61, p = 0.002), a result similar to the findings in white and Japanese populations. We then analysed haplotypes, which is suggested to be more powerful than individual single nucleotide polymorphism analyses. Analysis by haplo.stats showed that people carrying the +894T allele were at a lower risk of AMI than were those not carrying this allele in the presence of −786T>C polymorphism.

In summary, we focused on the relation between two variants (−786T>C, +894G>T) in the eNOS gene and AMI in the Chinese population. Our study shows that polymorphisms of −786T>C and +894G>T alone appear to have no significant association with AMI in the Chinese population. Haplotype analysis shows that the two polymorphisms together, however, may more efficiently predict a patient's susceptibility to AMI than either of them alone in the Chinese population.

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