Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1125–1129
Metabolism
Clinical and Experimental
www.metabolismjournal.com

The association of endothelial nitric oxide synthase G894T polymorphism with C-reactive protein level and metabolic syndrome in a Chinese study group

Ming-Chia Hsieh[a,b,c], Jeng-Yueh Hsiao[a], Kai-Jen Tien[a], Shun-Jen Chang[d], Pi-Chen Lin[a], Shih-Chieh Hsu[a], Hui-Ting Liang[a], Hung-Chun Chen[e], Shiu-Ru Lin[b,c,f,g,*]

[a]*Division of Endocrinology and Metabolism, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[b]*Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c]*Graduate Institute of Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan*
[d]*Department of Public Health, Faculty of Medicine, College of Medicine, Kaohsiung, Taiwan*
[e]*Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[f]*Bio-Medical Technology Developmental Center, Fooyin University*
[g]*Department of Medical Research, Fooyin University Hospital*

Received 29 August 2007; accepted 18 March 2008

Abstract

Some studies have reported a possible relationship between endothelial nitric oxide synthase (eNOS) and metabolic syndrome (MS), which is associated with an increased risk for cardiovascular disease. A recent meta-analysis study found the eNOS G894T polymorphism to be associated with ischemic heart disease. Here, we examine the association of eNOS G894T polymorphism with MS in a Chinese population (n = 397). The eNOS T+ (TT and GT) genotypes (56.92% vs 38.86%; odds ratio, 2.08; 95% confidence interval, 1.21-3.56; $P = .007$) and T allele (33.08% vs 23.34%; odds ratio, 1.62; 95% confidence interval, 1.08-2.44; $P = .019$) were significantly more frequent in subjects who had MS. Furthermore, subjects with eNOS T+ genotypes had significantly higher plasma C-reactive protein levels as compared with GG subjects ($P = .004$). This study shows that, in a Chinese population, eNOS G894T polymorphism is associated with an elevated C-reactive protein level and MS.

1. Introduction

Metabolic syndrome (MS) is associated with increased risk for cardiovascular disease; and in humans, it is expressed as a cluster of metabolic abnormalities that include obesity, elevated triglyceride (TG) concentrations, low high-density lipoprotein cholesterol (HDL-C), hypertension, and elevated fasting glucose level [1]. Prevalence of MS varies among ethnic groups [2,3]. According to twin studies, the individual components of MS are to some extent influenced by genetic factors [4,5]. In addition to lifestyle features such as poor diet and physical inactivity, several studies have suggested a heritable basis to the etiology of MS [6,7]. Taken together, these findings suggest that genetic factors might play an important role in MS.

Recently, Duplain et al reported that endothelial nitric oxide synthase (eNOS) knockout mice present a cluster of cardiovascular risk factors comparable with those of MS [8]. Endothelial nitric oxide synthase is involved in the production of nitric oxide (NO), a ubiquitous molecule responsible for the maintenance of normal endothelial function [9]. When NO metabolism is impaired, endothelial dysfunction results, which is likely to be an early step in the development of insulin resistance [10,11]. In turn, insulin resistance has been considered an underlying pathogenic link between the various components of MS, thus explaining the presence of MS even in nonobese individuals [12,13].

* Corresponding author. Department of Medical Research, Fooyin University Hospital, Pingtong 92847, Taiwan. Tel.: +886 8 8323146x9212; fax: +886 8 8325264.
E-mail address: srlin@ms2.hinet.net (S.-R. Lin).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.03.018

The eNOS human gene is on chromosome 7q35-36. Of the currently described polymorphisms [14], the exon 7 G894T polymorphism is the only known common variant in the coding region. This variant has previously been associated with eNOS activity [15,16] or endothelial dysfunction [17,18]. In a recent meta-analysis study, Casas et al [19] found that this variant was associated with ischemic heart disease. In this study, we hypothesize that the same eNOS G894T gene polymorphism is associated with MS, a syndrome that carries high risk for cardiovascular disease. To test this hypothesis, we genotyped 397 subjects for this polymorphism and examined a range of MS indicators.

2. Subjects and methods

Subjects for this cross-sectional study were individuals visiting Kaohsiung Medical University Hospital for routine health examinations in 2005 from January to June. The study design was approved by the hospital Human Research Ethics Committee, and informed consent was obtained from each individual.

Data collected on each subject included demographics and clinical history. Eleven health indicators were assessed. Blood pressure, height, weight, and waist circumference (WC) were measured using standard techniques. Fasting blood samples were obtained to determine plasma glucose, insulin, TG, total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, and C-reactive protein (CRP) concentrations. We excluded subjects who had high CRP levels (≥10 mg/L), a diagnosis of diabetes, or high fasting glucose level (≥126 mg/dL), or those who were taking hyperglycemic medications. Consequently, the current analysis was confined to the 145 men and 252 women who matched our study criteria. In this study, subjects with at least 3 MS risk factors were considered to have MS.

Characteristics of MS [1] include (1) blood pressure ≥130/85 mm Hg; (2) TG ≥150 mg/dL; (3) HDL-C of <40 mg/dL for men and <50 mg/dL for women; (4) fasting plasma glucose of ≥110 mg/dL; and (5) WC ≥ 90 cm in men and ≥80 cm in women. To determine the WC criteria, we followed the International Obesity Task Force criteria for the Asia-Pacific population.

Insulin resistance was assessed from fasting plasma glucose and insulin values using the homeostasis model assessment (HOMA) calculation: insulin resistance = [fasting insulin (in micro–international units per milliliter)× fasting glucose (in millimoles per liter)]/22.5 [20].

2.1. Genotype determination of eNOS G894T polymorphism

Genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism, with nucleotides 5′-GATGAGGCTCAGCCCCAGAAC-3′ and 5′-AGTCA -#ATCCCTTTGGTGCTCAC-3′ as primers. The amplified product was digested with Mbo I enzyme and size-fractionated by electrophoretic separation on 3% agarose gels.

2.2. Biochemical analyses

Biochemical analyses were done on a Beckman Coulter (Fullerton, CA) biochemical analyzer (SYNCHRON CX-5CE). Serum cholesterol (cholesterol oxidase phenol 4-aminoantipyrine peroxidase [CHOD-PAP] method), TG (glycerol 3-phosphate oxidase phenol 4-aminoantipyrine peroxidase [GPO-PAP] method), and HDL-C (direct method; polyethylene glycol–penetrated enzymes) levels were measured. C-reactive protein was measured with a highly sensitive assay (DPC, Los Angeles, CA).

2.3. Statistical analysis

The clinical and biochemical features of the population were presented as mean ± SD, median (interquartile range [IQR]), or percentages. Because the distributions of CRP, insulin, TG, and HOMA were highly skewed, these variables were natural log-transformed for all other analyses. Odds ratios (OR) and 95% confidence intervals (95% CIs) were determined to assess the level of differences in distributions for allelic and genotype frequencies between those with and without MS. The observed frequencies of the genotypes were compared with the frequencies under Hardy-Weinberg equilibrium by χ^2 tests [21]. The *t* test and 1-way analysis of variance were used to detect mean differences in biochemical data and in MS distribution or eNOS genotype. The analysis of covariance was used to adjust for covariance. All *P* values were calculated based on 2-sided tests, with statistical significance defined as P value $\leq .05$.

3. Results

Based on our criteria, MS was identified in the study population as presented in Table 1. Age, sex, and smoking status were similar between the 2 (MS and non-MS) groups. Subjects with MS had significantly higher body mass index

Table 1
Clinical characteristics of subjects with or without MS

	MS (n = 65)	Non-MS (n = 332)	*P*
Age	49.11 ± 11.18	50.21 ± 13.07	.532
Sex (male/female)	24/41	121/211	.942
BMI (kg/m^2)	26.45 ± 3.99	23.63 ± 3.30	<.001
WC (cm)	87.77 ± 9.61	78.60 ± 9.39	<.001
SBP (mm Hg)	130.62 ± 12.18	123.44 ± 15.23	<.001
DBP (mm Hg)	84.17 ± 9.11	78.68 ± 10.70	<.001
Fasting glucose (mg/dL)	102.22 ± 14.74	91.11 ± 15.51	<.001
HOMA-IR, median (IQR)	1.99 (0.36-7.98)	1.14 (0.10-5.64)	<.001
Insulin, median (IQR)	8.00 (1.5-32.0)	5.00 (0.49-78.5)	<.001
Chol (mg/dL)	203.15 ± 48.57	192.51 ± 36.84	.101
TG, median (IQR)	158 (56-864)	78 (30-481)	<.001
LDL-C (mg/dL)	135.45 ± 40.27	122.07 ± 32.96	.012
HDL-C (mg/dL)	38.53 ± 8.94	53.05 ± 11.91	<.001
CRP, median (IQR)	1.49 (0.209-9.92)	0.82 (0.010-8.01)	<.001
Smoking status (%)	9 (13.8%)	43 (13.0%)	.846

Data are means ± SD. DBP indicates diastolic blood pressure; IR, insulin resistance; Chol, cholesterol.

Table 2
Distribution of NOS G894T genotypes in subjects with or without MS

	MS (n = 65)	Non-MS (n = 332)	*P*
Genotypes			
TT	6 (9.23%)	26 (7.83%)	.022
GT	31 (47.69%)	103 (31.02%)	
GG	28 (43.08%)	203 (61.14%)	
Allele			
T	43 (33.08%)	155 (23.34%)	.019
G	87 (66.92%)	509 (76.66%)	

(BMI), WC, blood pressure, fasting plasma glucose levels, HOMA-IR, LDL-C, TG, and high-sensitivity CRP than did subjects without MS. The HDL-C levels were lower in subjects with MS.

The distributions of genotypes and alleles differed significantly between subjects with and without MS (Table 2). The eNOS T+ (TT and GT) genotypes (56.92% vs 38.86%; OR, 2.08; 95% CI, 1.21-3.56; $P = .007$) and T allele (33.08% vs 23.34%; OR, 1.62; 95% CI, 1.08-2.44; $P = .019$) were significantly more frequent in subjects with MS indicators than in those without. The eNOS genotype distribution followed Hardy-Weinberg equilibrium ($\chi^2 = 0.2$, $P > .05$).

We summarized the characteristics of all subjects by eNOS genotype (Table 3). No difference was found in age, sex, BMI, WC, systolic blood pressure (SBP), HOMA, or HDL-C level. However, subjects with T+ genotypes (compared with GG) had significantly higher fasting glucose, cholesterol, TG, LDL-C, and CRP levels. In our study, eNOS genotypes, CRP, and MS were strongly correlated. Subjects with T+ genotypes (compared with GG) still had significantly higher CRP levels after adjustment for MS status. Age, sex, and BMI were adjusted in analyses of blood pressure, cholesterol, TG, LDL-C, HDL-C, insulin, and HOMA. Subjects with T+ genotypes (compared with GG) still had significantly higher cholesterol, TG, and LDL-C.

4. Discussion

Our data show an association between eNOS G894T gene polymorphism and MS in a sample of ethnic Chinese. For a given BMI, Asian populations have been reported to have a higher proportion of body fat than white subjects have, which may contribute to the increased metabolic complications observed in Asian [22-24]. Only a few studies have considered the relationship between eNOS gene polymorphism and MS, and none in an Asian study group. The eNOS C786G polymorphism in hypertensive patients is associated with MS [25]; and eNOS haplotypes, but not the eNOS G894T polymorphism, recently have been found to be associated with features of MS [26]. Inconsistencies in the findings about the relationship between eNOS polymorphism and MS could reflect either genetic heterogeneity or differences in environmental factors that influence phenotypic expression of the gene variant.

This study further demonstrated a relationship between the eNOS 894T+ genotype and high cholesterol, TG, and LDL-C levels. In one study with diabetic patients, Ukkola et al [27] found the eNOS TT genotype to be associated with higher plasma very low-density lipoprotein cholesterol and very low-density lipoprotein–TG concentrations, as compared with GG or GT genotypes. The relationship of the eNOS G894T polymorphism and plasma cholesterol and LDL-C levels has never been studied. However, a significant elevation of plasma cholesterol in eNOS knockout mice has been reported [8,28]. The mechanism linking the eNOS G894T polymorphism and plasma lipid levels remains unknown.

Subjects with eNOS 894T+ genotypes also had higher blood pressure (compared with GG genotype). Chen et al [29] speculated that the eNOS allelic variation (G894T), in conjunction with insulin resistance, contributes to the predisposition to hypertension. Another study (by Jia et al [30]) suggested that the same polymorphism might be a major risk factor of essential hypertension in Chinese. Recently, however, no association was detected between the polymorphism and hypertension [31]. Inconsistencies in the reported correlation with blood pressure may be attributed to many factors including ethnic and environmental differences. Future studies with large numbers of subjects should clarify the situation.

In the present study, we found that plasma glucose levels were significantly higher in subjects with eNOS 894T+ genotypes as compared with those with GG genotype. Comparing this finding with previous research, we find that, in a cross-sectional study of a white population, Monti et al [32] found an association between eNOS G894T polymorphism and diabetes. Recently, Tso et al [33] found in a

Table 3
Clinical characteristics of all subjects by eNOS genotypes

eNOS genotype	TT + GT (n = 166)	GG (n = 231)	*P*	*P*
Age (y)	50.9 ± 13.7	49.3 ± 12.3	.246	–
Sex (male/female)	104/62	156/75	.336	–
BMI (kg/m^2)	24.3 ± 4.0	23.9 ± 3.9	.413	–
WC (cm)	81.3 ± 11.3	79.3 ± 10.1	.121	–
SBP (mm Hg)	125.8 ± 15.8	124.5 ± 15.4	.462	.091 *
DBP (mm Hg)	82.6 ± 11.0	79.4 ± 11.1	.011	.002 *
Chol (mg/dl)	200.7 ± 40.3	189.6 ± 37.6	.005	.005 *
TG, median (IQR)	93 (65-136)	80 (54-113)	.048	.737 *
HDL (mg/dL)	49.8 ± 12.7	51.1 ± 12.6	.325	.657 *
LDL (mg/dL)	131.1 ± 35.7	119.3 ± 32.8	<.001	<.001 *
Fasting glucose (mg/dL)	97.9 ± 19.6	90.3 ± 15.9	<.001	–
Insulin, median (IQR)	6.08 (3.57-9.40)	5.49 (3.22-9.95)	.953	.657 *
HOMA-IR, median (IQR)	1.36 (0.81-2.30)	1.14 (0.72-2.07)	.510	.830 *
CRP, median (IQR)	1.21 (0.56-2.20)	0.82 (0.36-1.69)	.004	.011 †
Smoking status (%)	22 (13.3%)	30 (13.0%)	.938	–

Data are mean ± SD. C-reactive protein adjusted for age, sex, and BMI.
* Adjusted for age, sex, and BMI.
† Adjusted for MS.

5-year prospective study that this polymorphism appears to be predictive of persistent hyperglycemia in Chinese subjects with impaired glucose tolerance. Nitric oxide modulates peripheral and hepatic glucose metabolism [34]; as such, the role of NO as a modulator of physiological insulin secretion has been extensively evaluated [35]. Nitric oxide alterations may also affect glucose metabolism. The replacement of glutamine by aspartate at position 298 in eNOS G894T polymorphism renders the eNOS protein more susceptible to intracellular cleavage [36]. In culture with transfected cells, the T variant results in impaired NO production [37], decreased eNOS activity in human placental tissues [38], and reduced NO levels or activity [32,39]. However, other studies [40,41] found no association between the polymorphism and levels or activity of NO.

One independent predictor of cardiovascular disease is CRP, an acute-phase reactant and sensitive marker of subclinical inflammation [42,43]. Here, CRP levels were elevated in subjects with MS; and a relationship was found between CRP levels and polymorphisms. This finding might partially explain the increased prevalence of eNOS G894T polymorphism among patients with cardiovascular disease. Other studies report the same CRP-polymorphism relationship [44,45]. Several studies have estimated the heritability of CRP to be between 0.3 and 0.4 after adjusting for sex and age [46,47]. Evidence of a significant association between CRP gene and CRP levels was found in some [48,49] but not all [50] studies. These differences point to the contribution of other genetic factors. The eNOS G894T polymorphism has been found to be associated with other inflammatory markers, like fibrinogen and white blood count, but not CRP [51]. Nitric oxide might down-regulate CRP expression in vivo [52,53]. As well, strategies to lower plasma CRP might be effective through improving NO bioavailability [54]. The mechanisms affecting plasma CRP levels require further study.

Limitations of this study include the absence of functional studies. Future research is needed to determine whether the eNOS G894T polymorphism functionally underlies a mechanism leading to MS. In addition, the nature of cross-sectional studies limits results to intriguing correlations on which more stringent studies can be based. Long-term prospective studies on the eNOS G894T polymorphism and MS could greatly elucidate their relationship.

In conclusion, our results showed that eNOS G894T polymorphism was associated with an increased risk for MS in a Chinese population. We also suspect that this polymorphism may be a genetic marker for elevated CRP levels.

References

[1] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Executive summary of the third report of the National Cholesterol Education Program (NCEP). JAMA 2001;285:2486-97.

[2] Raji A, Seely EW, Arky RA, Simonson DC. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Clin Endocrinol Metab 2001;86:5366-71.

[3] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination Survey. JAMA 2002;287:356-9.

[4] Heller DA, de Faire U, Pedersen NL, Dahlen G, McClearn GE. Genetic and environmental influences on serum lipid levels in twins. N Engl J Med 1993;328:1150-6.

[5] Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U. Quantitative genetic analyses of insulin-like frowth factor I (IGF-I), IGF-binding protein–1, and insulin levels in middle-aged and elderly twins. J Clin Endocrinol Metab 1996;81:1791-7.

[6] Hong Y, Pedersen NL, Brismar K, de Faire U. Genetic and environmental architecture of the features of the insulin-resistance syndrome. Am J Hum Genet 1997;60:143-52.

[7] Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci U S A 2000;97:14478-83.

[8] Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, et al. Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. Circulation 2001;104:342-5.

[9] Liese AD, Mayer-Davis EJ, Haffner SM. Development of the multiple metabolic syndrome: an epidemiologic perspective. Epidemiol Rev 1998;20:157-72.

[10] Egashira K, Inou T, Hirooka Y, Yamada A, Maruoka Y, Kai H, et al. Impaired coronary blood flow response to acetylcholine in patients with coronary risk factors and proximal atherosclerotic lesion. J Clin Invest 1993;91:29-37.

[11] Drexler H, Zeiher AM, Wollschlager AM, Meinertz T, Just H, Bonzel T. Flow dependent coronary artery dilatation in humans. Circulation 1989;80:466-74.

[12] Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 1991;34:416-22.

[13] Cheal KL, Abbasia F, Lamendola C, McLaughlin T, Reaven GM, Ford ES. Relationship to insulin resistance of the Adult Treatment Panel III diagnostic criteria for identification of the metabolic syndrome. Diabetes 2004;53:1195-200.

[14] Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993;268: 17478-88.

[15] Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. Proc Natl Acad Sci U S A 2000;97:2832-5.

[16] Persu A, Stoenoiu MS, Messiaen T, Davila S, Robino C, El-Khattabi O, et al. Modifier effect of eNOS in autosomal dominant polycystic kidney disease. Hum Mol Genet 2002;11:229-41.

[17] Savvidou MD, Vallance PJ, Nicolaides KH, Hingorani AD. Endothelial nitric oxide synthase gene polymorphism and maternal vascular adaptation to pregnancy. Hypertension 2001;38:1289-93.

[18] Leeson CP, Hingorani AD, Mullen MJ, Jeerooburkhan N, Kattenhorn M, Cole TJ, et al. Glu298Asp endothelial nitric oxide synthase gene polymorphism interacts with environmental and dietary factors to influence endothelial function. Circ Res 2002;90: 153-1158.

[19] Casas JP, Bautista LE, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase genotype and ischemic heart disease. Circulation 2004;109:1359-65.

[20] Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and *b*-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

[21] Cannings C, Edwards AW. Expected genotypic frequencies in a small sample: deviation from Hardy-Weinberg equilibrium. Am J Hum Genet 1969;21:245-7.
[22] Kim Y, Suh YK, Choi H. BMI and metabolic disorders in South Korean adults: 1998 Korea National Health and Nutrition Survey. Obes Res 2004;12:445-53.
[23] Thomas GN, Ho SY, Lam KS, Janus ED, Hedley AJ, Lam TH. Impact of obesity and body fat distribution on cardiovascular risk factors in Hong Kong Chinese. Obes Res 2004;12:1805-13.
[24] Lee SH, Kim YS, Sunwoo S, Huh BY. A retrospective cohort study on obesity and hypertension risk among Korean adults. J Korean Med Sci 2005;20:188-95.
[25] Femandez ML, Ruiz R, Gonzalez MA, Ramirez-Lorca R, Couto C, Ramos A, et al. Association of NOS3 gene with metabolic syndrome in hypertensive patients. Thromb Haemost 2004;92:413-8.
[26] Gonzalez-Sanchez JL, Martinez-Larrad MT, Saez ME, Zabena C, Martinez-Calatrava MJ, Serrano-Rios M. Endothelial nitric oxide synthase haplotypes are associated with features of metabolic syndrome. Clin Chem 2007;53:91-7.
[27] Ukkola O, Erkkila PH, Savolainen MJ, Kesaniemi YA. Lack of association between polymorphisms of catalase, copper-zinc superoxide dismutase (SOD), extracellular SOD and endothelial nitric oxide synthase genes and macroangiopathy in patients with type 2 diabetes mellitus. J Intern Med 2001;249:451-9.
[28] Cook S, Hugli O, Egli M, Vollenweider P, Burcelin R, Nicod P, et al. Clustering of cardiovascular risk factors mimicking the human metabolic syndrome X in eNOS null mice. Swiss Med Wkly 2003; 133:360-3.
[29] Chen W, Srinivasan SR, Elkasabany A, Ellsworth DL, Boerwinkle E, Berenson GS. Combined effects of endothelial nitric oxide synthase gene polymorphism (G894T) and insulin resistance status on blood pressure and familial risk of hypertension in young adults: the Bogalusa Heart Study. Am J Hypertens 2001;14:1046-52.
[30] Jia CQ, Zhao ZT, Wang LH, Hao FR, Feng YQ, Wang SM, et al. Effects of G894T mutation in the endothelial nitric oxide synthase gene on blood pressure. Zhonghua Liu Xing Bing Xue Za Zhi 2003;24:36-9.
[31] Moe KT, Lim ST, Wong P, Chua T, DeSilva DA, Koh TH, et al. Association analysis of endothelial nitric oxide synthase gene polymorphism with primary hypertension in a Singapore population. J Hum Hypertens 2006;20:956-63.
[32] Monti LD, Barlassina C, Citterio L, Galluccio E, Berzuini C, Setola E, et al. Endothelial nitric oxide synthase polymorphisms are associated with type 2 diabetes and the insulin resistance syndrome. Diabetes 2003;52:1270-5.
[33] Tso Annette WK, Tan Kathryn CB, Wat Nelson MS, Janus ED, Lam TH, Lam Karen SL. Endothelial nitric oxide synthase G894T (Glu298Asp) polymorphism was predictive of glycemic status in a 5-year prospective study of Chinese subjects with impaired glucose tolerance. Metab Clin Exp 2006;55:1155-8.
[34] Pieper GM. Enhanced, unaltered and impaired nitric oxide–mediated endothelium dependent relaxation in experimental diabetes mellitus: importance of disease duration. Diabetologia 1999;42:204-13.
[35] Spinas GA. The dual role of nitric oxide in islet *b*-cells. News Physiol Sci 1999;14:49-54.
[36] Tesauro M, Thompson WC, Roglianai P, Qi L, Chaudhary PP, Moss J. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. Proc Natl Acad Sci U S A 2000;97:2832-5.
[37] Noiri E, Satoh H, Taguchi J, Brodsky SV, Nakao A, Ogawa Y, et al. Association of eNOS Glu298Asp polymorphism with end-stage renal disease. Hypertension 2002;40:535-40.
[38] Wang XL, Sim AS, Wang MX, Murrell GA, Trudinger B, Wang J. Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. FEBS Lett 2000; 471:45-50.
[39] Veldman BA, Spiering W, Doevendans PA, Vervoort G, Kroon AA, de Leeuw PW, et al. The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. J Hypertens 2002;20:2023-7.
[40] Ohtoshi K, Yamasaki Y, Gorogawa S, Hayaishi-Okano R, Node K, Matsuhisa M, et al. Association of −786C mutation of endothelial nitric oxide synthase gene with insulin resistance. Diabetologia 2002; 45:1594-601.
[41] Nagassaki S, Metzger IF, Souza-Costa DC, Marroni AS, Uzuelli JA, Tanus-Santos JE. eNOS genotype is without effect on circulating nitrite/nitrate level in healthy male population. Thromb Res 2005;115: 375-9.
[42] Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996; 144:537-47.
[43] Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000;321:199-204.
[44] Choi EY, Park EH, Cheong YS, Rheem I, Park SG, Yoo S. Association of C-reactive protein with the metabolic risk factors among young and middle-aged Koreans. Metabolism 2006;55:415-21.
[45] Florez H, Castillo-Florez S, Mendez A, Casanova-Romero P, Larreal-Urdaneta C, Lee D, et al. C-reactive protein is elevated in obese patients with the metabolic syndrome. Diabetes Res Clin Pract 2006; 71:92-100.
[46] Pankow JS, Folsom AR, Cushman M, Borecki IB, Hopkins PN, Eckfeldt JH, et al. Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. Atherosclerosis 2001;154:681-9.
[47] Best LG, North KE, Tracy RP, Lee ET, Howard BV, Palmieri V, et al. Genetic determination of acute phase reactant levels: the strong heart study. Hum Hered 2004;58:112-6.
[48] Suk HJ, Ridker PM, Cook NR, Zee RY. Relation of polymorphism within the C-reactive protein gene and plasma CRP levels. Atherosclerosis 2005;178:139-45.
[49] Smith GD, Lawlor DA, Harbord R, Timpson N, Rumley A, Lowe GD, et al. Association of C-reactive protein with blood pressure and hypertension: life course confounding and mendelian randomization tests of causality. Arterioscler Thromb Vasc Biol 2005;25: 1051-6.
[50] Lange LA, Burdon K, Langefeld CD, Liu Y, Beck SR, Rich SS, et al. Heritability and expression of C-reactive protein in type 2 diabetes in the Diabetes Heart Study. Ann Hum Genet 2006;70: 717-25.
[51] Chrysohoou C, Panagiotakos DB, Pitsavos C, Antoniades C, Skoumas J, Brown M, et al. Evidence for association between endothelial nitric oxide synthase gene polymorphism (G894T) and inflammatory markers: the ATTICA study. Am Heart J 2004;148:733-8.
[52] Kaesemeyer WH Caldwell RW. Atherosclerosis and nitric oxide production. Circulation 2000;102:E90.
[53] McCarty MF. AMPK activation may suppress hepatic production of C-reactive protein by stimulating nitric oxide synthase. Med Hypotheses 2004;63:328-33.
[54] Fichtlscherer S, Breuer S, Schachinger V, Dimmeler S, Zeiher AM. C-reactive protein levels determine systemic nitric oxide bioavailability in patients with coronary artery disease. Eur Heart J 2004; 25:1412-8.