Biochem Genet (2013) 51:92–100
DOI 10.1007/s10528-012-9545-x

NOTE

Lack of Association of *NOS3* and *ACE* Gene Polymorphisms with Coronary Artery Disease in Southern Tunisia

Rania Abdelhedi · Najla Kharrat · Nouha Abdelmoula Bouayed · Leila Abid · Wafa Abdelmouleh · Imen Trabelsi Sahnoun · Suad AlFadhli · Lobna Laroussi · Ahmed Rebai

Received: 29 December 2011 / Accepted: 18 July 2012 / Published online: 26 September 2012

Introduction

Endothelial nitric oxide synthase (eNOS, encoded by the *NOS3* gene) synthesizes NO from L-arginine and molecular oxygen in vascular endothelial cells (Kincl et al. 2009). Angiotensin-converting enzyme (ACE), present on the surface of vascular endothelial cells, generates the potent vasoconstrictor angiotensin II from angiotensin I and inactivates the vasodilator bradykinin (Erdös 1990). Angiotensin II modulates NO synthesis in cardiovascular tissue, and NO modulates the action of angiotensin II (Dubey et al. 1995; Nakagami et al. 1999). Many polymorphisms located in the *ACE* and *NOS3* genes have been reported to play a major role in the pathogenesis of coronary artery disease (CAD) and related outcomes (Cambien et al. 1992; Yoshimura et al. 2000; Bor-Kucukatay et al. 2010; Hamelin et al. 2011). The −T786C polymorphism in the *NOS3* gene causes a reduction of promoter activity and has been reported as a risk factor for coronary spasm in a Japanese population (Nakayama et al. 1999). The Glu298Asp polymorphism has been

R. Abdelhedi (✉) · N. Kharrat · W. Abdelmouleh · A. Rebai
Laboratory of Microorganisms and Biomolecules, Research Group on Molecular and Cellular Screening Processes, Center of Biotechnology of Sfax, University of Sfax, Sidi Mansour Road, P.O. Box 1177, 3018 Sfax, Tunisia
e-mail: rania.abdelhedi@gmail.com

N. A. Bouayed
Department of Histology, Faculty of Medicine of Sfax, Majida Boulila Avenue, 3028 Sfax, Tunisia

L. Abid · I. T. Sahnoun · L. Laroussi
Cardiology Service, Hedi Chaker Hospital, Sfax, El Ain Road, 3029 Sfax, Tunisia

S. AlFadhli
Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, P.O. Box 31470, 90805 Sulaibikhat, Kuwait

associated with reduced basal NO production (Veldman et al. 2002) and has been linked to the risk for CAD (Hingorani et al. 1999).

A polymorphic variant in intron 16 (ACE-I/D) of the *ACE* gene, characterized by an insertion (I) or deletion (D) of a repeat sequence of 287 noncoding base pairs (Zintzaras et al. 2008), has been shown to be associated with hypertension and other cardiovascular risk factors (Rigat et al. 1990). The ACE4 polymorphism in the 5′ UTR has been considered as a tag SNP in African population (Keavney et al. 1998; Zhu et al. 2001), a result that has also been established by a study of a Tunisian population (Rebai et al. 2006). Few studies have addressed the relationship between *NOS3* and *ACE* gene variants and the pathogenesis of CAD (Nakagami et al. 1999; Alvarez et al. 2001). In this work, we report a case–control study in southern Tunisia, investigating the association between CAD and four SNPs: T-786C (rs11771443) and Glu298Asp (rs1799983) polymorphisms in the *NOS3* gene, and ACE-I/D (rs4340) and ACE4 (rs4291) polymorphisms in the *ACE* gene.

Materials and Methods

Study Subjects

We enrolled 249 unrelated patients (185 men and 64 women, 38–81 years old, mean age 57.07 ± 10.6 years) who were diagnosed with CAD by the Cardiology Service of the Hedi Chaker University Hospital of Sfax, Tunisia, from May 2007 to December 2009. Angiography confirmed that each patient had stenosis >50 % of at least one major coronary artery. As a routine procedure, an informed written consent was obtained from all patients.

The control group consisted of 295 healthy unrelated volunteers (202 men and 93 women, 36–69 years old, mean age 55.2 ± 11.7 years) from the staff of the Sfax Center of Biotechnology and blood donors of the Sfax Center of Transfusion. They had no history of vascular disease. Cases and controls were matched by gender, age, and ethnicity.

Biochemical Analysis

The serum concentrations of glucose, triglycerides, total cholesterol, creatinine, urea, uric acid, and blood major ionic constituents (K^+, Cl^-, and Na^+) were measured by the standard methods used in the clinical laboratory of the hospital.

DNA Analysis

Genomic DNA was extracted using the standard phenol/chloroform method from EDTA anticoagulated peripheral blood samples (Marcadet et al. 1987).

The ACE4, −T786C, and Glu298Asp polymorphisms were typed by PCR amplification, followed by restriction enzyme digestion (PCR–RFLP), with the classical PCR for −T786C and Glu298Asp (using the conditions described by Fatini et al. 2004) and nested PCR for ACE4 (conditions of Zhu et al. 2001). PCR products

were digested with the appropriate enzymes and run on agarose gels (4 %). For the ACE-I/D polymorphism, no digestion was needed. This analysis included an additional PCR to confirm the absence of an I allele in DD individuals. The ACE alleles were visualized as fragments of 490 bp (I) and 190 bp (D) (Shanmugan et al. 1993).

Statistical Analysis

All statistical analyses were performed using SPSS version 13.0 (Chicago, USA). Haplotypes were inferred using Phase version 2.0.2 (Stephens et al. 2001) to estimate the haplotype frequencies in both groups (patients and controls). The differences in allele, genotype, and haplotype frequencies between the groups were tested by chi-square or Student's tests wherever appropriate. Hardy–Weinberg equilibrium was tested using the Genetic Data Analyses program, version 1.1 (Weir 1996). A logistic regression analysis was performed to determine independent predictors for CAD. One-way ANOVA was used to analyze the relationship between genotypes and the general characteristics and severity of CAD. A value of $p < 0.05$ was considered the cutoff for significance.

Results

Genotype, Allele, and Haplotype Frequencies

All genotype distributions of the polymorphisms studied were in agreement with Hardy–Weinberg expectation ($p > 0.05$). Genotype, allele, and haplotype frequencies were similar between patients and controls (Table 1).

Risk Factor for CAD

Binary logistic regression was used to test the association of the diseases (dyslipidemia or CAD) with SNPs after adjusting for confounding factors (age, sex, smoking, hypertension, and body mass index). No significant association was found for the four polymorphisms ($p > 0.05$), but hypertension was identified as an acquired risk factor of CAD ($p = 0.034$) and also of dyslipidemia ($p < 0.001$). In addition, smoking was identified as a risk factor only for CAD ($p < 0.001$). Furthermore, a multivariate analysis using one-way ANOVA between patient groups showed no significant association between the four polymorphisms and any of the clinical and biological parameters ($p > 0.05$; data not shown).

Association Between SNPs, Clinical Characteristics, and CAD Severity

Patients with CAD were classified into three subgroups (G1, G2, and G3) according to the number of affected coronary arteries. The calculation of allele, genotype, and haplotype frequencies revealed no association with CAD severity (data not shown); however, smoking habit ($p = 0.008$), dyslipidemia ($p = 0.028$), type 2 diabetes

Table 1 Frequency of *ACE* and *NOS3* gene SNPs in a Tunisian population

Polymorphism	Controls (%) Total 295	CAD patients (%) Total 249	χ^2 (p)
ACE4			
Genotype			
AA	114 (38.64)	102 (41)	0.45 (0.798)
AT	146 (50)	121 (48.6)	
TT	35 (11.86)	26 (10.4)	
Allele			
A	374 (63.38)	325 (65.26)	0.41 (0.521)
T	216 (36.61)	173 (34.73)	
ACE-I/D			
Genotype			
II	36 (12.2)	28 (11.24)	5.22 (0.072)
ID	134 (45.42)	137 (55)	
DD	125 (42.37)	84 (33.73)	
Allele			
I	206 (35)	193 (38.75)	1.71 (0.190)
D	384 (65)	305 (61.24)	
Haplotype			
AI	78 (29)	66 (29)	1.91 (0.59)
AD	94 (38)	70 (33)	
TI	45 (4)	48 (10)	
TD	78 (29)	65 (28)	
−T786C			
Genotype			
TT	134 (45.42)	120 (48.2)	2 (0.368)
TC	131 (44.4)	105 (42.16)	
CC	40 (13.55)	24 (9.6)	
Allele			
C	211 (35.76)	153 (30.72)	1.86 (0.172)
T	399 (67.62)	345 (69.27)	
Glu298Asp			
Genotype			
GluGlu	130 (44.06)	130 (52.2)	3.59 (0.166)
GluAsp	130 (44.06)	94 (37.75)	
AspAsp	35 (11.86)	25 (10)	
Allele			
Asp	200 (33.89)	144 (28.91)	3.10 (0.078)
Glu	390 (66.1)	354 (71.08)	
Haplotype			
C-Asp	61(15)	48 (11)	0.45 (0.90)
C-Glu	72 (23)	58 (20)	
T-Asp	64 (17)	60 (23)	
T-Glu	98 (45)	83 (46)	

Table 2 Characteristics of patients and their association with CAD severity

Characteristic	All patients ($n = 249$)	CAD severity group[a]			p
		G1 ($n = 127$)	G2 ($n = 54$)	G3 ($n = 68$)	
					χ^2
Gender (male/female)	185/64	94/30	37/17	54/14	0.375
Diabetes mellitus	118	53	27	36	0.357
Type 1 diabetes	62	34	17	11	0.086
Type 2 diabetes	47	15	10	22	0.006
Smoker	142	77	24	49	0.008
Dyslipidemia	114	49	31	38	0.028
Hypertension	142	65	28	45	0.142
					Student's
Age (years)	57.07 ± 10.2	56.50 ± 10.93	56.38 ± 8.3	58.33 ± 10.37	0.512
Body mass index (kg/m^2)	26.66 ± 4.2	25.85 ± 4	26.57 ± 4.16	28.12 ± 4.4	0.090
Systolic blood pressure (mmHg)	133.17 ± 21.56	134.42 ± 21	134.90 ± 23.2	130.6 ± 21.64	0.275
Diastolic blood pressure (mmHg)	78.2 ± 12.3	79.28 ± 12.8	78.80 ± 11.3	76.27 ± 12.1	0.449
Serum chemistry					
Glucose (mmol/l)	8.08 ± 3.7	7.36 ± 3.083	8.81 ± 4.405	8.67 ± 4.054	0.033
Creatinine (μmol/l)	114.79 ± 69.6	109.44 ± 59.816	126.56 ± 107.094	113.96 ± 41.521	0.364
Urea (mmol/l)	7.41 ± 4	7.06 ± 3.915	7.25 ± 3.294	8.07 ± 4.481	0.288
Triglycerides (mmol/l)	1.88 ± 1.1	1.73 ± 0.979	1.93 ± 1.161	2.07 ± 1.476	0.255
Total cholesterol (mmol/l)	4.67 ± 1.2	4.46 ± 1.342	4.86 ± 1.078	4.87 ± 1.294	0.116
Sodium (Na^+ mmol/l)	139.1 ± 4	139.59 ± 5.005	138.31 ± 3.095	138.94 ± 3.095	0.196
Chlorine (Cl^- mmol/l)	100.5 ± 14	100.51 ± 14.444	100.21 ± 16.794	100.56 ± 11.335	0.993
Potassium (K^+ mmol/l)	4.09 ± 0.5	4.09 ± 0.515	4.22 ± 0.492	4.01 ± 0.463	0.107
Uric acid (mmol/l)	328.50 ± 113.816	31,697 ± 125.126	341.28 ± 94.345	345.83 ± 104.36	0.720

[a] Based on number of affected coronary arteries: stenosis >50 % of one (G1), two (G2), or three (G3) major coronary arteries. Quantitative data presented as mean ± standard deviation

($p = 0.006$), and increased rate of glucose ($p = 0.033$) were found to differ among the three groups of patients and to correlate with increased risk of CAD severity (Table 2).

Discussion

In the present study, we examined the possibility of association between CAD and four polymorphisms in a southern Tunisian sample: ACE4 and ACE-I/D polymorphism in the *ACE* gene, and −T786C and Glu298Asp in the *NOS3* gene with CAD. We found no association, and this finding persisted after adjusting for several potential confounding factors.

Regarding the Glu298Asp polymorphism in the *NOS3* gene, no association has been reported with CAD in Asian and European populations (Aras et al. 2002; Fatini et al. 2004; Kim et al. 2007; Guldiken et al. 2009), which agrees with our finding. This polymorphism was not associated with hypertension in the Tunisian population (Sediri et al. 2010). In contrast, previous studies showed that this polymorphism seemed to be significantly and independently associated with the occurrence and severity of CAD in Italian and Japanese populations (Yoshimura et al. 1998; Ghilardi et al. 2002; Colombo et al. 2003). These contradictory results might lie in the ethnic origins of the populations studied or in differences in selection criteria and sample sizes.

Next, we found no association between the polymorphism −T786C in the *NOS3* gene and CAD. The results were the same in studies involving European and Australian populations, but the relation between this SNP and CAD remained controversial (Marroni et al. 2005). The meta-analysis reported by Casas et al. (2004) for the Glu298Asp and −T786C polymorphisms showed a difference in allelic frequencies for Asians versus non-Asians for both polymorphisms. These interethnic differences might in part explain the ethnic disparities in NO bioavailability, cardiovascular risk, and response to drugs (Marroni et al. 2005).

No association with CAD was found for haplotypes of the *NOS3* or the *ACE* gene in our population. In contrast, Sandrim et al. (2007) reported that the C-Glu haplotype decreased the risk of developing hypertension and was associated with higher nitrite/nitrate (NO_x) levels in hypertensive patients, whether combined or not with type 2 diabetes mellitus, although individual *NOS3* polymorphisms did not have significant effects. Moreover, this same specific haplotype was involved in the modulation of NO formation (Metzger et al. 2005, 2007, 2011) in healthy Caucasian subjects. Our study included more enrolled subjects, which might have increased the power of our study.

The patients who had dyslipidemia were taking statins, which increased NOS3 expression and up-regulated NO formation (Lacchini et al. 2010). No significant association was found between dyslipidemia and the SNPs using binary logistic regression. On the other hand, several studies carried out on Caucasians have shown that only healthy subjects with the CC genotype for T-786C receiving treatment with a low dose of atorvastatin for 2 weeks had augmented NO availability, produced antioxidant (Nagassaki et al. 2006) and anti-inflammatory (Souza-Costa

et al. 2007) effects, and reduced membrane fluidity of erythrocytes (Nagassaki et al. 2009). These studies included only a small number (30) of healthy male subjects, which might have limited power to detect the difference between groups and may restrict the conclusions to this specific population.

Regarding the *ACE* gene polymorphism, we found no association for ACE4 with CAD. Zhu et al. (2001), however, found a positive correlation between blood pressure and plasma concentration in an African population. This result was expected because allele frequencies in our population are different from those of Africans and Europeans (El Moncer et al. 2010). For the ACE-I/D polymorphism, a recent meta-analysis conducted by Zintzaras et al. (2008), including 118 studies (43,733 cases with CAD and 82,606 controls), reported a significant association for European populations (odds ratio 1.25, DD vs. II). Furthermore, Dzimiri et al. (2000) stated that no association has been reported to date in Arab or North African populations. We can explain this finding by the heterogeneous genetic profile of the Tunisian population, which is characterized by important emetic exchanges throughout history and frequent migration around the Mediterranean Sea (Maalej et al. 2004). The authors ranked the Tunisian population between the Sub-Saharan African and the Caucasian populations. Another explanation of our result may be the presence of linkage disequilibrium between the ACE-I/D polymorphism and other polymorphisms in this region. Indeed, Keavney et al. (1998) suggested the presence of functional polymorphisms located between intron 18 and the 3′ UTR and excluded the ACE-I/D marker within intron 16 in this region.

Furthermore, conventional risk factors were highly prevalent in patients, as expected (Vogel and Motulsky 1997; Wilson and Culletton 1998). These results were further supported by regression analysis, which demonstrated, after correction, that only smoking could be identified as a dependent acquired risk factor for CAD. Hypertension was identified as a risk factor for CAD and dyslipidemia. Our study lacks the assessment of NO formation in CAD patients and controls, which could improve our finding.

Acknowledgments This work was supported by the Ministry of Higher Education and Scientific Research, Tunisia. We would like to thank Mr. Nouri of the Cardiology Service of the Chedi Chaker Hospital for his technical assistance. We express also our gratitude to Prof. J. Jaoua, founder and former Head of the English Department at Sfax Faculty of Sciences, for proofreading the manuscript. We also would like to thank M. Choura for her help in improving the manuscript and her friendliness.

References

Alvarez R, González P, Batalla A, Reguero JR, Iglesias-Cubero G, Hevia S, Cortina A, Merino E, González I, Alvarez V, Coto E (2001) Association between the NOS3 (−786 T/C) and the ACE (I/D) DNA genotypes and early coronary artery disease. Nitric Oxide 5:343–348

Aras O, Hanson NQ, Bakanay SM, Tsai MY, Gulec S (2002) Endothelial nitric oxide gene polymorphism (Glu298Asp) is not associated with coronary artery disease in Turkish population. Thromb Haemost 87:347–349

Bor-Kucukatay M, Demir S, Akbay R, Dursunoglu D, Akdag B, Semiz E (2010) Relationship between hemorheology and Glu(298)Asp polymorphism of endothelial nitric oxide synthase gene in patients with coronary artery disease. Mol Biol Rep 37:171–178

Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F (1992) Deletion polymorphisms in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 359:641–644

Casas JP, Bautista LE, Humphries SE, Hingorani AD (2004) Endothelial nitric oxide synthase genotype and ischemic heart disease. Meta-analysis of 26 studies involving 23,028 subjects. Circulation 109:1359–1365

Colombo MG, Paradossi U, Andreassi MG, Botto N, Manfredi S, Masetti S, Biagini A, Clerico A (2003) Endothelial nitric oxide synthase gene polymorphisms and risk of coronary artery disease. Clin Chem 49:389–395

Dubey RK, Jackson EK, Luscher TF (1995) Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell: role of cyclic-nucleotides and angiotensin 1 receptors. J Clin Invest 96:141–149

Dzimiri N, Basco C, Moorji A, Meyer BF (2000) Angiotensin-converting enzyme polymorphism and the risk of coronary heart disease in the Saudi male population. Arch Pathol Lab Med 124:531–534

El Moncer W, Esteban E, Bahri R, Gayà-Vidal M, Carreras-Torres R, Athanasiadis G, Moral P, Chaabani H (2010) Mixed origin of the current Tunisian population from the analysis of Alu and Alu/STR compound systems. J Hum Genet 55:827–833

Erdös EG (1990) Angiotensin I converting enzyme and the changes in our concepts through the years. Lewis K. Dahl memorial lecture. Hypertension 16:363–370

Fatini C, Sofi F, Sticchi E, Gensini F, Gori AM, Fedi S, Lapini I, Rostagno C, Comeglio M, Brogi D, Gensini G, Abbate R (2004) Influence of endothelial nitric oxide synthase gene polymorphisms (G894T, 4a4b, T-786C) and hyperhomocysteinemia on the predisposition to acute coronary syndromes. Am Heart J 147:516–521

Ghilardi G, Biondi ML, De Monti M, Bernini M, Turri O, Massaro F, Guagnellini E, Scorza R (2002) Independent risk factor for moderate to severe internal carotid artery stenosis: T786C mutation of the endothelial nitric oxide synthase gene. Clin Chem 48:989–993

Guldiken B, Sipahi T, Guldiken S, Ustundag S, Budak M, Turgut N, Ozkan H (2009) Glu298Asp polymorphism of the endothelial nitric oxide synthase gene in Turkish patients with ischemic stroke. Mol Biol Rep 36:1539–1543

Hamelin BA, Zakrzewski-Jakubiak M, Robitaille NM, Bogaty P, Labbé L, Turgeon J (2011) Increased risk of myocardial infarction associated with angiotensin-converting enzyme gene polymorphism is age dependent. J Clin Pharmacol 51:1286–1292

Hingorani AD, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, Haydock S, Hopper RV, Stephens NG, O'Shaughnessy KM, Brown MJ (1999) A common variant of the endothelial nitric oxide synthase (Glu298 → Asp) is a major risk factor for coronary artery disease in the UK. Circulation 100:1515–1520

Keavney B, McKenzie CA, Connell JMC, Julier C, Ratcliffe PJ, Sobel E, Lathrop M, Farrall M (1998) Measured haplotype analysis of the angiotensin-I-converting enzyme gene. Hum Mol Genet 7:1745–1751

Kim IJ, Bae J, Lim SW, Cha DH, Cho HJ, Kim S, Yang DH, Hwang SG, Oh D, Kim NK (2007) Influence of endothelial nitric oxide synthase gene polymorphisms 8(−786T>C, 4a4b, 894G>T) in Korean patients with coronary artery disease. Thromb Res 119:579–585

Kincl V, Vasků A, Meluzín J, Panovský R, Seménka J, Groch L (2009) Association of the eNOS 4a/b and −786T/C polymorphisms with coronary artery disease, obesity and diabetes mellitus. Folia Biol (Praha) 55:187–191

Lacchini R, Silva PS, Tanus-Santos JE (2010) A pharmacogenetics-based approach to reduce cardiovascular mortality with the prophylactic use of statins. Basic Clin Pharmacol Toxicol 106:357–361

Maalej A, Rebai A, Ayadi A, Jouida J, Makni H, Ayadi H (2004) Allelic structure and distribution of 103 STR loci in a Southern Tunisian population. J Genet 83:65–71

Marcadet A, O'Connel P, Cohen D (1987) Standardized southern blot workshop techniques. In: Dupont B (ed) Histocompatibility testing. Springer, New York, pp 587–590

Marroni AS, Metzger IF, Souza-Costa DC, Nagassaki S, Sandrim VC, Correa RX, Rios-Santos F, Tanus-Santos JE (2005) Consistent interethnic differences in the distribution of clinically relevant endothelial nitric oxide synthase genetic polymorphisms. Nitric Oxide 12:177–182

Metzger IF, Souza-Costa DC, Marroni AS, Nagassaki S, Desta Z, Flockhart DA, Tanus-Santos JE (2005) Endothelial nitric oxide synthase gene haplotypes associated with circulating concentrations of nitric oxide products in healthy men. Pharmacogenet Genomics 15:565–570

Metzger IF, Sertório JT, Tanus-Santos JE (2007) Modulation of nitric oxide formation by endothelial nitric oxide synthase gene haplotypes. Free Radic Biol Med 43:987–992

Metzger IF, Ishizawa MH, Rios-Santos F, Carvalho WA, Tanus-Santos JE (2011) Endothelial nitric oxide synthase gene haplotypes affect nitrite levels in black subjects. Pharmacogenomics J 11:393–399

Nagassaki S, Sertório JT, Metzger IF, Bem AF, Rocha JB, Tanus-Santos JE (2006) eNOS gene T-786C polymorphism modulates atorvastatin-induced increase in blood nitrite. Free Radic Biol Med 41:1044–1049

Nagassaki S, Herculano RD, Graeff CF, Tanus-Santos JE (2009) eNOS T-786C polymorphism affects atorvastatin-induced changes in erythrocyte membrane fluidity. Eur J Clin Pharmacol 65:385–392

Nakagami H, Ikeda U, Maeda Y, Yamamoto K, Hojo Y, Kario K, Kuroki S, Shimada K (1999) Coronary artery disease and endothelial nitric oxide synthase and angiotensin-converting enzyme gene polymorphisms. J Thromb Thrombolysis 8:191–195

Nakayama T, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, Motoyama T, Saito Y, Ogawa Y, Miyamoto Y, Nakao K (1999) T-786C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 99:2864–2870

Rebai M, Kharrat N, Ayadi I, Rebai A (2006) Haplotype structure of five SNPs within the ACE gene in the Tunisian population. Ann Hum Biol 33:319–329

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86:1343–1346

Sandrim VC, de Syllos RW, Lisboa HR, Tres GS, Tanus-Santos JE (2007) Influence of eNOS haplotypes on the plasma nitric oxide products concentrations in hypertensive and type 2 diabetes mellitus patients. Nitric Oxide 16:348–355

Sediri Y, Kallel A, Ayadi I, Feki M, Elasmi M, Haj Taieb S, Sanhaji H, Souheil O, Jemaa R, Kaabachi N (2010) Lack of association between endothelial nitric oxide synthase gene G894T polymorphism and hypertension in the Tunisian population. Prev Med 51:88–89

Shanmugan V, Sell KW, Saha BK (1993) Mistyping ACE heterozygotes. PCR Methods Appl 3:120–121

Souza-Costa DC, Sandrim VC, Lopes LF, Gerlach RF, Rego EM, Tanus-Santos JE (2007) Anti-inflammatory effects of atorvastatin: modulation by the T-786C polymorphism in the endothelial nitric oxide synthase gene. Atherosclerosis 193:438–444

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989

Veldman BA, Spiering W, Doevendans PA, Vervoort G, Kroon AA, de Leeuw PW, Smits P (2002) The Glu298Asp polymorphisms of the NOS3 gene as a determinant of the baseline production of nitric oxide. J Hypertens 20:2023–2027

Vogel F, Motulsky AG (1997) Human genetics: problems and approaches, 3rd edn. Springer, Heidelberg

Weir BS (1996) Genetic data analysis, 2nd edn. Sinauer Associates, Sunderland

Wilson PWF, Culletton BF (1998) Epidemiology of cardiovascular disease in the United States. Am J Kidney Dis 32:56–65

Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Sumida H, Sugiyama S, Kugiyama K, Ogawa H, Ogawa Y, Saito Y, Miyamoto Y, Nakao K (1998) A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. Hum Genet 103:65–69

Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Saito Y, Miyamoto Y, Ogawa Y, Kaneshige T, Hiramatsu H, Yoshioka T, Kamitani S, Teraoka H, Nakao K (2000) Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786C and missense Glu298Asp variants. J Invest Med 48:367–374

Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, McKenzie CA, Luke A, Chen G, Elston RC, Ward R (2001) Linkage and association analysis of angiotensin I-converting enzyme (ACE) gene polymorphisms with ACE concentration and blood pressure. Am J Hum Genet 68:1139–1148

Zintzaras E, Raman G, Kitsios G, Lau J (2008) Angiotensin-converting enzyme insertion/deletion gene polymorphic variant as a marker of coronary artery disease: a meta-analysis. Arch Intern Med 168:1077–1089