

**Reply**

Gian Paolo Rossi, and Maurizio Cesari

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## REFERENCES

- Rossi GP, Maiolino G, Zanchetta M, et al. The T(-786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients. *J Am Coll Cardiol* 2006;48:1166-74.
- 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.
- Metzger IF, Souza-Costa DC, Marroni AS, et al. Endothelial nitric oxide synthase gene haplotypes associated with circulating concentrations of nitric oxide products in healthy men. *Pharmacogenet Genomics* 2005;15:565-70.
- Nagassaki S, Sertorio JTC, Metzger IF, Bern AF, Rocha JBT, Tanus-Santos JE. eNOS gene T-786C polymorphism modulates atorvastatin-induced increase in blood nitrite. *Free Radic Biol Med* 2006;41:1044-9.
- Abe K, Nakayama M, Yoshimura M, et al. Increase in the transcriptional activity of the endothelial nitric oxide synthase gene with fluvastatin: a relation with the-786T>C polymorphism. *Pharmacogenet Genomics* 2005;15:329-36.
- Souza-Costa DC, Sandrim VC, Lopes LF, Gerlach RF, Rego EM, Tanus-Santos JE. Anti-inflammatory effects of atorvastatin: modulation by the T-786C polymorphism in the endothelial nitric oxide synthase gene. *Atherosclerosis* 2007. In press.

# Effects of Endothelial Nitric Oxide Synthase Gene Polymorphisms on Oxidative Stress, Inflammatory Status, and Coronary Atherosclerosis: An Example of a Transient Phenotype

In their recent study, Rossi et al. (1) showed that the T786C polymorphism on the promoter region of the endothelial nitric oxide synthase (eNOS) gene modifies redox-sensitive inflammatory pathways, and may be a predictor for clinical outcome in high-risk patients with coronary atherosclerosis. Although this polymorphism was found to be in linkage disequilibrium with the functional polymorphism G894T ( $D' = 0.3$ ), the latter had no predictive value in this cohort, despite the results of a large meta-analysis suggesting the opposite (2). However, the observation that the 894T/786T haplotype leads to a worse cardiovascular death-free survival supports the hypothesis of a more complex association between these polymorphisms and eNOS function.

We have recently shown (3) that the presence of the 894T allele is associated with higher levels of oxidized low-density lipoprotein

and proinflammatory cytokines only under conditions of "biological stress," such as during the acute phase of myocardial infarction, an effect not observed in the same subjects 1 year after the event, or in healthy individuals. Moreover, the 894T allele seems to be associated with impaired endothelial function in high-risk patients (4) and in healthy smokers (5), but not in healthy, low-risk individuals (5).

In the present study, Rossi et al. (1) actually introduce the hypothesis that the previously observed transient effect of the 894T allele on oxidative stress, inflammatory process, and endothelial function (3-5) could actually be due to its linkage disequilibrium with the 786T allele (which has been suggested to modify eNOS expression). However, this could be due to the complex interaction of both polymorphisms constituting the 894T/786T haplotype. The combination of low eNOS expression (induced by the 786T allele) and increased susceptibility of eNOS to proteolytic cleavage (induced by the 894T allele) (6) could lead to a combined effect on the associated phenotype of nitric oxide bioavailability, and the subsequent alterations of oxidative stress and inflammatory status. Therefore, further molecular studies are required to explore the transient behavior of eNOS genotypes/haplotypes, leading to a different phenotype, depending on the underlying disease state.

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## REFERENCES

- Rossi GP, Maiolino G, Zanchetta M, et al. The T(-786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients. *J Am Coll Cardiol* 2006;48:1166-74.
- Casas JP, Bautista LE, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase genotype and ischemic heart disease: meta-analysis of 26 studies involving 23,028 subjects. *Circulation* 2004;109:1359-65.
- Antoniades C, Tousoulis D, Vasiliadou C, et al. Genetic polymorphism on endothelial nitric oxide synthase affects endothelial activation and inflammatory response during the acute phase of myocardial infarction. *J Am Coll Cardiol* 2005;46:1101-9.
- Antoniades C, Tousoulis D, Vasiliadou C, et al. Genetic polymorphism G894T on the eNOS gene is associated with endothelial function and vWF levels in premature myocardial infarction survivors. *Int J Cardiol* 2006;107:95-100.
- Leeson CP, Hingorani AD, Mullen MJ, et al. Glu298Asp endothelial nitric oxide synthase gene polymorphism interacts with environmental and dietary factors to influence endothelial function. *Circ Res* 2002;90:1153-8.
- Tesaro 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.

## Reply

To explain our (1) intriguing results on the effects of the T-786C (in the promoter) and the G894T (in exon 7) single nucleotide polymorphisms (SNP) of the endothelial nitric oxide (NO) synthase (eNOS)

gene on cardiovascular mortality, Tanus-Santos et al. recalled that the former SNP alters the gene responsiveness to statins: statins would up-regulate eNOS expression (2) more potently in -786C homozygous (3) and therefore, these subjects would generate more NO while on statins than subjects with the other genotypes. Accordingly, atorvastatin increased NO availability and reduced inflammatory marker concentrations in CC, but not in TT healthy men (4). However, we found no significant interaction between statin treatment and the T-786C SNP affecting cardiovascular mortality (1). Moreover, only a minority of our patients were on statins (5); therefore, this mechanistic explanation is unlikely.

As with Tanus-Santos et al., we also found that the T-786C SNP did not affect nitrite/nitrate levels; however, functional data (6) indicate that the T-786C SNP affects NO bioactivity by altering the gene responsiveness to shear stress (7). Thus, the "Janus" nature of eNOS might reveal itself under conditions of oxidant stress, leading to decreased plaque stability and cardiovascular events (1).

Antoniades et al. raised another appealing hypothesis: an interaction of the 786T with the 894T allele constituting the 894T/786T haplotype might lower eNOS expression and increase susceptibility of eNOS to proteolytic cleavage, resulting into transiently increased oxidant stress and inflammatory status during acute conditions (8). However, the G894T SNP lies within a loop on the external surface of eNOS and does not make contact with either the active site of the enzyme, or the dimerization interface, suggesting that, if functional, this SNP could act by a mechanism independent of eNOS catalysis. Moreover, the increased susceptibility to cleavage of the Asp298-encoded eNOS enzyme has been shown to be artifactual (reviewed by Casas et al.) (9). Therefore, whether the 894T allele bears functional consequences remains controversial. Nonetheless, the linkage disequilibrium of the T-786C and G894T SNP (1) can explain the association of the latter SNP with coronary heart disease (CHD), as we pointed out.

Antoniades et al. stated that an increased risk of CHD (odds ratio [OR] = 1.31) for the 894T allele carriers was reported; however, the excess risk deriving from meta-analysis of cross-sectional association studies, which are prone to stratification biases, should be viewed cautiously. In fact, a much larger meta-analysis led to a markedly reduced estimate of risk (OR = 1.17) (9).

Consistent with prospective study results in high-risk patients (1,10), we found no evidence for a prognostic effect of the 894T allele. Thus, even if the 894T homozygosity would imply a blunted NO production and/or higher levels of oxidized low-density lipoprotein and proinflammatory cytokines during acute coronary events, overall prospective cohort studies show no prognostic effect in high-risk patients.

Finally, although underlying the fact that intriguing results such as ours are crucial for generating novel hypotheses, we agree that the elucidation of the complex interplay between the eNOS gene haplotypes and environmental factors deserves further research.

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## REFERENCES

1. Rossi GP, Maiolino G, Zanchetta M, et al. The T(-786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients. *J Am Coll Cardiol* 2006;48:1166-74.
2. Laufs U, La FV, Plutzky J, et al. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 1998;97:1129-35.
3. Abe K, Nakayama M, Yoshimura M, et al. Increase in the transcriptional activity of the endothelial nitric oxide synthase gene with fluvastatin: a relation with the -786T>C polymorphism. *Pharmacogenet Genomics* 2005;15:329-36.
4. Souza-Costa DC, Sandrim VC, Lopes LF, Gerlach RF, Rego EM, Tanus-Santos JE. Anti-inflammatory effects of atorvastatin: modulation by the T-786C polymorphism in the endothelial nitric oxide synthase gene. *Atherosclerosis* 2007. In press.
5. Cesari M, Maiolino G, Colonna S, et al. Under treatment with lipid-lowering drugs of high-risk coronary heart disease patients of the GENICA study. *J Cardiovasc Pharmacol* 2007;42:484-90.
6. Rossi GP, Taddei S, Virdis A, et al. The T-786C and Glu298Asp polymorphisms of the endothelial nitric oxide gene affect the forearm blood flow responses of Caucasian hypertensive patients. *J Am Coll Cardiol* 2003;41:938-45.
7. Cattaruzza M, Guzik TJ, Slodowski W, et al. Shear stress insensitivity of endothelial nitric oxide synthase expression as a genetic risk factor for coronary heart disease. *Circ Res* 2004;95:841-7.
8. Antoniades C, Tousoulis D, Vasiliadou C, et al. Genetic polymorphism on endothelial nitric oxide synthase affects endothelial activation and inflammatory response during the acute phase of myocardial infarction. *J Am Coll Cardiol* 2005;46:1101-9.
9. Casas JP, Cavalleri GL, Bautista LE, et al. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: a HuGE review. *Am J Epidemiol* 2006;164:921-35.
10. Zhang C, Lopez-Ridaura R, Hunter DJ, et al. Common variants of the endothelial nitric oxide synthase gene and the risk of coronary heart disease among U.S. diabetic men. *Diabetes* 2006;55:2140-7.

## Noncardiac Findings in Computed Tomography Coronary Angiography

The report by Onuma et al. (1) on noncardiac findings in multidetector computed tomography (MDCT) and the accompanying editorial comment by Rumberger (2) raise interesting issues. Onuma et al. (1) found that approximately 23% of 503 patients undergoing CT coronary angiography demonstrated significant noncardiac pathology requiring follow-up. This included 2 lung and 2 breast malignancies. Similarly, Baum et al. (3) have recently reported a high prevalence of extracardiac disease, including malignancies, among over a thousand patients undergoing MDCT.

Rumberger (2) suggests medico-legal and moral imperatives to seek noncardiac pathology. The patient's entire chest and upper abdomen have been irradiated, after all, and the imaging data are there awaiting reconstruction. Although this approach seems very reasonable, I believe we need to keep an open mind, recognizing the absence of hard evidence that the pursuit of extracardiac pathology leads to overall improved patient outcomes. Much of the noncardiac pathology, such as liver and renal cysts, is relatively unimportant and probably unrelated to the symptom of chest pain. With regard to more serious pathology, several questions arise: When found, are the newly discovered malignancies curable or amenable to treatment that prolongs life or improves quality of

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