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Relationship between endothelial nitric oxide synthase gene polymorphisms and the risk of myocardial infarction in the Algerian population

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ABSTRACT

Introduction: Endothelial nitric oxide synthase (eNOS), the enzyme in charge of nitric oxide production, plays a crucial role in vascular biology. However, the impact of single nucleotide polymorphisms (SNPs) affecting the gene encoding for eNOS (eNOS) on coronary artery diseases remains under debate and no data were available at present in populations originating from Mahghreb.

Aim of the Study: Our purpose was to evaluate the association between the eNOS -786T/C and +894G/T SNPs and (i) the risk of myocardial infarction (MI) and (ii) variations in systolic (SBP) and diastolic (DBP) blood pressure values.

Patients and Methods: Concerning MI, the SNPs were characterised in a case-control study (70 cases vs 68 controls) based on the male population originating from Oran, Algeria.

Results: The associations with blood pressure values were assessed in an enlarged control group including 115 male subjects. Since the -786T/C SNP could not be associated to MI, the genotype distribution of the +894G/T genotypes significantly differed between MI cases and controls (p=0.025). The risk of MI (odds ratio) associated to the +894G/T SNP was estimated to 1.2 (95%CI=[1.03;1.32]). The haplotype analysis confirmed this association and the absence of impact of the -786T/C SNP. On the other hand, no consistent association was shown between the two SNPs and SBP or DBP.

Conclusion: As observed in other populations, the eNOS +894G/T SNP was associated with MI in the Algerian population but the mechanism underlying the effect could not be related to variations in blood pressure.

Key Words:

Endothelial nitric oxide synthase, myocardial infarction, blood pressure, genetic epidemiology.

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INTRODUCTION

Despite major advances in prevention and treatments, coronary artery disease (CAD) remains the major cause of mortality and morbidity in developed countries[1]. In Northern Africa, epidemiological data are poorly documented despite a lately reported impressive prevalence of CAD in these populations[2]. Atherosclerosis, the underlying cause of CAD, is partly heritable although the involved genes and the associated relative risks remain largely under debate[3]. Numerous studies support that decreased nitric oxide (NO) production related to endothelial dysfunction plays a crucial role in the initiation and progression of atherogenesis and the presence of endothelial dysfunction can predict the presence of CAD and provides prognostic information[4]. NO plays a key role in the relaxation of vascular smooth muscle cells (VSMC), reduces VSMC migration and proliferation, inhibits adhesion of platelets and leukocytes to the endothelium, and limits the oxidation of low-density lipoproteins, all these mechanisms being strongly involved in the atherogenic process[5]. Moreover, as a potent vasodilatator, NO is deeply engaged in the regulation of blood pressure.

In vascular endothelium, NO is constitutively produced from L-arginine by endothelial nitric oxide synthase (eNOS)[6]. It has been shown that eNOS inhibition accelerates atherosclerosis in animal models and that abnormalities in the endothelial NO pathway is present in atherosclerotic patients[7,8]. Moreover, numerous epidemiological studies pointed out short nucleotide polymorphisms (SNPs) within the eNOS gene (eNOS) as putative risk factors for CAD, but the results are often conflicting and data from populations originating from Maghreb are scattered. Therefore, in a case-control study carried in the Algerian population of Oran, we evaluated the impact of the -786T/C and the +894G/T SNPs, respectively located in the 5'-flancking region and in exon 7 of eNOS, on the risk of myocardial infarction (MI) and on systolic (SBP) and diastolic (DBP) blood pressure values.

PATIENTS AND METHODS

Study population:
The study population was composed of subjects aged 25 to 64 years, living in Oran (Algeria) and whose parents and grand-parents were born in the west of the country. The case group was composed of 68 subjects who had survived a MI defined on the basis of the multinational monitoring of trends and determinants of cardiovascular disease (MONICA) criteria[9,10]. Controls (n=115) were randomly selected from the Algerian National Census list of households. Anthropometric measurements, clinical and biological examinations, as well as prevalence of risk factors were systematically recorded. SBP and DBP were measured twice, with an interval of at least 5 min, in a sitting position, after resting for at least 5-10 min. Genomic DNA was extracted from peripheral blood leukocytes.

Analysis of the eNOS -786T/C and +894G/T SNPs:
For the -786T/C SNP, the forward and reverse primers were 5'-CCCATCACACAAAAC-3' and 5'-CTCAATACACAAACTAC-3'. The genotypes were deduced after an incubation step with the restriction endonuclease HpaII (New England Biolabs).

For the + 894G/T SNP, the forward and reverse primers used were 5'-GCTCCAGGGGCACCTCAA-3' and 5'GCATTCAGCACGGCTGGA3' respectively. The genotypes were deduced by using the BanII restriction enzyme (New England Biolabs).

Statistical analyses:

For case-control comparison, 68 out of 115 controls were selected in order to approximately match the age distribution of controls (54.3±8.0 years) to that of the cases (56.1±8.5 years). Genotype distributions between cases and controls were compared using the Pearson's chi-square test. The associations of the eNOS SNPs with the risk of MI were assessed by multiple logistic regression analysis. SBP and DBP were compared between genotypes by analysis of covariance that used a general linear model. The tests were adjusted for age, body mass index, diabetes, current smoking and alcohol consumption. These analyses were carried out with the SAS release V.8.2 software. Due to their closely location within the eNOS gene, we calculated the linkage disequilibrium (LD) between the two SNPs and we tested the impact of the various eNOS haplotypes on the risk of MI and on SBP and DBP by using a stochastic-EM algorithm implemented in the THESIAS software (freely available from http://www.genecanvas.org)[11]. $p<0.05$ was considered as statistically significant.

RESULTS

The comparisons of the eNOS -786T/C and +894G/T genotype distributions between cases of myocardial infarction and controls are presented in (Table 1). Both eNOS -786T/C and +894G/T genotype distributions fulfilled the Hardy-Weinberg equilibrium.

The eNOS -786T/C genotype distribution did not differ between cases and controls. Consistently, the risk of MI associated to the polymorphism, which was estimated to be 1.4 (95% CI=[0.86;2.38]), did not reach statistical significance after adjustment for covariates (OR[95%CI]=1.38 [0.80; 2.39]). Conversely, the distribution of the eNOS +894G/T SNP is statistically different between cases and controls (p=0.025). Subjects carrying the eNOS+894T allele, especially homozygotes, are more frequent in cases than in controls. The risk of MI associated to+894T allele was estimated to be closed to 1.2 (95%CI=[1.03; 1.32]), remaining unchanged after adjustment for covariates.

Table 1: Comparison of eNOS -786T/C and +894G/T genotype distributions between cases (n=68) and controls (n=68).

N (%)	-786T/C			+894G/T	
	Cases	Controls		Cases	Controls
TT	22 (32.3)	28 (41.2)	GG	30 (42.9)	41 (60.3)
TC	34 (50.0)	33 (48.5)	GT	28 (40.0)	24 (35.3)
CC	12 (17.7)	7 (10.3)	TT	12 (17.1)	3 (4.4)
	p=0.359			p=0.025	

Although being closely located within the eNOS gene, the LD between the two SNPs was unexpectedly weak (D'=0.038; r^2=0.097). However, we compared the distribution of the eNOS haplotypes between cases of MI and controls (Table 2). Data showed that, compared to the most frequent haplotype (-786T*+894G), the haplotypes carrying the +894T allele were consistently more frequent in cases than in controls. The risk of MI associated to the -786C*+894T haplotype was significantly two fold increased; the risk associated to the -786T* + 894T allele was comparable even if it did not reach statistical significance, probably due to the lack of power related to the low frequency of that haplotype.

Table 2: Comparison of eNOS haplotype distribution between cases (n=68) and controls (n=68).

Haplotypes	Cases %	Controls %	Crude OR [95%CI]	Adjusted* OR [95%CI]
-786T*+894G	0.45	0.56	-	-
-786C*+894T	0.23	0.13	2.3 [1.1;4.7]; p=0.018	2.2 [0.9;4.9]; p=0.038
-786C*+894G	0.19	0.22	0.9 [0.5;1.6]; p=0.613	0.8 [0.4;1.6]; p=0.584
-786T*+894T	0.13	0.09	1.7 [0.7;4.3]; p=0.204	2.2 [0.7;6.4]; p=0.132

* Tests adjusted for age, BMI, diabetes, current smoking and alcohol consumption.

The comparisons of SBP and DBP between subgroups of eNOS -786T/C and +894G/T genotypes are presented in Table 3. The -786T/C SNP was only slightly associated with a decreased DBP. The +894G/T was not associated with any variation in SBP and DBP. The comparison of SBP and DBP between subGroups of eNOS -786T/C and +894G/T haplotypes in 115 control subjects confirmed that none of the two SNPs could be associated with SBP or DBP (data not shown).

DISCUSSION

The present study investigated the impact of eNOS SNPs on myocardial infarction and on blood pressure values in a male sample of the Algerian population. This is the first report in which eNOS polymorphisms were analysed in a Northern African population. To date, the prevalence of eNOS polymorphisms has been established for Caucasian, African American, Hispanic, Korean and Japanese populations.

The frequency of the rare -786C allele in our population was 34.6%. The highest frequency was reported in Caucasians (38%)[12] and the allele is less common in Korean (8,6%)[13], Japanese (9,2%)[14], and African Americans (17,5%)[15]. The allele frequencies of the + 894G/T SNP was also determined by ethnicity. In the present study, the prevalence of the allele was 22,1%. The + 894T allele was found to be more frequent in Hispanics (30%)[16] and Caucasians (29%)[17], but less frequent in African Americans (15,5%)[15], Indians (12%)[18] and Japanese (9%)[14]. These findings further support the important role of ethnicity in determining the prevalence of genetic polymorphisms an their subsequent putative impacts in a given population.

Our data suggest that the -786T/C SNP cannot be associated with the risk of

MI, a result that was also obtained in many Caucasian populations[19] as well as in the population of Taiwan[20]. Conversely, we associated the rare allele of the + 894G/T polymorphism with a moderate but increased risk of MI. This result is in agreement with others obtained in many countries, from both Caucasian[21,22] and Japanese[23,24] origins but the present findings contrast with those obtained from the French subset of the ECTIM (Etude Cas-Temoins de l'Infarctus du Myocarde) study[25], the authors pointing out an increased risk of MI in individuals carrying the +894G allele. At the protein level, the +894G/T SNP encodes for the replacement of the Glu298 by a residue of Asp (Glu298-Asp). Interestingly, numerous studies have suggested that the risk for CAD would be confined to homozygous individuals for the + 894T allele, suggesting that homozygosity for Asp298 should produce a significant decrease in the amount of eNOS or in its enzymatic activity[22,26]. Compared with the eNOS Glu298 protein, it has been also recently shown that the eNOS Asp298 protein has enhanced susceptibility to intracellular proteolytic cleavage[26,27]. Other possible explanations for the association of the + 894G/T SNP with the risk of MI rely on putative linkage disequilibrium between the mutation and another still unknown functional polymorphism located in the eNOS locus or in another gene nearby.

Conversely, in spite of the crucial role of eNOS in NO production and the function of NO in the regulation of blood pressure, no effect of eNOS SNPs on SBP and DBP could be shown. Consistently, it as been recently reported that plasma NO level and blood pressure were not associated in different genotypes of -786T/C and + 894G/T SNP in African Americans.[28]

CONCLUSION

Our data, although being obtained from a low effective study, suggested that the + 894G/T would be an independent genetic marker of MI in the Algerian population. Therefore, further studies are needed to investigate whether the + 894G/T of the eNOS gene could represent useful genetic markers for identifying individuals at risk for development of CAD. Moreover, it is necessary to explore and clarify the putative effects of the eNOS + 894G/T SNP on endothelial NO function.

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