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Original Article

Genetic association study between T-786C *NOS3* polymorphism and essential hypertension in an Algerian population of the Oran city

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ABSTRACT

Background: Essential hypertension is an important risk factor for the development of cardiovascular disease. Important candidate genes such as *NOS3* gene have been widely studied and reported to be associated with essential hypertension (HTN) in human populations.
Aim: We aim in this study to analyze the relationship between *NOS3* -786T/C, a common genetic variant and HTN in a sample of the Algerian population of the Oran city.
Methods: A case-control study has been performed in 154 subjects including 77 hypertensives and 77 normotensives. The recruitment of these subjects was done in local Health Centers of the city of Oran, West Algeria. HTN was defined as elevated systolic blood pressure SBD≥140 mmHg and or sustained diastolic blood pressure DBP≥90 mmHg, measured using an Omron® Automatic BP Monitor - M-3W machine. Consents were obtained from all participants. Polymerase chain reaction (PCR) combined with restrictive fragment length polymorphism (RFLP) was used to genotype the NOS -786T/C variant.
Results: The distribution of the allelic frequencies did not differ between cases and controls (OR = 1.48; 95%CI [0.94–2.32], P = 0.09). However, after adjustment with the age, sex, and body mass index, we observed significant association between NOS -786C allele and HTN status (OR = 2.08; 95%CI [1.18–3.66], P = 0.01).
Conclusion: Our results indicate that the C allele of the *NOS3* gene is associated with increased risk of essential hypertension in this sample of the Algerian population of the Oran city. Further validation in larger samples is needed to confirm this finding.

1. Introduction

Hypertension is a chronic elevation of blood pressure, defined by systolic/diastolic blood pressure (SBP/DBP) above 140/90 mmHg. In 2008, it has been estimated that 40% of worldwide adult population, aged from 25 years old and above, has a raised blood pressure. In 2025, the number of adults with hypertension was predicted to increase by about 60% to a total of 1.56 billion [1]. Hypertension (HTN) is a multifactorial disorder which causes severe damage to human health. It has been estimated that HTN is responsible for at least 45% of deaths due to heart disease and 51% of deaths due to stroke. Arterial hypertension is the result of the combination of environmental factors and genetic factors with a heritability ranging from 30 to 50% [2]. Indeed, Genome-Wide Association Studies have identified more than 100 quantitative trait loci attributed to essential hypertension across the genome, mainly in chromosomes 1, 2, 3, 17 and 18 [3]. The endothelial nitric oxide synthase (*NOSe* or *NOS3*) gene is considered to be an important candidate gene because of its substantial contributions to the regulation of blood pressure. The *NOS3* gene is located on the chromosome 7q35-36 including 26 exons [4]. A single nucleotide variation appears on the promoter region of the *eNOS* gene (a T-to-C transition [T-786C], rs2070744). Many studies have reported the eventual association between the −786C allele and reduced eNOS activity in human platelets [5] besides reduced mRNA levels in

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human cardiomyocytes [6]. The −786C variant was also linked to the risk of coronary artery disease and hypertension [7]. However, the results among several populations are conflicting. The objective of this study is to investigate the relationship between NOS -786T/C variant and essential hypertension in a sample of Algerian population.

2. Methods

2.1. Population study

We have enrolled a case-control study from 2011 until 2013 where we have included 154 Algerian subjects. All the implicated subjects have already given their written consent to participate in this study which was conducted according to the declaration of Helsinki Principles and approved by the local ethics committee. The recruitment of the hypertensive subjects was carried out in local health centers.

Hypertension was defined as having an elevated systolic blood pressure SBD≥140 mmHg and sustained diastolic blood pressure DBP≥90 mmHg, or being currently under antihypertensive therapy. We used Omron Automatic BP Monitor - M-3W device to measure arterial pressure. Any subjects with possibility of a secondary hypertension were excluded. The control group was selected from blood donors. Normotensive was defined as those with a blood pressure of less than 140/90 mmHg. Anthropometric measurements, clinical characteristics and prevalence of some risk factors were systematically recorded in a standardized questionnaire.

2.2. T-786C NOS3 gene polymorphism detection

Genomic DNA was extracted using 5 mL of blood samples using DNA isolation kit (STRATAGENE Inc., Canada) and quantified following spectrophotometric analysis. DNA fragments including the −786T/C polymorphism of *NOS3* gene was amplified by polymerase chain reaction. The following primers were used to determine the studied polymorphism 5′-CACAGAACTACAAACCCC-3′ and 5′- GCAGGTCAGCAGAGAGACTA -3′. The cycling conditions were: initial denaturation for 7 min at 96 °C followed by 35 cycles of denaturation at 96 °C for 45 s, annealing at 58 °C for 45 s and chain elongation at 72 °C for 45 s followed by final extension at 72 °C for 10 min. Polymerase chain reaction (PCR) products were digested with 5U of *MspI* enzyme for 3 h at 37 °C and electrophoresed on a 3% agarose gel with ethidium bromide staining.

2.3. Statistical analysis

Data analysis was done with the help of an (SPSS Inc., Chicago, Illinois, USA) version 21.0. Clinical characteristics of all the subjects are expressed as means ± SD. Descriptive statistics were used to analyze all studied variables such as the socio-demographic characteristics, anthropometric measurements, biological parameters and family history of hypertension of all the subjects. A two-tailed student's *t*-test was used to compare continuous variables between the two groups. Allele frequencies were calculated from genotype frequencies and they were compared between the two groups using chi-squared (X^2) statistics. Hardy-Weinberg's equilibrium was checked by a χ^2 test. P value < 0.05 was considered statistically significant.

3. Results

3.1. The clinical and demographic characteristics of the studied population

The clinical characteristics of the studied population are given in http://jra.sagepub.com/content/15/1/1.long Table 1. There was a total of 154 subjects recruited in the study including 77 hypertensives and 77 normotensives. The mean age of patients was 49.29 ± 7.80 versus 41.21 ± 9.37 in the control group and it was statistically different between the two groups (P<0.0001). The present study showed also a significant difference between hypertensives and controls with respect to SBP, DBP and family history of hypertension, while there were no statistical significant differences between cases and controls in term of body mass index BMI (P > 0.05).

3.2. Distribution of the −786T/C NOS3 gene polymorphism

To evaluate the impact of the −786T/C polymorphism of the *NOS3* gene on the risk of developing hypertension in this sample of Algerian population of the Oran city. We compared the genotype and allelic frequencies characterized in 77 hypertensives and in 77 controls among the 154 subjects. The Fig. 1 shows the results of the *MspI* digestion on PCR products. Therefore, a band at 176 bp

Fig. 1. 3% agarose gel electrophoresis. Lane 2, 4, 5 correspond to RFLP pattern of heterozygous (CT), wild homozygous (TT) and mutant homozygous (CC) respectively. M is 50bp linear DNA ladder (O'GeneRuler).

Table 1
Demographic and clinical characteristics in hypertensive patients and in normotensive subjects.

Parameters	Hypertensive patients (n = 77)	Normotensive subjects (n = 77)	P
Age (years)	49.29 ± 7.80	41.21 ± 9.37	P<0.0001
Gender (male/female)	41//36	46/31	NS
Body Mass Index (Kg/m^2)	27.10 ± 3.20	26.98 ± 3.60	NS
Systolic Blood pressure (mmHg)	144.55 ± 8.90	126.32 ± 7.60	P<0.0001
Diastolic Blood Pressure (mmHg)	87.21 ± 9.91	72.47 ± 9.24	P<0.0001
Family history of hypertension (%)	30 (38.96%)	5 (6.49%)	P<0.01

Values are presented as mean ± SD or number.
NS: Not Significant.
n: number of individuals.

indicates homozygous wild-type (TT), a band at 135 bp and non clearly visible band of 41bp indicates homozygous mutated (CC) and three bands at 176 bp, 135 bp and non clearly visible band of 41bp indicates heterozygous mutation (CT).

All the genotype distributions were in accordance with the Hardy-Weinberg Equilibrium. The analysis of the allelic distribution of −786T/C *NOS3* gene variant show that the −786C allele is more frequent in hypertensive patients than in normotensive subjects P = 0.01; P<0.05 after adjustment with the sex, age and BMI with an OR (CI 95% = 2.08 (1.18–3.66)) (Table 2).

4. Discussion

Nitric oxide synthase endothelial (*NOS3*) plays an important role in the production of nitric oxide, a signaling molecule that acts as an important endothelium-derived vasorelaxant factor [8]. The inheritance of variants impairing nitric oxide production became an appealing mechanism by which variation at *NOS3* might modify the risk of human hypertension. Nevertheless, genetic association studies on the relationship between *NOS3* variants and hypertension led to conflicting results. These controversial findings prompted us to study the polymorphism −786T/C of the *NOS3* gene in an Algerian sample for the first time in relationship with hypertension.

In this study, the frequency of the rare allele was 33%. The highest frequency was reported in Caucasians (38%) [9–11] While the C allele is less common in Korean (8.6%) [12], Japanese [13](9.2%) and African Americans (29%) [14]. Our findings suggest that the −786T/C SNP is associated with the risk of hypertension (OR = 2.08 CI 95% (1.18–3.66)). This result was found in other populations such as in American subjects [15] and in Canadians where the risk associated to hypertension in Canadians was 2.1 (IC95% = [1.3–3.7]) [16]. Indeed, it has been previously reported that subjects with the −786CC genotype had significantly higher systolic blood pressures and were more likely to be hypertensive [17]. Moreover, −786C *NOS3* allele has been related with impaired activity of the eNOS enzyme, coronary spasm, and impaired endothelium-dependent vasodilatation in CAD patients [16,17]. Nevertheless, many other studies have found a negative association between this polymorphism and essential hypertension. For that purpose, important meta-analysis of 53 studies involving 40413 subjects revealed an absence of association between the 786C allele and essential hypertension [18]. The same result was reported in Japanese population [19], Afro-American and Caucaso-American population [14]. Indeed, the authors suggest more accurate studies taking in account haplotypes analyses as well as interactions genes-environment to reduce these discrepancies. On the other hand, the polymorphism −786 T/C of the *NOS3* gene was widely studied in association with myocardial infarction where a negative association was found between this polymorphism and myocardial infarction in two Caucasian populations of Australian origin [20] and in Taiwanese [21]. However, the −786C allele was associated with the cardiovascular risk in Italian subjects, Caucasian from the North of Spain [22], from Ukraine [23], Turkey [24] and from Korea [13]. Conversely, in 2004, a meta-analysis of 26 case-control studies of cardiovascular diseases, including 9,867 cases and 13,161 controls of Asian and non-Asian origin, revealed an absence of the association between the polymorphism −786T/C and the cardiovascular diseases [25]. The authors of this meta -analysis noticed that the studies with reduced size can affect the association of this polymorphism with the cardiovascular diseases.

Table 2
The distribution of the C allele of −786T/C polymorphism of the *NOS3* gene in the case and control subjects.

	Case n = 77	Control n = 77
Frequency of the C allele	0.44	0.33
P-value without adjustment	0.09	
P-value after adjustment*	0.01*	
OR (CI 95%) *	2.08 (1.18–3.66)*	

*: After adjustment for age, sex and BMI.
OR: odds ratio, CI: confidence intervals.

5. Conclusion

Our data, although being obtained from a small sample case control study, suggested that the −786T/C would be a genetic risk factor for HTN in the Algerian population of Oran city, independent of classical risk factors, mainly, age, sex and BMI. However, this study may be improved with further studies with well-designed larger sample size involving other polymorphisms in *NOS3* gene in relation with essential hypertension.

Competing interests

The Authors declare that there is no conflict of interest.

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