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ScaI Atrial Natriuretic Peptide Gene Polymorphism and Hypertension in the Tunisian Population

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Abstract

Numerous genetic variants have been linked to hypertension. Among these variants T2238C polymorphism in atrial natriuretic peptide gene has been investigated in the pathogeneses of hypertension, but studies have often generated controversial results. The aim of this study was to investigate the association between hypertension and the ANP/ T2238C variant gene that led to the loss of ScaI restriction site, thus eliminated the regular stop codon and involved an extension of the human ANP by two additional arginines. We genotyped 384 patients with hypertension and 435 healthy controls. The ScaI ANP gene polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism analysis. The results shows that the ScaI ANP gene polymorphism genotypes distribution and allele frequencies were not significantly different between the hypertensive and normotensive subjects ($p>0.05$). The frequencies of A2 wild allele and A1 mutant allele were 48% and 52% respectively in hypertensive patients and 49% and 51% in control group ($p=0.66$). This polymorphism is not associated with hypertension (OR= 1.55, 95% CI [0.82-2.92]; $p=0.17$) for TC and (OR=1.80, 95% CI [0.81-3.98]; $p=0.14$) for CC after adjustment for age, gender, body mass index, fasting glucose concentration, dyslipidemia and smoking. Furthermore, no relationship was found between clinical characteristics and ScaI ANP genotypes. As a conclusion; this study suggested that the ScaI ANP gene polymorphism is not associated with hypertension in the Tunisian population.

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Keywords: Atrial natriuretic peptide; ANP polymorphism; ANP/T2233C gene polymorphism; hypertension

1. Introduction

Blood pressure (BP) is influenced by genetic and environmental factors [1]. Atrial natriuretic peptide (ANP), a cardiac hormone with natriuretic, diuretic and vasodilatory properties [2,3] is one of factors that have an important role in the regulation of BP. Consequently, the ANP gene is considered as a candidate gene for hypertension (HTA). Several polymorphisms have been described in the human ANP gene. Among these variants, the rs5065 in exon three has been involved in vascular disease.This polymorphism consisted of theScaI restriction site loss in the ANP precursor gene as the substitution of a T for a C at position 2238 which eliminated the regular stop codon. A new stop codon arises six nucleotides further on, and translation results in a human ANP with two additional arginines [4,5]. This polymorphism has been shown associated particularly with salt sensitive hypertension [6,7] and vascular diseases, including stroke, left ventricular hypertrophy, and hypertension [8,9], but with variable results [10,11]. Indeed, some studies have reported association of ANP gene variant with left ventricular hypertrophy in hypertension [12]. However, other studies in a Japanese and Black South African populations [13,14] have reported no relationship between the exon 3 variant and hypertension. Moreover, other reports [15] have indicated that the rs5065 C allele is associated with a lower risk of blood pressure progression and in type 2 diabetes this polymorphism had a protective effect against coronary artery disease in patients of Afro-Carabbean population with type 2 diabetes[16].

However, the relationship between the ScaI ANP gene polymorphism and HTA is controversial and probably the role of this polymorphism on the HTA incidence could be different among ethnic groups with differences in genetic background and lifestyle. Therefore, in the present study, we examined the relationship between the ScaI ANP gene polymorphism and HTA in a sample of Tunisian population.

2. Materials and methods

2.1. Study population

The study population consisted of a total of 837 subjects living in the City of Tunis (Tunisia). We studied 384 hypertensive patients (144 men and 240 women, age 54.06 ± 8.14 years) and 453 normotensive subjects (264 men and 189 women, age 53.65 ± 8.45 years).The control group was volunteers, collected among the families of hospital staff without antihypertensive treatment, and their SBP and DBP were less than 140 and 90 mm Hg, respectively. Hypertension was defined according to World Health Organization Criteria [17]. These criteria include a sitting systolic blood pressure (SBP) of ≥ 140 mm Hg or diastolic blood pressure (DBP) of ≥ 90 mm Hg on three occasions spanning two months from the first medical examination, without administration of antihypertensive drugs. Patients and controls were homogeneous Tunisian subjects, all were from North Tunisia

Body weight and height were measured on the subjects barefooted and lightly clothed. Body mass index (BMI; kg/m²) was calculated and obesity was defined as BMI > 30 kg/m² [18,19]. Diabetes mellitus was defined as hyperglycemia, requiring antidiabetic drugs fasting blood sugar over 7.0 mmol/L. Dyslipidemia was defined as a total cholesterol (TC) level > 6.47mmol/L and/or triglyceride (TG) level > 2.26 mmol /L. Cigarettes smoking

was quantified based on daily consumption and duration of smoking.The ethical aspects of this study were approved by the local research committee (Rabta Hospital Ethics Committee, Tunis). For all participants, blood collection was performed after informed consent.

2.2. Biochemical analysis

Blood samples were obtained after an overnight fast. Fasting glucose, creatinine, uric acid concentrations, TG,TC, high-density lipoprotein-cholesterol (HDL-C) were determined by standardized enzymatic methods, using commercial kits (Roche Diagnostics, Mannheim, Germany), on a Hitachi 912 analyzer, low-density lipoprotein-cholesterol(LDL-C) was calculated according to Friedwald's formula.

2.3. DNA analysis

Genomic DNA was prepared from white blood cells by phenol extraction [23]. Genotyping of the ANP gene variant T2238C (rs5065) was determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism followed by restriction enzyme ScaI[5,13]. PCR was performed in a reaction volume of 50 µL containing 200 ng of genomic DNA and final concentrations of 250 pM for each primer (sens: 5'GGCACACTCATACATGAAGCTGACTTTT3' and antisens: 5'GCAGTCTGTCCCTAGGCCCA3'), 1.5 mM $MgCl_2$, 50 mMKCl, 0.2 mM of each dNTP and 1unity (U)Taq polymerase (FERMENTAS FRANCE). DNA was amplified on a thermal cycler (BIOMETRA UNO II) according to the following protocol : initial denaturation at 94°C for 3 min, than 30 cycles of denaturation at 94° C for 30 s, annealing at 63° C for 1 min and extension at 72° C for 60 s, followed by a final extension step at 72° C for 10 min. Afterwards, 20 µL of the amplification products were digested for 12 h at 37° C with5 U ScaI restriction enzyme (FERMENTAS FRANCE). Digestion products were separated in 2 % agarose gel electrophoresis and visualized with ethidium bromide and viewed under ultra-violet illumination. The different fragments obtained were 133 pb PCR in homozygous mutant that correspond to the absence of ScaI restriction site, two fragments 77 and 56 pb in homozygous wild type (presence of restriction site) and all of these fragments for heterozygous.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA) and Epi Info (version 6.04a). The Student t-test was used for continuous variables, and the chi-squared test was used for categorical variables to test for statistical significance. We calculated odds ratio (OR) together with their 95% approximate confidence intervals (95% CI) as estimators of the relative risk of hypertension for the T2238C genotypes. A binary regression analysis was performed to determine the independent predictors for hypertension. We calculated the power in our samples to detect associations with different odds rations, by using Quanto computer Program [21]. A two tailed p-value<0.05 was considered statistically significant.

2. Results

Clinical characteristics of hypertensive and normotensive groups are shown in Table 1. Compared to control

subjects, hypertensive patients had a statistically higher mean of SBP, DBP, BMI and biological parameters:fasting glucose, TC, TG, LDL-C, creatinine and uric acid concentrations (p<0.001), but there were

Table 1: Demographic and clinical characteristics of the study population

Variable	NT (n=453)	HT (n=384)	p value
Age (years)	53.65 ± 8.45	54.06 ± 8.14	<0.10
BMI (Kg/m^2)	27.26 ± 4.90	30.90 ± 6.76	<0.001
Systolic BP (mmHg)	119.06 ± 10.49	151.41 ± 18.35	<0.001
Diastolic BP(mmHg)	71.62 ± 6.80	87.30 ± 10.13	<0.001
Heart rate (beats/min)	78.83 ± 7.58	79.66 ± 7.96	0.09
Diabetes mellitus (%)	6.8	33.5	<0.001
Obesity (%)	27.3	47.5	<0.001
Dyslipidemia (%)	20.1	32	<0.001
Smoking (%)	38.3	20.6	<0.001
TC (mmol/L)	4.86 ± 0.98	6.30 ± 1.01	<0.001
TG (mmol/L)	1.50 ± 0.94	1.97 ± 1.00	<0.001
HDL-C (mmol/L)	1.24 ± 0.33	1.49 ± 0.33	0.3
LDL-C (mmol/L)	2.80 ± 0.85	3.22 ± 0.90	<0.001
Fasting glucose (mmol/L)	5.62 ± 1.68	6.90 ± 3.36	<0.001
Creatinine (µmol/L)	81.41 ± 13.34	84.98 ± 31.55	<0.001
Uric acid (µmol/L)	294.64 ± 86.63	322.90 ± 102.45	<0.001

NT: normotensive; HT: hypertensive; BMI: body mass index; BP: blood pressure; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein-cholesterol; LDL-C:low-density lipoprotein-cholesterol. The data presented are means ± SD or % of patients, p<0.05 was considered significant.

no significant differences between the two groups in HDL-C (p=0.3).

The genotype frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium conditions in healthy control (χ^2 =1.69; p=0.42) and in hypertensive group (χ^2 = 1.82; p=0.40).

In total population, the distribution of genotypes in patients with hypertension was not significantly different from that in the control subjects (p=0.17) (Table 2). No significant difference in the frequency of C risk allele was detected between the hypertensive group and controls (52% vs 51% p = 0.68). We also performed stratified analysis of population by gender, because sex hormones might influence the mechanism of cardiovascular risk in patients, but results revealed also no significant genotypic and allelic differences between the two groups (Table 2).After adjustment for confounding factors (age, sex, BMI, fasting glucose concentration, dyslipidemia and smoking), and in comparison to the TT homozygous, OR for hypertension remained no significant (OR

=1.55; 95% CI [8.82-2.92]; p=17) for TC heterozygous and (OR =1.80; 95% CI [0.81-3.98]; p=0.14) for CC homozygous (Table 2).

Table 2: Genotype and allele distributions of the ANP T2238C polymorphism in hypertensive patients and controls, with adjusted Odd ratios for hypertension in the total population and by gender.

	Controls (n = 453)	**Hypertensive patients (n = 384)**	**p**	**OR**	**95% CI**
Total population					
Genotype n (%)					
TT	50 (11%)	27 (7%)		1[a]	
TC	348 (76.8%)	318 (82.8%)	0.17	1.55	[0.82-2.92]
CC	55 (12.1%)	39 (10.2%)	0.14	1.80	[0.81-3.98]
Allele frequencyn (%)					
T	49%	48%		1[a]	
C	51%	52%	0.68	1.04	[0.86-1.27]
Men	**(n=264)**	**(n=144)**			
Genotype n (%) TT	29 (11%)	8 (5.5%)	1[a]		
TC	202 (76.5%)	12 (84.6%)	0.35	2.51	[1.06-5.93]
CC	33 (12.5%)	15(10.5%)	0.22	1.89	[0.68-5.31]
Allele frequency n (%)					
T	49%	47%	1[a]		
C	51%	53%	0.58	1.09	[0.81-1.46]
Women	**(n=189)**	**(n=240)**			
Genotypen (%) TT	21 (11.1%)	20 (8.3%)	1[a]		
TC	146 (77.2%)	196 (81.7%)	0.32	1.39	[0.73-2.67]
CC	22 (11.6%)	24(10.0%)	0.78	1.13	[0.48-2.62]
Allele frequency n (%)					
T	49.7%	49.1%	1[a]		
C	50.2%	50.8%	0.86	1.02	[0.77-1.35]

[a] Reference genotype
OR: odds ratio; CI, confidence interval
OR adjusted for age, sex, BMI, diabetes, dyslipidemia and smoking.

Additionally, no relationship was found between clinical parameters and ANP/T2238C genotypes (Table 3).

***Table 3*:** Clinical characteristics of hypertensive patients and normotensive subjects according to ANP T2238C genotype

Parameters	TT	TC	CC	p
Age (years)				
NT	50.10 ± 9.63	52.57 ± 8.49	56.08 ± 7.10	0.45
HT	53.38 ± 8.85	56.15 ±8.24	50.39 ± 7.89	0.80
BMI (Kg/m^2)				
NT	27.58 ± 4.53	27.51 ± 5.08	27.31 ± 5.45	0.96
HT	31.51 ± 8.53	31.12 ± 6.80	30.95 ± 6.94	0.94
SBP (mm Hg)				
NT	119.54 ± 11.90	118.47 ± 10.41	119.49 ± 9.86	0.71
HT	149.96 ± 20.88	150.94 ± 18.36	156.89 ± 20.41	0.09
DBP (mm Hg)				
NT	71.44 ± 6.53	71.29 ± 6.75	72.41 ± 6.66	0.55
HT	85.27 ± 10.11	87.59 ± 11.08	90.32 ± 12.58	0.19
Fastingglucose (mmol/L)				
NT	5.62 ± 1.90	5.39 ± 1.29	5.53 ± 1.13	0.25
HT	6.38 ± 2.05	7.71 ± 3.27	5.74 ± 2.10	0.21
Creatinine (µmol/L)				
NT	79.00 ± 12.64	80.53 ± 13.70	82.38 ± 12.02	0.80
HT	85.27 ± 25.37	87.16 ± 35.27	81.50 ± 14.58	0.55
Uric acid (µmol/L)				
NT	295.09 ± 80.50	293.69 ± 87.28	294.29 ± 66.93	0.48
HT	331.11 ± 96.73	312.67 ± 97.04	327.38 ± 105.73	0.40
TC (mmol/L)				
NT	5.96 ± 0.85	5.66 ± 0.53	5.68 ± 0.79	0.41
HT	6.45 ± 0.87	6.32 ± 0.89	7.27 ± 1.31	0.69
TG (mmol/L)				
NT	1.62 ± 1.11	1.50 ± 0.88	1.45 ± 0.82	0.52
HT	1.42 ± 0.91	1.87 ± 0.78	1.84 ± 0.81	0.84
HDL-C (mmol/L)				
NT	1.35 ± 0.43	1.19 ± 0.25	1.39 ± 0.48	0.78
HT	1.48 ± 0.53	1.31 ± 0.39	1.22 ± 0.27	0.88
LDL-C (mmol/L)				
NT	2.96 ± 0.76	2.89 ± 0.65	2.98 ± 0.60	0.69
HT	3.12 ± 0.77	3.21 ± 0.76	3.45 ± 1.02	0.58

BMI: body mass index; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; HT: hypertensive subjects; NT: normotensive subjects; SBP: systolic blood pressure; TC: total cholesterol; TG: triglycerides. Data are expressed as mean ± SD; <0.05 was considered significant.

4. Discussion

Previous studies analyzing the role of ANP polymorphisms in the pathogenesis of hypertension have been controversial. In this study, we have not found association between the ScaI ANP gene genotype and the HTA in our sample of Tunisian population. This result was in contrast to some studies [6,22,23], but in accordance with others. Indeed, a lack of association between the ANP/T2238C and hypertension in the Japanese population and in Black South African subjects was found [13,14]. Moreover, Conen et al. [15] have reported that 2238C allele is associated with lower BP progression and in another study in Afro-Carabbean population with type 2

diabetes, the rs5065 had a protective effect against coronary artery disease [16].

Otherwise, Nakayama et al. [24] have also reported that the mutation of 5' flanking region of the human ANP gene induced susceptibility to essential hypertension or left ventricular hypertrophy in Japanese population. However, another study in Italian subjects supported the role of T2238C mutation as a direct contributor to stroke [8]. So association studies suggest the significant impact of the stop codon polymorphism on pathologies linked to hypertension complications such as left ventricular hypertrophy, renal disease and cerebrovascular accidents. We could explain these controversial studies by differences in ethnicity, the heterogeneity of study designs, endpoints considered, data computing and analysis [6,13,22,25,26].

Furthermore, we observed that the prevalence of the 2238 C allele (52 %) in our study was more frequent than in Asians. Indeed, allelic frequency for the ScaI mutant was 2% in the Japanese population [27] and 12 % in Chinese hypertensive subjects [28]. In Italian population, the allelic frequency of C variant was 11 % in the Tuscany hypertensive patients [25] and 17 % in the Milanese population [22]. Besides, the prevalence of the A1 allele in the Black hypertensive was 42 % [6] and 44 % in South African people [14]. Therefore, there is considerably clear ethnic difference in allele frequency, depending on background populations and the different form of hypertension that could explain the inconsistent results concerning the association of ScaI ANP gene polymorphism with hypertension.

However, data on the function of the ANP/T2238C variant are limited and to understand the biological significance of the T2238C ANP gene polymorphism with hypertension, we must dissect out the ANP peptide wild role. In physiological situation, high BP induced atrial stretch, the main trigger factor for ANP secretion [29] which is involved in the regulation of electrolytes and water balance, thus contributing to BP homeostasis. In the hypertension status, the regulation mechanism would be disrupted in subject carrier of mutant ANP, thus inducing high perfusion pressure in heart which induces hypertension and involves left ventricular hypertrophy. The mutant peptide could not settle on natriuretic receptors, therefore the vasodilatation will be disturbed, causing endothelial damage on vessels by vasoconstrictor substances which will not be counterbalanced by ANP vasodilator effects. This mechanism could confer vessels damage particularly in brain, inducing stroke. Besides, the mutant peptide could disturb the regulation of glomerular filtration in kidney.

In another study, Pankaj et al. [30] have reported a novel mechanism of ANP to regulate PB and salt homeostasis. In this study, authors have identified micro-RNA, miR-425 which is expressed in human atria and ventricles, and binds at noncoding region to regulate ANP production. Indeed, they have shown that a single base pair change in the type A natriuretic peptide receptor gene prevents binding of miR-425 and results in higher ANP levels [30]. So, genetic variants can have an influence on physiologic response. Currently, real mechanisms of ANP gene mutations and disease are more to dissect.

Our findings illustrate lack of association between the ANP/T2238C variant and the hypertension, but we must acknowledge certain limitation because our study was conducted with a limited sample size that might lead to a low statistical power that requires a sample size 1756 patients and 2055 Controls to reach a power of 80 % at a p=0.05.

5. Conclusion

This study indicates that ScaI ANP gene polymorphism is not associated with hypertension in the Tunisian population. The lack of association does not exclude the relevance of the ANP polymorphism gene on the incidence of hypertension. Further investigations need to be performed in other polymorphisms of ANP gene and their interaction might be evaluated. We could also identify the mechanism underlying the effect of this polymorphism on the release or the activity of ANP by identifying micro-RNA and their interaction with this variant.

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Conflict of interest

None declared.

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