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Prevalence of genetic polymorphisms of methylenetetrahydrofolate reductase C677T and angiotensin I-converting enzyme (insertion/deletion) in Sétif population, Algeria

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ABSTRACT

The aim of the present study was to assess the frequencies of angiotensin I-converting enzyme (*ACE*; OMIM: 106180) and methylenetetrahydrofolate reductase (*MTHFR*; OMIM: 607093) polymorphisms in the Algerian population to further facilitate investigations on possible associations with various pathologies. The study was carried out on 146 apparently healthy individuals (65 males, 81 females) who were randomly selected from an Algerian population of the Setif region (north-eastern Algeria). Their age ranged from 24 to 48 years. Using polymerase chain reaction based methods, genotypic frequencies of *MTHFR* C677T (rs. 1801133) and *ACE* I/D (rs. 1799752) polymorphisms were determined. No significant difference was found between genders regarding the distribution of *ACE* I/D and *MTHFR* C677T polymorphisms. In general, the prevalence of the D allele of the *ACE* polymorphism was 70.5%. The study population was in the Hardy-Weinberg equilibrium ($\chi^2=0.55$, $df=1$, $P=0.758$). The allelic frequency of the 677T allele (for *MTHFR* C677T polymorphism) was 35.3%. The study population was in the Hardy-Weinberg equilibrium ($\chi^2=1.05$, $df=1$, $P=0.304$). The surprisingly high incidence of minor alleles of the polymorphisms among our population requires further studies in vascular and other diseases.

Key words: *ACE*; Algerian Population; *MTHFR*; Polymorphism

INTRODUCTION

Angiotensin I-converting enzyme (*ACE*; OMIM: 106180) is a dipeptidyl carboxy peptidase belonging to the class of zinc metalloproteases. It is found as a membrane-bound

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protein in endothelial, epithelial, and neuroepithelial cells in the liver, kidney, and brain. ACE is an ectoenzyme, a key enzyme in the renin-angiotensin system (RAS) which converts angiotensin I (Ang I) to angiotensin II (Ang II) [1], a potent vasoconstrictor and proliferative hormone. ACE also degrades bradykinin which is a vasodilator [2, 3].

ACE insertion/deletion (I/D) polymorphism (rs. 1799752), is characterized by the insertion or deletion of a 287-bp Alu sequence within intron 16 on the human chromosome 17q23, resulting in three genotypes as II, DD and ID [4, 5], and is responsible for inter-individual differences in ACE plasma levels [6]. The plasma ACE activity in people with the DD genotype is approximately twice as much as those for whom the II [6, 7] and DI types are intermediate [7]. The factors involved in the regulation of plasma ACE concentration in normal subjects are unknown [8].

A common gene variant of methylenetetrahydrofolate reductase (*MTHFR*, OMIM: 607093) (EC 1.7.99.5) is the enzyme that catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine [9], which is the most frequent genetic cause of mild hyperhomocysteinemia. The *MTHFR* gene is located on chromosome 1 at 1p36.3. So far 14 rare mutations of the *MTHFR* gene have been associated with severe *MTHFR* deficiency. The *MTHFR* 677C-T polymorphism (rs. 1801133) was identified in 1995 [10]. The allele causes an alanine-to-valine amino acid substitution (Ala222-Val) within the catalytic domain of the enzyme, resulting in the production of a thermolabile enzyme (10) with decreased activity, with TT homozygotes having ~35%-50% reduction in enzyme activity compared to control values [11].

The aim of the present study was to assess the frequencies of *ACE* and C677T *MTHFR* polymorphisms in an Algerian population (Sétif, north-eastern Algeria) to further facilitate investigations on the possible associations with various pathologies and also to compare this population with others.

MATERIALS AND METHODS

Study population: The study was carried out on 146 (65 males and 81 females; age 24-48 years) apparently healthy and randomly selected Algerian individuals. They originated mostly from the east of the country, around Sétif (north-eastern Algeria). The history of Algeria includes successive invasions from different origins, including the Phoenicians, Romans, Vandals, Byzantines, Arabs and Turks and from the northern shore of the Mediterranean Sea, Spain and France. Blood samples for the study were transferred to Ankara/Turkey. Written consent was obtained from all participants. The research protocol was approved by the Setif Medical Faculty Ethics Committee.

Sample collection and DNA extraction: Peripheral blood samples were collected by venipuncture, collected in test tubes that contained EDTA as an anticoagulant, and frozen at -20°C up to the extraction of DNA and genotyping times. DNA was extracted using the conventional phenol-chloroform method. After haemolysis of blood in a hypotonic solution, DNA was isolated by a simple proteinase K treatment at 65°C in the presence of

sodium dodecyl sulphate, followed by ammonium acetate precipitation of debris and ethanol precipitation of DNA. The amount of DNA and its purity were then quantified for each DNA sample by spectrophotometry (Nanodrop ND-1000). DNA samples were stored at -4°C until use.

Analysis of polymorphisms: The genetic analysis of the *MTHFR* C677T polymorphism was performed by real-time polymerase chain reaction (PCR) via a melting curve analysis performed on a Light Cycler (Roche Molecular Biochemicals, Mannheim, Germany) in borosilicate capillaries with an *MTHFR* C677T polymorphism detection kit (Roche Molecular Biochemicals). Primers and fluorescence-labelled hybridization probes designs were used. The primer sequences were: 5'-TGG CAG GTT ACC CCA AAG G-3' (forward) and 5'-TGA TGC CCA TGT CGG TGC-3' (reverse) and hybridization probe sequences were: 5'-TGA GGC TGA CCT GAA GCA CTT GAA GCA CTT GAA GGA GAA GGT GTC T-3'-Flu and 5'-

LC-640-CGG GAG CCG ATT TCA TCA T-3'-PHO (TIB Molbiol, Berlin Germany). The 20.0 µl amplification reaction was prepared, containing 5.0 µl genomic DNA, 1.6 µl MgCl₂, 4.0 µl Reagent Mix (Specific primers and probe, Tib molbion), 2 µl Fast Start DNA master HybProbe (Roche Diagnostics Mannheim, Germany), 7.4 µl H₂O (PCR-grade). Cycling conditions for *MTHFR* were initial denaturation at 95°C for 10 min, followed by 45 cycles with denaturation at 95°C for 5 s, annealing at 60°C for 10 s and extension at 72°C for 15 s. After amplification, melting curves were generated following denaturation of the reaction at 95°C for 20s, holding the sample at 40°C for 20s and then slowly heating the sample to 85°C with a ramp rate of 0.2°C/s and simultaneous monitoring of fluorescence decline. The identification of the *MTHFR* genotype was performed by analysing the melting peaks of the run of the real-time PCR. The presence of just 1 melting peak at 63.0°C indicates a wild-type genotype, 2 melting peaks at 54.5°C and 63.0°C indicate a heterozygous mutant, and 1 melting peak at 54.5°C indicates a homozygous mutant.

The *ACE* I/D polymorphism was detected by PCR in a thermal cycler (Biometra/Germany) using 5 µL of 10 X PCR Buffer, 25mM MgCl₂, 10mM of deoxynucleoside triphosphate mix, and 10 pmol of each primer, forward 5'-CTG CAG ACC ACT CCC ATC CTT TCT- 3'; and reverse 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', and 5U of Taq polymerase (Fermentas) in a total reaction volume of 50 µL. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, annealing at 94°C for 1 min, and extension at 58°C at 1 min by 30 cycles, followed by a final extension at 72°C for 7 min. A 8 µL aliquot of the PCR product was separated by 2% agarose gel electrophoresis, and bands were visualised with ethidium bromide staining under UV light in a transilluminator. Homozygosity for the *D* allele (*DD*) was identified by the presence of a single 190-bp PCR product, for the *I* allele (*II*), it was identified by the presence of a single 490-bp PCR product, and for (*ID*) it was identified by the presence of both 190-bp and 490-bp PCR products.

Statistical analysis: Allelic frequencies were deduced from genotype distribution. A Chi-square test was performed for *MTHFR* C677T and *ACE* I/D genetic polymorphisms to

determine if the sample groups demonstrated the Hardy-Weinberg equilibrium. The difference in genotypic frequencies between sex groups was determined using the Chi-square test of goodness of fit. A probability of $P < 0.05$ was considered to be statistically significant.

RESULTS

The genotypic and allelic frequencies of the *MTHFR* C677T and *ACE* I/D genetic polymorphisms are reported in Table 1.

Table 1: Genotypic and allelic frequencies of *MTHFR* C677T and *ACE* I/D genetic polymorphisms in Algerian population

Polymorphisms	Females (n=81)	Males (n=65)	Total (n=146)
<i>MTHFR</i> C677T polymorphism			
Genotypes			
CC	30 (37.0)	34 (52.3)	64 (43.8)
CT	37 (45.7)	24 (36.9)	61 (41.8)
TT	14 (17.3)	7 (10.8)	21 (14.4)
Alleles			
C	97 (59.9)	92 (70.8)	189 (64.7)
T	65 (40.1)	38 (29.2)	103 (35.3)
<i>ACE</i> I/D polymorphism			
Genotypes			
II	8 (9.9)	6 (9.2)	14 (9.6)
ID	30 (37.0)	28 (43.1)	58 (39.7)
DD	43 (53.1)	31 (47.7)	74 (50.7)
Alleles			
I	46 (28.4)	40 (30.8)	86 (29.5)
D	116 (71.6)	90 (69.2)	206 (70.5)

The greatest difference in the distribution of the genotypes of the *ACE* polymorphism between genders was found for the I/D polymorphism. The DD genotype was more frequent in women as compared to men (53.1% vs 47.7%). Carriers of the D allele (DD + ID) represented 90.4% of the subjects (Table 1). No significant difference was found between the two gender groups for the genotypic prevalence of the *ACE* I/D genetic polymorphism ($\chi^2=0.28$, $df=2$, $P=0.594$). Therefore, the gender groups were pooled.

The overall prevalence of the D allele of the *ACE* polymorphism was 70.5%. The study population was in the Hardy-Weinberg equilibrium ($\chi^2=0.55$, $df=1$, $P=0.758$).

As can be seen in Table 1, the allele frequency of *MTHFR* 677T allele was 35.3%, and for the *MTHFR* C677T genetic polymorphism, the TT genotype frequency was higher in

women as compared to men (17.3% vs 10.8%). Although some differences were observed between women and men, no significant difference was found between the two gender groups for the genotypic prevalence of the *MTHFR* C677T polymorphism ($\chi^2=3.64$, $df=2$, $P=0.162$). As a result, the gender groups were pooled. The allelic frequency for the 677T allele was 35.3%. The study population was in the Hardy-Weinberg equilibrium ($\chi^2=1.05$, $df=1$, $P=0.304$).

DISCUSSION

The present study is the first of its kind focusing on the association and interaction of the *ACE* I/D and *MTHFR* C677T genetic polymorphisms among the Algerian population from Setif region.

The insertion/deletion polymorphism of the *ACE* is defined as either the presence (insertion, I) or absence (deletion, D) of a 287 base pair insert in intron 16 of the gene [12]. The insertion appears to reduce *ACE* expression, thus the DD homozygotes have 65% more, and the ID heterozygotes 31% more *ACE* than the II homozygote genotype [6].

Our results for the Algerian samples were slightly different from previously reported D allele frequencies for an Algerian population [13]. Comparing the D allele results in our population with those obtained from other regions, it can be concluded that European populations have the lowest frequency [14, 15], whereas Arab populations such as ours have the highest [8, 16, 17].

A meta-analysis of prevalence studies indicates that the overall frequency of the D allele is 54%, being unrelated to gender but associated with ethnic differences [18, 19]. Our data showed that a high prevalence of DD genotype or D allele was observed in women as compared to men. Shimizu et al. [20] suggested that females may be more influenced by the *ACE* gene than males due to gonadal effects on *ACE* activity.

Several investigators have found that the D allele is related to the increased activity of *ACE* in the serum [6, 21]. The highest serum *ACE* activity was seen in the DD genotype while the lowest was observed in the II genotype [6]. Several investigations suggest the genetic predisposition of the *ACE* I/D polymorphism with several diseases including cardiovascular diseases [22, 23], hypertension [24, 25] and diabetes mellitus [26, 27]. However, conflicting results have been reported regarding the association between *ACE* polymorphism and disease. There are a number of studies, for example, in which no association was found between *ACE* I/D polymorphism and the prevalence of cardiovascular diseases [28, 29], hypertension [30] and diabetes mellitus [31].

In the present study, the frequency of the DD genotype was very high in our healthy population (50.7%) compared to Tunisian diabetes patients (51.2%) [8], Turkish patients with coronary artery disease (50.6%) [32] and Lebanese patients with hypertension (43.4%) [33]. It can be concluded therefore, that the DD genotype may be a significant predictor of several diseases in our population.

In the present study, no differences were found between men and women in the allele frequencies and genotype distributions of the *MTHFR* C677T genetic polymorphism. The T and C allele frequencies in the men and the women were the same. Despite the finding

regarding the higher TT genotype frequency the women as compared to the men (17.3% vs 10.8%), the difference was not found to be statistically significant. In other words, the results provide support for the idea that this frequency is not associated with gender [34]. On the other hand, total homocysteine levels were slightly higher in men than women but again, not significantly. Men carrying the TT genotype appear to be at higher risk of hyperhomocysteinemia than women with the same genotype [11].

Homozygosity for the *MTHFR* 677T allele has been associated with increased plasma homocysteine, and hyperhomocysteinemia has been confirmed as a risk factor for venous thromboembolic events and occlusive arterial disease [35]. However, more studies have indicated that the *MTHFR* 677TT genotype is not a risk factor for venous thromboembolism [36].

Some studies showed the synergistic role of *ACE* I/D and *MTHFR* C677T polymorphism to effect ischemic stroke [37], pregnancy induced hypertension [38], migraine [39] and diabetes [8]. However, Goracy [40] has found that neither *ACE* I/D nor C677T *MTHFR* polymorphisms are associated with the risk of myocardial infraction.

In conclusion, the purpose of the present study was to determine whether any of these polymorphisms, impact our population health sufficiently to be detected as a decrease in genotype or allele frequency. According to the present results, the surprisingly high incidence of *MTHFR* C677T and *ACE* I/D polymorphisms among our population requires further studies in the area of vascular as well as other diseases.

Conflict of Interest: Author has no financial or any non-financial competing interests.

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