ACE Gene in Egyptian Ischemic Stroke Patients

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Background: Angiotensin-1-converting enzyme (ACE) is a crucial player in vascular homeostasis and in the pathogenesis of atherosclerosis and hypertension. The present study was conducted to determine whether there is an association between the ACE insertion/deletion (I/D) polymorphism and ischemic stroke in Egyptian population. Also, we analyzed the *ACE* gene I/D polymorphism as a risk factor for small-vessel (SV) versus large-vessel (LV) disease. *Methods:* Sixty patients with ischemic stroke were included: 30 with SV disease and 30 with LV disease. In addition, a control group of 30 apparent healthy subjects were studied. Clinical assessment, computed tomography, magnetic resonance imaging brain, and genetic study using the polymerase chain reaction of *ACE* gene were done for all subjects. *Results:* We found that the distribution of *ACE* gene polymorphism frequency was significantly different between the 3 groups. The DD genotype was far more common in stroke patients compared to controls. It was also significantly more common in each of the patient groups compared to controls but rather similar in the 2 patient groups with SV and LV diseases. *Conclusion:* We found that the *ACE* gene deletion/deletion genotype is common in Egyptian patients with non–cardioembolic ischemic stroke but does not appear to be specific neither to SV nor to LV disease. **Key Words:** *ACE* gene—small-vessel disease—large-vessel disease—leucoaraiosis.

Angiotensin-1 converting enzyme (ACE) is a crucial player in vascular homeostasis and in the pathogenesis of atherosclerosis and hypertension. It is believed to be involved in the development of cerebrovascular and cardiovascular diseases.[1] The insertion/deletion (I/D) polymorphism of the *ACE* gene has been extensively studied in cardiovascular and cerebrovascular diseases. Results of studies on the role of this polymorphism in ischemic stroke are inconsistent. Even large meta-analyses provide conflicting data. This is partly because most studies have not considered the etiology of ischemic stroke despite differences in vascular risk factors and genetic background between different etiological subtypes of ischemic stroke. However, some studies analyzing subtypes of ischemic stroke suggest that the deletion/deletion (DD) genotype might be associated with lacunar stroke.[2]

The present study was conducted to determine whether there is an association between the ACE I/D polymorphism and ischemic stroke in Egyptian population. Also, we analyzed the *ACE* gene I/D polymorphism as a risk factor for small-vessel (SV) versus large-vessel (LV) disease.

Subjects and Methods

This is a case–control study conducted on 60 patients with ischemic stroke: 30 patients with SV disease and 30 patients with LV disease. In addition, a control group (control) of 30 apparent healthy subjects was studied.

We recruited consecutive ischemic stroke patients (age above 40 years) from Ain Shams University Hospital, Cairo, Egypt. Stroke subtypes were classified according to the

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Table 1. *Characteristics of patients and control in the 3 study groups*

Variant	SV disease (n = 30)	LV disease (n = 30)	Control (n = 30)	*P* value
Age (y)	61.1 ± 8.66	66.87 ± 9.3	62.23 ± 8.96	<.05
Gender				
Male	20 (66.7%)	23 (76.7%)	17 (56.7%)	NS
Female	10 (33.3%)	7 (23.3%)	13 (43.3%)	NS
Hypertension	27 (90.0%)	24 (80.0%)	—	NS
Diabetes mellitus	21 (70.0%)	11 (36.7%)	—	<.05
Smoking	9 (30.0%)	12 (40.0%)	—	NS
Ischemic heart disease	10 (33.3%)	5 (16.7%)	—	NS
Dyslipidemia	192.97 ± 43.49	187.53 ± 43.18	—	NS

Abbreviations: LV, large vessel; SV, small vessel; NS, not significant.

modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.[3,4] SV diseases were those with at least 1 lacunar syndrome without evidence of cerebral cortical dysfunction and computed tomography (CT)/magnetic resonance imaging (MRI) neuroimaging was either normal or showed a relevant brainstem or subcortical ischemic lesion with a diameter less than 1.5 cm. Also, large extracranial arteries did not demonstrate a stenosis greater than 50% of an ipsilateral artery. On the other hand, LV diseases were considered when there was a significant (more than 50%) atherosclerotic stenosis or occlusion of a major brain artery or a cortical artery, clinical findings of cerebral cortical impairment, or brainstem or cerebellar dysfunction. There should be associated cortical, cerebellar, brainstem, or subcortical hemispheric infarcts greater than 1.5 cm in diameter on CT or MRI imaging and duplex imaging or an arteriography showing stenosis more than 50% of an appropriate intracranial or extracranial artery.

The exclusion criteria for the patients groups were hemorrhagic stroke, age younger than 40 years, presence of other medical illnesses, mainly hepatic and renal diseases, and presence of cardiac source of embolization. A control group was also recruited comprising 30 clinically healthy subjects without a history of stroke or risk factors of stroke who matched patient groups as regards age and sex. Informed written consent was obtained from all control subjects, patients, or their families. The study was conducted according to the ethical requirements of our institute.

Neuroimaging

Imaging was done initially by CT brain to exclude intracerebral hemorrhage and then by MRI brain (including T1W, T2W, T2*W, fluid-attenuated inversion recovery, diffusion-weighted imaging, and magnetic resonance angiography). MRI was performed using a 1.5-T machine (GE Sigma LX, GE Healthcare, Little Chalfont, Buchinghamshire, United Kingdom). Leucoaraiosis on MRI was graded according to Fazekas criteria[5] in which grade 0 = absent, grade 1 = punctuate leucoaraiosis, grade 2 = early confluent, and grade 3 = confluent, and this analysis was applied for both patient groups.

Genetic Testing

Polymerase chain reaction (PCR) was done for all stroke patients and controls in the Molecular Genetics Laboratory, Department of Genetics, Ain Shams University, to detect the presence of genetic polymorphism and genotyping of *ACE* gene using standard techniques.[6] The sense primer was 5'CTGGACACCACTCCCATCCTTTCT3' and the antisense primer was 5'GATGTGQCCATCACA TTCGTCAGAT3'.

The *ACE* gene polymorphism was evident as a 490-bp product in the presence of insertion (I allele) and as a 190-bp fragment in the absence of the insertion (D allele). Thus, each DNA sample revealed 1 of 3 possible patterns: a 490-bp band (genotype II), a 190-bp band (genotype DD), or both a 490-bp and a 190-bp band (genotype ID).

Table 2. ACE *gene polymorphism in all groups*

	Control		Patients				
ACE genetic polymorphism	N	%	N	%	χ^2	*P* value	Significance
Insertion (II) genotype	2	6.7%	10	16.6%	23.74	<.001	HS
Deletion (DD) genotype	3	10.0%	32	53.3%			
Insertion/deletion (ID) genotype	25	83.3%	18	30.0%			

Abbreviations: ACE, angiotensin converting enzyme; HS, highly significant.