

The Insertion/Deletion Polymorphism of the Angiotensin Converting Enzyme (ACE) in Parkinson's Disease

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Abstract: Parkinson's disease (PDI) is a neurodegenerative disorder of unknown etiology. Both genetic and environmental factors are thought to be implicated to some extent. The ACE gene insertion/deletion (I/D) polymorphism has been associated with common neurodegenerative disorders that share similar clinical and neuropathological features with PD (Alzheimer's disease). In this study we set out to examine the role of the ACE gene insertion/deletion (I/D) polymorphism in Parkinson's disease (PD).

We conducted a case-control association study among 77 PD patients and 50 non-PD controls from Greece.

The genotype frequencies for II, ID, and DD were 39, 48, and 13%, respectively, in the PD group and 32, 50, and 18% in the control group. Although the DD frequency was higher in the case group statistical significance was not reached.

We conclude that although disease modifying effects cannot be excluded, the ACE insertion/deletion polymorphism is unlikely to be an important determinant of susceptibility to PD in this population.

Keywords: Angiotensin Converting Enzyme, ACE, Polymorphism, Insertion-deletion, Parkinson's disease, Association study.

1. INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders characterized by selective neurodegeneration, leading to extrapyramidal, hypokinetic motor dysfunction (tremor, rigidity, bradykinesia). However, parkinsonian disorders may present with the additional atypical features of neuropsychiatric (dementia, depression, psychosis) and autonomic involvement. Pathogenic mutations in at least seven different genes are implicated in parkinsonism highlighting the importance of genetic factors and heterogeneous nature of the syndrome [1]. This clinical heterogeneity is also noted with similar characteristics in the presentation of other neurodegenerative disorders like Alzheimer's disease (AD), which share clinical and pathological characteristics with PD [2].

One candidate gene investigated for its relevance in neurodegeneration in both AD [3, 4] and PD [5, 6] is the angiotensin-converting enzyme (ACE). ACE increases blood pressure through conversion of angiotensin I (inactive) to the potent vasoconstrictor angiotensin II [7]. Moreover, ACE is

a major pathway for the degradation of bradykinin [8], a peptide of the kinin family involved in the regulation of vascular tone that also participates in the peripheral inflammatory response [9]. Apart from angiotensin II and bradykinin, endogenously occurring neuro-peptides, like substance P and opioid peptides have been identified as ACE substrates. An insertion/deletion (I/D) polymorphism of the ACE gene [10] accounting for half the variance in serum ACE levels has been described. We set out to study this ACE gene polymorphism, in a well characterized cohort of PD patients and aged control subjects.

2. MATERIALS AND METHODS

2.1. Study Population

The study population consisted of 77 patients with idiopathic PD and 50 controls. PD patients were consecutively recruited in the Department of Neurology of the Regional University Hospital of Patras, Greece (a tertiary referral center) over a period of 2 years. The controls were randomly selected from patients seen in the Regional University Hospital Evangelismos, Athens, Greece and were identified as healthy (no identifiable medical condition including neurological disorder) by their treating physicians. All PD patients and controls were Caucasians, genetically unrelated and of Greek origin. Informed consent was obtained from all the subjects. Diagnosis of PD was made according to the UK PD

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Society Brain Bank diagnostic criteria [11]. Thus, only patients who had bradykinesia and at least two of rigidity, rest tremor and postural instability were included. All patients were investigated with either brain CT or MRI. Cases considered as atypical parkinsonism, vascular parkinsonism, or any PD plus syndrome, were excluded from the study.

During a semi-structured interview demographic data, complete past medical history, age of PD onset, modality of presentation, duration of PD, and dosage of levodopa therapy were collected. The Unified Parkinson's Disease Rating Scale (UPDRS) [12] was completed during clinical examination and was used for evaluation of disease severity. This includes UPDRS I (mentation, behaviour and mood), UPDRS II (activities of daily living), UPDRS III (motor examination), UPDRS IV (complications of therapy), Hoehn & Yahr scale [13] (measures the severity of the disease and is based on lateralization of the symptoms and balance evaluation) and Schwab & England scale [14] (also measures activities of daily living).

2.2. Laboratory Technique

Genomic DNA was isolated from whole blood using the NucleoSpin Blood L kit (Mecherey – Nagel) following the protocol recommended by the manufacturer. To determine the 287-bp insertion/deletion polymorphism in intron 16 of the ACE gene, a conventional PCR [10] was performed using

500 ng genomic DNA as template with a flanking primer pair 5' CTG GAG ACC ACT CCC ATC CTT TCT 3' and 5' GAT GTG GCC ATC ACA TTC GTC AGA T-3' in a solution of 25 µl containing 20 pmol of each primer, 0.5mM dNTP, 2mM Mg, 10mM Tris-HCl (pH 8.8), 50mM KCl, 0.08% Nonidet P40 and 1unit of Taq DNA Polymerase, (Fermentas, Lithuania). Amplification with this primer pair results in 490 bp and 190 bp products corresponding to I and D alleles, respectively. PCR was carried out on the Robo Cycler 96 (Stratagene, USA) with initial denaturation at 94 °C for 10 min, followed by 30 cycles at 94 °C for 1 min, at 58 °C (annealing) for 1 min and at 72 °C (extension) for 1 min and then by a final extension period at 72 °C for 10 min. PCR products were visualised on a 1.5 % agarose-gel containing ethidium bromide. To reduce the incidence of mistyping ID as DD, each DD genotype was subjected to a second run of PCR with a primer pair 5' TGG GAC CAC AGC GCC CGC CAC TAC 3' and 5' TCG CCA GCC CTC CCA TGC CCA TAA 3' that recognizes the insertion-specific sequence with the following program: 10 min denaturation at 94°C, followed by 30 cycles at 94 °C for 50 seconds, at 62 °C (annealing) for 50 seconds and at 72 °C (extension) for 1 min. Under these conditions, only the I allele produced a 335 bp amplicon. Genotyping was performed in a blinded fashion with respect to clinical status of patients and controls.

Table 1. ACE Polymorphisms in Patients with Idiopathic Parkinson's Disease (PD) and Non-PD Controls

		PD patients						non-PD controls		Total	
No. of subjects		77						50		127	
No. of chromosome		154						100		254	
Allele frequency											
	No.	%						No.	%	No.	%
D	97	63.0						57	57.0	154	60.6
I	57	37.0						43	43.0	100	39.4
									P=0.6*		
	No.	%	UPDRSm	UPDRS total	H&Y	ADL	Age at onset	No.	%	No.	%
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)				
Genotype frequency											
DD	30	39.0	34.4(21.3)	57.5(37.6)	2.8(1.1)	69.3%(22.7%)	61.8(12.7)	16	32.0	46	36.2
DI	37	48.1	26.8(19.8)	44.7(31.8)	2.3(1.0)	77.3%(19.7%)	63.5(10.7)	25	50.0	62	48.8
II	10	13.0	37.1(21.5)	60.3(39.4)	3.0(1.7)	55.0%(37.2%)	63.4(9.7)	9	18.0	19	15.0
			P=0.16**	P=0.23**	P=0.09**	P=0.16**	P=0.98**		P=0.6*		
DD	30	39.0	34.4(21.3)	57.5(37.6)	2.8(1.1)	69.3%(22.7%)	61.8(12.7)	16	32.0	46	36.2
DI&II	47	61.0	29.0(20.4)	48.0(33.7)	2.4(1.2)	72.5%(25.7%)	63.5(10.4)	34	68.0	81	63.8
			P=0.16***	P=0.46***	P=0.09***	P=0.31***	P=0.92***		P=0.5*		

UPDRSm: motor part of the UPDRS scale; H&Y: Hoehn and Yahr scale; ADL: Activities of daily living scale; SD: standard deviation.
 * p value for the comparison of allele frequencies between PD patients and controls.
 ** p value for the comparison UPDRSm, UPDRS total, H&Y, ADL and age at onset between PD patients of different genotype groups (DD, DI, II).
 *** p value for the comparison UPDRSm, UPDRS total, H&Y, ADL and age at onset between PD patients of different genotype groups (DD vs DI and II).
 DI: deletion/insertion polymorphism; PD: Parkinson's disease.

2.3. Statistical Analysis

Statistical analysis was performed using the SPSS for Windows release 11.0, run on an IBM-compatible computer. Mann-Whitney U test for two samples was used in non-parametric comparisons of continuous data. Chi-square with Yates's corrected p-value and 2-tailed Fischer's exact test were used, as appropriate, for the comparison of proportions. For corrections of associations, multivariate analysis was performed using stepwise logistic regression. Adjustments were made for gender, age at onset and disease stage. The level of statistical significance was set at $p < 0.05$. The study was approved by the local Institutional Review Board (IRB).

3. RESULTS

Our study population consisted of 77 PD patients (43 men and 34 women), age 35 to 89 years with the mean of 69.2 (SD 10.4) years, and 50 control subjects (35 men and 15) women, age 19 to 80 years with the mean of 49.5 (SD 21.6) years. PD patients had a mean age of onset of 62.84 (SD 11.30) years (the age at onset of PD was defined as the appearance of the first symptom estimated by medical interview). The mean duration of symptoms was 6.27 (SD 5.28) years.

The ACE I/D polymorphism genotypes and allele frequencies are presented in Table 1. The distribution of II, ID and DD genotypes was not significantly different between PD patients and control subjects ($p=0.6$). The most frequent genotype was ID. There were fewer II genotypes 10 (13.0%) and more DD genotypes 30 (39.0%) in the PD patients. ACE genotypes for our cohort, PD cases and controls independently, were in Hardy-Weinberg equilibrium. The D and I allele distributions in the PD and control groups also revealed no significant differences ($p=0.6$). The D alleles were more common in both groups (63.0% in the PD group and 57.0% in the control group). No significant associations were observed when adjustment using stepwise logistic regression was applied ($p < 0.05$).

The most predominant symptoms at onset were bradykinesia or rigidity related. At the time of examination, the mean total UPDRS was 51.7 (SD 35.4). The UPDRS subscores were: UPDRSI 6.27 (SD 3.53), UPDRSII 15.82 (SD

11.16), UPDRSII (or UPDRSm) 31.09 (SD 20.79), UPDRSIV 2.52 (SD 3.95). The mean Hoehn & Yahr score was 2.56 (SD 1.14) and mean Swab & England score was 71.30% (SD 24.46%). The main symptoms of PD at onset and examination, and the L-dopa induced motor side effects (dyskinesias, wearing-off and on-off phenomena), the vascular comorbid conditions (ischemic heart disease and cerebrovascular disease) and family history of PD patients are presented in Table 2. The subgroup analysis of main PD symptoms and L-dopa motor side effects according to the presence or absence of the DD genotype (Table 2) revealed higher frequency of the DD phenotype in patients with the akinetic-rigid form of the disease and L-dopa induced motor complications. These differences were not statistically significant. When results were analyzed by groups defined by phenotype and not by genotype, a marginally non-significant result was revealed: there were only 9 DD homozygotes (26.5%) in the patients with an initial tremor-dominant form of PD ($n=34$) compared to 21 DD homozygotes (48.8%) in the bradykinesia-rigidity dominant form ($n=43$) (Fisher exact test $p=0.061$ and Pearson χ^2 test $p=0.061$).

Finally, we compared the distribution of the genotypes (DD, ID, II) in PD patients and control subjects categorized by age group. For PD patients we used the age of onset (age < 50 , 50–59, 60–69 and ≥ 70 years). No statistically significant differences were noted in the age subgroup analysis although patients with younger onset PD tended to have the DD phenotype more frequently. The results of this analysis are presented in Table 3.

DISCUSSION

The ACE gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. While ACE is best known for the conversion of angiotensin I to angiotensin II and for the degradation of bradykinin, this dipeptidyl carboxypeptidase has additional substrates. A substrate of special interest with regard to PD is substance P [15], an undecapeptide that displays neuroprotective effects [16–18]. Substance P also increases rat locomotor activity when microinjected into discrete regions of the basal ganglia including the substantia nigra reticulata (SNr), substantia nigra compacta (SNc), ventral tegmental area (VTA), and globus pallidum

Table 2. Subgroup Analysis of Main PD Symptoms and L-Dopa Motor Side Effects According to ACE Genotypes

	N (n=77, % of total)*	DD(%)**	DI(%)**	II(%)**	p-value
Initial Symptoms					
Tremor dominant	34(44.2%)	9 (30.0%)	20(54.1%)	5(50.0%)	0.13
B-R dominant	43(55.8%)	21 (70.0%)	17(45.9%)	5(50.0%)	
Tremor at examination	52(67.5%)	18 (60.0%)	28(75.7%)	6(60.0%)	0.34
Rigidity at examination	58(75.3%)	23 (76.7%)	27(73.0%)	8(80.0%)	0.88
Instability at examination	38(49.4%)	18 (60.0%)	14(37.8%)	6(60.0%)	0.15
Dyskinesias	15(19.5%)	7 (23.3%)	5(13.5%)	3(30.0%)	0.40
On-off phenomena	22(28.6%)	13 (43.3%)	6(16.2%)	3(30.0%)	0.06

B-R: bradykinesia rigidity; DI: deletion/insertion polymorphism; PD: Parkinson's disease.

*Percentage (%) of all PD patients.

**Percentage of PD patients with each genotype.

Table 3. Comparison ACE Genotypes in PD Patients and Control Subjects Categorized by Age Group

	PD group (N = 77)				Control subjects (N = 50)				p-value
	N	DD	DI	II	N	DD	DI	II	
Total	77	30 (39.0%)	37 (48.0%)	10 (13.0%)	50	16 (32.0%)	25 (50.0%)	9 (18.0%)	0.63
< 50	12	7(58.3%)	4 (33.3%)	1 (8.3%)	25	9 (36.0%)	11 (44.0%)	5 (20.0%)	0.40
50–59	11	1 (9.1%)	7 (63.3%)	3 (27.3%)	3	0 (0.0%)	1 (33.3%)	2 (66.7%)	0.43
60–69	30	12 (40.0%)	15 (50.0%)	3 (10.0%)	10	2 (20.0%)	7 (70.0%)	1 (10.0%)	0.50
≥70	24	10 (41.7%)	11 (45.8%)	3 (12.5%)	12	5 (41.7%)	6 (50.0%)	1 (8.3%)	0.93

D/I: deletion/insertion polymorphism; PD: Parkinson's disease.

(GP) [19–22]. Several studies in rats have also shown that the substance P can increase dopamine outflow [23–27] as well as acetylcholine release [28] under normal conditions.

A deletion/insertion (D/I) polymorphism of ACE has been reported that arises from the presence or absence of a 287-base pair sequence of intron 16 resulting in three genotypes (DD/II homozygotes and ID heterozygote) [29]. Although ACE activity is found in plasma, the majority of the biologically active enzyme is tissue-bound [30]. Turet *et al.* [31] provided evidence that the insertion/deletion (ID) polymorphism is associated with higher plasma ACE activity. Later reports established that the DD genotype is also associated with a higher tissue ACE activity [32]. As reduced neuroprotection is implicated in the pathogenesis of PD [33], one could speculate that the increased ACE activity in subjects with a DD genotype could lower the levels of substance P, making them more susceptible to PD. Lower levels of substance P may also affect locomotion and dopamine modulatory mechanisms further contributing to the development of PD. Interestingly, substance P levels and immunoreactivity are decreased in nigral and striatal tissues of animals models of PD and in postmortem samples of PD patients [16].

To test the hypothesis that the ACE I/D polymorphism is associated with PD, possibly through altering substance P levels, we determined ACE genotypes in PD patients and compared them to non-PD controls. The frequency of the DD genotype and the D allele was higher in the PD population. The frequency of the DD phenotype was also higher in younger patients and in patients with the akinetic-rigid type of PD. However, statistical significance was not reached in any of the comparisons made throughout our study. However, the marginally non-significant increased prevalence of DD homozygotes in the akinetic-rigid predominant PD patients may suggest that the I/D polymorphism has disease modifying effects. Mellick *et al.* [6] were the first to investigate the possible involvement of the ACE gene in the pathogenesis of PD in a population of Caucasians. Based on evidence linking the DD ACE genotype to longevity [34] they suggested that D allele may confer some long-term protective effect as a result of ACE's action on neuropeptides and neuroendocrine function. In their study similar frequencies were reported for the D and I alleles in the PD (D 48% and I 52%) and control population (D 54% and I 46%). Furthermore, the genotype distribution was also similar in the PD

and control group (the most frequent phenotype was ID in 52% of PD patients and 56% of controls). Our results are in agreement with those of Mellick *et al.* that also failed to provide evidence that the ACE polymorphism is related to PD. Both our studies report similar allele frequencies in the control groups confirming previous results (the reported D allele frequency in Caucasians is 50–58% [35, 36].

In a different study, Lin *et al.* [5] also evaluated the possible relationship between the ACE polymorphism and PD in a Chinese population. The frequency of the homozygote DD genotype of the ACE gene was increased in the PD group (22% in the PD vs 12% in the control group), but the statistical significance was marginal ($p = 0.048$). The increase in the DD genotype in the PD group was also noted in our study (39% in the PD vs 32% in the control group) but significance was not reached. One could speculate that various reasons like ethnicity, (Caucasians and Chinese have different genetic backgrounds), sample size and different recruitment methods used for the control population could account for this difference in significance between the two studies. In normal Chinese the D allele frequency is 35–39% [36]. The frequency of D allele in the control subjects in the study by Lin *et al.* (33%) was markedly different from that in our report (57%) and Mellick's study (54%). However, this is in accordance with the frequencies of the D allele in Caucasian and Chinese populations.

Our study has certain strengths and limitations. We acknowledge the relatively small sample size and highlight the preliminary nature of these findings. Strengths of our study include its prospective nature and the homogeneity of the sample population.

Based on the evidence presented herein, we conclude that the ACE insertion/deletion polymorphism is not associated with PD and that ACE genotypes do not correlate significantly with any of the symptoms of the disease in Caucasians, although a disease modifying effect cannot be excluded. Our data suggest that ACE genotype is unlikely to be an important determinant of susceptibility to PD.

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