

## ANGIOTENSIN-CONVERTING ENZYME GENOTYPE AND ACUTE PANCREATITIS IN TURKEY

Kasap E<sup>1\*</sup>, Akyıldız M<sup>2</sup>, Tekin F<sup>2</sup>, Akarca U<sup>2</sup>

**\*Corresponding Author:** Elmas Kasap, Department of Gastroenterology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey; Tel.: +90-236-2330115; +90-542-2457238; Fax: +90-236-2370213; E-mail: elmaskasap@yahoo.com

### ABSTRACT

The renin-angiotensin system (RAS) has been implicated in the pathogenesis of acute and chronic pancreatitis. Angiotensin-converting enzyme (ACE) is the key enzyme which activates RAS. The ACE intron 16 insertion/ deletion (I/D) polymorphism is associated with ACE activity and is considered to be a risk factor for several inflammatory processes. We investigated this polymorphism in 68 patients with acute pancreatitis (AP) and 157 healthy Turkish control subjects. Patients were evaluated with ultrasonography, abdominal tomography and laboratory markers and grouped by status for diabetes mellitus (DM), hypertension (HT), and both these diseases and by etiology. Genotyping of the I/D polymorphism was performed by polymerase chain reaction (PCR). The DD genotype was more prevalent in healthy controls, however, genotype II was significantly more frequent in AP patients ( $p < 0.05$ ). In severe AP patients, the genotype II frequency was significantly higher than in controls ( $p < 0.05$ ). Acute pancreatitis patients with both DM and HT had lower frequencies of genotype DD and of the D allele, and higher frequencies of genotype II and of the I allele than patients with either DM or HT ( $p < 0.05$ ).

**Key words:** Acute pancreatitis (AP), Angiotensin-converting enzyme (ACE) gene polymorphism, Genetic polymorphism

### INTRODUCTION

Acute pancreatitis (AP) is an acute inflammatory process of variable of etiology [1]. While etiology and pathogenesis of AP have been intensively investigated, it is still unclear why some patients progress to organ failure and others do not [2,3]. Blockage of the duodenal papilla or ampulla of Vater is the common characteristic of the disease. In developed countries, obstruction of the common bile duct by stones (38%) and alcohol abuse (36%) are the most frequent causes [4,5], but iatrogenic factors, sphincter of Oddi dysfunction and eating disorders are also important [6]. Acute pancreatitis is viewed as an event and chronic pancreatitis as a process that is sequentially linked to AP and reflects a complex interaction between genetic and environmental factors [7]. The major common genetic risk factors have yet to be defined [8].

The renin-angiotensin system (RAS) has been investigated in the pathogenesis of several diseases. Local RAS components exist in brain, heart, kidney, pancreas, adrenal glands and gonads [9-11], and contribute in the regulation of cell growth, differentiation, proliferation and apoptosis, reactive oxygen species generation, tissue inflammation and fibrosis, and hormonal secretion [12]. Renin-angiotensin system has been implicated in pathogenesis

<sup>1</sup> Department of Gastroenterology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

<sup>2</sup> Department of Gastroenterology, Faculty of Medicine, Ege University, Bornova, İzmir, Turkey

of AP and chronic pancreatitis [8]. Although the etiology of AP is believed to be multifactorial, the activation of proteolytic enzymes, lipase, kinins and other active peptides may be responsible for alterations of RAS expression [13,14]. In fact, the activity of the plasma RAS is significantly increased in AP [15,16]. Renin-angiotensin system is important in regulation of electrolyte balance, fluid and blood pressure. Angiotensin-converting enzyme is the key enzyme which activates the RAS [8,9] by converting angiotensin I to angiotensin II, which is a potent vasoconstrictor. The ACE inactivates bradykinin, a vasodilator of the kallikrein-kinin system, which has major influence in inflammatory processes. Since angiotensinogen and angiotensin receptors may play a role in induction of inflammation and microcirculatory regulation in the pancreas, they may contribute to its injury in AP [16,17]. Association of severe AP and impairment of pancreatic microcirculation has been demonstrated in experimental models of AP [18]. Indeed, vasoconstriction, capillary stasis, decreased oxygen tension, and progressive ischemia occur early in the course of AP [19].

The ACE gene is located on human chromosome 17q23. Three genotypes are associated with an Alu repetitive sequence about 287 bp long on intron 16. These genotypes are insertion (I) and deletion (D) alleles, respectively [16,17].

The DD genotype has been linked to several inflammatory diseases [8,9] and results in higher levels of circulating ACE than the II and DI genotypes. It is also significantly more frequent in patients with myocardial infarction or diabetic proteinuria than in controls [10]. However, several studies have reported that the ACE I/D polymorphism was not a risk factor in AP and chronic pancreatitis [8,9]. In this study, we investigated the I/D polymorphism in AP.

## MATERIALS AND METHODS

Sixty-eight patients with AP and 157 healthy controls were recruited from the archives of the Department of Gastroenterology, Ege University Medical School, İzmir, Turkey. Informed written consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the declaration of Helsinki. Etiology, gender, age, clinical presentation, clinical course, genotype fre-

quency of the ACE I/D polymorphism and their association were compared.

Blood specimens from all participants were obtained with a standard venipuncture technique that used ethylenediamine tetra-acetic acid-containing tubes. DNA was isolated from peripheral blood by a standard phenol/chloroform extraction method [12].

For the ACE polymorphism, we used polymerase chain reaction (PCR) methodology [19], with the upstream primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and the downstream primer 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'. Amplification was performed for 35 cycles with denaturation, extension and annealing temperatures of 94.8°C, 60.8°C and 72.8°C, respectively. The resulting PCR products were separated on 2% agarose gel with ethidium bromide staining and were visualized under ultraviolet light. Homozygotes for the deletion or insertion genotypes were described as DD and II, respectively, and the heterozygous genotype as ID.

All statistical analyses were performed using the SPSS 11.0 statistical program. Genotypes, allele frequencies, clinical features at diagnosis were evaluated by the  $\chi^2$  test. Clinical data are reported as mean  $\pm$  SD (standard deviation) and as percentages. Statistical significance was taken as  $p < 0.05$ .

## RESULTS

The characteristics of the patients with AP and the healthy controls are summarized in Table 1. Etiology and severity of AP are summarized in Table 2. The etiology was classed as biliary in 44, and 24 were classed to be of other etiologies [six hypertriglyceridemia, 10 post endoscopic retrograde cholangiopancreatography procedure (ERCP), three alcoholic, five idiopathic]. Fifty-five patients were diagnosed to have mild AP, 13 patients were diagnosed with severe AP. Thirteen patients with AP had diabetes mellitus (DM) type 2, 23 had essential hypertension (HT), and 10 had both.

Genotype distribution of ACE gene for both groups is summarized in Table 3. The DD genotype was more prevalent among healthy controls than in AP patients, while the II genotype was more prevalent in AP patients than healthy controls ( $p < 0.05$ ).

Genotype distribution of the ACE gene in bil-

**Table 1.** Characteristics of the study groups

Parameters	Acute Pancreatitis	Healthy Controls
Number of subjects	68	157
Sex (males/females)	42/26	102/55
Age (years)	51 ± 5	24 ± 7
Diabetes mellitus	13	0
Hypertension	23	0
Diabetes mellitus + hypertension	10	0

**Table 2.** Etiology and severity of acute pancreatitis

Etiology	Mild AP (n)	Severe AP (n)
Biliary	34	10
Endoscopic retrograde cholangiopancreatography procedure (ERCP)	9	1
Hypertriglyceridemia	4	2
Alcohol	3	0
Idiopathic	5	0
Severity	55	13
Ranson Score (median range)	1 (0-4)	4 (1-8)

**Table 3.** Genotype distribution of the angiotensin-converting enzyme gene in the study groups

Genotype	Acute Pancreatitis n (%)	Healthy Controls n (%)	p Value
DD	17 (25.0)	61 (38.0)	<0.05
DI	37 (54.4)	82 (52.0)	N.S.
II	14 (20.0)	14 (8.0)	<0.05
D (allele frequency)	71 (52.2)	204 (64.9)	N.S.
I (allele frequency)	65 (47.8)	110 (35.1)	N.S.

N.S.: not significant.

iary and in non biliary AP is summarized in Table 4. There was no significant difference in the distribution of the I/D polymorphism or in allele frequency between biliary and non biliary AP.

Genotype distribution of the ACE gene in AP associated with DM and/or HT is summarized in Table 5. There is no significant difference in the distribution of the I/D polymorphism or allele frequency between AP with DM and AP with HT.

However in patients with both these diseases, the ACE II genotype and allele I frequency was significantly higher than in the healthy group or in patients with only one of these diseases ( $p < 0.05$ ). The distribution of ACE genotypes DD and DI were similar in patients with mild or severe AP. However, frequency of the II genotype in severe AP was significantly higher than healthy controls ( $p < 0.05$ ) (Table 6).

**Table 4.** Genotype distribution of the angiotensin-converting enzyme gene in biliary and non biliary of acute pancreatitis

Genotype	Biliary AP <i>n</i> (%)	Non Biliary AP <i>n</i> (%)
DD	11 (25.0)	6 (25.0)
DI	24 (54.0)	13 (54.0)
II	9 (20.0)	5 (20.0)
D (allele frequency)	46 (52.2)	25 (52.0)
I (allele frequency)	42 (47.8)	23 (48.0)

The *p* value was not significant.

**Table 5.** Genotype distribution of the angiotensin-converting enzyme gene in acute pancreatitis associated with diabetes mellitus and/or hypertension

Genotype	Diabetes Mellitus <i>n</i> (%)	Hypertension <i>n</i> (%)	DM + HT <i>n</i> (%)	<i>p</i> Value
DD	5 (38.0)	7 (30.0)	* 1 (10.0)	<0.05
DI	7 (53.8)	13 (56.5)	5 (50.0)	N.S.
II	1 (7.6)	3 (13.0)	* 4 (40.0)	<0.05
D (allele frequency)	17 (68.0)	27 (63.0)	* 6 (31.0)	<0.05
I (allele frequency)	8 (32.0)	19 (37.0)	* 13 (69.0)	<0.05

*p* <0.05; \*: AP associated with DM and HT and/or patients who had only one of these diseases; N.S.: not significant.

**Table 6.** Genotype distribution of the angiotensin-converting enzyme gene and severity of acute pancreatitis

Genotype	Mild AP <i>n</i> (%)	Severe AP <i>n</i> (%)	Healthy Controls <i>n</i> (%)	<i>p</i> Value
DD	13 (23.2)	4 (30.0)	61 (38.0)	N.S.
DI	32 (57.0)	6 (46.0)	82 (52.0)	N.S.
II	11 (19.0)	* 3 (23.0)	14 (8.0)	<0.05
D (allele frequency)	58 (51.0)	14 (53.0)	204 (64.9)	N.S.
I (allele frequency)	54 (49.0)	12 (47.0)	110 (35.1)	N.S.

*p* <0.05; \*: AP associated with DM and HT and/or patients who had only one of these diseases; N.S.: not significant.

## DISCUSSION

The subjects who had the homozygous deletion (DD) genotype had the highest ACE frequency, while subjects who had the homozygous insertion (II) genotype had the lowest ACE levels. Studies from the United States, German and Finnish popu-

lations did not find a relationship between AP and I/D polymorphisms [9,20]. We found the DD genotype to be more prevalent in healthy controls than in patients with AP, however, the II genotype was significantly more prevalent in AP patients than in healthy controls (*p* <0.05). Even so, the ACE II genotype was significantly higher in severe AP pa-

tients than in healthy controls, and ACE II was not related to the etiology of pancreatitis.

Studies in the European populations, revealed the ratio for the ACE genotype DD/ID/II to be 1:2:1. However, in Turkey, the DD genotype is the most frequent, and genotype II the least common in the healthy population [21-23]. This may be one of the reasons for the difference between our results and these from studies of the United States, German and Finish populations [9,20]. A relationship between the II genotype and inflammation and pancreatic injury needs further clarification.

It has been suggested that the DD genotype is related to essential HT and DM [23,24]. On the other hand, the association between the I/D polymorphism and HT and DM is still controversial [24-27]. A study from Turkey did not show a significant association between the ACE gene polymorphism and HT in diabetic patients [26]. A study from Sweden showed that the DD genotype increases the risk of HT in diabetic patients [28].

We did not find significant difference either in the distribution of the I/D genotype polymorphism or allele frequency in diabetic AP and hypertensive AP groups. We have found that the ACE II genotype and allele I frequencies are higher in the patients who have both diabetes and HT compared to patients who have only one of these diseases. In conclusion, the ACE gene polymorphism may play in role in AP. We need larger studies for investigating the relation of the ACE I/I genotype with other inflammatory parameters. By such studies we could evaluate the significance of the ACE I/I genotype in AP and its possible use as a genetic marker.

## REFERENCES

1. James HG. Acute pancreatitis. In: James HG, Friedman SL, McQuaid KR, Eds. *Current Diagnosis and Treatment in Gastroenterology*, 2nd ed. 2003; 489-495.
2. Pandol SJ. Acute pancreatitis. *Curr Opin Gastroenterol* 2006; 22(5): 481-486.
3. Papachristou GI. Prediction of severe acute pancreatitis: current knowledge and novel insights. *World J Gastroenterol* 2008; 14(41): 6273-6275.
4. Lankisch PG, Assmus C, Lehnick D, Maisonneuve P, Lowenfels AB. Acute pancreatitis: does gender matter? *Dig Dis Sci* 2001; 46(11): 2470-2474.
5. Spanier BW, Dijkgraaf MG, Bruno MJ. Epidemiology, etiology and outcome of acute and chronic pancreatitis: An update. *Best Pract Res Clin Gastroenterol* 2008; 22(1): 45-63.
6. Siqin D, Wang C, Zhou Z, Li Y. The key event of acute pancreatitis: pancreatic duct obstruction and bile reflux, not a single one can be omitted. *Med Hypotheses* 2009; 72(5): 589-591.
7. Grigorescu M, Grigorescu MD. Genetic factors in pancreatitis. *Rom J Gastroenterol* 2005; 14(1): 53-61.
8. Oruc N, Lamb J, Kutlu OC, Barmada MM, Money ME, Slivka A, Whitcomb DC. The functional angiotensin converting enzyme gene I/D polymorphism does not alter susceptibility to chronic pancreatitis *JOP* 2004; 5(6): 457-463.
9. Oruc N, Papachristou G, Avula H, Slivka A, Lamb J, Whitcomb DC. Angiotensin-converting enzyme gene DD genotype neither increases susceptibility to acute pancreatitis nor influences disease severity. *HPB (Oxford)* 2009; 11(1): 45-49.
10. Bhaskar S, Reddy DN, Mahurkar S, Rao GV, Singh L, Chandak GR. Lack of significant association of an insertion/deletion polymorphism in the angiotensin converting enzyme (ACE) gene with tropical calcific pancreatitis *BMC Gastroenterol* 2006; 6:42-44.
11. Paul M, Mehr AP, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev* 2006; 86(3): 747-803.
12. Leung PS. The peptide hormone angiotensin II: its new functions in tissues and organs. *Curr Protein Pept Sci* 2004; 5(4): 267-273.
13. Agarwal N, Pitchumoni CS. Acute pancreatitis: a multisystem disease. *Gastroenterologist* 1993; 1(2): 115-128.
14. Lembeck F, Griesbacher T. Pathophysiological and possible roles of kinins in the pancreas. *Immunopharmacology* 1996; 33(3): 336-338.
15. Greenstein RJ, Krakoff LR, Felton K. Activation of the renin system in acute pancreatitis. *Am J Med* 1987; 82(3): 401-404.
16. Knoefel WT, Kollias N, Warshaw AL, Waldner H, Nishioka NS, Rattner DW. Pancreatic microcirculatory changes in experimental pancreatitis of graded severity in rat. *Surgery* 1994; 116(5): 904-913.

17. Lai BS. Local renin-angiotensin system in the pancreas: the significance in acute pancreatitis. *J Pancreas* (Online) 2001; 2(1): 13-15.
18. Bassi D, Kollias N, Fernandez-del Castillo C, Foitzik T, Warshaw AL, Rattner DW. Impairment of pancreatic microcirculation correlates with severity of acute experimental pancreatitis. *J Am Coll Surg* 1994;179(3): 257-263.
19. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86(3): 1343-1346.
20. Hucl T, Kylanpää ML, Künzli B, Witt H, Lempinen M, Schneider A, Kempainen E, Löhr M, Haas SL, Friess H, Ockenga J, Rosendahl J, Schulz HU, Gress T, Singer MV, Pfützer RH. Angiotensin-converting enzyme insertion/deletion polymorphism in patients with acute and chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2009; 21(9): 1032-1035.
21. Celik US, Noyan A, Bayazit A K, Büyükçeklik M, Dursun H, Anarat A, Attila G, Matyar S. ACE gene polymorphism in Turkish children with nephrotic syndrome. *Renal Failure* 2006; 28(1): 401-403.
22. Oztürk O, Oztürk U, Toprak N. Anterior akut miyokard infarktüsü hastalarda anjiyotensin konvertir enzim (ACE) gen polimorfizminin geliş sol ventrikül diyastolik doluş parametreleri üzerindeki etkisi *Dicle Tip Dergisi* 2006; 3(2): 220-226.
23. Degirmenci I, Kebapci N, Basaran A, Efe B, Gunes HV, Akalin A, Kurt H, Urhan M, Demirustu C. Frequency of angiotensin-converting enzyme gene polymorphism in Turkish type 2 diabetic patients. *Int J Clin Pract* 2005; 59(10): 1137-1142.
24. Di Pasquale P, Cannizzaro S, Scalzo S, Maringhini G, Pipitone F, Fasullo S, Giubilato A, Ganci F, Vitale G, Sarullo FM, Paterna S. Cardiovascular effects of I/D angiotensin-converting enzyme gene polymorphism in healthy subjects. Findings after follow-up of six years. *Acta Cardiol* 2005; 60(1): 427-435.
25. Feng Y, Niu T, Xu X, Chen C, Li Q, Qian R, Wang G, Xu X. Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. *Diabetes* 2002; 51(1): 1986-1988.
26. Singh PP, Naz I, Gilmour A, Singh M, Mastana S. Association of APOE (Hha1) and ACE (I/D) gene polymorphism with type 2 diabetes mellitus in North West India. *Diabetes Res Clin Pract* (2006); 74(1): 95-102.
27. O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D. Evidence for association and genetic linkage of the angiotensin converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation* 1998; 97(18): 1766-1772.
28. Bengtsson K, Orho-Melander M, Lindblad U, Melander O, Bøg-Hansen E, Ranstam J, Råstam L, Groop L. Polymorphism in the angiotensin converting enzyme but not in the angiotensinogen gene is associated with hypertension and type 2 diabetes: the Skaraborg Hypertension and Diabetes Project. *J Hypertens* 1999; 17(11): 1569-1575.