

Discipline: Biotechnology Evaluation of Gene I/D Polymorphism in Pancreatitis

Rita Singh, Dr. Anshurani Patel, Snehlata Pandey

Work done at Sanjay Gandhi Post Graduate institute of medical Science, Lucknow U.P
Present Address: Centre for Biotechnology, APS University Rewa M.P.

Abstract- Pancreatitis is inflammation of pancreas, most characteristic symptoms is epigastric pain, radiating to back. The severity and occurrence of the pain tends to reduce with disease progression. The ACE (angiotensin converting enzyme) I/D a deletion polymorphism of 287bp fragment of intron 16 of the ACE gene allele, has been shown to result in higher levels of circulating enzyme in a dependent manner. The ACE may have a pathogenic role in the development of chronic pancreatitis. The RAS is a circulatory cascade system and the key enzyme in this system is ACE which converts Angiotensin-I to the potent vasoconstrictor Angiotensin-II.

The aim of the study was to investigate the occurrence of the ACE I/D polymorphism in chronic pancreatitis patients. The study included 50 patients. Polymorphism was examined by PCR. ACE genotype between patients 18(36%)II 12(24%)ID, 20(40%)DD and allele frequencies I allele 48(48%)and D allele 52(52%) were observed. We concluded that the ACE II genotype may modify the progression of disease.

Index Terms- ACE, DNA, Pancreatitis, Allele, PCR.

I. INTRODUCTION

Chronic pancreatitis is an inflammatory disease which leads to pancreatic fibrosis and the destruction of the exocrine and the endocrine pancreas [1]. Pancreatitis appears to be a complex disorder reflecting the interaction of various genetic and environmental factors [2, 3]. Pancreatic inflammation is initiated by pancreatic injury, most commonly through activation of trypsinogen and other pancreatic zymogens leading to autodigestion [4]. Tropical calcific pancreatitis (TCP) is a form of chronic pancreatitis of unknown etiology, more prevalent in the tropical regions of developing countries such as India whereas fibrocalculous pancreatic diabetes (FCPD) is a form of diabetes secondary to TCP [5]. The most characteristic symptom is epigastric pain, radiating to back. The initial presentation is as recurrent severe episodes of pain separated over long periods, followed later by frequent milder episodes or chronic persistent pain. The severity and occurrence of the pain tends to reduce with disease progression. The pain seems to 'burn off' perhaps due to loss of most pancreatic parenchymal tissue. It has been seen that pain usually disappears with onset of exocrine insufficiency and /or diabetes [5]. Both TCP and FCPD are associated with extensive fibrosis involving both intra and interlobular regions and not limited to one zone of the pancreas, although the extent of fibrosis varies and is usually more in FCPD [6].

II. GENETIC FACTOR

Angiotensin-converting enzyme:- The ACE gene insertion/deletion (I/D) polymorphism was first identified in 1990 [7]. The ACE-D, a deletion polymorphism of a 287-bp fragment of intron 16 of the ACE gene allele, has been shown to result in higher levels of circulating enzyme in a dose dependent manner [7]. ACE may have a pathogenic role in the development of chronic pancreatitis.

Renin-Angiotensin System (RAS):- The Renin-Angiotensin system (RAS) is a circulatory cascade system primarily involved in the regulation of blood pressure and serum, electrolytes[8,9]. The key enzyme in this system is the angiotensin converting enzyme (ACE) which converts angiotensin I to the potent vasoconstrictor angiotensin II [8,9,10]. The RAS has been said to be involved in the pathogenesis of several diseases including fibrosis in the heart, kidney, lung and liver during chronic inflammation through the regulation of cell growth, inflammation, oxidative stress and fibrosis [11,12,13,14]. Recent studies have shown that the RAS is intrinsically present in the pancreas [15] and its genetic expression is enhanced during acute pancreatitis and chronic pancreatic hypoxia in experimental animals [16,17]. Furthermore, the pharmacological blockage of ACE significantly attenuated pancreatic fibrosis in an experimental model of chronic pancreatitis in rats[18]. Polymorphism in chronic pancreatitis and its contribution to the course of the disease has not yet been defined. We therefore investigated the ACE genotype and allele frequency between patients.

III. METHODS

Patients:- Patients were recruited from the department of Gastroenterology at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow (U.P.).

TLaboratory Procedure:- Genomic DNA was purified from peripheral blood cells of the subjects using phenol-chloroform extraction method. A 287-bp I/D polymorphism in intron 16 of the ACE gene was examined by polymerase chain reaction (PCR), using forward primer 5' – CTG GAG ACC ACT CCC ATC CTT TCT- 3' and the reverse primer 5'- GAT GTC GCC ATC ACA TTC GTC AGA T -3'. The amplified PCR product were separated on 1% agarose gel and visualized by UV illuminator. Amplified ACE gene fragments without insertion (D allele) and with insertion (I allele) of approximate 190 and approximate 490 bp. PCR amplification were performed with 25

µL reactions (0.5 µg genomic DNA, 500 pmol of primers, 0.5 mM each deoxy-ATP, GTP, CTP, TTP, 1.5 mM MgCl₂, 0.5 U Taq DNA polymerase), with 1 min of denaturation at 94°C, followed by 30 cycles of 30 s at 94°C, 45 s at 67°C, and 2 min at 72°C.

IV. RESULT

Table 1: ACE Genotype and Allele Frequencies between Patients.

	Patients Group n=50
ACE genotype	
II	18 (36%)
ID	12 (24%)
DD	20 (40%)
I allele	48 (48%)
D allele	52 (52%)

V. DISCUSSION

The RAS, traditionally known as an endocrine system regulating blood pressure and body fluid homeostasis [19]. The ACE plays a central role in this system by converting angiotensin I to the potent vasoconstrictor angiotensin II [20].

The RAS system has been shown to play an important role in the regulation of pancreatic exocrine and endocrine functions [21]. During acute and chronic inflammation of the pancreas, both circulating ACE activity and intrinsic pancreatic ACE activity is markedly elevated [17, 22, 23]. Results of this study indicate association between ACE I/D polymorphism and pancreatic disease. The DD genotype of the ACE I/D polymorphism was significantly most frequent in chronic pancreatitis. The importance of the role of ACE in pancreatic diseases, numerous studies have researched the potential roles of ACE inhibitors as protective factors in angiogenesis control in pancreatic disease. It is possible that the ACE I/D polymorphism plays a role in the development of chronic pancreatitis.

ACKNOWLEDGEMENT

We are grateful to all the patients and the normal individuals for agreeing to participate in the study and for giving consent for genetic studies. Thanks are also due to Dr. Sarita Agarwal, Department of Genetics SGPGIMS Lucknow (U.P.) for her help in the study.

REFERENCES

[1] Ammann RW, Akovbiantz A, Largiader F, Schueler G. Course and outcome of chronic pancreatitis. Longitudinal study of a mixed medical-surgical series of 245 patients. *Gastroenterology* 1984; 86:820-8.

[2] Whitcomb DC. Hereditary pancreatitis. New insights into acute and chronic pancreatitis. *Gut* 1999; 45:317-22.

[3] Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; 14:141-5.

[4] Bhatia M, Brady M, Shokuhi S, Christmas S, Neoptolemos JP, Slavin J. (2000) Inflammatory mediators in acute pancreatitis. *J Pathol* 190:117–125.

[5] Mohan V, Chari ST, Hitman GA, Suresh S, Madanagopalan N, Ramachandran A, Viswanathan M: Familial aggregation in tropical fibrocalculous pancreatic diabetes. *Pancreas* 1989, 4:690-693.

[6] Nagalotimath SJ: Pancreatic pathology in pancreatic calcification with diabetes. In *Secondary diabetes: The spectrum of the diabetic syndromes* Edited by: Podolsky S, Viswanathan M. New York: Raven Press; 1980:117-145.

[7] Rigat B, Hubert C, Alhenc-Gelas F, Corvol P, Soubrier F. An insertion/deletion polymorphism in angiotensin converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86:1343-6.

[8] Stroth U, Unger T. The renin angiotensin system and its receptors. *J Cardiovascular Pharmacol* 1999; 33:S21-8.

[9] Pueyo ME, N'Diaye N, Michael JB. Angiotensin II elicited signal transduction via AT 1 receptors in endothelial cells. *Br J Pharmacol* 1996; 118:79-84.

[10] Mii S, Ware JA, Mallette SA, Kent KC. Effect of angiotensin II on human vascular smooth muscle cell growth. *J Surg Res* 1994;57:174-8.

[11] Frishman WH, Fozailoff A, Lin C, Dike C. Renin inhibition: a new approach to cardiovascular therapy. *J Clin Pharmacol* 1994;34:873-80.

[12] Ruiz-Ortega M, Ruperez M, Esteban V, Egido J. Molecular mechanisms of angiotensin II-induced vascular injury. *Curr Hypertens Rep* 2003; 5:73-9.

[13] Bataller R, Gines P, Nicolas JM, Gorbis MN, Garcia Ramealo E, Gasull X, et al. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; 118:1149-56.

[14] Marshall RP, McNulty RJ, Laurent GJ. Angiotensin II is mitogenic for human lung fibroblasts via activation of the type 1 receptor. *Am J Respir Crit Care Med* 2000; 161:1999-2004.

[15] Nobiling R. The renin-angiotensin system: from the renal basis to an organ-specific subsystem in the pancreas. *JOP. J Pancreas (Online)* 2001; 2:9-12.

[16] Leung PS, Chappell MC. A local pancreatic rennin-angiotensin system endocrine and exocrine roles. *Int J Biochem Cell Biol* 2003; 35:838-46.

[17] Chan WP, Fung ML, Nobiling R, Leung PS. Activation of local renin-angiotensin system by chronic hypoxia in rat pancreas. *Mol Cell Endocrinol* 2000;160:107-14.

[18] Kuno A, Yamada T, Masuda K, Ogawa K, Sogawa M, Nakamura S, et al. Angiotensin-Converting Enzyme attenuates pancreatic inflammation and fibrosis in male Bonn/Kabori rats. *Gastroenterology* 2003; 124:1010-9.

[19] Oparil S, Haber E. The renin-angiotensin system (first of two parts). *N Engl J Med* 1974; 291:389-401.

[20] Matsusaka T, Ichikawa I. Biological functions of angiotensin and its receptors. *Annu Rev Physiol* 1997;59:395-412.

[21] Leung PS, Carlsson PO. Tissue renin-angiotensin system: its expression, localization, regulation and potential role in the pancreas. *J Mol Endocrinol* 2001; 26:155-164.

[22] Leung PS, Chan HC, Nobiling R. Regulated expression of pancreatic renin-angiotensin system in experimental pancreatitis. *Mol Cell Endocrinol* 2000; 166:121-128.

[23] Ip SP, Kwan PC, Williams CH, Pang S, Hooper NM, Leung PS. Changes of angiotensin converting enzyme activity in the pancreas of chronic hypoxia and acute pancreatitis. *Int J Biochem Cell Biol* 2003;35:944-54.

AUTHORS

First Author – Rita Singh, Sanjay Gandhi Post Graduate institute of medical Science, Lucknow U.P, Email Id: biotechrita60@gmail.com

Second Author – Dr. Anshurani Patel, Sanjay Gandhi Post Graduate institute of medical Science, Lucknow U.P

Third Author – Snehlata Pandey, Sanjay Gandhi Post Graduate institute of medical Science, Lucknow U.P