Journal of Biological Research **6**: 231–234, 2006
J. Biol. Res. is available online at http://www.jbr.gr

— SHORT COMMUNICATION —

Potential influence of the pancreatitis related genes on the phenotype of cystic fibrosis patients

ATHANASIOS KALOGERIDIS[1,2], JOHN TSANAKAS[2] and ANASTASIA KOUVATSI[1*]

[1] *Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University, Thessaloniki 54124, Greece*
[2] *Hippokration General Hospital of Thessaloniki, Thessaloniki 54642, Greece*

Received: 29 August 2006 Accepted after revision: 1 November 2006

The DNA of 83 unrelated patients with cystic fibrosis (CF) from Northern Greece was analyzed for common mutations in the pancreatitis associated genes (PRSS1 and PSTI), in order to test the possible relative contribution of these two genes to the CF disease phenotype. PCR amplification of different regions of the two genes was followed by restriction digestion or sequencing analysis. One patient, homozygous for the ΔF508 mutation, was found to be homozygous for the A16V mutation of the PRSS1 gene. The existence of the A16V substitution in a single patient, with good pulmonary status, may represent a mere coincidence or may be an indication that the PRSS1 gene acts as a modifier gene in the pathogenesis of cystic fibrosis.

Key words: cystic fibrosis, PRSS1, PSTI, modifier genes.

INTRODUCTION

Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasian populations affecting about 1 in 2500 births. It is clinically characterized by obstructive lung disease, exocrine pancreatic insufficiency, elevated sweat electrolytes and male infertility. The disease is caused by mutations (> 1000) in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, on the long arm of chromosome 7 (Riordan *et al*., 1989). The most common mutation is a 3 base pair deletion, resulting in the loss of phenylalanine at position 508 (ΔF508).

The phenotype is highly heterogeneous among patients, even between siblings carrying the same mutations (Rowntree & Harris, 2003). The phenotypic variation may be caused by primary and secondary determinants. The primary source of variation is the high number of different mutations and the possible interactions between them (Kiesewetter *et al*., 1993). Furthermore, the modulation of the severity of the disease may be explained by secondary factors, such as other genes (modifier genes) and also environmental effects (e.g. diet, socio-economical status) (Kiesewetter *et al*., 1993; Rozmahel *et al*., 1996; Zielenski *et al*., 1999; Rowntree & Harris, 2003).

CFTR genotype seems to be predictive of the pancreatic status of the patients. Recent reports suggest that two severe mutations (class I, II or III) are required to cause pancreatic insufficiency (PI), whereas patients with pancreatic sufficiency (PS) have at least one mild mutation (class IV or V) (Kristidis *et al*., 1992; Rowntree & Harris, 2003). About 90% of the CF patients showed pancreatic insufficiency (The Cystic Fibrosis Genotype-Phenotype Consortium, 1993). Moreover, pancreatitis is a rare complication occurring in 1.24% of the CF patients, carrying a wide range of mutations (De Boeck *et al*., 2005).

The main objective of this study was to test for possible relative contribution of the pancreatitis associated genes (cationic trypsinogen gene – PRSS1 –

* Corresponding author: tel.: +30 2310 998361, fax: +30 2310 998374, e-mail: akouvats@bio.auth.gr

and pancreatic secretory trypsin inhibitor gene –PSTI–) to the CF disease.

MATERIALS AND METHODS

Clinical data

Eighty-three unrelated CF patients were included in this study. They originated from Northern Greece and attended the CF unit of the Pediatric Department of the Hippokration General Hospital of Thessaloniki, Greece. The diagnosis was based on clinical manifestation, pulmonary and gastrointestinal status, and on at least two abnormal values of the sweat chloride test. The patients were classified as pancreatic sufficient (PS) and pancreatic insufficient (PI) by biochemical tests in serum and by cationic trypsinogen levels.

Genetic analysis

Genomic DNA was extracted from whole blood samples of the patients by the phenol-chloroform protocol, and was screened for 11 common CFTR mutations reported previously (Kalogeridis *et al*., 2001).

In the present study, the DNA was analyzed for 4 common mutations of the PRSS1 gene (R122H, N29I, A16V and K23R) and 3 common mutations of the PSTI gene (N34S, P55S and -215G > A). In particular, exons 2 and 3 of the PRSS1 gene, and exon 3 and promoter region of the PSTI gene were amplified by PCR. Primers for the PRSS1 gene were designed in the lab, while primers for the PSTI gene were those from the literature (Keneko *et al*., 2001)

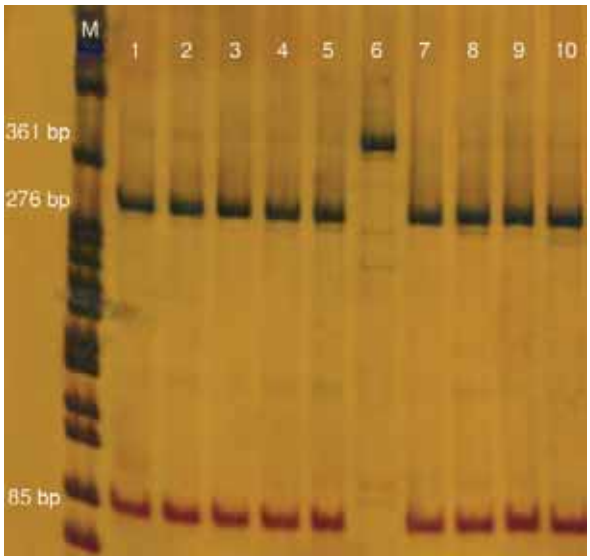

FIG. 1. Detection of the A16V mutation (PRSS1 gene) on 8% polyacrylamide gel. Sample 6 is homozygous for the mutation, all the others are normal. M: marker pBR322.

(Table 1). PCRs were carried out in 50 μl reaction mixtures and the conditions used for 30 cycles were denaturation at 94˚C for 35 sec, annealing at 58-65˚C (depending on primer pair) for 35 sec and extension at 72˚C for 60 sec. For the detection of mutations, the PCR products of exons 2 and 3 of the PRSS1 gene and the promoter region of the PSTI gene were digested with specific restriction enzymes (Table 1). The digested products were analyzed on 8% polyacrylamide gels. Sequencing analysis was performed for the PCR products of exon 3 of the PSTI gene by the Sanger method.

TABLE 1. PCR primers and detection methods used

Mutation	Gene	Primers	Method
R122H	PRSS1	5′ tggctgtgggagaaggtctt 3′	RFLPs
	exon 3	3′ tggtgcccagtgcagagtct 5′	(*Afl*III)
N29I	PRSS1	5′ tagcagaaagcaatcacagg 3′	RFLPs
	exon 2	3′ gttttcttctccaccacctc 5′	(*Hpy*CH4III)
A16V	PRSS1	5′ tagcagaaagcaatcacagg 3′	RFLPs
	exon 2	3′ gttttcttctccaccacctc 5′	(*Fnu*4HI)
K23R	PRSS1	5′ tagcagaaagcaatcacagg 3′	RFLPs
	exon 2	3′ gttttcttctccaccacctc 5′	(*Alw*I)
N34S	PSTI	5′ ccaatcacagttattccccagag 3′	Sequencing
	exon 3	3′ gtttgcttttctcggggtgag 5′	
P55S	PSTI	5′ ccaatcacagttattccccagag 3′	Sequencing
	exon 3	3′ gtttgcttttctcggggtgag 5′	
-215G>A	PSTI	5′ tttgagttcatcttacaggtgag 3′	RFLPs
	promotor	3′ tatggcagatggcagcaagg 5′	(*Bgl*I)

RESULTS AND DISCUSSION

From the 83 CF patients, only one was found to be homozygous for the A16V mutation (Fig. 1). This is a C to T transition in exon 2 of the PRSS1 gene, leading to a change of alanine to valine at codon 16. It is a substitution at the signal peptide cleavage site of the cationic trypsinogen.

Cystic fibrosis is a disorder that results in a complex spectrum of phenotypes. The amount of CFTR protein required for each organ involved in the disease to remain phenotypically "normal" varies, and similarly, the extent to which each organ contributes to the CF phenotype varies substantially. This heterogeneity suggests that, except for the CFTR genotype, there are more factors involved in the determination of the phenotype, e.g. other genetic determinants. A CF modifier gene for meconium ileus was detected in 1999 on chromosome 19 (Zielenski *et al*., 1999), and there is an ongoing effort to determine other modifier genes. The aim of this study was to test for the possible modifying role of the pancreatitis associated genes (cationic trypsinogen gene –PRSS1– and pancreatic secretory trypsin inhibitor gene –PSTI–) to the CF disease.

Our single patient, who was found homozygous for the A16V mutation (Truninger *et al*., 2001) of the PRSS1 gene, was also homozygous for the ΔF508 mutation in the CFTR gene (Kalogeridis *et al*., 2001). He was a 20-year-old male, who belonged to the pancreatic insufficiency group, with positive sweat chloride test (90 mmol/l). According to the "Huang score method" (Matouk *et al*., 1997) and other tests, his lung function was extremely normal for a CF patient. His PI status was in accordance with the fact that he was homozygous for a severe CFTR mutant allele (Kristidis *et al*., 1992). Moreover, it is known that among patients with this genotype, a wide variability of pulmonary function has been observed (Kerem *et al*., 1990). Comparing the clinical data of all our 20 patients that were homozygous for the ΔF508, the certain patient showed more severe pancreatic defects.

What is the role of the A16V mutation identified in a single CF patient? There could be two possibilities: a) The A16V mutation represents a mere coincidence, providing that it does not have any phenotypic-modifying effect on the CF disease. Thus, the clinical phenotype of the CF patient is the result of his homozygous status for the common ΔF508 CF mutation; b) The PRSS1 gene may act as a modifier gene in the pathogenesis of the CF disease. Even in CF patients with the most severe PI genotype (ΔF508/ΔF508), the pancreatic status may be the result, not only of a specific CFTR genotype, but also of the co-existence of mutations in the CFTR gene and the PRSS1 gene related to pancreatitis.

Although it seems more likely that the PRSS1 gene is not involved as a modifier in cystic fibrosis, co-examination of CFTR and PRSS1 gene mutations in a large number of CF patients could shed light on possible gene interactions in the CF disease. Identification of modifier genes will permit better understanding of the clinical heterogeneity of CF and, thereby provide insights into prognosis and management.

REFERENCES

De Boeck K, Weren M, Proesmans M, Kerem E, 2005. Pancreatitis among patients with cystic fibrosis: Correlation with pancreatic status and genotype. *Pediatrics*, 115: 463-469.

Kalogeridis A, Kouvatsi A, Tsanakas I, 2001. CFTR mutations in Northern Greece. High frequency of mutation 621+1 G>T. *Balkan journal of medical genetics*, 4: 11-14.

Keneko K, Nagasaki Y, Furukawa T, Mizutamari H, Sato A, Masamune A, Shimosegawa T, Horii A, 2001. Analysis of the human pancreatic secretory trypsin inhibitor (PSTI) gene mutations in Japanese patients with chronic pancreatitis. *Journal of human genetics*, 46: 293-297.

Kerem E, Corey M, Kerem BS, Rommens J, Markiewicz D, Levison H, Tsui LC, Durie P, 1990. The relation between genotype and phenotype in cystic fibrosis - analysis of the most common mutation (deltaF508). *New England journal of medicine*, 323: 1517-1522.

Kiesewetter S, Macek MJr, Davis C, Curristin S, Chu C-S, Graham C, Shrimpton A, Cashman S, Tsui L-C, Mickle J, Amos J, Highsmith W, Shuber A, Witt D, Crystal R, Cutting G, 1993. A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nature genetics*, 5: 274-278.

Kristidis P, Bozon D, Corey M, Markiewicz D, Rommens J, Tsui LC, Durie P, 1992. Genetic determination of exocrine pancreatic function in cystic fibrosis. *American journal of human genetics*, 50: 1178-1184.

Matouk E, Ghezzo RH, Gruber J, Hidvegi R, Gray-Donald K, 1997. Internal consistency reliability and predictive validity of a modified N. Huang clinical scoring system in adult cystic fibrosis patients. *European respiratory journal*, 10: 2004-2013.

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel L, Grzelchak Z, 1989. Identification of the cystic fi-

brosis gene: cloning and characterization of complementary DNA. *Science*, 245: 1066-1073.

Rowntree RK, Harris A, 2003. The phenotypic consequences of CFTR mutations. *Annals of human genetics*, 67: 471-485.

Rozmahel R, Wilschanki M, Matin A, Plyte S, Oliver M, Auerbach W, Moore A, Forstner J, Dune P, Nadeau J, Bear Ch, Tsui L-C, 1996. Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. *Nature genetics*, 12: 280-287.

The Cystic Fibrosis Genotype-Phenotype Consortium, 1993. Correlation between genotype and phenotype in patients with cystic fibrosis. *New England journal of medicine*, 329: 653-658.

Truninger K, Ammann RW, Blum HE, Witt H, 2001. Genetic aspects of chronic pancreatitis: insights into aetiopathogenesis and clinical implications. *Swiss medicine weekly*, 131: 565-574.

Zielenski J, Corey M, Rozmahel R, Markiewicz D, Aznarez I, Casals T, Larriba S, Mercier B, Cutting G, Krebsova A, Macek MJr, Langfelder-Sahwind E, Marshall B, Germana J, Clausters M, Palacio A, Bal J, Nowakawska A, Ferec C, Estivill X, Durie P, Tsui LC, 1999. Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. *Nature genetics*, 22: 128-129.