Polymorphism of Cytokine Genes (*TGF-β1*, *IFN-γ*, *IL-6*, *IL-10*, and *TNF-α*) in Patients With Chronic Pancreatitis

Maria Teresita Bendicho, MS, Jorge Carvalho Guedes, MD, PhD,† Naize Nara Silva, MS,* Genoile Oliveira Santana, MS, MD,† Raquel Rocha dos Santos, MS,† André Castro Lyra, MD, PhD,† Luiz Guilherme Lyra, MD, PhD,† Roberto Meyer, MD, PhD,‡ and Denise Carneiro Lemaire, PhD‡§*

Objectives: The polymorphisms in cytokine genes have allowed for the understanding of the genetic determinants of diseases. The aims of this study were to describe and compare the frequencies of polymorphisms on the *interleukin* (*IL*)*-6*, *IL-10*, *tumor necrosis factor* (*TNF*)*-α*, *transforming growth factor* (*TGF*)*-β1*, and *interferon* (*IFN*)*-γ* genes between patients with chronic pancreatitis (CP) and healthy individuals from Bahia, Brazil.

Methods: Twenty-eight individuals were evaluated at a university gastroenterology outpatient service (4 women and 24 men), all diagnosed with CP based on clinical and radiologic aspects. The control group was composed of 94 (11 women and 83 men) blood donors. The polymorphisms studied were *TNF-α* (−308G/A), *TGF-β1* (codon 10C/T, codon 25C/G), *IL-10* (−1082A/G; −819T/C; −592A/C), *IL-6* (−174G/C), and *IFN-γ* (+874T/A).

Results: A statistically significant difference was observed in the frequency of the polymorphisms between the group of patients with CP and the group of healthy individuals with the polymorphism of the *TGF-β1* gene on codon 10. No statistically significant differences were found for the allele and genotypic frequencies on the genes that code *TNF-α*, *IFN-γ*, *IL-10*, and *TGF-β1* codon 25, and *IL-6* between the control and case groups.

Conclusion: The genotypes corresponding to the high TGF-β1 producer phenotypes can be associated with the fibrogenesis shown with CP.

Key Words: chronic, pancreatitis, cytokines, genetic polymorphism, transforming growth factor-β1

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Chronic pancreatitis (CP) is a disease that affects both endocrine and exocrine functions of the pancreas. The main histopathologic alterations seen in this disease are irregularly distributed fibrosis, decrease in the number and size of acinus and Langerhans islets, and obstruction of pancreatic ducts. The presence of pancreatic acinus groups surrounded by proliferating fibroblasts, collagenous fibers, lymphocytes, and plasmocytes and the fibrotic narrowing of the ductile system with squamous metaplasia of the epithelial lining and minuscule ruptures surrounded by areas of necrotic remains or cystic spaces can also be evidenced. These modifications cause pain and permanent loss of function.[1–4]

The mechanism of disease development is still not clear, but some studies have shown an association with cytokines,[5] whose importance on the inflammation and/or on the formation of fibrosis is well defined for other organs.[6–8]

Variations in the amino acid sequence of cytokines and cytokine receptors were described in healthy individuals, showing polymorphisms associated with changes of the expression of the protein and of interindividual differences on the profile of its production. Such polymorphisms have been associated with human diseases, becoming an area of interest for the possibility of identifying genetic markers of the diseases.[9–11]

This study aimed to describe and compare the frequency of polymorphisms of the *transforming growth factor* (*TGF*)*-β1* (codon 10; codon 25), *interleukin* (*IL*)*-6* (−174G), *IL-10* (−1082A; −819T; 592A), *tumor necrosis factor* (*TNF*)*-α* (−308A), and *interferon* (*IFN*)*-γ* (+874T) genes between patients with CP and healthy individuals in Bahia, Brazil.

MATERIALS AND METHODS

Sample

The study group was composed of 28 patients with CP, diagnosed from March 2002 to August 2004 in the Pancreas ambulatory of the University Hospital Professor Edgard Santos from the Federal University of Bahia (UFBA), in Salvador, Brazil. The service offers appointments to low-income population of the public health sector, and it is a regional reference center for this disease.

The patients had a mean age of 47 ± 10 years (4 women and 24 men). The mean time from diagnosis of the disease was 35 ± 34 months and varied between 1 and 120 months. The disease etiology was characterized as alcoholism in 25 patients, as nutritional deficiency in 2 patients, and as obstructive in 1 patient (Table 1). The patients had family relationship among themselves.

The patients with CP were defined through clinical criteria (abdominal pain, diabetes, and/or steatorrhea),

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From the *Laboratório de Imunogenética e Transplante de Órgãos, Hospital Universitário Prof. Edgar Santos/Instituto de Ciências da saúde, Universidade Federal da Bahia, Brazil; †Serviço de Gastroenterologia Hospital Universitário Prof. Edgar Santos, Fac de Medicina da Universidade Federal da Bahia, Brazil; ‡Instituto de Ciências da Saúde; Universidade Federal da Bahia, Brazil; and §Departamento de Ciências da Vida, Universidade do Estado da Bahia, Brazil.

Reprints: M. Teresita Bendicho, Lot. Aquarius, 523, ap. 801, Salvador, Bahia, Brazil CEP-41 (e-mail: mtb@ufba.br).

TABLE 1. Demographic and Clinical Data From Patients With CP

Patient	Age (yr)	Sex	Probable Etiology	Predicted Phenotypes				
				TGF-β1	IL-10	IL-6	INF-γ	TNF-α
1	46	M	Alcoholism	H	L	H	I	L
2	52	M	Alcoholism	H	L	H	I	L
3	55	F	Alcoholism	L	L	H	L	H
4	44	M	Alcoholism	H	I	H	I	L
5	26	F	Nutritional deficiency	H	L	H	I	L
6	36	M	Obstructive	I	L	H	I	L
7	41	M	Alcoholism	H	I	H	I	L
8	50	M	Alcoholism	H	I	H	I	L
9	44	M	Alcoholism	H	I	H	L	L
10	53	F	Alcoholism	H	I	H	I	H
11	41	M	Alcoholism	H	L	H	L	H
12	62	M	Alcoholism	H	I	H	L	L
13	50	M	Alcoholism	H	I	H	I	L
14	65	M	Alcoholism	L	L	H	I	L
15	42	M	Alcoholism	H	I	H	L	L
16	50	M	Alcoholism	H	I	H	L	L
17	33	M	Alcoholism	H	L	H	I	L
18	67	M	Alcoholism	H	I	H	L	L
19	53	M	Alcoholism	H	H	H	H	H
20	39	M	Alcoholism	I	L	H	I	L
21	38	M	Alcoholism	H	L	H	L	L
22		M	Alcoholism	H	L	H	L	L
23		M	Alcoholism	H	L	H	L	L
24	51	M	Alcoholism	H	L	H	I	L
25		F	Alcoholism	I	L	H	H	L
26		M	Alcoholism	I	L	H	H	L
27	48	M	Alcoholism	H	L	H	H	L
28		M	Alcoholism	H	L	H	H	H
29	48	M	Nutritional deficiency	H	I	H	I	H

H, high; I, intermediate; L, low.

associated to radiologic criteria (Wirsung dilation, amputation of secondary branches of the pancreas secretory ducts, and pancreatic calcifications on ultrasound, computerized tomography, and/or endoscopic colangio-pancreatography).

The etiologic classification was based on clinical criteria, familial history, and nutritional evaluation, defining alcoholism when ethanol consumption was more than 80 g/d.[12]

The control group was composed by 94 voluntary blood donors from a Hemotherapy Service from Salvador (11 women and 83 men; age, 31 ± 8 years; age range, 18–54 years). The donors did not have family relationship with the group of patients with CP in this study.

Genotyping

Blood was collected from each individual in a tube containing 0.5 mol/L k3EDTA, and the genomic DNA was obtained using the modified "salting-out" method (Dynal, Oslo, Norway). The kit for genotyping, "Cytokine Genotyping Tray," (One Lambda, Canoga Park, CA) was used. After polymerase chain reaction (PCR), an electrophoretic run was realized in agarose gels at 1%. The amplified products were visualized using ultraviolet light and photographed with the help of a transilluminator. The interpretation of the results was based on the presence or absence of a specific amplified DNA fragment, and the results were obtained using genotyping plaque maps from cytokines provided by the PCR kit manufacturer.

Statistical Analysis

The genotypic frequency was obtained by the direct counting of the data obtained through PCR, and the allele frequency was calculated through the division of the number of alleles found by the total number of genes analyzed. The predicted phenotypes were obtained by the addition of the absolute values found on genotypes with similar patterns of cytokine expression.

The comparison between the allelic, genotypic, and predicted phenotypes frequencies with the different groups was performed with the Fisher exact test, establishing the significance level at $P < 0.05$.

RESULTS

There was no significant difference for the polymorphisms of *IFN-γ, TNF-α, IL-6*, and *IL-10* between the 2 groups evaluated; however, there was for *TGF-β1*. The data are shown on Table 2. A statistically significant difference between patients with CP and controls was observed for the polymorphism on *TGF-β1* on codon 10, both in the alleles [$P = 0.0245$; relative risk (RR) = 1.755; IC = 1.088–2.830] and in the genotypes ($P = 0.0470$; RR = 2.676; IC = 0.9816–7.298). The group of patients with CP showed frequencies of 0.7931, 0.1379, and 0.0690 for high, intermediate, and low producers of TGF-β1, respectively, whereas in the group of healthy individuals, the frequencies were 0.5213, 0.3830, and 0.0346. The analysis of the inferred phenotypes in the patients with CP compared with the healthy individuals showed a significant difference between the high and intermediate TGF-β1 producer phenotype ($P = 0.0108$; RR = 3.194; IC = 1.188–8.590), as well as the high producer phenotype compared with the lower intermediate TGF-β1 producer.

The differences observed between the groups of patients with CP and the controls were not statistically significant compared with genotypes and predicted phenotypes for the polymorphism on the *IFN-γ* ($P = 0.4983$; RR = 1.505; IC = 0.6506–3.482), where the frequencies were 0.1724, 0.4828, and 0.3428 in the group of patients with CP and 0.0957, 0.4787, and 0.4256 in the healthy group for the predicted phenotypes of high, intermediate, and low production of this cytokine, respectively. The same occurs with the genotypes ($P = 0.5900$; RR = 1.497; IC = 0.5696–3.936) and predicted phenotypes ($P = 1.0000$; RR = 0.9632; IC = 0.4383–2.117) in the polymorphism on the *TNF-α*, with frequencies of 0.2069 and 0.7931 in the group of patients with CP and 0.2234 and 0.7760 in the healthy group of individuals for the predicted phenotypes of high, intermediate, and low production of TNF-α, respectively, as well as for the genotypes ($P = 0.1564$; RR = 1.940; IC = 0.8022–4.691) and predicted phenotypes ($P = 0.5727$) on

TABLE 2. Genotypic Frequency of the Polymorphism of *IFN-γ*, *TNF-α*, *IL-6*, *IL-10*, and *TGF-β1* in Patients With CP and Healthy Individuals

Genotypic Polymorphism (phenotype*)	CP	Healthy Individuals	*P*
INF-γ + 874A/T	n = 29	n = 94	
T/T (high)	(05) 0.1724	(09) 0.0957	0.4983
T/A (intermediate)	(14) 0.4828	(45) 0.4787	
A/A (low)	(10) 0.3448	(40) 0.4256	
TNF-α−308G/A	n = 29	n = 94	
G/A; A/A (high)	(06) 0.2069	(20) 0.2234	0.0000
G/G (low)	(23) 0.7931	(73) 0.776	
IL-6−174G/C	n = 28	n = 94	
G/G;G/C (high)	(28) 1.000	(90) 0.9574	0.5727
C/C (low)	(0) 0.000	(04) 0.0426	
IL-10−1082G/A:−819C/T, and −592C/A	n = 29	n = 94	
GCC/GCC (high)	(01) 0.0345	(13) 0.1383	0.4351
GCC/ACC; GCC/ATA (intermediate)	(10) 0.3448	(43) 0.4575	
ACC/ACC; ACC/ATA;ATA/ATA (low)	(18) 0.6207	(38) 0.4042	
TGF-β1 codon 10 and codon 25	n = 29	n = 94	
T/T G/G; T/C G/G (high)	(23) 0.7931	(49) 0.5213	0.0108
T/C G/C; C/C G/C; T/T G/C (intermediate)	(04) 0.1379	(36) 0.3830	
C/C G/C; C/C C/C; T/T C/C; T/C C/C (low)	(02) 0.0690	(09) 0.0346	

*Phenotype of cytokine production according to Hoffman et al,[13] Pravica et al,[14] Turner et al,[15] and Wilson et al.[16]

the polymorphism on *IL-6*, with frequencies from 1 and 0 in the group of patients with CP and 0.9574 and 0.0426 in the group of healthy individuals for the predicted phenotypes of high, intermediate, and low IL-6 producers, respectively. The absence of a low IL-6 producer predicted phenotype on patients with CP was observed.

The possible combinations of the polymorphisms of *IL-10* on the positions −1082, −819, and −592 form 6 possible genotypes: GCC/GCC, GCC/ACC, GCC/ATA, ACC/ACC, ACC/ATA, and ATA/ATA. For statistical analysis, the genotypes were grouped according to the predicted phenotypes. The analysis showed that there are no significant differences between the predicted phenotypes of high, intermediate, and low production in the compared groups ($P = 0.4351$; RR = 0.3786; IC = 0.0528–2.714).

The correlation of the genotypes with the probable phenotype producers of each cytokine was not done in this study because this has already been described in the literature.[13–16]

DISCUSSION

This study shows the association between genetic predisposition to the high production of TGF-β1 and the risk of developing CP in Brazilian mixed-raced people. This risk was characterized by a higher frequency of the allele *+869T* (codon 10) and of the high producers of TGF-β1 predicted phenotypes in individuals with CP compared with controls. This association was also suggested by Scheneider et al,[17] who compared the genotypic frequency of the polymorphisms on positions −509, +869, and +915 between individuals with alcoholic CP and healthy controls. These authors did not find a statistically significant difference between these groups; however, they observed a tendency of individuals with alcoholic CP of being homozygous for the allele *T* on position +869.[17]

This data, when compared with studies that show the increase of TGF-β1 expression in the tissue from the pancreas of individuals or animals with CP, allow for the hypothesis that the polymorphism on the gene for this cytokine can be a determinant factor of its production on CP and that the polymorphism on the *TGF-β1* gene can be correlated to the physiopathology of the events that result in the fibrogenesis of CP. TGF-β1, because of its properties in the regulation of production, degradation, and accumulation of proteins of the extracellular matrix, is described as having an important role in the formation of fibrosis.[18] Studies done in patients with CP and normal controls showed an increased expression of TGF-β1 in pancreatic tissue of ill individuals in relation to tissue from the normal pancreas.[19,20] In the same way, a higher expression of this cytokine was obtained in pancreatic tissue of transgenic hamsters after CP induction.[21]

Our study, similar to the studies of Scheneider et al[17] and Sargen et al,[22] did not find any association between the polymorphism of the *IL-10* gene and CP, even though this is a potent anti-inflammatory cytokine. Experimental studies, however, proved the participation of IL-10 on the limitation of fibrogenesis, acinar necrosis, parenchyma infiltration by polymorphonucleus cells and macrophages, and of the freeing of proinflammatory mediators and chemokines.[23,24] The analysis of the predicted phenotypes of IL-10 in this work has provided evidence that the 4 patients with CP who did not possess the TGF-β1 high producer phenotype had low IL-10 producer phenotypes. The high frequency found in genotypes of TGF-β1 production and the high number of haplotypes involved in the IL-10 production hindered the stratification and

separate analysis of the polymorphisms for both cytokines. These data suggest that the polymorphisms of TGF-β1 can act as a confounder variable on the analysis of the association between the polymorphisms of IL-10 and CP.

Mews et al[25] showed that the quiescent stellate pancreatic cells, observed on normal pancreas, could be activated by cytokines such as TNF-α, IL-1, IL-6, and IL-10 and suggested that the persistent activation of these cells could be a factor involved in the progression of CP. Vaccaro et al[26] verified that the TNF-α expression on acinar pancreas cells is not typical but occurs at the beginning of pancreatitis. They also suggested that these cells are a source of TNF-α, which is, in part, the response of acinar cells to aggression during the course of pancreatitis. Zhang et al[27] did not find any correlation between the polymorphism on position −308 of the *TNF-α* gene and acute pancreatitis. In this study and that from Scheneider et al,[17] no association between these polymorphisms and CP was observed. Mitsue et al[28] verified that pancreatic periacinar myofibroblasts produced high quantities of IL-6 in response to proinflammatory cytokines and concluded that they could have an important role in the pathogenesis of acute pancreatitis. In this study, an association between the polymorphism for *IL-6* and CP was not observed.

IFN-γ increases the synthesis of collagen and its deposition on the extra cellular matrix, besides facilitating the transcription of other genes from the matrix, such as fibronectin.[29,30] The role of this cytokine in fibrogenesis suggests an association between the high producers of IFN-γ phenotypes/genotypes and CP. However, the data from this study and from Scheneider et al[17] do not corroborate this hypothesis.

In summary, this study showed the association between genetic predisposition to the high production of TGF-β1 and CP. It was not possible to show an association between the polymorphisms of the *IFN-γ* (+874T/A), *TNF-α* (−308), and *IL-6* (−174G/C) genes and this disease. These results emphasize the relevant role of the TGF-β1 in the pathogenesis of CP.

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