

Relevance of transforming growth factor- β 1, interleukin-8, and tumor necrosis factor- α polymorphisms in patients with chronic pancreatitis

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ABSTRACT. Cytokine regulation may be an important factor in the susceptibility for the development of chronic pancreatitis; transforming growth factor- β 1 (TGF- β 1) plays a central role in the pathogenesis of pancreatic fibrogenesis. The aim of our study was to analyse the relevance of TGF- β 1, interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) polymorphisms in patients with chronic pancreatitis. **Patients:** of the 83 patients enrolled in the study, 43 were treated medically and 40 patients underwent surgical intervention. Healthy blood donors ($n = 75$) served as controls. **Methods:** the polymorphisms of TGF- β 1 +869 T $\rightarrow$ C and IL-8 -251 T $\rightarrow$ A were determined by the ARMS method, while that of TNF- α -308 was investigated using NcoI RFLP. **Results:** there was a higher frequency (50%) of the TT genotype of TGF- β 1 +869, with a concomitantly higher TGF- β 1 level in the plasma (5.2 ± 1.7 ng/mL) of patients with chronic pancreatitis than in healthy blood donors (28% and 2.8 ± 0.9 ng/mL respectively). The number of TT homozygotes differed significantly between the patients who underwent surgical intervention and the controls, and even between the surgical and the non-surgical patients. The frequency of the T/A genotype with higher IL-8 production, was significantly higher in both groups of patients than in the controls (58% and 58% versus 40%). No correlation was found between the TNF- α -308 polymorphism and chronic pancreatitis. **Conclusions:** correlations of the TGF- β 1 and IL-8 single nucleotide polymorphisms (SNPs) with chronic pancreatitis underline the importance of these cytokines in the pathomechanism of the disease. Moreover, it seems that the TT genotype of +869 TGF- β 1 might be a risk factor for the development of a severe form of chronic pancreatitis, and could serve as a prognostic sign for any future surgical intervention or even repeat surgery. Further studies on a larger group of patients, in addition to a follow-up study, are necessary to confirm this preliminary observation.

Keywords: chronic pancreatitis, TGF- β , IL-8, TNF- α polymorphism

Chronic pancreatitis (CP) is characterized by dysplastic ducts, foci of proliferating ductal cells, acinar cell degeneration, and fibrosis. There are a number of underlying conditions that can contribute to an increased incidence of CP. They include, but are not limited to, an excessive ethanol intake, pancreatic stone formation, and recurrent episodes of acute pancreatitis [1].

In the course of chronic pancreatitis, the pancreatic acinar and ductal cells undergo continuous destruction and are replaced by fibrous tissue. An overproduction of extracellular matrix molecules may further compromise the remaining parenchyma leading to an ongoing process of tissue destruction, which finally results in an exocrine, and sometimes endocrine insufficiency. Recent studies have furnished evidence of an overproduction of various growth factors, or members of the transforming growth factor- β family [2-4]. The overproduction of TGF- β in chronic pancreatitis in particular, appears to be an important factor

in the production of extracellular matrix molecules, inducing the connective tissue growth factor (CTGF) and subsequently leading to the production of collagen, fibronectin and proteoglycan.

The cytokine TGF- β 1, which plays a central role in the pathogenesis of pancreatic fibrogenesis, not only exerts immunomodulatory functions but is also a potent fibrogenic mediator, promoting the proliferation of fibroblasts and the production of connective tissue. TGF- β is likely to be involved in the fibrogenetic processes occurring in chronic pancreatitis, thereby contributing to the loss of functional, exocrine pancreatic tissue [5]. An upregulated expression of TGF- β in mononuclear and ductal epithelial cells has been observed in tissue sections from patients with chronic pancreatitis [6].

The inflammatory processes that characterize chronic pancreatitis are regulated by a variety of other cytokines, including tumor necrosis factor- α (TNF- α), and also

by chemokines [7]. Thus, chemokines of the C-X-C family, such as interleukin-8 (IL-8), and the C-C-chemokine family, such as MCP-1, can be identified in chronic pancreatitis [8, 9].

The regulation of cytokines may be important with regard to susceptibility to the development of chronic pancreatitis. Recent work has demonstrated a high degree of polymorphism in the cytokine genes involved in inflammation and immunity [10].

The production of TGF- β 1 varies from individual to individual, and depends, in part, on the polymorphisms of this gene. The human gene encoding TGF- β 1 is located on chromosome 19q13. A number of polymorphisms have been described in the TGF- β 1 gene, including a T-to-C transition at nucleotide 29 at position +869, in the region encoding the signal sequence, which results in a leucine-proline substitution at the 10th amino acid. It has been demonstrated that TT homozygous genotypes are high TGF- β 1 producers [11-13].

It has been reported that a T-A mutation in the -251 promoter region is accompanied by increased IL-8 production [14]. The G-to-A transition at position -308 in the promoter region is associated with an elevated expression of TNF- α [15, 16].

There are conflicting results concerning the association between TNF- α and TGF- β 1 polymorphisms and chronic pancreatitis [17-19].

We have therefore investigated this in a Central-East European (Hungarian) population, in addition to an investigation of IL-8 polymorphisms in order to determine whether polymorphisms of the TGF- β 1 gene at position +869 (codon 10), the TNF- α gene at position -308, and the IL-8 gene at -251 position are associated with chronic pancreatitis.

PATIENTS AND METHODS

Patients

Our study involved 83 patients (24 females and 59 males; mean age 52.7 years, range 22-70), who underwent medical or surgical treatment for chronic pancreatitis at the Department of Internal Medicine and/or Department of Surgery of the University of Szeged, between 2003 and 2006. The diagnosis of chronic pancreatitis was based on the typical history (daily alcohol intake), abdominal complaints (pain, bloating, steatorrhea, etc.) and characteristic morphological and/or functional alterations of the pancreas. The morphological changes due to chronic inflammation of the pancreas (pancreatic calcification on ultrasonography (US) and/or computed tomography (CT), mild to moderate or marked ductal lesions during endoscopic retrograde cholangio-pancreatography- (ERCP) examination) were assessed in each case. Pancreatic calcifications were found in 31 (37.5%) patients on US or CT. According to the etiology, 65 of the patients (78.6%) had a history of alcohol abuse (consumption of > 50 g/day) and 12 (21.4%) patients has idiopathic CP. Exocrine pancreatic insufficiency was assessed by means of a stool elastase test [20]. Forty seven patients with stool elastase values of less than 200 μ g were considered to have pancreatic insufficiency.

The endocrine function was evaluated in non-diabetic patients by means of the oral glucose tolerance test (OGTT). Fifty six patients (67.9%) had impaired endocrine function (latent or manifest diabetes).

Surgical intervention

The indications for surgery were intractable pain, loss of body weight, and obstruction of the ductal system (pancreatic duct, common bile duct or the duodenum) caused by an inflammatory enlargement of the pancreatic head. These are generally accepted indications for surgery [21-23]. Duodenum-preserving, pancreatic head resection [24] was performed in eight cases, organ-preserving, pancreatic head resection [25] in 15 cases, pylorus-preserving, pancreatic head resection in four cases, and Wirsungojejunostomy in 13 cases. Rehospitalization and repeat surgery was necessary in eight cases.

The control group consisted of 75, age- and gender-matched, healthy blood donors, who had no gastrointestinal or liver diseases, and who were selected locally from consecutive blood donors in Szeged, Hungary. The study protocol was approved by the Ethics and Science Committee of the Ministry of Health and the University of Szeged Regional and Institutional Committee of Science and Research Ethics.

All participating subjects were of Hungarian ethnic origin and resident in Hungary.

DNA extraction

For the study of TNF- α , TGF- β 1 and IL-8 polymorphisms, genomic DNA purified from peripheral blood was used. Leukocyte DNA was isolated using the High Pure PCR Template Preparation Kit according to the manufacturer's instructions (Roche Diagnostic GmbH, Mannheim, Germany) and the genomic DNA was stored at -20°C until further use.

Determination of TGF- β 1 +869 T→C polymorphism

The defined, single-nucleotide polymorphism T²⁹-C in exon 1 of the human TGF- β 1 gene was determined with an amplification refractory mutation system -ARMS- [26], using a generic primer (sense), (5'-TCCGTGGGA-TACTGAGACACC-3'); and with two allele-specific anti-sense primers, differing from each other in only one base at the 3'-end- primer C: 5'-GCAGCGGTAGCAGCAGCG-3' and primer T: 5'-AGCAGCGGTAGCAGCAGCA-3'. The reaction mixture of 50 μ l contained 100 ng of genomic DNA, 20 pmol each of the sense and the anti-sense primer, 1.25 U Taq DNA polymerase, 1.5 mM MgCl₂, 1xPCR Taq polymerase buffer + (NH₄)₂SO₄, and 25 mM of each dNTP (Fermentas, Vilnius, Lithuania). The thermocycling procedure was as follows: initial denaturation at 94 °C for 5 minutes; 35 cycles of 94 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes. The PCR products were analyzed using 1.5% agarose (Sigma-Aldrich, St. Luis, MO, USA) gel electrophoresis, visualised under UV illumination and stained with 0.4 mg/L ethidium bromide. The expected size of the specific amplification product was 241 bp. Samples from two known homozygous individuals and one heterozygous individual,

confirmed by sequencing, were included in each reaction. Sequencing was performed with an automated sequencer (ABI Prism; Applied Biosystems, CA, USA).

Determination of IL-8 -251 polymorphisms

A single nucleotide T→A polymorphism at -251 nt, relative to the transcription start site, accompanied by increased IL-8 production was typed by amplification refractory mutation system (ARMS) [14]. Allele specific primers were: 5' CCACAATTGGTGAATTATCAAT 3' (-251A) and 5' CACAATTTGGTGAATTATCAAA 3' (-251T). The consensus primer was: 5' TGCCCCTTCACTCTGT-TAAC 3', giving a PCR product of 336bp. In each reaction, a second set of primers for exon 3 of the HLA-DRB1 gene (forward: 5' TGCCAAGTGGAGCACCCAA 3', reverse: 5' GCATCTTGCTCTGTGCAGAT 3', product size (796 bp) was used as a control for PCR efficiency. Reactions were carried out using Advantage-GC cDNA polymerase mix and buffer (Clontech, Palo Alto, CA, USA), under the following conditions: 96 °C for 120 s; four cycles of 96 °C for 35 s, 68 °C for 45s, 72 °C for 35s; four cycles of 96 °C for 35 s, 65 °C for 45s, 72 °C for 45s; four cycles of 96 °C for 35 s, 62 °C for 45s, 72 °C for 55s; ten cycles of 96 °C for 35 s, 58 °C for 45s, 72 °C for 65s; ten cycles of 96 °C for 35 s, 55 °C for 45s, 72 °C for 75s; four cycles of 96 °C for 35 s, 52 °C for 45s, 72 °C for 85s; four cycles of 96 °C for 35 s, 50 °C for 45s, 72 °C for 90s; 72°C for 5 min.

Determination of TNF- α -308 G→A polymorphism

This SNP of TNF- α at position -308 in the promoter region was analyzed by PCR-RFLP (restriction fragment length polymorphism) [15]. A single base change at the 3' end of primer A1 was required for the formation of an NcoI (Fermentas, Vilnius, Lithuania) recognition sequence CCATGG (instead of GCATG originally found on the gene investigated) (primer A1:5'-AGGCAATAGGTTTTG-AGGGCCAT-3' and primer A2:5'-TCCTCCCTGCTC-CGAT TCCG-3'). The reaction mixture of 100 μ L contained 100 ng of genomic DNA, 20 pmol each of the A1 and the A2 primer, 2.5 U Taq DNA polymerase, 1.5 mM MgCl₂, 1xPCR Taq polymerase buffer + (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania) and 25 mM of each dNTP (Fermentas, Vilnius, Lithuania). The PCR conditions were as follows: initial denaturation at 94 °C for 3 minutes; 36 cycles of 94 °C for 1 minute, 60 °C for 1 minute and 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes.

The amplified product was digested with the endonuclease NcoI and analyzed on a 12% polyacrylamide gel under UV illumination. The TNF G allele gave two fragments of 87 bp and 20 bp, while the TNF A allele gave a single, 107 bp fragment.

TGF- β ELISA

Venous blood was collected from healthy blood donors and from patients with chronic pancreatitis into EDTA-containing tubes for collecting plasma. Blood was collected from surgical patients before the operation. Centrifugation was carried out at 2000g for 10 min at 4°C. All samples were stored at -20 °C. Plasma concentrations of TGF- β 1 were determined by enzyme-linked immunosorbent assay kit (R&D System Inc., Minneapolis, USA) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses for comparison of allele and genotype frequencies between groups were performed using the χ^2 test and Fisher's exact test if one cell had $n < 5$. The probability level of $p < 0.05$ indicated statistical significance. The relationship between genotypes and disease severity is presented as the odds ratio (OR), with a 95% confidence interval (95% CI). The genotype frequencies for each polymorphism were tested for deviation from the Hardy-Weinberg equilibrium by means of the χ^2 test, with one degree of freedom used [27]. The levels of TGF- β 1 in the plasma were compared by means of one-way ANOVA. The Bonferroni test was used for *post hoc* pairwise multiple comparisons. In all tests, an α level of $p < 0.05$ was taken as an indication of statistical significance. All statistical calculations were performed with the GraphPad Prism4 (GraphPad Software Inc., San Diego, CA, USA) statistical program.

RESULTS

TGF- β 1 +869 T→C polymorphism

The genotypic distributions of the +869 T→C polymorphism of the TGF- β 1 gene are shown in *table 1*. The distribution of the TGF- β 1 genotypes was in accordance with the Hardy-Weinberg equilibrium in the control population ($\chi^2 = 2.95$; $p = 0.2676$), but not in the patient group with chronic pancreatitis ($\chi^2 = 5.215$, $p = 0.022$).

Table 1
TGF- β 1 +869 genotype distribution in patients with chronic pancreatitis and in control subjects

	TT	TC	CC	
Operated	25/40 (62%) $p = 0.0007$ versus control ^b	10/40 (25%)	5/40 (13%)	$p = 0.001^a$
Non operated	17/43 (39.5%) $p = 0.223$ versus control ns ^b $p = 0.0486$ versus operated ^b	17/43 (39.5%)	9/43 (20%)	
Total	42/83 (50%) $p = 0.005$ versus control ^b	27/83 (33%)	14/83 (17%)	$p = 0.009^a$
Control	21/75 (28%)	30/75 (40%)	24/75 (32.%)	

ns = non-significant.

^a chi-square test versus control.

^b Fisher test.

There was a significant difference in genotypic distribution between the chronic pancreatic patients overall and the healthy controls ($p = 0.009$, $\chi^2 = 9.409$). When the patients were stratified according progression of the disease - *i.e.* medical treatment or surgical treatment – a significant difference was observed only between the controls and the surgical patients ($p = 0.0012$), and not between the controls and the patients receiving only medical treatment. To elucidate the reason for this difference, we compared the numbers of TT homozygotes among the patients and the healthy controls. The frequency of TT homozygotes (high TGF- β 1-producing phenotype) was significantly higher in the patient group overall (50%) than in the controls (28%) ($p = 0.005$; OR = 2.634; 95% CI = 1.358-5.111). There was an even higher frequency of the TT genotype among patients undergoing surgical intervention as compared with the controls, 62% versus 28%, $p = 0.0007$, OR = 4.018, 95% CI = 1.796 - 8.987. There was also a significant difference between the surgical patients, and those treated medically ($p = 0.0486$, OR = 2.549, 95%CI = 1.052-6.178). Although the frequency of the TT genotype was still higher among the patients in the medically treated (medical) group (39.5%) than in the controls, the difference was not statistically significant. No further significant differences were observed as regards the SNPs when the patients were stratified according to the presence or absence of calcification.

TGF- β 1 plasma levels

Plasma levels of TGF- β 1 were higher in the patients overall than in controls (3.98 ± 1.26 ng/mL versus 2.1 ± 0.85 ng/mL), and higher in the patients with the TT genotype than in those with the CT and the CC genotypes

(5.2 ± 1.7 ng/mL versus 3.8 ± 1.1 ng/mL and versus 1.5 ± 0.5 ng/mL respectively; $p < 0.001$ ANOVA). A similar tendency was observed in the control group; the subjects with the TT genotype demonstrated the highest plasma TGF- β 1 levels (2.8 ± 0.9 ng/mL) (figure 1). However, the plasma TGF- β 1 concentrations differed significantly between the patients and the controls, both in the TT homozygote groups and in the TC heterozygote groups. ($p < 0.001$ statistically significant according the Bonferroni post-test.) No significant difference was observed between the “low-level” TGF- β 1 concentrations when the patients and controls were CC homozygotes.

IL-8 -251 polymorphism

The genotypic distribution of the -251 polymorphism of the IL-8 gene is shown in table 2.

The distribution of IL-8 genotypes was in accordance with the Hardy-Weinberg equilibrium both in the control population ($\chi^2 = 2.083$, $p = 0.148$) and in the patient group with chronic pancreatitis ($\chi^2 = 2.413$, $p = 0.120$).

There was a significant difference in genotype distribution between the patients and the healthy controls ($\chi^2 = 11.8298$, $p = 0.0027$). There was a higher frequency of the A/T genotype (high IL-8 producers) among those patients with chronic pancreatitis as compared with the controls: 48 of the 83 patients (58%) versus 30 of the 75 healthy controls (40%), $p = 0.0271$; OR = 2.057, 95% CI = 1.090 – 3.882). In spite of this difference, the statistical power is only 73%. Conversely, the prevalence of the IL-8 TT, wild type genotype was significantly lower in the group of patients (15%) than in the control group (40%) ($p = 0.007$, OR = 3.590, 95% CI = 1.694-7.607).

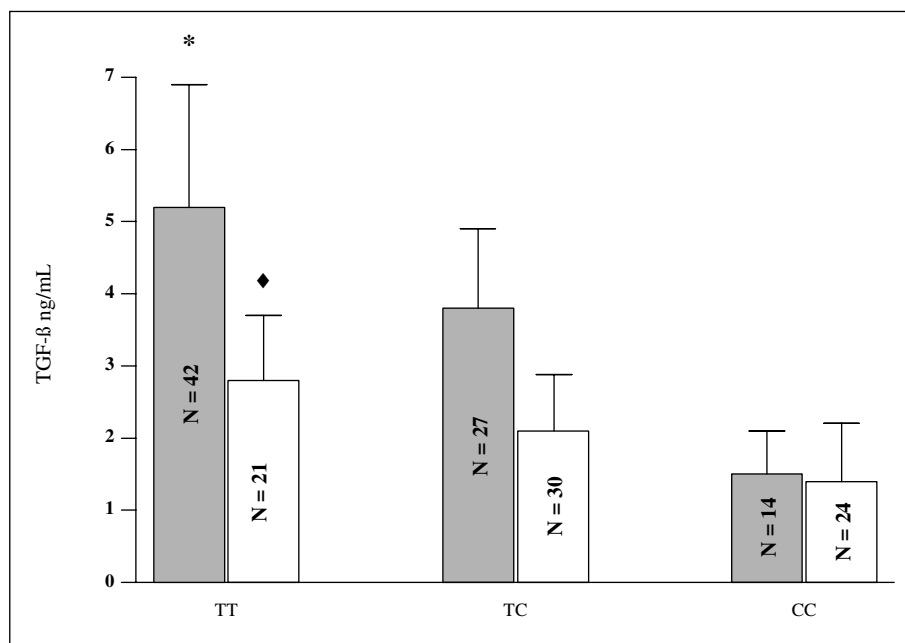


Figure 1

TGF- β 1 plasma levels in patients with chronic pancreatitis (filled bars) and in control subjects (open bars) with different genotypes. Data are means $\pm$ SD.

* $p < 0.001$ ANOVA test on patients

♦ $p < 0.05$ ANOVA test on controls

Bonferroni post-test analysis revealed statistically significant differences between the columns, apart from between the patients and controls with the CC genotype, and between the controls with the TC and the CC genotype.

Table 2
IL-8 -251 genotype distribution in patients with chronic pancreatitis and in control subjects

	TT	TA	AA	
Operated	7/40 (17%)	23/40 (58%)	10/40 (25%)	
Non operated	6/43 (14%)	25/43 (58%)	12/43 (28%)	
		ns versus operated ^b		
Total	13/83 (15%)	48/83 (58%)	22/83 (27%)	p = 0.0027 ^a
	p = 0.007 versus control ^b	p = 0.0271 versus control ^b		
Control	30/75 (40%)	30/75 (40%)	15/75 (20%)	

ns = non significant.

^a chi-square test versus control.

^b Fisher test.

There was no significant difference in the genotypic distribution of IL-8 polymorphism between the two groups of patients.

TNF- α -308 G→A polymorphism

The distribution of the TNF- α -308 genotypes was in accordance with the Hardy-Weinberg equilibrium both in the control population ($\chi^2 = 0.0167$, p = 0.897) and in the patient group ($\chi^2 = 3.483$, p = 0.062).

There were no significant differences in the TNF- α -308 promoter genotypic distribution between the patients with CP and healthy controls (table 3).

DISCUSSION

An association between genetic predisposition to high production of TGF- β 1 and the risk of developing chronic pancreatitis was found in Brazilian, mixed-raced people [17]. A similar association was suggested by Schneider *et al.*, who compared the genotypic frequencies of polymorphism at position +869 in individuals with alcoholic chronic pancreatitis and in healthy controls, but they did not find a statistically significant difference between the groups. However, they did observe a tendency for individuals with alcoholic chronic pancreatitis to be homozygous for the T allele [18].

We investigated the frequency of the TT genotype in patients with chronic pancreatitis, relative to that in healthy controls, and also compared the genotypes between patients treated medically and those undergoing surgery. The latter patients were regarded as a “severe” group, with considerable progression of the disease. The differences in TT genotype frequency proved significant between the surgical group and the controls, and between the surgical and the medical group. The frequency of the TT genotype was relatively high among the medically-treated patients, but only as a tendency and was without statistical significance.

This means that chronic pancreatitis patients, who do not need surgery, rather carry the “protective” C allele, while the TT genotype seems to be a risk factor for surgery. It is noteworthy, that repeat surgery was necessary within three years in eight patients, all of whom were TT homozygotes. The highest TGF- β concentrations (5.2–7.4 ng/mL) were detected in the plasma of these patients. It is noteworthy, that these patients were heavy drinkers.

The plasma levels of TGF- β 1 were significantly increased among the chronic pancreatic patients overall as compared with the group of healthy blood donors (3.98 ± 1.26 ng/mL versus 2.1 ± 0.85 ng/mL). Higher concentrations of TGF- β 1 were detected in the plasma of those subjects with the TT and TC genotypes as compared with those with the CC genotype, both among the patients and among the controls (figure 1). The frequency of high producers (TT) was higher among the patients with chronic pancreatitis than among the controls (table 1), and the TGF- β 1 levels differed in the patient and control groups (figure 1). It is tempting to speculate that in the “high producer” patients, the inflammatory stimuli resulted in elevated levels of TGF- β 1, which further increased the fibrotic processes in the pancreatic tissue.

The results of other studies support the idea of a role of TNF in chronic pancreatitis [7, 28]. In our present study, we could not confirm this result; there was no association found between TNF- α polymorphism and chronic pancreatitis in our studies. One of the possible explanations for the conflicting findings might be the small size of our study. Our results are in line however, with the report of Schneider and colleagues [18, 29] and with Beranek *et al.* [30]. In their study, no association between the TNF- α promoter polymorphism and chronic pancreatitis was found.

We detected a considerable difference in the IL-8 polymorphism between the chronic pancreatitis patients and the controls. Howell *et al.* [31] reported a non-significant decrease in frequency of the IL-8 -251 polymorphism in patients with chronic pancreatitis as compared to the con-

Table 3
TNF- α -308 genotype distribution in patients with chronic pancreatitis and in control subjects

	GG	GA	AA	
Operated	24/40 (60%)	13/40 (32%)	3/40 (8%)	
Non operated	32/43 (74%)	8/43 (19%)	3/43 (7%)	
Total	56/83 (67%)	21/83 (25%)	6/83 (7%)	p = 0.417 ns ^a
Control	52/75 (69%)	21/75 (28%)	2/75 (3%)	

ns = non significant.

^a chi-square test versus control.

trols, which was unexpected because others have described an increased expression of IL-8 in chronic pancreatitis [9]. Our results concerning the association of a higher frequency of the high IL-8-producing genotype (A/T) with CP is in accordance with the finding of these authors. No significant difference in frequencies of the IL-8 polymorphism was observed however, between the two groups of patients. Accordingly, we presume that the high IL-8-producing phenotype may be a predisposing factor for the development of inflammatory processes in chronic pancreatitis, but this SNP might not be connected with the severity of the disease.

We earlier observed a correlation between the IL-8 -251 polymorphism and acute pancreatitis [32]. The relationship between acute and chronic pancreatitis has long been debated. It is currently, generally assumed that the onset of CP is closely linked with recurrent episodes of acute pancreatitis [33]. It is tempting to speculate that a high IL-8-producing genotype - together with other predisposing factors (e.g. alcohol consumption) may be a risk factor for the progression of relapsing alcoholic pancreatitis into irreversible fibrosis observed in CP.

There was no connection between the IL-8 and TGF- β SNPs. However, it is noteworthy, that seven of the eight patients who underwent repeat surgery simultaneously carried the mutant alleles of IL-8 -251, and were TT homozygotes for TGF- β +869. We hypothesize that this observation may possibly be of prognostic value.

In conclusion, it is very likely that both TGF- β 1 and IL-8 polymorphisms contribute to the genetic susceptibility to chronic pancreatitis. The IL-8 -251 polymorphism, with its high IL-8-producing phenotype may be a risk factor for the development of chronic pancreatitis, but the presence of this SNP does not influence the outcome as regards progression, with the necessity for surgery. In contrast, the TGF- β 1 genetic polymorphism with higher TGF- β 1 production appeared relevant among those patients with chronic pancreatitis who underwent surgery, particularly when repeat surgery was necessary.

As chronic pancreatitis is a multifactorial disease, overproduction of cytokines is an important, but not an absolute factor in the pathogenesis of the disease. Moreover, expression of TNF- α and TGF- β 1 is complex, and is probably modified by haplotype, cell type and stimulus, therefore care has to be taken not to over-exaggerate the robustness of the association of SNPs and this disease.

The small number of subjects in our preliminary study is an indicator of the need for caution. Although there were significant differences in the TGF- β TT genotype distributions (table 1) the statistical power of the results was 88% only in the case of comparison the group of patients overall ($n = 83$) with the controls. Following the stratification of patients to surgical ($n = 40$) and medically-treated groups ($n = 43$), the statistical power was 97%, comparing the data for controls with the patients who underwent surgery. However, when the frequency of the TT genotype in the two groups of patients was compared, the power of statistics was only 66%. For a strong (80%) power, the number of patients in each group should be increased to 80, which requires a multicenter approach.

Therefore, our results can be regarded as preliminary results, drawing attention to the possible prognostic value of TGF - β 1 polymorphisms and the associated TGF- β 1

levels in chronic pancreatitis, which should be confirmed in a future, multicenter study on a larger series of patients.

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REFERENCES

1. Kloppel G. Progression from acute to chronic pancreatitis. A pathologist's view. *Surg Clin North Am* 1999; 79: 801.
2. Lee MS, Gu D, Feng L, Curriden, *et al.* Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor β 1. *Am J Pathol* 1995; 147: 42.
3. Van Laethem JL, Deviere J, Resibois A, *et al.* Localization of transforming growth factor-beta 1 and its latent binding protein in human chronic pancreatitis. *Gastroenterology* 1995; 108: 1873.
4. Yamanaka Y, Friess H, Büchker MW, *et al.* Synthesis and expression of transforming growth factor beta-1, beta-2, and beta-3 in the endocrine and exocrine pancreas. *Diabetes* 1993; 42: 746.
5. Van Laethem JL, Robberecht P, Resibois A, *et al.* Transforming growth factor promotes development of fibrosis after repeated courses of acute pancreatitis. *Gastroenterology* 1996; 110: 576.
6. Slater SD, Williamson RC, Foster CS. Expression of transforming growth factor-beta 1 in chronic pancreatitis. *Digestion* 1995; 56: 237.
7. Berberat PO, Friess H, Buchler MW. Chronic pancreatitis – new pathophysiological concepts. *Swiss Surg* 2000; 6: 227.
8. Saurer L, Reber P, Schaffner T, *et al.* Differential expression of chemokines in normal pancreas and in chronic pancreatitis. *Gastroenterology* 2000; 118: 356.
9. Di Sebastiano P, di Mola FF, di Febbo C, *et al.* Expression of interleukin 8 (IL-8) and substance P in human chronic pancreatitis. *Gut* 2000; 47: 423.
10. Bidwell J, Keen L, Gallagher G, *et al.* Cytokine gene polymorphism in human disease; on-line databases, supplement 1. *Genes Immun* 2001; 2: 61.
11. Arkwright PD, Laurine S, Super M, *et al.* TGF-beta(1) genotype and accelerated decline in lung function of patients with cystic fibrosis. *Thorax* 2000; 55: 459.
12. Awad MR, El-Gamel A, Hasleton P, *et al.* Genotype variation in the transforming growth factor-beta gene: association with transforming growth factor-beta 1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation* 1998; 66: 10140.
13. Hinke V, Seck T, Clanget C, *et al.* Association of transforming growth factor-beta 1 (TGF-beta1) T29 $\rightarrow$ C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-beta 1 in a population-based sample of postmenopausal German women. *Calcified Tissue Intern* 2001; 69: 315.
14. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax* 2000; 55: 10237.
15. Wilson AG, Giovine FS, Blakemore AIF, *et al.* Single base polymorphism in the human tumour necrosis factor alpha gene detectable by *NcoI* restriction of PCR product. *Hum Mol Gene* 1992; 1: 353.
16. Mira JP, Cariou A, Grall F, *et al.* Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA* 1999; 282: 579.

17. Bendicho MT, Guedes JC, Silva NN, *et al.* Polymorphism of cytokine genes (TGF- β 1, IFN- γ , IL-6, IL-10, and TNF- α) in patients with chronic pancreatitis. *Pancreas* 2005; 30: 333.
18. Schneider A, Barmada MM, Slivka A, *et al.* Analysis of tumor necrosis factor- α , transforming growth factor- β 1, interleukin-10, and interferon- γ polymorphisms in patients with alcoholic chronic pancreatitis. *Alcohol* 2004; 32: 19.
19. Bohm K, Teich N, Hoffmeister A, *et al.* Transforming growth factor-beta-1 variants are not associated with chronic nonalcoholic pancreatitis. *Pancreatol* 2005; 5: 75-80.
20. Keim V, Teich N, Moessner J. Clinical value of a new fecal elastase test for detection of chronic pancreatitis. *Clin Lab (Zaragoza)* 2003; 49: 209.
21. Beger HG, Büchler M, Bittner R, *et al.* Duodenum-preserving resection of the head of the pancreas in severe chronic pancreatitis. *Ann Surg* 1989; 209: 273.
22. Russel C. Indications for Surgery. In: Beger H, Warshaw A, Büchler M, Carr-Locke DL, Neoptolemos JP, Russel C, Sarr MG, eds. *The Pancreas*. Blackwell-Science, 1998: 815; (vol. 1).
23. Izbicki JR, Bloechle C, Knoefel WT, *et al.* Surgical treatment of chronic pancreatitis and quality of life after operation. *Surg Clin North Am* 1999; 79: 913.
24. Beger HG, Krautzberger W, Bittner R, *et al.* Duodenum-preserving resection of the head of the pancreas in patients with severe chronic pancreatitis. *Surgery* 1985; 97: 467.
25. Farkas G, Leindler L, Daróczy M, *et al.* Prospective randomised comparison of organ-preserving pancreatizing head resection with pylorus-preserving pancreaticoduodenectomy. *Langenbecks Arch Surg* 2006; 391: 338.
26. Perrey C, Pravica V, Sinnott PJ, *et al.* Genotyping for polymorphisms in interferon-gamma, interleukin-10, transforming growth factor-beta 1 and tumour necrosis factor-alpha genes: a technical report. *Transplant Immunol* 1998; 6: 193.
27. Kocsis I, Vászrhelyi B, Györfly A, *et al.* Missed Calculations and New Conclusions: Re-Calculation of Genotype Distribution Data Published in Journal of Investigative Dermatology, 1998–2003. *Neurology* 2004; 63: 357.
28. Hanck C, Rossol S, Hartmann A, *et al.* Cytokine gene expression in peripheral blood mononuclear cells reflects a systemic immune response in alcoholic chronic pancreatitis. *Int J Pancreatol* 1999; 26: 137.
29. Schneider A, Pogue-Geile K, Barmada MM, *et al.* Hereditary, familial, and idiopathic chronic pancreatitis are not associated with polymorphisms in the tumor necrosis factor (TNF-alpha) promoter region or the TNF receptor 1 (TNFR 1) gene. *Genet Med* 2003; 5: 120.
30. Beranek H, Teich N, Witt H, *et al.* Analysis of tumour necrosis factor alpha and interleukin 10 promotor variants in patients with chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2003; 15: 12237.
31. Howell WM, Pead PJ, Shek FW, *et al.* Influence of cytokine and ICAM-1 gene polymorphisms on susceptibility to chronic pancreatitis. *J Clin Pathol* 2005; 58: 595.
32. Hofner P, Balog A, Gyulai Z, *et al.* Polymorphism in the IL-8 gene, but not in the TLR4 gene, increases the severity of acute pancreatitis. *Pancreatol* 2006; 5: 542.
33. Lankisch PG. Chronic pancreatitis: development from acute pancreatitis? In: Büchler MW, Friess H, Uhl W, Malfertheiner P, eds. *Chronic Pancreatitis. Novel Concepts in Biology and Therapy*. Blackwell Publ. Co, 2002: 101.