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TNF-alpha gene (*TNFA*) variants increase risk for multi-organ dysfunction syndrome (MODS) in acute pancreatitis

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Abstract

Background/Objectives—Acute pancreatitis (AP) is a complex inflammatory syndrome with unpredictable progression to systemic inflammation and multi-organ dysfunction syndrome (MODS). Tumor necrosis factor alpha (TNF–α) is a cytokine that may link inflammation to the systemic inflammatory response syndrome (SIRS), which usually precedes MODS. Small genetic cohort studies of the *TNFA* promoter in AP produced ambiguous results. We performed a comprehensive evaluation of *TNFA* promoter variants to assess both susceptibility to AP and risk of progression to MODS.

Methods—We prospectively ascertained 401 controls and 211 patients with AP that were assessed for persistent SIRS (>48 hours) and MODS. MODS was defined as failure of ≥2 organ systems (cardiovascular, pulmonary, and/or renal) persisting more than 48 hours. Subjects were genotyped by DNA sequencing and analyzed for SNPs at −1031 C/T (rs1799964), −863 A/C (rs1800630), −857 C/T (rs1799724), −308 A/G (rs1800629), and −238 A/G (rs361525).

Results—Twenty-three of 211 AP patients (11%) developed MODS. *TNFA* promoter variants were not associated with susceptibility to AP, but progression to MODS was associated with the minor allele at −1031C (56.5% vs. 32.4% P=0.022, OR: 2.7; 95%CI: 1.12–6.51) and −863A (43.5% vs. 21.8% P=0.022, OR: 2.76; 95%CI: 1.12–6.74).

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Conclusion—*TNFA* promoter variants do not alter susceptibility to AP, but rather the TNF–α expression-enhancing −1031C and −863A alleles significantly increased the risk of AP progression to MODS. These data, within the context of previous studies, clarify the risk of specific genetic variants in *TNFA* and therefore the role of TNF–α in the overall AP syndrome.

Keywords

systemic inflammatory response syndrome (SIRS); genetic epidemiology; shock; modeling; pancreas

Introduction

Acute pancreatitis (AP) is a syndrome of sudden onset pancreatic inflammation recognized in humans by abdominal pain, elevation of serum amylase and lipase levels, and abdominal imaging findings (1, 2). The inflammatory process is usually limited to the pancreas and surrounding adipose tissues. However, in about 20% of patients the inflammatory process overflows the pancreas leading to systemic inflammation, with almost one-third of these patients developing severe local and systemic complications, including the multi-organ dysfunction syndrome (MODS) (1, 3).

The specific mechanisms and variables responsible for the transition from local to systemic inflammation and then to MODS have not been clearly deciphered in humans because the outcome is unpredictable until after early complications have already developed, which requires 24–48 hours of observation (4). However, a number of pro-inflammatory cytokines, including TNF-α, have been shown to be increased early in the clinical course and are associated with the inflammatory cascade that leads to a systemic inflammatory response syndrome (SIRS), which is then linked, in a subset of patients with SIRS, to MODS (3, 5, 6).

TNF–α, a key regulator of pro-inflammatory cytokines, is thought to play a pivotal role in the pathogenesis of AP. Animal studies show that inhibition of this protein reduces tissue damage, severity, and mortality (reviewed in (7)), suggesting that the end-organ dysfunction observed in the severe form of the disease is mediated, at least in part, by TNF–α. Clinical studies indicate that TNF–α levels are associated with a more severe course of AP, including SIRS and MODS (8, 9), although association does not prove causality. TNF–α is also thought to play a central role in the pathogenesis of SIRS in sepsis and multiple trauma (5, 10) disorders in which most of the features of systemic inflammation are similar to severe AP (11, 12).

TNF–α reaches the circulatory peak within a few hours after the onset of AP and is rapidly cleared from the bloodstream (7), which reduces TNF–α utility as biomarker that can be used as a prognostic factor in the clinical setting since most patients are admitted several hours or even days after the onset of pain. Furthermore, TNF–α is one of many cytokines released in the inflammatory cascade, making it difficult to determine the specific effects on susceptibility to clinical AP and independent contribution to SIRS and MODS.

The expression of the TNF–α gene (*TNFA*) varies among individuals and is affected by genetic factors (13–15), which may explain some of the unpredictability in progression from

local to systemic inflammation in AP. Five single nucleotide polymorphisms (SNPs) within the promoter region of *TNFA* variant alleles at positions −238, −308, −857, −863, and −1031, have been reported to influence transcriptional activation and cytokine production (14–16) and are shown to be related to either the susceptibility or severity of several other diseases (17–22). However, the effects of the individual *TNFA* polymorphisms on susceptibility to and severity of AP remain unclear. Reported studies in AP focus mainly on the polymorphism at −308 (9, 23–26), the results of which are contradictory. The polymorphism at −238 locus was recently suggested to be associated with organ dysfunction and severe AP (27). The question of which of the SNPs alter TNF–α expression and in which direction, and if the functional SNPs are associated with AP susceptibility or with progression from local AP to SIRS / MODS has not been clarified.

The purpose of the current study is to systematically analyze *TNFA* SNPs at positions −238, −308, −857, −863, and −1031 of the *TNFA* promoter region in a well-characterized cohort of prospectively ascertained AP patients and population controls at a single tertiary university-based hospital in Pittsburgh, PA. After genotyping and haplotype analysis we examined the association of these polymorphisms with regard to susceptibility to AP and progression to MODS in our population, and compared our results with findings of previously published reports. These data confirm the hypothesis TNF-α is a key variable in the mechanism linking local inflammation to systemic inflammation in humans, but is not critical for the initial injury.

Methods

Patients and controls

The Severity of Acute Pancreatitis Study / Pancreatitis-associated Risk Of Organ Failure (28) protocol was approved by the institutional review board of the University of Pittsburgh Medical Center. Informed written consent was obtained from all patients before their enrollment in the study. The diagnosis of AP was made when two out of the following three criteria were met: abdominal pain, amylase and/or lipase levels elevated at least 3 times greater than the upper limit of normal, and imaging. Patients with AP (109 men, 102 women; mean age, 51.3 y; SD, 18.9) who were admitted to the University of Pittsburgh Medical Center between June 2003 and Feb 2008 were enrolled consecutively. Blood samples obtained at admission were collected in ethylene ediaminetetraacetic acid-containing tubes for DNA extraction.

All patients were followed until discharge. MODS was defined as persistent (>48 h) dysfunction of more than one remote organ system: cardiovascular (systolic pressure <90 mm Hg), pulmonary (arterial PO2 <60 mm Hg at room air or mechanical ventilation), or renal (serum creatinine level <2 mg/dL after rehydration or hemodialysis). Controls included 401 phenotyped individuals without pancreatic disease who were recruited as controls in the North American Pancreatitis Study 2 (NAPS2) (29). The demographics of cases and controls were not different (data not shown).

Genotyping

DNA was extracted by using the Gentra Systems Puregene system (Minneapolis, MN) and the QIAGEN Flexigene DNA Kit (Qiagen, Valencia, CA) according to manufacturer instructions. The DNA extracts were stored at −20 °C until analysis. Two sets of PCR were used to analyze the 5 SNPs. Each polymerase chain reaction was performed in a total reaction volume of 25 OL containing 50 ng of genomic DNA. For SNPs at −308 and − 238 loci, we used primers 5′-ACTGAAACCAGCATTATGAGTCT C-3′ and 5′-GGACATATAAAGGCAGTTGTTGG-3′ with PCR condition of 12 min at 95° C, followed by 30 cycles of 20 sec at annealing temperature of 59° C, 45 sec at 72 C (extension), and 15 sec at 95° C (denaturation). The reaction was ended by a 2 min extension at 72° C. To analyze SNPs at −1031, −863, and −857 loci, primers 5′-GGAAAGGCTCTGAAAGCCAGC-3′ and 5′-GGTATGGAATACAGGGGACGTTTA-3′ were used with the following cycling reaction: 12 min at 95° C, followed by 35 cycles of 30 sec at annealing temperature of 54° C, 30 sec at 72° C (extension), and 15 sec at 95° C (denaturation). The reaction was ended by a 2 min extension at 72° C. All PCRs were performed using the AmpliTaqGold polymerase (ABI, Foster City, CA). Cycle sequencing was performed using the ABI Prism Big Dye Terminator Sequencing Kit v3.1 diluted 1:4 (ABI, Foster City, CA). Reaction products were purified by ethanol ethylenediaminetetraacetic acid precipitation after the ABI sequencing kit protocol. Sequence products were run on an ABI Prism 3730 Genetic Analyzer, and sequence data were analyzed using Sequencher 4.1.4 (ABI).

Statistical analyses

Statistical comparisons between groups were performed by χ^2 tests. When the expected value was<5, two-tailed Fisher's exact test was used. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated. SPSS version 16 (SPSS, Inc., Chicago, IL, USA) was used for all analyses. The control population was in Hardy–Weinberg equilibrium of the polymorphisms by comparing the expected with the observed genotype frequencies using a 2 x 3 table ^{2} test. Haplotype estimation was done using fast PHASE, Version: 1.2.3 (30). Additional haplotype analysis was performed using R version 2.13.0 (31). All P values are two-sided; P<0.05 was considered to be statistically significant.

Results

In total, 211 patients with AP and 401 healthy controls were studied for *TNFA* promoter SNP over the locus on −1031(T/C), −863(C/A), −857(C/T), −806(C/T), and −308(G/A). The etiologies of pancreatitis were ascertained in all 211 patients, of whom 23 (11%) developed MODS (Table 1). Patients with MODS had higher BMI compared to patients without MODS (non-MODS AP) (32.3 vs. 28.3, p=0.02).

Table 2 shows genotype frequency between cases and controls. None of the variant alleles at the 5 tested loci was associated with susceptibility to AP when comparing cases with controls. To evaluate the association of the *TNFA* promoter polymorphisms with MODS, we compared the frequency of observed genotypes in cases with MODS to cases without MODS (Table 3). The variant allele (C) carriers at −1031 were significantly more frequently

observed among patients with MODS compared with those without MODS (56.5% vs. 32.4% P=0.022, OR: 2.7; 95%CI: 1.12–6.51). At the −863 locus, carriers of variant allele (A) were seen more frequently in MODS cases than in cases with no or only single organ dysfunction (43.5% vs. 21.8% P=0.022, OR: 2.76; 95%CI: 1.12–6.74). We did not find an association between the analyzed alleles at other loci (−857, −308, −238) and the presence of MODS.

Higher BMI is a known risk factor for severe pancreatitis (2), and was associated with MODS in our series as reported above. The BMI in individuals carrying the variant allele at −1031 (T/C+C/C) was not different from individuals with genotype T/T in cases and controls (27.9 (SD 6.5) vs. 28.1 (SD 7.2), P=0.70), among cases (28.3 (SD 7.7) vs. 29 (SD 7.2), P=0.55) or among controls (27.7 (SD 5.7) vs. 27.68 (SD 7.2), P=0.97). Similarly, there was no difference in BMI between variant allele carriers (C/A+A/A) and genotype C/C at −863 among cases and controls (27.8 (SD 6.5) vs. 28.1 (7.1), P=0.59), among AP (28.9 (8.2) vs. 28.7 (7.1), P=0.84) and among controls (27.3 (SD 5.4) vs. 27.8 (SD 7.1), P=0.44). These data show that higher BMI does not account for the observed association between the −1031 and −863 SNPs with MODS.

Eight common haplotypes (<1%) where identified at the *TNFA* locus defined by −1031T/C, −863A/C, −857C/T, −308G/A, −238G/A loci. The distribution of haplotypes was not different between cases and controls and was consistent with the finding that the *TNFA* promoter SNPS were not associated with AP susceptibility (data not shown). On the other hand, the CACGG haplotype was significantly associated with presence of MODS in patients with AP (23.9% in MODS vs. 12.2% in non-MODS AP, P=0.039).

Discussion

The current study represents the largest prospective series studying the relation of AP susceptibility and severity with common *TNFA* promoter polymorphisms and provides new insights into the role of TNF-α in human acute pancreatitis. In comparison to previous studies, our analysis of SNPs at positions −238, −308, −857, −863, and −1031 of the *TNFA* promoter confirms reports that these SNPs do not influence susceptibility to AP. However, of greater importance, we demonstrated that progression to MODS was specifically associated with the *TNFA* promoter variants −1031C and −863A that are known to alter TNF–α expression (15, 19, 33). Haplotype analysis of the 5 SNPs also revealed that the (containing −1031C and −863A) carries the risk for progressing to MODS. The observation that *TNFA* −1031C and −863A promoter variants specifically alter the risk of MODS indicates that TNF–α is not only associated with severe acute pancreatitis (along with many other biomarkers of acute inflammation), but that it plays a significant deterministic role in the mechanism linking pancreatic inflammation to systemic inflammation and MODS in humans with AP.

Functional studies on human samples from patients with *TNFA* 1031C/−863A alleles demonstrate that these SNPs alter TNF-α expression. Peripheral blood mononuclear cells carrying −1031C/−863A, when induced by concanavalin A, produce higher levels of TNF-α, and have two-times higher transcriptional promoter activity compared to cells carrying

the dominant allele (15). TNF-863 was also shown to be related to the nuclear factor-kappa beta (NF-κB) pathway. This site normally binds both p65-p50 and p50-p50 dimers, but a single base change from C to A inhibits p50-p50 binding. The ability to bind p50-p50 reduces the enhancer effect of this NF-κB site, inhibits the transcription of p65-p50 and subsequently expresses less TNF-α upon LPS induction. Therefore, the allele TNF-863A through inhibiting this binding is associated with higher expression of the TNF-α (33). Using the LPS-stimulated monocyte/macrophage assay, Soga et al have shown that TNF-α productivity in variants of −1031 and −863 tend to be elevated compared with the dominants, though the difference did not reach significance (19).

However, results of the functional studies of polymorphisms at −1031 and/or −863 are not consistent between tissues. Skoog et al demonstrated that TNF-863A variant had a lower transcriptional activity in chloramphenicol acetyltransferase (CAT) reporter gene studies in human hepatoblastoma (HepG2) cells, and was associated with a lower TNF-α level in healthy men (34). Heesen et al also showed that C homozygotes at −863 are associated with higher TNF-α response of human whole blood of healthy volunteers to endotoxin (35) stimulation. More recently, Kao et al showed that TNF-α secretion levels by peripheral mononumclear cells in vitro after hepatitis B antigen stimulation was lower in individuals carrying the TNF-863A variant (22). Together, these studied demonstrate that the −1031 and/or −863 are functional, and that the effect is dependent on the context. The current study provides strong evidence that these promoter SNPs increase the severity of systemic response to pancreatic injury, and the association of the −1031C and −863A with MODS suggest that the response is through a mechanism similar to that of stimulated peripheral blood mononuclear cells described in the previous paragraph.

AP is a complex process that evolves through various phases over time. This indicates that different steps, with different mechanisms and variables are sequentially linked, but have independent features. The current study seeks to determine if TNF–α is indeed a major effector of severe AP by comparing low and high expressing *TNFA* genotypes, and clarifying the step in the sequential AP process that TNF–α mediates. Most previous case-control studies of *TNFA* promoter polymorphisms in AP focused on the −308 locus in relation to susceptibility of disease (Table 4), though a report by de-Madaria et al. (27) tested the association of SNPs at both −308 and −238 loci with disease risk. None of these studies focusing on a limited number of SNPs, (9, 23–27), nor the current study with expanded genotype evaluation identified an effect of *TNFA* variants on susceptibility to AP. In a study from Taiwan, recently, Chang et al (36) reported a small study of a homogenous Taiwanese population with hypertriglyceridemia with (n=46) or without (n=80) pancreatitis. Five *TNFA* promoter SNPS (positions −1031, 863, 857, 308, and 238) and extensive cystic fibrosis transmembrane conductance regulator (CFTR) genotyping was conducted. Pancreatitis was reported to be associated with a complex *CFTR* haplotype and the *TNFA* −863A variant in this small study without evidence of epistasis, meaning there was no suppression of the effect of one gene by the other. This finding has not been replicated and there is a possibility that TNF–α is associated with a different disease susceptibility mechanism in patients with hypertriglyceridemia than the focus of the current investigation. With only 8 cases caused by hypertriglyceridiemia, we could not replicate these findings.

However, based on our findings and previous reports on non-hypertriglyceridemia AP studies, we conclude that *TNFA* variants are not associated with susceptibility to AP.

Few studies report a comprehensive testing of *TNFA* promoter polymorphisms in association with AP severity (Table 4). Most of the existing literature focuses on polymorphism at the −308 locus. Zhang et al. (9) studied the *TNFA* −308 variant in 102 controls and 127 consecutive patients in Jiangsu Province, China with a first attack of unequivocal acute biliary pancreatitis. Of the 127 patients, 61 (48%) developed severe pancreatitis (Acute Physiology and Chronic Health Examination (APACHE II) ≥8 or computed tomography severity index (CTSI) score ≥4), and among severe AP 18 (30%) developed “septic shock” (classified by Zhang et al using the definition of Muckart and Bhagwanjee as “hypotension refractory to adequate restoration of intravascular volume, when associated with perfusion abnormalities and requiring the use of inotropes” (37)). They found no association between the *TNFA* −308A allele and susceptibility or severity, but they did report an association with the risk allele and shock (p=0.023). Balog et al. (25) in a study of 77 patients with pancreatitis divided in to mild (n=29) and severe (n=48) based on Ranson’s criteria, which included multiple markers of organ dysfunction, found that the *TNFA* −308A allele was associated with risk of severe AP (P = 0.046). On the other side, no association between the *TNFA* −308A allele and disease severity was found by Powell et al. (n=190) (24), Sargen et al. (n=135) (23), Tukiainen (n= 397) (26), or de-Madaria (n=84) (27), or in the present study. However, the association reported by Balog et al. (25) could be potentially derived by the high prevalence of ancestral haplotype 8.1 in the eastern European and Hungarian population that includes *TNFA* −308A as well as other pro-inflammatory genetic variants (38, 39). In this case, *TNFA* −308A would be associated with more severe disease since it is inherited with a high-risk DNA haplotype that contains other pro-inflammatory genes that contain the true severity risk variants. Finally, while de-Madaria et al. (27) observed an association between −238 A/G genotype and organ failure (including shock and respiratory failure), we did not observe any association between MODS and the *TNFA* −308G allele.

Although not previously tested in AP, variant alleles of *TNFA* at −1031 and −863 have been shown to affect disease course in other inflammatory conditions. Basu et al (32) investigated five *TNFA* SNPs (−1031T/C, −857C/T, −308G/A, −238G/A and intronic variant 489G/A (rs1800610)) in malaria patients in India. They found that the TNFA −1031C allele and CC genotype were risk factors for more severe clinical indices. Using *in silico* analysis the C-C-G-G and C-C-G-A promoter haplotype (corresponding to the a C-x-C-G-A or C-x-C-G-G in the current report) was predicted to have enhanced binding of NF-κB and Oct-1 compared to the reference haplotype (T-C-G-G), which was predicted to have reduced NF-κB binding and no Oct-1 binding, which is a biologically plausible explanation for the varied response. Both *TNFA* −1030C and *TNFA* −863A alleles were found to be associated with a clinically distinct subset of scleroderma patients at risk for pulmonary vascular disease (20). The variant alleles at these loci were also shown to be associated with the severe periodontitis, which has been shown to be influenced by cytokine productivity (19). Lu et al. (40) evaluated *TNFA* promoter SNPs in H-pylori infection and reported that subjects with *TNFA* −1031C or −863A increased TNF–α expression and gastric neutrophil infiltration. The authors proposed that SNPs at these loci may influence the risk of gastric ulcer in H-pylori

infected patients by altering TNF–α levels in tissue and causing gastric inflammation. All of these reports suggest disease-modifying effects of the −1031 and −863 alleles. Taken together, these data are consistent with the observation that *TNFA* SNPs −1031C and −863A increase risk of MODS in AP, and this effect is likely mediated by increased TNF–α levels that amplify the inflammatory response and contributing to development of systemic inflammation and associated complications.

Another assessment of the functional significance is correlation of the secreted protein level with the observed genotype. However, in AP, testing the association of the *TNFA* polymorphisms with the TNF–α serum protein level is greatly limited by the rapid clearance of the TNF–α protein from the bloodstream within the earliest stages of the disease, and long before organ dysfunction and transfer to tertiary centers occurs. Further studies are thus needed to test the *in vitro* and *in vivo* significance of the *TNFA* −1031 and/or −863 variants on TNF–α production and levels in patients wit AP.

In conclusion, our data suggest that polymorphisms at −1031 C/T, −863 A/C, −857 C/T, −308 A/G and −238 A/G of *TNFA* promoter site are not associated with susceptibility to AP. However, the *TNFA* minor alleles −1031C and −863A significantly increase the risk of progression to severe AP and are specifically associated with MODS. These findings establish the importance of *TNFA* sequence variants in AP and implicate TNF–α in the development of MODS. Knowledge of the importance the *TNFA* sequence variants could also identify, prior to injury, those patients at increased risk of SIRS, MODS and a poor outcome, and help identify targets for therapy.

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Table 1

Characteristics of AP patients based on the presence of MODS.

	Non-MODS AP	MODS	P
Sex, F (%)	93 (49.5%)	9 (39.1%)	0.35
Age, mean (SD)	50.7(19.3)	55.6 (14.8)	0.25
Race, white (%)	156 (83.0%)	16(69.6%)	0.65
BMI, mean (SD)	28.3 (7.4)	32.2 (6.0)	0.02
Etiology			
Biliary	75 (39.9%)	8 (34.8%)	
Idiopathic	43 (22.9%)	7 (30.4%)	
Post-ERCP	25 (13.3%)	2 (8.7%)	
Alcohol	27 (14.4%)	1 (4.3%)	
Hypertriglycerdemia	5 (2.7%)	3 (13%)	
Drugs	8 (4.2%)	1 (4.3%)	
Pancreatic mass/divisum	4 (2.1%)	0	
Hypercalcemia	1 (0.5%)	1 (4.3%)	

Table 2

Genotypes of *TNFA* promoter in controls compared with patients with AP.

		Controls (n=401)	Cases (all AP) (n=211)	P value, OR (95%CI)
	Genotype			
TNFA-1031	T/T	255 (63.6%)	137 (64.9%)	0.74, 0.94 (0.66–1.33)
	Minor allele (C) carrier (T/C + C/C)	146 (36.4%)	74 (35.1%)	
TNFA-863	C/C	291 (72.6%)	160 (75.8%)	0.38, 0.84 (0.57–1.23)
	Minor allele (A) carrier (C/A+A/A)	110 (27.4%)	51 (24.2%)	
TNFA-857	C/C	323 (80.5%)	171 (81%)	0.88, 0.96 (0.63–1.48)
	Minor allele (T) carrier (C/T+T/T)	78 (19.5%)	40 (19%)	
TNFA-308	G/G	306 (76.3%)	147 (71%)*	0.16, 1.31 (0.9–1.91)
	Minor allele (A) carrier (G/A+A/A)	95 (23.7%)	60 (29%)	
TNFA-238	G/G	369 (92%)	186 (89.9%)*	0.37,1.3 (0.73–2.32)
	Minor allele (A) carrier (G/A+A/A)	32 (8%)	21 (10.1%)	

* In four patients with AP, genotypes at −308 and −238 locus could not be determined.

Table 3

Genotypes of *TNFA* promoter in AP patients with and without MODS

		non-MODS AP (n=188)	MODS (n=23)	P value, OR (95%CI)
	Genotype			
TNFA-1031	T/T	127 (67.6%)	10 (43.5%)	0.022, 2.7 (1.12–6.51)
	Minor allele (T/C+C/C)	61 (32.4%)	13 (56.5%)	
	T/C	54 (28.4%)	12 (52.2%)	
	C/C	7 (3.7%)	1 (4.3%)	
TNFA-863	C/C	147 (78.2%)	13 (56.5%)	0.022, 2.76 (1.12–6.74)
	Minor allele (A/C+A/A)	41 (21.8%)	10 (43.5%)	
	A/C	35 (18.6%)	9 (39.1%)	
	A/A	6 (3.2%)	1 (4.3%)	
TNFA-857	C/C	153 (81.4%)	18 (78.3%)	0.71, 1.21 (0.42–3.49)
	Minor allele (C/T+T/T)	35 (18.6%)	5 (21.7%)	
	C/T	35 (18.6%)	4 (17.4%)	
	T/T	0 (0%)	1 (4.3%)	
TNFA-308	G/G	130 (70.6%)*	17 (73.9%)	0.92, 0.84 (0.31–2.27)
	Minor allele (G/A+A/A)	54 (29.3%)	6 (26.1%)	
	G/A	54 (29.3%)	6 (26.1%)	
	A/A	0 (0%)	0 (0%)	
TNFA-238	G/G	164 (89.1%)*	22 (95.7%)	0.54, 0.37 (0.04–2.91)
	Minor allele (G/A+A/A)	20 (10.9%)	1 (4.3%)	
	G/A	19 (10.3%)	1 (4.3%)	
	A/A	1 (0.6%)	0 (0%)	

*In four patients with non-MOD AP, genotypes at −308 and −238 locus could not be determined.

Table 4

Published studies of TNF-α promoter polymorphisms and AP

SNP(s)	First Author	Year	Ethnicity (Country)	Design	Severity Criteria	Main Findings
TNFA 308 A/G	Powell JJ	2001	UK	Case (190 AP)-Control (102 healthy)	Atlanta	a-No association with pancreatitis b-No association with severity
TNFA 308 A/G	Zhang DL	2003	China	Case (208 AP)-Control (116 healthy)	APCHE >8 or CT index> 4	a-No association with pancreatitis b-No association with severe pancreatitis but variant allele was associated with shock among severe cases
TNFA 308 A/G	Sargen K	2000	UK (caucasian)	Case (135 AP)-Control (107 healthy)	Atlanta or Organ Failure Score	a-No association with pancreatitis b-No association with severity c-No association with etiology
TNFA 308 A/G	Balog A	2005	Hungary	Case (77 AP)-Control (71 healthy)	Ranson	*TNFA* 308 A allele is associated with risk of severe acute pancreatitis
TNFA 308 A/G& 238 A/G	de-Madaria	2008	Spain	Cohort of pancreatitis (84 cases)	Atlanta	a-*TNFA* 308 polymorphism is not associated with either etiology and/or severity b-*TNFA* 238 AG genotype is associated with organ dysfunction (including shock and respiratory failure)
TNFA 1031 T/C, 863 C/A, 857 T/C, 308 A/G & 238 A/G	Chang YT	2008	Taiwan (Chinese)	Case (46, Hyperlipidemic pancreatitis) Control (80 Hypertriglyceridemia without pancreatitis)	Not done	Among 5 SNPs studied, only 863A variant was associated with HLP
*TNFA*308 A/G	Tukiainen E	2008	Finland	Case (397 AP enriched in alcohol-induced AP) Control (300 healthy	Atlanta	*TNFA* 308 polymorphism is not associated with either etiology and/or severity