Single Nucleotide Polymorphisms of Genes for EGF, TGF-β and TNF-α in Patients with Pancreatic Carcinoma

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Abstract. *Aim: To show whether single nucleotide polymorphisms (SNPs) of Epidermal growth factor (EGF)-61*A/G, Transforming growth factor beta 1 (TGF-B1) -509*T/C and Tumor necrosis factor-alpha (TNF-A) -308*A/G are associated with the survival rate after pancreatic cancer surgery and with the frequency of post-operative complications. Patients and Methods: EGF 61*A/G, TGF-B1-509*T/C and TNF-A-308*A/G genotypes were analyzed in patients who underwent pylorus-preserving pancreaticoduonectomy for pancreatic carcinoma and were determined by means of Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP). The association of each genetic polymorphism with clinical and pathological data of the patients and early tumor recurrence were evaluated. Results: A significantly lower median survival duration was found in EGF 61*AA homozygotes, as compared to the AG heterozygous group. There was also a significantly lower median survival duration in the TNF-A-308* AA homozygote group as compared to the AG and GG groups. Survival duration in patients had no correlation with TGF-B1 -509*T/C polymorphism. There was a significantly lower median survival duration in the TNF-A -308* AA homozygous group, as compared to the AG and GG group in a Cox proportional hazard model. The frequency of the TGF-B1 T-allele was higher among patients with leakage of the pancreatic anastomosis. The frequency of the TGF-B1 TC genotype was significantly higher among patients who developed leakage of the biliodigestive anastomosis as compared with the TGF-B1 CC genotype. The frequency of TGF-B1 T-carriers (i.e. TT+TC) was significantly higher among patients with leakage of the biliodigestive anastomosis, as compared to these with the TGF-B1 CC genotype. In a Cox proportional hazard model, only wound infection had a significant correlation with long-term survival duration of patients with pancreatic cancer. Conclusion: There appears to be a significant correlation of the EGF-61* AA and of the TNF-A -308* AA polymorphism with lower survival duration in patients with resectable pancreatic carcinoma. The presence of wound infection was associated with poor prognosis. TGF-B1-509* T-carrying genotypes were more frequent in paitents with severe post-operative complications.*

Pancreatic carcinoma is a highly malignant tumor and is among the solid tumors with the worst prognosis (1). The five-year survival rates range from 5 to 15% in Europe and the United States (2). Traditional treatment of early-stage cancer is surgical resection. Chemotherapy is used in a palliative or adjuvant setting (3).

Patients with the same tumor stage have different outcomes (4). Despite the apparent progress in diagnostic techniques, it is often difficult to distinguish patients with a poor prognosis from others with a better prognosis. Therefore, molecular markers which may serve as prognostic factors are desirable to identify more aggressive pancreatic cancer phenotypes and to individually tailor therapy.

In recent decades, various factors have been associated with the development of complications after pancreatic surgery, with some being patient-related and pre-existing, such as advanced age, duration of surgical procedure, tumor diameter and long-standing diabetes (5). To assess these risk factors, scoring systems have been implemented to better explain and select patients at-risk (6). However, despite these improvements, it is still difficult to determine patients at-risk who may develop severe post-operative complications. Although perioperative mortality following pancreatic resection has apparently decreased in recent years (7), the overall complications rate is still high, with a wide variation in existing literature. Major complications, such as leakage of

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anastomosis or wound infection, are a significant reason for post-operative mortality, especially if they require re-operation (8).

Single nucleotide polymorphisms (SNPs) are the most common source of human genetic variations, and they may contribute to the prediction of the clinical outcome of patients with pancreatic carcinoma. SNPs have been described for Epidermal growth factor (*EGF*), Transforming growth factor beta 1 (*TGF-B*1) and Tumor necrosis factor-alpha (*TNF-A*) (9).

EGF is a member of the EGF superfamily which activate DNA synthesis and stimulate cellular proliferation and mitogenesis. Furthermore, its receptor is overexpressed in pancreatic cancer, and the EGF-EGFR pathway is involved in pancreatic cancer growth and progression. Previous studies have reported that the *EGF*61* A/G SNP is associated with pancreatic cancer and colorectal carcinoma (9, 10).

TGF-B acts not only as a potent inhibitor of proliferation of epithelial, endothelial and hematopoietic cells, but also as an important pro-inflammatory cytokine (11). The TGF-B pathway has important roles in cellular proliferation, angiogenesis, differentiation, migration and apoptosis (12). In non-cancer cells, TGF-B stops cell proliferation, induces differentiation, and promotes apoptosis (13, 14). In cancer cells, mutations of proteins in the TGF-B pathway confer resistance to growth inhibition by TGF-B, resulting in uncontrolled cell proliferation. The increase of TGF-B production in cancer cells also stimulates angiogenesis and suppresses the activities of infiltrating immune cells, thereby facilitating the tumor in escaping immunosurveillance. Among the three TGF isoforms (TGF-B1, B2 and B3), TGF-B1 is known to be expressed in endothelial, hematopoietic and connective tissues, and has an effect on the growth of pancreatic carcinoma (15, 16). It has been reported that TGF-B expression levels have been reported to correlate with post-operative survival duration in various malignancies (17-19).

TNF-A is an important member of the TNF-A superfamily, and plays roles in immunity, cellular remodeling, apoptosis and cell survival. It is produced by diverse kinds of cells, such as macrophages, neutrophils, fibroblasts, keratinocytes, natural killer (NK) cells, T- and B-cells, and tumor cells (20). TNF-A has been reported to play an important role in the pathogenesis of cancer with a significant role in the inflammatory etiology of pancreatic cancer. The TNF-A-308 polymorphism has been identified as a risk factor for various types of cancer, such as hepatocellular carcinoma, gastric cancer and breast cancer (21-23). Although previous studies have confirmed that TNF-A-308 A/G gene polymorphisms are not related to pancreatic cancer (9), the TNF-A-308 polymorphism might play an role in postoperative survival duration and complications.

EGF-61 A/G, *TGF*-B -509 T/C, and *TNF-A* -308 A/G gene polymorphisms have been analyzed in pancreatic cancer and colorectal carcinoma (9, 10). However, it remains unknown if any of these genetic polymorphisms are associated with post-operative survival duration and complications after surgery for pancreatic cancer. Therefore, the aim of this study was to determine the association between these gene polymorphisms and the post-operative outcome.

Materials and Methods

Sample collection for clinical experiments. This prospective study was conducted at the Surgical Department of the University Hospital Mannheim, Germany from October 2000 to August 2009. Blood samples were collected after informed patient consent was given and sample acquisition and subsequent use was also approved by the Institutional Review Board of the local Ethics Committee. All patients were histologically diagnosed as having pancreatic carcinoma by experienced pathologists.

All patients were Caucasians who underwent an oncological pylorus-preserving pancreatico-duodenectomy. Tumor pathology stages were classified according to the tumor-node-metastasis (TNM) classification of the Union International Control Cancer (UICC) (24). Pathology grades were determined according to the criteria of the World Health Organization (WHO) (25). Regular follow-up was performed according to our hospital's protocol. Patients who entered the case cohort were followed up every 3 months prospectively by personal or family contact until death or being lost to follow-up. The maximum duration of follow-up was 72 months (last follow-up: August 2009). As a result, 126 patients with pancreatic cancer who had complete clinical information and adequate DNA samples were included and genotyped.

Genotyping. For genetic analyses, whole blood was collected from patients with pancreatic cancer, at the time of enrollment. Genomic DNA was extracted from peripheral blood by standard methods. Genomic DNA was isolated from peripheral blood of pancreatic cancer patients in EDTA using the QIAamp DNA Mini and QIAamp DNA Blood Mini Kits (Qiagen GmBH, Hilden, Germany), according to the manufacturer's instructions. DNA concentrations were determined by A280 using an ultraviolet spectrophotometer. The amount and quality of extracted DNA allowed the analysis of the *EGF* genotypes in 77 patients *TGF-B* genotyping was performed using full blood samples of 107 patients. *TNF-A* genotypes were analyzed using samples derived from 110 patients.

Gene polymorphisms were detected using polymerase chain reaction–restriction fragment length polymorphism analysis (PCR–RFLP). Primers and lengths of the amplified PCR fragments are given in Table I. PCR conditions are summarized in Table II.

The *EGF* (61*A/G) PCR product was digested with restriction endonuclease *Alu* I (sequence of restriction site: AG▼CT) for 2 h. *TGF-B*1 (-509*T/C) PCR products were digested with the restriction endonuclease Bsu36 I (sequence of restriction site: CC▼TNAGG) for 2 h. *TNF-A* (-308*A/G) PCR products were digested with restriction endonuclease *Nco*I (sequence of restriction site: C▼CATGG) for 2 h. DNA fragments were analyzed on 2-3% agarose gels stained with ethidium bromide (26-28).

Table I. *PCR Primer sequences and resulting fragment lengths for growth factor gene.*

Gene polymorphism	Primer direction	Primers	Amplified fragment(bp)	Reference
EGF	Forward	5'-TGTCACTAAAGGAAAGGAGGT-3'	242	28
	Reverse	5'TTCACAGAGTTTAACAGCCC-3'		
*TGF-B*1	Forward	5'-CGGACACCCAGTGATGGG-3'	530	26
	Reverse	5'-CCTCCTGGCGGCCAAGCGC-3'		
TNF-A	Forward	5'-AGGCAATAGGTTTTGAGGGCCAT-3'	345	27
	Reverse	5'-GAGCGTCTGCTGGCTGGGTG-3'		

Table II. *Polymerase chain reaction (PCR) conditions to detect growth factor gene polymorphisms.*

Gene	Method		PCR reaction conditions			
	Temperature	Cycles				
EGF	94˚C 5 min	1	10*PCR buffer	5	*Taq* polymerase (5U/µl)	0.4
	94˚C 1 min	35	dNTP (10 mM)	2	Restriction enzyme	*Alu*I
	57˚C 1 min		Primer forward (10 µM)	3	Restriction time (hours)	2
	72˚C 1 min		Primer reverse (10 µM)	3	Restriction pattern length (bp)	A: 102+91+34+15
	72˚C 10 min	1	$MgCl_2$ (50 mM)	3		G: 193+34+15
*TGF-B*1	94˚C 1 min	1	10*PCR buffer	5	*Taq* polymerase (5U/µl)	0.4
	94˚C 1 min	30	dNTP (10 mM)	1	Restriction enzyme	*Bsu*36 I
	60˚C 1 min		Primer forward (10 µM)	2	Restriction time (hours)	2
	72˚C 1.5 min		Primer reverse (10 µM)	2	Restriction pattern lengt (bp)	C: 273+257
	72˚C 10 min	1	$MgCl_2$ (50 mM)	1.5		T: 530
TNF-A	94˚C 5 min	1	10*PCR buffer	5	*Taq* polymerase (5U/µl)	0.4
	94˚C 1 min	30	dNTP (10 mM)	1	Restriction enzyme	*Nco*I
	60˚C 1 min		Primer forward (10 µM)	1	Restriction time (hours)	2
	72˚C 1 min		Primer reverse (10 µM)	1	Restriction pattern length (bp)	G: 325+20
	72˚C 5 min	1	$MgCl_2$ (50 mM)	1.5		A: 345

Statistical analysis. Survival duration was calculated from the date of diagnosis of pancreatic cancer to the date of death. The associations between survival duration and clinical features and SNPs in *EGF* 61*A/G, TGF-B1-509*T/C and *TNF-A* -308*A/G were estimated using the Kaplan–Meier method. Mean survival duration was presented when the median survival duration (MST) could not be calculated. We treated only one potential prognostic factor each time using a multivariate technique, *i.e.* the Cox proportional hazard model, to obtain the results of the univariate analysis. The estimated relative risk (hazard) ratio was calculated by the exponential of coefficient obtained from the Cox stepwise hazard model.

Genotype proportions among groups with and without complication were compared with the Fisher's exact test. A chi-square test was conducted, when one or more of the cells had an expected frequency of five or less. Differences were regarded significant at $p<0.05$. All statistical analyses were carried out using SPSS 19.0 (IBM Corporation, NY 10589). All statistical evaluations were made assuming a two-sided test with the significant level of 0.05, unless stated otherwise.

Results

The distribution of all genotypes are shown in Table III.

For the analysis of the *EGF* genotypes 77 patient samples were included. *TGF-B* genotypes were determined in 107 samples, the *TNF-A* genotypes were analyzed in 110 samples. The median post-operative survival of the patients in the three groups of SNPs was consistent with expected survival after pancreatic cancer resection.

Overall survival of the genotyped groups was analyzed using Kaplan-Meier survival curves. There was a significantly lower MST for the *EGF*61* AA homozygous group as compared to the AG heterozygous group (12 months for AA *versus* 22 months for AG, $p=0.028$ log-rank test).

The *TNF-A* -308*AG genotype was significantly associated with a better post-operative survival duration. There was a significantly lower MST for the *TNF-A*-308* AA homozygote group compared to the AG and GG groups (2.5 months for

Table III. *Associations between Epidermal growth factor (EGF), Transforming growth factor beta 1 (TGF-B1) and Tumor necrosis factor-alpha (TNF-A) genotypes and post-operative survival time of patients with pancreatic cancer.*

Genotype	Log-rank test			Cox stepwise hazards regression			
	Cases (n)	MST (months)	*p*-Value (log-rank)	*p*-Value (adjusted)	RR	95% CI	
						Lower	Upper
EGF-61							
AA	28	12	0.085	0.187	0.679	0.382	1.206
GG	18	13.5		0.871	0.945	0.478	1.870
AG	31	22		.	1.000		
AA *versus* GG			0.559				
AA *versus* AG			0.028				
GG *versus* AG			0.168				
*TGF-B*1 509							
TT	14	33	0.23	0.254	1.496	0.749	2.985
CC	40	15		0.764	0.926	0.562	1.528
TC	53	20		.	1.000		
TT *versus* CC			0.076				
TT *versus* TC			0.115				
CC *versus* TC			0.969				
TNF-A 308							
AA	3	2.5	0	0.018	0.194	0.050	0.758
GG	80	20		0.913	1.030	0.605	1.753
AG	27	23.5		.	1.000		
AA *versus* GG			0.000				
AA *versus* AG			0.000				
GG *versus* AG			0.812				

MST, Median survival time; RR, relative risk; CI, confident interval. p-Values were adjusted for gender UICC stages and age.

AA *versus* 20 months for GG, $p<0.001$ and 23.5 months for AG, $p<0.001$, log-rank test). There was also a significantly lower MST for the *TNF-A*-308* A/A homozygous group as compared to the GG and AG heterozygote group in a Cox proportional hazard model ($p=0.018$).

None of *TGF-B*1 -509*T/C genotypes was significantly associated with post-operative survival duration. The survival analysis according to the three SNPs is summarized in Table III.

In order to determine a correlation of genotypes with adverse events, the genotype analysis included patients with (n=11) and patients without (n=97) leakage of the pancreatic anastomosis; patients with (n=4) and patients without (n=104) leakage of the biliodigestive anastomosis; and patients with (n=10) and patients without (n=98) wound infection.

Depending on the presence of post-operative complications, the survival rate for patients after resection of pancreatic cancer was studied using Kaplan–Meier survival curves. In the group of patients with wound infection, the MST was 10.5 months. In the group of patients without wound infection, the MST was 20.7 months (Table IV). In Cox proportional hazard models, after adjusting for the demographic and clinical characteristics including age, gender and disease stage, only wound infection was found to have a significant influence on the survival rate for patients with pancreatic cancer, ($p=0.026$). The survival analysis according to the three types of complications is summarized in Table IV.

Post-operative complications were correlated with genotypes using the Pearson chi-square test. The distribution of the genotypes of the patients with complications and those with an uncomplicated course is given in Tables V-VII. The group of patients, who carried leakage of the pancreatic anastomosis, had a higher frequency of the *TGF-B*1 T-allele (16%, 14/14+72) than the frequency of the C-allele (8%, 12/12+138), ($p=0.043$). The distribution of *TGF-B*1 -509 T/C SNPs in patients was significantly associated with leakage of the biliodigestive anastomosis ($p=0.043$). The frequency of the *TGF-B*1 TC genotype (10%) was significantly higher among patients who developed a leakage of the biliodigestive anastomosis, when compared to those with the *TGF-B*1 CC genotype (0%) ($p=0.035$). The frequency of the *TGF-B*1 T-bearing genotypes (*i.e.* TT+TC) was also significantly higher among patients with leakage of the biliodigestive anastomosis when compared those with *TGF-B*1 CC genotype ($p=0.049$).

Table IV. *Association between complications and post-operative survival time of patients with pancreatic cancer.*

Complication	Cases (n)	MST (months)	*p*-Value (Log-rank)	RR	95% CI		*p*-Value (adjusted)
					Lower	Upper	
Leakage of the pancreatic anastomosis							
Yes	11	18.5	0.83	2.268	0.946	5.438	0.066
No	97	20					
Leakage of the biliodigestive anastomosis							
Yes	4	20	0.152	2.800	0.961	8.157	0.059
No	104	19					
Wound infection							
Yes	10	10.5	0.131	2.477	1.117	5.496	0.026
No	98	20					

MST, Median survival time; RR, relative risk; CI, confident interval. *p*-Values were adjusted for gender UICC stages and age.

Table V. *Epidermal growth factor (EGF), Transforming growth factor beta 1 (TGF-B1) and Tumor necrosis factor-alpha (TNF-A) genotypes and allelic frequencies in patients with leakage of the pancreatic anastomosis.*

Genotypes	Leakage of the pancreatic anastomosis		Total	Fisher's exact test	
	yes (%)	no (%)		*p*-Value (2-Sided)	*p*-Value (1-Sided)
EGF-61			Censored=2		
AA	3 (75%)	26 (33.3%)	29	0.191	
GG	0 (0%)	20 (25.6 %)	20		
AG	1 (25%)	32 (41%)	33		
A allele	7 (87.7%)	84 (53.8%)	91	0.077	0.062
G allele	1 (12.5%)	72 (46.2%)	73		
AA *versus* GG				0.260	0.198
AA *versus* AG				0.332	0.259
GG *versus* AG				1.000	0.623
TGF-B1-509			Censored=2		
TT	3 (23.1%)	10 (9.5%)	13	0.112	
CC	2 (15.4%)	43 (41%)	45		
TC	8 (61.5%)	52 (49.5%)	60		
T allele	14 (53.8%)	72 (34.3%)	86	0.055	0.043
C allele	12 (46.2%)	138 (65.7%)	150		
TT *versus* CC				0.069	0.069
TT *versus* TC				0.401	0.304
CC *versus* TC				0.182	0.113
TNF-A-308			Censored=2		
AA	0 (0%)	4 (3.7%)	4	1.000	
GG	10 (76.9%)	77 (72%)	87		
AG	3 (23.1%)	26 (24.3%)	29		
A allele	3 (11.5%)	34 (15.9%)	37	0.596	0.405
G allele	23 (88.5%)	180 (84.1%)	203		
AA *versus* GG				1.000	0.623
AA *versus* AG				1.000	0.670
GG *versus* AG				1.000	0.584

Discussion

EGF, TGF-B1 and TNF-A are three important growth factors that play key roles in tumor biology. They have been shown to be involved in growth, differentiation and epithelial transformation in the multistep processes of tumorigenesis (29-31). Up-regulation and overexpression of growth factors and growth factor receptors has been correlated to many processes associated with cancer, including uncontrolled cellular proliferation, autocrine stimulation of tumors and prevention of apoptosis. High expression of *EGF*, *TGF-B*1 and *TNF-A* has been observed in pancreatic cancer (32, 33). Some studies have

Table VI. *Epidermal growth factor (EGF), Transforming growth factor beta 1 (TGF-B1) and Tumor necrosis factor-alpha (TNF-A) genotypes and allelic frequencies in patients with leakage of the biliodigestive anastomosis.*

Genotypes	Leakage of the biliodigestive anastomosis		Total	Fisher's exact test	
	yes (%)	no (%)		*p*-Value (2-Sided)	*p*-Value (1-Sided)
EGF-61			Censored=1		
AA	2 (100%)	28 (34.6%)	30	0.184	
GG	0 (0%)	20 (24.7%)	20		
AG	0 (0%)	33 (40.7%)	33		
A allele	2 (100%)	89 (54.9%)	91	0.503	0.306
G allele	0 (0%)	73 (45.1%)	73		
AA *versus* GG				0.510	0.355
AA *versus* AG				0.223	0.223
GG *versus* AG				N/A	N/A
TGF-B1-509			Censored=1		
TT	0 (0%)	13 (11.5%)	13	0.043	
CC	0 (0%)	46 (40.7%)	46		
TC	6 (100%)	54 (47.8%)	60		
T allele	6 (50%)	80 (35.4%)	86	0.360	0.233
C allele	6 (50%)	146 (64.6%)	152		
TT *versus* CC				N/A	N/A
TT *versus* TC				0.359	0.294
CC *versus* TC				0.035	0.029
CC *versus* TC +TT				0.081	0.049
TNF-A-308			Censored=1		
AA	0 (0%)	4 (3.5%)	4	1.000	
GG	5 (83.3%)	83 (72.2%)	88		
AG	1 (16.7%)	28 (24.3%)	29		
A allele	1 (8.3 %)	36 (15.7%)	37	0.698	0.427
G allele	11 (91.7%)	194 (84.3%)	205		
AA *versus* GG				1.000	0.797
AA *versus* AG				1.000	0.879
GG *versus* AG				1.000	0.537

shown that the maximal capacity of cytokine production varies among individuals and correlates with SNPs such as those of *EGF*, *TNF-A* and *TGF-B*1 (34-36). However, an association of these genotype polymorphisms with survival duration of patients after resection of pancreatic cancer and the rate of perioperative complications has not yet been elucidated.

Currently, surgical resection is the only chance of long-term survival for patients with pancreatic carcinoma. A high rate of post-operative morbidity is reported (37). Presence of post-operative complications after pancreatic resections have negative influence on the survival rate for patients with pancreatic cancer (38).

In our study, we found no correlation of insufficiency of the pancreatic anastomosis, or biliodigestive anastomosis with post-operative survival. Our findings are in general agreement with previous descriptions where anastomotic leakage showed no effect on the oncological outcome (39, 40). Only wound infection had a negative influence on the long-term survival duration of patients with pancreatic cancer. Since anastomotic break down is a major source of morbidity for the patients we investigated if variability at gene *loci* may influence the risk of post-operative complications. We found no significant differences in allelic frequency and genotype distribution of *EGF*-61, *TNF-A*-308 polymorphisms and the occurrence of anastomotic insufficiency and wound infections.

For *TGF-B*, the TT genotype was more frequent in patients with leakage of the pancreatic anastomosis. Furthermore, patients with T-bearing genotypes had a higher frequency of leakage of the biliodigestive anastomosis compared with CC homozygotes.

Given its important role in cellular proliferation, survival, migration and differentiation, a functional polymorphism in *TGF* genes has been reported to be associated with the gene expression. *TGF-B*1 is a key player in wound healing with a negative effect on regeneration of the bowel wall mucosa and angiogenesis in the course of intestinal anastomotic wound healing. Interestingly, Grainger *et al*. reported that *TGF* -509 TT-homozygous individuals had higher plasma concentrations of *TGF-B*1 than TC heterozygous or CC

Table VII. *Epidermal growth factor (EGF), Transforming growth factor beta 1 (TGF-B1) and Tumor necrosis factor-alpha (TNF-A) genotypes and allelic frequencies in patients with wound infection.*

Genotypes	Wound infection		Total	Fisher's exact test	
	yes (%)	no (%)		*p*-Value(2-Sided)	*p*-Value (1-Sided)
EGF			Censored=0		
AA	4(40%)	26 (35.1%)	30	1.000	
GG	2 (20%)	18 (24.3%)	20		
AG	4 (40%)	30 (40.5%)	34		
A allele	12 (60%)	82 (55.4%)	94	0.812	0.444
G allele	8 (40%)	66 (44.6%)	74		
AA *versus* GG				1.000	0.544
AA *versus* AG				1.000	0.572
GG *versus* AG				1.000	0.609
*TGF-B*1			Censored=0		
TT	1 (7.1%)	12 (11.3%)	13	0.788	
CC	5 (35.7%)	42 (39.6%)	47		
TC	8 (57.1%)	52 (49.1%)	60		
T allele	10 (35.7%)	76 (35.8%)	86	1.000	0.583
C allele	18 (64.3%)	136 (64.2%)	154		
TT *versus* CC				0.690	0.495
TT *versus* TC				0.690	0.495
CC *versus* TC				0.771	0.454
TNF-A			Censored=0		
AA	2 (14.3%)	2 (1.9%)	4	0.086	
GG	9 (64.3%)	80 (74.1%)	89		
AG	3 (21.4%)	26 (24.1%)	29		
A allele	7 (25%)	30 (13.9%)	37	0.157	0.107
G allele	21 (75%)	186 (86.1%)	207		
AA *versus* GG				0.067	0.067
AA *versus* AG				0.099	0.099
GG *versus* AG				1.000	0.606

homozygous individuals (41). Our findings are in general agreement with previous descriptions which showed negative effects of *TGF-B*1 on anastomotic healing and tissue regeneration (42).

The present study showed that patients with *EGF*-61 AG genotypes had a significantly longer survival than those with *EGF*-61 AA genotypes. Individuals with the G/G genotype have a higher production of *EGF* than those with *EGF* AA (28). The *EGF*-61* GG genotype and G allele have been described to be significantly correlated with pancreatic carcinoma (9). The EGF-EGFR signaling pathway plays an important role in regulating cell proliferation, migration, adhesion, and inflammatory processes, and is therefore tightly linked to tumorigenesis and tumor progression. Both are dependent on the levels of circulating EGF. The expression of *EGF* or *EGF*-R alone in pancreatic cancer does not reflect the prognosis of patients; however, the co-expression of *EGF* and *EGF*-R may be a potential prognostic factor for pancreatic cancer (43). It will be a topic of future studies to show if EGF levels are associated with a bad prognosis.While no *TGF-B* genotype had a correlation with survival, patients homozygous for *TNF-A*-308 AA had a lower postoperative survival duration than AG heterozygous and GG homozygous individuals. The *TNF-A*-308* A allele has been shown to increase the constitutive and inducible expression of TNF-A protein, possibly by the differential binding of a nuclear protein to the *TNF-A*-308 A allele (44, 45). It has also been reported that the TNF-A protein inhibits apoptosis of pancreatic cancer cells. Moreover, higher TNF-A levels are measured in the serum of patients with pancreatic cancer, especially in those with advanced disease, and these levels are associated with poor nutritional status. This is consistent with previous studies about the association between *TNF-A* and the long term prognosis of pancreatic carcinoma (33).

In summary, we observed a significant association of the *EGF* -61*AG and the *TNF-A* -308* AG polymorphisms with poor survival duration of patients with resectable pancreatic cancer. The presence of wound infection was associated with a poor prognostis. The *TGF-B*1-509* T allele-bearing genotypes were significantly associated with the occurrence of insufficiency of the biliodigestive and pancreatic anastomosis. All genotype distribution and allelic frequencies in the present

study were in agreement with those quoted in literature (33, 42). Certainly, cancer is a multi-factorial disease. Nevertheless, the present research may be helpful in establishing prognostic and predictive factors for pancreatic cancer. Genetic profiling of patients may provide the fundamental information required for future individualized therapy. Further studies on larger numbers of samples are required to strengthen these results and guide future investigations.

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