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TGFB1 and *TGFBR1* polymorphic variants in relationship to bladder cancer risk and prognosis

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Abstract

The TGF-β signalling pathway plays an important role in tumor development and progression. We aimed at analyzing whether seven different common variants in genes coding for two key members of the TGF-β signalling pathway (*TGFB1* and *TGFBR1*) are associated with bladder cancer risk as well as prognosis. A total of 1,157 cases with urothelial cell carcinoma of the bladder and 1,157 matched controls where genotyped for three SNPs in *TGFB1* (rs1982073, rs1800472, rs1800471) and an additional three SNPs and one indel polymorphism in *TGFBR1* (rs868, rs928180,

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rs334358, and rs11466445, respectively). In the case-control study, we estimated odds ratios and 95% confidence intervals for each individual genetic variant using unconditional logistic regression adjusting for age, gender, study area and smoking status. Survival analysis was performed using the Kaplan-Meier method and Cox models. The endpoints of interest were tumor relapse, progression, and death from bladder cancer. All the SNPs analyzed showed a similar distribution among cases and controls. The distribution of the TGFBR1*6A allele (rs11466445) was also similar among cases and controls, indicating no association with bladder cancer risk. Similarly, none of the haplotypes was significantly associated with bladder cancer risk. Among patients with muscle-invasive tumors, we found a significant association between *TGFBR1*-rs868 and disease-specific mortality with an allele dosage effect (p-trend=0.003). In conclusion, the genetic variants analysed were not associated with an increased risk of bladder cancer. The association of *TGFBR1*-rs868 with outcome should be validated in independent patient series.

Keywords

Urinary bladder cancer; genetic susceptibility; Transforming Growth Factor beta; Transforming Growth Factor beta receptor; prognosis; survival; recurrence; progression

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The Transforming Growth Factor-beta (TGF-β) pathway plays an important role in tumor development and progression. There is extensive evidence that TGF-β has antiproliferative effects and can hinder the early steps of tumor progression in several cell types. By contrast, recent evidence has suggested that TGF-β can also play an important role in later steps of tumor progression where it accelerates invasion and metastasis (1,2).

The TGF-β pathway is altered in a wide variety of cancers, including urinary bladder tumors. TGF-β receptors (TGFBR) as well as other proteins involved in TGF-β signalling, such as Smads, have been shown to be targets of somatic mutations in several cancers including those of the colorectum, pancreas, and lung. Furthermore, altered levels of ligand and receptors have been described in the neoplastic tissue (3).

More recently, there has been interest in the contribution of genetic variation in this pathway to cancer susceptibility. Several polymorphic variants in genes involved in this pathway have been studied. Single nucleotide polymorphisms (SNP)in *TGFB1* (exon 1: 327 C>T, rs1982073; exon 5: 73 C>T, rs1800472), coding for one of the secreted ligands, have been reported to be associated with breast, colorectal, lung, and nasopharyngeal carcinomas and non-Hodgkin lymphomas (4–7). A larger number of studies have dealt with genetic variation in *TGFBR1*, which codes for one of the two subunits of the heterodimeric membrane receptor kinase (8). The most extensively studied variant allele is *TGFBR1*6A*, rs11466445, which corresponds to a trinucleotide repeat coding for an Ala repeat. The most common allele among Caucasians contains 9 Ala residues whereas the 6 Ala variant is less common and has been reported to be a susceptibility allele for colorectal and breast cancer (9–10). Somatic acquisition of this trinucleotide variant has also been reported in colon cancer (11). Very few studies have analyzed its association with bladder cancer risk and their results are not conclusive (12).

In this study, we analyzed in a prospectively recruited large case-control population whether seven different common polymorphisms in genes coding for two key members of the TGF-β signalling pathway (*TGFB1* and *TGFBR1*) are associated with bladder cancer risk as well as with patient's prognosis.

MATERIAL AND METHODS

Study population

A detailed description of the study population has been reported elsewhere (13). In brief, 1,219 patients with a new diagnosis of bladder cancer, aged 21–80 years (mean=66 years, SD=10), were recruited in 18 hospitals in Spain between 1998–2001. Sections of paraffin-embedded blocks from the initial tumor were reviewed by a panel of pathologists using the 1999 World Health Organization classification for urothelial lesions (14) with modifications as described elsewhere (15). Controls were 1,271 subjects selected from participating hospitals with diagnoses thought to be unrelated to the exposures of interest, individually matched to the cases by age at diagnosis (± 5 years), gender, ethnicity, and study area. Blood and/or buccal-cell samples were provided by 1,188 cases and 1,173 controls. Exclusions were made to reduce heterogeneity (cases with non-urothelial histology and non-white subjects) or because of low amounts of DNA. The final study population available for the case-control analysis was 1,157 cases and 1,157 controls (16). Mean age of cases and controls was 66 and 65 years, respectively; 13% of subjects were female. Fourty six and 47% of cases and controls, respectively, had attained primary education and there were no significant differences among the two groups regarding this variable. Fourteen percent and 28% of cases and controls were non-smokers, respectively. Eighty-two percent and 63% of cases and controls were former or current smokers, respectively. The remaining subjects in each group were classified as occasional smokers.

The survival analyses included the 1,105 cases for whom pathological review of the initial tumor was possible, of whom 859 (77.7%) had non-muscle invasive and 246 (22.3%) had muscle-invasive tumors. All study subjects were interviewed during their first hospital admission. Patient's clinical records were reviewed annually in order to obtain information about the outcomes of interest and any treatment change. Furthermore, a telephone interview was performed on an annual basis to complete information on tumor progression and patient's vital status. Last complete follow-up was as of December 31, 2005 (15).

For the survival analyses, the endpoints of interest were: tumor relapse, defined as reoccurrence of a tumor of any stage or grade after transurethral resection (TUR); tumor progression, defined as development of a muscle-invasive tumor - for patients with non-muscle invasive bladder cancer - or any tumor progression event for patient with muscle-invasive bladder cancer; and disease-specific mortality (DSM), considering the time from TUR to death from bladder cancer. Patients who died because of bladder cancer without having been diagnosed with a tumor progression were considered uncensored at the midpoint period between TUR and death for estimation of time to progression. For patients with non-muscle invasive tumors, recurrence was defined as the reappearance of a non-muscle invasive tumor (pTa or pT1) and progression as the development of a muscle invasive mass (≥pT2). For patients whose tumors were initially classified as muscle-invasive, any

tumor reappearance after treatment was considered progression, regardless of its location. The median follow-up time for patients who were free-of-disease at the end of follow-up was 64.4 months (range 0.2–90.2 months).

Genotype assays

A total of six SNPs in *TGFB1* (rs1982073, rs1800472, and rs1800471) and *TGFBR1* (rs868, rs928180, and rs334358) were selected from the SNP500Cancer database (http://snp500cancer.nci.nih.gov) for genotyping. In addition, an insertion/deletion polymorphism (indel) in *TGFBR1* (rs11466445) was studied.

SNPs in *TGFB1* and *TGFBR1* were investigated using germline DNA as previously described (16). The SNP genotype assays were performed at the Core Genotyping Facility at the US National Cancer Institute using TaqMan® assays (Applied Biosystems, Foster City, CA, USA) for *TGFB1* Ex1–327C>T (rs1982073) and Ex5–73C>T (rs1800472), and a GoldenGate® assay (Illumina®, San Diego, CA, USA) for *TGFB1* Ex1–282C>G (rs1800471), *TGFBR1* IVS3-intronic SNPs 2409A>G (rs928180), and IVS8+547G>T (rs334358) and Ex9+195A>G (rs868) in the 3' untranslated region.

In addition, the *TGFBR1*6A* (rs11466445) exon 1 polymorphic variant allele, leading to the deletion of three Ala residues from a nine-Ala (9A) stretch in the wild-type allele, was genotyped. The length of this GCG repeat was determined by PCR analysis with a 5´ fluorescence-labelled forward primer and capillary electrophoresis. Details on genotyping assays have been reported elsewhere (10,11).

Statistical analysis

Hardy-Weinberg equilibrium was checked among the control population. In the case-control study, we estimated odds ratio (OR) and 95% confidence interval (95%CI) for each individual SNP using unconditional logistic regression adjusting for age, gender, study area, and smoking status (non-smoker, occasional, former, and current smoker). Interaction effects between SNPs and smoking status were assessed by fitting the model with and without the interaction parameters and conducting a likelihood ratio test. Haplotype frequencies, OR and 95%CI for genes showing blocks of linkage disequilibrium were estimated using SNPStats (http://bioinfo.iconcologia.net/SNPstats) (17).

Survival curves were estimated using the Kaplan-Meier product limit method and the differences between categories of each variable were assessed using the log-rank and Breslow tests. Hazard ratios (HR) and 95%CI were estimated using Cox models. The following variables were considered for adjustment: geographical area, gender, age, stage, grade, tumor size, tumor location in the bladder, number of tumors, presence of metastases, and treatment. For each of the analyses reported, variables used to adjust the final model are specified in the table footnotes. Survival analyses were performed separately for non-muscle invasive and muscle-invasive tumors. Haplotype effects in censored data analysis were estimated using the Thesias 3.1 software (http://genecanvas.ecgene.net) (18).

To correct for false discovery rate (FDR), the Benjamini-Hochberg test was applied for each of the p-trend values in Tables 1, 3, and 4 (19).

RESULTS

Genetic variation in *TGFB1* and *TGBR1* and bladder cancer susceptibility

Genotype distribution in the control population did not deviate significantly from that expected for a population in Hardy-Weinberg equilibrium. The three *TGFB1* SNPs analyzed showed a similar distribution among cases and controls; a lower, non-significant, risk of bladder cancer was found for the rs1800471 genotype (p for trend=0.081) (Table 1). Regarding the *TGFBR1* polymorphisms analyzed, several were in strong positive linkage disequilibrium: rs868 with rs334358 (D'=0.99, r^2=0.99) and rs928180 with rs11466445 (D'=0.98, r^2=0.91). Therefore, only the results for those with the least amount of missing data (rs334358 and rs11466445) were included in the haplotype analysis. All the SNPs analyzed showed a similar distribution among cases and controls (Table 1). The distribution of the *TGFBR1*6A* allele was also similar among cases and controls (Table 1) (p=0.744), indicating no association with bladder cancer risk. Similarly, none of the haplotypes was significantly associated with bladder cancer risk (Table 2).

Genetic variation in *TGFB1* and *TGFBR1* and bladder cancer prognosis

In the survival analyses, the Cox model for *TGFB1* and *TGFBR1* polymorphisms rendered no significant association with tumor relapse and progression among cases with non-muscle invasive bladder tumors (Table 3). By contrast, in patients with muscle-invasive tumors, we found a significant association between *TGFBR1* rs868 and DSM. The G allele was an independent predictor of bladder cancer mortality with an allele dosage effect: the HR were 1.85 (95%CI: 1.15–2.97) for heterozygous patients (A/G) and 3.00 (95%CI: 1.15–7.82) for homozygous G/G patients (p-trend= 0.003) (Table 4). SNP rs334358 also showed an association with DSM, as expected because of the linkage disequilibrium with rs868. FDR values for these two SNP associations were 0.036 and 0.054, respectively, based on the p-values for trend (1 df) of all variants evaluated in this study. Regarding haplotype analyses, we found a statistically significant increase in risk of death from bladder cancer only for patients with invasive tumors carrying alleles IVS3–2409A (rs928180) and IVS8+547T (rs334358): HR=1.50 (95%CI: 1.04–2.16) (p=0.030) (Supplementary Table 1).

DISCUSSION

There is extensive evidence that the TGF-β pathway plays an important role in cancer development and progression. While TGF-β has an antitumor role in early carcinogenesis, there is extensive evidence that it can foster progression at later stages of tumor evolution (1,2). For these reasons, there is a great interest in analyzing the role of genetic variation in *TGFB* and the *TGFB* receptor in cancer susceptibility and disease progression.

In this study, we selected *TGFB1* polymorphisms leading to non-synonymous amino acid changes. SNPs rs1982073 (Leu10Pro) and rs1800471 (Arg25Pro), coding for residues located in the signal peptide, have been related with TGF-β1 expression levels in leukocytes (20,21). In addition, an association between these polymorphisms and the risk of developing breast, colorectal, lung and nasopharyngeal carcinomas has been reported (4,7). SNP rs1800472 (Thr263Ile) is located at exon 5 and the corresponding residue lies near the site

where the latency-associated peptide is cleaved from the active peptide. Therefore, this polymorphism may be related to the activation of TGF-β1, as was previously suggested (22).

The *TGFBR1* polymorphisms analyzed include two intronic SNPs (IVS3–2409A>G and IVS8+54G>T) and one in the 3′ untranslated region (Ex9+195A>G). To our knowledge, these polymorphisms have not previously been associated with cancer risk. The fourth polymorphism analyzed in this gene is a coding indel in exon 1 within the leader peptide sequence. The *TGFBR1*6A* allele functions as a less effective mediator of TGF-β-antiproliferative signals (10). There have been many studies analyzing cancer susceptibility associated with this allele in patients with a variety of tumors. Individuals carrying the *TGFBR1*6A* allele have been reported to have an increased risk for breast and ovarian cancers (9). Whether this allele confers an increased susceptibility to colorectal cancer is controversial (9–12, 23). Until now, the evidence on the risk of bladder cancer associated with the *TGFBR1*6A* allele has been inconclusive. Only a few studies have been reported, all of which included a very small number of cases and controls (12). Van Tilborg et al (24) examined 146 patients with transitional carcinoma of the bladder and 183 controls not matched for age, sex, or ethnicity and found no association with bladder cancer risk. Our study, the largest of this association reported to date, does not show an association for any of the polymorphisms analyzed and bladder cancer risk. Because of its large size and the largely unbiased nature of the patient population, the results of this study are strongly suggestive of the null effect of the studied polymorphisms in bladder cancer susceptibility. However, we cannot rule out that other genetic variants in these genes may confer a risk for this cancer.

The finding that the *TGFBR1* rs868 allele is strongly and independently associated with DSM is potentially relevant. The strong experimental evidence that TGF-β plays an important role fostering progression at late stages of tumor evolution would be consistent with this association (1,2). This result warrants further investigation in larger series to test possible explanations such as the selective involvement in regional vs. distant metastatic spread and an association with treatment response. Little is known regarding the functional role of the rs868 variant that would support its causal association with DSM; it is also possible that other genetic variants in linkage disequilibrium with rs868 account for the association observed.

Our study was hypothesis-driven and involved a small number of genetic variants. Based on FDR calculations, the associations with DSM are likely not to be due to chance (19) although further work is required to confirm this association in independent patient series. The establishment of polymorphic variants of genes in the TGF-β pathway as prognostic markers may contribute to an improved management of bladder cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

DSM	disease-specific mortality
TGF	Transforming Growth Factor
TGFBR	Transforming Growth Factor-beta Receptor
TUR	transurethral resection

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Statement of clinical relevance

In this manuscript we provide two important pieces of information. First, we show in a large case-control study carried out in Spain that genetic variants in *TGFB1* and *TGFBR1* that have been proposed to play a role as susceptibility factors in a wide variety of cancers do not seem to confer an increased risk for development urinary bladder cancer. In addition, we show that – among patients with muscle-invasive tumors - the variant *TGFBR1*-rs868 is independently associated with disease-specific mortality with an allele dosage effect (p-trend=0.003). If confirmed in independent series, the latter finding might be applicable to identify subjects with muscle-invasive bladder tumors who are at greater risk of death from bladder cancer.

Annex1. Participating Study Centers in Spain

Institut Municipal d'Investigació Mèdica, Universitat Pompeu Fabra, Barcelona – Coordinating Center (M. Sala, G. Castaño, M. Torà, D. Puente, C. Villanueva, C. Murta-Nascimento, J. Fortuny, E. López, S. Hernández, R. Jaramillo); Hospital del Mar, Universitat Autònoma de Barcelona, Barcelona (J. Lloreta, S. Serrano, L. Ferrer, A. Gelabert, J. Carles, O. Bielsa, K. Villadiego), Hospital Germans Trias i Pujol, Badalona, Barcelona (L. Cecchini, J.M. Saladié, L. Ibarz); Hospital de Sant Boi, Sant Boi de Llobregat, Barcelona (M. Céspedes); Consorci Hospitalari Parc Taulí, Sabadell (D. García, J. Pujadas, R. Hernando, A. Cabezuelo, C. Abad, A. Prera, J. Prat); Centre Hospitalari i Cardiològic, Manresa, Barcelona (M. Domènech, J. Badal, J. Malet); Hospital Universitario de Canarias, La Laguna, Tenerife (J. Rodríguez de Vera, A.I. Martín); Hospital Universitario Nuestra Señora de la Candelaria, Tenerife (FJ. Taño, F. Cáceres); Hospital General Universitario de Elche, Universidad Miguel Hernández, Elche, Alicante (F. García-López, M. Ull, A. Teruel, E. Andrada, A. Bustos, A. Castillejo, J.L. Soto); Universidad de Oviedo, Oviedo, Asturias; Hospital San Agustín, Avilés, Asturias (J.L. Guate, J.M. Lanzas, J. Velasco); Hospital Central Covadonga, Oviedo, Asturias (J.M. Fernández, J.J. Rodríguez, A. Herrero), Hospital Central General, Oviedo, Asturias (R. Abascal, C. Manzano, T. Miralles); Hospital de Cabueñes, Gijón, Asturias (M. Rivas, M. Arguelles); Hospital de Jove, Gijón, Asturias (M. Díaz, J. Sánchez, O. González); Hospital de Cruz Roja, Gijón, Asturias (A. Mateos, V. Frade); Hospital Alvarez-Buylla (Mieres, Asturias): P. Muntañola, C. Pravia; Hospital Jarrio, Coaña, Asturias (A.M. Huescar, F. Huergo); Hospital Carmen y Severo Ochoa, Cangas, Asturias (J. Mosquera).

Table 1.

Association of *TGFB1* and *TGFBR1* polymorphisms with bladder cancer risk.

SNP	Cases	Controls	OR†	95%CI	p-LRT*	p-trend
TGFB1						
rs1982073 Ex1–327C>T					0.648	0.768
TT	376	377	1.00	Ref		
CT	517	463	1.09	0.90–1.33		
CC	189	175	1.01	0.78–1.31		
rs1800472 Ex5–73C>T					0.270	NA
CC	1013	949	1.00	Ref		
CT	81	71	1.10	0.77–1.55		
TT	0	1	NA	NA		
rs1800471 Ex1–282C>G					0.220	0.081
GG	976	914	1.00	Ref		
CG	129	142	0.80	0.61–1.05		
CC	7	9	0.70	0.25–1.94		
TGFBR1						
rs868 Ex9+195A>G					0.102	0.475
AA	680	639	1.00	Ref		
AG	351	316	1.07	0.88–1.30		
GG	41	60	0.66	0.43–1.02		
rs928180 IVS3–2409A>G					0.918	0.779
AA	905	849	1.00	Ref		
AG	175	179	0.96	0.75–1.22		
GG	5	5	1.14	0.29–4.42		
rs334358 IVS8+547G>T					0.239	0.682
GG	683	650	1.00	Ref		
GT	359	327	1.07	0.88–1.29		
TT	42	56	0.73	0.47–1.12		
TGFBR1 exon 1					0.744	0.704
*9A/*9A	887	812	1.00	Ref		
*6A/*9A	199	191	0.99	0.79–1.25		
*6A/*6A	8	11	0.68	0.26–1.81		

†Adjusted for geographical area, gender, age and smoking status

NA, not applicable

*p-LRT, p value from the likelihood ratio test

Table 2.

Association of *TGFB1* and *TGFBR1* haplotypes with bladder cancer risk.

Polymorphisms			Haplotype frequency		OR†	95%CI	p-value#	Global p-value
			Cases	Controls				
TGFB1								
rs1982073	rs1800472	rs1800471						0.270
T	C	G	0.59	0.60	1.00	Ref		
C	C	C	0.31	0.29	1.07	0.93–1.23	0.38	
C	C	C	0.06	0.07	0.83	0.65–1.05	0.13	
C	T	G	0.04	0.04	1.06	0.75–1.50	0.75	
TGFBR1								
rs928180	rs334358							0.730
A	G		0.71	0.69	1.00	Ref		
A	T		0.20	0.21	0.95	0.82–1.11	0.52	
G	G		0.09	0.09	0.93	0.74–1.17	0.56	

†Adjusted for geographical area, gender, age and smoking status

#Simulated maximum score p-value

Table 3.

Cox model for *TGFB1* and *TGFBR1* polymorphisms and outcome in patients with non-muscle invasive bladder tumors.

SNP	Cases	Tumor relapse†					Progression‡				
		N	HR	95%CI	p-LRT	p-trend	N	HR	95%CI	p-LRT	p-trend
TGFB1											
rs1982073					0.379	0.182				0.364	0.236
TT	280	110	1.00	Ref			25	1.00	Ref		
CT	375	128	0.93	0.72–1.20			34	0.96	0.57–1.61		
CC	143	41	0.78	0.54–1.12			10	0.61	0.29–1.29		
rs1800472					0.674	NA				0.671	NA
CC	743	256	1.00	Ref			62	1.00	Ref		
CT	62	21	1.10	0.70–1.73			5	0.82	0.33–2.06		
TT	0	0	NA	NA			0	NA	NA		
rs1800471					0.916	0.709				0.643	NA
GG	713	247	1.00	Ref			58	1.00	Ref		
CG	100	36	1.08	0.76–1.54			11	1.24	0.65–2.39		
CC	6	2	1.00	0.25–4.05			0	NA	NA		
TGFBR1											
rs868					0.088	0.811				0.143	0.799
AA	509	174	1.00	Ref			39	1.00	Ref		
AG	259	95	1.19	0.92–1.54			28	1.38	0.84–2.24		
GG	26	5	0.52	0.21–1.28			1	0.33	0.04–2.41		
rs928180					0.947	0.889				0.199	NA
AA	669	230	1.00	Ref			54	1.00	Ref		
AG	131	47	0.99	0.72–1.36			15	1.22	0.68–2.16		
GG	3	1	0.73	0.10–5.30			0	NA	NA		
rs334358										0.172	0.860
GG	508	171	1.00	Ref	0.107	0.551	39	1.00	Ref		
GT	267	99	1.23	0.95–1.58			28	1.33	0.82–2.17		
TT	27	6	0.63	0.28–1.43			1	0.33	0.05–2.42		
TGFBR1 exon 1										0.232	NA
*9A/*9A	655	249	1.00	Ref	0.363	0.922	51	1.00	Ref		
*6A/*9A	142	53	1.07	0.79–1.45			17	1.31	0.75–2.30		
*6A/*6A	6	1	0.33	0.05–2.39			0	NA	NA		

†Adjusted for gender, stage-grade, tumor size, number of tumors and treatment

‡Adjusted for stage-grade and tumor site

NA, not applicable

Table 4.

Cox model for *TGFB1* and *TGFBR1* polymorphisms and outcome in patients with muscle invasive bladder tumors.

SNP	Cases	Progression†					Disease-specific mortality‡				
		N	HR	95%CI	p-LRT	p-trend	N	HR	95%CI	p-LRT	p-trend
TGFB1											
rs1982073					0.931	0.796				0.533	0.718
TT	78	41	1.00	Ref			33	1.00	Ref		
CT	116	65	1.09	0.69–1.71			49	0.89	0.56–1.39		
CC	35	17	1.05	0.56–1.98			15	1.26	0.67–2.35		
rs1800472					0.059	NA				0.073	NA
CC	218	113	1.00	Ref			88	1.00	Ref		
CT	16	12	2.02	1.03–3.97			11	1.90	0.99–3.65		
TT	-	0	NA	NA			0	NA	NA		
rs1800471					0.102	NA				0.080	NA
GG	215	119	1.00	Ref			96	1.00	Ref		
CG	22	7	0.46	0.20–1.06			4	0.40	0.15–1.11		
CC	1	0	NA	NA			0	NA	NA		
TGFBR1											
rs868					0.531	0.341				**0.013**	**0.003**
AA	134	67	1.00	Ref			52	1.00	Ref		
AG	81	44	1.29	0.83–2.03			35	1.85	1.15–2.97		
GG	10	6	1.16	0.44–3.03			5	3.00	1.15–7.82		
rs334358					0.801	0.547				**0.032**	**0.009**
GG	137	70	1.00	Ref			54	1.00	Ref		
GT	80	42	1.16	0.74–1.82			34	1.67	1.05–2.68		
TT	10	6	1.13	0.43–2.95			5	2.83	1.09–7.34		
rs928180					0.259	0.259				0.813	-
AA	191	99	1.00	Ref			77	1.00	Ref		
AG	36	19	1.39	0.80–2.41			16	1.07	0.61–1.87		
GG	0	0	NA	NA			0	NA	NA		
TGFBR1 exon 1					0.752	NA				0.943	NA
*9A/*9A	165	86	1.00	Ref			81	1.00	Ref		
*6A/*9A	41	21	1.09	0.65–1.81			21	1.02	0.61–1.69		
*6A/*6A	0	0	NA	NA			0	NA	NA		

†Adjusted for stage, localization, metastasis and treatment

‡Adjusted for age, stage, localization and metastasis

NA, not applicable