

## Significant Associations of Mismatch Repair Gene Polymorphisms With Clinical Outcome of Pancreatic Cancer

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Terms in blue are defined in the glossary, found at the end of this article and online at [www.jco.org](http://www.jco.org).

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### ABSTRACT

#### Purpose

DNA mismatch repair (MMR) is critical in maintaining genomic stability and may modulate the cellular response to gemcitabine. We hypothesized that genetic variations in MMR may affect the clinical outcome of patients with pancreatic cancer.

#### Patients and Methods

We evaluated 15 single-nucleotide polymorphisms (SNPs) of eight MMR genes in 154 patients with potentially resectable pancreatic adenocarcinoma who were enrolled onto phase II clinical trials for preoperative gemcitabine-based chemoradiotherapy from 1999 to 2006. Associations of genotypes with tumor response to therapy (change of tumor size by radiologic evaluation at restaging), margin-negative tumor resection, and overall survival were evaluated using logistic regression and Cox proportional regression models.

#### Results

Five, six, and 10 genotypes were significantly associated with tumor response to preoperative chemoradiotherapy, tumor resectability, and overall survival, respectively, in univariable analysis. *TREX1* EX14-460C>T and *TP73* Ex2+4G>A genotypes remained as significant predictors for tumor response, *MLH1* IVS12-169C>T and *TP73* remained as significant predictors for tumor resectability, and *EXO1* R354H, *TREX1*, and *TP73* remained as significant predictors for overall survival in multivariable models that included all clinical factors and genotypes examined. A strong combined genotype effect on each clinical end point was observed. For example, 20 of the 25 patients with zero to one adverse genotypes were alive, those with two, three, four, five, and six to seven adverse genotypes had median survival times of 36.2, 23.9, 16.3, 13.0, and 8.3 months, respectively ( $P < .001$ ).

#### Conclusion

SNPs of MMR genes have a potential value as predictors for clinical response to chemoradiotherapy and as prognostic markers for tumor resectability and overall survival of patients with resectable pancreatic cancer.

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### INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer deaths in this country and has the lowest 5-year survival rate among all malignancies.<sup>1</sup> Pancreatic tumors are highly aggressive and resistant to most treatments. Gemcitabine has been used as a major chemotherapeutic choice for pancreatic cancer, but factors influencing individual variations in response to gemcitabine are not well defined. Recent developments in pharmacogenetics have shown that genetic variations in drug metabolism, drug resistance, and cellular response to genotoxic stress affect individual responses to cytotoxic therapies, and these variations may serve as novel prognostic markers for stratifying pa-

tients in clinical trials or for predicting response to cytotoxic therapy.<sup>2,3</sup>

The DNA repair pathways responsible for the repair of gemcitabine-induced DNA damage are not clearly understood.<sup>4</sup> Gemcitabine inhibits DNA synthesis by targeting ribonucleotide reductase, which results in deoxyadenosine triphosphate depletion, causing DNA replication errors.<sup>5</sup> Gemcitabine is also incorporated into DNA and arrests DNA replication via a "masked chain termination" mechanism.<sup>6</sup> Both the mispaired bases and gemcitabine-modified DNA bases can be the substrates for postreplicative DNA mismatch repair (MMR) machinery. If MMR were responsible for the removal and repair of gemcitabine-induced DNA damage, one would expect deficient MMR to confer a better

response to gemcitabine. Indeed, MMR-deficient cells were more sensitive in vitro to gemcitabine-mediated radiosensitization.<sup>7</sup> MMR also plays a key role in maintaining genome stability, so MMR deficiency could lead to genome-wide instability and aggressive tumor phenotypes through rapid accumulation of genetic alterations. However, the role of MMR in pancreatic cancer is not clear. Germline mutations or inactivation of MMR genes by promoter hypermethylation is uncommon in this disease.<sup>8</sup> Some investigators suggested that pancreatic cancers with MMR deficiency have characteristic histologic features and may have an improved prognosis,<sup>9</sup> but others found no association between MMR and long-term survival among patients with pancreatic cancer.<sup>10</sup>

Our previous work showed that polymorphic variants of DNA homologous recombination genes (eg, *RecQ1* and *RAD54L*) had a significant effect on the overall survival of patients with pancreatic cancer treated with preoperative gemcitabine and radiation.<sup>11</sup> *RecQ1* has been reported to interact with DNA MMR proteins<sup>12</sup>, which raised the question of whether the effect of *RecQ1* genotype on patient survival was related to DNA MMR capacities. The current study evaluated 15 single-nucleotide polymorphisms (SNPs) of eight MMR genes: exonuclease I (*EXO1*), mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 3 (*MSH3*), mutS homolog 6 (*MSH6*), postmeiotic segregation increased 1 (*PMS1*), three prime repair exonuclease 1 (*TREX1*), and tumor protein 73 (*TP73*). SNPs were selected if they met at least two of the following three criteria: (1) the minor allele frequency is greater than 5% among white patients, (2) nonsynonymous SNP or SNP occurs at the 3'UTR, 5'UTR, or the splicing site, and (3) SNPs have been associated with cancer risk or clinical outcome in previous investigations.

## PATIENTS AND METHODS

### Patient Recruitment and Data Collection

Our study involved 154 patients who, at the time of their diagnosis, had potentially resectable adenocarcinoma of the head of the pancreas and

had not received any treatment for pancreatic cancer. All patients had been enrolled in one of the two phase II clinical trials (ID98-020 and ID01-341) of preoperative chemoradiotherapy that were conducted sequentially from February 1999 through June 2006,<sup>13,14</sup> with continuing observation through June 2008. A DNA sample was collected from 154 patients for genotyping assays. The protocols of these trials were approved by the institutional review board of The University of Texas M. D. Anderson Cancer Center (Houston, TX) and were conducted in accordance with all current ethical guidelines.

The patients from the ID98-020 trial (N = 70) had received chemoradiotherapy with seven weekly intravenous (IV) infusions of gemcitabine (400 mg/m<sup>2</sup> IV over 30 minutes) plus radiation therapy (30 Gy in 10 fractions over 2 weeks). For the patients from the ID01-341 trial (N = 84), chemotherapy consisted of gemcitabine (750 mg/m<sup>2</sup>) and cisplatin (30 mg/m<sup>2</sup>) given every 2 weeks for four doses. Chemoradiotherapy consisted of four weekly infusions of gemcitabine (400 mg/m<sup>2</sup>) combined with radiation therapy (30 Gy in 10 fractions administered over 2 weeks) delivered 5 days per week. The same eligibility criteria for patient recruitment had been applied in both trials, and no significant difference in any clinical feature was observed between the two patient populations.<sup>13,14</sup>

Tumor response to therapy was evaluated by computed tomography scan before and after completion of the preoperative chemoradiotherapy. Tumor response was defined according to the Response Evaluation Criteria in Solid Tumors as partial response, stable disease, or progressive disease. Tumor resectability was defined by achievement of margin-negative resection. Postsurgical treatment or treatment received after tumor recurrence was not considered in this study. Overall survival and disease-free survival times were calculated from the date of enrollment on the protocol or date of tumor resection, respectively, to the date of death or date of last follow-up.

### DNA Extraction and Genotyping

DNA was extracted from peripheral-blood lymphocytes of 127 patients and from paraffin sections of normal adjacent tissues of 27 patients with resected tumors (20 from the ID98-020 trial) using Qiagen DNA isolation kits (Valencia, CA). Normal and tumor tissues are expected to have the same genotype for these germline common polymorphic sequence variants. Genotyping used the mass spectroscopy-based MassArray method (Sequenom Inc, San Diego, CA). Twenty percent of the samples

**Table 1.** SNPs Examined and Genotype Frequency

Gene	SNP	Function	RS No.	No. of Patients		Minor Allele Frequency*		
				M/M	M/m	m/m	Observed	Reported
<i>EXO1</i>	Ex11+20G>A	R354H	735943	47	71	36	0.464	0.485
	Ex12+105G>A	V458M	4149965	98	50	6	0.201	0.242
	Ex15+59C>T	P757L	9350	111	40	3	0.149	0.121
<i>MLH1</i>	-92G>A	Promoter	1800734	87	55	12	0.256	0.200
	IVS12-169C>T	Intron	2286940	52	68	34	0.452	0.414
<i>MSH2</i>	Ex8-41G>A	V213M	2308317	152	2	0	0.001	0.020
	Ex6+23G>A	G322D	4987188	135	18	1	0.064	0.032
	IVS12-6T>C	Splicing site	2303428	97	52	5	0.201	0.145
<i>MSH3</i>	Ex4-100G>A	P231P	1805355	128	23	3	0.094	0.032
	Ex23+3A>G	T1045A	26,279	80	60	14	0.286	0.345
<i>MSH6</i>	Ex1-145G>A	G39E	1042821	124	27	3	0.107	0.207
<i>PMS1</i>	Ex1-4G>C	5'UTR	5742933	98	46	10	0.214	0.161
<i>TREX1</i>	Ex14-460C>T	3'UTR	11,797	41	81	32	0.470	0.431
	Ex2-9A>C	K125Q	11925638	148	4	0	0.001	0.045
<i>TP73</i>	Ex2+4G>A	5'UTR	2273953	88	52	14	0.260	0.234

Abbreviations: SNP, single-nucleotide polymorphism; RS No., reference SNP identification number; MM, Major/major allele of the SNP; Mm, Major/minor allele of the SNP; mm, minor/minor allele of the SNP.

\*The reported minor allele frequency was from SNP500Cancer database.

were analyzed in duplicate, with 100% concordance in genotype calling. The genes, nucleotide substitutions, function (such as encoding amino acid changes), reference SNP identification numbers, and reported allele frequencies of the 15 SNPs evaluated in this study are summarized in Table 1.

### Statistical Analysis

The distribution of genotypes was tested for Hardy-Weinberg equilibrium with the goodness-of-fit  $\chi^2$  test. The heterozygous and homozygous genotypes were collapsed in the analysis if the frequency of the homozygous mutant was low or if the homozygous and heterozygous genotypes had the same direction of effect (eg, both had reduced survival time compared with the referent group). The associations between genotypes and tumor response to therapy or tumor resectability were estimated with odds ratios using multivariable unconditional logistic regression with adjustment for all clinical factors, including sex, age, race, tumor size, preoperative treatment (ie, the two clinical protocols), and serum CA19-9 levels at diagnosis, as well as all 15 SNPs examined in this study. The overall survival time and disease-free survival time were estimated using the Kaplan-Meier method. The associations between genotypes and survival time were tested using the log-rank test. The median follow-up time was computed using censored observations only. The effect of genotypes on overall survival time were estimated using hazard ratios (HRs), and 95% CIs were estimated using the multivariate Cox proportional hazards regression model, with adjustment for tumor response and resectability and other clinical factors and all genotypes examined. The effect of genotype on disease-free survival was further adjusted for node status and tumor grade. All statistical testing was conducted with SPSS software version 15.0 (SPSS Inc, Chicago, IL). All tests were two-sided, and a *P* value of less than .05 was considered statistically significant.

We estimated the false-positive report probability (FPRP) for the observed statistically significant associations using the methods described by Wacholder et al.<sup>15</sup> Odds ratio and HR values of 2.0 to 4.0 were considered as a likely threshold values. The prior probability used was 0.25 for all SNPs. The FPRP value for noteworthiness was set at 0.2.

## RESULTS

### Patients' Characteristics and Genotype Frequencies

The patients' characteristics and tumors' clinical features are summarized in Table 2. In the 154 patients included in the current study, age, race, sex, and tumor size were not significantly associated with overall survival in the univariable analysis. The primary tumor was surgically resected in 116 patients (75.3%), and nine of the 116 patients had margin-positive resection (7.7%). At the end of our study (June 2008), 117 (76%) of the 154 patients had died. The median follow-up time was 49.9 months for the patients still alive. The median survival time (MST) of the 154 patients was 21.7 months (95% CI, 17.7 to 25.6 months). The factors that significantly associated with reduced overall survival time included poor tumor differentiation, serum CA19-9 level at diagnosis more than 47 units/mL, unresected tumor, increased tumor size after treatment, and node-positive resection.

Genotype frequencies of this patient population are presented in Table 1. There was a statistically significant difference in the racial distribution of *MLH1* -92G>A, *EXO1* V458M, and *MSH3* P231P, with a higher frequency of the AA (90.1% v 41.7%), GG (18.4% v 5.4%), and GA/AA (30.8% v 10.2%) in nonwhites compared with that of whites, respectively.

**Table 2.** Characteristics of the Study Population

Variable	No. of Patients	No. of Deaths	MST (months)	<i>P</i> *
Age, years				.060
≤ 50	14	14	16.9	
51-60	47	31	33.6	
61-70	62	47	21.5	
> 70	31	25	21.2	
Sex				.510
Male	96	73	20.9	
Female	58	44	24.5	
Race				.690
White	133	103	23.9	
Hispanic	10	7	17.5	
Black	7	4	33.6	
Other	4	3	10.7	
Tumor size, cm				.100
≤ 2	56	39	28.1	
> 2	98	78	20.9	
Tumor differentiation				.040
Well to moderate	89	85	28.7	
Poor	29	23	19.8	
CA19-9, U/mL				.001
≤ 47	40	23	52.8	
48-500	78	61	22.7	
500-1,000	14	13	17.6	
> 1,000	22	20	16.9	
Preoperative treatment				.016
ID98-020	70	51	27.8	
ID01-341	84	66	17.6	
Tumor resection				< .001
No	38	38	10.5	
Yes	116	79	32.6	
Node status				.002
Negative	61	35	39.2	
Positive	55	44	26.4	
Tumor size changes				< .001
PR	48	32	27.8	
SD	83	63	24.5	
PD	23	22	11.0	

Abbreviations: MST, median survival time; PR, partial response; SD, stable disease; PD, progressive disease.  
\**P* values were calculated using the log-rank test.

### Association of Genotypes With Tumor Response to Preoperative Therapy

Five SNPs were significantly associated with tumor response to preoperative therapy, and a combined effect of the five genotypes was observed (Table 3). The effects of *MSH2* IVS12-6T>C, *MSH3* P231P, *TP73* Ex2+4G>A, and the combined genotype on tumor response were statistically significant after adjusting for clinical factors. The effects of *TREX1* Ex14-460C>T, *TP73* Ex2+4G>A SNP, and preoperative treatment on tumor response were statistically significant after further adjusting for all SNPs examined. The FPRP was 0.183 and 0.240 for *TREX1* Ex14-460C>T and *TP73* Ex2+4G>A, respectively, indicating noteworthiness for *TREX1* but not *TP73*.

### Association of Genotypes With Tumor Resectability

Six SNPs were significantly associated with tumor resectability in the univariable analysis (Table 4). A significant combined genotype

**Table 3.** Association of Genotype With Tumor Response to Preoperative Therapy

Genotype	PR/SD		PD		$\chi^2$ P	Odds Ratio*	95% CI	P	Odds Ratio†	95% CI	P
	No.	%	No.	%							
<i>MSH2</i> G322D					.004						
GG	119	88.1	16	11.9		1.00			1.00		
GA/AA	12	63.2	7	36.8		3.20	0.84 to 12.2	.088	1.45	0.17 to 11.8	.730
<i>MSH2</i> IVS12-6T>C					< .001						
TT	90	92.8	7	7.2		1.00			1.00		
TC/CC	41	71.9	16	28.1		4.70	1.51 to 14.7	.008	4.29	0.96 to 19.3	.057
<i>MSH3</i> P231P					.002						
GG	114	89.2	14	10.9		1.00			1.00		
GA/AA	17	65.4	9	34.6		3.63	1.10 to 12.0	.035	3.85	0.65 to 22.8	.138
<i>TREX1</i> Ex14-460C>T					.047						
TT/CT	100	88.5	13	11.5		1.00			1.00		
CC	31	75.6	10	24.4		1.73	0.58 to 5.17	.325	5.24	1.21 to 22.7	.027
<i>TP73</i> Ex2+4G>A					< .001						
GG	83	94.3	5	5.7		1.00			1.00		
GA/AA	48	72.2	18	27.3		6.32	1.79 to 22.3	.004	7.34	1.26 to 42.6	.026
No. of at-risk genotypes					< .001						
0-1	89	94.7	5	5.3		1.00					
2	28	84.8	5	15.2		2.88	0.61 to 13.6	.182			
3-4	14	51.9	13	48.1		13.5	3.33 to 55.0	< .001			

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.

\*Odds ratios were adjusted for all clinical factors. P values are from logistic regression.

†Odds ratios were adjusted for all clinical factors and genotypes examined. P values are from logistic regression.

effect on the rate of margin-negative resection was observed. The effects of *MLH1* IVS12-169C>T, *TP73* Ex2+4G>A, and the combined genotype were statistically significant after adjusting for clinical factors. The *MLH1* and *TP73* SNPs and tumor response to

therapy remained as significant predictors for margin-negative resection after further adjusting for other SNPs. The FPRP for *MLH1* and *TP73* was 0.034 and 0.051, respectively, indicating noteworthy for both SNPs.

**Table 4.** Associations of Genotype With Tumor Resectability (R0 resection)

Genotype	Yes		No (No.)	$\chi^2$ P	Odds Ratio*	95% CI	P	Odds Ratio†	95% CI	P
	No.	%								
<i>EXO1</i> P757L										
CC	83	74.8	28		1.00			1.00		
CT/TT	24	55.8	19	.022	2.27	0.95 to 5.44	.065	1.78	0.64 to 4.96	.270
<i>MLH1</i> IVS12-169C>T										
CT/TT	77	75.5	25		1.00			1.00		
CC	30	55.7	22	.023	2.87	1.23 to 6.69	.015	4.07	1.50 to 11.0	.006
<i>MSH2</i> G322D										
GG	99	73.3	36		1.00			1.00		
GA/AA	8	42.1	11	.006	3.06	0.95 to 9.91	.062	3.81	0.89 to 16.4	.073
<i>MSH2</i> IVS12-6T>C										
TT/TC	75	77.3	22		1.00			1.00		
CC	32	56.1	25	.006	2.27	0.99 to 5.20	.053	1.41	0.52 to 3.82	.496
<i>MSH3</i> P231P										
GG	94	73.4	34		1.00			1.00		
GA/AA	13	50.0	13	.006	2.19	0.77 to 6.20	.140	3.32	0.86 to 12.8	.081
<i>TP73</i> Ex2 + 4G>A										
GG	75	83.0	15		1.00			1.00		
GA/AA	34	51.5	32	< .001	3.38	1.44 to 7.90	.005	4.02	1.41 to 11.4	.009
No. of at-risk genotype										
0	28	96.6	1		1.00‡					
1	41	83.7	8		1.00‡					
2-3	32	52.5	29		4.95	1.67 to 14.7	.004			
4-5	6	40.0	9	< .001	9.01	3.18 to 25.5	< .001			

\*Odds ratios were adjusted for all clinical factors.

†Odds ratios were adjusted for all clinical and genetic factors.

‡Patients with zero or one adverse genotype were combined as the referent group in the logistic regression.

### Associations of Genotype With Overall and Disease-Free Survival

Nine SNPs had statistically significant effects ( $P < .05$ ) and one had a borderline significant effect ( $P = .05$ ) on overall survival by log-rank test (Table 5). A strong gene-dosage effect was observed when the 10 genotypes were analyzed in combination (Fig 1). The MST decreased as the number of variant alleles increased. Five SNPs and the combined genotype remained as significant predictors for survival after adjusting for clinical factors (Table 5). *EXO1* R354H, *TREX1* Ex14-460C>T, *TP73* Ex2+4G>A, and margin-negative resection remained as significant predictors for overall survival after further adjustment for other genotypes. The FPRP was 0.085 for *EXO1*, 0.009 for *TREX1*, and 0.046 for *TP73*, all indicating noteworthiness.

The combined genotype was a significant predictor for disease-free survival among the 107 patients with R0 resection (Fig 1). The

MST could not be calculated for 13 patients with zero to one variant genotype because 10 patients were still alive; MST was 50.1, 22.9, and 13.9 months for those with two ( $n = 34$ ), three ( $n = 36$ ), and four to seven ( $n = 24$ ) adverse genotypes, respectively ( $P < .001$ ). Carrying more than three adverse genotypes (HR = 4.02; 95% CI, 1.15 to 14.1;  $P = .03$ ) and having poorly differentiated tumors (HR = 2.06; 95% CI, 1.05 to 4.04;  $P = .035$ ) were the only significant predictors for disease-free survival in multivariable Cox regression models that included all clinical and genetic factors. None of the genotypes were significantly related to tumor grade (data not shown).

## DISCUSSION

In this study, we found that SNPs of DNA MMR genes were significantly associated with tumor response to preoperative therapy, tumor

**Table 5.** Association of Genotypes With Overall Survival

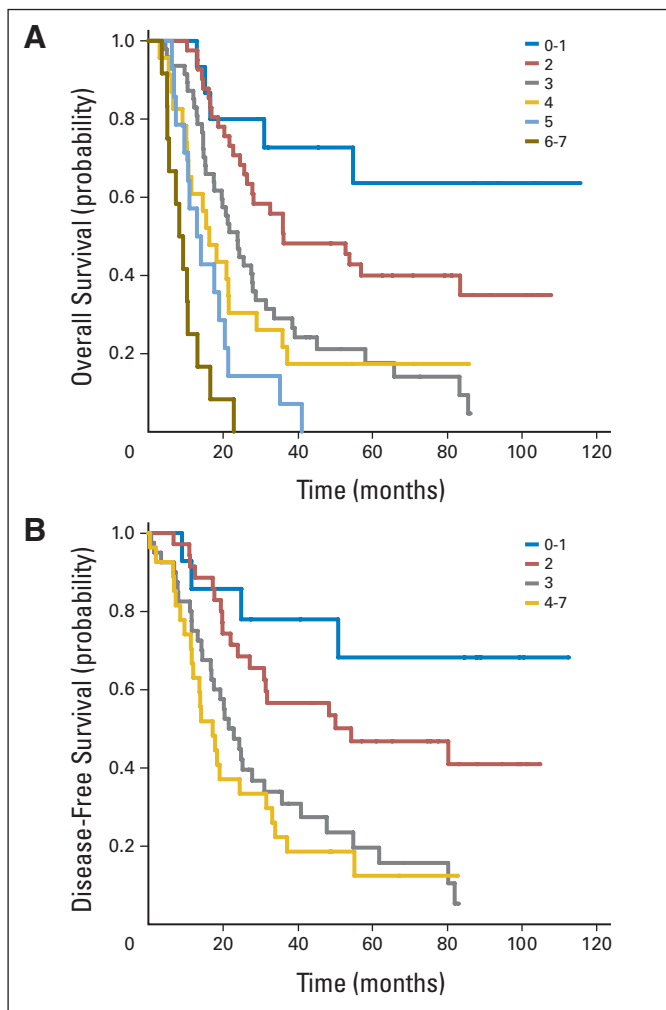
Genotype	No. of Patients	No. of Deaths	MST (months)	$P^*$	HR	95% CI	$P^\dagger$	HR	95% CI	$P^\ddagger$
<i>EXO1</i> R354H				.050						
GG	47	31	31.0		1.0			1.0		
GA/AA	107	86	21.2		1.38	0.89 to 2.15	.155	1.78	1.05 to 3.00	.032
<i>EXO1</i> P757L				< .001						
CC	111	78	27.8		1.0			1.0		
CTTT	43	39	14.6		1.89	1.25 to 2.87	.003	1.52	0.93 to 2.47	.093
<i>MSH2</i> G322D				.001						
GG	135	99	24.5		1.0			1.0		
GA/AA	19	17	11.0		1.86	1.03 to 3.36	.039	1.92	0.94 to 3.93	.074
<i>MSH2</i> IVS12-6T>C				.014						
TT	97	70	25.5		1.0			1.0		
TC/CC	57	47	17.6		1.22	0.83 to 1.82	.305	1.04	0.66 to 1.66	.862
<i>MSH3</i> P231P				.007						
GG	128	94	24.3		1.0			1.0		
GA/AA	26	23	16.6		1.60	0.99 to 2.60	.054	1.38	0.64 to 2.98	.417
<i>MSH6</i> G39E				.015						
GG	124	90	25.7		1.0			1.0		
GA/AA	30	27	17.5		2.04	1.29 to 3.22	.002	1.45	0.85 to 2.46	.175
<i>PMS1</i> Ex1-4G>C				.030						
GG	98	73	22.7		1.0			1.0		
GC/CC	56	44	18.9		2.13	0.78 to 5.86	.141	2.92	0.93 to 9.11	.066
<i>TREX1</i> K125Q				.010						
AA	148	111	22.9		1.0			1.0		
AC	4	4	10.3		2.77	0.84 to 9.11	.093	2.35	0.57 to 9.78	.240
<i>TREX1</i> Ex14-460C>T				.002						
TT/CT	113	81	26.4		1.0			1.0		
CC	41	36	15.5		1.59	1.06 to 2.38	.026	2.01	1.26 to 3.19	.003
<i>TP73</i> Ex2 + 4G>A				< .001						
GG/GA	140	103	25.5		1.0			1.0		
AA	14	14	7.4		2.64	1.35 to 5.18	.005	2.73	1.28 to 5.84	.010
No. of at-risk genotypes				< .001						
0-1	15	5	-		1.0					
2	41	25	36.2		2.30	0.87 to 3.78	.092			
3	47	40	23.9		4.55	1.75 to 11.8	.002			
4	23	19	16.3		5.52	2.00 to 15.3	.001			
5	14	14	13.0		6.40	2.19 to 18.7	.001			
6-7	12	12	8.3		19.3	5.92 to 63.1	< .001			

Abbreviations: MST, median survival time; HR, hazard ratio.

\* $P$  values were calculated using the log-rank test.

†HRs were adjusted for all clinical factors.

‡HRs were adjusted for all clinical and genetic factors.



**Fig 1.** Combined effect of 10 genotypes on (A) overall survival and (B) disease-free survival among patients with pancreatic cancer. The Kaplan-Meier method was used to assess the combined effect of the *EXO1* R354H GA/AA, *EXO1* P757L CT/TT, *MSH2* G322D GA/AA, *MSH2* IVS12-6T>C TC/CC, *MSH3* P231P GA/AA, *MSH6* G39E GA/AA, *PMS1* Ex1-4G>C GC/CC, *TREX1* K125Q AC, *TREX1* Ex14-460C>T CC, and *TP73* Ex2+4G>A AA genotypes on overall and disease-free survival. The numbers from 0 to 7 indicate the numbers of at-risk genotypes. *P* value from log-rank test was less than .001 for both A and B.

resectability, and overall and disease-free survival of patients with potentially resectable pancreatic adenocarcinoma who had received preoperative chemoradiotherapy, thus validating our hypothesis. A strong combined-genotype effect on each clinical end point was observed. These observations, for the first time, to our knowledge, demonstrated a potential value of DNA MMR gene variants as both predictors for clinical response to chemoradiotherapy and prognostic factors for survival in patients with resectable pancreatic cancer.

Of the 15 SNPs evaluated, *EXO1* R354H, *MLH1* IVS12-169C>T, *TREX1* Ex14-460C>T, and *TP73* Ex2+4G>A showed significant associations with clinical outcome after adjusting for all clinical and other genotypes. *MSH2* G322D, *MSH2* IVS12-6T>C, *MSH3* P231P, and *PMS1* -4G>C showed significant associations after adjusting for clinical factors but borderline significant associations after further adjusting for other genotypes. It is known that gemcitabine incorporation causes DNA replication arrest, and ATR/Chk1 signaling pathway plays a crucial role in cellular response to stalled DNA replication

fork.<sup>16,17</sup> Cells that lack *ataxia-telangiectasia mutated and rad3-related (ATR)* or checkpoint kinase 1 (*Chk1*) genes have been shown to be more sensitive to gemcitabine.<sup>17,18</sup> Our previous study in the same patient population has shown a significant combined genotype effect of *ataxia telangiectasia mutated (ATM)*, *ATR*, *Chk1*, and checkpoint kinase 2 (*Chk2*) on overall survival.<sup>19</sup> The observed effects of *MSH2*, *TREX1*, *TP73*, and *EXO1* genotypes in the current study may all be associated with interference of the ATR/Chk1 signal pathway. For example, *MSH2* not only plays a key role in recognizing the base-base mismatches and initializes the MMR process,<sup>20</sup> but also directly interacts with *ATR* to regulate the DNA damage response.<sup>21</sup> *TREX1* is a major 3' to 5' exonuclease in mammalian cells, and *TREX1* deficiency resulted in intracellular accumulation of single-stranded DNA and chronic activation of the DNA damage response ATM/Chk2 network.<sup>22</sup> *TREX1*-null cells failed to phosphorylate *Chk1* after cells were exposed to hydroxyurea, which suggests a compromised ATR signaling pathway function.<sup>22</sup> *TP73* is a member of the *p53* family of transcription factors involved in cellular responses to stress and development.<sup>23</sup> *TP73* accumulation was shown after a variety of chemotherapeutic agents,<sup>24</sup> but it was not induced in MMR-deficient cells after cisplatin.<sup>25</sup> *EXO1* is a 5' to 3' exonuclease that directly interacts with *MSH2*, *MSH3*, and *MLH1* and possibly acts in MMR by catalyzing 5' to 3' excision and by stabilizing higher-order complexes of MMR proteins.<sup>26-28</sup> Previous studies have shown that *P73* is a target of *Chk1* kinase,<sup>29</sup> and *EXO1* stability is dependent on *ATR* kinase.<sup>30</sup> It is conceivable that *MSH2* deficiency may lead to failure in DNA damage recognition and activation of ATR/Chk1 machinery. *TREX1* and *TP73* deficiency may further compromise the ATR/Chk1 signaling transduction. *EXO1* deficiency would reduce the MMR capacity. Thus cells with these variant genotypes may not be able to undergo apoptosis in response to gemcitabine-induced DNA damage, which is a critical determinant of the efficacy of cytotoxic therapies. The poor response to therapy would result in reduced tumor resectability or persistent micrometastatic disease, which in turn contribute to reduced overall survival and disease-free survival.

The *EXO1* R354H and *MSH2* G322D are nonsynonymous SNPs that result in replacement of amino acids, in turn, possibly affecting the protein functions. The *TREX1* Ex14-460C>T and *TP73* Ex2+4G>A SNPs are located at 3'UTR and 5'UTR, respectively. Although these sequences do not translate into proteins, the 3'UTR may contain sequence motifs crucial for the regulation of transcription, mRNA stability, and cellular location of the mRNA or the binding of microRNA.<sup>31</sup> The 5'UTR may contain regulatory sequences that serve as binding sites for proteins or affect the mRNA's stability and translation.<sup>32</sup> Further investigations into the functional significance of the MMR gene SNPs and the genotype-phenotype associations are warranted.

*MLH1* is frequently mutated in hereditary nonpolyposis colorectal cancer. The exact role of *MLH1* in MMR is not fully understood.<sup>33</sup> Tumors with inactivated *MLH1* gene by loss of heterozygosity or promoter methylation show higher rate of microsatellite instability and higher tendency for lymphocytic infiltration or poor differentiation.<sup>34-37</sup> The fact that *MLH1* SNP was significantly associated with tumor resectability but not with tumor response or survival in this study suggests that pancreatic tumors with *MLH1* variant alleles may have some unidentified pathologic characteristics. Further study is required to fully understand the role of *MLH1* in pancreatic cancer.

Although some SNPs in this study showed weak effect individually, the combined-genotype effect on each clinical end point was dramatic, suggesting that these genes act in concert, and the combined action of many genes may have a greater influence on the phenotype, (eg, the chance of survival) than individual SNPs. Identification of a panel of genetic markers that can be applied in the clinic to help predict tumor response to therapy, tumor resectability, and patient survival would be the ultimate goal of this research. In addition, some of the genes identified in these studies may serve as a therapeutic target to improve the efficacies of cytotoxic agents in cancer treatment.<sup>38,39</sup>

Our study was conducted in a relatively homogenous patient population with an adequate sample size. Although some differences in the clinical outcomes were observed for patients who were enrolled on the two different protocols, this was partially explained by a sampling bias. For patients enrolled in ID98-020 (N = 70), DNA was extracted from 50 blood samples and 20 paraffin sections from normal adjacent tissue of resected tumors when blood sample was not available, which resulted in a higher tumor resection rate (87%) than the true resection rate of 74% in this trial.<sup>14</sup> To overcome this bias, we have adjusted for this variable (preoperative treatment) in the multivariable models. Some of the associations we observed could have occurred by chance, although the results of FPRP test indicate noteworthiness for most SNPs that are significant in the multivariable models. Our observations need to be confirmed in separate patient populations. If confirmed, these

findings may provide opportunities for discovery of novel markers that can help in choosing therapy for pancreatic cancer and in predicting a patient's tolerance and response to treatment, tumor resectability, and overall clinical outcome.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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## Glossary Terms

**Mismatch repair:** One of four major pathways of DNA repair in mammalian cells. Mismatch repair recognizes and corrects errors in DNA replication leading to single base-pair mismatches or insertions/ deletions in small repetitive tracts of DNA known as microsatellites.

**SNP (single nucleotide polymorphism):** Genetic polymorphisms are natural variations in the genomic DNA sequence present in greater than 1% of the population, with SNP representing DNA variations in a single nucleotide. SNPs are being widely used to better understand disease processes, thereby paving the way for genetic-based diagnostics and therapeutics.

**ATR (ataxia telangiectasia and Rad3-related):** ATR is a member of the phosphoinositol-3 kinases like protein kinase (PIKK) family of proteins that play key roles in signal transduction

pathways following DNA damage. Unlike ATM (ataxia telangiectasia mutated protein), which is activated following DNA double-strand breakage, ATR responds to single-strand regions of DNA exposed during stalling of DNA replication forks and during the repair of certain DNA adducts. Homozygous mutation of the *ATR* allele can result in the rare, autosomal recessive condition Seckel syndrome, which is characterized by developmental abnormalities, chromosomal instability, and a predisposition to cancer.

**TP73:** Family member of the tumor suppressor gene TP53. Although p73 was initially described as a tumor suppressor protein, nowadays it is accepted that it could exert both a tumor suppressor or an oncogenic function depending on its isoforms.

**MLH1 (MutL homolog 1):** The DNA mismatch repair enzyme, MLH1 is responsible for overall fidelity of DNA replication.