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ORIGINAL ARTICLE – UROLOGIC ONCOLOGY

Genetic Variations in Glutathione Pathway Genes Predict Cancer Recurrence in Patients Treated with Transurethral Resection and Bacillus Calmette–Guerin Instillation for Non-muscle Invasive Bladder Cancer

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ABSTRACT

Background. Glutathione (GSH) is an important molecule involved in cell detoxification and antioxidation and may affect cancer development or outcome. We hypothesized that genetic variation in the GSH pathway might influence the clinical outcome of patients who have non-muscle invasive bladder cancer (NMIBC).

Methods. A total of 114 single nucleotide polymorphisms (SNPs) in 21 GSH pathway genes were genotyped in 414 NMIBC patients treated with transurethral resection alone (TUR) and both TUR and intravesical bacillus Calmette–Guérin instillation (BCG) therapy. The effect of each SNP on time to recurrence was estimated using the multivariate Cox proportional hazards model. Cumulative effect and survival tree analyses were performed to determine the joint effects of unfavorable genotypes and gene–gene interactions on bladder cancer prognosis.

Results. Seven SNPs showed significant associations with cancer recurrence in the TUR group and 15 SNPs showed significant associations with recurrence in the BCG group. The most significant SNP in the TUR group was rs3746162 in *GPX4*, whose variant genotype conferred a 5.4-fold increased risk of recurrence compared with wild-type (hazard ratio [HR] = 5.43, 95 % confidence interval [CI] = 2.19–13.46), whereas the most significant SNP in the BCG group was rs7265992 in *GSS* (HR 3.43, 95 % CI 1.56–7.56). The risk of recurrence increased with the number of unfavorable genotypes in both groups. Within treatment group, stratified analyses by tumor grade also indicated predictive variants.

Conclusions. Genetic variants in GSH pathway may influence cancer recurrence in NMIBC patients receiving curative treatment.

Bladder cancer accounts for approximately 4.4 % of new primary cancers and 2.6 % of cancer deaths in the United States, with an estimated 72,570 new cases and 15,210 deaths in 2013.[1] Most bladder cancers (75–85 %) are non-muscle invasive at diagnosis.[2] Although multimodal treatments have been standard treatment for many years, the prognosis for non-muscle invasive bladder cancers (NMIBCs) remains variable, as seen in the 30–80 % recurrence rate and 1–45 % progression rate within 5 years.[2] Tumor cell implantation after transurethral resection (TUR), the standard treatment for NMIBC, is attributed to many early recurrences.[3] To reduce the risk of recurrence, clinicians use perioperative intravesical therapy to prevent tumor reimplantation.[4] The major choice for

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intravesical therapy is immunotherapy with bacillus Calmette–Guérin (BCG), which results in a massive local immune response characterized by the induced expression of cytokines in the urine and bladder wall and the accumulation of granulocytes and mononuclear cells.[5] BCG is considered the best adjuvant therapy after TUR for preventing recurrence and delaying progression in NMIBC; however, clinical response is variable ranging from 36–71 %.[6–8] Moreover, intravesical BCG administration commonly causes side effects that affect the patient's quality of life and thus the success of treatment. Most patients experience urinary frequency and urgency, and serious sequelae and rare deaths have occurred.[9] Therefore, it is imperative to identify new biomarkers that accurately predict a patient's response to BCG therapy and post-treatment prognosis.

Glutathione (GSH) is the most abundant low-molecular-weight peptide present in eukaryotic cells and is one of the major compounds involved in cellular antioxidation and detoxification.[10] It is synthesized intracellularly from glutamic acid, cysteine, and glycine in two steps; this process is catalyzed by γ-glutamylcysteine synthetase and GSH synthetase (GSS). In antioxidation, GSH, a reduced compound, is oxidized to GSH disulfide by GSH peroxidase (GPX), by GSH-dependent transhydrogenases, or by direct interaction with free radicals. GSH disulfide is then reduced to GSH by NADPH-dependent GSH reductase (GSR). Through this oxidation process, GSH can effectively scavenge free radicals and other reactive oxygen species.[11] The detoxification role of GSH involves GSH-S-transferases (GSTs), a family of phase II detoxification enzymes. These enzymes conjugate GSH with various electrophiles, physiological metabolites, and xenobiotics to form mercapturates, which can then be eliminated via an ATP-dependent GSH S-conjugate export pump.[12] GSH also is required in many aspects of the immune response. It is essential for cytotoxic T lymphocyte activation, proliferation, and differentiation and also is involved in the activation of polymorphonuclear leukocytes as well as cytokine production.[11]

It is clear that GSH plays important role in antioxidation and detoxification and that there is strong evidence linking bladder cancer development with exposure to chemical carcinogens. Because intravesical immunotherapy with BCG instillation is the standard treatment for NMIBC, we performed this study to test the hypothesis that genetic variants in the GSH pathway may affect patients' clinical response to BCG instillation therapy.

MATERIALS AND METHODS

Details of the methodologies used for this study are described online in Supplementary Methods.

Study Population and Epidemiologic Data

The study population and enrollment procedures were depicted previously.[13] Briefly, a total of 414 patients with incident and histologically confirmed NMIBC were recruited. The primary study end point was time to cancer recurrence, defined as a newly diagnosed intravesical tumor subsequent to a previous negative cystoscopy follow-up or a newly found extravesical metastasis. Median follow-up time of the cohort is 48 months (53.3 months for recurrent cases and 38.6 months for nonrecurrent cases). The standard surveillance procedure may vary but usually consists of cystoscopic screening every 3 months for 2 years and then every 6 months for 3 years followed by yearly cytology and imaging screening. This study was approved by the Institutional Review Boards of MD Anderson Cancer Center and Baylor College of Medicine (online Method S1).

Gene/SNP Selection and Genotyping

A total of 114 SNPs from 21 genes (Supplementary Table S1) were identified by data mining the International HapMap Project, dbSNP, and the UCSC Genome Browser. DNA was extracted from peripheral blood, and SNP genotyping was performed using Illumina iSelect custom array platform (online Method S2).

Statistical Analysis

The main effect of a SNP on time to recurrence was estimated using the multivariate Cox proportional hazards model adjusted for potential covariates. Statistical analyses were performed using Stata 10.0 (StataCorp LP, College Station, TX) and STREE program (online Method S3).[14]

RESULTS

As shown in Table 1, no significant differences were identified between subjects with and without recurrence based on age ($P = 0.45$), sex ($P = 0.15$), smoking status ($P = 0.44$), cancer stage ($P = 0.13$), cancer grade ($P = 0.19$), or focality ($P = 0.25$); however, significant differences were found for treatment ($P = 3.42 \times 10^{-16}$).

Individual SNPs and Cumulative Effects of Unfavorable Genotypes in Cancer Recurrence after TUR Treatment Only

Among the 114 SNPs examined, 7 were significantly associated with bladder cancer recurrence in TUR-only patients (Table 2). The most significant SNP was

TABLE 1 Demographic and clinical characteristics of the NMIBC patients

Variables	Recurrence, *N* (%)	No recurrence, *N* (%)	*P*
Age, mean (SD)	62.63 (11.04)	63.49 (11.57)	0.445
Sex			0.153
Male	193 (85.0)	149 (79.7)	
Female	34 (15.0)	38 (20.3)	
Smoking status[a]			0.444
Never	63 (27.8)	54 (28.9)	
Former	116 (51.1)	85 (45.5)	
Current	48 (21.1)	48 (25.7)	
Stage			0.131
Tis	17 (7.5)	6 (3.2)	
Ta	104 (46.0)	84 (44.9)	
T1	105 (46.5)	97 (51.9)	
Grade[b]			0.192
G1	5 (2.3)	11 (5.9)	
G2	77 (36.2)	66 (35.5)	
G3	131 (61.5)	109 (58.6)	
Focality[c]			0.254
1	76 (52.1)	86 (31.1)	
2	19 (13.0)	11 (7.4)	
Multiple	51 (34.9)	51 (34.5)	
Treatment			3.42×10^{-16}
TUR only	91 (40.1)	45 (24.1)	
TUR + iBCG	90 (39.6)	30 (16.0)	
TUR + iBCG + mBCG	31 (13.7)	51 (27.3)	
Others[d]	15 (6.6)	61 (32.6)	
Total	227	187	

SD standard deviation, *TUR* transurethral resection, *iBCG* induction bacillus Calmette–Guérin, *mBCG* maintenance bacillus Calmette-Guérin

[a] Individuals who had smoked less than 100 cigarettes in their lifetime were defined as having never smoked. Individuals who had quit smoking at least 1 year before diagnosis were categorized as former smokers

[b] For equivalence with two-grade system, G1 and G2 are considered low grade and G3 high grade

[c] Not counting patients with missing information: 81 with recurrence; 39 without recurrence

[d] Patients who received intravesical chemotherapy but no BCG

rs3746162 in *GPX4* (HR 5.43, 95 % CI 2.19–13.46, $P = 0.0003$), and after adjustment for multiple comparisons, this SNP remained statistically significant with false discovery rate set at 5 % ($Q = 0.05$), indicating that this finding was robust. In stratified analysis by tumor grade, we found rs3746162 was significantly associated with recurrence in patients with low grade but not high grade tumors (HR 6.97, 95 % CI 2.69–18.05, $P = 6.25 \times 10^{-5}$ and HR 1.32, 95 % CI 0.52–3.35, $P = 0.564$, respectively; Supplementary Table S2). In contrast, rs1006771 of *GSTT2* and rs17614751 of *GSTA4* were significantly associated with recurrence in high-grade but not low-grade patients.

We assessed the cumulative effect of unfavorable genotypes identified from the individual SNP analysis on bladder cancer recurrence (*GPX4*:rs3746162 (AA); *GPX5*:rs451774 (GG); *GSTT2*:rs1006771 (GG); *GSTA2*:rs2144698 (GG); *GSTA4*: rs367836 (AC+CC); *GSTA2*:rs2180314 (CC); *GSTA4*:rs17614751 (GG)). We observed that increased risk of cancer recurrence was associated with increased numbers of these genotypes. Table 2 shows results for recurrence risk and recurrence-free survival in patients in the TUR group. Compared with the low-risk group (patients with 0–2 unfavorable genotypes), the medium-risk group (patients with three unfavorable genotypes) and high-risk group (patients with 4–6 unfavorable genotypes) had HRs for recurrence of 2.44 (95 % CI 1.34–4.44, $P = 0.003$) and 2.53 (95 % CI 1.51–4.25, $P = 0.0004$), respectively ($P_{\text{trend}} = 0.0003$). Kaplan–Meier recurrence-free survival curves (Supplementary Fig. S1) showed that the high-risk group and the medium-risk group had recurrence-free median survival times (MSTs) of 5.9 months and 5.3 months, respectively, which were much shorter than that of the low-risk group (MST = 21.7 months, $P_{\text{log-rank}} = 0.003$).

Individual SNPs and Cumulative Effects of Unfavorable Genotypes in Cancer Recurrence after TUR and BCG Treatment

In the 191 patients who received intravesical BCG therapy, 15 SNPs showed significant effects on recurrence (Table 3). The most significant SNP was rs7265992 in *GSS* (HR 3.43, 95 % CI 1.56–7.56, $P = 0.002$). Four SNPs remained significance after adjusting for multiple comparisons. These four SNPs were all located in the *GSS* gene, and two of them (rs7260770 and rs4911455) were strongly linked with $r^2 = 1.0$. When stratified by tumor grade, rs6060124 of *GSS* was significantly associated with recurrence in patients with both low- and high-grade tumors (HR 4.11, 95 % CI 1.25–13.58, $P = 0.02$ and HR 2.79, 95 % CI 1.28–6.06, $P = 0.01$, respectively), whereas two SNPs in *GPX5* and *GSTM3* and eight variants in *GSS*, *GSR*, *GSTT2*, *GSTM3*, and *GSTM4* were associated with recurrence in low-grade and high-grade patients, respectively (Supplementary Table S3).

Table 3 also shows the cumulative effects of unfavorable genotypes on recurrence in the BCG group (*GSS*: rs7265992 (AA); *GSS*:rs6060124 (AA); *GSS*:rs7260770 (AA); *GSS*:rs4911455 (CC); *GPX5*:rs377514 (TT); *GSS*: rs6088662 (TT); *GSR*:rs8190996 (CC+CT); *GSTM3*: rs4970737 (CC+CG); *GSTM3*:rs4970774 (AA+AC);

TABLE 2 Selected SNPs and the cumulative effect of unfavorable genotypes on recurrence in NMIBC patients receiving treatment of TUR alone

SNP	Gene	Genotype	MOI*	Recurrence WW/WV/VV	No recurrence WW/WV/VV	HR (95 % CI)[b]	*P*	*Q*
rs3746162	**GPX4**	**G/A**	**rec**	**59/22/7**	**26/19/0**	**5.43 (2.19–13.46)**	**0.0003**	**0.013**
rs451774	GPX5	A/G	rec	41/36/11	21/24/0	2.45 (1.24–4.84)	0.010	0.237
rs1006771	GSTT2	T/G	rec	38/38/12	17/26/2	2.24 (1.17–4.27)	0.015	0.237
rs2144698	GSTA2	G/T	dom	53/26/9	16/19/10	0.61 (0.40–0.95)	0.029	0.248
rs367836	GSTA4	A/C	dom	19/50/19	15/22/8	1.81 (1.06–3.08)	0.029	0.248
rs2180314	GSTA2	G/C	add	44/32/12	14/15/16	0.72 (0.54–0.97)	0.031	0.248
rs17614751	GSTA4	G/A	dom	80/8/0	35/10/0	0.45 (0.21–0.96)	0.038	0.248

Group (number of unfavorable genotypes[a])	Recurrence	No recurrence	HR (95 % CI)[b]	*P*	MST (mo)
Low-risk group (0–2)	30	29	1 (reference)		21.7
Medium-risk group (3)	7	24	2.44 (1.34–4.44)	0.003	5.3
High-risk group (4–6)	8	38	2.53 (1.51–4.25)	0.0004	5.9
			P_{trend}	0.0003	

SNPs that continued to have a significant effect after correcting for multiple comparisons by *Q* value with a false discovery rate of $\leq$ 10 % are in boldface

rec recessive, *dom* dominant, *add* additive, *WW* homozygous wild-type genotype, *WV* heterozygous variant genotype, *VV* homozygous variant genotype, *HR* hazard ratio, *CI* confidence interval, *MST* median survival time, *MOI* model of inheritance

* The model with the smallest *P* value

[a] Unfavorable genotypes: *GPX4* rs3746162 (vv); *GPX5* rs451774 (vv); *GSTT2* rs1006771 (vv); *GSTA2* rs2144698 (ww); *GSTA4* rs367836 (wv+vv); *GSTA2* rs2180314 (vv); *GSTA4* rs17614751 (ww)

[b] Adjusted by age, sex, smoking status, cancer stage, and cancer grade

GSTM3:rs4970776 (TT+TA); *GPX2*:rs10133290 (CC); *GSTM3*:rs11101992 (CC); *GSTM3*:rs1571858 (GG+GA); *GSTM4*:rs560018 (GG); *GSTM3*:rs15864 (GG+GC)). Compared with the low-risk group (patients with 1–4 unfavorable genotypes), the medium-risk group (patients with five or six unfavorable genotypes), and high-risk group (patients with 7–10 unfavorable genotypes) had HRs of recurrence of 2.38 (95 % CI 1.39–4.07, $P = 0.002$) and 6.20 (95 % CI 3.15–12.18, $P = 1.22 \times 10^{-07}$), respectively ($P_{trend} = 1.37 \times 10^{-07}$). Kaplan–Meier recurrence-free survival curves (Fig. 1) indicated the high-risk group had a recurrence-free MST of 7.1 months, whereas the medium-risk group had MST of 8.6 months and the low-risk group had MST of greater than 100 months (more than half of the patients were recurrence-free throughout the study period; $P_{log\text{-}rank} = 0.0005$).

Survival Tree Analysis

To explore the potential high-order gene–gene interactions between the GSH pathway SNPs, we performed a survival tree analysis using the significant SNPs identified in the individual SNP analysis. In the TUR group, no interaction was found between the seven significant SNPs. In the BCG group, the tree structure resulted in four terminal nodes with a range of low- to high-risk subgroups (Fig. 2; Supplementary Table S4). The initial split was rs377514 in *GPX5* followed by splits at rs8190996 in *GSR* and rs6088662 in *GSS*. Using the low-risk group (node 1) as a reference, the HR was 2.36 (95 % CI 0.26–21.33, $P = 0.445$) for the medium-risk group (node 2), and 7.97 (95 % CI 1.10–57.80, $P = 0.040$) for the high-risk group (nodes 3 and 4; $P_{trend} = 0.003$).

DISCUSSION

In this report, we have identified several genetic variants in GSH pathway with possible predictive value in NMIBC patients treated with TUR and BCG treatment. We systematically evaluated the effects of 114 SNPs in 21 GSH pathway genes on NMIBC recurrence. One SNP (rs3746162) in *GPX4* was significantly associated with bladder cancer recurrence after TUR treatment. Four SNPs (rs7265992, rs6060124, rs7260770, and rs4911455) in *GSS* were significantly associated with bladder cancer recurrence after TUR and BCG treatment. To our knowledge, this is the first study to systemically evaluate the association between GSH pathway variants and NMIBC outcome.

The enzyme glutathione synthetase, encoded by the *GSS* gene, is involved in GSH synthesis. It is known that

TABLE 3 Selected SNPs and the cumulative effect of unfavorable genotypes on recurrence in NMIBC patients receiving BCG after TUR

SNP	Gene	Genotype	MOI*	Recurrence WW/WV/VV	No recurrence WW/WV/VV	HR (95 % CI)[b]	*P*	*Q*
rs7265992	**GSS**	**G/A**	**rec**	**81/21/8**	**60/20/1**	**3.43 (1.56–7.56)**	**0.002**	**0.050**
rs6060124	**GSS**	**C/A**	**rec**	**61/36/12**	**44/34/3**	**2.80 (1.44–5.47)**	**0.003**	**0.050**
rs7260770[c]	**GSS**	**G/A**	**rec**	**56/41/13**	**41/35/5**	**2.56 (1.34–4.90)**	**0.005**	**0.050**
rs4911455[c]	**GSS**	**A/C**	**rec**	**56/40/13**	**40/35/5**	**2.54 (1.32–4.87)**	**0.005**	**0.050**
rs377514	GPX5	T/C	dom	93/15/2	56/24/1	0.50 (0.29–0.87)	0.013	0.104
rs6088662	GSS	T/G	add	77/30/3	44/31/6	0.64 (0.45–0.93)	0.017	0.115
rs8190996	GSR	C/T	rec	31/59/20	17/41/23	0.56 (0.34–0.92)	0.022	0.121
rs4970737	GSTM3	C/G	rec	54/50/6	41/32/8	0.39 (0.16–0.92)	0.031	0.121
rs4970774	GSTM3	A/C	rec	26/68/16	20/47/14	0.55 (0.31–0.96)	0.036	0.121
rs4970776	GSTM3	T/A	rec	34/62/14	29/40/12	0.53 (0.29–0.97)	0.038	0.121
rs10133290	GPX2	A/C	rec	64/36/10	49/28/4	2.08 (1.04–4.17)	0.039	0.121
rs11101992	GSTM3	A/C	add	65/37/8	56/21/4	1.39 (1.02–1.91)	0.040	0.121
rs1571858	GSTM3	G/A	rec	50/49/11	36/36/9	0.51 (0.26–0.98)	0.043	0.121
rs560018	GSTM4	A/G	rec	52/42/16	32/36/11	1.76 (1.01–3.08)	0.046	0.121
rs15864	GSTM3	G/C	rec	49/53/8	40/32/9	0.47 (0.22–0.99)	0.048	0.121

Group (number of unfavorable genotypes[a])	Recurrence	No recurrence	HR (95 % CI)[b]	*P*	MST (mo)
Low-risk group (1–4)	30	20	1 (reference)		
Medium-risk group (5–6)	39	70	2.38 (1.39–4.07)	0.002	8.6
High-risk group (7–10)	9	29	6.20 (3.15–12.18)	1.22×10^{-07}	7.1
			P_{trend}	1.37×10^{-07}	

SNPs that continued to have a significant effect after correcting for multiple comparisons by *Q* value with a false discovery rate of ≤ 10 % are in boldface

rec recessive, *dom* dominant, *add* additive, *WW* homozygous wild-type genotype, *WV* heterozygous variant genotype, *VV* homozygous variant genotype, *HR* hazard ratio, *CI* confidence interval, *MST* median survival time, *MOI* model of inheritance

*The model with the smallest *P* value

[a] Unfavorable genotypes: *GSS* rs7265992 (vv); *GSS* rs6060124 (vv); *GSS* rs7260770 (vv); *GSS* rs4911455 (vv); *GPX5* rs377514 (ww); *GSS* rs6088662 (ww); *GSR* rs8190996 (ww + wv); *GSTM3* rs4970737 (ww + wv); *GSTM3* rs4970774 (ww + wv); *GSTM3* rs4970776 (ww + wv); *GPX2* rs10133290 (vv); *GSTM3* rs11101992 (vv); *GSTM3* rs1571858 (ww + wv); *GSTM4* rs560018 (vv); *GSTM3* rs15864 (ww + wv)

[b] Adjusted by age, sex, smoking status, cancer stage, and cancer grade

[c] rs4911455 is in strong linkage with rs7260770, $r^2 = 0.99$

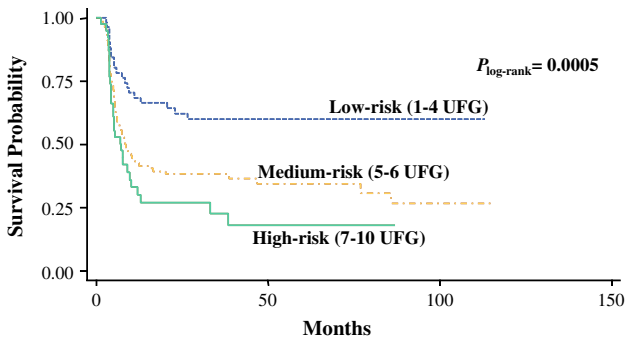

FIG. 1 Kaplan–Meier estimate of NMIBC recurrence-free survival by level of risk according to the number of unfavorable genotypes (UFG) in the TUR- and BCG-treated group

overexpression of GSS failed to increase intracellular GSH, but GSS deficiency could result in decreased GSH levels and cause dramatic metabolic consequences.[10] Genetic variations in *GSS* may affect GSS production and/or function, leading to a deficiency in GSH. In our study, two of the significant SNPs in *GSS* that were related to poor outcomes for NMIBC patients after BCG therapy were located in the intron region. Their functional significance is not yet understood. One of them (rs7265992) has already been found to be associated with the survival of small-cell lung cancer patients after platinum-based chemotherapy.[15]

GPX is an enzyme family whose function is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. This reducing

function helps protect cells from oxidative damage.[16] The eight isozymes in this family are encoded by various genes (*GPX1-8*).[17] SNP caused by transversion of cytosine to thymine at codon 198 of *GPX1* (resulting in an amino acid substitution of proline to leucine) was associated with an increased risk of bladder and lung cancers but prolonged recurrence-free survival in NMIBC.[18,19] According to Chiong et al., the *GPX1* CT genotype was associated with decreased recurrence time after intravesical BCG therapy.[20] The same group had previously found that the combination of BCG and antioxidants caused a reduction in reactive oxygen species and increased cytotoxicity in bladder cancer cells, suggesting that a nonimmunological effect of BCG treatment also may contribute to the antitumor response.[20,21]

GPX4 is a selenium-containing enzyme. Selenium can be incorporated at amino acid 21 to form selenocysteine, which is in the catalytic center of GPX4. Selenium incorporation requires a specific RNA structure in the 3′ untranslated region (3′UTR) of the *GPX4* mRNA. Therefore, the SNPs in the *GPX4* 3′UTR may affect the function of GPX4. Bermano et al. reported that the SNP rs713041, located in *GPX4* 3′UTR, was associated with the risk of colorectal cancer. They also showed that different genotypes of rs713041 led to different mRNA expression and enzyme activities.[22] In our study, the most significant SNP for the TUR group (rs3746162) also was located in the 3′UTR of *GPX4*. This may provide the functional basis for the association of this SNP, although its biological significance remains to be determined in future experiments. Interestingly, the association of rs3746162 was highly significant in patients with low-grade but not high-grade tumors ($Q < 0.05$), whereas the association of rs1006771 of *GSTT2* was significant in high-grade but not low-grade patients, indicating the predictive value of these SNPs based on treatment and tumor characteristic.

There are several significant SNPs in GST genes related to cancer recurrence in the TUR or BCG groups. GSTs play

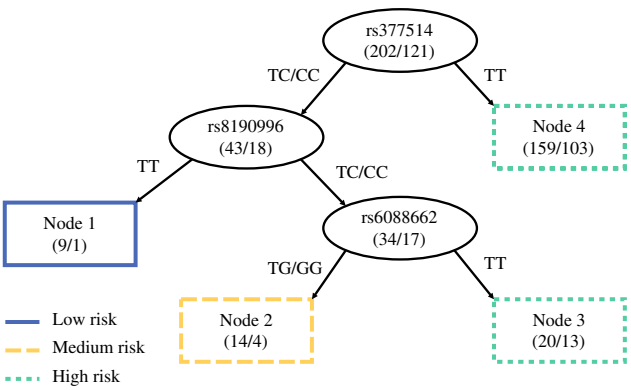

FIG. 2 Survival tree analysis of NMIBC recurrence after TUR and BCG therapy. Four nodes were generated which could be divided into three groups of low, medium, and high risk

a major role in phase II detoxification by catalyzing the conjugation of GSH with a number of different compounds, many of which are carcinogenic.[11] Human cytosolic GSTs are highly polymorphic and can be divided into six classes: alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT), and zeta (GSTZ).[10] SNPs in GST genes have been widely studied and are correlated with an increased risk of various cancers. We found that two SNPs in *GSTA2* (rs2144698 and rs2180314) were significantly correlated with NMIBC recurrence after TUR treatment. In a previous study, Ning et al. did not find any association with prostate cancer after evaluating various *GSTA2* genetic polymorphisms.[23] Andonova et al. genotyped two SNPs in *GSTA2* (rs2180314 and rs6577), and they did not find any association with breast cancer risk.[24] However, the same gene, and even the same SNP, may have different influences on different cancer types. More studies are needed to understand how *GSTA2* may impact cancer risk and prognosis. *GSTM3* SNPs have been more widely studied in various cancer types. Schnakenberg et al. reported that homozygous wild-type genotypes of a *GSTM3* SNP (rs36120609) in intron 6 were significantly protective against bladder cancer.[25] However, the same SNP in *GSTM3* was reported to have no impact on bladder cancer risk in a study by Golka et al.[26] In a study by Yu et al. investigating breast cancer risk in a Han Chinese population, the GG genotype in *GSTM3*:rs4970737 was a protective factor.[27] We also found that the GG genotype in *GSTM3*:rs4970737 could protect NMIBC cancer patients from early recurrence after TUR and BCG therapy.

We also evaluated the cumulative effects of GSH pathway SNPs on the risk of bladder cancer recurrence. We observed a dose-dependent correlation between the number of unfavorable genotypes and recurrence risk. Altogether, these results support the hypothesis that NMIBC recurrence after definitive treatment is a polygenic process.

Besides recurrence, we evaluated the association of the GSH SNPs with NMIBC progression, which is defined as recurring tumor progressing in tumor grade or stage or leading to cystectomy. Overall, there were a total of 105 cases showing progression and 307 cases without progression. Our findings showed a few SNPs with significant association, but none of the associations were significant after consideration of multiple testing. This might be due to the small sample size or suggests the unique role of the GSH pathway genes in NMIBC recurrence.

There are a few limitations in our study. First, our sample size, although adequately powered for overall analysis, may be limited in subgroup analyses. Nevertheless, several top significant associations with biological plausibility were found in patients stratified by treatment, even after adjustment for multiple testing to minimize false discoveries. Second, the SNPs genotyped in this study are

mostly tagging SNPs and most likely not the true causal variant. Therefore, functional characterizations are needed to ascertain the underlying biological mechanisms for the significant associations. Due to the significant challenges of obtaining a comparable clinical cohort with similar study design and treatment regimens with follow-up protocol, we were unable to conduct a replication analysis at this time. Further independent or external validation will be necessary to verify our findings.

CONCLUSIONS

We have conducted the first study to evaluate systemically the association between genetic variation in GSH pathway and NMIBC recurrence. We also determined the effect of GSH pathway SNPs on clinical outcome in various treatment and tumor grade subgroups. Once validated, these findings may provide urologists additional genetic information for clinical assessment and treatment decisions. Nevertheless, the underlying mechanisms through which these genes or SNPs affect the clinical behavior of bladder cancers require further study. Future investigations in other populations and detailed functional characterization are needed to establish GSH pathway variants as predictive or prognostic markers for NMIBC.

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CONFLICT OF INTEREST All authors declare no conflict of interest.

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