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Clinical significance of runt-related transcription factor 1 polymorphism in prostate cancer

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OBJECTIVE

To investigate the association of *RUNX1* rs2253319 with clinicopathological characteristics of prostate cancer (PCa) and disease recurrence after radical prostatectomy (RP).

PATIENTS AND METHODS

Taking advantage of the systematic stage and grade for each tumor in a cohort of 314 patients with localized PCa receiving RP, we evaluated the associations of *RUNX1* rs2253319 with age at diagnosis, preoperative prostate-specific antigen (PSA) level, Gleason score, surgical margin, pathologic stage, status of lymph node metastasis, and PSA recurrence after RP.

RESULTS

The minor allele, T, and the minor homozygote TT genotype of *RUNX1* rs2253319 were significantly associated with a 1.49- to 2.76-fold higher risk for advanced pathologic stage and a 3.35- to 9.52-fold higher risk for lymph node metastasis. *RUNX1* rs2253319 TT genotype was also associated with poorer PSA-free survival compared with the major homozygote CC genotype in Kaplan–Meier analysis (log-rank test, $P = 0.038$) and multivariate Cox proportional hazards model adjusting for age and PSA concentration ($P = 0.045$).

CONCLUSION

RUNX1 rs2253319 is associated with adverse clinicopathological features and might be a prognostic factor for the recurrence of PSA in patients with PCa receiving RP.

What's known on the subject? and What does the study add?
Although most clinically localized prostate cancer patients who underwent radical prostatectomy have a favorable outcome, molecular markers capable of providing prognostic information are still urgently needed to identify high-risk patients who might benefit from aggressive treatment. In this study, we found that *RUNX1* rs2253319 polymorphism was significantly associated with higher risks of advanced pathological stage, lymph node metastasis, and time to disease recurrence. Our results suggest that a simple and pretreatment analysis of genetic variants might add prognostic value to the currently used indicators for outcome prediction in patients receiving radical prostatectomy.

KEYWORDS

lymph node metastasis, prostate cancer, runt-related transcription factor 1, single nucleotide polymorphism

INTRODUCTION

The runt-related transcription factor (RUNX) family, also known as the acute myeloid leukemia (AML) and core-binding factor-α (CBFα), consists of three DNA-binding α subunits, RUNX1, RUNX2 and RUNX3, each of which is capable of forming heterodimers with the common CBFβ subunit. RUNX heterodimers bind to their consensus target sequence, TGT/CGGT, and either activate or repress the transcription of the target genes. Potential molecular switches controlling these activities seem to involve promoter-specific features such as the proximity of binding sites for co-activators, ETS, p300/CBP, and SMADs, or co-repressors, NCoR, SMRT, and mSIN3A, the availability of cofactors in the nucleus,

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TABLE 1 Demographic and clinicopathological characteristics of 314 patients with prostate cancer (PCa) who received radical prostatectomy (RP)

Characteristics	*n*	(%)	Median	(IQR)
Age at diagnosis, years			66.5	(61.8–70.0)
Body mass index, kg/m^2			24.6	(22.9–26.4)
Preoperative PSA level, ng/mL			11.4	(7.2–19.8)
Gleason score				
2–7	265	(86.6)		
8–10	41	(13.4)		
Surgical margin				
Negative	193	(70.2)		
Positive	82	(29.8)		
Pathologic stage*				
Localized	200	(64.9)		
Locally advanced	108	(35.1)		
Lymph node metastasis				
Negative	284	(93.1)		
Positive	21	(6.9)		
Status of PSA recurrence†				
No PSA recurrence	201	(64.0)		
PSA recurrence	113	(36.0)		

*IQR, Interquartile range. *TNM staging by AJCC in 1997: localized, T1/T2 N0 M0; locally advanced, T3/T4 N+ M0. †With mean follow-up 38.5 months and median follow-up 30.8 months.*

and the post-translational modifications, phosphorylation and acetylation, on RUNX [1]. Despite their structural similarity, the RUNX genes have divergent tissue-specific functions, with RUNX1 being essential for hematopoiesis, RUNX2 for osteogenesis and RUNX3 for neurogenesis. Moreover, RUNX genes are also associated with different neoplasias, and they could function as both dominant oncogenes and tumor suppressors in a context-dependent manner [2].

Interest in the RUNX genes in cancer began with the discovery of *RUNX1* as an important translocation breakpoint in leukemias, with the TEL–RUNX1 fusion accounting for 20% of acute lymphoblastic leukemia (ALL) and the RUNX1-ETO fusion accounting for 12% of AML [3]. Dysregulation of RUNX-mediated gene expression has also been linked to cell transformation and tumor progression. Recently, RUNX1 and RUNX2 were found in both normal and cancerous prostate cells [4]. RUNX1 has long been implicated in the regulation of cell cycle genes, such as p21$^{WAF1/CIP1}$, which encodes a cyclin-dependent kinase inhibitor for checkpoint control and cell differentiation [5]. Several studies also showed that RUNX2 activated expression of bone matrix proteins, matrix metalloproteinases, and angiogenic factors that were associated with tumor progression and metastasis [6]. Therefore, RUNX genes might play pivotal roles during prostate cancer (PCa) progression.

Recent Cancer Genetic Markers of Susceptibility (CGEMS) and other genome-wide association studies have identified several PCa susceptibility loci (http://cgems.cancer.gov/) [7]. However, the prognostic value of those identified PCa susceptibility variants has not been determined. In this study, we evaluated 40 single-nucleotide polymorphisms (SNPs) associated with genes that have been implicated in cancer progression and had low *P* values ($P < 0.01$) among CGEMS (see the Supporting Information, Table S1) in a cohort of 314 patients with clinically localized PCa who underwent radical prostatectomy (RP). Taking advantage of our patient population treated with RP, each patient's tumor was extensively and accurately graded and staged [8–12]. This offered the ability to assess association of these genetic variants with clinicopathological features and prognosis of PCa. Interestingly, our primary allelic analysis revealed that only *RUNX1* rs2253319 was associated with lymph node metastasis, suggesting an important role of this genetic polymorphism in PCa progression.

PATIENTS AND METHODS

PATIENT RECRUITMENT AND DATA COLLECTION

The study population was expanded from our hospital-based PCa case–control study that has been described previously [8–12]. Briefly, patients with diagnosed and pathologically confirmed PCa were actively recruited from the Kaohsiung Medical University Hospital, Kaohsiung Veterans General Hospital, and National Taiwan University Hospital. A subset of patients with clinically localized PCa who underwent RP was followed up prospectively to evaluate the potential association of genetic variants with clinicopathological characteristics of the disease. As the prostate gland was entirely removed from each patient, each tumor could be accurately graded using the Gleason scoring system [13]. As presented in Table 1, 41 (13.4%) patients had Gleason scores of 8–10, according to previous risk assessment models for recurrence of PSA after RP [14,15]. Pathology analyses were performed on the whole specimens with step sections (2–3 mm) and the positive surgical margin was defined as tumor cells present at the inked margin. Positive surgical margins were found in 82 specimens (29.8%). Disease stage was determined by pathologic findings, pelvic CT or MRI and radionucleotide bone scans, according to the criteria outlined by the American Joint Committee on Cancer [16] TNM classification. Tumors with pathologic stage T1/T2 were defined as localized PCa ($n = 200$, 64.9%), and tumors with pathologic stage T3/T4 were defined as locally advanced PCa ($n = 108$, 35.1%). Of these, 21 (6.9%) patients had lymph node metastasis. Recurrence of PSA was defined as two consecutive PSA measurements of >0.2 ng/mL at an interval of >3 months [17], and a PSA concentration of >0.2 ng/mL at the first follow-up was considered the date of recurrence. For more precise analysis of the effect of disease recurrence after RP, patients who received neo-adjuvant hormone therapy or radiotherapy were excluded, thus leaving 314 cases in the final analysis. Overall, 113 (36.0%) patients experienced recurrence of PSA during the 38.5-month (mean) and 30.8-month (median) follow-up periods. This study was approved by the Institutional Review

TABLE 2 Association between RUNX1 rs2253319 and clinicopathological characteristics among patients with prostate cancer (PCa) who received RP

	Frequency		Age at diagnosis		Preoperative PSA level		Gleason score 8–10*	
rs2253319	*n*	(%)	β (95% CI)	*P*	β (95% CI)	*P*	aOR (95% CI)	*P*
Alleles								
C	389	(61.9)	0.00		0.00		1.00	
T	239	(38.1)	−0.40 (−1.46–0.67)	0.467	1.95 (−1.74–5.64)	0.601	1.05 (0.65–1.70)	0.834
Genotypes								
CC	121	(38.5)	0.00		0.00		1.00	
CT	147	(46.8)	−0.34 (−1.94–1.26)	0.675	−2.26 (−7.74–3.23)	0.419	0.75 (0.36–1.56)	0.443
TT	46	(14.6)	−0.82 (−3.08–1.44)	0.478	6.38 (−1.34–14.1)	0.105	1.31 (0.52–3.31)	0.575

	Positive surgical margin*		Locally advanced pathologic stage*		Positive lymph node metastasis*		PSA recurrence*	
rs2253319	aOR (95% CI)	*P*	aOR (95% CI)	*P*	aOR (95% CI)	*P*	aOR (95% CI)	*P*
Alleles								
C	1.00		1.00		1.00		1.00	
T	1.44 (0.99–2.09)	0.059	**1.49 (1.06–2.10)**	**0.022**	**3.35 (1.67–6.70)**	**0.001**	1.34 (0.96–1.88)	0.089
Genotypes								
CC	1.00		1.00		1.00		1.00	
CT	1.28 (0.72–2.27)	0.403	1.00 (0.59–1.70)	0.990	1.93 (0.49–7.63)	0.351	1.21 (0.72–2.02)	0.471
TT	**2.33 (1.04–5.21)**	**0.040**	**2.76 (1.35–5.65)**	**0.006**	**9.52 (2.43–37.3)**	**0.001**	1.89 (0.94–3.82)	0.075

*β, β Estimates; aOR, age-adjusted odds ratio; CI, confidence interval. Units: age at diagnosis, years; preoperative PSA level, ng/mL. *Comparison of Gleason score between 8–10 vs 2–7; comparison of surgical margin between positive vs negative; comparison of pathologic stage between locally advanced vs localized PCa; comparison of lymph node metastasis between positive vs negative; comparison of PSA recurrence between recurrence vs no recurrence. Values of* P ≤ *0.05 are in bold type.*

Boards at these three hospitals, and informed consent was obtained from each participant.

GENOTYPING

DNA samples prepared from blood were available for all patients. Genotyping was performed using Sequenom iPLEX matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry technology at the National Genotyping Center, Academia Sinica, Taiwan. Briefly, primers for locus-specific PCR and allele-specific extension were designed by MASSARRAY ASSAYDESIGN 3.0 software (Sequenom, San Diego, CA, USA). The sample DNAs were amplified by primers flanking the targeted sequence, followed by dephosphorylation and allele-specific primer extension. The extension products were purified, loaded into a 384-format SpectroChip, and subjected to MALDI-TOF mass spectrometry. The resulting data were analysed by the Sequenom MASSARRAY TYPER software. Quality control included genotyping of 39 blind duplicate samples, revealing a 100% agreement on genotyping calls. The SNP was in Hardy–Weinberg equilibrium ($P > 0.05$) and the genotyping call rate was 100%.

STATISTICAL ANALYSIS

The linear regression model was used to estimate the effects of alleles and genotypes on age at time of first diagnosis and age-adjusted preoperative PSA concentrations. Logistic regression analyses were performed to compute odds ratios (OR) and the 95% confidence intervals (CI) for estimating the associations of individual SNP alleles as well as genotypes to the risk of clinicopathological features and PSA recurrence, while adjusting for age (age-adjusted OR (aOR)). The Kaplan–Meier method was used to compare the influence of genotypes in the PSA-free survival interval, and significance was determined using the log-rank test. Univariate and multivariate analyses to determine the interdependency of genotypes and the risk parameters, such as age, preoperative PSA concentration, Gleason score, pathologic stage and surgical margin, were carried out using Cox proportional hazards regression. The Statistical Package of the Social Sciences software version 16.0.1 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. A two-sided *P*-value of <0.05 was considered statistically significant.

RESULTS

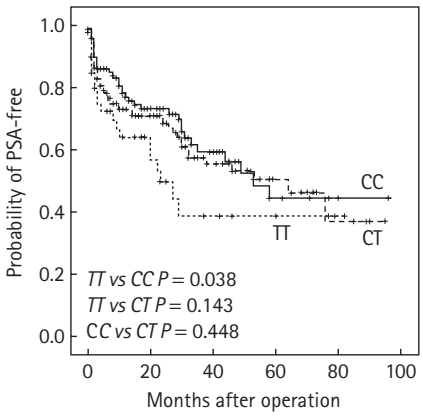
A total of 40 SNPs (associated with genes that have been implicated in cancer progression and had low *P*-values ($P < 0.01$) in CGEMS, see Supplementary Table S1) were selected and evaluate their prognostic significance on disease progression in a cohort of 314 patients with clinically localized PCa who underwent RP. Our primary allelic analysis revealed that only RUNX1 rs2253319 was associated with lymph node metastasis. Taking advantage of the accurate tumor grading in patients with PCa that underwent RP, associations of RUNX1 rs2253319 with age at diagnosis, preoperative PSA level, Gleason score, surgical margin, pathologic stage, lymph node metastasis and PSA recurrence were evaluated in Table 2. We found no allele and genotype associations with patients' age at diagnosis, preoperative PSA level, and Gleason score. However, the minor homozygous genotype (TT) of RUNX1 rs2253319 was significantly associated with

TABLE 3 Logistic regression analysis of factors associated with lymph node metastasis

	Univariate analysis		Multivariate analysis*			
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, years	0.96 (0.90–1.03)	0.212	0.93 (0.85–1.03)	0.154	0.95 (0.84–1.06)	0.335
Preoperative PSA, ng/mL	**1.03 (1.01–1.05)**	**0.005**	1.01 (0.99–1.04)	0.236	1.00 (0.96–1.04)	0.911
Gleason score						
2–7	1.00		1.00		1.00	
8–10	**6.23 (2.29–16.9)**	**<0.001**	**3.75 (1.12–12.6)**	**0.033**	3.36 (0.73–15.4)	0.120
Surgical margin						
Negative	1.00				1.00	
Positive	**5.00 (1.46–17.1)**	**0.010**			1.37 (0.27–6.83)	0.704
Pathologic stage						
Localized	1.00				1.00	
Locally advanced	**20.9 (4.77–91.9)**	**<0.001**			6.47 (0.65–64.4)	0.111
Status of PSA recurrence						
No PSA recurrence	1.00		1.00		1.00	
PSA recurrence	**20.8 (4.75–91.3)**	**<0.001**	**12.0 (2.15–67.2)**	**0.005**	**11.3 (1.09–117)**	**0.042**
RUNX1 rs2253319						
CC	1.00		1.00		1.00	
CT	1.67 (0.49–5.70)	0.410	1.73 (0.39–7.77)	0.475	1.74 (0.32–9.33)	0.520
TT	**7.06 (2.05–24.3)**	**0.002**	**6.26 (1.23–31.8)**	**0.027**	3.17 (0.37–27.4)	0.294

*OR, Odds ratio. *Age, Preoperative PSA, Gleason score, Surgical margin, Pathologic stage, Status of PSA recurrence, or* RUNX1 *rs2253319 genotypes were included in the multivariate analysis. Values of* P ≤ *0.05 are in bold type.*

FIG. 1. Kaplan–Meier analysis revealed that RUNX1 rs2253319 TT genotype was associated with a significantly poor PSA-free survival after radical prostatectomy (RP) than the CC genotype.



higher relative risks of positive surgical margin (aOR 2.33, 95% CI 1.04–5.21), locally advanced pathologic stage (aOR 2.76, 95% CI 1.35–5.65) and positive lymph node metastasis (aOR 9.52, 95% CI 2.43–37.3), as well as weakly associated with higher risks for PSA recurrence (aOR 1.89, 95% CI 0.94–3.82). Accordingly, higher relative risks of locally advanced pathologic stage ($P = 0.022$) and positive lymph node metastasis ($P = 0.001$) were also observed in patients with minor allele (T) of this polymorphism.

As lymph node metastasis is generally considered a poor prognostic indicator in PCa, we used multivariate logistic regression models to further investigate the extent to which RUNX1 rs2253319 contributes to the risk of lymph node metastasis. On univariate analysis, preoperative PSA level, Gleason score 8–10, positive surgical margin, advanced pathologic stage, PSA recurrence, and the minor homozygous TT genotype of RUNX1 rs2253319 were associated with lymph node metastasis ($P \leq 0.010$; Table 3). The multivariate analysis revealed that patients carrying rs2253319 TT genotype had a significantly greater likelihood of developing lymph node metastasis ($P = 0.027$) in the model adjusted for age, preoperative PSA level, Gleason score 8–10 and PSA recurrence. However, RUNX1 rs2253319 did not reach significance after adjusting for all clinicopathological features because of its strong correlation with some features (Table 2).

Although the minor homozygous genotype (TT) of RUNX1 rs2253319 was just weakly associated with increased risk of PSA recurrence after RP compared with the homozygote of the major allele (CC) in the logistic regression model ($P = 0.075$; Table 2), it showed a significant association between the RUNX1 rs2253319 TT genotype and time to PSA recurrence in the Kaplan–Meier survival analysis (log-rank test $P = 0.038$; Fig. 1) and in the univariate Cox proportional hazard model ($P = 0.047$; Table 4). The median estimated cumulative PSA-free survivals were significantly lower in TT genotype carriers than in those with the CC genotype (23 months vs 53 months).

In addition to RUNX1 rs2253319, our univariate analyses showed that high preoperative PSA level, Gleason score 8–10, positive surgical margin, advanced pathologic stage and positive lymph node metastasis significantly influenced post-RP PSA-free survival time ($P < 0.001$; Table 4). As the TT genotype of RUNX1 rs2253319 was in a strong correlation with those clinicopathological features of PCa (Table 2), this polymorphism did not reach significance after adjusting for all clinicopathological risk factors in the multivariate analyses (Table 4). However, RUNX1 rs2253319 still retained its significance ($P = 0.045$) in the multivariate

TABLE 4 Cox proportional hazards analysis of factors associated with PSA recurrence after radical prostatectomy (RP)

	Univariate analysis		Multivariate analysis*			
Variables	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, years	0.99 (0.96–1.02)	0.494	1.01 (0.98–1.04)	0.721	0.99 (0.96–1.02)	0.598
Preoperative PSA, ng/mL	**1.02 (1.01–1.03)**	**<0.001**	**1.02 (1.01–1.03)**	**<0.001**	**1.03 (1.02–1.04)**	**<0.001**
Gleason score						
2–7	1.00				1.00	
8–10	**3.67 (2.33–5.78)**	**<0.001**			**2.41 (1.39–4.17)**	**0.002**
Surgical margin						
Negative	1.00				1.00	
Positive	**2.76 (1.84–4.16)**	**<0.001**			1.29 (0.77–2.13)	0.331
Pathologic stage						
Localized	1.00				1.00	
Locally advanced	**3.48 (2.36–5.12)**	**<0.001**			**1.96 (1.18–3.26)**	**0.009**
Lymph node metastasis						
Negative	1.00				1.00	
Positive	**10.6 (6.16–18.1)**	**<0.001**			**4.20 (1.88–9.38)**	**<0.001**
RUNX1 rs2253319						
CC	1.00		1.00		1.00	
CT	1.17 (0.77–1.78)	0.454	1.34 (0.87–2.07)	0.190	1.11 (0.70–1.77)	0.660
TT	**1.72 (1.01–2.93)**	**0.047**	**1.75 (1.01–3.04)**	**0.045**	1.01 (0.50–2.03)	0.984

*HR, Hazard ratio. *Age, Preoperative PSA, Gleason score, surgical margin, pathologic stage, lymph node metastasis or* RUNX1 *rs2253319 genotypes were included in the multivariate analysis. Values of* P ≤ *0.05 are in bold type.*

analyses when adjusted with age at diagnosis and preoperative PSA level.

DISCUSSION

The present study represents the first association analysis of RUNX1 polymorphism on clinicopathological characteristics and prognosis of PCa after RP. Results from this study revealed that RUNX1 rs2253319 was significantly associated with higher relative risks of locally advanced pathologic stage and lymph node metastasis for the minor allele T vs major allele C carriers as well as the minor allele homozygote TT vs the major allele homozygote CC carriers (Table 2). In addition, metastasis is the most critical complication for PCa, and the adverse impact of lymph node metastasis on disease prognosis has been reported [18]. According to our logistic regression, Kaplan-Meier, and Cox regression analyses, our results suggest that RUNX1 rs2253319 might be prognostic factors not for only lymph node metastasis (Table 3) but also PSA recurrence after RP (Fig. 1 and Table 4). These findings might thus be beneficial for selecting adjuvant therapy for high-risk patients, and might shed light on the molecular biological mechanisms underlying the development of lymph node metastasis and PSA recurrence.

Generally, African-American men tend to have higher incidence, more aggressive disease and worse outcomes for PCa than Caucasians and Chinese [19,20]. High prevalence of the RUNX1 rs2253319 T allele in the African ancestry compared with the European and Chinese ancestry was also observed in the HapMap database (CC 22.6%, CT 47.2% and TT 30.2% for those with African ancestry in south-west USA; CC 50.4%, CT 42.5% and TT 7.1% for Utah residents with northern and western European ancestry; CC 34.1%, CT 54.1% and TT 11.8% for those with Chinese ancestry in Metropolitan Denver, CO, USA) [21]. These data are in accord with our finding that indicates higher risks for advanced pathologic stage and lymph node metastasis, as well as a poorer PSA-free survival in RUNX1 rs2253319 TT carriers (Table 2 and Fig. 1). Thus, this polymorphism might partly explain variations in the progression of PCa among different ethnic groups.

As the rs2253319 polymorphism is located in the sixth intron of the RUNX1 gene, it is plausible that this SNP might alter the consensus splicing site sequence or the binding site for transcription factors, thereby influencing RUNX1 splicing and expression. Interestingly, the risk allele T creates a putative transcription factor binding site for RUNX1 itself, according to the functional prediction by FASTSNP [22], whereas the role of RUNX1 in PCa progression has not yet been explored. Recently, homeobox C6 (HOXC6) has been correlated with the progression of PCa and been identified as the gene most strongly correlated with Gleason score [23]. A genome-wide transcriptional network analysis also demonstrated that RUNX1 is one of the HOXC6 direct targets [24], suggesting that their expression might work together to regulate the aggressiveness and metastasis of PCa. Androgen deprivation therapy is a widely used treatment for patients with advanced PCa, but several studies have found that prolonged exposure to reduced levels of androgen resulted in a marked acceleration of PCa progression. Notably, RUNX1 expression was found to be increased both in samples treated with reduced level of androgen and in hormone-refractory tumors [25], further implying its relevance to the progression of PCa. However, as described previously, RUNX–CBFβ complexes can either activate or repress transcription of target genes and lead to

activation of both growth-promoting and growth-suppressing signals in a context-dependent manner [2]. Therefore, further investigations are required to determine the potential roles of RUNX1 and its genetic variants during the progression of PCa.

In conclusion, most RUNX1 studies have focused on leukemia [26] and arthritis [27], so we provide the first evidence for the association of RUNX1 rs2253319 with higher risks of PCa in its advanced pathologic stage, positive lymph node metastasis and shorter time to PSA recurrence after RP. However, this study is limited by sample size in analyses of outcomes and in subset analyses. Our homogeneous Chinese Han population may also make our findings less generalizable to other ethnic groups. Furthermore, although each tumor could be accurately graded and staged in our cohort, all the patients examined were those who received RP for curative treatment. Thus, the clinical significance of RUNX1 polymorphism should be further assessed in large independent studies with clinically non-organ-confined PCa and in other ethnic populations.

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CONFLICT OF INTEREST

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Abbreviations: **CI**, confidence interval; **OR**, odds ratio; **PCa**, prostate cancer; **RP**, radical prostatectomy; **RUNX1**, runt-related transcription factor 1; **SNP**, single nucleotide polymorphism.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table S1. List of single-nucleotide polymorphisms (SNPs) evaluated and their associations with lymph node metastasis.

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