NRP-1 and *KDR* polymorphisms are associated with survival time in patients with advanced gastric cancer

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Abstract. Neuropilin-1 (*NRP-1*), a member of the NRP-family, has been reported to be vital for tumor angiogenesis, growth and metastasis. As a co-receptor of vascular endothelial growth factor (*VEGF*), *NRP-1* can bind to *VEGF* and meditate vascular development through the *VEGF-VEGF* receptor 2 (*VEGFR2*) signaling pathway. Furthermore, *NRP-1* is capable of binding with platelet-derived growth factor (*PDGF*) to regulate the *PDGF-PDGF* receptor (*PDGR*) signaling pathway in tumor angiogenesis. In the present study, The DNA was obtained from the paraffin-embedded tissues of patients with advanced gastric cancer (AGC), amplified using PCR and subsequently sequenced to determine the polymorphisms within *NRP-1, VEGFR2* [kinase insert domain receptor (*KDR*)] and *PDGF*. The effect of the functional polymorphism of the aforementioned genes on the overall survival (OS) and progression-free survival (PFS) of 81 patients with advanced gastric cancer was examined. Three single nucleotide polymorphisms (SNPs) of *KDR* were significantly associated with clinical outcomes. The rs1870377 TT genotype was positively associated with longer OS and PFS times compared with the AA+AT genotype (PFS, P=0.012; OS, P=0.038), the rs7692791 wild-type TT genotype was positively associated with longer PFS time and the rs2034965 AA+GA genotype was associated with shorter OS time (P=0.034). With regards to the SNPs of *NRP-1*, the rs2065364 AA genotype was significantly associated with improved OS and PFS times (PFS, P=0.023; OS, P=0.045). Following multivariate analysis using Cox proportional hazards regression models, patients with the *KDR* rs7692791 TT genotype experienced a longer PFS time compared with those with the CT genotype (P=0.016), and patients with the *NRP-1* rs2065364 variant-type AA genotype still experienced a longer PFS time compared with those patients with the AG+GG genotypes (P=0.006). Regarding OS, the results demonstrated that the *KDR* rs2034965 AG+GG genotypes presented with a significant reduction in OS time (P=0.029), and that the *KDR* rs1870377 AT+AA genotypes had worse OS times compared with the wild-type TT genotype (P=0.021). In addition, increased mortality risk and AGC progression were significantly associated with the number of adverse alleles for combinations of *NRP-1* rs2065364 and *KDR* rs1870377. In conclusion, the data from the present study demonstrated that the selected *KDR* and *NRP-1* gene polymorphisms may be potential prognostic biomarkers in AGC.

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Introduction

Gastric cancer is one of the most common cancer types worldwide and remains the third leading cause of cancer-associated mortality, accounting for >783,000 deaths worldwide in 2018 (1). The diagnostic rate of early gastric cancer is only 10% in China, and most patients are at an advanced stage when clinically diagnosed, which confers a poor prognosis (2).

Systematic chemotherapy is the major treatment for patients with advanced gastric cancer (AGC). Platinum-fluoropyrimidine- and paclitaxel-fluoropyrimidine-based chemotherapy regimens are recommended as the first-line treatments in line with the Chinese Society of Clinical Oncology guidelines (3).

However, patients with the same tumor stage and receiving similar treatment can exhibit different clinical outcomes, and gastric cancer is a complex disease and its prognosis and progression are significantly affected by genetic and environmental factors (4). Identifying predictive genetic biomarkers could therefore contribute to the development of individualized therapy and follow-up strategies (5).

Neuropilin-1 *(NRP-1)* is a type I transmembrane glycoprotein distributed at the surface of cells that has been reported to affect neuronal axon guidance and embryonic angiogenesis (6), and to serve as a co-receptor regulating tumorigenesis in the vascular endothelial growth factor (*VEGF*)-*VEGF* receptor 2 (*VEGFR2*) [kinase insert domain receptor (*KDR*)] or platelet-derived growth factor (*PDGF*)-*PDGF* receptor (PDGFR) signaling pathways (7,8). *VEGFRs* are a type of tyrosine kinase receptor, and include *VEGFR1* and *VEGFR2*

(KDR), which can be activated by binding with *VEGF* ligands (9). The *VEGF-VEGFR2* signaling pathway is the leading pathway that activates the proliferation and migration of endothelial cells, therefore promoting angiogenesis and stimulating tumor growth and invasion (10,11). Furthermore, *PDGF* isoforms can transduce signals via binding to structurally similar α- and β-tyrosine kinase receptors, known as *PDGFRα* and *PDGFRβ*, respectively. The *PDGF-PDGFR* signaling pathway serves critical roles in regulating proliferation and survival of certain cell types (e.g. hematopoietic stem cell, vascular endothelial cell and vascular smooth muscle cell) during embryogenesis, and overexpression or mutation of the *PDGF-PDGFR* pathway can stimulate tumor cell proliferation (12,13). Previous studies have reported that polymorphisms within *VEGF* and *KDR* impacted their expression at the gene level (14,15). Thus, polymorphisms of these two signaling pathways may affect AGC prognosis by regulating the expression of the aforementioned genes and therefore affect the survival of patients with AGC. The present study investigated the association between polymorphisms of the *NRP-1, KDR, PDGFβ, PDGFRβ* and *PDGFRα* genes and the prognosis of patients with AGC.

Materials and methods

Study population. A total of 100 patients with AGC from the Second Affiliated Hospital of Dalian Medical University (Dalian, Liaoning, China) were recruited between January 2011 and June 2016. The inclusion criteria were as follows: i) Patients were histopathologically diagnosed with gastric adenocarcinoma; ii) patients had inoperable locally advanced, metastatic or recurrent gastric cancer (AGC); iii) patients had an Eastern Cooperative Oncology Group performance status (ECOG-PS) ≤2 (16); and iv) patients underwent at least 2 cycles of chemotherapy at the Second Affiliated Hospital when diagnosed with postoperative recurrence or inoperable advanced gastric cancer. The exclusion criteria were as follows: i) Patients who received chemotherapy, radiotherapy and/or biological treatment previously; ii) patients with an ECOG-PS >2; and iii) patients with multiple primary malignant neoplasms. The 100 patients were followed up by clinic visits and phone calls every 2 months, and clinical outcomes were recorded until October 2018. Genotype information was not available for 8 patients, 5 cases were lost to follow-up and 6 patients failed to receive the protocol treatment. Therefore, 81 patients were analyzed in the present study. Unresectable patients were staged according to imaging and gastroscopy when histopathologically diagnosed by biospy, and the postoperative recurrence patients were staged according to postoperative pathology. Tumors were staged using the 7th edition of the Tumor-Node-Metastasis (TNM) staging system of the International Union Against Cancer/American Joint Committee on Cancer (17). Chemotherapy was given prior to the present study, and regimens included platinum and fluoropyrimidine [cisplatin (D) 80 mg/m^2 on day 1 and fluorouracil (F) 750 mg/m^2 from day 1-4; D 80 mg/m^2 on day 1 and capecitabine (X) 1,000 mg/m^2 from day 1-14], and paclitaxel (P) and fluoropyrimidine [P, 150 mg/m^2 on day 1 and F 750 mg/m^2 from day 1-5; P 150 mg/m^2 on day 1 and X 1,000 mg/m^2 from day 1-14]. The regimens were repeated every 21 days. Chemotherapy was stopped in case of disease progression, patient refusal or grade 3-4 toxicity according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (18).

SNP selection. The SNP loci of the target genes were selected from the public SNP database of the 1,000 Genome Project in the National Center for Biotechnology Information (NCBI) using minor allele frequency (MAF) >0.1 in the Chinese Han population and the Hardy-Weinberg equilibrium with a P-value of >0.1, then tagSNPs with a cut-off value of R^2>0.8, and covering the gene and flanking 3 kb either side of the gene regions were chosen by the Genome Variation Server (https://gvs.gs.washington.edu). In total, 66 tagSNPs (27 from *KDR* gene, 32 from *NRP-1* gene and 7 from *PDGFβ*) were selected, however due to financial constraints, 10 SNPs (rs7692791, rs6838752, rs2034965, rs1531290, rs13109660 from *KDR*, rs2070296, rs2804495, rs2065364 from *NRP-1*, and rs*4*821877, and rs9622978 from *PDGFβ*) were randomly selected from the tagSNPs. In addition, five disease-associated SNPs (rs1870377 and rs2305948 from *KDR*, rs6554162 and rs1800812 from *PDGFRα*, and rs2302273 from *PDGFRβ*) were selected according to their use in previous literature (19-24). Finally, the 15 SNPs (Table SI) of *KDR* rs7692791, rs2305948, rs6838752, rs2034965, rs1531290, rs13109660 and rs1870377, of *NRP-1* rs2070296, rs2804495 and rs2065364, of *PDGFβ* rs4821877 and rs9622978, of *PDGFRα* rs6554162 and rs1800812, and of *PDGFRβ* rs2302273, were obtained from the SNP database of the NCBI (http://www.ncbi.nlm.nih.gov/SNP).

SNP genotyping. The tissues from patients with AGC were obtained via biopsy or surgery, fixed with 10% neutral buffer formalin for 24 h at room temperature, immersed in 60˚C paraffin, embedded in a paraffin block and stored at 4˚C. Genomic DNA was extracted from paraffin-embedded tissues of patients with AGC using the QIAamp DNA FFPE Tissue kit (Qiagen GmbH) according to the manufacturer's instructions. The polymerase chain reaction (PCR) primers for SNPs were designed using Sequenom Assay Design 3.1 software (Sequenom) and are listed in Table SII. A thermocycler (PTC-100PCR; MJ Research) and KAPA Taq HotStart DNA polymerase (Kapa Biosystems; Roche Diagnostics) were used for PCR amplification, the thermal cycling program employed was as follows: 94˚C for 5 min, followed by 35 cycles of 30 sec at 94˚C, then 30 sec of annealing at 60˚C, 30 sec of extension at 72˚C, and a final elongation step at 72˚C for 10 min. The PCR products were sequenced using a 3730XL DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.).

Statistical analysis. In the present study, the genetic model was divided into 3 types, namely, general, dominant and recessive models, as follows: Dominant model, MW+MM vs. WW; recessive model, WW+WM vs. MM; and general model, MM vs. WM vs. WW, where W indicates the wild-type allele and M the mutant allele). Before analysis, the Hardy-Weinberg equation for the equilibrium of allele distributions was tested by the χ^2 test (Table I) and the SNPs with a P-value of <0.05 were excluded. The progression-free survival (PFS) and overall survival (OS) probabilities were estimated using

Table I. Hardy-Weinberg equilibrium test results of selected SNPs.

Gene	SNP	χ^2	P-value
KDR	rs7692791	0.11	0.739
	rs2305948	0.02	0.902
	rs6838752	0.65	0.418
	rs2034965	1.66	0.197
	rs13109660	0.03	0.860
	rs1870377	0.58	0.455
	rs1531290	3.37	0.067
NRP-1	rs2070296	3.46	0.062
	rs2804495	0.08	0.775
	rs2065364	1.00	0.317
PDGFβ	rs9622978	11.54	0.007[a]
	rs4821877	0.00	0.998
PDGFRα	rs6554162	0.00	0.951
	rs1800812	30.3	<0.001[a]
PDGFRβ	rs2302273	7.04	0.007[a]

[a]P<0.05. KDR, kinase insert domain receptor; NRP-1, neuropilin-1; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; SNP, single nucleotide polymorphism.

the Kaplan-Meier method. The association between SNPs and PFS and OS were analyzed by log-rank tests and Cox regression analyses. Hazard ratios (HR) and 95% confidence intervals (CIs) were estimated for the uivariate and multivariate analyses usingy Cox regression analyses. Bonferroni's correction was applied for multiple comparisons (with the significance level set at P<0.025). Statistical analyses were conducted using SPSS v.21.0 (IBM Corp.). All tests were two-sided, and P<0.05 was considered to indicate a statistically significant difference.

Results

Patient clinical characteristics. A total of 81 patients were recruited in the present study, including 56 men (69.1%) and 25 women (30.9%). The age of the patients ranged from 30 to 83 years, and the mean age was 60.7±10.1 years. By October 2018, 79 patients were deceased, 2 had been lost during follow-up, and the median PFS and OS times were 5.5 and 11.0 months, respectively. The association between clinical pathological features and survival time are listed in Table II. The results demonstrated that TNM stage analyzed by Kaplan-Meier analysis was significantly associated with longer OS time (log-rank, P=0.047), and the platinum-based chemotherapy regimen was significantly associated with longer PFS time (log-rank, P=0.025). Associations between survival time and other clinical characteristics were not identified.

Associations between genotype and survival time. Associations between genotype and prognosis were estimated by Kaplan-Meier analysis, statistical significance was determined by the log-rank test and the genotype information are listed in Table SIII. The associations between the three types of genetic models (general, dominant and recessive) and survival time were analyzed (Table III). The results demonstrated that of all the selected SNPs, five SNPs (*KDR* rs7692791, *KDR* rs1870377, *KDR* rs2034965, *NRP-1* rs2065364 and *NRP-1* rs2804495) were significantly associated with PFS or OS; however, SNPs from *PDGF* and *PDGFR* genes were not associated with clinical outcomes.

Following univariate analysis (Tables IV and V), the dominant model of *KDR* rs1870377 indicated that AA+AT carriers were associated with shorter PFS and OS times compared with TT carriers [PFS: HR, 2.618; 95% CI, 1.235-5.550; P=0.012; OS: HR, 2.041; 95% CI, 1.042-3.999; P=0.038) (Fig. 1). Furthermore, in a recessive model of *NRP-1* rs2065364, AA genotype carriers exhibited more favorable PFS and OS times compared with the GG+AG genotypes (PFS: HR, 2.896; 95% CI, 1.159-7.237; P=0.023; OS: HR, 2.367; 95% CI, 1.019-5.496; P=0.045) (Fig. 2). However, *KDR* rs1870377 variant AA and AT genotype were significantly associated with poor PFS times compared with wild-type TT (AA vs. TT: HR, 3.221; 95% CI, 1.356-7.651; P=0.008; AT vs. TT: HR, 2.545, 95% CI, 1.159-5.589; P=0.020; Table IV) (Fig. S1). Furthermore, the *KDR* rs7692791 CT genotype was associated with lower PFS times compared with the wild-type TT genotype (HR, 1.829, 95% CI, 1.091-3.066, P=0.022; Table IV) (Fig. 3). In addition, in the dominant model of *KDR* rs2034965, AA+GA genotypes were significantly associated with reduced OS times (HR, 1.687; 95% CI, 1.039-2.738; P=0.034; Table IV) (Fig. 3). Statistical significance between SNPs and survival time in other polymorphisms was not found.

For multivariate analysis (Tables IV and V), adjustments were performed for different variables in PFS and OS. Variables that were considered clinically relevant, such as age and TNM stage, or that presented an association with survival time following univariate analysis as listed in Table II were entered into a multivariate Cox proportional-hazards regression model. *KDR* rs7692791 remained significantly associated with PFS, and the TT genotype was associated with better prognosis compared with the CT genotype (HR, 1.969; 95% CI, 1.150-3.369; P=0.013). Furthermore, the association between *NRP-1* rs2065364 AG+GG genotypes and shorter PFS remained significant following adjustment (HR, 3.905; 95% CI, 1.485-10.268; P=0.006). Furthermore, *KDR* rs2034965 AA+GA genotypes remained significantly associated with worse OS following adjustment (HR, 1.978; 95% CI, 1.193-3.280, P=0.008), and the *KDR* rs1870377 AA+AT genotypes were also associated with shorter OS compared with the wild-type TT genotype following adjustment (HR, 2.264; 95% CI, 1.130-4.536; P=0.021).

Effect of risk allele combinations on PFS and OS. To study the combined effects of polymorphisms on survival time, risk alleles were selected according to the aforementioned results. The *NRP-1* rs2065364G allele and the *KDR* rs1870377 A allele were found to be unfavorable for PFS and OS. Subsequently, the *NRP-1* rs2065364/*KDR* rs1870377 combination was tested for its association with survival time and numbers of 'risk alleles' (Tables VI and VII). The results suggested that patients carrying >2 risk alleles were more likely to have shorter PFS

Table II. Association between characteristics and prognosis of patients with advanced gastric cancer.

Variables	n	mPFS (95% CI)	mOS (95% CI)	Log-rank P-value PFS	Log-rank P-value OS
Sex				0.433	0.703
Male	56	5.0 (3.3-6.7)	11 (9.6-12.4)		
Female	25	6.0 (3.6-8.4)	12 (9.4-14.5)		
Age, years				0.773	0.898
>60	33	5.5 (2.8-8.2)	10.2 (6.0-14.4)		
≥60	48	6.0 (4.8-7.2)	11.0 (10.0-12.0)		
N stage				0.590	0.081
N1+N2	49	5.0 (3.6-6.4)	11.6 (10.4-12.8)		
N3	32	5.0 (2.8-7.2)	10.2 (8.6-11.8)		
TNM stage				0.080	0.047[a]
I, II and III	26	6.8 (5.9-7.7)	12.0 (8.5-15.5)		
IV	55	4.5 (2.8-6.2)	10.5 (8.5-12.5)		
Tumor size, cm				0.803	0.916
>5	31	5.0 (2.9-7.1)	11.0 (9.0-13.0)		
≥5	50	6.0 (4.4-7.6)	11.0 (9.5-12.5)		
Differentiation				0.415	0.079
Well to moderate	27	6.0 (4.5-7.5)	14.8 (8.0-21.6)		
Poor	54	4.5 (3.5-5.5)	10.2 (8.2-12.2)		
Platinum chemotherapy regimen				0.025[a]	0.359
Platinum included	38	6 (4.6-7.4)	11.6 (9.3-13.9)		
Non-platinum included	43	4.5 (3.3-5.7)	10.5 (9.0-12.0)		
Paclitaxel chemotherapy regimen				0.393	0.484
Paclitaxel included	39	4.4 (2.9-5.9)	11.0 (7.1-14.9)		
Non-paclitaxel included	42	6 (4.6-7.4)	11.0 (10.2-11.8)		

[a]P<0.05. CI, confidence interval; OS, overall survival (months); mOS, median overall survival; PFS, progression-free survival (months); mPFS, median progression-free survival; TNM, Tumor-Node-Metastasis.

Table III. Effect of SNPs in selected genes on the prognosis in patients with advanced gastric cancer.

Gene	SNP	Allelic change	Log-rank P-value for PFS General	Dominant	Recessive	Log-rank P-value for OS General	Dominant	Recessive
KDR	rs7692791	T/C	0.032[a]	0.009[a]	0.281	0.227	0.093	0.364
	rs2305948	C/T	0.619	0.329	0.871	0.277	0.109	0.748
	rs6838752	T/C	0.097	0.137	0.053	0.203	0.254	0.095
	rs2034965	G/A	0.155	0.065	0.240	0.065	0.031	0.883
	rs13109660	G/A	0.795	0.522	0.687	0.365	0.376	0.481
	rs1870377	T/A	0.030[a]	0.008[a]	0.256	0.091	0.032[a]	0.250
	rs1531290	A/G	0.236	0.128	0.313	0.451	0.845	0.207
NRP-1	rs2070296	G/A	0.498	0.417	0.486	0.993	0.964	0.909
	rs2804495	G/T	0.064	0.150	0.028[a]	0.085	0.308	0.029
	rs2065364	G/A	0.052	0.300	0.015[a]	0.113	0.587	0.037[a]
PDGFβ	rs4821877	C/T	0.712	0.490	0.862	0.949	0.933	0.747
PDGFRα	rs6554162	G/A	0.513	0.322	0.751	0.501	0.413	0.561

[a]P<0.05. KDR, kinase insert domain receptor; NRP-1, neuropilin-1; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; OS, overall survival; PFS, progression-free survival; SNP, single nucleotide polymorphism.

Table IV. Associations of SNPs in selected genes and PFS in patients with advanced gastric cancer.

					Univariate analysis		Multivariate analysis	
SNP	Outcome	mPFS, months	Model	Log-rank P-value	HR (95% CI)	P-value	HR (95% CI)[a]	P-value[a]
KDR rs7692791	PFS		General	0.032		0.020		0.012
		4.2	CC	0.099[b]	1.926 (0.859-4.319)	0.112	2.053 (0.855-4.929)	0.107
		5.0	CT	0.018[b]	1.829 (1.091-3.066)	0.022	1.969 (1.150-3.369)	0.013
		6.0	TT		Reference		Reference	
			Dominant	0.009		0.010		0.006
		6.0	TT		Reference		Reference	
		5.0	CC+CT		1.892 (1.156-3.098)	0.011	1.982 (1.196-3.284)	0.008
KDR rs1870377	PFS		General	0.030		0.017		0.127
		4.0	AA	0.005[b]	3.221 (1.356-7.651)	0.008	2.892 (0.987-8.474)	0.053
		5.5	AT	0.015[b]	2.545 (1.159-5.589)	0.020	1.778 (0.724-4.366)	0.209
		10.0	TT		Reference		Reference	
			Dominant	0.008		0.009		0.051
		10.0	TT		Reference		Reference	
		4.5	AA+AT		2.618 (1.235-5.550)	0.012	1.970 (0.861-4.503)	0.108
NRP-1 rs2065364	PFS		Recessive	0.015		0.017		0.004
		8.0	AA		Reference		Reference	
		4.5	AG+GG		2.896 (1.159-7.237)	0.023	3.905 (1.485-10.268)	0.006

[a]Adjusted for age, sex, N stage, TNM stage, platinum included or not and differentiation. [b]Bonferroni-adjusted P-value=0.05/2, so P<0.025 was considered statistically significant. CI, confidence interval; HR, hazard ratio; KDR, kinase insert domain receptor; NRP-1, neuropilin-1; PFS, progression-free survival; mPFS, median progression-free survival; SNP, single nucleotide polymorphism.

Table V. Associations of SNPs in selected genes and OS in patients with advanced gastric cancer.

					Univariate analysis		Multivariate analysis	
SNP	Outcome	mOS, months	Model	Log-rank P-value	HR (95% CI)	P-value	HR (95% CI)[a]	P-value
KDR rs2034965	OS		Dominant	0.031		0.032		0.029
		11.6	GG		Reference		Reference	
		10.3	AA+GA		1.687 (1.039-2.738)	0.034	1.978 (1.193-3.280)	0.008
NRP1 rs2065364	OS		Recessive	0.037		0.039		0.105
		17.8	AA		Reference		Reference	
		11.0	AG+GG		2.367 (1.019-5.496)	0.045	2.048 (0.847-4.952)	0.112
KDR rs1870377	OS		Dominant	0.032		0.034		0.035
		16.0	TT		Reference		Reference	
		10.8	AA+AT		2.041 (1.042-3.999)	0.038	2.264 (1.130-4.536)	0.021
NRP1 rs2804495	OS		Recessive	0.029		0.031		0.084
		8.8	TT		Reference		Reference	
		12.0	GT+GG		1.710 (1.046-2.796)	0.033	1.570 (0.924-2.667)	0.095

[a]Adjusted for age, gender, N stage, TNM stage and differentiation. CI, confidence interval; HR, hazard ratio; KDR, kinase insert domain receptor; NRP-1, neuropilin-1; OS, overall survival; mOS, median overall survival; SNP, single nucleotide polymorphism.

and OS times compared with carriers with 1-2 risk alleles (PFS: HR, 0.427; 95% CI, 0.260-0.701; P=0.008; OS: HR, 0.523; 95% CI, 0.323-0.845; P=0.008; Tables VI and VII) (Fig. 4). Following adjustment, this association was also significant (PFS: HR, 0.427; 95% CI, 0.257-0.709; P=0.001; OS: HR, 0.511; 95% CI, 0.314-0.833; P=0.007).

Discussion

The results from the present study demonstrated that polymorphisms of *NRP-1* and *KDR* genes were associated with clinical outcome in patients with AGC. Following univariate analysis, *KDR* rs1870377 AA+AT genotypes were found to be associated with shorter PFS and OS times compared with the wild-type TT genotype, and the *KDR* rs1870377 variant AA and AT genotypes were significantly associated with poor PFS time compared with wild-type TT genotype. Furthermore, the *NRP-1* rs2065364 homozygous mutant AA genotype was significantly associated with higher PFS and OS times compared with the GG+AG genotypes. The genotypes of *KDR* rs7692971 and *KDR* rs2034965 were also significantly associated with higher PFS and OS times, respectively. Following adjustment, the *KDR* rs7692791 TT genotype was associated with increased PFS time compared with the CT genotype, and the *NRP-1* rs2065364 AG+GG genotypes were associated with shorter PFS times compared with the AA genotype. The *KDR* rs2034965 AA+GA genotypes were associated with worse OS times compared with the GG genotype. The *KDR* rs1870377 AA+AT genotypes were associated with shorter OS times compared with the TT genotype. Additionally, increasing number of risk alleles with the *NRP-1* rs2065364/*KDR* rs1870377 combination was significantly associated with shorter OS and PFS times. These results demonstrated that *NRP-1* rs2065364, *KDR* rs7692791, *KDR* rs2034965 and *KDR* rs1870377 may be considered as independent indicators of prognosis in patients with AGC.

NRP-1 was originally found to be crucial for neuronal axon guidance and embryonic angiogenesis, and was identified as a novel receptor involved in angiogenesis (6-8). Previous studies reported that the *NRP-1* gene is associated with tumorigenesis and progression. One study reported that *NRP-1* overexpression is associated with the promotion of gastric cancer migration, invasion and growth (25). Lin *et al* (26) demonstrated that *NRP-1* is a novel TEA domain transcription factor target that serves a crucial role in hepatocellular carcinoma tumorigenesis. A previous demonstrated that *NRP-1* is abnormally highly expressed in non-small cell lung tumor tissue, and is associated with patient prognosis (27). Another study reported that *NRP-1* affects the chemosensitivity of cancer cells (28), Wey *et al* (28) demonstrated that *NRP-1* overexpression in pancreatic cancer cell lines is associated with increased chemoresistance to gemcitabine *in vitro*. Yue *et al* (29) reported that NRP-1 overexpression increases osteosarcoma cell survival following exposure to doxorubicin. To the best of our knowledge, no study has demonstrated the association between *NRP-1* SNPs and cancer. The present study confirmed that the *NRP-1* rs2065364 AA genotype was associated with increased PFS time compared with the AG+GG genotypes. Further molecular investigation is required to reveal the underlying mechanisms involved.

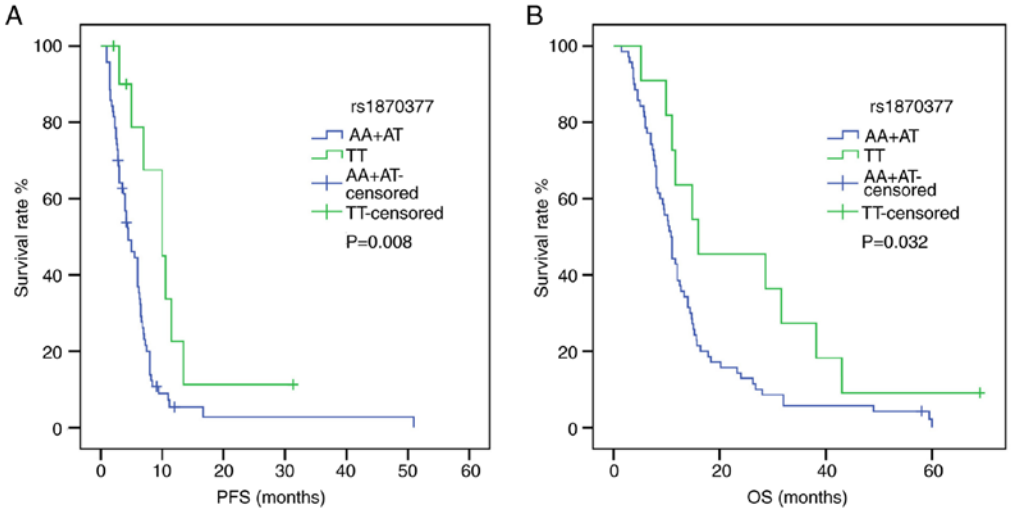

Figure 1. Effect of kinase insert domain receptor rs1870377 on survival time in patients carrying the AA+AT and TT genotypes. (A) PFS curve. (B) OS curve. P-values were obtained by log-rank tests. OS, overall survival; PFS, progression-free survival.

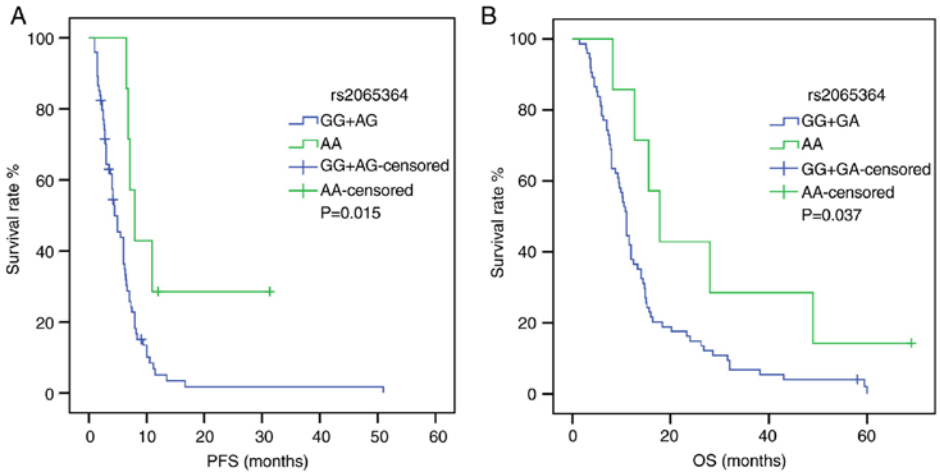

Figure 2. Effect of neuropilin-1 rs2065364 on survival time in patients carrying GG+AG and AA genotypes. (A) PFS curve. (B) OS curve. P-values were obtained by log-rank tests. OS, overall survival; PFS, progression-free survival.

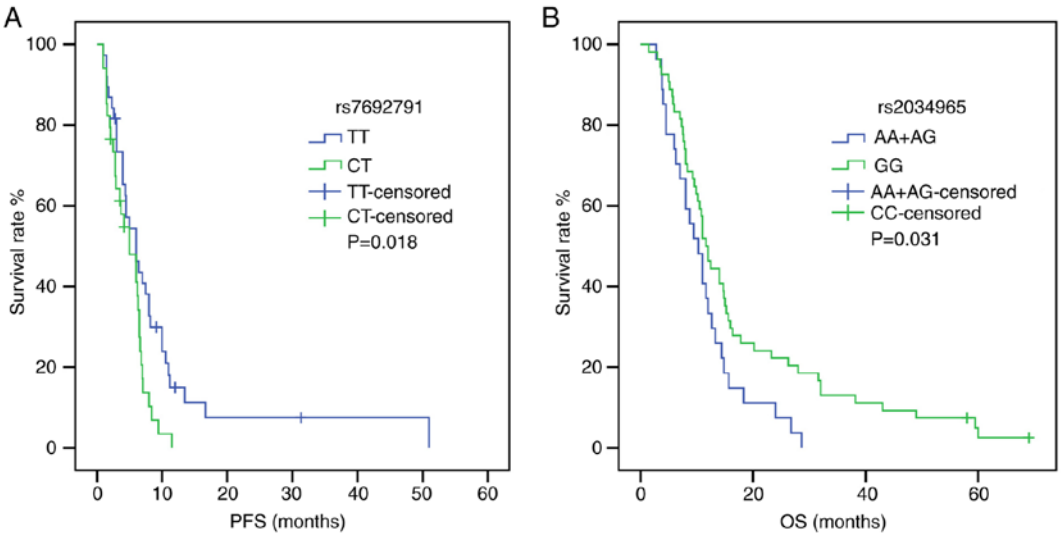

Figure 3. Effect of kinase insert domain receptor rs7692791 and rs2034965 on survival time. (A) PFS curve of rs7692791 in patients with TT and CT genotypes. (B) OS curve of rs2034965 in patients with AA+AG and GG genotypes. P-values were obtained by log-rank tests. OS, overall survival; PFS, progression-free survival.

Table VI. Association between number of risk alleles and overall survival in patients with advanced gastric cancer.

		Univariate analysis		Multivariate analysis[a]	
Alleles combination	n	HR (95% CI)	P-value	HR (95% CI)	P-value
rs2065364/rs1870377					
1-2 risk alleles	39	0.523 (0.323-0.845)	0.008	0.511 (0.314-0.833)	0.007
3-4 risk alleles	42	Reference		Reference	

[a]Adjusted for age, sex, N stage, TNM stage, platinum included or not and differentiation. CI, confidence interval; HR, hazard ratio; OS, overall survival.

Table VII. Association between number of risk alleles and progression-free survival in patients with advanced gastric cancer.

		Univariate analysis		Multivariate analysis[a]	
Alleles combination	n	HR (95% CI)	P-value	HR (95% CI)	P-value
rs2065364/rs1870377					
1-2 risk alleles	39	0.427 (0.260-0.701)	0.008	0.427 (0.257-0.709)	0.001
3-4 risk alleles	42	Reference		Reference	

[a]Adjusted for age, sex, N stage, TNM stage, platinum included or not and differentiation. CI, confidence interval; HR, hazard ratio; PFS, progression-free survival.

KDR (*VEGFR-2*) is a tyrosine kinase receptor that can regulate signal transduction by binding to *VEGF* via its extracellular domain (9). *VEGF/VEGFR2* is an important signaling pathway that can promote proliferation, survival and migration of vascular endothelial cells and increase vascular permeability (9,10). The cellular processes mediated by the *VEGF-VEGFR2* signaling cascade can lead to angiogenesis and therefore regulate tumor growth and invasion, and therapeutic resistance (10,11). Previous studies reported that *KDR* gene polymorphisms are associated with clinical outcomes in various types of cancer, including colorectal cancer, glioma, hepatocellular carcinoma and gastric cancer. Torben *et al* (30) reported that *VEGFR2* 1192C>T and -604T>C polymorphisms were associated with increased microvessel density in colorectal cancer. A previous study of glioma in the Chinese population demonstrated that three SNPs of *VEGFR2* (rs7667298, rs2305948 and rs1870377) are correlated with an increased risk of a glioma when homozygous (31). Another study described that the *VEGFR-2* rs2305948 T polymorphism frequency is higher in patients with gastroenteropancreatic neuroendocrine neoplasms compared with that in the healthy population (19). In the present study, among the genetic variations of the *VEGFR2* gene, the *KDR* rs1870377 and *KDR* rs7692791 TT genotypes were found to be associated with a better prognosis, and the *KDR* rs2034965 GG genotype was associated with increased OS time. Zhu *et al* (20) demonstrated that the *VEGFR2* rs1870377 TT genotype confers a favorable prognosis in gastric cancer. Furthermore, Wang *et al* (21) investigated the correlation between polymorphisms of four genes from the epidermal growth factor receptor *(EGFR*) pathway and the clinical outcome of 363 patients with hepatocellular carcinoma, and reported that *EGFR* rs2034965 with the AA genotype is negatively correlated with disease-free survival. These results were consistent with the results from the present study; however, inconsistent results were reported in other types of cancer, and Kim *et al* (22) reported that the *VEGFR2* rs1870377 TT genotype is associated with shorter OS time in patients with diffuse large B cell lymphoma. Furthermore, it was reported that rs7692791 C allele is significantly correlated with increased OS and DFS in hepatocellular carcinoma (21). These discordances may be partly attributed to the different types of cancer, the different clinical characteristics of the patients and the study sizes. The rs1870377 mutation is located in the coding region of *KDR* and is a missense mutation. The functional role of this gene polymorphism remains unclear.

Proteins from the *PDGF* family are crucial to stimulate the proliferation, survival and migration of mesenchymal cells (32), This family consists of 5 different isoforms, named disulphide-bonded homodimers of A-, B-, C- and D-polypeptide chains and the heterodimer *PDGF-AB*. *PDGFR* is classified as a receptor tyrosine kinase, and the 5 *PDGF* isoforms can activate cellular responses via *PDGFRα* and *PDGFRβ* (32,33). Overactivation of the *PDGF-PDGFR* signaling pathway has been reported to be associated with tumorigenesis (34). *PDGFR* gene mutations have been found in malignancies. Point mutations in *PDGFRα* were found in ~5% of gastrointestinal stroma tumors, which led to amino acid residue changes, therefore activating *PDGFR* kinase activity (35). In addition, a study reported that rs1800812 T allele and rs6554162 G allele in *PDGFRα* were related to decreased frequency in patients with papillary thyroid cancer compared with that in the healthy population (23). A previous study demonstrated that two SNPs in *PDGFβ* (rs5757573 T>C and rs6001516 C>T) were associated with an increased risk of pancreatic cancer (36). Furthermore, Volz *et al* (24) found that the SNP (rs2302273 C>T) in *PDGFRβ* gene was associated

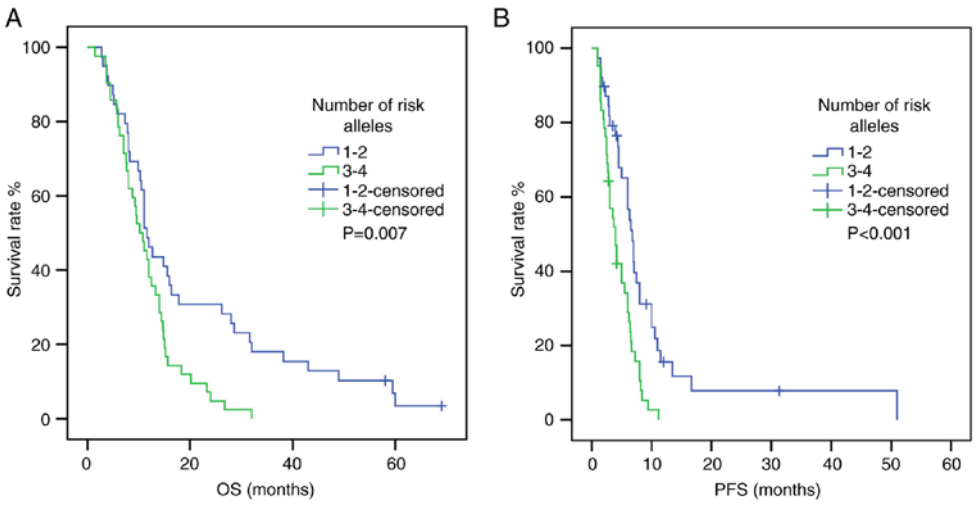

Figure 4. Association between number of risk alleles (rs1870377/rs2065364 combination) and survival time. (A) PFS curve. (B) OS curve. P-values were obtained by log-rank tests. OS, overall survival; PFS, progression-free survival.

with a significantly longer PFS time in patients with metastatic colorectal cancer. However, in the present study, no relevance was found between SNPs and prognosis.

The present study had some limitations. Firstly, only 81 patients with AGC were eligible for statistical analysis. Since the sample size was relatively small, the results from this study should be considered as preliminary data and for generation of a hypothesis for subsequent investigation. Secondly, since the patients studied had AGC, it is not known whether the results could be applicable to patients with other types of gastric cancer. Further investigation should therefore be conducted to validate the results.

In conclusion, the results from the present study demonstrated that *KDR* rs7692791 and *NRP-1* rs2065364 were positively associated with PFS. Furthermore, *KDR* rs2034965 and *KDR* rs1870377 significantly negatively correlated with OS time following multivariate analysis in patients with AGC. In addition, the numbers of 'risk alleles' of *NRP-1* rs2065364/*KDR* rs1870377 combination were significantly associated with survival time. These results suggested that genetic variants in *NRP-1* and *KDR* genes may affect the biological features and prognosis of patients with AGC. Due to limited funding, the underlying mechanisms were not explored, and further investigation is required to verify these results.

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Availability of data and materials

All data and materials generated and/or used during the study are available from the corresponding author upon reasonable request.

Authors' contributions

TW and YS designed the study. YS collected the patients' clinical data. YJZ and YS performed the experiments. YJZ and YS analyzed the data and wrote the manuscript. TW contributed to the revision of the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Clinical Research Ethics Committee of The Second Hospital Affiliated to Dalian Medical University and was conducted in accordance with The Declaration of Helsinki. Participants were fully informed of the procedures and provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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