

# Association between potentially functional polymorphisms of chemokine family members and the survival of esophageal cancer patients in a Chinese population

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**Background:** The chemokine family plays an important role in the growth, invasion, and metastasis of tumors. However, most studies have only focused on a few genes or a few gene loci, and thus could not reveal the associations between functional polymorphisms of chemokine family members and tumor progression. This study aimed to determine the associations between single nucleotide polymorphisms (SNPs) of chemokine family members and the prognosis of esophageal cancer (EC).

**Methods:** The Cox risk proportional model and log-rank test were used to analyze the associations of 16 potentially functional SNPs in 13 genes from the chemokine family with the survival of 729 Chinese patients with EC.

**Results:** Prognostic analysis on the 16 SNPs showed that different genotypes of 5 SNPs were associated with patients' survival and the risk of death. Multivariate Cox regression analysis showed that the risk of death was higher in CCL26rs2302009 genotype A/C carriers than in A/A carriers and it was also higher in CX3CL1rs2239352 genotype T/T carriers than in C/C carriers. Stepwise Cox regression analysis showed that CCL26rs2302009 genotype A/C was an independent prognostic factor of EC, and its association with increased risk of death was stronger in patients who were ≤60 years old, female, with tumors located in the middle part of esophagus, with undifferentiated or poorly differentiated tumors, with early-stage pathologic type disease, with the longest diameter of tumor ≤5cm than in their counterparts.

**Conclusion:** These findings suggest that CCL26rs2302009 may be a candidate biomarker for EC and its effect on death risk are associated with the histological grade, pathologic type, and the longest diameter of tumor.

**Keywords:** esophageal cancer, prognosis, single nucleotide polymorphism, chemotactic factor

## Introduction

China has the highest incidence and mortality of esophageal cancer (EC) in the world, with over 50% of new cases and deaths of EC occurring in China every year. More than 90% of patients are diagnosed at middle or late stages.<sup>1</sup> Although rapid progress has been made in the treatments of EC, its prognosis remains poor, with a 5-year survival rate of approximately 20% for patients with middle-stage or advanced EC.<sup>2</sup> Currently, the main prognostic factors of EC are TNM stage and treatment methods. However,

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heterogeneity in tumor progression and prognosis has been observed among EC patients who had the same clinical features and underwent the same treatment regimens, which suggests the existence of other important factors that may affect the prognosis. Therefore, the identification of other potential prognostic factors of EC, such as specific biomarkers, is now under investigation.

Dysregulated inflammatory response is known to be associated with increased risk of some chronic diseases, including cancers. Chemokines are a group of small-molecule cytokines (relative molecular weight, 8–14kDa) that have chemo attractant activity. These cytokines all contain 4 conservative cytokines and are classified into CXC, CC, CX3C, and C subfamilies. Chemokine receptors, a group of G protein-coupling transmembrane receptors (GPCR) which mediate functions of chemokines, are usually expressed on the membrane of immunocytes, endothelial cells, and other cells. Chemokine receptors are also classified into 4 subfamilies according to their ligands.<sup>3</sup> By binding to specific GPCR, chemokines play roles in many normal biological processes, such as hematopoietic cell genesis, leukocyte migration and homing, and embryonic development, and some pathologic processes, such as inflammatory diseases, virus infection, graft rejection, and malignant tumorigenesis.<sup>4</sup> Some studies showed that chemokines play important roles in the growth, angiogenesis, and metastasis of many types of tumor.<sup>5</sup> Tumor-associated chemokines not only can promote the proliferation of tumor cells and inhibit apoptosis but also are involved in the migration of tumor cells, angiogenesis of tumors, infiltration of lymphocytes in tumor tissues, and organ-specific metastasis of tumor cells.<sup>5</sup> For example, some members of the CC and CXC subfamilies are involved in the angiogenesis of tumors and can promote the invasion and organ-specific metastasis of breast cancer, prostatic cancer, malignant melanoma, and lung cancer.<sup>6–9</sup> Abnormal expression of chemokines, such as CXC ( $\alpha$ ) and CC ( $\beta$ ), may affect the tumorigenesis, progression, and prognosis of many types of tumors, including EC.<sup>8–11</sup> For example, the high expression of CXCL12 and its receptor CXCR4 was shown to be associated with the invasion and metastasis of EC, and continuous expression of CXCR4 after preoperative radio chemotherapy was associated with early relapse and poor prognosis of EC.<sup>9–11</sup>

The associations between the polymorphisms of a few genes in the chemokine family and the progression and prognosis of tumors have been studied. CXCL12rs1801157 has

been reported to be associated with the risk of breast, lung, rectal, and liver cancers and may be a predictive marker of the lymph node metastasis of colon cancer and distant metastasis of lung cancer.<sup>12–14</sup> CXCL14rs2237062 was associated with the progression of HBV-related liver cancer in Chinese populations.<sup>15</sup> The C>A mutation of CCL22rs4359426 may aggravate *H. pylori* infections, increasing the risk of gastric cancer.<sup>16</sup> Schimanski et al found that the genotype GA/AA of CXCL12rs1801157 was associated with distant metastasis of EC, which suggested that CXCL12rs1801157 may be a potential prognostic marker of EC.<sup>17</sup> Although these studies have shown the associations between polymorphisms of some genes in the chemokine family and the prognosis of some tumors, most studies have only focused on a few genes/polymorphisms, and thus could not reveal associations between functional polymorphisms of chemokine family members and tumor progression. In addition, the results from European and American populations may not be used to predict the situation in Chinese populations.

In the present study, we used the improved multiple ligase detection reaction (iMLDR) genotyping to detect potentially functional SNPs of chemokine family members and to determine their associations with the prognosis of Chinese patients with EC.

## Materials and methods

### Study population

A total of 729 patients with newly diagnosed EC between January 1, 2004 and December 31, 2013 at Cixian People's Hospital (Handan, Hebei, People's Republic of China) were selected. All the diagnoses were confirmed pathologically. No patients had a history of other tumors as well as a history of chemotherapy or radiotherapy. Tumor tissue sampling was performed after the doctors explained the potential usage and risks in detail to patients and/or family members. The informed consent was signed by patients and/or family members before sampling. Patients or their family members were interviewed after discharge every 6 months for the first year and every year thereafter until death. The follow-up was ended on August 1, 2015. Patient data were strictly conducted in accordance with the Declaration of Helsinki.

Cixian People's Hospital affiliated with Guangdong Medical University. Permission to use the data and follow-up results were granted by the Institutional Ethics Review Board of Guangdong Medical University (No. PJ2015042KT).

## SNP selection and genotyping

Potentially functional polymorphisms in chemokine family genes were screened using HapMap database ([www.hapmap.org/](http://www.hapmap.org/), Phase 2 and 3, Release 27, NCBI Build 36.3). Candidate genes included all the genes and their receptors (a total of 77 genes) in the chemokine family. Firstly, we downloaded the relevant SNP information from HapMap, and then selected polymorphisms according to the following criteria: 1) they would be located at potentially functional regions, such as 5'-untranslated regions (UTRs), exons, and 3'-UTRs; 2) they would be with a minor allele frequency (MAF)  $\geq 0.05$  in Chinese populations; 3) one locus would be selected as a representative when a high linkage disequilibrium (LD) ( $r^2 \geq 0.80$ ) existed among multiple polymorphisms. The selected polymorphisms were re-examined through the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/snp/>) to identify the potentially functional SNPs. The candidate SNPs were further assessed through the improved multiple ligase detection reaction (iMLDR) and identified SNaPshot sequencing.

The genomic DNA was extracted from the 729 EC samples using the AxyPrepMultisource Genomic DNA Minprep Kit (Axygen Bioscience, Union City, CA, USA). SNPs were typed using the imLDR TMMultisource SNP Typing Kit. Blank control in 1 well and repeated samples in 5 parallel wells were set in every 96-well plate for quality control. The SNPs with inconsistent genotypes in repeated samples were excluded, and those with consistent genotypes and with a call rate  $>95\%$  were considered qualified. MAFs of the SNPs were compared with those in the HapMap-HCB (Han Chinese) database: the SNPs with a MAF difference  $<0.05$  were considered qualified, whereas those with a MAF difference  $>0.05$  were subjected to re-typing to rule out the mistakes in typing. The  $D'$  values between every two SNPs were analyzed using the Haplo View software to rule out the mistakes in typing.

## Statistical analysis

Hardy–Weinberg equilibrium of the genotypes of identified SNPs among patients was assessed using a goodness-of-fit chi-square test. The median survival was calculated using the Kaplan–Meier method. Survival curves were plotted using the Kaplan–Meier method, and survival data were compared using the log-rank test. Univariate and multivariate Cox proportional hazard regression analyses were performed to estimate the crude or adjusted HR and their 95% CIs, with adjustment of age, gender, smoking history, histological type, clinical stage, and treatment. Cox stepwise regression analysis was also conducted to determine predictive factors of the

prognosis of EC, with a significance level of 0.05 for entering and 0.10 for removing the respective explanatory variables. The associations between CCL26 rs2302009 genotypes and EC survival were evaluated by stratified analysis of age, gender, histological types, anatomical site, pathologic grade, pathologic type, TNM stage, and longest diameter of tumor. All the statistical analyses were carried out using SAS statistical package version 9.1 (SAS Institute Inc., Cary, NC, USA).

## Results

### Clinicopathologic characteristics of patients

Of the 729 patients, 676 were followed, with a follow-up rate of 92.7%. Among the followed-up patients, 241 died. The median survival time was 63.0 months. Age, histological type, and tumor location had no relationship with the patient survival. Gender, histological grade, pathologic type (according to the classification of International Union Against Cancer, the pathological types of early EC include erosive type, plaque type, hidden type and nodule type according to its morphology, while the middle and late EC included medullary type, mushroom type, ulcerative type, constrictive type, intraluminal type and undefined type), TNM stage, and the longest diameter of tumor were associated with the patient survival (Table 1).

### Associations of SNPs of chemokine family members with the patient survival

We identified 17 potentially functional SNPs in 14 genes from the chemokine family. Among the 17 SNPs, rs3136667 showed three genotypes (C, G, and A) according to the data in the NCBI database. In addition, the primers for extension could not be designed because both ends of its sequence were G. Therefore, rs3136667 was excluded, and 16 SNPs in 13 genes were finally selected. For the 16 SNPs, the call rates of genotyping were all above 90%, and their MAF and Hardy–Weinberg equilibrium values were all above 0.05. These genes and SNPs are presented in Table 2. Prognostic analysis on the 16 SNPs showed that different genotypes of 5 SNPs were associated with the patient survival.

Multivariate Cox regression analysis on the prognostic values of the 5 SNPs showed that CCL26rs2302009 and CX3CL1rs2239352 were associated with the prognosis of EC patients (Table 3). After adjustment of age, gender, histological type, tumor location, histological grade, pathologic type, TNM stage, and the longest diameter of tumor, the

**Table 1** Patient characteristics and features

Variable		Patients		Deaths		MST (months)	Log-rank P	HR (95% CI)
		n	(%)	n	(%)			
Age (years)	≤60	419	62.0	150	62.3	67.0	0.430	1.000
	>60	257	38.0	91	37.7	61.0		0.900(0.692–1.171)
Gender	Male	434	64.2	172	71.4	58.0	0.008	1.000
	Female	242	35.8	69	28.6	67.0		0.686(0.519–0.908)
Histological type	Squamous cells	623	92.2	222	92.1	62.0	0.935	1.138(0.561–2.305)
	Adenocarcinoma	28	4.1	11	4.6	–		1.113(0.447–2.770)
	Others	25	3.7	8	3.3	–		1.000
Anatomical site	Proximal end of esophagus	88	13.0	40	16.6	63.0	0.687	1.184(0.757–1.853)
	Middle esophagus	478	70.7	163	67.7	62.0		1.025(0.720–1.460)
	Distal esophagus	110	16.3	38	5.7	66.0		1.000
Histological grade	Well-differentiated	106	15.7	30	12.4	108.0	0.032	0.611(0.395–0.946)
	Moderately differentiated	417	61.7	145	60.2	63.0		0.719(0.537–0.963)
	Poorly undifferentiated	153	22.6	66	27.4	53.0		1.000
Pathologic type	Early stage	171	25.3	27	11.2	81.0	<0.001	1.000
	Middle–late stage	505	74.7	214	88.8	55.0		3.095(2.058–4.653)
TNM stage	I	145	21.4	38	15.8	108.0	0.026	1.000
	II	351	51.9	133	55.2	62.0		1.561(1.084–2.248)
	III/IV	180	26.7	70	29.0	62.0		1.674(1.123–2.493)
Longest diameter of tumor(cm)	≤5	552	81.7	182	75.5	63.0	<0.001	1.000
	>5	124	18.3	59	24.5	42.0		1.712(1.273–2.301)

**Abbreviation:** MST, media survival time.

heterozygous mutation genotype A/C of CCL26rs2302009 and the homozygous mutation genotype T/T of CX3CL1rs2239352 were associated with increased risk of death. The risk of death among patients who carried both risk genotypes was increased by 303.7% as compared with that among patients who carried only one or none of the two risk genotypes.

## Independent predictors of the patient survival

To identify independent prognostic factors, age, gender, histological type, tumor location, histological grade, pathologic type, TNM stage, the longest diameter of tumor, CCL26rs2302009 genotypes, and CX3CL1rs2239352 genotypes were included in stepwise Cox regression analysis. Histological grade, pathologic type, the longest diameter of tumor, and CCL26rs2302009 genotype A/C were identified as independent prognostic factors (Table 4).

## Stratified analysis on the prognostic value of CCL26rs2302009 genotype A/C

Stratified analysis showed that the association between CCL26rs2302009 genotype A/C and the increased risk of

death was stronger in patients who were ≤60 years old, female, with tumors located in the middle part of esophagus, with undifferentiated or poorly differentiated tumors, with early-stage pathologic type disease, with the longest diameter of tumor ≤5cm than in their counterparts (Table 5). Kaplan–Meier plots of EC-specific survival by rs2302009 genotypes and histological grade, pathologic type or the longest diameter are shown in Figures 1, 2 and 3.

## Discussion

In the present study, we analyzed the associations between potentially functional SNPs of genes from the chemokine family and prognosis of EC patients after surgery. Multivariate Cox regression analysis showed that CCL26rs2302009 and CX3CL1rs2239352 genotypes were associated with the prognosis. Stepwise Cox regression analysis showed that histological grade, pathologic type, the longest diameter of tumor, and CCL26rs2302009 genotype A/C were the independent prognostic factors. These findings suggest that, in addition to pathologic stage, pathologic type, and the longest diameter of tumor, CCL26 rs2302009 may be a candidate biomarker for the prediction of the survival of EC patients and may affect the risk of death.

**Table 2** Genotyping of selected SNPs of chemokine family and their associations with esophageal cancer survival in genetic models

SNPs	Location	No.(%) of genotyping	HWE#	MAF in patients	Hapmap-HCB	Log-rank P			
						Dominant model	Recessive model	Additive model	Co-dominant model
CCL16rs2063979	3'-UTR (T>C)	95.08	0.629	0.555	0.496	0.436	0.535	0.478	0.655
CLI19rs2227302	3'-UTR (G>A)	99.47	0.378	0.100	0.073	0.882	0.264	0.490	0.275
CCL23rs1003645	nonsynon_exon4 (T>C)	99.47	0.526	0.37	0.433	0.047	0.539	0.138	0.219
CCL25rs1129763	nonsynon_exon4 (C>T)	99.20	0.846	0.151	0.157	0.175	0.281	0.142	0.398
CCL26rs2302009	3'-UTR (A>C)	94.02	0.181	0.112	0.084	0.013	0.902	0.028	0.945
CCR6rs3093005	3'-UTR (T>C)	96.68	0.270	0.053	0.069	0.408	0.242	0.266	0.251
CX3CLIrs2239352	3'-UTR (C>T)	99.34	0.801	0.074	0.084	0.234	0.000	0.000	0.000
CXCL2rs9131	3'-UTR (C>T)	99.47	1.000	0.322	0.333	0.064	0.437	0.177	0.223
CXCL9rs373236	3'-UTR (G>A)	99.47	0.206	0.07	0.056	0.028	0.610	0.070	0.617
CXCL10rs3921	3'-UTR (G>C)	99.47	0.117	0.054	0.044	0.237	0.323	0.397	0.318
CXCL12rs1029153	3'-UTR (A>G)	90.03	0.599	0.25	0.224	0.523	0.453	0.689	0.406
CXCL12rs1801157	3'-UTR (C>T)	99.20	0.847	0.216	0.252	0.078	0.471	0.101	0.753
CXCL12rs2522	3'-UTR (A>G)	99.47	0.724	0.085	0.066	0.670	0.190	0.331	0.189
CXCL16rs1050998	nonsynon_exon4 (G>A)	99.47	0.976	0.374	0.412	0.626	0.653	0.714	0.856
CXCL16rs1051009	3'-UTR (A>G)	98.94	0.422	0.448	0.500	0.246	0.986	0.473	0.491
CXCR6rs936939	intron1 (C>A)	99.47	0.575	0.351	0.379	0.320	0.990	0.579	0.726

Note: #Hardy-Weinberg equilibrium was examined by goodness-of-fit  $\chi^2$  test.

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms.

**Table 3** Polymorphisms of chemokine family and their associations with esophageal cancer survival

Genotype		Patients	Deaths	MST (mo)	Crude HR(95% CI)	Adjusted*HR(95%CI)
CCL26rs2302009		n=629	n=231			
	A/A	499	170	66.00	1.000	1.000
	A/C	117	56	43.00	1.504(1.109–2.038)	1.466(1.078–1.993)
	C/C	13	5	61.00	1.031(0.424–2.509)	0.927(0.378–2.270)
	Additive model				1.241(1.064–1.446)	1.249(1.070–1.459)
	A/A	499	170	66.00	1.000	1.000
	(A/C)/(C/C)	130	61	44.00	1.518(1.128–2.042)	1.525(1.131–2.056)
CX3CL1rs2239352		n=668	n=240			
	C/C	574	200	66.00	1.000	1.000
	C/T	91	37	58.00	1.150(0.809–1.635)	1.230(0.859–1.762)
	T/T	3	3	15.00	7.185(2.279–22.656)	7.164(2.184–23.499)
	Additive model				1.109(0.929–1.324)	1.124(0.940–1.344)
	C/C	509	200	63.84	1.000	1.000
	(C/T)/(T/T)	83	40	58.22	1.271(0.897–1.801)	1.295(0.912–1.838)
Combined effect (Number of risk genotypes carried) <sup>b</sup>		n=628	n=231			
	0	442	146	66.87	1.000	1.000
	1	167	72	48.86	1.313(0.989–1.744)	1.276(0.960–1.697)
	2	19	13	23.75	3.365(2.052–6.349)	4.337(2.427–7.750)
					<i>P</i> for trend <0.001	
	0–1	609	218	64.01	1.000	1.000
	2	19	13	23.75	3.350(1.905–5.888)	4.037(2.276–7.161)

**Note:** \*Adjusted for age, gender, anatomical site, histological type, histological grade, pathologic type, TNM stage and the longest diameter of the tumor.

**Abbreviations:** MST, media survival time.

**Table 4** Multivariate Cox regression analysis on esophageal cancer related survival

Variables	$\beta$	SE	Wald $\chi^2$	<i>P</i>	HR*	HR 95%CI
Histological grade	0.257	0.109	5.535	0.019	1.293	1.044–1.601
Pathologic type	0.939	0.217	18.806	<0.001	2.558	1.673–3.911
Longest diameter of tumor	0.358	0.157	5.189	0.023	1.431	1.051–1.948
rs2302009(A/A)	-	-	6.330	0.042	-	-
rs2302009(A/C)	0.386	0.156	6.142	0.013	1.471	1.084–1.995
rs2302009(C/C)	-0.099	0.455	0.048	0.827	0.905	0.371–2.208

**Note:** \*Adjusted for age, gender, anatomical site, histological type, histological grade, pathologic type, TNM stage and the longest diameter of the tumor.

**Abbreviations:** SE, standard error.

In 2001, Muller et al found that tumor cells may achieve organ-specific metastasis through the binding of chemokines and their receptors.<sup>18–20</sup> Since then, accumulating evidence showed that the specific binding of chemokines and their receptors played a key role in the growth, invasion, and metastasis of malignant tumors.<sup>20,21</sup> The poor prognosis of malignant tumor is associated with tumor metastasis, which is a highly tissue- and organ-selective, complex process.<sup>22</sup> CCL26 is a member of the chemokine CC subfamily and acts on

eosinophil granulocytes and T lymphocytes.<sup>22</sup> Its receptor CCR3 is produced by epithelial cells and may contribute to the accumulation and local infiltration of eosinophil granulocytes. As the activator of CCR3, CCL26 attracts and activates eosinophil granulocytes, basophils, and Type 2 helper (Th2) T lymphocytes. CCL26 is also an antagonist of CCR1, CCR2, and CCR5.<sup>23</sup> Heresi et al found that CCL26 inhibited the function of monocyte chemotactic protein-1 (MCP-1, also termed CCL2).<sup>23–25</sup> Therefore, we consider that CCL26 may regulate the inflammation.

**Table 5** Stratified analysis of variants genotypes associated with EC survival

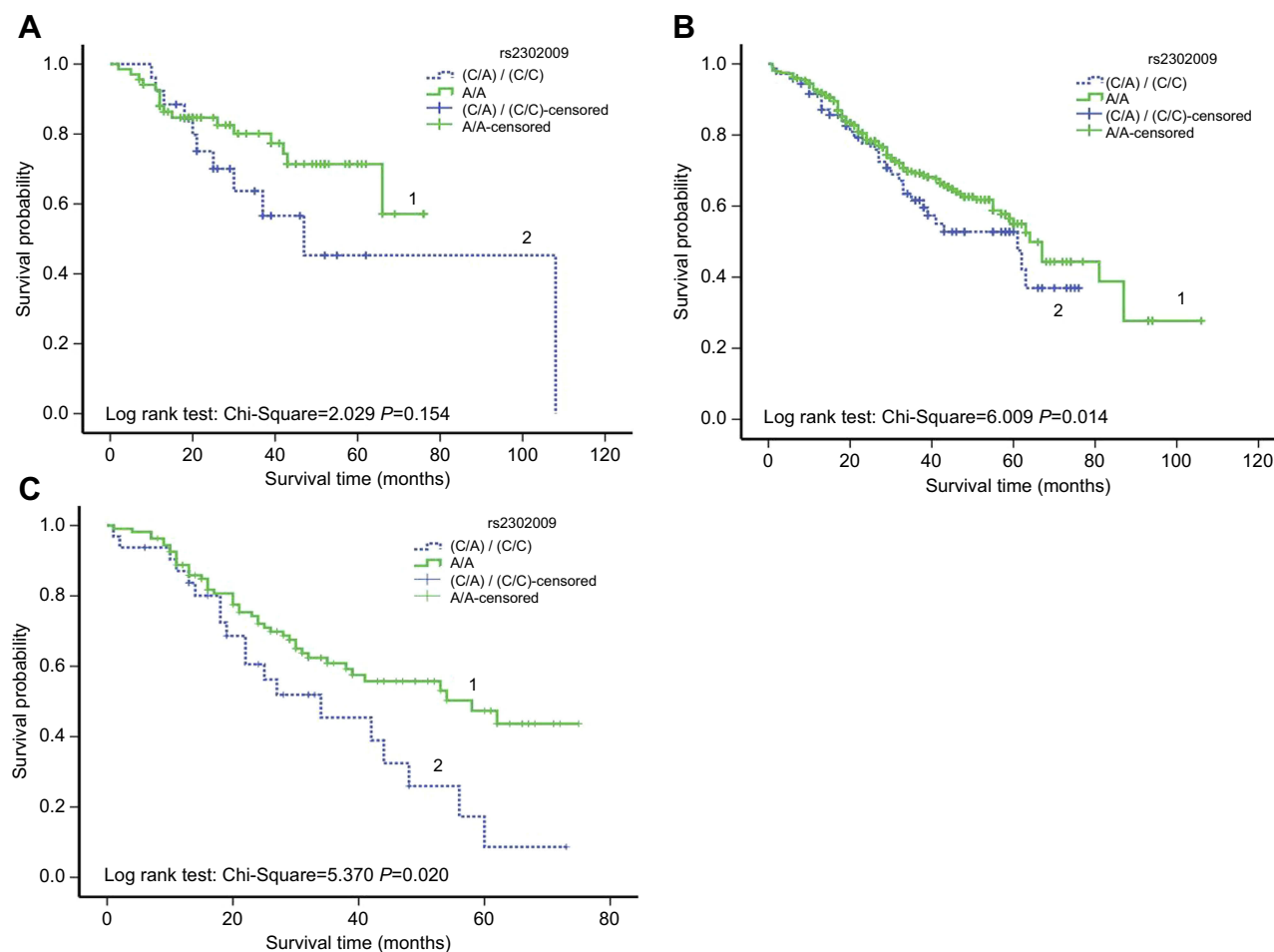
Variables		CCL26rs2302009(deaths/ patients)		Adjusted HR	Adjusted HR 95%CI
		A/A	(A/C)/(C/C)		
Age (year)	≤60	105/312	39/79	1.606	1.104–2.336
	>60	65/187	22/51	1.198	0.727–1.977
Gender	Male	126/318	38/85	1.114	0.768–1.646
	Female	44/181	23/45	2.442	1.434–4.160
Histological types	Squamous cells	158/461	55/119	1.348	0.987–1.842
	Adenocarcinoma	6/19	4/7	6.445	0.632–65.767
	Others	6/19	2/4	0.525	0.014–19.245
Anatomical site	Proximal end of esophagus	30/68	10/16	1.226	0.550–2.735
	Middle esophagus	114/351	42/94	1.448	1.011–2.073
	Distal esophagus	26/80	9/20	1.219	0.552–2.690
Histological grade	Well-differentiated	16/68	11/26	1.161	0.486–2.770
	Moderately differentiated	110/323	31/72	1.209	0.804–1.817
	Poorly undifferentiated	44/108	19/32	1.788	1.013–3.155
Pathologic type	Early stage	16/129	10/27	2.936	1.150–7.498
	Middle–late stage	154/370	51/103	1.278	0.928–1.760
TNM stage	I	24/109	12/25	1.642	0.746–3.614
	II	100/259	26/62	1.138	0.732–1.769
	III/IV	46/131	23/43	1.590	0.916–2.758
Longest diameter of tumor(cm)	≤5	129/411	46/105	1.434	1.019–2.017
	>5	41/88	15/25	1.277	0.659–2.475

**Abbreviation:** EC, esophageal cancer.

CCL26 has been reported to be expressed in several types of solid tumor and plays important roles in the migration, infiltration, metastasis, and prognosis of the tumor.<sup>24,25</sup> Blanchard et al reported that external stimulation or damage may induce the activation of CCL26.<sup>26</sup> CCL26 gene products have a chemotactic effect on eosinophil granulocytes. The secretion from eosinophil granulocytes contains vascular endothelial growth factor, and the accumulation of eosinophil granulocytes may improve angiogenesis in tumor tissues. This may explain, in part, the role of CCL26rs2302009 mutation in facilitating tumor metastasis and causing poor prognosis. CCL26 may attract macrophages to secrete abundant cytokines, induce angiogenesis, and lead to the deterioration of the tumor.<sup>27</sup> It is noteworthy that CCL26 is also an activator of CX3CR1. When the expression of CX3CR1 is low, both CCL26 and CX3CL1 attract eosinophil granulocytes to accumulate in tumor tissues.<sup>28</sup> In the present study, we found that CCL26 mutations were associated with poor prognosis of EC patients after surgery. A similar result was reported by Yang et al in a tissue microarray study.<sup>29</sup>

In the present study, we found that CCL26rs2302009 genotype A/C could be an independent prognostic factor of

EC after surgery. Stratified analysis showed that the association of CCL26rs2302009 genotype A/C with increased risk of death was stronger in patients who were ≤60 years old, female, with tumors located in the middle part of the esophagus, with undifferentiated or poorly differentiated tumors, with early-stage pathologic type disease, and with the longest diameter of tumor ≤5cm than in their counterparts. This finding may be used to guide the individualized therapy for EC. In an extensive genomic study on 117 patients with eosinophilic esophagitis conducted by Blanchard et al, the CCL26rs2302009 mutation was found in people who were susceptible to eosinophilic esophagitis, and this mutation increased the stability of CCL26 mRNA.<sup>26</sup> Therefore, we speculate that CCL26rs2302009 genotype A/C may increase the stability of CCL26 mRNA in EC patients, and affect the transcription and expression of the CCL26 gene. The continuous expression of CCL26 in esophageal tissues may induce severe infiltration of the eosinophil granulocytes and angiogenesis in the tumor tissues, leading to the metastasis of the tumor and resulting in poor prognosis. However, the biological effects of CCL26 and its receptor CCR3 are complex and may be affected by other factors. For example, abnormal expression of CCL26 is regulated by the signal



**Figure 1** Kaplan-Meier plots of survival by CCL26rs2302009 genotypes and histological grade in EC-specific survival. **(A)** Strata with the well-differentiated histological grade (log-rank  $P=0.154$ ); "1" means patients with genotype (A/A) and with the well-differentiated histological grade; "2" means patients with genotype (C/A)/(C/C) and with the well-differentiated histological grade. **(B)** Strata with the moderately-differentiated histological grade (log-rank  $P=0.014$ ); "1" means patients with genotype (A/A) and with the moderately-differentiated histological grade; and "2" means patients with genotype (C/A)/(C/C) and with the moderately-differentiated histological grade. **(C)** Strata with the poorly-undifferentiated histological grade (log-rank  $P=0.020$ ); "1" means patients with genotype (A/A) and with the poorly-undifferentiated histological grade; and "2" means patients with genotype (C/A)/(C/C) and with the poorly-undifferentiated histological grade.

transducer and activator of transcription 6 (STAT6) or other signaling pathways.<sup>30</sup> The synergetic effect of CCL26 and CX3CL1 may induce high expression of CX3CR1, attracting eosinophil granulocytes to accumulate in the tumor tissues.<sup>28</sup> However, the exact biological mechanisms and associated with signaling pathways need to be determined in future functional studies.

Several limitations of the present study need to be mentioned. First, EC patients were selected from a single hospital, with limited representativeness for general application. Second, due to the genotyping failure, other SNPs were not included in statistical analyses to analyze their associations with the prognosis of EC, which suggested the existence of information bias. Third, the exact biological mechanisms are still unclear and need to be validated through functional analyses in large cohort studies involving multiple races.

## Conclusion

In the chemokine family, CCL26rs2302009 genotype A/C is associated with the poor prognosis of EC patients and may be a candidate prognostic factor of EC.

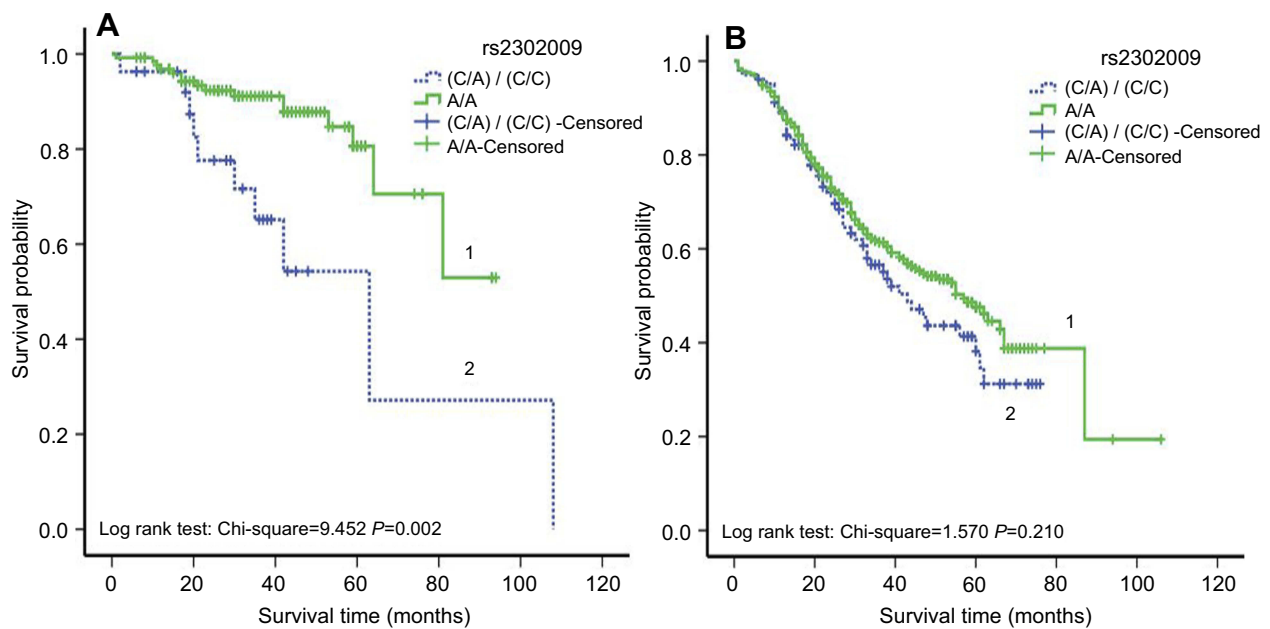
## Ethics approval and consent to participate

This manuscript was approved by the Institutional Ethics Review Board of Guangdong Medical University.

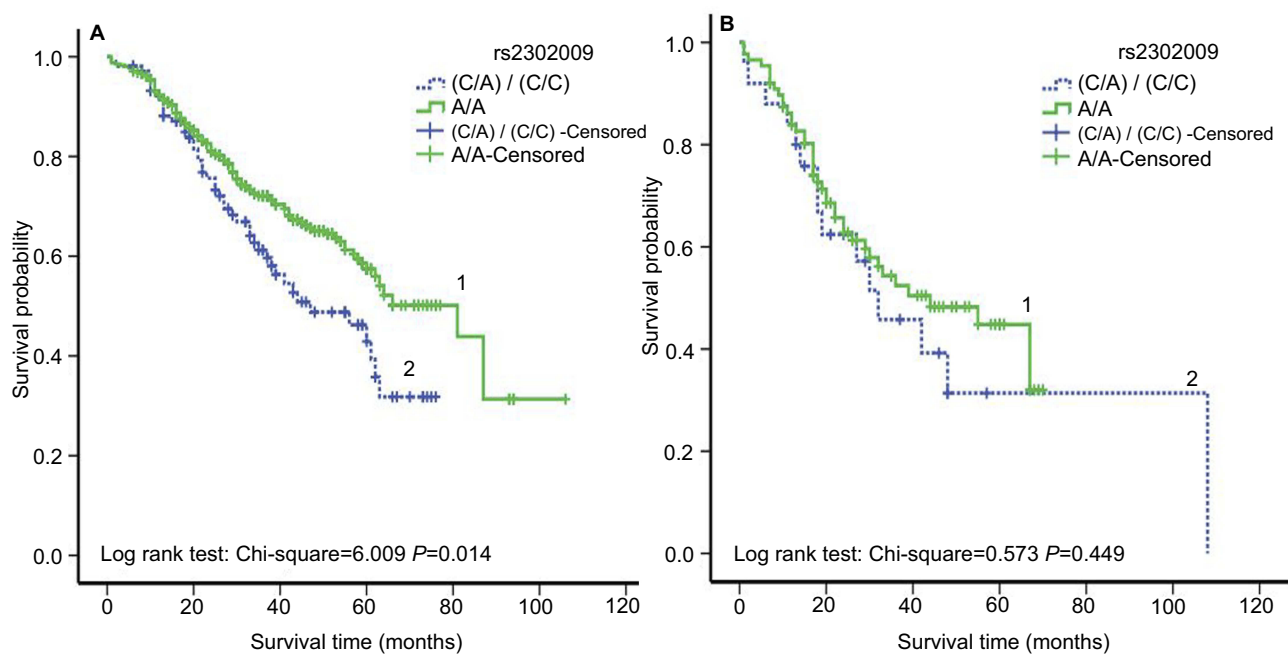
## Abbreviation list

SNPs, single nucleotide polymorphisms; EC, esophageal cancer; GPCR, G protein-coupling transmembrane receptors; MAF, a minor allele frequency; iMLDR, the improved multiple ligase detection reaction.





**Figure 2** Kaplan-Meier plots of survival by CCL26rs2302009 genotypes and pathologic type in EC-specific survival. **(A)** Strata with the early stage of pathologic type (log-rank  $P=0.002$ ); “1” means patients with genotype (A/A) and with the early stage of pathologic type; “2” means patients with genotype (C/A)/(C/C) and with the early stage of pathologic type. **(B)** Strata with the middle-late stage of pathologic type (log-rank  $P=0.210$ ); “1” means patients with genotype (A/A) and with the middle-late stage of pathologic type; and “2” means patients with genotype (C/A)/(C/C) and with the middle-late stage of pathologic type.



**Figure 3** Kaplan-Meier plots of survival by CCL26rs2302009 genotypes and the longest diameter of tumor in EC-specific survival. **(A)** Strata with  $\leq 5$ cm of the longest diameter (log-rank  $P=0.014$ ); “1” means patients with genotype (A/A) and with  $\leq 5$ cm of the longest diameter; “2” means patients with genotype (C/A)/(C/C) and with  $\leq 5$ cm of the longest diameter. **(B)** Strata with  $> 5$ cm of the longest diameter (log-rank  $P=0.449$ ); “1” means patients with genotype (A/A) and with  $> 5$ cm of the longest diameter; and “2” means patients with genotype (C/A)/(C/C) and with  $> 5$ cm of the longest diameter.

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## Author contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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