Original Paper

The Relationship Between *MMP-2* -1306C>T and *MMP-9* -1562C>T Polymorphisms and the Risk and Prognosis of T-Cell Acute Lymphoblastic Leukemia in a Chinese Population: A Case-Control Study

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Key Words

T-cell acute lymphoblastic leukemia • Polymorphism • *MMP-2* -1306C>T • *MMP-9* -1562C>T • Risk • Prognosis

Abstract

Background: T-cell acute lymphoblastic leukemia (T-ALL) is a malignant hematological disease and is often accompanied by a variety of genetic abnormalities. Hence, our study aims to investigate the relationship between *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms and the risk and prognosis of T-ALL. **Methods:** From April 2009 to February 2011, a total of 376 T-ALL patients were chosen as the case group. Meanwhile, 352 healthy people who passed routine health examinations were selected as the control group. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was used to detect the frequency of *MMP-2* -1306C>T (rs243865) and *MMP-9* -1562C>T (rs3918242) polymorphisms in the study subjects. The serum levels of *MMP-2* and *MMP-9* were detected using enzyme-linked immunosorbent assay (ELISA). A Kaplan-Meier analysis was employed to analyze the event-free survival (EFS) rates of the T-ALL patients with different *MMP-2* and *MMP-9* genotypes. A multivariate COX model was applied to analyze the relationship between *MMP-2* and *MMP-9* polymorphisms and the prognosis of T-ALL patients. A C-statistic and net reclassification index (NRI) was carried out to evaluate the predictive value of *MMP-2* and *MMP-9* gene polymorphisms using the Cox model. **Results:** Compared to the control group, the genotypic frequency of *MMP-2* -1306C>T (CT + TT) and *MMP-9* -1562C>T (CT + TT) in the case group C.-M. Lin, Y.-L. Zeng and M. Xiao are regarded as co-first author.

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was significantly higher. The serum level of MMP-9 was markedly elevated in T-ALL patients with the CT + TT genotype compared to patients with the CC genotype. The results of the Kaplan-Meier analysis showed that the median EFS was lower in T-ALL patients with the CT + TT genotype of *MMP-9* -1562C>T compared to patients with the CC genotype. The results of a multivariate analysis using the Cox proportional hazard model indicated that the *MMP-9* -1562C>T polymorphism was associated with the prognosis of T-ALL patients. **Conclusion:** These results indicated that *MMP-2* -1306C/T and *MMP-9* -1562C/T polymorphisms might be associated with an increased risk of T-ALL. The *MMP-9* -1562C>T polymorphism may also be related to the prognosis of T-ALL patients.

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Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a malignant hematological disease characterized by high white blood cell counts, accumulation of immature T cells and tissue infiltration [1]. It progresses rapidly and is often accompanied by a variety of genetic abnormalities [2]. Currently, T-ALL accounts for 25% of all acute lymphoblastic leukemia (ALL) cases in adults worldwide and approximately 10-15% of ALL cases occurring in children [3]. Aberrant cell proliferation, elevated cell survival and blocked cell differentiation in immature T-cell progenitors induced by genetic alterations contribute to the occurrence of T-ALL [4]. The combination of high strength chemotherapies has greatly improved the prognosis of T-ALL, and the cure rate in children and adults could reach 75% and 50%, respectively [1]. Nonetheless, patients who have failed conventional therapy have a very poor prognosis with a death rate of 20% in children and more than 50% in adults [5]. Recent studies on the molecular mechanisms involved in T-ALL have strengthened our understanding of the pathogenesis of T-ALL [6]. New treatment strategies to combat aberrant genetic expressions in T-ALL are urgently needed for these patients who have poor prognoses. Matrix metalloproteinases (MMPs) are well known for their critical role in the metastasis, apoptosis, proliferation and angiogenesis of many malignant tumors and some pernicious lymphocytes [7-9]. Among them, MMP-2 and MMP-9 belong to the zinc ion-dependent endopeptidases are found in a wide variety of tissues [10-12]. MMP-2 and MMP-9 expressions have been correlated with cell invasion and tumor size in several cancers such as lung cancer, breast cancer, cervical cancer and ovarian cancer [13-15]. MMP-2 and MMP-9 have also been strongly connected with extramedullary infiltrates in adult acute lymphoblastic leukemia (ALL) [16]. A previous study of childhood T-ALL reported that higher expressions of MMP-2 and MMP-9 were closely related with the pathogenesis of T-ALL [17]. The abnormal expression of MMP-2/-9 was also a potential prognostic factor in acute myeloid leukemia (AML); thus, these proteins represent possible targets for experimental treatments from the clinical perspective [18-21]. *MMP-2* (72 kDa), also called gelatinase A, is located in the gene-coding region on chromosome 16q21 [22]. *MMP-9* (92 kDa), also known as gelatinase B, is located in the gene-coding region on chromosome 20q11.2-q13.1 [23]. *MMP-2* -1306C>T polymorphisms have been shown to affect susceptibility to lung cancer in Asian populations [24]. Several MMP-9 genetic polymorphisms, which are correlated with gastric cancer, have been identified and exert allele-specific influences on MMP9 protein expression [25]. There is also a significant association between the *MMP-9* -1562C>T polymorphism and myelodysplastic syndrome (MDS) and AML [26]. However, a previous study indicated no association between lymphoid malignancy formation and the genotypic distributions of *MMP-9* -1562 C>T and *MMP-2* -1306C>T [27]. Currently, not enough data has been found regarding these SNPs and their association with T-ALL. Therefore, looking at *MMP-2* and *MMP-9* polymorphisms as a starting point, this study measured the frequency of *MMP-2* and *MMP-9* polymorphisms in the case and control groups to determine whether there is an association between *MMP-2/MMP-9* polymorphisms and the risk and prognosis of T-ALL.

Materials and Methods

Ethics statement

This study was approved by the Ethics Committee of Fujian Institute of Hematology, Fujian Provincial Key Laboratory of Hematology, Fujian Medical University Union Hospital and Nanfang Hospital, Southern Medical University. Written informed consent was obtained from all subjects.

Study subjects

From April 2009 to February 2011, a total of 376 T-ALL patients were chosen as the case group, among which, 154 patients with splenomegaly, 131 patients with hepatomegaly and 181 patients with chromosomal abnormalities were chosen. All patients met the following inclusion criteria: (1) Patients were diagnosed with T-ALL according to the immunological classification standard devised by the European Group for the Immunological Characterization of Leukemias (EGIL) [28]; (2) Patients were 14 years of age or greater; (3) Patients had cells that were mostly nucleated on the bone marrow smear, showed obvious or severe hyperplasia and could exhibit hypocellularity, which was mainly due to lymphocyte proliferation of naive lymphocytes $\geq 30\%$. The exclusion criteria included the following: (1) any serious complications such as lung infections; (2) severe organ dysfunction or disease, such as heart, liver, kidney, pancreas and other severe diseases and dysfunction; (3) pregnancy; and (4) a previous history of cancer. Meanwhile, 352 healthy people who had passed routine health examinations were selected as the control group. There was no significant difference in the sex ratio, age or other aspects between the case group and the control group, and no blood relationship was found in the two groups. All patients signed an informed consent document and were approved by the Ethics Committee of Fujian Institute of Hematology, Fujian Provincial Key Laboratory of Hematology, Fujian Medical University Union Hospital and Nanfang Hospital, Southern Medical University.

Enzyme-linked immunosorbent assay (ELISA)

Peripheral venous blood (6 ml) was collected after overnight fasting from the control group and the case group. Ethylenediaminetetraacetic acid (EDTA) anticoagulation was added to 2ml for DNA extraction and was stored at -80°C . Another 3 ml were kept at room temperature for 30 min and then centrifuged for 15 min at 2000 r/min to collect serum samples. An ELISA was used to test the serum levels of MMP-2 and MMP-9. The extraction kit was purchased from Wuhan Boster Biological Engineering Co., Ltd. (Wuhan, China).

DNA extraction

A total of 2 ml of EDTA-anticoagulated blood was extracted. A Blood Genomic DNA Extraction Kit (centrifugal column type, Tiangen Biotech Co., Ltd. Beijing, China, model: DP318-03) was used to extract DNA according to the manufacturer's instructions. The concentration and purity of the recovered DNA fragments were detected using a UV spectrophotometer. The ratio of OD260/OD280 was between 1.7 and 1.9, and the extracted DNA was stored at -20°C .

SNP sequencing

The polymerase chain reaction (PCR) amplification primers for the *MMP-2* -1306C/T and *MMP-9* -1562C/T loci were designed and validated with the Primer Premier 5.0 Software and were synthesized by the Shanghai Biological Engineering Technology Co., Ltd. (Shanghai, China). The primer sequences and PCR conditions are shown in Table 1. The PCR reaction included 200 ng of DNA template, 1 μl each of upstream and downstream primers, 25 μl of PCR Master Mix and nuclease free water. The total reaction volume was 50 μl . The conditions for the PCR reaction were as follows: 94°C for 2 min, 94°C for 1 min, 52°C for 1 min,

Table 1. Primer sequences used for the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Notes: SNP, single nucleotide polymorphism

SNP	Sequence
<i>MMP-2</i> -1306C>T	Forward: 5'-CTTCCTAGGCTGGTCCTTAC-3'
	Reverse: 5'-AGACCTGAAGAGCTAAAGACT-3'
<i>MMP-9</i> -1562C>T	Forward: 5'-GCCTGGCACATAGTAGGCCC-3'
	Reverse: 5'-CTTCCTAGCCAGCCGGCATC-3'

72°C for 1 min, cycled for 35 times, extension at 72°C for 5 min, and stored at 4°C. The specific restriction endonucleases Pae I (Sph I) (MBI Fermentas Company, Shanghai, China) and Xsp I (Jinmai Biotech Co., Ltd. Shanghai, China) were used to cut the PCR products of *MMP-2* -1306C>T and *MMP-9* -1562C>T. Gel electrophoresis was used to detect the PCR products. A DNA sequencer (model ABI370, ABI Company, Oyster Bay, NY, USA) was used to determine the genotypes.

Therapy regimens

All patients were treated with a chemotherapy based on VDCP (Vincristin (VCR), Daunorubicin (DNR), Cyclophosphamide (CTX), Prednisone (Pred)) regimen (cyclophosphamide + vincristine + daunorubicin + dexamethasone). 315 patients were treated with a CODP regimen and 61 patients with a VDCLP (VCR, DNR, CTX, L-asparaginase (L-Asp), Pred) regimen (VDCP regimen + L-ASP/Lasparaginase). During the process of consolidation, methotrexate and dexamethasone were intrathecally injected at regular intervals to prevent central nervous system leukemia (CNS-L). The remission condition of the bone marrow was assessed after one course of treatment. A complete response (CR) was defined as blast making up less than 5% of total white blood cells on the 28th day of the induction treatment.

Follow-up

The follow-up were done periodically through telephone, or by consulting case data at the time of treatment. All follow-up had received prior consent from the patients or their relatives. The deadline for the follow-up was February 2016, and the length of the follow-up was from 0.5 months to 60 months with a rate of 100%. Through regular follow-up, any change in the patients' conditions could be monitored and the event free survival (EFS) rate was evaluated from the date of diagnosis to the occurrence of an event, including death, disease progression, change of chemotherapy regimens, addition of other treatments or having side effects related to death or intolerance.

Statistical analysis

The data were analyzed using the SPSS 21.0 statistical software and R version 3.0.2(<http://www.R-project.org>), which includes survival package, Rcmdr package (survival plug-in), Hmisc package, RMS package and surcIDINRI package. Measurement data were compared by t-test and presented using mean $\pm$ standard deviations ($\bar{x} \pm s$). Count data were presented with a ratio or rate and were validated using a χ^2 test. Gene frequencies were tested for Hardy-Weinberg equilibrium and were examined using a χ^2 test. $P = P^*2$ (2 SNPs) < 0.05 was considered statistically significant after a Bonferroni correction. The genotype relative risk was calculated using an odds ratio (OR) and a 95% confidence interval (CI). The Kaplan-Meier method was performed for univariate survival analysis. The Multivariate Cox proportional hazard model was applied to predict prognosis of T-ALL. A C-statistic was carried out to assess the accuracy of the prognostic predictions for *MMP-2* and *MMP-9* gene polymorphisms. A Net Reclassification Index (NRI) was estimated for the predictive value of *MMP-2* and *MMP-9* gene polymorphisms on multivariate Cox proportional hazard model. All *P* values are two-tailed, and a *P* value of < 0.05 indicated that the difference was statistically significant.

Results

Comparisons of baseline characteristics in the case group and the control group

The control group consisted of 376 cases, with 212 males and 164 females aged between 24 to 74 years old. The mean age in the control group was 47.3 ± 8.0 years. The baseline characteristics of the two groups indicated that there was no significant difference in sex ratio and sex composition, age, history of smoking or history of alcoholism between the case group and the control group (all $P > 0.05$). While the hemoglobin and platelet counts in the case group were significantly lower than those in the control group (both $P < 0.05$), the number of white blood cells and the serum levels of MMP-9 and MMP-2 in the case group were higher than in the control group (both $P < 0.05$) (Table 2).

Genotype analysis of the *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms

The PCR amplified fragment of *MMP-2* -1306C>T was 188 bp. After Pae I (Sph I) enzyme cutting, there was the homozygous wild-type genotype CC (188 bp and 5 bp), the mutant

Table 2. Comparisons of baseline characteristics between the case group and the control group

Characteristic	Control group (n = 352)	Case group (n = 376)	t / χ^2	P
Age (years)	47.3 $\pm$ 8.0	48.7 $\pm$ 8.2	0.717	0.474
Gender (male/female)	189/163	212/164	0.532	0.466
Smoking history (Yes/No)	222/130	254/122	1.616	0.204
Drinking history (Yes/No)	225/127	241/135	0.002	0.961
White blood cells ($\times 10^9/L$)	8.2 $\pm$ 1.1	34.6 $\pm$ 8.4	60.390	< 0.001
Hemoglobin (g/L)	132.7 $\pm$ 7.6	62.1 $\pm$ 24.0	54.210	< 0.001
Platelet ($\times 10^9/L$)	169.3 $\pm$ 22.3	83.8 $\pm$ 16.4	58.610	< 0.001
MMP-2 ($\mu g/L$)	156.2 $\pm$ 14.6	208.5 $\pm$ 15.3	54.21	< 0.001
MMP-9 ($\mu g/L$)	182.7 $\pm$ 19.8	486.8 $\pm$ 26.2	58.61	< 0.001

Fig. 1. Primer sequences of *MMP-2* -1306C>T (A) and *MMP-9* -1562C>T (B) by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) assay.

heterozygous genotype CT (188 bp, 162 bp, 26 bp and 5 bp) and the mutant homozygous genotype TT (162 bp, 26 bp, and 5 bp). In the results of gel electrophoresis, the 26 bp and 5 bp fragments were not displayed because they were too small to be recognized. The PCR amplified fragment of *MMP-9* -1562C>T was 435 bp. After Xsp I enzyme cutting, the homozygous wild-type genotype CC (435 bp), the mutant heterozygous genotype CT (435 bp, 247 bp, 26 bp and 188 bp) and the mutant homozygous genotype TT (247 bp and 188 bp) were observed (Fig. 1).

*Comparison of the *MMP-2* -1306C>T and *MMP-9* -1562C>T genotype and allele frequencies in the case group and the control group*

In the control group, a goodness-of-fit test was carried out for the Hardy-Weinberg equilibrium. The results indicated that the frequency distribution of the *MMP-2* -1306C>T and *MMP-9* -1562C>T genotypes in the control group was consistent with a Hardy-Weinberg equilibrium ($P > 0.05$); thus, the samples in this study were indeed random samples and could represent the population.

After a Bonferroni correction, the genotype and allele frequencies of *MMP-2* -1306C>T between the case and control groups were significantly different (all $P < 0.05$). Compared to the CC genotype, the CT genotype (OR = 2.155, 95% CI = 1.515-3.066, $P < 0.001$) and the TT genotype (OR = 3.394, 95% CI = 1.491-7.730, $P = 0.004$) might both increase T-ALL risk. Compared with the C allele, the T allele might increase T-ALL risk (OR = 2.155, 95% CI = 1.610-2.886, $P < 0.001$). Similarly, the genotype and allele frequencies of *MMP-9* -1562C>T between the case and the control groups were significantly different ($P < 0.05$). Compared with the CC genotype, the CT genotype (OR = 1.999, 95% CI = 1.441-2.774, $P < 0.001$) and the TT genotype (OR = 3.100, 95% CI = 1.624-5.920, $P = 0.001$) might both increase T-ALL risk. Compared with the C allele, the T allele might increase T-ALL risk (OR = 1.986, 95% CI = 1.536-2.569, $P < 0.001$) (Table 3).

*Comparison of baseline characteristics of the T-ALL patients with the different genotypes of *MMP-2* -1306C>T and *MMP-9* -1562C>T in the case group*

Compared to T-ALL patients with the CC genotype of *MMP-2* -1306C>T, patients with the CT + TT genotype showed no significant difference in hemoglobin, splenomegaly,

Table 3. Comparisons of the genotype and allele frequencies of *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms between the case and control groups (n/%). Notes: *P*, *P* value assessed by Bonferroni; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI: 95% confidence intervals

SNP	Control group (n = 352)	Case group (n = 376)	<i>P</i>	OR (95% CI)
<i>MMP-2</i> -1306C>T				
CC	281 (79.8)	238 (63.3)	Ref.	
CT	63 (17.9)	115 (30.6)	< 0.001	2.155 (1.515-3.066)
TT	8 (2.3)	23 (6.1)	0.004	3.394 (1.491-7.730)
CT + TT				
C	625 (88.8)	591 (78.6)	Ref.	
T	79 (11.2)	161 (21.4)	< 0.001	2.155 (1.610-2.886)
<i>MMP-9</i> -1562C>T				
CC	253 (71.9)	204 (54.3)	Ref.	
CT	85 (24.1)	137 (36.4)	< 0.001	1.999 (1.441-2.774)
TT	14 (4.0)	35 (9.3)	0.001	3.100 (1.624-5.920)
CT + TT				
C	591 (83.9)	545 (72.5)	Ref.	
T	113 (16.1)	207 (27.5)	<0.001	1.986 (1.536-2.569)

Table 4. Comparisons of baseline characteristics of T-ALL patients with different genotypes of *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms in the case group. Notes: *P*, *P* value assessed by Bonferroni

Characteristic	<i>MMP-2</i> -1306C>T			<i>MMP-9</i> -1562C>T		
	CC	CT + TT	<i>P</i>	CC	CT + TT	<i>P</i>
White blood cells ($\times 10^9/L$)	33.6 $\pm$ 8.3	36.4 $\pm$ 8.4	0.004	32.6 $\pm$ 7.5	37.1 $\pm$ 8.8	< 0.001
Hemoglobin (g/L)	61.2 $\pm$ 25.1	63.7 $\pm$ 25.1	0.702	62.7 $\pm$ 23.2	61.4 $\pm$ 24.9	1
Platelet ($\times 10^9/L$)	86.0 $\pm$ 19.2	79.9 $\pm$ 19.2	0.006	86.3 $\pm$ 13.5	80.8 $\pm$ 18.9	0.002
<i>MMP-2</i> (ng/mL)	207.2 $\pm$ 17.3	210.6 $\pm$ 18.3	> 0.073	210.2 $\pm$ 19.3	206.6 $\pm$ 19.8	0.076
<i>MMP-9</i> ($\mu g/L$)	489.8 $\pm$ 30.07	494.8 $\pm$ 25.3	> 0.106	436.8 $\pm$ 31.2	563.8 $\pm$ 33.6	< 0.001
Splenomegaly			1			0.03
Yes	95	59		72	82	
No	143	79		132	90	
Hepatomegaly			0.758			0.54
Yes	79	52		66	65	
No	159	86		138	107	
Karyotype			0.672			0.024
Normal	125	70		118	77	
Abnormal	113	68		86	95	
Risk stratification			0.078			0.004
Low risk	75	28		71	32	
Moderate risk	131	83		104	110	
High risk	32	27		29	30	

hepatomegaly, rate of chromosomal abnormalities or risk stratification (all $P > 0.05$). Compared to patients with CC genotype, the number of white blood cells in patients with the CT + TT genotype was significantly higher ($P < 0.05$), while the platelet count was significantly lower ($P < 0.05$). In comparison to T-ALL patients with the CC genotype of *MMP-9* -1562C>T, patients with the CT + TT genotype did not display a significant difference in hemoglobin and hepatomegaly (all $P > 0.05$). However, the white blood cell counts, the rate of splenomegaly, the rate of chromosomal abnormalities, the serum level of *MMP-9* and the ratio of high and moderate risk stratification were all significantly higher than in patients with the CC genotype ($P < 0.05$). Furthermore, the platelet counts were significantly lower than that in patients with CC genotype ($P < 0.05$) (Table 4).

Relationship between MMP-2-1306C/T and MMP-9-1562C/T polymorphisms and the prognosis of T-ALL patients

A total of 217 T-ALL patients reached CR after 1 course of chemotherapy (57.7%). Patients who failed to reach CR were re-induced with a regimen containing Ara-C in the

Fig. 2. Event-free survival (EFS) rates of patients with T-cell acute lymphoblastic leukemia carrying different genotypes of *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms. Notes: A: EFS rates of patients carrying different genotypes of *MMP-2* -1306C>T polymorphism; B: EFS rates of patients carrying different genotypes of *MMP-9* -1562C>T polymorphism.

Table 5. Association of *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms with the prognosis of T-ALL patients. Notes: *P*, *P* value assessed by Bonferroni; SNP, single nucleotide polymorphisms; CR, complete response; EFS, event-free survival

SNP	CR (%)	<i>P</i>	Median EFS (month)	<i>P</i>
<i>MMP-2</i> -1306C>T		0.618		0.678
CC	76.5		41.0 ± 21.2	
CT + TT	71.7		38.8 ± 21.9	
<i>MMP-9</i> -1562C>T		< 0.001		< 0.001
CC	82.4		44.1 ± 21.1	
CT + TT	65.7		35.6 ± 21.1	

Table 6. Multivariate analysis by Cox proportional hazard model. Notes: S.E, standard error; OR, odds ratio; 95% CI, 95% confidence intervals

Factor	B	S.E.	Wald	<i>P</i>	OR	95% CI
White blood cells ($\times 10^9/L$)	0.028	0.009	10.577	0.001	1.028	1.011-1.045
Platelet ($\times 10^9/L$)	-0.004	0.004	0.764	0.382	0.996	0.987-1.005
Splenomegaly	-0.01	0.143	0.005	0.942	0.99	0.748-1.309
Karyotype	0.069	0.143	0.233	0.629	1.071	0.810-1.418
Risk stratification	0.361	0.114	10.085	0.001	1.434	1.148-1.792
Serum MMP-2 level	0.069	0.106	0.254	0.023	1.282	0.921-1.526
Serum MMP-9 level	0.262	0.142	6.604	0.014	1.092	0.867-1.346
<i>MMP-9</i> -1562C>T	0.457	0.154	8.839	0.003	1.579	1.169-2.135

second course of chemotherapy upon which 64 T-ALL patients reached CR. The total CR rate was 74.7%. The CR rates in patients with the CC and CT + TT genotypes of *MMP-2* -1306C>T were 76.5% and 71.7%, respectively, which indicated no significant difference ($P = 0.618$). The CR rates in patients with the CC and CT + TT genotypes of *MMP-9* -1562C>T were 82.4% and 65.7%, respectively. The CR rate in T-ALL patients with the CT + TT genotype was significantly lower than T-ALL patients with the CC genotype ($P < 0.001$) (Table 5).

The median EFS of T-ALL patients with the CC and CT + TT genotypes of *MMP-2* -1306C>T was (41.0 ± 21.2) months and (38.8 ± 21.9) months, respectively, which indicated no significant difference ($P = 0.678$). The median EFS of T-ALL patients with the CC genotype of *MMP-9* -1562C>T was (41.0 ± 21.2) months, which was significantly higher than the median EFS of (35.6 ± 21.1) months in T-ALL patients with CT + TT ($P < 0.001$) (Table 5, Fig. 2).

Multivariate analysis in Cox proportional hazard model

The clinical factors (white blood cell count, platelet count, splenomegaly, karyotype, serum MMP-9 and MMP-2 levels and risk stratification) and polymorphisms in *MMP-2* -1306C>T and *MMP-9* -1562C>T were all included in the Cox proportional hazard model.

The results suggested that platelet, splenomegaly, karyotype and the *MMP-2* -1306C>T polymorphism were not associated with the prognosis of T-ALL patients (all $P > 0.05$). While the white blood cell counts ($P = 0.001$, OR = 1.028, 95% CI = 1.011-1.045), risk stratification ($P = 0.001$, OR = 1.434, 95% CI = 1.148-1.792), serum level of *MMP-2* ($P = 0.023$, OR = 1.282, 95% CI = 0.921-1.526), serum level of *MMP-9* ($P = 0.014$, OR = 1.092, 95% CI = 0.867-1.346) and *MMP-9* -1562C>T polymorphism ($P = 0.003$, OR = 1.579, 95% CI = 1.169-2.135) were all significantly associated with the prognosis of T-ALL patients (Table 6). The C-statistic in *MMP-9* -1562C>T is 0.726 (95% CI = 0.661-0.795). To confirm whether there was additional value of the *MMP-9* -1562C>T polymorphism on the prognosis of the multivariate Cox proportional hazard model, a Cox model, which eliminated the *MMP-9* -1562C>T polymorphism, was established, and an NRI analysis was conducted to analyze the two Cox models. The results indicated that there was a predictive value of *MMP-9* -1562C>T polymorphism on multivariate Cox proportional hazard model (NRI = 0.042, 95% CI = 0.028, $P < 0.001$).

Discussion

This case-control study investigated the relationship between the *MMP-2* and *MMP-9* polymorphisms and the risk and prognosis of T-ALL in a Chinese population. The results revealed that the *MMP-2* -1306C/T and *MMP-9* -1562C/T polymorphisms may be associated with an increased risk of T-ALL. The *MMP-9* -1562C>T polymorphism may also be related to the prognosis of T-ALL patients.

The results showed that the hemoglobin and platelet counts in T-ALL patients were significantly lower than those in the normal controls. Consistent with our study, Seibel et al. noted that hemoglobin and platelet counts have been conventional prognostic factors for ALL patients [29]. This study also found that the number of white blood cells and the serum levels of *MMP-9* and *MMP-2* in the case group was higher than that in the control group, which supports the conclusions of previous studies. The high white blood cell counts were significantly correlated with a low survival rate of T-ALL patients compared to the group with moderate counts [30] and that the high expressions of *MMP-2* and *MMP-9* were correlated with childhood T-ALL [17].

The results indicated that the frequency of *MMP-2* -1306C/T genotypes and alleles were significantly different between the case and control groups. Compared with the CC genotype, the CT and TT genotypes both increased the risk of T-ALL. *MMP-2* is one of the factors in the *MMP* family that is closely associated with tumor progression [31, 32]. In addition to the degradation of type IV collagen in the basement membrane, *MMP-2* interacts with other biologically active molecules, such as growth factors binding proteins and growth factor receptors [33]. The 1306C>T polymorphism is located upstream of the *MMP-2* transcription initiation point and may affect protein expression by changing the activity of gene transcription, hence leading to the occurrence of many diseases [34-36]. The promoter region of *MMP-2* gene contains multiple cis-acting elements including activator protein-1 (AP-1), specificity protein-1 (SP-1) and activator protein-2 (AP-2), which suggested that a variety of transcription factors are involved in the transcription of the *MMP-2* gene [37, 38]. When the C nucleotide is substituted by T at 1306C>T, the SP-1 binding region is inactivated, thus inhibiting the promoter activation and affecting the level of gene transcription and protein expression [39]. Our study also revealed that the frequency of *MMP-9* -1562C>T genotypes and alleles was significantly different between the case group and the control group. Compared with the CC genotype, the CT and TT genotypes may increase the risk of T-ALL. *MMP-9* -1562C>T is located in the promoter region and is at a recognition site for transcription inhibitors [40]. Therefore, the conversion from C to T destroyed the binding site for inhibitor recognition and significantly increased the promoter activity [40]. Medley et al. [41] have shown that the expression of *MMP-9* protein is significantly higher in the -1562T genotype than that in the -1562C genotype. Similar to the findings in this paper, Matsumura et al. [42] have found that there is no obvious difference in the *MMP-9* genotype

frequency between gastric cancer patients and the controls. However, because SNPs are closely associated with tumor invasion, clinical progression and lymph node metastasis, the -1562T mutation may relate to the occurrence and development of gastric cancer [42].

Combined with the univariate survival analysis, our results indicated that *MMP-2* -1306C>T and *MMP-9* -1562C>T loci were largely correlated, indicating that the strong promoter activity of *MMP-2* -1306C>T and *MMP-9* -1562C>T may exert certain synergistic effects in the risk of T-ALL. In addition, multivariate analysis by Cox proportional hazard model suggested that the *MMP-9* -1562C>T polymorphism was associated with the diagnosis of T-ALL patients, while *MMP-2* -1306C>T was not related to the diagnosis of T-ALL patients. This may be due to relatively limited experimental technology and the small sample size. Thus, such conclusions require additional verification in the future.

The predictive value of the *MMP-2* and *MMP-9* gene polymorphisms on a Cox proportional hazard model was assessed using C-statistic and a net reclassification index (NRI). The results demonstrated that there was additional prognostic value of *MMP-2* and *MMP-9* gene polymorphisms on the Cox model. C-statistic is often used to demonstrate the prognostic value of certain indexes in a model [43, 44]. By implementing several biomarkers to existing risk prediction models, the net reclassification index (NRI) draws conclusions about improvements in prediction model performance [45].

In summary, the *MMP-2* -1306C>T and *MMP-9* -1562C>T gene polymorphisms may be associated with the risk of T-ALL. The experimental results have confirmed that the CT genotype and the T allele in the two loci may increase the risk of T-ALL, but this study only considered the relationship between the gene polymorphism and T-ALL. Thus, to obtain a more comprehensive and reasonable conclusion, more and larger multi-center comparative studies involving large sample sets, more genes, and environmental interactions are required to further investigate the interaction between the *MMP-2* -1306C>T and *MMP-9* -1562C>T polymorphisms with the mechanisms of T-ALL.

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Disclosure Statement

The authors have declared that no competing interests exist.

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