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ORIGINAL ARTICLE

Polymorphisms in cytokine genes as prognostic markers in diffuse large B cell lymphoma patients treated with (R)-CHOP

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Abstract To investigate whether cytokine genetic polymorphisms influence the outcome of diffuse large B cell lymphoma (DLBCL), we tested 337 consecutive DLBCL treated with CHOP or rituximab-CHOP (R-CHOP) from *interleukin 10* (*IL10*), *Bcl-2*, and *tumor necrosis factor* (*TNF*)-α polymorphisms. Patients who carried the *IL10* rs1800871 TT or rs1800872 AA genotype showed higher complete response (CR) and overall response rate (ORR) significantly. A longer progression-free survival (PFS) was observed in patients with *IL10* rs1800871 TT ($P = 0.017$) or rs1800872 AA ($P = 0.017$) genotype after rituximab-based chemotherapy, and better PFS was also noted with *Bcl-2* rs1801018 AA genotype in the CHOP group ($P = 0.048$). Furthermore, the R-CHOP group patients who carried the *IL10* non-CCA haplotype had longer PFS ($P = 0.030$). Cox proportional hazards analyses demonstrated that the genotype TT of *IL10* rs1800871 and AA plus AC of rs1800872 were predictive of longer PFS and event-free survival (EFS) in DLBCL patients treated with R-CHOP. And the *Bcl-2* rs2279115 AA plus AC genotypes and rs1801018 GG genotype were risk factors for EFS in DLBCL patients treated with CHOP. In conclusion, the results reminded us those DLBCL patients with *IL10* rs1800871 TT, rs1800872 AA, or *IL10* non-CCA haplotype are likely to benefit from the therapy of rituximab-based chemotherapy.

Key words Diffuse large B cell lymphoma · Rituximab · Cytokine genes · Prognosis

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Introduction

Diffuse large B cell lymphoma (DLBCL) is the most commonly diagnosed subtype of non-Hodgkin lymphoma (NHL). The addition of rituximab (R) to the chemotherapy combination of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) has significantly improved the chemotherapy response rates and survival times in patients with DLBCL [1]. However, a significant fraction of DLBCL patients do not achieve complete remission or relapse after chemotherapy [2–4]. There is a growing appreciation that cytokines and related immune factors may be linked to the incidence and proliferation, differentiation, and movement of lymphoma by the development of inflammatory reactions at tumor sites [5–7].

Interleukin 10 (IL10) is an important immunoregulatory cytokine mainly produced by normal and neoplastic B cells and T cells, as well as stimulated monocytes and macrophages. IL10 has strong immunosuppressive effects by inhibition of proinflammatory Thelper 1 (Th1) lymphocytes and stimulates the proliferation and differentiation of B and Th2 cells [8, 9]. K. Bogunia-Kubik et al. [10] observed *IL10* rs1800896 A homozygosity was found to be associated with poor prognosis in DLBCL patients. Some studies also showed the *IL10* single nucleotide polymorphisms (SNPs) were associated with treatment outcomes for survival and progression in the patients with DLBCL [11–13], but no differences were found in other studies [14–18]. *Bcl-2* and *tumor necrosis factor* (*TNF*-α) have also been proposed as potential prognostic markers in DLBCL

patients. Only one study found the *Bcl-2* rs2279115 AA genotype significantly affected overall survival [11]. Moreover, some studies reported that the *TNF-α* rs1800629 A allele treated in the pre-rituximab era had inferior outcome [13–15, 19]. Krzysztof Warzocha et al. [20] observed the TNF/LTa haplotype status was to be an independent risk factor for the outcome of NHL, but no association was found in another study [21].

Up to now, the impact of *IL 10*, *Bcl-2*, and *TNF-α* polymorphisms on the outcome of DLBCL in China remains unclear. Thus, we should evaluate the potential role of polymorphisms in DLBCL patients in order to define specific subgroups more likely to benefit from such a treatment approach.

Materials and methods

Patient characteristics

A total of 337 DLBCL patients who received CHOP (n = 148) or R-CHOP (n = 189) as a frontline therapy for DLBCL were included in the current study. The patients who had been newly diagnosed, were histopathologically confirmed and untreated. A detailed questionnaire, including requests for demographics information, was administered to all subjects. At our department, patients with DLBCL are followed and monitored through their treatment and post-treatment courses with regularly scheduled clinical and radiographic examinations. In addition, clinicopathological data including age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, B symptom, bulky, Extranodal sites, LDH level, bone marrow involvement, IPI scores, Ann Arbor stage, and subtype were collected from medical records. The molecular classification of DLBCL was analyzed by immunohistochemistry [22]. Also, the clinical data were collected, including total number of courses per patient, each course of chemotherapy, date and type of response, date of progression, red blood cell, and platelet transfusion and toxicity.

Treatment protocol

All patients included in this study received CHOP or R-CHOP protocol, and the addition of a rituximab (375 mg/m^2/d) intravenous infusion was over 4 to 6 h at day 1 before administration of CHOP drugs. Patients in complete remission or partial remission should receive a total of six or eight courses of chemotherapy, respectively. Follow-up visits were scheduled every 3 months for the first 2 years and every 6 months thereafter. Dose reduction and support treatment were prescribed according to R-CHOP 21 guidelines [3, 23].

DNA extraction and genotyping

The strategy for candidate gene selection involved in those related to cytokine regulation and function according to the following criteria: (1) SNPs known to be relevant for prediction of outcome or toxicity in DLBCL; (2) SNPs affecting regulatory regions and predicting to alter expression level of gene or protein functions; (3) SNPs with minor allele frequency (MAF) of more than 5 % in the study population. Six polymorphisms were analyzed as follows: (1) *IL10* (rs1800871, rs1800872, rs1800896); (2) *Bcl-2* (rs2279115, rs1801018), and (3) *TNF-α* (rs1800629).

DNA samples were obtained from stored blood samples using Qiagen standard protocols (QIAamp DNA Blood Midi or Maxi kit: http://www.qiagen.com/default.aspx). SNP genotyping was carried out using the Multiplex SNaPshot method. Polymerase chain reactions (PCRs) contained 10–50 ng of DNA, 1× HotStarTaq buffer, 3 mM $MgCl_2$, 300 μM of each dNTP, 0.08 μM of each primer, and 1 U of HotStar Taq polymerase (Qiagen, Hamburg, Germany) in a 20 μl reaction volume. The following touchdown PCR program was used: denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 15 s, annealing at 55 °C for 15 s, and extension at 72 °C for 30 s. This was followed by 30 cycles of denaturation at 96 °C for 10 s, annealing at 52 °C for 5 s, and extension at 60 °C for 30 s and a final extension at 95 °C for 3 min. The PCR products were purified by treatment with Exonuclease I (USB Corporation, Cleveland, Ohio, USA) and Shrimp Alkaline Phosphatase (USB Corporation, Cleveland, Ohio, USA) at 37 °C for 15 min followed by incubation at 80 °C for 15 min. The extension reaction contained 1× ABI Prism SNaPshot Multiplex ready reaction mix (Applied Biosystems, Grand Island, NY, USA), 0.5 μM of each primer, and 1 μl of each PCR product and was carried out as recommended (Applied Biosystems, Grand Island, NY, USA). The extension PCR products were purified using 1 U of Shrimp Alkaline Phosphatase and then analyzed using an ABI 3730xl Genetic Analyzer. SNP calling was carried out using GENEMAPPERTM software v.4.0 (Applied Biosystems, Grand Island, NY, USA). The genotyping has been successfully done in 334 of 337 (99.1 %) samples. For quality control, 15 % of the assays were randomly selected for sequencing. These results of the quality control analysis confirmed 100 % concordance.

PRIMER5 (http://frodo.wi.mit.edu/) was used to design primers to amplify a different sized fragment for each SNP within a multiplex. Extension primers, again differing in length within a multiplex, were chosen from the sequence immediately up- or down-stream of each SNP. The primers for PCR and SNaPshot are listed in Table 1.

Definitions

Treatment responses were scored according to the International Working Group criteria [24]. Progression-free survival (PFS) was defined as time to disease progression, relapse, or death. Event-free survival (EFS) was defined as the time from the beginning of therapy to disease progression, relapse, death, or initiation of additional (off-protocol) or salvage therapy.

Biostatistical analysis

The frequencies of genotypes were calculated using the Haploview program [25]. Haplotype frequencies were estimated with the linkage disequilibrium (LD) coefficient, D. D' is the value of D as a percentage of the maximum calculated value based on the observed allele frequencies. D' values of 0 denoted complete linkage equilibrium, while D' values of 1 denoted complete LD.

Statistical analysis

Clinical characteristics and treatment outcomes were stratified according to the SNPs and were compared between two treatment groups (CHOP and R-CHOP) using Pearson's chi-square test, Fisher exact test, or Armitage trend test. The statistical endpoints included complete response (CR), partial response (PR), overall response rate (ORR), stable disease (SD), progressive disease (PD), PFS, and EFS. Survival distributions were estimated with the Kaplan–Meier method and were compared with the log-rank test. Multivariate analyses were done using Cox proportional hazard models to estimate hazard ratios and their 95 % confidence interval (CI) for having an event. Analysis for toxicity was performed by Binary-logistic regression analysis with SNP genotypes as the explainable variables.

Statistical significance was established at $P < 0.05$ and all tests were two-sided. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 13.0 software.

Results

Patients' characteristics

Demographic characteristics and treatment outcomes of the cohort at DLBCL diagnosis are displayed in Table 2. A total of 337 patients who received CHOP ($n = 148$) or R-CHOP ($n = 189$) as a frontline therapy for DLBCL were included in the current study. All the subjects (178 males and 159 females) of the study were Chinese Han, with median age at diagnosis of 55.3 years (range 13–84). No differences were identified between the two groups in demographic characteristics.

The CR was significantly different between CHOP and R-CHOP groups ($P = 0.009$) according to frontline chemotherapy. The CR was 78.8 % in the R-CHOP group, which was higher than that in the CHOP group (65.5 %).

Clinical characteristics and variant genotypes and haplotype

The LD analysis showed a strong LD among *IL10* rs1800871, rs1800872, and rs1800896 SNPs (D' = 0.864), and another D' was 0.856 between *Bcl-2* rs1801018 and rs2279115. Therefore, generating the haplotypes were ATA (frequency 0.678), CCA (0.229), and CCG (0.084) for the proximal polymorphisms *IL10* rs1800871, rs1800872, and rs1800896. In addition, for *Bcl-2* rs1801018 and rs2279115, polymorphisms were AC (0.664), AA (0.246), and GA (0.082).

Moreover, we detected the association between variant genotypes and the clinicopathological data of DLBCL. However, the SNPs were not correlated with clinical characteristics according to B symptom, Bulky, bone marrow involvement, ECOG (PS = 0 vs >1), extranodal sites (0 vs >1),

Table 1 Genotyping primers of *IL10*, *Bcl-2*, and *TNF-α* SNPs

SNP	Allele	PCR primer	SNaPshot primer
IL10 rs1800871	C/T	F: TCATTCTATGTGCTGGAGATG R: GGTAAGAGTAGTCTGCACTTG	SR: GAGCAAACTGAGGCACAGAGAT
IL10 rs1800872	A/C	F: AGCTGAAGAGGTGGAAACATG R: CTTGCTAACTTAGGCAGTCAC	SR: TTTTTTTTTTCACATCCTGTGACCCCGCCTGT
IL10 rs1800896	A/G	F: AAGACAACACTACTAAGGCTT R: GATAGGAGGTCCCTTACTTTC	SR: CTTACCTATCCCTACTTCCCC
Bcl-2 rs2279115	A/C	F: GTCCTGCCTTCATTTATCCAG R: CTGTGATGTTTTCCCCTTCTC	SR:TTTTTTTTTTTTTTTTTTTTTCCCCGGCTCCTTCATCGTCCC
Bcl-2 rs1801018	A/G	F: GGGAAACACCAGAATCAAGTG R: CATCACTATCTCCCGGTTATC	SR: TTTTTTTTTTTTTTTGGATGGCGCACGCTGGGAGAAC
TNF-α rs1800629	A/G	F: AGGACTCAACACAGCTTTTCC R: ACTGATTTGTGTGTAGGACCC	SR: GCAATAGGTTTTGAGGGGCATG

PCR polymerase chain reaction, *SNP* single nucleotide polymorphism

Table 2 Clinical characteristics of the DLBCL cohort at diagnosis

Clinical characteristics	Overall ($n = 337$)	CHOP-14 ($n = 148$)	R-CHOP-14 ($n = 189$)	P^a
Age (years)	55.3 (13–84)	54.5 (13–84)	55.9 (13–84)	NS
Age >65 years	71/337 (21.1 %)	28/148 (18.9 %)	43/189 (22.8 %)	NS
Male to female	178:159	76:72	102:87	NS
ECOG PS > 1	29/337 (8.6 %)	9/148 (6.1 %)	20/189 (10.6 %)	NS
B symptom	71/337 (21.1 %)	31/148 (20.9 %)	40/189 (21.2 %)	NS
Bulky	99/337 (29.4 %)	41/148 (27.7 %)	58/189 (30.7 %)	NS
Extranodal sites > 1	38/337 (11.3 %)	11/148 (7.4 %)	27/189 (14.3 %)	NS
LDH positive	86/337 (25.5 %)	42/148 (28.4 %)	44/189 (23.3 %)	NS
Bone marrow involvement	22/337 (6.6 %)	9/148 (6.1 %)	13/189 (6.9 %)	NS
IPI				
0	135/337 (40.1 %)	68/148 (45.9 %)	67/189 (35.4 %)	NS
1	116/337 (34.4 %)	51/148 (34.5 %)	65/189 (34.4 %)	
2	44/337 (13.1 %)	19/148 (12.8 %)	25/189 (13.2 %)	
3	33/337 (9.8 %)	6/148 (4.1 %)	27/189 (14.3 %)	
4, 5	9/337 (2.7 %)	4/148(2.7 %)	5/189 (2.6 %)	
Ann Arbor stage				
I–II	162/337 (48.1 %)	73/148 (49.3 %)	89/189 (47.1 %)	NS
III–IV	175/337 (51.9 %)	75/148 (50.7 %)	100/189 (52.9 %)	
Subtype				
GCB	102/337 (30.3 %)	40/148 (27.0 %)	62/189 (32.8 %)	
Non-GCB	235/337 (69.7 %)	108/148 (73.0 %)	127/189 (67.2 %)	
Response				
CR, *n* (%)	246/337 (73.0 %)	97/148 (65.5 %)	149/189 (78.8 %)	0.009
PR, *n* (%)	12/337 (3.6 %)	9/148 (6.1 %)	3/189 (1.6 %)	
SD or PD, *n* (%)	79/337 (23.4 %)	42/148 (28.4 %)	37/189 (19.6 %)	
ORR (CR+PR), *n* (%)	258/337 (76.6 %)	106/148 (71.6 %)	152/189 (80.4 %)	NS

Abbreviations: *NS* indicates non-significant, *ECOG PS* Eastern Cooperative Oncology Group performance status, *LDH* lactate dehydrogenase, *IPI* international prognostic index, *GCB* germinal center B cell, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *ORR* overall response rate

[a] Comparison the frequencies of treatment in CHOP and R-CHOP groups using Pearson's χ^2

LDH level (positive vs negative), IPI scores (0 to 5), Ann Arbor stage (I, II vs III, IV), and subtype (non-GCB vs GCB). In the R-CHOP group, the patients with TT genotype of *IL10* rs1800871 showed a significant higher CR (86.5 %) than those with CT (74.3 %) and CC (66.7 %) genotypes ($P = 0.048$). In addition, a better ORR was observed in patients with homozygous T genotype compared to those with CT and CC genotype ($P = 0.037$). A strong LD was found between *IL10* rs1800871 and rs1800872 (D' = 1.000); thus, trend was also found in rs1800872 (Table 3). Furthermore, the patients with *IL10* non-CCA haplotype showed a significant higher CR (88.1 %) and ORR (90.5 %) than those with CCA haplotype ($P = 0.042$).

Single-marker analysis for treatment outcomes

We compared treatment outcomes to determine whether SNPs were associated with different treatment outcomes when adding rituximab to CHOP chemotherapy. In our study, there was a difference of the survival curve with respect to *IL10* variants for patients treated with R-CHOP. R-CHOP group patients who carried the *IL10* rs1800871 TT genotype showed significantly longer PFS (log-rank test, $P = 0.033$; Fig. 1a). The estimated median PFS for patients who had the CC, CT, and TT genotype of *IL10* rs1800871 was 22.7 months (95 % CI = 15.3-30.1), 63.1 months (95 % CI =43.5-82.6), and 102.1 months (95 % CI = 90.9–113.3), respectively. Meanwhile, patients with *IL10* rs1800872 AA had longer PFS than patients with AC or CC genotype (log-rank test, $P = 0.033$; Fig. 1b). In addition, there was a significant better EFS for the patients with *Bcl-2* rs1801018 AA genotype in the CHOP group (log-rank test, $P = 0.048$; Fig. 2). Furthermore, we found a trend that R-based chemotherapy is effective to improve the outcome than CHOP chemotherapy for the patients with *Bcl-2* rs1801018 AA genotype (Fig. 3). However, there was no association between *TNF-α* rs1800629 polymorphism and the survival of patients

Table 3 Clinical response to R-CHOP therapy according to *IL10* polymorphisms

Response	*IL10* rs1800871 genotype *n* (%)				*IL10* rs1800872 genotype *n* (%)			
	CC	CT	TT	*P* value*	AA	AC	CC	*P* value*
No. (*N* = 182)	18	74	90		90	74	18	
CR	12 (66.7 %)	55 (74.3 %)	77 (86.5 %)	0.048**	77 (86.5 %)	55 (74.3 %)	12 (66.7 %)	0.048**
PR	1 (5.6 %)	0 (%)	2 (2.2 %)		2 (2.2 %)	0 (%)	1 (5.6 %)	
SD/PD	5 (27.8 %)	19 (25.7 %)	10 (11.2 %)		10 (11.2 %)	19 (25.7 %)	5 (27.8 %)	
ORR (CR+PR)	13 (72.2 %)	55 (74.3 %)	79 (88.8 %)	0.037***	79 (88.8 %)	55 (74.3 %)	13 (72.2 %)	0.037***

Abbreviations: *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *ORR* (CR+PR), overall response
*χ^2 test; **$P = 0.048$ when comparing CR, PR, and SD+PD; ***$P = 0.037$ when comparing ORR with SD/PD

with DLBCL. With respect to the occurrence of side effects, no SNPs were found to be associated with grade 3–4 hematologic toxicity (data are not shown).

Haplotype genotypes associate with treatment outcomes

We generated two risk groups according to patient SNP information on the *IL10* and *Bcl-2* haplotypes to evaluate treatment outcomes. The analysis revealed that the estimated median PFS of the patients who carried the *IL10* non-CCA haplotype were 107.1 months (95 % CI, 98.6–115.6), which is significantly longer than the CCA haplotype patients in the R-CHOP group (log-rank test, $P = 0.030$; Fig. 4). This reminded the *IL10* haplotype could be a prognostic factor on chemotherapy response rate in the DLBCL patients with R-CHOP chemotherapy.

Multivariate analysis

To estimate the independent impact of each variable on PFS and EFS, a descriptive Cox proportional hazard model was performed adjusting for International Prognostic Index (Table 4). Analysis identified that the patients with *IL10* rs1800871 TT genotype had longer PFS ($P = 0.017$; HR = 0.537, 95 % CI 0.322–0.895), while *IL10* rs1800872 CC genotype had shorter PFS ($P = 0.017$; HR = 1.863, 95 % CI 1.117–3.107) in the R-CHOP group. Moreover, a better EFS was found in the patients with *Bcl-2* rs2279115 CC genotype ($P = 0.037$; HR = 0.618, 95 % CI 0.394–0.971) while the patients with *Bcl-2* rs1801018 GG genotype had poor EFS ($P = 0.050$; HR = 2.174, 95 % CI 1.001–4.722) in the CHOP group.

Discussion

Although some studies have focused on the role of polymorphic features in development of DLBCL, to date, only a few studies examined the association between the *IL10*, *Bcl-2*, and *TNF-α* polymorphisms and therapeutic outcome with clinicopathological parameters. The current study documented a potential effect of the cytokine genes as targets of R-CHOP

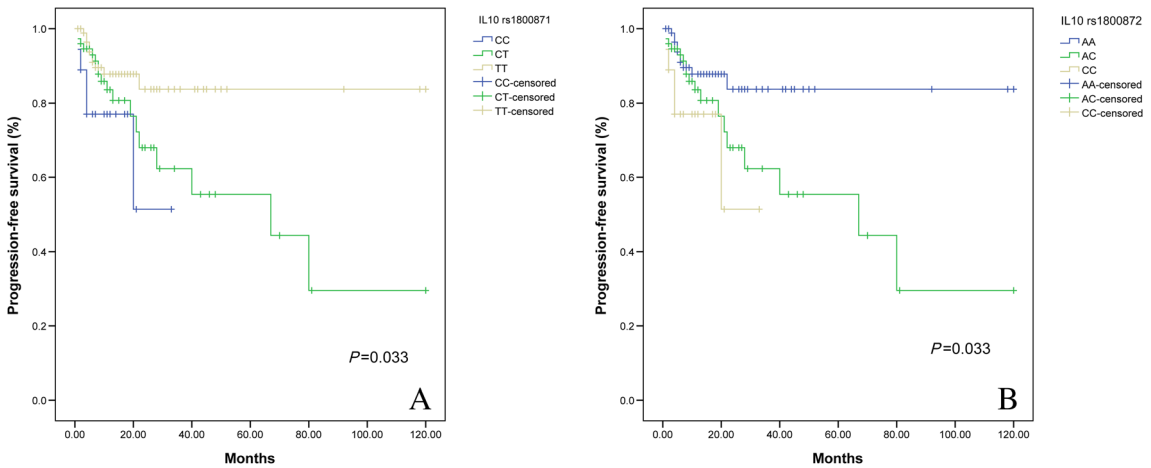

Fig. 1 These Kaplan–Meier curves illustrate progression-free survival of diffuse large B cell lymphoma patients with R-CHOP chemotherapy according to the variant genotypes of *IL10* rs1800871 (**a**) and rs1800872 (**b**)

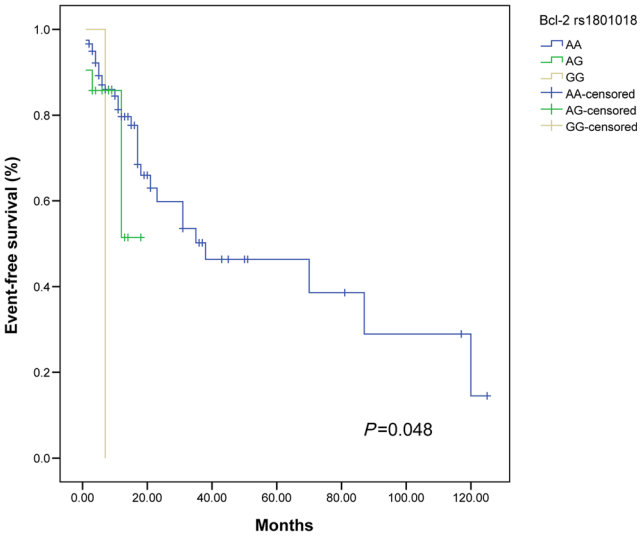

Fig. 2 Kaplan–Meier curves illustrating the event-free survival of diffuse large B cell lymphoma patients who received CHOP chemotherapy according to the variant genotypes of *Bcl-2* rs1801018

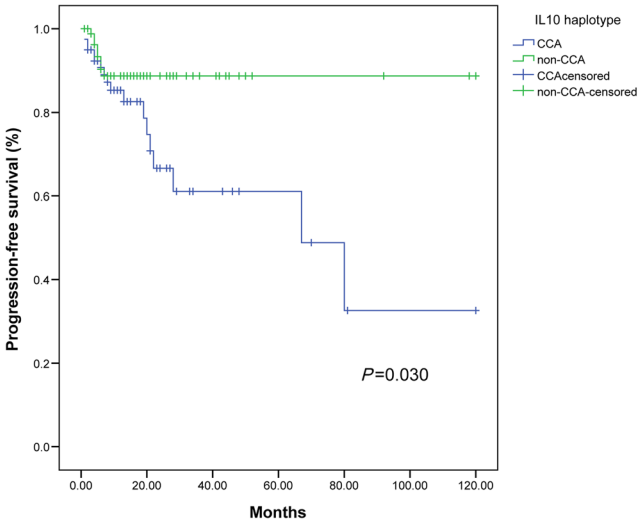

Fig. 4 Kaplan–Meier curves illustrating the progression-free survival of diffuse large B cell lymphoma patients who received R-CHOP chemotherapy according to the *IL10* CCA haplotype

therapy on overcoming chemo-resistance in patients with DLBCL.

IL10 plays a crucial role in normal ontogenesis and function of the immune system and is involved in the inflammatory responses that accompany various lymphoproliferative disorders [8]. In most studies, the *IL10* rs1800871, rs1800872, and rs1800896 polymorphisms or the haplotypes were found to be related to the IL10 production capacity in vitro [26–32]. We found the patient with the *IL10* rs1800871 TT or rs1800872 AA genotype had longer PFS and significant higher CR and ORR in the R-CHOP group. These results were concurred with Yeon Hee Park et al.' s study; they observed the patients with *IL10* rs1800871 TT/TC or rs1800872 AA/AC genotypes correlated with improved ORR in the CHOP group [11]. The *IL10* rs1800896 gene variation is the most intensively studied variation in this cytokine gene promoter. Cunningham et al. [26] showed that the frequency of the low-IL10 producing AA allele (rs1800896), ATA, and ACC haplotypes were higher in DLBCL patients; meanwhile, Turner et al. [27] found *IL10* rs1800896 promoter polymorphism correlates with IL10 protein production in vitro. As associations of *IL10* rs1800896 AA with unfavorable prognosis were identified by other three studies in Europe and Australia populations [10, 12, 26], we performed a study in Asian but not found significant results. Park et al. [11] suggested that *IL10* haplotype was associated with clinical outcomes after R-CHOP for failure-free survival. And our study found the patients who carried the *IL10* non-CCA haplotype had better response and PFS in the R-CHOP group.

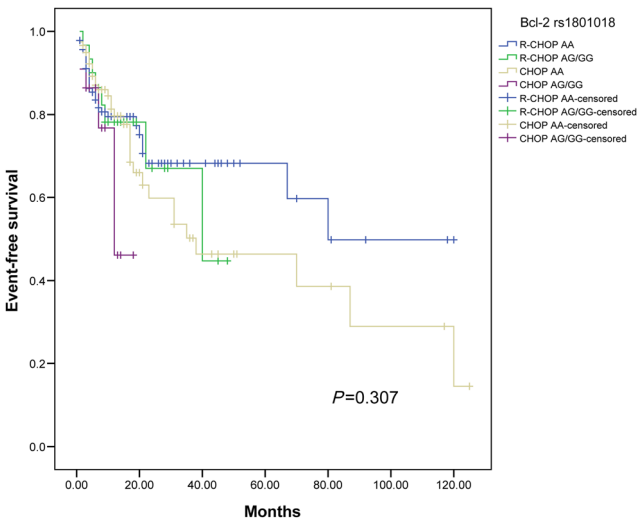

Fig. 3 Kaplan–Meier curves illustrating the event-free survival of diffuse large B cell lymphoma patients who received CHOP or R-CHOP chemotherapy according to the variant genotypes of *Bcl-2* rs1801018

Three references described the genotype distribution of the *IL10* rs1800871, rs1800872, and CCA haplotype polymorphisms in healthy control subjects [10, 12, 27]. A higher frequency of the *IL10* rs1800871 C allele was found both in patients and healthy control subjects in Poland and UK. However, Park et al. reported the frequency of the *IL10* rs1800871 C allele was lower than T allele in the patients with NHL. Furthermore, we detected the allele frequency in the DLBCL patients between CHOP and R-CHOP groups. The differences of genotype distributions were not found between two groups, and the patients' allele frequency of the *IL10* rs1800871, rs1800872 in China is similar to the results in Korea.

Studies suggest that IL-10 production has strong immunosuppressive effects through inhibition of Th1-type cytokines, and increased IL-10 production within tumor microenvironment might be of protective value [33, 34]. In addition,

Table 4 Cox regression analysis of potential factors for PFS and EFS survival in DLBCL patients adjusting for International Prognostic Index

Variable	CHOP			R-CHOP		
	P^a	Hazard ratio[a]	95%CI	P^a	Hazard ratio[a]	95%CI
PFS survival						
Rs1800871 (TT vs. CC/CT)	0.399	0.784	0.446–1.380	*0.017*	*0.537*	*0.322–0.895*
Rs1800872 (CC vs. AA/AC)	0.399	1.275	0.725–2.244	*0.017*	*1.863*	*1.117–3.107*
Haplotype 1 (ATA vs. non-ATA)	0.786	1.224	0.285–5.259	0.070	2.478	0.929–6.657
Haplotype 2 (ACC vs. non-ACC)	0.472	0.768	0.374–1.578	*0.036*	*0.410*	*0.178–0.943*
Haplotype 3 (GCC vs. non-GCC)	0.976	0.981	0.292–3.302	0.905	1.057	0.423–2.641
Haplotype 4 (AC vs. non-AC)	0.546	1.452	0.432–4.881	0.312	1.728	0.598–4.993
Haplotype 5 (AA vs. non-AA)	0.161	0.571	0.261–1.249	0.278	0.666	0.319–1.388
Haplotype 6 (GA vs. nNon-GA)	0.475	0.636	0.184–2.201	0.652	0.812	0.329–2.005
EFS survival						
Rs1800871 (TT vs. CC/CT)	0.630	0.885	0.539–1.453	*0.050*	*0.645*	*0.416–1.000*
Rs1800872 (CC vs. AA/AC)	0.630	1.130	0.688–1.854	*0.050*	*1.551*	*1.000–2.403*
Rs2279115 (CC vs. AA/AC)	*0.037*	*0.618*	*0.394–0.971*	0.641	0.895	0.560–1.429
Rs1801018 (GG vs. AA/AG)	*0.050*	*2.174*	*1.001–4.722*	0.672	1.180	0.548–2.542
Haplotype 1 (ATA vs. non-ATA)	0.899	0.911	0.216–3.835	0.113	2.040	0.844–4.933
Haplotype 2 (ACC vs. non-ACC)	0.573	0.840	0.457–1.542	0.084	0.546	0.275–1.084
Haplotype 3 (GCC vs. non-GCC)	0.555	1.431	0.435–4.706	0.526	1.308	0.570–3.001
Haplotype 4 (AC vs. non-AC)	0.100	2.096	0.868–5.061	0.827	1.122	0.398–3.166
Haplotype 5 (AA vs. non-AA)	0.129	0.606	0.318–1.157	0.475	0.795	0.423–1.493
Haplotype 6 (GA vs. non-GA)	0.128	0.468	0.176–1.243	0.713	0.864	0.396–1.884

[a] *P*, HR, and 95 % CI were assessed using univariate Cox regression analysis adjusting for International Prognostic Index

Helminen et al. [35] reported that *IL-10* genotypes influence susceptibility to EBV infection, but the incidence of virus positivity (EBV or otherwise) in NHL is relatively low. Thus, the significance of *IL-10* polymorphisms in malignancy was likely to be more complex.

As a member of the Bcl-2 family of proteins, Bcl-2 plays an important role in regulating apoptosis and highly expressed at the onset of DLBCL [36]. Previous studies have suggested that inhibition of IL10-mediated loops could down-regulate Bcl-2 expression and sensitize lymphoma cells to cytotoxic chemotherapy [37]. Only one study found that *Bcl-2* rs2279115 AA genotype affected overall survival, and the interactive effect between *Bcl-2* and *IL10* SNPs was associated with treatment outcomes significantly after R-CHOP [11]. In the present study, a longer EFS was noted in the patients with *Bcl-2* rs1801018 AA genotype than AG or GG genotype in the CHOP group. However, patients with R-CHOP chemotherapy had better outcome, especially to the patients with *Bcl-2* rs1801018 AA genotype. It is well established that R-based chemotherapy is effective to improve the outcome for DLBCL patients.

To date, only a few studies have been published to study the association of *TNF-α* rs1800629 polymorphism and prognosis of patients with DLBCL [14, 15, 19]. Olivera Tarabar et al. found *TNF-α* rs1800629 A allele showed a less favorable survival in patients treated with R-CHOP therapy significantly [14] while Olav E. Yri suggested patients receiving rituximab had equal outcome regardless of their genotype

[15]. And there was no significant difference observed in response rate or survival between in DLBCL patients with chemotherapy or without rituximab in the present study.

Recent data reminds us that polymorphisms at the regulatory or structural regions of certain cytokine genes could have effects on prediction of outcome in DLBCL subtly. We also recognize the potential limitations of this study. The current study has been done with a small number of patients, and our patient cohort only included Chinese Han population with a short duration of follow-up. Accordingly, a larger number of patients, a longer follow-up period, and further functional studies will be performed to investigate the association of gene polymorphisms and the clinical characteristics of DLBCL and to provide the useful biomarker for individual therapy and prognosis to DLBCL.

In conclusion, the observations suggest that SNPs involved in the cytokine genes may have effect on predicting failure of therapy in DLBCL. A highly cost-effective DLBCL treatment is what we hope these accompanying predictive and prognostic biomarker tests will result in.

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Compliance with ethical standards The study was approved by the Ethics Committee of Harbin Medical University with the following reference number, HMUIRB20160004. All participants provided written informed consents and all efforts had been made to protect patient privacy and anonymity. The study was conducted according to the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

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