FCGR3A 158V/F Polymorphism and Response to Frontline R-CHOP Therapy in Diffuse Large B-Cell Lymphoma

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The influence of Fc gamma receptor IIIA (*FCGR3A*) 158V/F polymorphisms on the response to rituximab (R) plus CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP) therapy in diffuse large B-cell lymphoma (DLBCL) is uncertain. Thus, a retrospective study and a meta-analysis were performed to examine the possible correlation between *FCGR3A* 158V/F polymorphism and the response rate of R-CHOP regimen in patients with newly diagnosed DLBCL. The genotypes of *FCGR3A* 158V/F in 164 newly diagnosed DLBCL patients treated with R-CHOP were determined in this retrospective study. Additionally, a meta-analysis of current and previously published studies was conducted. Overall response rate (complete and partial response, ORR) and complete response rate (CR) were evaluated. The results of our retrospective study showed lack of correlation between *FCGR3A* 158V/F polymorphism and ORR ($p=0.78$) or CR ($p=0.76$) with R-CHOP therapy. A meta-analysis of 731 cases also showed lack of significant association of ORR and CR in all genetic models with *FCGR3A* 158V/F polymorphism. In survival analysis, the homozygous F genotype correlated with a shorter progression-free survival than that of non-F/F genotype ($p=0.05$), this was significant for the non-GC subset of DLBCL ($p=0.04$), but no association was found between overall survival and *FCGR3A* 158V/F polymorphism. Further analysis with nonsuperiority test ($p<0.0001$) suggested that *FCGR3A* 158V/F polymorphism was not associated with better ORR or CR in newly diagnosed DLBCL patient treated with R-CHOP. No clear relationship was found between *FCGR3A* 158V/F polymorphism and response to frontline R-CHOP therapy in patients with DLBCL.

Introduction

RITUXIMAB (R) PLUS CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP) is the standard frontline therapy for diffuse large B-cell lymphoma (DLBCL) (Feugier *et al.*, 2005; Habermann *et al.*, 2006; Pfreundschuh *et al.*, 2008). However, not all patients with DLBCL respond to R-CHOP therapy. The precise mechanisms of rituximab and its resistance have not been clearly defined. Antibody-dependent cellular cytotoxicity (ADCC) is one of the major mechanisms of rituximab activity (Cartron *et al.*, 2004; Jazirehi and Bonavida, 2005). Fc gamma receptor IIIA (*FCGR3A*) is the major key factor of ADCC. Previous *in vitro* studies indicated that the level of ADCC activity depends on the genetic polymorphism of *FCGR3A* 158V/F (rs396991 G/T) (Koene *et al.*, 1997). Recently, several studies reported the relationship of *FCGR3A*

158V/F polymorphism with response to R-CHOP in patients with DLBCL (Kim *et al.*, 2006; Mitrovic *et al.*, 2007; Zhang *et al.*, 2010; Ahlgrim *et al.*, 2011; Fabisiewicz *et al.*, 2011; Varoczy *et al.*, 2012). The reported results appear to be controversial since two reported that this polymorphism is predictive for the response of R-CHOP in patients with DLBCL (Kim *et al.*, 2006; Zhang *et al.*, 2010), whereas the other four studies disagreed (Mitrovic *et al.*, 2007; Ahlgrim *et al.*, 2011; Fabisiewicz *et al.*, 2011; Varoczy *et al.*, 2012).

We performed a retrospective analysis of this relationship in 164 patients with DLBCL who were treated with R-CHOP in our center. Further, we did a meta-analysis of published data to delineate this plausible association. We conclude that no clear relationship exists between *FCGR3A* 158V/F polymorphism and response to frontline R-CHOP therapy in patients with DLBCL.

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Patients, Materials, and Methods

Retrospective study

Patients. This clinical research protocol was approved by our Institutional Review Board (IRB) and by the Research and Ethical Committee of Peking University School of Oncology.

This study included 164 patients with CD20⁺ DLBCL confirmed by our Department of Pathology according to the World Health Organization classification. All patients received standard R-CHOP or R-CHOP-like chemotherapy regimen between June 2007 and December 2010 at Beijing Cancer Hospital, Peking University School of Oncology (Jin *et al.*, 2012; Lin *et al.*, 2013). R-CHOP-like regimen consists of R-CHOP and etoposide or methotrexate (MTX). Among the 164 patients, 129 received frontline R-CHOP and were evaluable for clinical efficacy. Of the 129 patients, 31 patients received involved-field radiation. The response to R-CHOP therapy was evaluated after completion of 2–3 courses of therapy and 1–2 months after completion of all therapies. All responding patients were followed up every 3 months for the first year and every 6 months thereafter until progression.

FCGR3A gene polymorphism study. One single-nucleotide polymorphism (SNP) of *FCGR3A* gene was evaluated in the current study. Blood samples were obtained from all lymphoma patients before the initiation of therapy for genetic analysis. Genomic DNA was prepared from peripheral blood mononuclear cells using Blood Genomic DNA Extraction kit following the manufacturer's instructions (Bio-teeke Corporation).

The *FCGR3A* gene polymorphism was detected by polymerase chain reaction (PCR)–sequencing assay as previously described (Huang *et al.*, 2013). The first PCR primers for the *FCGR3A* gene SNP at locus 158 were 5-ATA TTT ACA GAA TGG CAC AGG-3 and 5-GAC TTG GTA CCC AGG TTG AA-3[8], while the second PCR primers for the *FCGR3A* gene at locus 158 were 5-ATA TTT ACA GAA TGG CAC AGG-3 and 5-ATG CTG CAG AGT GAA TGA CAC-3, generating a 394-bp fragment. PCR was done on a thermocycler (Gene Cycloer™; Bio-Rad) in a 30 μ L reaction volume containing 30 ng genomic DNA. The PCR program for first-step amplification for the *FCGR3A* gene at locus 158 was as the following: denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min 45 s, and the final elongation step at 72°C for 7 min. And second-step amplification for the *FCGR3A* gene at locus 158 was as follows: denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 45 s, and the final elongation step at 72°C for 7 min. Amplified products were analyzed by gel electrophoresis on 2% agarose gels. All fragments of the second-step amplification were purified with the AxyPrep DNA Gel Extraction kit according to the manufacturer's instructions (Axygen Sci, Inc.). Those purified products were sequenced using an ABI 3730XL Avant Genetic Analyzer (Applied Biosystems, Inc.). Finally, the sequences were analyzed with the software Seqman (DNASTAR, Inc.).

Definitions. Clinical responses were determined following the criteria formulated by International Working Group (Cheson *et al.*, 1999) and as previously published (Chauchet *et al.*, 2012; Jin *et al.*, 2012; Lin *et al.*, 2013).

Statistical analysis. The clinical characteristics and response rate were compared using χ^2 test and Fisher's exact test. The association of the polymorphism with overall survival (OS) and progression-free survival (PFS) was analyzed using Kaplan–Meier plotting and the log-rank test. The prognostic factors were evaluated by the Cox regression model. Differences between groups were regarded as significant with a $p < 0.05$. All tests of statistical significance were two-sided. The analysis was performed using the SPSS software package (v.16.0; SPSS).

Meta-analysis

Selection of studies for meta-analysis. We carefully searched Medline (through PubMed), ISI Web of Science databases, Cochran Library, Chinese Wanfang Data, and CNKI (China National Knowledge Infrastructure) before December 30, 2013, without language limitation. The search strategy was designed by two researchers and included the following terms: “diffuse large B-cell lymphoma” or “DLBCL” and “*FCGR3A*” or “Fc γ receptor IIIA.” Further, hand searches of bibliographies in these studies and other published reviews were conducted. All of the identified records were examined independently by two reviewers.

Studies were considered eligible if the publications met all of the following criteria: (1) *FCGR3A* gene 158V/F (rs396991) polymorphism, (2) specified the histological subtype as DLBCL, (3) compared relationship of *FCGR3A* SNP and response to R-CHOP group, and (4) the genotype distribution of the studies had to be consistent with a Hardy–Weinberg equilibrium (HWE) ($p > 0.05$). For the studies that investigated the same or subset populations, the studies with the most recent or larger sample size were included (Zhang *et al.*, 2009, 2010). Studies were excluded for the reasons indicated in Figure 1 (following the PRISMA 2009 Flow Diagram www.prisma-statement.org). As a result, a total of seven studies were included.

Data extraction and quality assessment. The following information from each publication was carefully extracted by two investigators independently: the first author's last name, year of publication, country of study, median age (range), gender, stages, chemotherapy, response indicators, genotyping method, sample size, and genotype frequency.

To evaluate the quality of the included studies, we followed the checklist based on the Graphic Appraisal Tool for Epidemiological studies (GATE) (Jackson *et al.*, 2006). The quality of each article was independently appraised by two reviewers and assigned a score. Each item was rated as +1 (well reported and reliable), 0 (unclear, insufficient detail provided), or –1 (poorly reported, not useful or reliable). The overall validity of each study was also rated by a similar system: +1 (most of the quality items were fulfilled), 0 (some criteria were not fulfilled), or –1 (few or none of the items were fulfilled) (Lau *et al.*, 1997).

Statistical analyses. The meta-analysis was performed with Review Manager (v5.2; Oxford) and Stata statistical software (v.12.0; Stata Corp). The pooled odds ratios (ORs) and 95% confidence interval (CI) for the response rate associated with V/V allele compared with non-V/V allele of *FCGR3A* gene 158V/F polymorphisms were calculated for total subjects. A χ^2 -based Q test was performed to assess the

FIG. 1. PRISMA 2009 Flow Diagram in our study.

study heterogeneity (Lau *et al.*, 1997; Higgins *et al.*, 2003). Following previously published methods, a fixed-effect model using the Mantel-Haenszel method was used to analyze the pooled data, p -value >0.1 and I^2 value is $<25\%$ (Ma *et al.*, 2012; Rafiyath *et al.*, 2012; He *et al.*, 2013). The significance of the pooled OR was determined by the Z test; a p -value of <0.05 was considered significant. Publication bias was investigated by Begg's funnel plot and the Egger's regression test (Egger *et al.*, 1997).

The Power and Sample Size Program software was applied to calculate for power analysis (Dupont and Plummer, 1990), and the commonly observed relative risk (RR) of 1.10 was used to estimate risk, which represents a "weak" gene effect (Li *et al.*, 2013).

An equivalence-based method in a nonsuperiority mode was applied to confirm the hypothesis of absence of association between *FCGR3A* gene 158V/F SNP and response rate to R-CHOP (Gourraud, 2011; Li *et al.*, 2013).

Results

Retrospective study

Patient characteristics. The general features of the patients in this study are summarized in Table 1, including 81 women and 83 men. The median age at diagnosis was 53 years

(range, 15–90 years). Eighty-nine (54%) patients were in stage III or IV, and 50 (30%) patients had intermediate-high or high International Prognostic Index (IPI) scores. Bone marrow was involved by lymphoma in six patients (4%) at diagnosis. R-CHOP followed by involved-field radiation was given to 31 (19%) patients. R-CHOP as a front-line regimen was administered to all patients, and 129 patients were evaluable for clinical efficacy in this study. The median doses of rituximab delivered were six (range, 4–14), and a median of six cycles of chemotherapy was given (range, 2–8 cycles).

***FCGR3A* 158V/F polymorphism.** The frequency of the *FCGR3A* [158F] allele among all patients was 0.73, whereas the frequency of the *FCGR3A* [158V] allele was 0.27. Ninety-one patients (55%) were homozygous F, 14 patients (8%) were homozygous V, and 59 patients (36%) were heterozygous. The genotype distribution of DLBCL population enrolled in our study was in HWE with regard to the *FCGR3A* [158] polymorphism examined ($p=0.32$). There was no significant difference in the disease features between *FCGR3A* gene polymorphism groups (Table 1).

Clinical responses and *FCGR3A* 158V/F polymorphism. Among the 129 patients evaluable for response to R-CHOP,

TABLE 1. PATIENTS' CHARACTERISTICS AND THEIR CORRELATIONS WITH *FCGR3A* ALLELES

Clinical parameters	No.	Genotype			p ^a	Clinical parameters	No.	Genotype			p ^a
		FF	FV	VV				FF	FV	VV	
Gender						Bulky mass					
Male	83	42	36	5	0.10	≥ 10 cm	18	9	6	3	0.42
Female	81	49	23	9		< 10 cm	146	82	53	11	
Age						Localized					
≤ 60	102	56	39	7	0.53	Yes	25	10	13	2	0.18
> 60	62	35	20	7		No	139	81	46	12	
B symptoms						No extra nodal					
Positive	62	33	19	10	0.02	≤ 1	122	63	48	11	0.23
Negative	102	58	40	4		> 1	42	33		9	
LDH						Incidence site					
Positive	77	47	23	7	0.31	Lymph node	93	54	32	7	0.72
Negative	87	44	36	7		Extralymph	71	37	27	7	
β ₂ -MG						IPI					
Positive	49	29	14	6	0.29	0–2	114	61	44	9	0.56
Negative	106	55	43	8		3–5	50	30	15	5	
Stage						Molecular subtypes					
I–II	75	41	29	5	0.65	GCB	28	18	10	0	0.22
III–IV	89	50	30	9		Non-GCB	113	63	39	11	

^aχ² test.

LDH, lactate dehydrogenase level; MG, macroglobulin; IPI, international prognostic index; GCB, germinal center B cell.

the ORR was 87.59% (113 of 129 patients) with a CR of 62.01% (80 of 129 patients), and a partial response rate of 25.58% (33 of 129 patients). As shown in Table 2, there is no statistical difference in CR rates in the *FCGR3A* V/V allele (60.00%) group compared with V/F (62.00%) and F/F allele (77.8%; $p=0.76$) groups. The difference in ORR was not significant in the *FCGR3A* V/V allele (85.71%) compared with V/F (90.00%) and F/F alleles (88.89%; $p=0.78$).

Survival analysis according to *FCGR3A* 158V/F polymorphism status. After a median follow-up of 524 days (range, 60–2073 days), 32 (25%) patients relapsed or progressed, and 18 (14%) died. Number of events in the survival analysis is summarized in Table 3. Seven patients participated in a clinical trial evaluating everolimus (RAD001) as maintenance therapy and were censored for PFS analysis. Seven patients were censored for lacking follow-up data on progression. The patients with homozygous F/F genotype had a median PFS of 426.5 days (range, 41–1275 days) versus 487 days (range, 50–1230 days) for V/V and V/F genotypes.

TABLE 2. CLINICAL RESPONSE TO R-CHOP THERAPY ACCORDING TO *FCGR3A* ALLELES

Response	FCGR3A genotype no.(%)			p-Value ^a
	FF	FV	VV	
No. (N = 129)	70	50	9	
CR	42 (60.0)	31 (62.0)	7 (77.8)	0.76
PR	18 (25.7)	14 (28.0)	1 (11.1)	
SD/PD	10 (14.3)	5 (10.0)	1 (11.1)	
ORR (CR + PR)	60 (85.7)	45 (90.0)	8 (88.9)	0.78

^aχ² test.

CR, complete response; ORR (CR + PR), overall response; PD, progressive disease; PR, partial response; SD, stable disease.

This difference was borderline significant ($p=0.05$) (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/dna). When patients were further subdivided into germinal center (GCB) and non-GCB lymphoma groups, the difference reached a statistical significance in non-GCB group ($p=0.04$) (Supplementary Fig. S2). Survival data were available for 129 patients. The OS was 532.5 days (range, 152–1364 days) for homozygous F/F patients, and 668 days (range, 152–1350 days) for V carriers ($p=0.48$, Supplementary Fig. S3). This difference was not statistically significant between GCB and non-GCB groups (data not shown).

Multivariate analysis. A multivariate analysis was done to evaluate the effect of the following variables on OS and PFS: age (≤ 60 vs. > 60 years), stage (stages 1, 2 vs. 3, 4), B symptoms (positive vs. negative), β₂-microglobulin level (normal vs. abnormal), lactate dehydrogenase level (LDH) (normal vs. abnormal), IPI score (0–2 vs. 3–5), number of extranodal sites (≤ 1 site vs. ≥ 2 sites), bulky mass (≥ 10 cm vs. < 10 cm), and Exon2_[216] (homozygous C vs. T carriers). Among these, LDH ($p=0.004$, HR 6.21, 95% CI 1.78–21.65) was the only parameter as a poor prognostic factor.

Meta-analysis results

Characteristics of the studies and assessment of quality. The search identified 17 abstracts, of which 6 met the inclusion criteria (Table 4). Eleven were omitted because of the following reasons: (1) the article was a meeting abstract, (2) the data was already published in another article identified in the search, and (3) data did not include the response rate (Fig. 1).

So, the data for this analysis were obtained from seven studies (six that met the inclusion criteria plus the present study) (Kim *et al.*, 2006; Mitrovic *et al.*, 2007; Zhang *et al.*,

TABLE 3. SURVIVAL ANALYSIS OF DLBCL SUBJECTS BY *FCGR3A* ALLELES

<i>Comparisons (no.)</i>	<i>Subtypes (no.)</i>	<i>FCGR3A 158V/F allele</i>	<i>Total no.</i>	<i>No. of event</i>	<i>Survival (%)</i>	<i>p-Value^a</i>
Overall survival (129)		FF	70	11	59 (84.3)	0.48
		VV + VF	59	7	52 (88.1)	
Progression-free survival (115)		FF	62	22	40 (64.5)	0.05
		VV + VF	53	10	43 (81.1)	
	GCB(25)	FF	16	0	16 (100.0)	—
		VV + VF	9	0	9 (100.0)	
	Non-GCB(78)	FF	41	21	20 (48.8)	0.04
		VV + VF	37	8	29 (78.4)	

^aLog rank (Mantel-COX) test.
DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell.

TABLE 4. CHARACTERISTICS OF STUDIES INCLUDED IN THE META-ANALYSIS

Reference	Country	Diagnosis	Sample size	Median age years (range)	Gender (male/female)	Stages 3, 4 (%)	Chemotherapy cycle (range)	Evaluation criteria	Outcomes	Genotyping methods	Genotype frequency		
											FF	FV	VV
Kim <i>et al.</i> (2006)	Asian (Korea)	DLBCL	113	60 (18–84)	64/49	45 (40)	6 (1–8)	International working group criteria 1999	RR, PFS, and OS	PCR-RFLP	6	54	53
Mitrovic <i>et al.</i> (2007)	Caucasian (Croatia)	DLBCL	58	NR (18–84)	37/21	34 (59)	NR	International working group criteria 1999	RR, PFS, and OS	PCR-RFLP	10	32	16
Zhang <i>et al.</i> (2010)	Asian (China)	DLBCL	34	42 (19–73)	NA	18 (53)	6 (4–8)	International working group criteria 1993	RR and OS	PCR	5	18	11
Ahlgrimm <i>et al.</i> (2011)	Caucasian (German)	DLBCL	263	68 (61–80)	141/122	128 (49)	NR	International Working Group criteria 2008	RR, PFS, and OS	Real-time PCR	47	140	76
Fabisiewicz <i>et al.</i> (2011)	Caucasian (Poland)	DLBCL	87	57 (25–81)	41/46	64 (74)	6 (4–8)	International working group criteria 1999	RR, PFS, and OS	Taqman SNR genotyping assays and sequencing	37	35	15
Varoczy <i>et al.</i> (2012)	Caucasian (Hungary)	DLBCL	51	53.1 (19–83)	19/32	NR	NR (1–8)	NR	RR, PFS, and OS	Real-time PCR	10	29	12
Present study	Asian (China)	DLBCL	129	53 (15–90)	83/81	89 (69)	6 (2–8)	International working group criteria 1999	RR, PFS, and OS	PCR-sequencing	70	50	9

NR, not reported; OS, overall survival; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; PFS, progression-free survival; RR, response rate.

TABLE 5. SUMMARY OF POOLED RISK RATIOS WITH 95% CONFIDENCE INTERVALS AND HETEROGENEITY FOR VARIOUS GENETIC CONTRASTS PERFORMED WHEN INVESTIGATING THE ASSOCIATION OF *FCGR3A* 158V/F POLYMORPHISM WITH THE RESPONSE RATE OF R-CHOP IN DLBCL

Contrast groups	Allele no.	Pooled RR of 158V/F		Heterogeneity	
		p-Value	RR (95% CI)	I ² (%)	p-Value
CR vs. SD/PD					
FF vs. VV	127/152	0.12	1.11 (0.97–1.26)	37	0.15
FF vs. FV	127/268	0.28	0.95 (0.85–1.05)	35	0.16
FF vs. FV + VV	127/420	0.20	0.99 (0.86–1.13)	52	0.05
VV vs. FV	152/268	0.34	1.04 (0.96–1.12)	0	0.83
VV vs. FV + FF	152/395	0.15	1.06 (0.98–1.14)	0	0.58
ORR vs. SD/PD					
FF vs. VV	157/166	0.13	1.09 (0.97–1.23)	42	0.11
FF vs. FV	157/305	0.26	0.95 (0.87–1.04)	40	0.13
FF vs. FV + VV	157/481	0.17	0.95 (0.87–1.02)	58	0.03
VV vs. FV	166/305	0.36	1.03 (0.96–1.11)	0	0.83
VV vs. FV + FF	166/462	0.16	1.05 (0.98–1.13)	0	0.54

2010; Ahlgrimm *et al.*, 2011; Fabisiewicz *et al.*, 2011; Varoczy *et al.*, 2012).

Table 4 presents the main characteristics of these studies. There are three groups of Asians and four Caucasians. All except one of the seven studies scored the response to R-CHOP according to International Working Group criteria (Varoczy *et al.*, 2012); most studies provided complete outcome data and full reporting of results. Most studies did not state whether the laboratory was blinded. Regarding the quality appraisal of the included studies, four were rated as +1 and 3 as 0 (Supplementary Table S1). All of the studied SNPs fulfilled the HWE (most studies used calculated HWE according to the original data).

Association between polymorphism and response rate. Seven data sets were finally included in this meta-analysis with a total sample size of 731 patients. Table 5 summarizes the pooled RR estimates and the corresponding 95% CI of this meta-analysis. Data showed that individuals with the V/V allele did not have a significantly higher CR rate or ORR compared with FF, FV, or non-V/V (FF+FV). No significant associations with CR rate or ORR were found for FF allele compared with all genetic models (Table 5).

The OS analysis according to the *FCGR3A* 158V/F genotypes of these patients showed no difference in the published studies (Kim *et al.*, 2006; Mitrovic *et al.*, 2007; Zhang *et al.*, 2010; Ahlgrimm *et al.*, 2011; Fabisiewicz *et al.*, 2011; Varoczy *et al.*, 2012), except for a borderline significant association with PFS in our present study. Because the raw survival data were not available in studies included, the *FCGR3A* 158V/F genotype comparisons for PFS analysis were not stratified for GCB and non-GCB subgroups in our meta-analysis.

Publication bias. We found no evidence of publication bias using the Begg's funnel plots and Egger's test for all genetic models of *FCGR3A* 158V/F polymorphism (data not shown).

Power analysis. A *post hoc* power analysis on the total sample size used for the meta-analysis was calculated using

the following parameters: 187 patients with V/V allele and 374 controls of non-V/V allele, two-tailed $\alpha=0.05$, and for retrospective cohort study, prior data indicate that the response rate among controls is 0.84. Given the inconsistent relative risk of *FCGR3A* 158V/F polymorphism for R-CHOP in DLBCL patients, we determined the commonly observed relative risk to be 1.10 in genetic association studies. The results showed in a power of 82% for ORR for the present sample size.

Equivalence-based test to confirm the nonassociation between 158V/F SNP and response to R-CHOP. Non-superiority mode was used to confirm the nonassociation between *FCGR3A* gene 158V/F SNP and response rate to R-CHOP. The null hypothesis of the test is that the frequency of 158V allele is greater by 3% compared with the frequency in controls. The specific 2% was set, since the lowest difference of 158V allele between ORR and SD/PD in seven independent populations was 2.3%. Thus, a 2% advantage in response group can be regarded as a fairly stringent cut-off.

Based on the just explained hypothesis, the nonsuperiority *p*-value was <0.0001, indicating the absence of association between 158V/F SNP and response rate to R-CHOP in DLBCL patients.

Discussion

Our retrospective study of the role of *FCGR3A* polymorphism in DLBCL is by far the largest for Chinese patients, and the results showed that there were no significant association between *FCGR3A* 158V/F polymorphism and ORR ($p=0.78$) or CR ($p=0.76$) with R-CHOP therapy. These results differed from those of two prior reports on Asian patients (Kim *et al.*, 2006; Zhang *et al.*, 2010). The two prior reports, however, had fewer patients included. Moreover, we conducted a meta-analysis of the data pooled from seven published reports to further delineate the predictive value of *FCGR3A* gene F158V polymorphism in DLBCL therapy. The results of meta-analysis of 731 cases agreed with our single-center data and confirmed that

FCGR3A 158V/F polymorphism did not affect ORR and CR of R-CHOP therapy (Table 5).

Our patients revealed higher frequency of F allele than the V allele. This frequency distribution is not significantly different from that reported in Japanese cases ($p > 0.05$) (Hirakawa *et al.*, 2011), but is quite different from another two reports—one in a smaller sample of Chinese patients (34 patients, $p = 0.003$) and one in Korean cases ($p < 0.001$) (Kim *et al.*, 2006; Zhang *et al.*, 2010). Other specific factors, such as patient stratification, genotyping errors, and environmental exposure, may also contribute to this observed discrepancy (Gourraud, 2011).

In our retrospective study, we analyzed the survival data of our DLBCL patients. Although there was no difference in OS, patients carrying homozygous F genotype had a shorter PFS ($p = 0.05$) (Table 3). When the patients were subdivided into GCB and non-GCB lymphoma groups, the inferior survival time was observed in the latter lymphoma group ($p = 0.04$). Although not significant, as to PFS and/or event-free survival, a trend toward an unfavorable outcome for carriers of *FCR3A* 158F/F was reported in R-CHOP-treated patients in the German and Hungary studies (Ahlgrimm *et al.*, 2011; Varoczy *et al.*, 2012). According to this, the *FCR3A* 158V/F polymorphisms likely affect duration of response by altering the degree and depth of malignant B cell depletion. And the difference of depletion appears more prominent in non-GCB. Different mechanisms for anti-CD20-mediated depletion depending on the B-cell subpopulations may be one reason (Hamaguchi, 2009; Leandro, 2013). Ongoing studies need to explore the association of this SNP with survival in a larger population of DLBCL and verify it with laboratory experiment. Among clinical variables, only LDH was a poor prognostic factor ($p = 0.004$, HR 6.21, 95% CI 1.78–21.65).

We have showed that our sample size has enough power to detect significance using relative risk corresponding to small effect size (relative risk = 1.10). However, if we used the observed relative risk (1.05) in the meta-analysis, then the present sample size would only have 25.5% power of detecting significant association. The sample size would have to be increased to more than 811 cases and 1622 controls in order for the sample to have 80% power of detecting a significant association ($\alpha < 0.05$). Therefore, the sample size for the meta-analysis remains small, which is a significant limitation. To avoid such possible false-negative association from this study, equivalence-based method that minimizes the probability of observing a lack of association by chance was used. p -Value of the nonsuperiority test for *FCGR3A* 158V/F SNP was < 0.0001 , which strengthens the probability of nonassociation between *FCGR3A* polymorphism and R-CHOP response rate.

In conclusion, our retrospective study together with the meta-analysis demonstrated nonassociation between *FCGR3A* 158V/F gene polymorphism and response to frontline R-CHOP in DLBCL patients.

Author Contributions

Z.J. and S.Y.Q. designed the study and reviewed the final article. L.F. collected and analyzed the data for meta-analysis. D.H.R. performed and evaluated the experiments. J.X. and D.N. helped to perform the experiments. F.Z.Y. helped to

collect the samples. H.Y. helped to perform meta-analysis. F.L., S.Y.Q., and D.H.R. wrote the article. L.F. and D.H.R. contributed equally to this work. All authors read and approved the final article.

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Disclosure Statement

The authors have no conflicts of interest.

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