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Original Article

The single nucleotide polymorphism and haplotype analysis of *MDR1* in Chinese diffuse large B cell lymphoma patients

Ying Ni [1], Zhengrui Xiao [1], Guangli Yin, Lei Fan, Li Wang, Huayuan Zhu, Hanxin Wu, Sixuan Qian, Wei Xu, Jianyong Li, Kourong Miao *

Department of Hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing Medical University, Nanjing, 210029 PR China

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ABSTRACT

We investigated whether the *MDR1* (multidrug resistance 1) gene single nucleotide polymorphism (SNP) and haplotype variants were associated with the susceptibility to diffuse large B-cell lymphoma (DLBCL). A total of 129 DLBCL patients and 208 healthy controls from Jiangsu Han population were enrolled in this study. They were genotyped by polymerase chain reaction-allele specific primers (PCR-ASP) method or DNA direct sequencing at three common loci: C1236T, G2677T/A and C3435T. At locus G2677T/A, allele G and genotype GT were significantly more common in DLBCL (G: OR = 1.48, 95% CI: 1.08–2.02, $Pc = 0.03$; GT: OR = 1.96, 95% CI: 1.25–3.07, $Pc < 0.01$), while genotype AT in this locus seemed to be protective (OR = 0.29, 95% CI: 0.02–0.72, $Pc = 0.03$). TT genotype at locus C3435T showed a risk factor in DLBCL (OR = 2.38, 95% CI: 1.52–3.74, $Pc < 0.01$). The frequency of T-G-T haplotype was significantly increased in DLBCL group (OR = 5.21, 95% CI: 2.58–10.54, $Pc < 0.01$); haplotype of G-T in 2677–3435 and diplotype of 2677GT/3435TT were significantly more frequent in DLBCL group (G-T: OR = 3.97, 95% CI: 2.31–6.85, $Pc < 0.01$; 2677GT/3435TT: OR = 4.55, 95% CI: 2.02–10.22, $Pc < 0.01$). Our findings demonstrate that G, GT at locus G2677T/A, and TT at locus C3435T might contribute to the susceptibility to DLBCL, as well as haplotype of T-G-T, G-T in 2677–3435 and diplotype of 2677GT/3435TT, while AT at locus G2677T/A might be a protective genotype. These findings could provide evidence that the *MDR1* SNPs may modify the susceptibility to DLBCL and shade new lights in disease association studies.

1. Introduction

MDR1 (multidrug resistance 1), located on chromosome 7q21.1, is composed of 28 exons and involved in the efflux of drugs. It encodes a 170 kDa membrane transporter named P-glycoprotein (P-gp), which is a member of the adenosine triphosphate (ATP) binding cassette (ABC) transporter of the MDR/TAP subfamily and is also called ATP-binding cassette subfamily B member 1 (ABCB1) [1,2]. The P-gp is the first defence of the body against oral exposure to drugs and toxins, reducing the intercellular concentrations of different chemotherapeutic agents. Moreover, the *MDR1* gene is indicated highly polymorphic, and up to now a total of 50 *MDR1* single nucleotide polymorphisms (SNPs) have been discovered [2,3]. Among the reported SNPs, a majority of investigators have paid close attentions to three most common SNPs, C1236T (Exon 12, rs1128503), G2677T/A (Exon 21, rs2032582) and C3435T (Exon 26, rs1045642), to explore the potential associations with disease development and drug response in different populations [4–8].

There is growing evidence that the *MDR1* SNPs are highly relevant to the susceptibility to hematologic diseases, involved in leukemia, multiple myeloma and lymphoma fields [6,9,10]. Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) in the world, and it accounts for around 40% of all adult NHLs [11]. Although potentially curable, approximately 40% of patients with DLBCL will die of relapsed or refractory disease, and the exact molecular mechanism for DLBCL development remains unclear [12]. Due to the different ethnic and geographic populations are of high variance, we were interested and determined to explore the potential associations between *MDR1* SNPs and DLBCL development in Jiangsu Han population of China.

* Corresponding author. Department of Hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, 210029 Nanjing, PR China. Tel.: +86 25 83781120; fax: +86 25 83781120.
E-mail address: kourongmiao@163.com (K. Miao).
[1] Ying Ni and Zhengrui Xiao contributed equally to this paper.

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2. Materials and methods

2.1. Patients and controls

A total of 129 patients, with a confirmed histological diagnosis of DLBCL, were invited to participate in this study, including 59 (45.7%) males and 70 (54.3%) females, with a median age of 60 years (range = 17–86 years). These patients were Han ethnic, belonging to Jiangsu population, and their characteristics were summarized in Table 1. The control group consisted of 208 healthy donors with the same ethnic and geographic background from Jiangsu province. Informed consents were individually obtained prior to each blood sample collection.

2.2. MDR1 genotyping by PCR-ASP

PCR-ASP (polymerase chain reaction-allele specific primers) at the positions of C1236T (rs1128503), G2677T/A (rs2032582) and C3435T (rs1045642) was employed to identify the genotypes of *MDR1* for both patients and controls. Basically, after genomic DNA was extracted from peripheral blood using salting out method (Lot# N3113, Tiangen Company, Beijing, China), touchdown PCR-ASP technique was performed. Set up included mixing a PCR Master Mix reaction buffer (Lot# KT201-02, Tiangen Company, Beijing, China) with a human genomic DNA sample and Taq DNA polymerase; dispensing the mixture into the PCR reaction wells; and thermal cycling; after PCR was completed, the PCR products underwent electrophoresis on a 2% agarose gel. The ethidium bromide stained gel was photographed and interpreted for genotyping. PCR primers and reaction conditions were referenced in the previous literature [13]. DNA direct sequencing was used to confirm the genotype of G2677T/A (rs2032582).

3. Statistic analyses

Allele, genotype and diplotype frequencies in three loci of *MDR1* were calculated and the maximum-likelihood haplotype frequencies were computed by the expectation-maximization (EM) algorithm using Arlequin software 3.01 [14]. The significance of differences in allele, genotype, diplotype and haplotype frequencies between DLBCL patients and controls were assessed using the χ^2 test, described by odds ratio (OR) with 95% confidence interval (CI), as well as the probability values. Chi-squared with Fisher's exact test was also employed, if appropriate. Generally, $P < 0.05$ was considered significant. According to the Bonferroni correction algorism [15], if an experimenter is testing n independent hypotheses on a set of data, then the statistical significance level that should be used for each hypothesis separately is $1/n$ times what it would be if only one hypothesis were tested. Bonferroni inequality method was employed to correct the original P value (Pc).

Table 1
The characteristics of 129 patients with DLBCL.

Characteristics	Number of patients (%)
Age (years)	
>60	69 (53.49)
≤60	60 (46.51)
Gender	
Male	59 (45.73)
Female	70 (54.27)
Staging	
I–II	45 (34.88)
III–IV	84 (65.12)
Group	
A	96 (74.42)
B	33 (25.58)
IPI	
0–2	88 (68.22)
3–5	41 (31.78)

IPI: international prognostic index.

4. Results

4.1. Allele and genotype frequencies

Allele and genotype frequencies of all three common *MDR1* SNPs (C1236T, G2677T/A and C3435T) in DLBCL patients and

Table 2
The allele and genotype distributions at three common loci (C1236T, G2677T/A and C3435T) inpatients and controls.

MDR1	DLBCL patients, *n* (%)	Controls, *n* (%)	OR	95% CI	*P*	*Pc*
Allele						
C1236T						
C	82 (31.78)	145 (34.86)	0.87	0.63–1.21	0.41	
T	176 (68.22)	271 (65.14)	1.15	0.82–1.60	0.41	
G2677T/A						
A	20 (7.75)	55 (13.22)	0.56	0.32–0.94	0.03	0.09
G	**143 (55.43)**	**190 (45.67)**	**1.48**	**1.08–2.02**	**0.01**	**0.03**
T	95 (36.82)	171 (41.11)	0.84	0.60–1.15	0.27	
C3435T						
C	139 (53.88)	250 (60.10)	0.78	0.57–1.06	0.11	
T	119 (46.12)	166 (39.90)	1.29	0.94–1.76	0.11	
Genotype						
C1236T						
1236CC	11 (8.53)	25 (12.02)	0.68	0.32–1.44	0.31	
1236CT	60 (46.51)	85 (45.67)	1.26	0.81–1.96	0.31	
1236TT	58 (44.96)	88 (42.31)	1.11	0.72–1.73	0.63	
G2677T/A						
2677AA	4 (3.10)	3 (1.44)	2.19	0.48–9.93	0.44	
2677AG	6 (4.65)	19 (9.13)	0.49	0.19–1.25	0.13	
2677AT	**6 (4.65)**	**30 (14.42)**	**0.29**	**0.12–0.72**	**0.005**	**0.03**
2677GT	**65 (50.39)**	**71 (34.13)**	**1.96**	**1.25–3.07**	**0.003**	**0.018**
2677GG	36 (27.91)	50 (24.04)	1.22	0.74–2.02	0.43	
2677TT	12 (9.30)	35 (16.83)	0.51	0.25–1.02	0.05	
C3435T						
3435CC	46 (35.66)	78 (37.50)	0.92	0.59–1.46	0.73	
3435CT	47 (36.43)	94 (45.19)	0.70	0.59–1.46	0.11	
3435TT	**72 (27.91)**	**72 (17.31)**	**2.38**	**1.52–3.74**	**<0.01**	**<0.01**

OR: odds ratio; CI: confidence interval; *Pc*: *P* corrected. The significant allele and genotype were bolded in this table.