



Relationship of *MDR1* gene polymorphism and P-glycoprotein expression in Chinese refractory lupus nephritis

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Abstract

To evaluate the association of multidrug resistance 1 (*MDR1*) polymorphism and the expression of P-glycoprotein (Pgp) in Chinese refractory lupus nephritis (LN) patients. Polymerase chain reaction-direct sequencing was used to analyze *MDR1* polymorphism. The genotype distribution of *MDR1* polymorphism in 132 SLE (systemic lupus erythematosus) patients was evaluated. ELISA was used to measure the expression of Pgp. Relationship among Pgp expression, *MDR1* polymorphism, SLEDAI (SLE disease activity index), and kidney pathological score was analyzed by using One-way ANOVA and Pearson linear correlation. The frequency distribution of the *MDR1* gene was consistent with the Hardy-Weinberg equilibrium. Compared with CT and CC, patients with T/T homozygote in *MDR1* C3435T had significantly increased Pgp expression in the refractory group ($p < 0.05$). Additionally, SLEDAI score was positively correlated with Pgp expression ($r = 0.481$, $p < 0.05$). Also, Pgp expression was positively correlated with renal pathological activity index ($r = 0.76$, $p < 0.05$). *MDR1* C3435T polymorphism is significantly associated with Pgp expression in patients with refractory LN. Pgp expression is closely related to SLEDAI and renal pathological score. Thus, Pgp may be useful in evaluation of the prognosis of patients with refractory LN.

Keywords Multidrug resistance (*MDR*) · Systemic lupus erythematosus (SLE) · Refractory lupus nephritis

Abbreviations

MDR1	Multidrug resistance 1
Pgp	P-glycoprotein
SLE	Systemic lupus erythematosus
LN	Lupus nephritis
SLEDAI	SLE disease activity index
Hb	Hemoglobin
BUN	Urea nitrogen
Scr	Serum creatinine
ALB	Albumin
IgG	Immunoglobulin
ANA	Antinuclear antibodies
dsDNA	Anti-double-stranded DNA
anti-ENA antibody	Anti-nuclear extract antibody

anti-CL antibody	Anti-cardiolipin antibody
AI	Activity Index

Background

Systemic lupus erythematosus (SLE) is a diffuse, systemic autoimmune disease. When renal involvement occurs, it is known as lupus nephritis (LN). About 50% of patients with SLE gradually develop LN (Lanata et al. 2018). Renal pathological biopsy results show almost 100% of SLE patients have kidney disease, and about 20% of SLE patients eventually progress to end-stage renal disease (Shao 2017). Hormones and immunosuppressants are the main drugs for the treatment of LN, and the reactivity to drugs is an important indicator to measure the prognosis of LN (Bawazier 2017). In recent years, the survival rate of SLE patients has been significantly improved due to early diagnosis and comprehensive treatment (Timlin et al. 2017). However, some patients with refractory SLE still have poor or no response to traditional immunosuppressive therapy. Non-response or low response to hormones and immunosuppressive agents, with appearance of multidrug resistance (MDR), are important factors affecting the treatment of SLE (Chen et al. 2016).

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Multidrug resistance 1 (*MDR1*) and the metabolic enzyme CYP3A are two major factors affecting pharmacokinetic metabolism. The protein product encoded by *MDR1*, P-glycoprotein (Pgp), is an ATP-dependent export-oriented transport pump composed of 1280 amino acids. The main function of Pgp is to actively pump out various chemicals and drugs, which play an important role in the metabolism of drugs in the body (Zhao et al. 2015). Studies have shown that *MDR1* gene polymorphism affects the expression and function of Pgp (Ayaz et al. 2013; Wincewicz et al. 2016). There are more than 50 single nucleotide polymorphisms in *MDR1*, and the two common gene polymorphisms affecting *MDR1* gene function are in exon 26 (C3435T) and exon 12 (C1236T) (Subhani et al. 2015; Uludag et al. 2014). The C1236T is a synonymous mutation that does not cause amino acid change (Kozhakhmetov et al. 2013). While, C3435T is the only silent polymorphism currently identified, which may affect the expression of Pgp in different organs/tissues and even in different races (Aydos et al. 2015).

Pgp is one of the most important causes of drug resistance (Hansen et al. 2015; Zoghbi et al. 2017). Wang et al. (2017) found that Pgp overexpression in hormone-resistant SLE patients may be the cause of steroid resistance. Many drugs commonly used to treat SLE, such as methotrexate, glucocorticoids, etc., are Pgp substrates, which could be pumped out of the cell by Pgp, thereby reducing their intracellular concentrations (Feng et al. 2017; García-Carrasco et al. 2015). Some drugs can inhibit the function of Pgp, such as cyclosporine (Gohla 2018; Llaudó et al. 2013). As a transport substrate of Pgp, cyclosporine exerts competitive inhibition and reverses steroid resistance (Yigitaslan et al. 2016). Also, mycophenolic acid has shown inhibitory effect on Pgp activity of lymphocyte in vitro, but its effect is significantly weaker than Pgp-specific blocking agents and cyclophosphamide (Wang et al. 2017). Based on the mentioned study, hormones and some immunosuppressive agents are substrates of Pgp, which may be involved in the formation of MDR in SLE patients. Therefore, it is important to explore the role of *MDR1* genotype and Pgp in patients with refractory SLE.

Here, we designed this experiment to investigate different *MDR1* genotype at C3435T and C1236T site as well as its corresponding Pgp expression. Understanding the role of *MDR1* resistance gene and Pgp in refractory LN may provide a new therapeutic target for refractory LN.

Materials and methods

Patients

A total of 132 patients with SLE were enrolled from Haikou People's Hospital between January 2012 and December 2015. They were all Han patients. All cases were diagnosed in

accordance with the SLE classification standard revised by the American College of Rheumatology in 1997. SLE patients involved in this research had at least four positive items of the 11 criteria. Besides, all participants were in SLE active period. The disease activity was evaluated by SLE disease activity index (SLEDAI) (Ward et al. 2000). Patients with other connective tissue diseases, tumors, severe trauma, serious infections and other diseases were excluded. Besides the diagnosis of SLE, patients with persistent proteinuria (> 0.5 g/day) or multiple urinary protein (+++), and/or cellular casts (including red cell, hemoglobin, granular, tubular or mixed) were diagnosed as LN. LN patients who were without remission after treatment with at least one immunosuppressive agent for more than 6 months, or with LN accompanied by pathological transformation, or with continued positive immunological markers were considered to have refractory LN (Bonilla-Abadía et al. 2013). Renal biopsy puncture was required for refractory LN, and the pathological type was determined according to the 2003 ISN/RPS classification criteria. Age and gender matched patients were enrolled as the control group. The inclusion criteria were as follows: i. SLE patients without renal involvement; ii. SLE patients with normal serum creatinine, negative proteinuria, and negative hematuria; iii. SLE patients without other connective tissue diseases, tumors, severe trauma, or serious infections, etc.

All subjects volunteered to participate in this study and signed informed consent. This study was approved by the ethics committee of Haikou People's Hospital (Approval no. 2017-082).

Laboratory test

Hemoglobin (Hb), Urea nitrogen (BUN), Serum creatinine (Scr), Albumin (ALB), Immunoglobulin (IgG), C3, C4, anti-nuclear antibodies (ANA), anti-double-stranded DNA (dsDNA), anti-nuclear extract antibody (anti-ENA antibody), anti-cardiolipin antibody and Pgp were tested in blood of patients with SLE. In addition, 24-h urine protein was also tested.

Polymerase chain reaction-restriction fragment length polymorphism assay

The leukocytes were isolated from peripheral blood by hypotonic hemolysis, and DNA was further extracted by phenol chloroform extraction. The *MDR1* gene at C3435T and C1236T site was detected by polymerase chain reaction-restriction fragment length polymorphism assay, respectively. The primer for C3435T and C1236T sites were listed as follows: C3435T Forward primer 5'-TGTTTTTCAGCTGCTTGATGG-3' and Reverse primer 5'-AAGGATGTATGTTGGCCTC-3'; and, C1236T Forward primer 5'-TTGAATGAAGAGTTTCTGATGTTTTC-3' and Reverse

primer: 5'-CCTGACTCACCACACCAATG-3'. Then, the PCR products were digested and then DNA electrophoresis (2.5% agarose gel) was performed to analyze the results.

Statistical analysis

Data analysis was performed using the SPSS 16.0 software package. The measurement data were expressed as mean ± SD. The data was compared by *t*-test or one-way ANOVA. Hardy-Weinberg equilibrium and rate comparison were performed by chi-square test. The comparison of different genotype parameters also used one-way ANOVA. The correlation between Pgp expression and SLEDAI score or Activity Index (AI) was analyzed by using Pearson linear correlation analysis. *P* < 0.05 was considered statistically significant.

Results

LN patients have obvious abnormalities in Scr, SLEDAI, Hb and ALB

A total of 132 SLE patients were enrolled, including 17 males and 115 females. This may be caused by the fact that SLE mainly affects female, with the ratio of female to male more than 8:1 (Kotzin 1996). The average age of SLE patients was 35.06 ± 13.45 years old. Among them, 88 patients were with LN, 46 patients were with refractory LN, and 44 patients were non-LN (control). With comparison to the non-LN group, Scr and SLEDAI were significantly increased in the LN and refractory LN group (*p* < 0.05); however, Hb and ALB were significantly decreased (*p* < 0.05) (Table 1).

Allele and genotype frequencies

The *MDR1* allele and gene frequency was analyzed. The *MDR1* C1236T gene frequency was consistent with the Hardy-Weinberg equilibrium (wild homozygous CC = 9.8%,

heterozygous CT = 36.8%, and mutant homozygous TT = 51.9%; *p* = 0.999). Also, *MDR1* C3435T gene frequency was consistent with Hardy-Weinberg Equilibrium (wild homozygous CC = 33.1%, heterozygous CT = 52.6% and mutant homozygous TT = 12.8%; *p* = 0.660). There were no statistical differences between *MDR1* C1236T genotype (*p* = 0.878) and C3435T (*p* = 0.285) genotype in skin, mucosa, arthritis, serous effusion, renal system, blood system and central nervous system, which indicates no significant difference in genotype of extrarenal involvement.

Compared with CT and CC, *MDR1* C3435T significantly increases Pgp expression in refractory group

As shown in Table 2, there was no significant difference in gene frequency between patients with refractory LN and non-refractory LN. Compared with CT and CC, *MDR1* C3435T in the refractory LN group had a significant increase in Pgp expression (17.51 ± 29.08 vs 3.6 ± 3.09 vs 6.42 ± 9.97; *p* = 0.047), and the Pgp expression level in different genotypes as follows: TT > CC > CT (Table 3), indicating that the *MDR1* C3435TT genotype can increase Pgp expression level or its activity. Other biochemical items showed no significant differences. In addition, immunological parameters such as ANA, dsDNA, anti-SM antibody, anti-RNP antibody and anti-CL antibody among different (*MDR1* C1236T or C3435T) genotypes (*p* > 0.05) also showed no statistical significance (Table 4).

The distribution of genotypes in the different pathological types of refractory LN

As shown in Table 5, in the refractory LN group, there were 5 cases of type III LN, 3 cases of type III + IV LN, 25 cases of type IV LN, 8 cases of type IV + V LN, and 5 cases of type V LN. There were no significant differences in gender, age, biochemical indexes, immunological factors, SLEDAI, and Pgp expression levels among different pathological types. Also, no

Table 1 The characteristics of patients in LN and non-LN group

	LN group (n = 88)	Refractory LN (n = 46)	non-LN group (n = 44)
Female (N): male (N)	74:14	41:5	41:3
Age (years old)	34.65 ± 13.64	35.25 ± 14.24	35.89 ± 13.02
Hemoglobin (g/L)	95.9 ± 26.25*	92.89 ± 31.16*	107.27 ± 25.54
Serum creatinine (umol/L)	171.69 ± 243.78*	213.66 ± 210.16*	72.86 ± 18.59
Serum albumin (g/L)	25.51 ± 8.38*	24.07 ± 9.56*	31.94 ± 6.14
IgG (g/L)	18.71 ± 16.73	16.08 ± 16.69	24.38 ± 34.34
C3 (mmol/L)	0.84 ± 0.28	0.82 ± 0.27	0.9 ± 0.27
SLEDAI	12.51 ± 6.88*	12.48 ± 8.35*	7.25 ± 7.24

SLEDAI SLE disease activity index

*indicated there was a statistical difference between the LN group and the non-LN group (*p* < 0.05)

Table 2 Comparison of *MDR1* gene frequency and genotype in patients with refractory and non-refractory LN

	Refractory LN (n = 46)	Non-refractory LN (n = 86)	P value
Dominant model (<i>MDR1</i> C1236T)			
TC + CC	51	86	0.37
TT	21	47	
Recessive model			
CC	15	24	1
TC + TT	57	109	
Dominant model (<i>MDR1</i> C3435T)			
TC + TT	29	57	0.966
CC	15	29	
Recessive model			
TT	5	12	0.679
TC + CC	39	74	

significant difference was found between *MDR1* C1236T ($P=0.184$) and C3435T ($P=0.578$) genotypes in the distribution of five different pathological types, indicating no correlation was found between different *MDR1* genotype distribution and the pathological type.

SLEDAI score and AI are positively correlated to Pgp expression level

Pearson linear correlation analysis was used to determine the relationship of Pgp level with SLEDAI and AI. The results showed that SLEDAI score was closely related to the Pgp expression. Patients with higher SLEDAI score showed a stronger Pgp expression ($r=0.481$, $p=0.000$, Fig. 1). Moreover, there was a positive correlation between SLEDAI score and Pgp expression in the LN group ($r=0.608$, $p=0.000$) (Fig. 2) and refractory LN group ($r=0.744$, $p=0.000$) (Fig. 3), suggesting that lupus activity was positively correlated with Pgp expression ($p<0.01$). In addition, Pgp expression was also positively correlated with AI. This indicates that Pgp expression is positively correlated with SLE activity and AI.

Discussion

LN is a common complication of SLE, hormone insensitivity or hormone resistance is still a tricky issue for LN treatment (Kalloo et al. 2013). Excessive expression of Pgp in peripheral lymphocytes is thought to be a reason of hormone resistance (Laberge et al. 2014). Kansal A et al. (Kansal et al. 2016) found that the expression of Pgp in peripheral monocytes of SLE patients was positively correlated with SLEDAI score,

and its expression could lead to a decrease in hormone content in lymphocytes, which was considered to be related to the poor efficacy of SLE treatment. Ragab et al. (Ragab and Soliman 2013) found that Pgp expression was positively correlated with SLE disease activity in CD5 and CD7 lymphocyte of SLE children. Compared with hormone alone therapy, hormone combined with cyclophosphamide treatment could significantly reduce Pgp expression (Xuan et al. 2014). The expression level of Pgp in peripheral blood lymphocytes of patients with SLE is closely related to disease activity or the drug treatment efficacy. The imbalance of T cell subsets is not only an important part of the development of SLE, but also closely related to Pgp-mediated drug resistance (Zhang et al. 2018). Studies (Mu et al. 2014) have found that regulatory T cells (Tregs) selectively increased the sensitivity to drugs due to the lack of Pgp expression. Henmi et al. (Henmi et al. 2008) found that Pgp was overexpressed in peripheral blood CD4+ T cells in patients with poor SLE response. These studies demonstrate that down-regulation of Pgp expression is beneficial in improving the efficacy of patients with high-activity SLE, and that Pgp can be used as an important predictor of drug resistance to help patients with SLE to choose appropriate initial treatment strategies.

Many factors may affect Pgp expression, such as drugs, cytokines and gene polymorphisms, etc. (Cuppen et al. 2015). Here, in this study, we found no significant difference in the expression of Pgp among *MDR1* C1236T genotypes. The possible mechanism is that *MDR1* C1236T is a synonymous mutation and does not affect the function of Pgp. Compared with CT and CC, the TT genotype of *MDR1* C3435T in refractory LN group showed a significant increase in Pgp expression ($TT>CC>CT$), suggesting TT genotype can significantly increase the expression level or activity of Pgp. Although *MDR1* C3435T is a synonymous mutation, it can affect the protein folding process by changing the choice and use of codons, and finally lead to the local structural change of Pgp, which affects its affinity for each substrate (Brambila-Tapia 2013). This study also found that *MDR1* CC and CT genotype had low Pgp expression level. *MDR1* C3435T polymorphism is an extensively studied polymorphism. It is shown that *MDR1* TT genotype is associated with low Pgp expression in different tissues and cells (such as enterocytes and peripheral blood mononuclear cells) whereas *MDR1* CC genotype is associated with increased Pgp expression (Owen et al. 2004). This is not consistent with our results, which may be because of different study populations.

At present, the evaluation system commonly used in the international evaluation of lupus activity (Wang et al. 2016). The SLEDAI score currently has certain limitations including poor correlation in subjective assessment of patients, low score sensitivity and inability to reflect the severity of some activities (Zhou and Jiang 2012). Renal pathology is the gold standard that reflects the activity of LN. However, renal biopsy is an invasive test.

Table 3 Clinical characteristics of *MDR1* C3435T genotypes in refractory and non-refractory patients

	TT	TC	CC	P value
Total number	17	70	44	0.926
Refractory LN (n = 46)	5	24	15	
Non-refractory LN (n = 86)	12	46	29	
Age (years old)	30.35 ± 14.30	37.46 ± 13.46	33.55 ± 12.23	0.084
Refractory LN (n = 46)	35.4 ± 17.90	36.08 ± 15.29	33.87 ± 12.01	0.898
Non-refractory LN (n = 86)	28.25 ± 12.84	38.17 ± 12.52	33.38 ± 12.55	0.057
IgG(g/L)	14.58 ± 16.01	21.01 ± 29.31	21.52 ± 14.99	0.566
Refractory LN (n = 46)	9.62 ± 6.14	16.46 ± 20.31	17.80 ± 12.64	0.646
Non-refractory LN (n = 86)	16.65 ± 18.53	23.29 ± 32.89	23.45 ± 15.95	0.724
IgM(g/L)	0.94 ± 0.78	1.18 ± 1.04	1.21 ± 0.90	0.603
Refractory LN (n = 46)	0.56 ± 0.45	1.1 ± 0.89	1.21 ± 0.74	0.304
Non-refractory LN (n = 86)	1.10 ± 0.85	1.22 ± 1.11	1.20 ± 0.99	0.934
IgA(g/L)	4.20 ± 6.43	1.69 ± 1.00	2.48 ± 1.43	0.075
Refractory LN (n = 46)	2.56 ± 3.17	1.45 ± 1.17	2.62 ± 1.99	0.107
Non-refractory LN (n = 86)	3.71 ± 8.35	2.86 ± 1.47	2.58 ± 1.58	0.341
SLEDAI	11.12 ± 9.99	9.50 ± 6.47	12.39 ± 7.42	0.122
Refractory LN (n = 46)	13.29 ± 10.37	11.06 ± 5.90	14.20 ± 6.87	0.126
Non-refractory LN (n = 86)	9.00 ± 5.80	9.15 ± 7.20	11.07 ± 6.24	0.446
Pgp	7.22 ± 16.12	4.32 ± 3.95	4.64 ± 6.23	0.344
Refractory LN (n = 46)	17.51 ± 29.08	3.60 ± 3.09	6.42 ± 9.97	0.047*
Non-refractory LN (n = 86)	2.93 ± 1.49	4.70 ± 4.33	3.71 ± 2.70	0.242
C3(mmol/L)	0.75 ± 0.24	0.85 ± 0.24	0.90 ± 0.34	0.191
Refractory LN (n = 46)	0.58 ± 0.17	0.85 ± 0.23	0.84 ± 0.33	0.125
Non-refractory LN (n = 86)	0.82 ± 0.23	0.85 ± 0.25	0.93 ± 0.35	0.426
24-h urine protein quantification (g/L)	1.20 ± 1.50	1.23 ± 1.51	1.52 ± 1.67	0.588
Refractory LN (n = 46)	2.10 ± 2.30	1.96 ± 1.67	2.57 ± 2.08	0.608
Non-refractory LN (n = 86)	0.83 ± 0.92	0.83 ± 1.29	0.98 ± 1.11	0.884
Hemoglobin (g/L)	93.94 ± 30.30	99.61 ± 28.14	102.82 ± 21.73	0.497
Refractory LN (n = 46)	88.20 ± 30.90	84.42 ± 33.62	108 ± 21.82	0.063
Non-refractory LN (n = 86)	96.33 ± 31.09	107.54 ± 21.18	100.13 ± 21.57	0.203
Urea nitrogen (mmol/L)	9.47 ± 8.29	7.88 ± 7.87	7.72 ± 7.6	0.714
Refractory LN (n = 46)	16.01 ± 10.57	14.03 ± 9.65	10.84 ± 10.06	0.495
Non-refractory LN (n = 86)	6.75 ± 5.65	4.67 ± 4.07	6.1 ± 5.49	0.272
Serum creatinine (μmol/L)	161.94 ± 160.20	158.39 ± 262.81	100.34 ± 66.09	0.7303
Refractory LN (n = 46)	314.40 ± 238.00	242.08 ± 245.45	134.6 ± 93.75	0.157
Non-refractory LN (n = 86)	98.42 ± 41.88	114.72 ± 263.49	82.62 ± 36.88	0.784
Blood albumin (g/L)	27.20 ± 7.33	28.06 ± 8.78	27.23 ± 7.99	0.849
Refractory LN (n = 46)	24.99 ± 5.69	22.55 ± 10.45	26.21 ± 9.10	0.505
Non-refractory LN (n = 86)	28.12 ± 7.96	30.94 ± 6.14	27.76 ± 7.47	0.119

Notice: SLEDAI SLE disease activity index

*indicated that there is a statistical difference in the comparison of genotypes

Thus, there is an urgent need for a better system to evaluate the activity of SLE disease. This study found that the higher the SLEDAI score, the stronger the Pgp expression, suggesting that the activity of lupus is positively correlated with Pgp expression. The study also found that the expression of Pgp was positively correlated with the AI of kidney disease. The higher the AI, the stronger the expression of Pgp, suggesting that the detection of

Pgp levels may predict the disease activity of LN patients to some extent. In this study, *MDR1* TT genotype had higher Pgp expression level whereas *MDR1* CC and CT genotype had low Pgp level in refractory LN group. The *MDR1* C3435T may also predict the disease activity of refractory LN patients to some extent. *MDR1* TT genotype may predict greater lupus activity while *MDR1* CC and CT genotype may predict weak lupus activity.

Table 4 Comparison of immunological parameters among different (*MDR1* C1236T and (not or) C3435T) genotypes

	TT	TC	CC	<i>P</i> value
<i>MDR1</i> C1236T				
ANA	70	49	13	0.965
Positive	65	46	2	
Negative	5	3	1	
dsDNA	70	49	13	0.768
Positive	55	37	12	
Weakly Positive	5	2	0	
Negative	10	10	1	
SM	70	49	13	0.409
Positive	39	28	10	
Weakly Positive	21	15	3	
Negative	10	6	0	
RNP	70	49	13	0.246
Positive	29	23	5	
Weakly Positive	33	22	5	
Negative	8	4	3	
CL	70	49	13	0.504
Positive	14	10	2	
Weakly Positive	49	32	11	
Negative	7	7	0	
<i>MDR1</i> C3435T				
ANA	18	70	44	0.493
Positive	16	66	41	
Negative	2	4	3	
dsDNA	18	70	44	0.448
Positive	12	57	34	
Weakly positive	2	2	4	
Negative	4	11	6	
SM	18	70	44	0.338
Positive	9	39	29	
Weakly Positive	7	20	12	
Negative	2	11	3	
RNP	18	70	44	0.331
Positive	8	29	20	
Weakly Positive	9	33	18	
Negative	1	8	6	
CL	18	70	44	0.299
Positive	1	13	12	
Weakly Positive	13	50	29	
Negative	4	7	3	

MDR multidrug resistance, *ANA* Antinuclear antibodies, *dsDNA* Anti-double-stranded DNA, *SM* Anti-Sm antibody, *RNP* Anti-ribonuclear protein antibody, *CL* Anti-cardiolipin antibody

This study has some limitations. First, besides Pgp, other factors may also affect SLE hormone resistance. Second, there is a lack of placebo control group. Third,

Table 5 The distribution of genotypes in the different pathological types of refractory LN

	TT	TC	CC	<i>P</i> value
<i>MDR1</i> C1236T				
Type III LN	2	3	0	0.184
Type IV LN	16	8	1	
Type V LN	2	2	1	
Type III-(A) + IV LN	1	1	1	
Type IV-G(A) + V LN	4	1	3	
<i>MDR1</i> C3435T				
Type III LN	0	4	1	0.578
Type IV LN	6	12	7	
Type V LN	0	1	4	
Type III-(A) + IV LN	0	2	1	
Type IV-G(A) + V LN	2	2	4	

the sample size is small. To better explain the mechanism of *MDR1* gene polymorphism on Pgp expression at the gene and cellular levels, we will further detect Pgp mRNA and protein expression in kidney tissue in the following work.

Conclusion

MDR1 C3435T polymorphism is significantly associated with Pgp expression in patients with refractory LN in Chinese Han SLE patients. Pgp expression is closely related

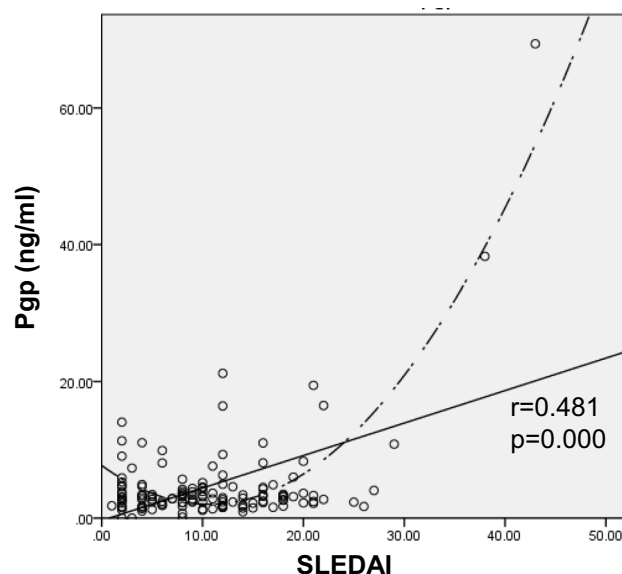


Fig. 1 Relationship between SLEDAI score and Pgp expression level in SLE patients. The results showed that SLEDAI score was closely related to the Pgp expression ($r = 0.481$, $p = 0.000$)

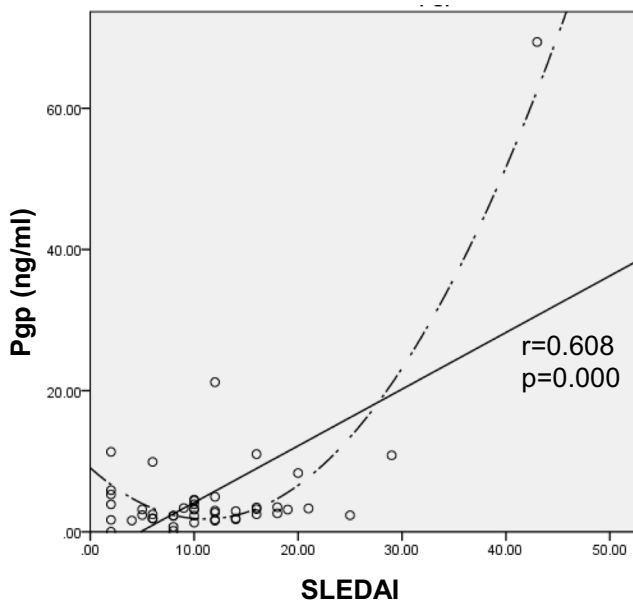


Fig. 2 Relationship between SLEDAI score and Pgp expression level in patients with refractory lupus nephritis. The results showed that SLEDAI score was closely related to the Pgp expression in patients with lupus nephritis ($r = 0.608$, $p = 0.000$)

to SLEDAI and renal pathological score. Thus, Pgp may be useful in evaluation of the prognosis of patients with refractory LN. Additionally, it is suggested that a more accurate drug regimen should be applied in accordance with different *MDR1* C3435T genotype and Pgp expression level. Also, hormone therapy should be individualized. Moreover, blockage of Pgp may become an ideal intervention for refractory LN treatment.

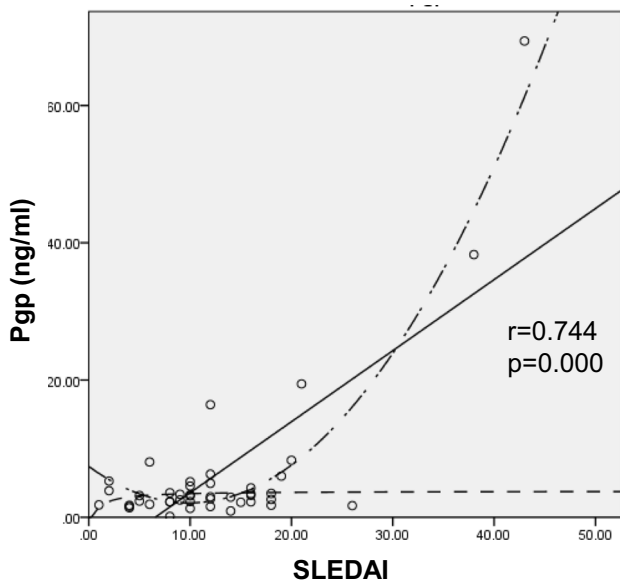


Fig. 3 Relationship between SLEDAI score and Pgp expression level in patients with refractory lupus nephritis. The results showed that SLEDAI score was closely related to the Pgp expression in patients with refractory lupus nephritis ($r = 0.744$, $p = 0.000$)

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethics committee of Haikou People’s Hospital (Approval no. 2017-082) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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