

Expression and Polymorphisms of Lysosome-Associated Protein Transmembrane 5 (LAPTM5) in Patients with Systemic Lupus Erythematosus in a Chinese Population

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Abstract Lysosome-associated protein transmembrane 5 (LAPTM5) have been demonstrated a role in the prevention of lymphocyte hyperactivation, and its deficiency is involved in the immunological dysfunction of mouse models. The aim of this study was to detect mRNA expression of *LAPTM5* in peripheral blood mononuclear cells (PBMCs) from patients with systemic lupus erythematosus (SLE), and to assess association between *LAPTM5* single nucleotide polymorphisms (SNPs) (rs10798801, rs4614309, rs1188348, and rs1188349) and SLE in a Chinese population. Real-time transcription-polymerase chain reaction analysis was used to determine expression of *LAPTM5* mRNA in PBMCs from 132 patients with SLE and 62 healthy controls. *LAPTM5* mRNA expression decreased in SLE patients ($n = 71$) compared with healthy controls ($n = 58$) ($p = 3.68 \times 10^{-5}$). The expression of *LAPTM5* mRNA in SLE patients with lupus nephritis (LN) ($n = 35$) was lower than in those without LN ($n = 36$) ($p = 0.004$). The expression level of *LAPTM5* correlated with serum total protein ($r_s = 0.41$, $p = 0.027$) and negatively correlated with 24-h proteinuria ($r_s = -0.45$, $p = 0.027$). *LAPTM5* SNPs (rs10798801, rs4614309, rs1188348, and rs1188349) was also analyzed by restriction fragment length polymorphism (RFLP) in 380 SLE patients and 460 healthy controls. No significant difference in the genotype or allele frequencies for

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LAPTM5 SNPs was detected in 380 SLE patients and 460 healthy controls ($p > 0.05$). Substantially low frequency of GGAT haplotype was observed in SLE patients ($p < 0.001$). It is concluded that insufficient expression of *LAPTM5* may take part in the pathogenesis of SLE and contribute to the severity of the disease, and none of *LAPTM5* polymorphisms contributes significantly to SLE susceptibility in a Chinese population.

Keywords Lysosome-associated protein transmembrane 5 · Systemic lupus erythematosus · Polymorphism · Lupus nephritis · Autoimmune disease

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting the joints, skin, kidneys, and brain. Women of child bearing age are preferentially affected at a rate nine times that of men, and those of African American and Asian ancestries are affected more frequently and manifest more severe disease than those of European ancestry (Lessard et al. 2012). It is characterized by autoantibody production by dysregulated B cells, target organ infiltration by inflammatory T cells, and aberrant immune cell activation. Autoreactive B cells and T cells are critical immune cells that have been implicated in the pathogenesis of SLE, and abnormal lymphocyte signaling and activation are the characteristic features (Chan et al. 2013; Vaughn et al. 2012). The exact cause of SLE remains unknown and physicians still have difficulties in diagnosing SLE accurately at initial stages.

There is great potential in using genetic studies to investigate lupus pathogenesis and the identification of single nucleotide polymorphisms (SNPs) that correlate with lupus holds promise toward the goal of risk assessment and treatment (Onel et al. 2009). To date, several genome-wide associations (GWA) in SLE have been described in genes that participate in the lymphocyte activation, immune response, apoptosis, and immune complex clearance (Lessard et al. 2012; Rullo and Tsao 2013). Over 50 genetic variants have been statistically associated with the development of SLE and the lymphocyte antigen receptor signaling pathways are rich in SLE susceptibility genes (Manjarrez-Orduno et al. 2012). Therefore, it seems that the gene expression and the polymorphic variants of such gene need to be investigated.

LAPTM5 encodes a transmembrane protein that resides in late endosomes and lysosomes and is expressed preferentially in hematopoietic tissues. It has been demonstrated that *LAPTM5* has an essential role for the lysosomal degradation of T cell and B cell antigen receptors (TCR/BCR) through the transport endosomes to lysosomes (Ishihara et al. 2011). It physically interacted with BCR complex and promoted its degradation in the lysosomal compartment in B cells. The *LAPTM5*-deficient mice had increased titers of serum IgM and autoantibodies and immune complex deposition in the kidney (Ouchida et al. 2010). In addition, the *LAPTM5* deficiency in pre-B cells led to the augmented expression level of surface pre-BCR. Pre-B cells in *LAPTM5*-deficient mice showed augmented pre-BCR expression levels, illustrating the importance of *LAPTM5* in the autonomous downmodulation

of the pre-BCR (Kawano et al. 2012). *LAPTM5* negatively regulated surface TCR expression and *LAPTM5* deficiency could also result in elevated TCR expression on both CD4⁺CD8⁺ thymocytes and spleen T cells, as well as enhance T cell responses in vitro and in vivo (Ouchida et al. 2008). These results demonstrate a role for *LAPTM5* in the negative regulation of immune response and the prevention of lymphocyte hyperactivation and autoimmunity.

Table 1 Demographic characteristics, clinical features, and laboratory measurements of SLE patients

	SLE patients (n = 71)
<i>Demographic characteristics</i>	
Female, n (%)	62 (87%)
Male, n (%)	9 (13%)
Age (years)	33 (16–50)
<i>Clinical features</i>	
Fever, n (%)	29 (41%)
Baldness, n (%)	32 (45%)
Light sensitivity, n (%)	9 (12%)
Facial erythema, n (%)	36 (51%)
Oral ulcer, n (%)	17 (24%)
Raynaud's phenomenon, n (%)	7 (10%)
Arthritis, n (%)	44 (62%)
Lupus nephritis, n (%)	35 (49%)
Serositis, n (%)	16 (23%)
CNS disease, n (%)	6 (8%)
<i>Laboratory measurements</i>	
Hematuria, n (%)	45 (63%)
Cylindruria, n (%)	15 (21%)
ANA, n (%)	60 (84%)
Anti-dsDNA, n (%)	58 (82%)
Anti-Sm, n (%)	32 (45%)
ANCA, n (%)	15 (21%)
C3 (g/l)	0.66 (0.24–1.51)
C4 (g/l)	0.13 (0.03–0.37)
IgG (g/l)	15.69 (9.33–31.6)
IgM, (g/l)	1.30 (0.2–3.56)
IgA (g/l)	3.06 (1.05–6.37)
ESR (mm/h)	32.7 (3–104)
Serum total protein (g/l)	60.98 (10.1–85.7)
Serum albumin (g/l)	35.9 (13.2–65)
Serum creatinine (μmol/l)	65.23 (37–252)
Serum CRP (mg/l)	13.64 (0.14–74)
Serum LDH (U/l)	242.85 (79–878)
24-h proteinuria (g/day)	18.28 (0.016–436)

CNS central nervous system,
ANA anti nuclear antibody,
ANCA anti-neutrophil
cytoplasmic antibodies, ESR
erythrocyte sedimentation rate,
LDH lactate dehydrogenase,
CRP C-reactive protein

* Values are the mean (range)

This study was to detect the mRNA expression of *LAPTM5* in peripheral blood mononuclear cells (PBMCs) from patients with SLE and to assess the association between *LAPTM5* SNPs (rs10798801, rs4614309, rs1188348 and rs1188349) and SLE in a Chinese population. It was the first attempt to investigate the expression and polymorphisms of *LAPTM5* in SLE patients in a Chinese population.

Materials and Methods

Human Subjects

380 patients with SLE diagnosed according to the criteria of the 1982 American College of Rheumatology were enrolled. At the same time, 460 healthy controls without autoimmune disease or cancer were recruited, who were sex- and age-matched with the patients. The informed consents were obtained from all donors, and the study was approved by the human ethics committee of China Medical University. The demographic characteristics, clinical features, and laboratory measurements of 71 SLE patients are recorded and shown in Table 1. The kidney biopsy tissues were evaluated for routine histology and immunofluorescence, and lupus nephritis (LN) was classified according to the RPS/ISN criteria (Renal Pathology Society Working Groups/International Society of Nephrology).

Quantitative Real-Time PCR

PBMCs were separated by density gradient centrifugation. Total RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's protocol and then quantified by photometrical measurement. One microgram of total RNA was reverse transcribed to cDNA in a total volume of 20 μ l system using a RT reaction kit (Promega). Real-time PCR was performed using the Express SYBR greener qPCR supermix Universal Kit (Invitrogen) on a Rotor-gene 6000 system (Qiagen). The 25- μ l PCR mixture contained 2 μ l reverse-transcribed product 12.5 μ l SYBR Green supermix, 8.5 μ l RNase-free water, 1 μ l forward, and 1 μ l reverse primers. The reaction was performed in a 72-well optical plate in triplicate. The first step of PCR protocol is 95°C for 10 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s as the second step. A melting-curve analysis was performed to ensure specificity of the PCR products, and all the PCR products were subjected to electrophoresis in an agarose gel to confine to a single band of the expected size. The expression of *LAPTM5* was normalized to β -actin and determined using the comparative ($2^{-\Delta\Delta C_t}$) method (Livak and Schmittgen 2001). Primers were as follows: *LAPTM5* forward: 5'-gtcctctgcaaatcatggact-3', and reverse: 5'-tgatagtggtggcacttcc-3'; β -actin forward: 5'-tacagcttcaccaccacg-3', and reverse: 5'-aaggaaggctgaaaagagc-3'.

SNP Selection and Genotyping

Genomic DNA was extracted via the conventional phenol–chloroform extraction method. Both the quality and quantity of the extracted DNA were then determined

using spectrophotometry. SNPs genotyped in this study were selected by an integration of the publicly available tools, HapMap Project, Haploview 4.2 and FastSNP search (Available online: <http://www.hapmap.org>, <http://www.broadinstitute.org/mpg/haploview> and <http://fastsnp.ibms.sinica.edu.tw>). TagSNPs were chosen based on rare variants [minor allele frequency (MAF) > 0.1] and the potentially functional effects, which were separately predicted by the tools of Haploview 4.2 and FastSNP search. The *LAPTM5* SNPs (rs10798801, rs4614309, rs1188348, and rs1188349) were analyzed by restriction fragment length polymorphism (RFLP). (Wilson et al. 1992) Primers used in PCR–RFLP were: for rs10798801, 5'-ccaaagccactgtgatagg-3' (forward) and 5'-aggccagaagtcaggaat-3' (reverse); for rs4614309, 5'-actttggaagccaggactgttac-3' (forward) and 5'-ctgtggaagattattgaccaccta-3' (reverse); for rs1188348, 5'-ctgttagaactgccacctt-3' (forward) and 5'-gttactgaatcccacttgacc-3' (reverse); for rs1188349, 5'-caagggaagtgcacaaagcacc-3' (forward), and 5'-cacgatgtggaacctgcggatag-3' (reverse). The restriction enzymes for the SNPs above were XmnI, HincII, XcmI, and BsaWI, respectively (New England Biolabs). Direct sequencing was performed using randomly selected subjects to validate the accuracy of the SNP genotyping assays.

Statistical Analysis

Data were managed and stored using the SPSS 16.0 statistical software package (SPSS, Chicago, IL). The differences of relative mRNA levels between patient and control groups were tested by the Mann–Whitney *U* test. Correlations were calculated using Spearman's rank coefficient. Hardy–Weinberg equilibrium (HWE) of the *LAPTM5* tagSNPs in the control subjects was evaluated using chi-square test. The frequencies of the alleles and genotypes were also compared between patient and control groups by the chi-square test. The odds ratio (OR) and 95% confidence intervals (95% CI) were estimated. The haplotypes comprising rs10798801, rs4614309, rs1188348, and rs1188349 for each individual were assigned using the online software platform SHEsis (<http://analysis2.bio-x.cn/myanalysis.php>). All tests were two-tailed, and *p* values <0.05 were considered as statistically significant.

Results

Laboratory Measurements of the Patients with SLE

The demographic characteristics, clinical manifestation, and laboratory measurements in the SLE patients are presented in Table 1. Of the 71 patients included in the study, the majority were women ($n = 62$). The LN, serositis, arthritis, and CNS disease were found in 35, 16, 44, and 6 patients, respectively. The ANA, anti-dsDNA, anti-Sm, and ANCA autoantibodies were detected in 60, 58, 32, and 15 patients. The mean values of C3, C4, IgG, IgM, and IgA for the patients were 0.66 (range 0.24–1.51), 0.13 (range 0.03–0.37), 15.69 (range 9.33–31.6), 1.30 (range 0.2–3.56), and 3.06 (range 1.05–6.37) g/l. The mean values of serum total protein and serum albumin were 60.98 (range 10.1–85.7) and 35.9 (range 13.2–65) g/l,

respectively. The mean value of 24-h proteinuria was 18.28 g/day with the range from 0.016 to 436 g/day. Kidney biopsy tissues from SLE patients were evaluated for routine histology and immunofluorescence and were classified according to the RPS/ISN criteria. There were 6 (17%) patients with class II, 3 (9%) with class III, 20 (57%) with class IV, 4 (11%) with class V, and 2 (6%) with class IV + V LN (Table 2).

Quantification of *LAPTM5* mRNA Expression in PBMC from SLE Patients and Healthy Controls by Real-Time PCR

The expression of *LAPTM5* mRNA in PBMCs from 71 SLE patients and 58 healthy controls was assessed by quantitative real-time PCR. Comparing with healthy subjects, the collected PBMCs from SLE patients had lower expression levels of mRNA for *LAPTM5* than controls [median (range); control = 1.28 (0.34–3.65) vs. SLE = 0.74 (0.12–2.28), $p = 3.68 \times 10^{-5}$] (Fig. 1). Besides, the expression of *LAPTM5* mRNA in SLE patients with LN was significantly lower than in those without LN [median (range); SLE with LN = 0.87 (0.60–1.90) vs. SLE without LN = 1.18 (0.61–2.93), $p = 0.004$] (Fig. 2).

Analysis of Relationships Between *LAPTM5* mRNA Expression and Laboratory Parameters in SLE Patients

Association of *LAPTM5* with laboratory parameters was analyzed. The results showed that the mRNA expression of *LAPTM5* significantly correlated with serum total protein ($r_s = 0.41$, $p = 0.027$) and inversely correlated with 24-h proteinuria ($r_s = -0.45$, $p = 0.027$). No substantial correlation was observed between the *LAPTM5* mRNA expression and other laboratory parameters (Table 3).

Association Between *LAPTM5* Gene Polymorphisms and SLE in the Chinese Population

In order to test for the genetic association of *LAPTM5* with SLE in Chinese population, we further investigated *LAPTM5* SNPs using the publicly available tools (HapMap Project, Haploview 4.2, and FastSNP search) and selected four functional tagSNPs (rs10798801, rs4614309, rs1188348, and rs1188349) to genotype in 380 SLE patients and 460 healthy controls by PCR–RFLP. In addition, we sequenced

Table 2 Kidney biopsy tissues were evaluated for routine histology and immunofluorescence and were classified according to the RPS/ISN criteria

RPS/ISN class	Lupus nephritis ($n = 35$)
I	0
II	6 (17%)
III	3 (9%)
IV	20 (57%)
V	4 (11%)
IV + V	2 (6%)

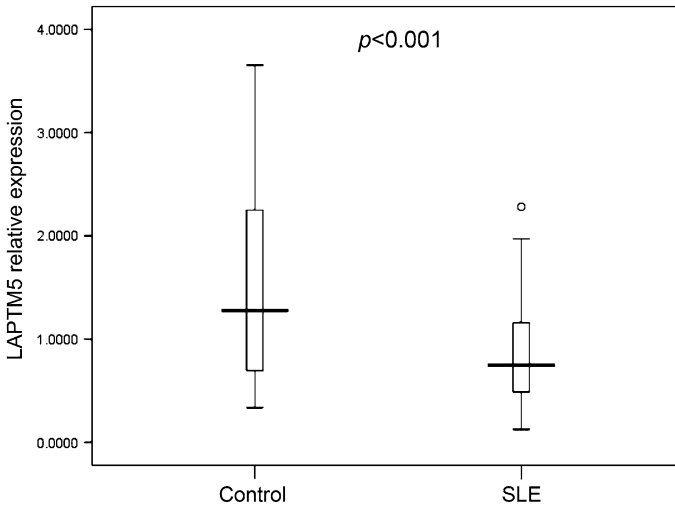


Fig. 1 *LAPTM5* mRNA expression levels in PBMC from SLE patients and healthy controls. The results are shown in box plots given medians, quartiles, and outsiders (*circle points*). *p* values of differences between the SLE patients ($n = 71$) and healthy controls ($n = 58$) are defined by Mann–Whitney statistics ($p = 3.68 \times 10^{-5}$)

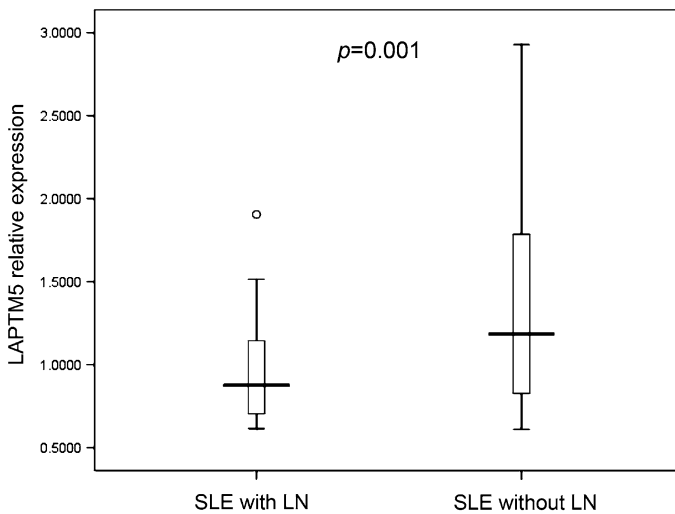


Fig. 2 *LAPTM5* mRNA expression levels in PBMC from SLE patients with LN and those without LN. The results are shown in *box plots* given medians, quartiles, and outsiders (*circle points*). *p* values of differences between the SLE patients with LN ($n = 35$) and those without LN ($n = 36$) are defined by Mann–Whitney statistics ($p = 0.001$)

the region of each SNP in 12 randomly selected individuals (six patients and six controls) and validate the accuracy of the SNP genotyping assays.

Among the control subjects, the genetic distributions of the SNPs did not deviate from Hardy–Weinberg equilibrium ($p = 0.32, 0.18, 0.44,$ and $0.52,$ respectively).

Table 3 Correlation between *LAPTM5* mRNA expression and clinical variables in SLE patients

SLE patients (<i>n</i> = 30)	r_s	<i>p</i>
C3	0.07	n.s.
C4	−0.06	n.s.
IgG	0.06	n.s.
IgM	−0.21	n.s.
IgA	0.24	n.s.
ESR	−0.06	n.s.
Serum total protein	0.41	<0.05
Serum albumin	0.28	n.s.
Serum creatinine	−0.05	n.s.
Serum CRP	−0.11	n.s.
Serum LDH	−0.2	n.s.
24-h proteinuria	−0.45	<0.05

* *ns* no significance

We found no significant difference in the genotype or allele frequencies for any of the SNPs examined (Table 4). The online software platform SHEsis were used to analyze the haplotype data and probabilities. The results show that the GGAT haplotype frequency was significantly low in the SLE patients, unlike that observed in the healthy controls ($p < 0.001$, OR 0.365, 95% CI 0.203–0.656). No difference was detected between the SLE patients and the healthy controls with respect the other haplotypes (Table 5).

Discussion

SLE is an idiopathic multisystem inflammatory disease characterized by lymphocyte hyperactivation and autoantibody production. Appropriate BCR and TCR signaling is crucial for the normal function of lymphocytes and induction of an optimal immune response. Altered expression and function of the signal transduction molecule in lymphocytes is involved in the development of SLE (Renaudineau et al. 2004; Moulton and Tsokos 2011). *LAPTM5* encodes a lysosomal protein that targets both TCR and BCR and involves in their signaling. We hypothesized that abnormal expression and genetic variation in *LAPTM5* resulting in subtle alterations of BCR and TCR signaling and lymphocyte hyperactivation could be associated with altered susceptibility to SLE. Here we tested for the first time the mRNA expression and the genetic association of *LAPTM5* with SLE in Chinese population.

Our results suggested that *LAPTM5* transcript level significantly decreased in PBMCs from SLE patients compared to healthy controls. The reduction of *LAPTM5* mRNA expression may contribute to the pathogenesis of SLE. Lymphocyte surface antigen receptor is normally internalized after antigen stimulation and then degraded in lysosomes (Naramura et al. 2002). Abnormality in the receptor degradation could affect the receptor levels and lymphocyte function. Few lysosomal proteins involved in the process of the BCR and TCR degradation have been identified. At present no paralogues with homology to *LAPTM5* have been

Table 4 Frequencies of alleles and genotypes of *LAPTM5* polymorphisms in SLE patients and healthy controls

SNP	Subjects	Genotype/ Allele	Control		SLE		χ^2 (<i>p</i>)	Odds ratio [95% CI]
			<i>n</i>	%	<i>n</i>	%		
rs10798801	809	GG	271	60.9	233	64.0	2.826 (0.243)	0.876 [0.658–1.166]
		AG	157	35.3	124	34.1		
		AA	17	3.8	7	1.9		
		G	699	78.5	590	81.0		
		A	191	21.5	138	19.0		
rs4614309	805	GG	235	52.7	198	55.2	1.729 (0.421)	0.906 [0.685–1.197]
		TG	185	41.5	147	40.9		
		TT	26	5.8	14	3.9		
		G	655	73.4	543	75.6		
		T	237	26.6	175	24.4		
rs1188348	641	AA	167	46.4	121	43.1	2.312 (0.315)	1.152 [0.872–1.522]
		AG	161	44.7	125	44.5		
		GG	32	8.9	35	12.4		
		A	495	68.8	367	65.3		
		G	225	31.2	195	34.7		
rs1188349	814	CC	250	55.4	192	52.9	0.607 (0.738)	1.108 [0.839–1.462]
		CT	168	37.3	141	38.8		
		TT	33	7.3	30	8.3		
		C	668	74.1	525	72.3		
		T	234	25.9	201	27.7		

Table 5 Frequencies of the haplotypes formed by rs10798801, rs4614309, rs1188348, and rs1188349 SNPs in SLE patients and healthy controls

Haplotype	SLE (%)	Control (%)	χ^2	Fisher's <i>p</i>	Pearson's <i>p</i>	Odds ratio [95% CI]
ATAC	90.09 (0.161)	118.31 (0.167)	0.122	0.726642	0.726638	0.948 [0.701–1.281]
GGAC	223.48 (0.400)	262.54 (0.370)	0.908	0.340721	0.340665	1.119 [0.888–1.411]
GGAT	15.22 (0.027)	49.86 (0.070)	12.223	0.000476	0.000475	0.365 [0.203–0.656]
GGGC	53.88 (0.097)	64.89 (0.091)	0.058	0.809608	0.809592	1.048 [0.716–1.533]
GGGT	125.09 (0.224)	133.63 (0.188)	2.164	0.141359	0.141284	1.230 [0.933–1.620]
GTAC	25.64 (0.046)	40.79 (0.057)	0.934	0.333917	0.333859	0.779 [0.469–1.294]

identified in either mouse or human (Ouchida et al. 2010). It has been demonstrated that *LAPTM5* promotes CD3zeta degradation to control surface TCR expression and T cell activation (Ouchida et al. 2008). *LAPTM5* negatively regulates cell surface BCR levels in vitro and in vivo, and its deficiency in B cell causes enhanced B cell activation and antibody production. These results suggest that *LAPTM5* could inhibit T and B cell activation to fine-tune the immune responses.

LN represents one of the most serious manifestations of SLE. It has been reported that *LAPTM5*^{-/-} mice produce increased levels of serum IgM and anti-dsDNA antibody and have immune complex deposition in the kidney, leading to the injury of kidney (Ouchida et al. 2010). We found that there was a significant difference in *LAPTM5* mRNA expression between SLE patients with LN and those without LN. The association of *LAPTM5* mRNA expression with LN in SLE patients indicates its decrease was involved in severity of SLE. In addition, the correlation between *LAPTM5* mRNA expression and serum total protein level, and the inverse correlation between *LAPTM5* mRNA expression and 24-h proteinuria suggest that the insufficient expression of *LAPTM5* may contribute to severity of the disease.

Taken together, the deficiency of *LAPTM5* expression may contribute to the pathogenesis of SLE through the mechanisms below: one of possible mechanisms is that the insufficient expression of *LAPTM5* may cause hyperactivation of T and B cell, which is one of hallmarks of SLE. Another possible mechanism is that the decreased expression of *LAPTM5* may enhance the deposition of autoimmune complexes, which is also a key marker of SLE.

Since the cause of abnormal expression may be the polymorphism in the gene and some polymorphic variants can influence the gene expression (Jennes et al. 2012; Li et al. 2012), we further investigated the association between *LAPTM5* tagSNPs (rs10798801, rs4614309, rs1188348, and rs1188349) and SLE in a Chinese population. The distribution of both allelic and genotypic frequencies of the *LAPTM5* polymorphisms showed no significant association between the SLE patients and healthy controls. These results indicated that downregulation of *LAPTM5* expression might be a result of interaction and involvement of other factors and genes. (Ishihara et al. 2011; Pak et al. 2006) Interestingly, haplotype analysis revealed that the GGAT haplotype frequency was significantly low in the SLE patients compared with the healthy controls, indicating that the haplotype GGAT confers a reduced risk of SLE and may be protective against this disease. As with other studies regarding the association of gene polymorphisms in SLE, our study also has limitations. Our study only tested the limited tagSNPs and investigated SLE patients from a Chinese population. Expanding the range of *LAPTM5* SNPs and performing the test on multiethnic populations should be accomplished.

In conclusion, we report that the insufficient expression of *LAPTM5* may take part in the pathogenesis of SLE and contribute to the severity of the disease. Although the haplotype GGAT was found to protect against SLE, we did not observe any significant association between *LAPTM5* polymorphisms and SLE in Chinese population. Our data support that *LAPTM5* gene may be a target for therapy of SLE, but an additional comprehensive genotyping of *LAPTM5* SNPs in other ethnic groups will provide more clues to understanding the pathogenesis of SLE.

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