Paper

Association of PER2 gene single nucleotide polymorphisms with genetic susceptibility to systemic lupus erythematosus

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Abstract

The circadian clock plays a crucial role in the progress of systemic lupus erythematosus (SLE). In this study, we performed a case-control study to explore the association between *Period* 2 (PER2) gene single nucleotide polymorphisms (SNPs) and the susceptibility of systemic lupus erythematosus (SLE). A total of 492 SLE patients and 493 healthy controls were included. The improved multiple ligase detection reaction (iMLDR) was used for genotyping. The correlations between four SNPs of PER2 (rs10929273, rs11894491, rs36124720, rs934945) and the genetic susceptibility and clinical manifestations of SLE were analyzed. Significant differences were observed in the distributions of allele frequencies and genotype under dominant model in rs11894491 between SLE patients and controls ($p = 0.030$, $p = 022$, respectively). We hypothesized that PER2 gene SNPs was related to the genetic susceptibility and clinical manifestations, implying the potential role of PER2 in the pathogenesis of SLE.

Keywords

systemic lupus erythematosus, Period 2, single nucleotide polymorphisms

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Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease caused by multiple factors. This disease occurs due to the disruption of balance between immune tolerance and immune activation, which promotes the formation of immune complexes, autoantibodies, inflammation in multiple organs and tissues, and proinflammatory cytokines in the blood fluid.[1] Although, the pathogenesis of SLE has not been fully elucidated, immunological disarrangement, environmental and genetic factors have been shown to be closely related to the pathogenesis of SLE.[2]

Biorhythm, which is regarded as a primary factor in the pathological and physiological condition, has become an important field of medical research. Circadian rhythms are regulated by clock genes (like CLOCK, BMAL1, PER1, PER2, PER3) that interact in complex ways to produce oscillations in gene

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expression. Many studies have shown a biphasic effect between clock genes and inflammation.[3–5] Disruption of circadian rhythms significantly affects the function of immune system, and cellular expression of core clock genes directly alters inflammatory responses.[5,6]

Period 2 (PER2), a member of the Period family, is principally generated in the suprachiasmatic nucleus (SCN). It is a key clock gene that regulates circadian rhythms of mammals on the level of pathology, physiology and gene expression.[7] Interruption in the expression of PER2 can lead to reduction in the transcription level of other clock genes.[8] In addition, a previous study which has also shown an interaction between PER2 and inflammation cytokine, shows the expression of PER2 reduced in synchronized fibroblasts after TNF-α treatment.[9] The production of IL-10 and IFN-γ were reduced in PER2 mutated mice.[10] Lee et al. indicated that PER2 gene single nucleotide polymorphisms (SNPs) would increase rheumatoid arthritis (RA) susceptibility through regulating the protein expression of PER2.[11] In the experiment of autoimmune encephalomyelitis (EAE) mice involving a wide-used animal model of multiple sclerosis, Abigail et al. discovered significant variability of PER2 expression against control.[12]

PER2 has a potential role in the occurrence and progression of autoimmune diseases. In order to study the function of PER2 in SLE and also establish clarification to the function of PER2 in SLE, we carried out a case-control study to explore the relationship between PER2 gene SNPs and genetic susceptibility of SLE, as well as their correlation with clinical manifestations.

Materials and methods

Subjects selection

A total of 985 participants (492 SLE patients and 493 health controls) were enrolled in our study. All included patients were recruited from the Department of Rheumatology and Immunology at the First Affiliated Hospital of University of Science and Technology of China, and the First Affiliated Hospital of Anhui Medical University, and were diagnosed according to the criteria of American College of Rheumatology (ACR) revised in 1997.[13] Subjects were excluded if they had other autoimmune diseases and (or) cancer. In addition, 493 health controls were extracted from the Health Examination Centre of the First Affiliated Hospital of Anhui Medical University. All normal individuals had no family history of any autoimmune diseases or any serious diseases (e.g., cancer, diabetes and hypertension). Demographic profiles and clinical characteristics were extracted from questionnaires and medical records. The consent of all participants was sought, and the Medical Ethics Committee of Anhui Medical University approved this study.

SNPs selection

The Ensemble gene database was used to acquire information of PER2 gene SNPs. Subsequently, tag SNP selection was implemented through Haploview 4.2 software, according to three criteria: a) minor allele frequency (MAF) $\geq$ 5% in Chinese Han population; b) linkage disequilibrium (LD) coefficient $r^2 \geq 0.8$; c) consulting relative studies and estimating predicted function of tag SNPs through the SNP Function Prediction (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm), and gave preference to those SNPs located in the important areas. Four SNPs (rs10929273, rs11894491, rs36124720, rs934945) were finally included in our study for further analysis (Table 1).

DNA extraction and genotyping

We collected 5 ml peripheral venous blood of subjects, and then abstracted genomic DNA consulting the manufacturer's instruction (QIAGEN, Hilden, Germany). We employed NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA) to detect the quantification and concentration of DNA. Quantified sample: a) had concentration $\geq$ 50 ng/μl; b) concentration $\geq$ 600 ng; c) no obvious degradation. Genotyping of four SNPs were analyzed through improved multiple ligase detection reaction (iMLDR), which technically supported by Genesky Biotechnologies Inc., Shanghai.

Statistical analysis

All statistical analyses were conducted by using the SPSS 23.0 software. Age was described as mean $\pm$ standard deviation (XXXX$x \pm$ s) after carrying out Shapiro-Wilk normal test and was compared with t-test between two groups. Distribution of genotype and allele frequencies between case and control groups were analyzed using Chi-square or Fisher's exact probability test. Hardy - Weinberg equilibrium (HWE) was performed to detect the genetic balance. Moreover, online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php) was employed for haplotype analyses, excluded haplotype with frequency < 0.03. The inspection level was determined as two-tailed ($\alpha = 0.05$).

Table 1. Characteristics of PER2 gene SNPs.

SNP	Chr	Chr. position	Allele	*P* value for HWE test
rs10929273	2	239174706	G/A	0.595
rs11894491	2	239198325	G/A	0.984
rs36124720	2	239155971	C/G	0.843
rs934945	2	239155053	C/T	0.999

Chr: chromosome; HWE: Hardy-Weinberg equilibrium.

Table 2. Demographic profiles and clinical characteristics of SLE patients.

Characteristics	Patients with SLE (n = 492)	Health controls (n = 493)
Demographic profiles		
Age, year	37.58 ± 11.46	38.45 ± 11.32
Sex, male/female	58/434	59/434
Clinical characteristics		
Oral ulcers, n (%)	119 (24.2)	–
Immunologic abnormality, n (%)	359 (73)	–
Serositis, n (%)	45 (9.1)	–
Renal disorder, n (%)	183 (37.2)	–
Photosensitivity, n (%)	193 (39.2)	–
Malar rash, n (%)	222 (45.1)	–
Neurological abnormality, n (%)	21 (4.3)	–
Discoid rash, n (%)	93 (18.9)	–
Arthritis, n (%)	244 (49.6)	–
Hematological abnormality, n (%)	336 (68.3)	–

n: number; SLE: systemic lupus erythematosus.

Results

Basic characteristics

Our study enrolled 985 participants, including 492 SLE patients with a mean age of 37.58 ± 11.46 years and 493 health controls with a mean age of 38.45 ± 11.32 years. The proportion of male to female was 58/434 in patients and 59/434 in controls. The differences of age and gender between SLE patients and controls were not statistically significant ($p > 0.05$). The main clinical features of SLE patients, referred to ACR classification criteria (1997) of SLE, were oral ulcers (24.2%), immunologic abnormality (73%), renal disorder (37.2%), photosensitivity (39.2%), malar rash (45.1%), arthritis (49.6%) and hematological abnormality (68.3%) (Table 2). The genotype distribution of these 4 SNPs conformed to HWE in SLE group and control group ($p > 0.05$).

Association between PER2 gene polymorphisms and SLE susceptibility

The genotype and allele frequencies of PER2 gene in SLE patients and controls are detailed in Table 3. No significant difference of genotype distribution in these four SNPs was detected between SLE patients and controls ($p > 0.05$). As for allele frequencies, the result indicated a relationship between rs11894491 and SLE susceptibility that A allele elevated the risk of SLE ($P = 0.030$, $OR = 1.230$, 95% CI:1.020-1.482). Moreover, in dominant models, AA+GA genotype of rs11894491 was associated with the increased risk of SLE compared to GG ($P = 0.022$, $OR = 1.343$, 95% CI:1.043-1.729).

Association between PER2 gene polymorphisms and major clinical characteristics of SLE

A case-only study was further performed to detect the impact of PER2 gene polymorphisms on major clinical manifestations. CC/GC/GG genotype and C/G allele of rs36124720 distributed significantly less in SLE patients with photosensitivity than those without ($p = 0.003$, $p = 0.002$, respectively) (Table 4). However, no effective relationships were found between all included SNPs and oral ulcers, immunologic abnormality, serositis, renal disorder, malar rash, neurological abnormality, discoid rash, arthritis and hematological abnormality in SLE patients (all $p > 0.05$).

Table 3. Genotype and allele frequencies of PER2 gene in SLE patients and controls.

SNP	Analyze model	SLE patients [n (%)]	Control [n (%)]	*P* value	OR (95% CI)
Rs10929273	Genotype				
	GG	134 (27.2)	119 (24.1)	0.288	
	GA	237 (48.2)	262 (53.1)		
	AA	121 (24.6)	112 (22.7)		
	Allele				
	G	505 (51.3)	500 (50.7)	0.786	1.025 (0.859–1.223)
	A	479 (48.7)	486(49.3)		
	Dominant model				
	GG	134 (27.2)	119 (24.1)	0.266	0.850 (0.638–1.132)
	GA+AA	358 (72.8)	374 (75.9)		
	Recessive model				
	GG+GA	371 (75.4)	381 (77.3)	0.489	1.109 (0.827–1.489)
	AA	121 (24.6)	112 (22.7)		
	Additive model				
	GG	134 (52.5)	119 (51.5)	0.820	1.042 (0.730–1.489)
	AA	121 (47.5)	112 (47.5)		
rs11894491	Genotype				
	GG	195 (39.6)	231 (46.9)	0.070	
	GA	236 (48.0)	211 (42.8)		
	AA	61 (12.4)	51 (10.3)		
	Allele				
	G	626 (63.6)	673 (68.3)	0.030	1.230 (1.020–1.482)
	A	358 (36.4)	313 (31.7)		
	Dominant model				
	GG	195 (39.6)	231 (46.9)	0.022	1.343 (1.043–1.729)
	GA+AA	297 (60.4)	262 (53.1)		
	Recessive model				
	GG+GA	431 (87.6)	442 (89.7)	0.310	1.227 (0.826–1.820)
	AA	61 (12.4)	51 (10.3)		
	Additive model				
	GG	195 (76.2)	231 (81.9)	0.101	0.706 (0.465–1.072)
	AA	61 (23.8)	51 (18.1)		
rs36124720	Genotype				
	CC	219 (44.5)	207 (42.0)	0.425	–
	GC	218 (44.3)	218 (44.2)		
	GG	55 (11.2)	68 (13.8)		
	Allele				
	C	656 (66.7)	632 (64.1)	0.231	1.120 (0.930–1.349)
	G	328 (33.3)	354 (35.9)		
	Dominant model				
	CC	219 (44.5)	207 (42.0)	0.424	0.902 (0.701–1.161)
	GC+GG	273 (55.5)	286 (58.0)		
	Recessive model				
	CC+GC	437 (88.8)	425 (86.2)	0.215	0.787 (0.538–1.150)
	GG	55 (11.2)	68 (13.8)		
	Additive model				
	CC	219 (79.9)	207 (75.3)	0.191	0.765 (0.511–1.144)
	GG	55 (20.1)	68 (24.7)		
rs934945	Genotype				
	CC	243 (49.4)	272 (55.2)	0.191	–
	CT	211 (42.9)	188 (38.1)		
	TT	38 (7.7)	33 (6.7)		
	Allele				
	C	697 (70.8)	732 (74.2)	0.090	1.187 (0.973–1.447)
	T	287 (29.2)	254 (25.8)		

(continued)

Table 3. Continued.

SNP	Analyze model	SLE patients [n (%)]	Control [n (%)]	*P* value	OR (95% CI)
	Dominant model				
	CC	243 (49.4)	272 (55.3)	0.069	1.261 (0.982–1.620)
	CT+TT	249 (50.6)	221 (44.8)		
	Recessive model				
	CC+CT	454 (92.3)	460 (93.3)	0.532	1.167 (0.719–1.893)
	TT	38 (7.7)	33 (6.7)		
	Additive model				
	CC	243 (86.5)	272 (89.2)	0.316	1.289 (0.784–2.120)
	TT	38 (13.5)	33 (10.8)		

Table 4. The positive findings of association between genotype frequencies in PER2 and clinical characteristics.

			Genetypes, n				Allele, n		
Gene (SNP)	Clinical features	Group	CC	GC	GG	*P* value	C	G	*P* value
rs36124720	Photosensitivity	Positive	98	84	11	0.003	280	106	0.002
		Negative	121	134	44		376	222	

Haplotype analysis

Seven haplotypes (AACC, AACT, AGCC, AGGC, GACT, GGCC and GGGC) of PER2 gene were analyzed through SHEsis software. Results showed no significant difference in the distribution of all haplotypes between cases and controls (all $p > 0.05$) (Table 5).

Discussion

Circadian clock genes are essential for maintaining circadian rhythms in behavior, physiologic and endocrine systems. In mammals, SCN, in the hypothalamus, is a key point that regulates circadian rhythms. PER2 is generated at SCN and has been proven to impact the circadian rhythm.[5] PER2 can bind to the enhancers of promoters to suppress the transcription of CLOCK/BMAL1, two essential molecules regulating mammals' circadian rhythms on the level of gene expression, physiology and pathology.[14] Furthermore, bidirectional interactions have been found between PER2 and some immune markers (IFN-γ, IL-6, TNF-α).[15–17] Recent studies have implied potential functions of PER2 in the pathogenesis and development of several autoimmune diseases.[11,12]

SLE is a chronic autoimmune disease with unclear etiology. Researchers have hypothesized that circadian rhythms may participate in the pathogenesis of SLE. Wang et al. analyzed the gene polymorphisms of melatonin pathway in SLE patients and found that genotypes of rs3760138 and rs8150 are related to SLE genetic susceptibility.[18] The present case-control study investigated the association between PER2 polymorphisms and genetic susceptibility to SLE as well as major clinical manifestations. Results showed that allele frequency of rs11894491 distributed differently between SLE patients and health controls, and its A allele might increase the risk of SLE. In dominant models, GA + GG genotype of rs11894491 was related to elevated risk of SLE. Also, rs36124720 GG/GC/CC genotype and G/C allele were related to photosensitivity. These findings indicate that PER2 might be involved in the occurrence and progression of SLE.

Presently, glucocorticoids are widely used in the treatment of SLE for the imbalance of cortisol level and neuroendocrine-immune pathway playing a crucial role in autoimmune diseases. Cortisol level peaks in early morning and touches the bottom in midnight. Circadian rhythm was strongly correlated with cortisol levels. Circadian release of corticosterone was regulated by SCN, leading to rhythmic expression of PER2 mRNA in hepatocytes and fibroblasts.[19,20] A previous study suggested that glucocorticoids and their receptors participated in the negative feedback regulation of PER2mRNA and protein expression.[21] Thus, PER2 might work in the treatment of SLE through affecting glucocorticoids and their receptors. Moreover, autophagy, a process of self-preservation and life-sustaining. Its important role in the development and pathogenesis of SLE has been clarified through the studies of genetics, cell culture, animal experiment and clinic.[22] Kalfalah et al. demonstrated that elevating PER2 expression in primary dermal fibroblasts recovered attenuate autophagy levels.[23] A study by Liu et al. in oral squamous cell carcinoma cells showed

Table 5. Haplotype analysis of four SNPs in PER2 gene in SLE patients and controls.

Haplotype	Case [n (%)]	Control [n (%)]	χ^2	*P* value	OR (95% CI)
rs10929273- rs11894491- rs36124720- rs934945					
AACC	36.76 (3.7)	40.01 (4.1)	0.121	0.728	0.922 (0.584–1.457)
AACT	53.93 (5.5)	42.82 (4.3)	1.427	0.232	1.285 (0.851–1.939)
AGCC	266.64 (27.1)	269.04 (27.3)	0.001	0.977	0.997 (0.816–1.219)
AGGC	110.78 (11.3)	122.29 (12.4)	0.563	0.453	0.900 (0.684–1.185)
GACT	221.09 (22.5)	193.76 (19.7)	2.565	0.109	1.196 (0.961–1.488)
GGCC	46.34 (4.7)	56.67 (5.7)	1.023	0.312	0.814 (0.546–1.214)
GGGC	191.26 (19.4)	208.59 (21.2)	0.811	0.368	0.903 (0.724–1.127)

Total χ^2=5.557, df = 6, P = 0.475.
Note: All the haplotypes with a frequency <0.03 were ignored in the analysis.

overexpression of PER2 motivated autophagy.[24] Another study also revealed that PER2 overexpression facilitated the expression of Beclin1, which was necessary for autophagy and apoptosis.[25] Consequently, PER2 played a crucial role in the process of autophagy and might impact the pathogenesis of SLE through autophagy pathway.

The functions of PER2 in other autoimmune diseases have been explored before. Lee et al. estimated the relationship of PER2 gene polymorphisms with RA susceptibility and compared the protein levels of PER2 in synoviocytes. CC genotype of rs2304674 was associated with the reduction in the risk of RA, but no relationships were found between PER2 gene polymorphisms and clinical features of RA patients. Moreover, they found that inflammation caused by lipopolysaccharide restrains the expression of PER2 in RA synoviocytes.[11]

Our study was the first to investigate the association between PER2 polymorphisms and genetic susceptibility of SLE in the Chinese population. However, there were some limitations that should be acknowledged. First, all subjects of our study were collected from two tertiary hospitals in Anhui Province, which might generate selection bias. Again, we lacked some more detailed information for subjects (e.g., body mass index). Furthermore, we just provided clues for the potential role of PER2 in the pathogenesis of SLE, and no mechanisms were explored. Finally, the sample size of our study was limited, which might result in the weak association between rs11894491 and SLE, and between rs36124720 and photosensitivity

In conclusion, this study indicated that, PER2 gene SNPs were related to genetic susceptibility and clinical manifestations of SLE in the Chinese population, implying the feasible role of PER2 in the pathogenesis of SLE. In the future, studies with large sample size in other population exploring the impact of PER2 gene on the pathogenesis and progress of SLE are warranted.

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