Journal of Clinical Immunology, Vol. 26, No. 6, November 2006 (© 2006)
DOI: 10.1007/s10875-006-9048-9

Programmed Death-1 Gene Polymorphisms in Patients With Systemic Lupus Erythematosus in Taiwan

SHU-CHEN WANG,[1,5] YI-JING CHEN,[2,5] TSAN-TENG OU,[3] CHENG-CHIN WU,[3] WEN-CHAN TSAI,[2,3] HONG-WEN LIU,[2,3] and JENG-HSIEN YEN[3,4,6]

Received August 7, 2006; accepted September 8, 2006
Published online: 6 October 2006

To investigate the role of programmed cell death-1 (PD-1) gene polymorphisms in the development of systemic lupus erythematosus (SLE) in Taiwan, 109 patients with SLE and 100 healthy controls were enrolled in this study. The PD-1 gene polymorphisms were determined by the method of polymerase chain reaction/restriction fragment length polymorphism. This study showed that the genotype distributions of *PD-1* 7209 C/T polymorphisms were significantly different between the patients with SLE and controls ($P = 0.002$, $Pc = 0.018$). The frequencies of the *PD-1* 7209 C/C genotype and *PD-1* 7209 C allele were significantly higher in the patients with SLE than those of the controls ($P = 0.001$, OR = 2.6, 95% CI = 1.5–4.6, and $P = 0.002$, OR = 2.1, 95% CI = 1.3–3.4, $Pc = 0.018$, respectively). Moreover, the association of *PD-1* 7209 C with susceptibility to SLE was independent of the *PD-1* ligand. This study also showed that the *PD-1*-536 A 7146 G 7209 C 7499 G haplotype was associated with the development of SLE in Taiwan.

KEY WORDS: PD-1; systemic lupus erythematosus; polymorphism; haplotype.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease involving multiple organs and systems. Although the detailed etiology is still obscure, many genes are involved in the pathogenesis of this disease (1–4).

Programmed cell death-1 (PD-1) was isolated from a thymic T-cell line by apoptotic stimuli (5). PD-1 is a 55-kDa transmembrane protein with one extra-cellular IgV-like domain and a 97-amino acid cytoplasmic tail containing one immunotyrosine inhibitory motif (ITIM) and one immunotyrosine switch motif (6). PD-1 can be expressed on activated T-cells, B-cells, and myeloid cells (7). PD-1 conducts negative signals to T-cells during interaction with its ligands, programmed death-1 ligands (PD-L). PD-1–PD-L interactions lead to cell cycle arrest in G0/G1 but do not increase cell death (8). PD-1 is a new member of the CD28 family and shared 23% amino-acid sequence homology with CTLA-4 (7). Mice deficient in PD-1 gene exhibit a breakdown of peripheral tolerance and develop autoimmune diseases. Disruption of the PD-1 gene in C57BL/6 mice resulted in a late onset of SLE (9). NOD mice with deficient PD-1 gene would develop type 1 diabetes (10, 11). BALB/c mice deficient in the PD-1 gene developed an early onset autoimmune dilated cardiomyopathy associated with an auto-antibody against heart tissue (12, 13). In 2C-$PD^{-/-}$ H-$2^{b/d}$ mice, 2 C^{+} T-cells were activated, and the mice died of a graft-versus-host-like disease (9).

The PD-1 gene is located on chromosome 2q37, which is a SLE susceptible locus in whole genome-wide linkage studies (14, 15). Several polymorphisms have been demonstrated in the PD-1 gene. A polymorphism in intron 4 affected the binding of transcription factor RUNX1 and then decreased the expression of PD-1 (16). Many studies showed that the PD-1 gene polymorphisms were associated with the development of SLE (17, 18), rheumatoid arthritis (19–21), type 1 diabetes (22), and disease progression of multiple sclerosis (23). However, these associations have been proved to be controversial. The purpose of the present study is to investigate the associations of PD-1 gene polymorphisms with susceptibility to SLE in Taiwan.

[1]Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.
[2]College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.
[3]Department of Internal Medicine, Kaohsiung Medical University Hospital, Division of Rheumatology, 100 Zihyou 1st Road, Kaohsiung City, 807, Taiwan.
[4]Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.
[5]Both Authors Contributed equally to this work.
[6]To whom correspondence should be addressed at e-mail: jehsye@kmu.edu.tw.

0271-9142/06/1100-0506/0 © 2006 Springer Science+Business Media, LLC

MATERIALS AND METHODS

Study Subjects

One hundred and nine patients with SLE (100 females and 9 males, mean age ± SD = 39.1 ± 10.9 years) and 100 healthy controls (84 females and 16 males, mean age ± SD = 37.2 ± 7.4 years) were enrolled in this study. Healthy controls consisted of the volunteer of the local residents. All of the patients and controls are Taiwanese. The diagnosis of SLE was in accordance with the American College of Rheumatology 1997 revised criteria for the classification of SLE. This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital.

Genotyping

Nine single nucleotide polymorphisms of the PD-1 gene, which were obtained from nucleotide sequence AF 363458 (NCBI web resource), were determined in this study, including *PD-1*-536 G/A (promoter), 5708 C/T (intron 1), 6438 G/A (intron 2), 7146 G/A (intron 4), 7209 C/T (intron 4), 7499 G/A (intron 4), 7625 C/T (exon 5, amino acid 215, Ala → Val), 7785 C/T (exon 5, amino acid 268, Ala → Ala, synonymous), and 8737 A/G (3'-UTR). The methods of polymerase chain reaction (PCR)/restriction fragment length polymorphism were used to determine these polymorphisms. The sequences of primers (Mission Biotech Co. Ltd., Taiwan) and restriction enzymes are shown in (Table I). All of the restriction enzymes used in this study were purchased from New England BioLab Inc., USA. Besides primers and DNA templates, Taq DNA polymerase (Bertec enterprise Co. Ltd., Taiwan) and dNTP (Promega Co., USA) were used during PCR amplification. To determine the polymorphisms of *PD-1*-536 A/G and 5708 T/C, the amplification conditions consisted of initial denaturation at 96°C for 3 min, followed by 5 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, and 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, and then a final extension phase at 72°C for 7 min. The restriction enzymes, Nci I and Hha I, were used to determine *PD-1*-536 A/G and 5708 T/C polymorphisms, respectively. The amplification conditions for determining *PD-1* 6438 G/A polymorphisms were initial denaturation at 96°C for 3 min and 30 cycles of denaturation at 95°C for 1 min, annealing at 63°C for 1 min, and extension at 72°C for 1 min, and then a final extension phase at 72°C for 7 min. The PCR product was digested with MsP I. To determine the polymorphisms of *PD-1* 7146 G/A and 7209 C/T, PCR was carried out under the following conditions: initial denaturation at 96°C for 3 min and 30 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. A final extension phase was also performed at 72°C for 7 min. Then the PCR product was digested with Bsa I and BstU I, respectively. To determine the polymorphisms of *PD-1* 7499 G/A, the amplification conditions consisted of initial denaturation at 96°C for 3 min, followed by 5 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, and 30 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min, and then a final

Table I. Sequences of Primers and Restriction Enzymes in Determining PD-1 Gene Polymorphisms

PD-1 polymorphisms	Primers	Restriction enzymes
−536 G/A	5′-CCGATTAGCCATGGACAG-3′ 5′-TTCCTGGGCTGCAGTGAG-3′	Nci I
5708 C/T	5′-CCAGAGCAGTGCAGACAG-3′ 5′-TCATTGAGAAGTCTCTGCTG-3′	Hha I
6438 G/A	5′-TGCACAGGATCAGGAGCTC-3′ 5′-CCCAGACTAGCAGCACCAG-3′	Msp I
7146 G/A	5′-ACGGCCTGCAGGACTCAC-3′ 5′-AGGCAGGCACATATGTG-3′	Bsa I
7209 C/T	5′-ACGGCCTGCAGGACTCAC-3′ 5′-AGGCAGGCACATATGTG-3′	BstU I
7499 C/T	5′-AGCTCAGGGTAAGCAGCAC-3′ 5′-TAGTCCACAGAGAACACAGG-3′	BsaA I
7625 C/T	C/T 5′-GATCACACAGAGGGCAGTG-3′ 5′-CACAGAGAACACAGGCCCG-3′	Msp I
7785 C/T	5′-CGGAGTATGCCACCATTGTC-3′ 5′-GGTCTGCAGAACACTGGTG-3′	Alu I
8737 A/G	5′-AGGCCCCTCCCAGCTGTG-3′ 5’-CCACTCAGGTGCCTGCTG-3’	Nde I

Table II. Genotype Frequencies of PD-1 Gene Polymorphisms in the Patients with SLE and Controls

PD-1 polymorphisms	SLE ($n = 109$, %)	Controls ($n = 100$, %)	P	Pc
−536 A/A	43 (39.4)	35 (35.0)	NS	
A/G	45 (41.3)	40 (40.0)		
G/G	21 (19.3)	25 (25.0)		
5708 T/T	75 (68.8)	70 (70.0)	NS	
T/C	31 (28.4)	23 (23.0)		
C/C	3 (2.8)	7 (7.0)		
6438 A/A	25 (22.9)	19 (19.0)	NS	
A/G	67 (61.5)	63 (63.0)		
G/G	17 (15.6)	18 (18.0)		
7146 G/G	103 (94.5)	92 (92.0)	NS	
G/A	5 (4.6)	7 (7.0)		
A/A	1 (0.9)	1 (1.0)		
7209 C/C	76 (69.7)[a]	47 (47.0)	0.002	0.018
C/T	32 (29.4)	50 (50.0)		
T/T	1 (0.9)	3 (3.0)		
7499 G/G	62 (56.9)	45 (45.0)	NS	
G/A	45 (41.3)	49 (49.0)		
A/A	2 (1.8)	6 (6.0)		
7625 C/C	33 (30.3)	29 (29.0)	NS	
C/T	53 (48.6)	59 (59.0)		
T/T	23 (21.1)	12 (12.0)		
7785 C/C	62 (56.9)	47 (47.0)	NS	
C/T	42 (38.5)	47 (47.0)		
T/T	5 (4.6)	6 (6.0)		
8737 A/A	42 (38.5)	42 (42.0)	NS	
G/A	60 (55.0)	53 (53.0)		
G/G	7 (6.4)	5 (5.0)		

Note. NS: not sihnificant; Pc: corrected P-value.
[a]SLE vs. controls: $P = 0.001$, OR = 2.6, 95% CI = 1.5–4.6

extension phase at 72°C for 7 min. The PCR product was digested with BsaA I. The PCR conditions for determining *PD-1* 7625 C/T polymorphisms were initial denaturation at 96°C for 3 min and 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, and then a final extension phase at 72°C for 7 min. Then the PCR product was subjected to digestion with Msp I. The PCR conditions for determining *PD-1* 7785 C/T polymorphisms were initial denaturation at 96°C for 3 min and 30 cycles of denaturation at 95°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min, and then a final extension phase at 72°C for 7 min. Then the PCR product was digested with Alu I. To determine the polymorphisms of *PD-1* 8737 A/G, the amplification conditions consisted of initial denaturation at 96°C for 3 min, followed by 5 cycles of denaturation at 95°C for 1 min, annealing at 66°C for 1 min, and extension at 72°C for 1 min, and 30 cycles of denaturation at 95°C for 1 min, annealing at 64°C for 1 min, and extension at 72°C for 1 min, and then a final extension phase at 72°C for 7 min. The PCR product was digested with Nde I.

Statistics

The chi-square test or Fisher's exact test was used for statistical analysis. Odds ratio (OR) and its 95% confidence interval (CI) were calculated by using the SPSS statistic program. The P-value was corrected by multiplying the number of comparisons (Pc). The Mantel–Haenszel test was used in a statistic with stratification. The EH program was used for calculating estimated haplotype frequencies (Web Resources of Genetic Linkage Analysis).

RESULTS

The genotype distributions of *PD-1* 7209 C/T polymorphisms were significantly different between the patients with SLE and controls (Table II, $P = 0.002$, Pc = 0.018). Furthermore, the patients with SLE have a significantly higher genotype frequency of *PD-1* 7209 C/C than the controls (Table II, $P = 0.001$, OR = 2.6, 95% CI = 1.5–4.6).

The allele frequency of *PD-1* 7209 C was also significantly increased in the patients with SLE compared with that of the controls (Table III, $P = 0.002$, OR = 2.1, 95% CI = 1.3–3.4, Pc = 0.018).

In our previous study, *PD-L2* 47103 T was associated with the development of SLE (submitted for publication). To clarify the association between *PD-1* 7209 C/C and SLE, the genotype frequencies of *PD-1* 7209 C/C in the patients with SLE and controls were stratified with *PD-L2* 47103 T (Table IV). The Mantel–Hanszel test was used for statistical analysis. This study revealed that the association of *PD-1* 7209 C/C with SLE was independent of *PD-L2* 47103 T ($P = 0.001$, adjusted OR = 2.57, 95% CI = 1.5–4.6).

This study also demonstrated that the estimated haplotype frequency of *PD-1*-536A 7146G 7209C 7499G was significantly increased in the patients with SLE in comparison with that of the controls (Table V, $P < 0.0001$, OR = 2.06, 95% CI = 1.38–3.08, Pc < 0.0014).

DISCUSSION

Based on the results of this study, it is evident that *PD-1* 7209 C is associated with susceptibility to SLE in Taiwan. The haplotype *PD-1*-536 A, 7146 G, 7209 C, 7499 G is also involved as a susceptible gene for SLE in Taiwan.

PD-1, containing an ITIM, inhibits autoimmune response and preserves self-tolerance. PD-1 defect may lead to the breakdown of peripheral tolerance and the

Table III. Allele Frequencies of PD-1 Gene in the Patients with SLE and Controls

PD-1	SLE (2*n* = 218, %)	Controls (2*n* = 200, %)	*P*	OR (95% CI)	Pc
−536 A	131 (60.1)	110 (55.0)	NS		
G	87 (39.9)	90 (45.0)			
5708 T	181 (83.0)	163 (81.5)	NS		
C	37 (17.0)	37 (18.5)			
6438 A	117 (53.7)	101 (50.5)	NS		
G	101 (46.3)	99 (49.5)			
7146 G	211 (96.8)	191 (95.5)	NS		
A	7 (3.2)	9 (4.5)			
7209 C	184 (84.4)	144 (72.0)	0.002	2.1(1.3–3.4)	0.018
T	34 (15.6)	56 (28.0)			
7499 G	169 (77.5)	139 (69.5)	NS		
A	49 (22.5)	61 (30.5)			
7625 C	119 (54.6)	117 (58.5)	NS		
T	99 (45.4)	83 (41.5)			
7785 C	166 (76.1)	141 (70.5)	NS		
T	52 (23.9)	59 (29.5)			
8737 A	144 (66.1)	137 (68.5)	NS		
G	74 (33.9)	63 (31.5)			

Note. NS: not significant; Pc: corrected *P*-value.

development of autoimmune disorders (7). There are several polymorphisms in the PD-1 gene. These polymorphisms may result in dysfunction of the PD-1 gene. The *PD-1* 7146 G/A polymorphisms are located in an enhancer-like structure of PD-1. The transcription factor RUNX1 binds to the wild type PD-1 gene enhancer (*PD-1* 7146 G) to modulate the PD-1 gene transcription. A G → A nucleotide substitution at *PD-1* 7146 will abolish the binding of transcription factor RUNX1 to this site, leading to decreased expression of PD-1 and the breakdown of self-tolerance. The PD-1 7209 C/T polymorphisms are also in the intronic enhancer, and near the binding sites of transcription factors NFkB and RUNX1 (16). These polymorphisms may also affect the binding affinity and transcription activity of NFkB. Therefore, the associations of PD-1 7209 C with susceptibility to SLE in Taiwan may be due to lower binding affinity of NFkB and RUNX1 and decreased expression of PD-1.

Prokunina *et al*. have showed that *PD-1* 7146 A contributes to the development of SLE in Europeans and Mexicans (16). However, a similar finding could not be found in our study. The associations of *PD-1* 7146 G/A polymorphisms with SLE are still controversial (18). Our study, which is compatible with the study of Johnson *et al*. (18), revealed that there was no significant difference in the genotype distribution of *PD-1* 7146 between SLE patients and controls. Johansson *et al*. demonstrated that *PD-1* 7146 A was not associated with susceptibility to SLE in northern Sweden. On the other hand, Ferreiros-Vidal *et al*. showed that the *PD-1* 7146 A allele was significantly less frequent in Spanish female SLE patients than in female controls (17). The discrepancies may be due to ethnic differences. A previous study conducted by Lin showed there was no association of *PD-1* C + 872T (the same as *PD-1* 7785 C/T in this study) with SLE (21). Only one polymorphic site was studied by Lin *et al*.; however, more polymorphisms were investigated in this study.

An association between *PD-1* 7146 A and lupus nephritis was demonstrated by Prokunina and Johansson (18, 24). Nonetheless, the association of *PD-1* 7146 G/A or 7209 C/T polymorphisms with lupus nephritis could not be found in the present study. There were no associations between PD-1 gene polymorphisms and other clinical manifestations of SLE in Taiwan (data not shown). Our previous study also demonstrated that the *PD-L2* 47103

Table IV. Genotype Frequencies of PD-1 7209 C/C in the Patients with SLE and Controls Stratified with PD-L2 47103 T

	PD-L2 47103 T(+)		*PD-L2* 47103 T(-)			
	SLE (*n* = 52, %)	Controls (*n* = 30, %)	SLE (*n* = 57, %)	Controls (*n* = 70, %)	*P*	Adjusted OR (95% CI)
PD-1 7209 C/C(+)	36 (69.2)	15 (50.0)	40 (70.2)	32 (45.7)	0.001	2.57 (1.5–4.6)
(−)	16 (30.8)	15 (50.0)	17 (29.8)	38 (54.3)		

Note. The Mantel–Hanszel test was used for statistic analysis.

Table V. Estimated Haplotype Frequencies of PD-1 Gene in the Patients with SLE and Controls

Haplotype of *PD-1*								
−536	7146	7209	7499	SLE	Controls	*P*	OR (95% CI)	Pc
A	G	C	G	0.519658	0.341496	< 0.0001	2.06 (1.38–3.08)	< 0.0014
A	G	C	A	0.028559	0.025988	NS		
A	G	T	G	0.000000	0.023100	0.036	2.12 (1.91–2.35)	NS
A	G	T	A	0.042252	0.085607	NS		
A	A	C	G	0.000007	0.011595	NS		
A	A	C	A	0.009523	0.001874	NS		
A	A	T	A	0.000000	0.010340	NS		
G	G	C	G	0.215622	0.341496	0.004	0.52 (0.33–0.81)	NS
G	G	C	A	0.064732	0.025988	NS		
G	G	T	G	0.040910	0.023100	NS		
G	G	T	A	0.073981	0.085607	NS		
G	A	C	G	0.004755	0.011595	NS		
G	A	C	A	0.000000	0.001874	NS		
G	A	T	A	0.000000	0.010340	NS		

Note. NS: not significant; Pc: corrected *P*-value.

T was associated with the development of SLE in Taiwan (submitted for publication). *PD-L2* 47103 C/T are non-synonymous polymorphisms. *PD-L2* 47103 C encodes an amino acid serine in the transmembrane region. However, *PD-L2* 47103 T encodes amino acid phenylalanine, which contains an aromatic ring. The expression of protein molecule on the cell surface may be regulated by its trans-membrane domain (25). Trans-membrane domain also plays a role in dimerization or oligomerization for surface molecules (26, 27). The amino acid substitution of PD-L2 in the transmembrane domain may affect the expression and oligomerization of PD-L2 molecules, and then influences the inhibiting effect of PD-1-PD-L pathway on autoimmunity. In this study, we found that the association of *PD-1* 7209 C/C with SLE was independent of *PD-L2* 47103 T.

In summary, the *PD-1* 7209 C and estimated haplotype *PD-1*-536 A, 7146 G, 7209 C, 7499 G are associated with susceptibility to SLE in Taiwan.

REFERENCES

1. Tsao BP: Update on human systemic lupus erythematosus genetics. Curr Opin Rheumatol 16(5):513–521, 2004
2. Prokunina L, Alarcon-Riquelme M: The genetic basis of systemic lupus erythematosus—Knowledge of today and thoughts for tomorrow. Hum Mol Genet 13 (Special Issue 1):R143–148, 2004
3. Moser KL, Neas BR, Salmon JE *et al.*: Genome scan of human systemic lupus erythematosus: Evidence for linkage on chromosome 1q in African-American pedigrees. Proc Natl Acad Sci USA 95(25):14869–14874, 1998
4. Croker JA, Kimberly RP: Genetics of susceptibility and severity in systemic lupus erythematosus. Curr Opin Rheumatol 17(5):529–537, 2005
5. Ishida Y, Agata Y, Shibahara K, Honjo T: Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 11(11):3887–3895, 1992
6. Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T: PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci USA 98(24):13866–13871, 2001
7. Freeman GJ, Long AJ, Iwai Y *et al.*: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 192(7):1027–1034, 2000
8. Latchman Y, Wood CR, Chernova T *et al.*: PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2(3):261–268, 2001
9. Nishimura H, Nose M, Hiai H, Minato N, Honjo T: Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 11(2):141–151, 1999
10. Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T: Establishment of NOD-Pdcd1 −/− mice as an efficient animal model of type I diabetes. Proc Natl Acad Sci USA 102(33):11823–11828, 2005
11. Ansari MJ, Salama AD, Chitnis T *et al.*: The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. J Exp Med 198(1):63–69, 2003
12. Nishimura H, Okazaki T, Tanaka Y *et al.*: Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science 291(5502):319–322, 2001
13. Okazaki T, Tanaka Y, Nishio R *et al.*: Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. Nat Med 9(12):1477–1483, 2003
14. Lindqvist AK, Steinsson K, Johanneson B *et al.*: A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. J Autoimmun 14(2):169–178, 2000
15. Magnusson V, Lindqvist AK, Castillejo-Lopez C *et al.*: Fine mapping of the SLEB2 locus involved in susceptibility to systemic lupus erythematosus. Genomics 70(3):307–314, 2000
16. Prokunina L, Castillejo-Lopez C, Oberg F *et al.*: A regulatory polymorphism in PDCD1 is associated with susceptibility to

systemic lupus erythematosus in humans. Nat Genet 32(4):666–669, 2002

17. Ferreiros-Vidal I, Gomez-Reino JJ, Barros F *et al.*: Association of PDCD1 with susceptibility to systemic lupus erythematosus: Evidence of population-specific effects. Arthritis Rheum 50(8):2590–2597, 2004
18. Johansson M, Arlestig L, Moller B, Rantapaa-Dahlqvist S: Association of a PDCD1 polymorphism with renal manifestations in systemic lupus erythematosus. Arthritis Rheum 52(6):1665–1669, 2005
19. Prokunina L, Padyukov L, Bennet A *et al.*: Association of the PD-1.3A allele of the PDCD1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope. Arthritis Rheum 50(6):1770–1773, 2004
20. Kong EK, Prokunina-Olsson L, Wong WH *et al.*: A new haplotype of PDCD1 is associated with rheumatoid arthritis in Hong Kong Chinese. Arthritis Rheum 52(4):1058–1062, 2005
21. Lin SC, Yen JH, Tsai JJ *et al.*: Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. Arthritis Rheum 50(3):770–775, 2004
22. Nielsen C, Hansen D, Husby S, Jacobsen BB, Lillevang ST: Association of a putative regulatory polymorphism in the PD-1 gene with susceptibility to type 1 diabetes. Tissue Antigens 62(6):492–497, 2003
23. Kroner A, Mehling M, Hemmer B *et al.*: A PD-1 polymorphism is associated with disease progression in multiple sclerosis. Ann Neurol 58(1):50–57, 2005
24. Prokunina L, Gunnarsson I, Sturfelt G *et al.*: The systemic lupus erythematosus-associated PDCD1 polymorphism PD1.3A in lupus nephritis. Arthritis Rheum 50(1):327–328, 2004
25. Chou WC, Liao KW, Lo YC, Jiang SY, Yeh MY, Roffler SR: Expression of chimeric monomer and dimer proteins on the plasma membrane of mammalian cells. Biotechnol Bioeng 65(2):160–169, 1999
26. Choi S, Lee E, Kwon S *et al.*: Transmembrane domain-induced oligomerization is crucial for the functions of syndecan-2 and syndecan-4. J Biol Chem 280(52):42573–42579, 2005
27. Bourguignon LY, Singer SJ: Transmembrane interactions and the mechanism of capping of surface receptors by their specific ligands. Proc Natl Acad Sci USA 74(11):5031–5035, 1977