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ORIGINAL ARTICLE

Genetic influence of DNAM-1 (DNAX accessory molecule-1) gene polymorphism (rs 763361) on susceptibility and disease activity of systemic lupus erythematosus and rheumatoid arthritis in Egyptian patients

Rabab A. Mohamed[1] · Mervat I. Abd Elazeem[2]

Abstract Genome-wide association studies have led to the involvement of multiple susceptibility genes in the development of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The present case control study was conducted to determine the possible association of DNAM-1(C/T, rs 763361) single nucleotide polymorphism (SNP) with RA and SLE in the Egyptian population. This study aimed to determine the possible genetic influence of SNP on disease activity in SLE (represented by SLEDAI score) and in RA patients (represented by DAS28). Fifty SLE patients compared to 43 healthy controls and 35 RA patients compared to 30 healthy controls were included in the study. The PCR-RFLP technique was used to detect DNAM-1(C/T rs 763361) gene polymorphism. Analysis of the frequency distribution of both allele and genotype frequency distribution of SLE and RA patients compared to healthy control subjects did not reveal a significant association between SLE and RA patients and the rs763361. Analysis of the disease activity status in lupus nephritis (LN) and non-lupus nephritis patients revealed a significant association between DNAM-1(C/T) SNP and LN patients carrying the TT and CT genotypes (P = 0.009 and 0.004; respectively). DNAM-1(C/T rs763361) SNP is not associated with the development of SLE and RA in Egyptian population. The SNP might be associated with disease activity status represented by SLEDAI score in LN patients carrying CC and CT genotypes, respectively.

Keywords Rheumatoid arthritis (RA) · Systemic lupus erythematosus (SLE) · PCR-RFLP · DNAM-1

Introduction

Autoimmune diseases have widely confirmed complex pathogenesis. The association between genetic and environmental factors is associated with the development of autoimmunity (Ceccarelli et al. 2016). Systemic lupus erythematosus (SLE) and rheumatoid arthritis are systemic multifactorial heterogeneous autoimmune diseases. Genome-wide association studies have led to the involvement of multiple susceptibility genes in the development of SLE and RA (Suzuki et al. 2011).

SLE is a multisystem autoimmune disease that has a broad range of clinical manifestations that is characterized by remissions and exacerbations. Tissue damage in SLE is due to the production of autoantibodies and complement fixing immune complex deposition, which can affect multiple organ systems, resulting in a broad range of immunologic and clinical manifestations (Keishan et al. 2007; Narayanan et al. 2010). The major histocompatibility gene complex (MHC) represents an important genetic risk factor associated with SLE (Sanchez et al. 2011). However, the HLA system constitutes 40% of the overall estimated genetic risk for SLE (Wandstrat and Wakeland 2001).

Rheumatoid arthritis (RA) is a systemic, chronic autoimmune inflammatory disorder that primarily attacks the synovial joints resulting in an inflammatory response in the synovium with hyperplasia of the synovial cells and excess

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synovial fluid (Majithia and Geraci 2007). The disease manifests clinically by joint pain, stiffness, and swelling. The HLA region contributes to about 60% of the genetic pathogenesis of the disease; similarly, the non-HLA genes contribute to disease manifestations (Kiani et al. 2015). Recently, much progress has been made in defining non-HLA-related susceptibility loci for RA (Du et al. 2012).

DNAX accessory molecule 1(DNAM-1), also known as CD226, is a type-I membrane protein that is involved in the process of adhesion and costimulation of T cells. DNAM-1 is constitutively expressed on natural killer (NK) cells, CD4$^+$ and CD8$^+$ T cells, a subset of B cells, monocytes, and platelets (Du et al. 2010).

The CD226 gene is detected on chromosome 18q22-23, and it is a 65-KDa transmembrane glycoprotein that consists of an extracellular region that has two Ig-V-like domains, a transmembrane region, and a cytoplasmic region that has tyrosine and serine-phosphorylated sites (Sherrington et al. 1979).

DNAM-1 expression deficiency was reported to cause high sensitivity to apoptosis in NK T cells which were obtained from SLE patients, thus providing supporting evidence for DNAM-1 role in autoimmune diseases (Tao et al. 2005). Furthermore, the GLy307Ser variant could change the expression or signaling of CD226 due to the fact that it is present in the molecule's cytoplasmic tail (Todd et al. 2007).

The rs 763361 single nucleotide polymorphism in the CD226 gene has been described as a novel susceptibility locus for several autoimmune diseases such as type 1 diabetes (TID) (Douroudis et al. 2009). In addition to TID, the non-synonymous rs 763361 variant was found to be related to several other autoimmune diseases as rheumatoid arthritis, celiac disease, Graves' disease, systemic lupus erythematosus, multiple sclerosis, and psoriasis mainly in Caucasian populations (Wieczorek et al. 2009).

Song et al. reported an association between DNAM-1 (CD226) gene SNP and a subgroup of autoimmune diseases in Europeans, South Americans, and Asians (Song et al. 2012). The variation in the genetic susceptibility in different ethnic groups has led us to attempt to study the genetic influence of DNAM-1(C/T, rs763361) SNP on the development of SLE and RA in Beni-Suef Governorate in Upper Egypt. In addition, attempts were made to detect the possible genetic association between the polymorphism and the disease activity in both SLE and RA patients.

Patients and methods

Patients

The present case control study was conducted in BENI-SUEF UNIVERSITY HOSPITAL. All SLE and RA patients and control subjects were informed of the purpose of the study, and their consent was obtained, and the study was approved by the local ethics committee. The SLE and RA patients were recruited from the outpatients' clinic of Rheumatology and Rehabilitation department, between September 2014 and December 2015. SLE group consisted of 50 SLE patients and 43 unrelated, age- and sex-matched, healthy control subjects with no family history of autoimmune diseases. The patients were diagnosed by a rheumatologist according to American College of Rheumatology (ACR) revised criteria (Hochberg 1997). The rheumatoid arthritis group consisted of 35 RA patients and 30 unrelated, age- and sex-matched, healthy control subjects with no family history of autoimmune diseases. The diagnosis of the patients was done according to 2010 ACR/EULAR (formerly, the American Rheumatism Association) classification criteria (Aletaha et al. 2010). All patients were subjects to standard hospital questionnaire and thorough clinical examination. Relevant data on history and pathological findings of patients were obtained from hospital medical records.

Clinical assessment of systemic lupus erythematosus patients

The SLE patients' disease activity was assessed using SLE Diseases Activity Index (SLEDAI) score (Bomardier et al. 1995). The SLE patients were arbitrarily classified according to disease activity status into three groups based on the rheumatologist clinical judgment. The first group represented patients with mild disease activity (scores ≤8), the second group represented patients with moderate disease activity (scores 9–18), and the third group represented the patients with severe disease activity (scores >18). The SLE patients were further stratified into two groups. The first group, non-lupus nephritis patients (non-LN), lacked evidence of renal involvement, tested positive for antinuclear antibodies (ANAs) or anti-double-stranded deoxyribonucleic acid antibodies (anti-ds-DNA antibodies). The second group had LN and was defined according to clinical and laboratory manifestations that met the ACR criteria (proteinuria levels higher than 0.5 g/day or greater than +++ by dip stick and/or cellular casts including red blood cells, hemoglobin, granular, tubular, or mixed) and/or renal biopsy samples demonstrating immune complex-mediated glomerulonephritis compatible with LN (Petri et al. 2012).

Clinical assessment of rheumatoid arthritis patients

The RA patients were divided into groups according to disease activity as indicated by DAS 28 scoring system. The modified disease activity score (DAS 28) was

calculated from hospital medical records for all patients. DAS 28 score of cutoff higher than 5.1 is indicative of high disease activity, whereas a cutoff >3.2–<5.1 indicates moderate disease activity, cutoff ≥2.6–<3.2 is indicative of mild disease activity, and DAS 28 cutoff <2.6 indicated remission state according to EULAR criteria (Van der Heijde et al. 1990).

Laboratory assessment of SLE and RA patients

Two milliliters of EDTA samples was collected from each patient for determination of erythrocyte sedimentation rate (ESR; Western green). At least 3 ml of venous blood was collected from each patient and placed in tubes without anticoagulant. The serum samples were used for determination of C-reactive protein (CRP) by semiquantitative latex (AVITEX® CRP, Omega Diagnostics). CRP was considered positive >6 mg/L. C3 and C4 were measured by radial immune diffusion (Biocientifica SA). For SLE patients, ANA was detected by indirect immunofluorescence (IF) using HEp-2 cells (Kallestad™, BIO RAD) and tests were considered positive for titers above 1/40. Anti ds DNA was determined by IF, with Crithidia lucilia (Kallestad™, BIO RAD), and tests were considered positive for titers above 1/10. For RA patients, rheumatoid factor (RF) was performed by semiquantitative latex (AVITEX® RF, Omega Diagnostics), RF is considered positive ≥8 IU/ml. Anticyclic citrullinated peptide (Anti-CCP) was determined using enzyme-linked immunosorbent technique (ELISA) using (QUANTA Lite® CCP3IgG ELISA, INOVA Diagnostics). According to the manufacturer's protocol, serum was considered positive when the reading was ≥20 U.

Genotyping

Genomic DNA was extracted from EDTA anticoagulated whole blood using QIAamp DNA Mini Kit (cat. no. 51104, QIAGEN) according to the manufacturer's protocol. The sequences flanking the DNAM1 (C/T, rs 763361) SNP were amplified by PCR. Genotyping of the polymorphism was determined by restriction fragment length polymorphism (RFLP) (Avis 1994).

The 213-bp fragment of the CD226 gene, encompassing the CD226 C/T (rs 763361) polymorphic site, was amplified via PCR using the sense (5′-CTGCGAGAGAAGGT TGGATAGTTGAC-3′) and the anti-sense primer (5′-CTTG TCCCATATCATGCCTGCATT-3′) primer pair. The total reaction volume was 25 µl: first, 2.5 µl of DNA was placed in an Eppendorf tube with a reaction mixture containing 0.5 µl of each primer; 2.5 µl of 10× Pfu PCR Mgso$_4$ buffer, 0.5 µl of dNTP Mix, 10 mM each (no. R0191), and 0.7 µl PFU DNA polymerase (no. EP0571), and adjusted to a final volume of 25 µl. The PCR reaction was performed in T-personal thermal

cycler (Biometra) under the following conditions: initial denaturation at 95 °C for 1–3 min, 35 cycles of amplification (30 s at 95 °C, 30 s at 60 °C, 2 min at 72 °C), and a final extension phase for 5 min. The PCR products were separated on 2% agarose gel using 1× TAE buffer for 40 min at 100 V, followed by ultraviolet visualization. The PCR generated a 213-bp fragment carrying the polymorphic site.

The PCR products were digested using MspI fast digest restriction enzyme (no. FD0554 Thermo Scientific) according to the manufacturer's protocol. The digested products were separated on 4% agarose gel using 1× TAE buffer for 40 min at 100 V followed by ultraviolet visualization. After digestion, CD 226 T allele gave rise to 140 and 73 bp fragments, while the C allele gave rise to 213 bp fragment (Figs. 1 and 2).

Statistical methods

The collected data review, coding, and statistical analysis was done by using SPSS program (statistical package of social science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows. Mean, median, range, and standard deviation were calculated to measure central tendency and dispersion of quantitative data while frequency of occurrence was calculated to measure qualitative data. Student t test was used to determine the significance in difference between two means. Chi-square test (χ^2) was done for comparison of qualitative data, and fisher's exact test was used instead when the count of cell was less than 5. Odds ratios (ORs) with 95% confidence intervals (CI) were calculated whenever applicable, to test association between genotype and SLE and RA. Mann–Whitney U test was used to determine the significant difference between two non-parametric variables. Genotype distributions were compared with those expected for samples from populations in Hardy-Weinberg equilibrium using a χ^2 test (1 df). The level of significance was taken at P value of <0.05.

Fig. 1 PCR-RFLP analysis of DNAM1 gene polymorphism using MspI restriction enzyme in SLE patients. *M* DNA molecular weight marker; *Lanes 1*, *2*, *3*, *4*, *6*, *7*, and *11* represent homozygous wild-type (CC) genotype. *Lanes 8*, *9*, and *10* represents heterozygous (CT) genotype. *Lane 5* represents homozygous mutant (TT) genotype

Fig. 2 PCR- RFLP analysis of DNAM1 gene polymorphism using MspI restriction enzyme in RA patients. *M* DNA molecular weight marker; *Lanes 1*, *3*, *4*, *5*, *7*, *8*, and *10* represent homozygous wild-type (CC) genotype. *Lanes 2*, *6*, and *9* represents heterozygous (CT) genotype. *Lane 11* represents homozygous mutant (TT) genotype

Results

Genetic influence of DNAM-1 (C/T, rs 763361) SNP on SLE patients

In the current case–control study, the percentage of SLE female patients was 88% and the percentage of male patients was 12%. As for the control group, the percentage of females was 76.7% and the percentage of males was 23.3%. As regards age, SLE patients' ages ranged between 12 and 52 years with a mean value of 27.48 ± 9.58, while the healthy control subjects' ages ranged between 17 and 53 years with a mean value of 29.23 ± 8.47. The demographic, clinical, and laboratory data of the SLE patients and the control group are summarized in Table 1.

The frequency distribution of DNAM-1 genotypes in SLE patients and healthy control subjects conformed to Hardy-Weinberg equilibrium (HWE) ($P = 0.117$ and $P = 0.641$; respectively). Similarly, the frequency distribution of DNAM-1 genotypes in both LN and non-LN patients conformed to HWE ($P = 0.141$ and $P = 0.377$; respectively).

We first attempted to analyze the association of the SLE with the SNP and its association with LN. Analysis of the frequency distribution of DNAM-1 (rs763361) genotypes revealed a non-significant differences in both allele ($X^2 = 1.65$, $P = 0.20$) and genotype distribution among the SLE patients and control group ($X^2 = 2.32$, $P = 0.313$). Further on, analysis of the frequency distribution of allele and genotype frequencies in both LN and non-LN patients revealed a non-significant difference in both allele ($X^2 = 1.56$, $P = 0.212$) and genotype distribution ($X^2 = 2.30$, $P = 0.323$) in both patients' groups (Table 2).

Next, the patients were grouped arbitrarily into three groups according to the SLEDAI scoring system to investigate the association between the degree of disease activity and

DNAM-1 (C/T rs 763361) gene polymorphism. The grouping was done according to the rheumatologist clinical opinion. The first group represented the patients with mild disease activity (score ≤ 8), the second group represented patients with moderate disease activity (score 9–18), and the third group represented the patients with severe disease activity (score > 18). However, we did not detect an association between the degree of disease activity and the frequency distribution of DNAM-1 (C/T rs 763361) genotypes in SLE patients ($X^2 = 1.49$ and $P = 0.920$) (Table 3 and Fig. 3).

On the other hand, on analysis of the disease activity status in LN and non-LN patients and its association with DNAM-1 (C/T rs 763361) gene polymorphism, we used the median to compare the difference in disease activity in patients carrying different genotypes, due to the presence of extreme values in SLEDAI score, in our data. Our results indicated that LN patients carrying the wild-type (CC) genotype had a higher median compared to non-LNs carrying the same genotype, and the difference between the two groups was statistically significant ($P = 0.009$). Similarly, LN patients carrying the heterozygous (CT) genotype had a higher median compared to non-LN patients carrying the CT genotype and the difference between the two groups was statistically significant ($P = 0.009$) (Table 4 and Fig. 4).

Genetic influence of DNAM-1 (C/T, rs 763361) on RA patients

In the present case–control study, the percentage of RA female patients was 97.15% and the percentage of male patients was 2.85%. As regards the control group, the percentage of females was 86.67% and the percentage of males was 13.33%. RA patients' ages ranged between 20 and 70 years with a mean value of 45.62 ± 13.93, while on the other hand, the healthy control subjects' ages ranged between 22 and 65 years with a mean value of 40.80 ± 6.41. The demographic, clinical, and laboratory data of SLE patients and control group are represented in (Table 5).

Regarding consistency with HWE, the frequency distribution of DNAM-1 genotypes in RA patients and healthy control subjects were consistent to HWE ($P = 0.869$ and $P = 0.900$; respectively). We first attempted to detect an association between the SNP and RA by comparing the frequency distribution of the SNP in both RA patients and the healthy control group. Analysis of the frequency distribution of DNAM-1 (rs763361) genotypes in the RA patients' group revealed a non-significant difference in both allele ($X^2 = 0.253$, $P = 0.615$) and genotype distribution among RA patients and the healthy control group ($X^2 = 0.30$, $P = 0.865$) (Table 6).

For our final analysis, we analyzed the frequency distribution of DNAM-1 (C/T rs 763361) genotypes and its

Table 1 Demographic, clinical, and laboratory data of patients and control group in SLE patients, and LN and non-LN patients

Clinical and lab. data	SLE cases ($N = 50$)	Controls ($N = 44$)	LN ($N = 33$)	Non-LN ($N = 17$)	Significance test and P value
Age (years)					
Mean ± SD	27.48 ± 9.58	25.86 ± 4.47	24.29 ± 9.20	27.34 ± 8.88	$P = 0.308$[a]
					$P = 0.267$[b]
Sex					
Female	44 (88%)	34 (76.7)			$P = 0.167$[a]
Male	6 (12%)	10 (23.3)			
Disease duration (months)					
Mean ± SD	25.76 ± 17.98		27.52 ± 19.2	22.35 ± 15.27	$P = 0.342$[b]
SLEDAI					
Score (mean ± SD)	12.18 ± 8.03		15.48 ± 7.84	5.76 ± 4.42	$P < 0.001$[b]*
SLICC					
Score (mean ± SD)	1.68 ± 1.73				
ESR (mm/h)					
Mean ± SD	67.98 ± 41.66				
CRP (mg/L)					
Positive	15(30.0%)				
Negative	35(70.0%)				
HB (gm/dl)					
Mean ± SD	10.30 ± 2.06				
ANA (IF)					
Positive	50 (100%)				
Negative	0 (0%)				
Anti-DNA					
Positive	31(62%)				
Negative	19 (38%)				
C3 and C4					
Consumed	23 (46%)				
Normal	27 (54%)				
24 h urinary proteins(mg/dl)					
Mean ± SD	1097.40 ± 10-38.				

N number, *SLICC* Systemic Lupus International Collaborating Clinics (damage index), *SLEDAI* Systemic Lupus Disease activity Index, *IF* indirect immunofluorescence, *LN* lupus nephritis, *Non-LN* non-lupus nephritis

*Significance level ($P < 0.05$)

[a] SLE versus controls

[b] LN versus non-LN

association with disease activity in RA patients as represented by DAS 28 score. The rheumatoid arthritis patients were divided into two groups according to the status of disease activity: the first group consisted of the non-remission patients, which according to the EULAR criteria included patients with low disease activity (DAS $28 \geq 2.6- < 3.2$), moderate-activity patients (DAS $28 \geq 3.2– \leq 5.2$), and high-disease-activity patients (DAS $28 > 5.1$). The second group included the patients that are in remission state (DAS $28 < 2.6$) (van der Heijde et al. 1990). However, our results did not reveal a significant association between the degree of disease activity and the frequency distribution of DNAM-1 (C/T rs 763361) genotypes ($X^2 = 4.949$, $P = 0.0841$) (Table 7).

Discussion

Genome-wide association scans in common human autoimmune diseases have lately detected multiple loci associated with disease susceptibility. Understanding allele heterogeneity and homogeneity among diseases gives insight into common gene function and pathways (Hafler et al. 2009).

Table 2 Genotype and allele frequency of DNAM 1(C/T, rs763361) SNP in All SLE patients, control group, and LN and non-LN patients

	SLE patients (N = 50%)	Controls (N = 43%)	X^2 and P value	Lupus nephritis (N = 33 (%))	Non-lupus nephritis (N = 17 (%))	X^2 and P value
Genotype						
CC	20 (40.0%)	24 (55.8%)		15 (45.5%)	5 (29.4%)	
CT	27 (54.0%)	17 (39.5%)	$X^2 = 2.32$	17 (51.5%)	10 (58.8%)	$X^2 = 2.30$
TT	3 (6.0%)	2 (4.7%)	$P = 0.313$	1 (3.0%)	2 (11.8%)	$P = 0.323.$
Allele						
C	67 (67.0%)	65 (75.6%)	$X^2 = 1.65$	19 (28.8%)	14 (41.18%)	$X^2 = 1.56$
T	33 (33.0%)	21 (24.4%)	$P = 0.20$	47 (71.2%)	20 (58.82%)	0.212

Significance level ($P < 0.05$)

N number

The studies on the association between CD226 C/T polymorphism and autoimmune diseases yielded conflicting results. To the best of our knowledge, this is the first case–control study conducted in Egypt to detect a possible genetic association between SLE and RA and DNAM-1 (CD226) C/T gene polymorphism.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that has been associated with the involvement of multiple tissues and organs. Epidemiological evidence with studies concerning linkage association studies has suggested that susceptibility of SLE is associated with genetic factors (Du et al. 2010).

In the current case–control study, analysis of the association CD226 (C/T) polymorphism revealed that the frequency distribution of the C and the T alleles were not significantly different when compared in SLE patients and the healthy control group ($P = 0.20$). Moreover, the genotype frequency distribution of rs 763361 SNP SLE patients was not significantly different from the healthy control group ($P = 0.313$).

However, in contrast to our results, a case–control study conducted by Du et al. (2010) reported that the CD226 polymorphism showed an association of the T allele with increased SLE susceptibility on comparing cases and healthy control subjects ($P = 0.018$) and the mutant (TT) genotype was more frequently represented among SLE patients as compared to the normal population ($P < 0.025$). Similarly, Nie et al. reported that SLE risk was significantly higher in carrier T allele of rs 76331 within CD 226 gene compared to carriers of the CC genotype (CT + TT vs CC) (Nie et al. 2016).

The discrepancy between the results of the current study and similar studies regarding the association between CD226 C/T polymorphism and SLE might be due to the clinical heterogeneity of SLE, different ethnicities, and real genetic heterogeneity (Bollain-y-Goytia et al. 2014). In addition, SLE is a complex disease; individual exposure to different environmental factors in combination with genetic susceptibility may have resulted in such conflicting results (Lu et al. 2012). Another possible explanation is the small sample size in the current study and different genotyping techniques used for detection of the polymorphism in different studies.

Renal involvement is one of the serious complications of SLE, because it can result in high rates of morbidity and mortality (Iwata et al. 2011). The diagnosis of glomerulonephritis

Table 3 Association of disease activity (represented by SLEDAI Score) in SLE patients and DNAM-1 (C/T, rs 763361) polymorphism genotype frequency distribution

Variable	Mild[a] (≤8) N = 20 (%)	Moderate[a] (9–18) N = 15 (%)	Severe[a] (>18) N = 14 (%)	P value
Genotype				
CC	7 (35.0%)	6 (40.0%)	7 (50.0%)	$X^2 = 1.49$
CT	12 (60.0%)	8 (53.3%)	6 (42.9%)	$P = 0.920$
TT	1 (5.0%)	1 (6.7%)	1 (7.1%)	

Significance level ($P < 0.05$)

N number

[a] Patients were grouped arbitrarily according to disease activity status (represented by SLEDAI score) according to the rheumatologist clinical opinion, N number
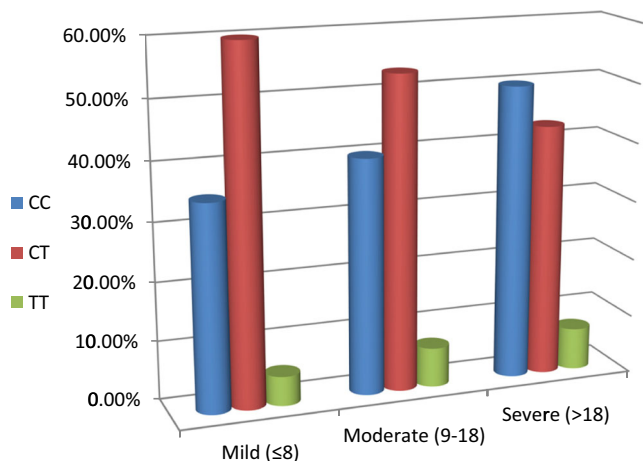

Fig. 3 Disease activity (represented by SLEDAI Score) in SLE patients and DNAM 1 (C/T, rs 763361) SNP genotype frequency distribution

Table 4 Comparison of SLEDAI score in LN patients and non-LN patients in DNAM 1 (C/T, rs763361) SNP genotypes

Variable	SLEDAI in LN Patients (median)	SLEDAI in non-LN patients (median)	Mann–Whitney U test	P value
Genotype				
Wild type				
CC	18.00	5.00	Z = 02.62	0.009*
Mutant type				
TT	16.00	23.50	Z = 0.00	1.00
Heterozygous				
Mutant type				
CT	16.00	4.00	Z = 2.87	0.004*

*Significant difference ($P < 0.05$)

is suspected when proteinuria and urinary sediments are associated with arterial hypertension. These data may predict kidney involvement, although renal biopsy continues to be the gold standard for diagnosis and classification of LN (Bollain-y-Goytia et al. 2014).

To investigate the possible role of CD226 (C/T) gene polymorphism in the development of lupus nephritis, SLE patients were stratified into two groups; the first group represented the LN patients (33 patients), while the second group represented the non-lupus nephritis patients (17 patients). The results of the current study indicated that there was no statistically significant association between the developments of lupus nephritis and CD226 (C/T) gene polymorphism.

Global scores like SLEDAI can be problematic at times in that the score may be the same whether the patients are improving, stable, or worsening. For instance, a rash can improve and still be present or deteriorate and yet the score may be same (Narayanan et al. 2010).

In the current study, we investigated the influence of CD226 (C/T) gene polymorphism on disease activity in LN and non-LN patients, we detected that LN patients carrying the wild-type (CC) genotype and heterozygous (CT) genotype

had higher median of disease activity compared to non-LN patients ($P = 0.009$ and $P = 0.004$, respectively).

The results of the current study regarding the genetic association of CD226 (C/T) gene polymorphism with the degree of disease activity represented by SLEDAI score with LN and

Table 5 Demographic, clinical, and laboratory data of RA patients and control group

Variable	Patients (N = 35)	Healthy controls (N = 30)	P value
Age (years)			
Mean ± SD	45.62 ± 13.93	40.80 ± 6.41	0.081
Range	20–70	22–65	
Sex			
Male (N/%)	1 (2.85%)	4 (13.33%)	
Female (N/%)	34 (97.15%)	26 (86.67%)	0.114
Disease			
Duration (years)			
Mean ± SD	8.48 ± 6.95		
Range	10–30		
CRP (mg/l)			
Positive	17 (48.6%)		
Negative	18 (51.4%)		
Anti-CCP (U/ml)			
Positive	29 (82.9%)		
Negative	6 (17.1%)		
RF (IU/ml)			
Positive	25 (71.4%)		
Negative	10 (28.6%)		
ESR (mm/h)			
Mean ± SD	56.40 ± 22.79		
Range	10.00–100-00		

Significant difference ($P < 0.05$)

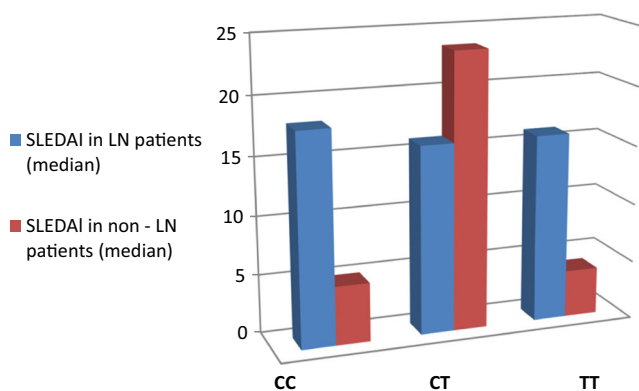N number, *Anti-CCP* anticyclic citrullinated peptide, *RF* rheumatoid factor

Fig. 4 SLEDAI score in LN patients and non-LN patients in DNAM 1 (C/T, rs 763361) SNP genotypes

Table 6 Genotype and allele frequency distribution of DNAM 1 (C/T, rs 763361) gene polymorphism in rheumatoid arthritis patients and healthy control subjects

Variable	Patients N = 35 (%)	Controls N = 30 (%)	X^2 and P value
Genotype			
CC	21(60.6%)	16(53.3%)	
TT	2(5.7%)	2(6.7%)	$X^2 = 0.30$
CT	12(34.3%)	12(40.0%)	$P = 0.863$
Allele			
C	54(77.1%)	44(73.3%)	$X^2 = 0.253$
T	16(22.9%)	16(26.7%)	$P = 0.615$

Significant value ($p < 0.05$)

N number

non-LN might be caused by the heterogenic nature of SLE pathogenesis. In addition, Lozano et al. demonstrated that CD226 provides a positive costimulatory signal for the proliferation of T cells and augments proinflammatory cytokine production, leading to shifting the balance between Th1/Th17 and Th2 cells toward a proinflammatory response (Lozano et al. 2013).

Hence, we postulate that the C allele of CD226 might be involved in the augmentation of the inflammatory response which might be responsible for an increased disease activity in LN patients and might also be responsible for the association between disease activity with SLE patients in TT and CT genotype subgroups.

Despite the fact that the full etiology of RA is unclear, evidence suggests that multiple genetic and environmental risk factors contribute to breaking of immune tolerance, resulting in the autoimmune manifestations of RA (Liu et al. 2012).

Table 7 Comparing genotype frequency distribution of DNAM 1 (C/T) gene polymorphism and disease activity in RA patients (represented by DAS 28 score)

Variable	DAS 28[a] Non-remission group N = 33 (%)	DAS 28[b] Remission group (N = 2) (%)	P value
Genotype			
CC	11(33.33%)	0.0 (0.0%)	
TT	20(60.60%)	1.0 (50.0%)	$P = 0.0841$
CT	2(6.06%)	1.0(50.0%)	

Significance level ($P < 0.05$)

N number, *DAS28* disease activity score 28

[a] Non-remission group includes low-activity patients (DAS 28 ≥ 2.6-< 3.2), moderate-activity patients (DAS 28 ≥ 3.2-≤ 5.2), and high-activity group (DAS 28 > 5.1)

[b] Remission group (DAS 28 < 2.6)

In the present case–control study, there was no statistically significant difference detected on investigating the allele frequency distribution of DNAM-1 (C/T rs 763361) in RA patients and the control group ($P = 0.615$). Similarly, no statically significant difference was detected on analysis of genotype frequency distribution in both patients and healthy controls ($P = 0.863$).

In accordance with our results, Liu et al. (2012) could not detect a genetic association between DNAM-1 (rs 763361) and rheumatoid arthritis in a sample of the Chinese population. Similarly, these results were in agreement with the results of several studies performed in other countries (Kiani et al. 2015 and Myethianou et al. 2016).

However, in contrast to the results of the current study, Du et al. (2012) demonstrated that the rs 763361 variant in DNAM-1 is associated with RA in the Chinese Han population. This was in agreement with the results of Maiti et al. (2010) and Hashemi et al. (2013) that indicated the presence of a genetic association between rs 763361 and RA.

Further on, in the current study, we did not detect a statistically significant difference, on investigating the genetic association between DNAM-1(C/T, rs 763361) polymorphism and the disease activity state in RA patients (represented by DAS 28 score) in both remission and non- remission patient groups ($P = 0.0841$).

There is a plethora of evidence suggesting that autoimmune diseases share a common genetic background, and several studies suggested that autoimmune phenotypes involve pleiotropic outcomes of non-specific disease genes (Lettre and Rioux 2008; Anaya 2010). Multiple susceptibility genes for autoimmune diseases have been detected without confirmation of their role in the development in the pathogenic phenotype; therefore, it was mandatory to investigate their functional validation in vivo. The contribution of CD 226 to the phenotype of multiple autoimmune diseases has been demonstrated in vivo, in some murine models (Anaya et al. 2006; Dardalhon et al. 2005). In these murine models, the blocking of DNAM-1 was associated with decreased infiltration of T cells, implying that it could reflect a general effect of DNAM-1 on inflammation rather than an effect linked to the genetic association of DNAM-1 with the susceptibility for the considered autoimmune disease (Elhai et al. 2015).

Hence, the results of the current study regarding the lack of association between DNAM-1 (C/T rs 763361) variant and RA could be explained by highlighting the results of the study conducted by Elhai et al. (2015), on collagen-induced arthritis (CIA) murine model that demonstrated that blocking CD 226/DNAM-1 has an effect on the development CIA despite a decrease in infiltrating T cells, which suggests that other cells are involved in the pathophysiology of CIA. Thus, they indicated that DNAM-1 may be implicated in the development of a specific pathogenic phenotype, as encephalomyelitis, dermal fibrosis, and graft versus host diseases, but not RA.

Finally, the present work should be regarded as a hypothesis testing with its limitations, and further studies on larger population samples and in other populations of similar ethnic origin are needed to establish the results of the current study.

In conclusion, the current study suggests that there is lack of genetic association between DNAM-1 (C/T rs 763361) SNP and the development of SLE and RA. However, this SNP might be associated with a high SLEDAI score in LN patients in patients carrying TT and CT genotypes, respectively.

References

Aletaha D, Neogi T, Silman AJ et al (2010) American College of European League against Rheumatism classification criteria for rheumatoid arthritis. Arthritis Rheum 62:2582–2591

Anaya J-M (2010) The autoimmune tautology. Arthritis Res Ther 12(6): 147

Anaya J-M, Gómez L, Castiblanco J (2006) Is there a common genetic basis for autoimmune diseases? Clin Dev Immunol 13(2–4):185–195

Avis JC (1994) Molecular markers. Natural history and evolution. Chapman and Hall, New York 511pp

Bollain-y-Goytia JJ, Mariela AR, de JT F, Leonel DB, Jose FM, Esperanza AD, Rafael HE (2014) Soluble fas and the −670 polymorphism of Fas in lupus nephritis. Int J Nephrol . doi:10.1155/2014/780406Article ID 780406,10 pages

Bomardier C, Gladmsn FF, Urowit MB, Caron D, Chang CH (1995) (1995): Derivation of SLEDAI: a disease activity index for lupus patients. The committee on prognosis studied in SLE. Arthritis Rheum 35:630–640

Ceccarelli F, Agmon-Levin N, Perricone (2016) Genetic factors of autoimmune diseases. J Immunol Res . doi:10.1155/2016/3476023 Article ID 3476023, 2 pages

Dardalhon V, Schubart AS, Reddy J, Meyers JH, Monney L, Sabatos CA et al (2005) CD226 is specifically expressed on the surface of Th1

cells and regulates their expansion and effector functions. J Immunol 175(3):1558–1565. doi:10.4049/jimmunol.175.3.1558

Douroudis K, Nemvalts V, Rajaslu T et al (2009) The CD226 gene in susceptibility of type1 diabetes. HLA Immune Response Genetics (Tissue Antigens formerly) 74:417–419. doi:10.1111/j.1399-0039.2009.01320.x

Du Y, Tian L, Shen LX, Wang F, Yu LK, Song Y et al (2010) Association of CD226 single nucleotide polymorphism with systemic lupus erythematosus in the Chinese Han population. HLA Immune Response Genetics (Tissue Antigens formerly) 77:65–67. doi:10.1111/j.1399-0039.2010.01568.x

Du Y, Shen L-X, Yu L-K, Song Y, Zhu J-F, Du R (2012) The CD226 gene in susceptibility of rheumatoid arthritis in Chinese Han population. Rheumatol Int 32:1299–1304

Elhai M, Chiocchia G, Marchio C, Lager F, Renault G, Colonna M et al (2015) Targeting CD 226/DNAX accessory molecule-1 (DNAM-1) in collagen-induced arthritis mouse models. J Inflamm 12:9. doi:10.1186/s12950-015-0056-5

Hafler JP, Maier LM, Cooper JD et al (2009) CD226 Gly307Ser association with multiple autoimmune diseases. Genes Immun 10:5–10. doi:10.1038/gene

Hashemi M, Zakeria Z, Eskandrani-Nasab E, Atabaki M, Pourhosseini ESM, Jahntigh M et al (2013) CD226 rs 763361 (Gly307Ser) polymorphism is associated with susceptibility to rheumatoid arthritis in Zahden, South Iran. Iran Biomed J 17(4):194–199

Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. [Letter] [see comments] Arthritis Rheum 41:1326–1327

Iwata Y, Furuichi K, Kaneko S and Wada T (2011) The role of cytokine in the lupus nephritis. J Biomed Biotechnol 2011: Article ID 594809, 7 pages. doi: 10.1155/2011/594809

Keishan S, Chowdhury B, Jaung YT, Tsokos GC (2007) Overview of the pathogenesis of systemic lupus erythematosis. In: Tsokos GC, Gordon C, Smolen JS (eds) Systemic lupus erythematosis: a companion to rheumatology. Mosby Inc, Philadelphia, pp 55–63

Kiani AK, Jahngir S, John P, Bhatti A, Zia A, Wang X et al (2015) Genetic link of type 1 diabetes susceptibility loci with rheumatoid arthritis in Pakistani patients. Immunogenetics 67:277–282

Lettre G, Rioux JD (2008) Autoimmune diseases: insights from genome-wide association studies. Hum Mol Genet 17(R2):R116–R121. doi:10.1093/hmg/ddn246

Liu R, Nanwei X, Wang X, Shen L, Zhao N, Wang H et al (2012) Influence of MIF, CD40 and Cd226 polymorphisms on the risk of rheumatoid arthritis. Mol Biol Rep 39:6915–6922

Lozano E, Joller N, Cao Y, Kuchroo VK, Hafler DA (2013) The CD226/CD155 interaction regulates the proinflammatory (Th1/Th17)/anti-inflammatory (Th2) balance in humans. J Immunol 191(7):3673–3680

Lu MM, Ye QL, Feng CC, Yang J, Zhang T et al (2012) Association of FAS gene polymorphisms with systemic lupus erythematosus: a case–control study and meta- analysis. Exp Ther Med 4(3):497–502

Maiti AK, Kim-Howard X, Viswanathan P, Guillen L, Qian X, Rojas-Villarraga A et al (2010) Non-synonymous variant (Gly307Ser) in CD226 is associated with susceptibility to multiple autoimmune diseases. Rheumatology (Oxford) 49(7):1239–1244

Majithia V, Geraci SA (2007) Rheumatoid arthritis; diagnosis and management. Am J Med 120(11):936–939

Myethianou E, Zervou MI, Budu-Aggrey A, Eliopoulas E, Kardrassis D et al (2016) Investigation of genetic overlap between rheumatoid arthritis and psoriatic arthritis in a Greek population. Scand J Rheumatol. doi:10.1080/03009742.2016.1199734 1–7 pages

Narayanan CK, Marwaha CV, Shanmuganandan CK, Shanker GCS (2010) Correlation between systemic lupus erythematosus disease activity index, C3,C4 and anti-dsDNA antibodies. MJAFI 66:102–107

Nie D, Li H, Yang G, Wang Z, He Z, Zhou W (2016) Gene-gene interaction between CD40 and CD226 on systemic lupus erythematosus in the Chinese Han population. Rheumatol Int 36(12):1657–1662

Petri M, Ortabi AM, Gracida SA, Caroline G, Joan TM et al (2012) Deriviation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheuma 64(8):2677–2686

Sanchez E, Nadig A, Richardson BC (2011) Phenotypic associations of genetic susceptibility loci in systemic lupus erythematosus. Ann Rheum Dis 70(10):1752–1777

Sherrington PD, Scot JL, Jin B, Simmons D, Dorahy PJ, Lloyd J et al (1979) TLiSA1 (PTA1) activation antigen implicated in T cell differentiation and platelet activation is a member of immunoglobulin superfamily exhibiting distinctive regulation of expression. J Biol Chem 272:21735–21744

Song G, Bea SC, Choi S, Ji J, Lee Y (2012) Association between the CD226 rs 763361 polymorphism and susceptibility to autoimmune diseases, a meta-analysis. Lupus 21(14):1522–1530

Suzuki A, Kochi Y, Okada Y, Yammato K (2011) Insight from genome wide association studies in rheumatoid arthritis and multiple sclerosis. FEBS Lett 585:3627–3632

Tao D, Shangwu L, Qun W et al (2005) CD226 expression deficiency causes high sensitivity to apoptosis in NK T cells. J Immunol 174(3): 1281–1290

Todd JA, Walker NM, Cooper JD, Symth DJ, Downes K, Plagnol V et al (2007) Robust associations of four new chromosome regions from genome-wide analysis of type 1 diabetes. Nat Genet 39:857–864

Van der Heijde DM, van t Hof MA, van Riel PL et al (1990) Judging diseases activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 49(11):916–920

Wandstrat A, Wakeland E (2001) The genetics of complex autoimmune diseases: non-MHC susceptibility genes. Nat Immunol 2(9):802–809

Wieczorek S, Hoffjan S, Chan A et al (2009) Novel association of the CD226 (DNAM-1) Gly 307 Ser polymorphism in Wegener's granulomatosis and confirmation for multiple sclerosis in German patients. Genes Immun 10(6):591–595