Overexpression and Secretion of the Soluble *CTLA-4* Splice Variant in Various Autoimmune Diseases and in Cases with Overlapping Autoimmunity

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Aim: To explore the potential genetic association of *CTLA-4* Exon1 +49A/G and 3'UTR (AT)_n to susceptibility to systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and overlapping (OP) autoimmunity; affected with more than one autoimmune disease. Expression of two major *CTLA-4* isoforms; full length (m*CTLA-4*) and soluble (s*CTLA-4*) were explored in all subjects. A total of 680-age/gender/ethnically matched Kuwaitis were recruited and polymerase chain reaction (PCR)-fragment analysis was employed for genotyping both markers. m*CTLA-4* and s*CTLA-4* mRNA expression were analyzed using quantitative real time-PCR. The enzyme-linked immunosorbent assay (ELISA) was used to screen s*CTLA-4* in all subjects' sera. **Results:** Only two *CTLA-4* 3'UTR (AT)_n allelotypes, (AT)₁₅ and (AT)₆ were detected. The heterozygous (AT)_{15/6} genotype confers protectivity rather than susceptibility to SLE ($p=0.01$, odds ratio=0.43, and confidence interval=0.21–0.86). No significant association was observed between Exon 1 +49A/G and any of the tested diseases. A consistently high serum s*CTLA-4* level was observed in RA (6.8 ng/mL, $p=0.005$), SLE (6.34 ng/mL, $p=0.007$), and OP (8.75 ng/mL, $p=0.012$) compared to healthy control. A significant increase in the expression of s*CTLA-4* mRNA was observed in OP ($p=0.05$) and SLE ($p=0.047$), while a significant increase in the expression of m*CTLA-4* ($p=0.01$) was observed only in OP. **Conclusion:** The present study is the first to report a statistically significant association between OP and serum s*CTLA-4*. The novelty of our study is the significance of *CTLA-4* in the pathogenesis of OP besides SLE and RA.

Introduction

AUTOIMMUNE DISEASES ARE presumed to be the result of a malfunctioning immune system that fails to maintain nonresponsiveness or tolerance to self (Lesage and Goodnow, 2001). Most frequent autoimmune diseases, such as systemic lupus erythematosus (SLE) (MIM#152700) and rheumatoid arthritis (RA) (MIM# 180300), cluster within the same families, and their inheritance pattern in general is very complex. For the development of efficient disease-preventative and modifying therapies, a deep insight into the genetic and environmental causes that lead to the diseases is imperative.

SLE is a complex prototype of systemic autoimmune disease characterized by sustained abnormal immune activation and autoantibody production. A high concordance rate in monozygotic twins and an increased risk of SLE in first degree relatives compared to the general population demonstrates the underlying genetic basis of this disease (Vyse and Kotzin, 1996; Harley *et al.*, 1998; Bengtsson *et al.*, 2002). RA is a polygenic autoimmune disease that primarily affects the synovial tissue in diarthroidal joints causing inflammation disrupting joint integrity (Lee *et al.*, 2003). Both SLE and RA are believed to be

T-cell-mediated autoimmune diseases and thus may share a similar pathway of etiology or even steps in a branched pathway. About 25% of patients diagnosed with autoimmune disorders may have a tendency to develop one or more additional autoimmune diseases leading to overlapping autoimmune disease. Such cases share a common causative gene or, more probably, polygenes leading to the assumption that a common pathway of etiology leads to the development of autoimmune diseases in genetically susceptible individuals.

Our previous study on genome scan meta-analysis for non-HLA type candidate loci has confined the risk factor for these autoimmune diseases to locus 2q33, which contains the T lymphocyte regulatory genes, such as *CTLA-4*, CD28, and ICOS (AlFadhli *et al.*, 2010). Cytotoxic T-lymphocyte-associated antigen-4 (*CTLA-4*) is found to be a negative regulator of an immune response, while the other two genes appear to positively regulate immune responses. Hence, we primarily decided to select *CTLA-4* as a candidate gene for the present study.

CTLA-4 plays a significant role in the immune response, by regulating the adaptation of T cells to a state of proliferative responsiveness and tolerance. It belongs to the

immunoglobulin super family and exists in multiple molecular forms and membrane fractions. It generates two major isoforms—membrane bound (mCTLA-4) and soluble form (s-CTLA-4) (Magistrelli *et al.*, 1999). The full length *CTLA-4* mRNA contains four exons encoding the *CTLA-4* molecule expressed at the membrane surface. The soluble form, lacking exon three encoding the transmembrane region, is the most widely studied alternate splice variant of the *CTLA-4* gene (Oaks and Hallet, 2000; Wan *et al.*, 2006; Saverino *et al.*, 2007; Daroszewski *et al.*, 2009; Chan *et al.*, 2010). Several studies have previously implicated the elevated levels of sCTLA-4 proteins in sera of patients with autoimmune thyroiditis (Saverino and Brizzolara, 2007), SLE (Wong *et al.*, 2005), Myasthenia gravis (Wang *et al.*, 2002), systemic sclerosis (Sato *et al.*, 2004), celiac disease (Simone *et al.*, 2009), and autoimmune pancreatitis (Umemura *et al.*, 2008). Similarly, *CTLA-4* polymorphisms are found to be associated with several autoimmune disorders; namely, type I diabetes, autoimmune thyroid disease, celiac disease, grave's disease, RA, and multiple sclerosis.

In the present study, we aimed to evaluate the genetic association and contribution of two individual markers from the *CTLA-4* gene known to be involved in susceptibility to various autoimmune diseases, the *CTLA-4* Exon 1 + 49 A/G SNP (rs231775) resulting in a threonine to alanine substitution in the signal peptide of the molecule (Anjos *et al.*, 2002) and the *CTLA-4* 3'UTR (AT)_n repeat proposed to influence *CTLA4* mRNA stability and turnover (Conne *et al.*, 2000; Lowe *et al.*, 2000; Kuersten and Goodwin, 2003), in patients with individual autoimmune diseases, such as SLE, RA and in a group of patients with overlap autoimmune diseases. We further aim to explore the functional role of the *CTLA-4* gene at the mRNA and protein level.

Materials and Methods

A total of 680 subjects were recruited in this study; 236 SLE, 124 RA, 38 overlap syndrome, and 282 healthy controls. Blood samples were collected from outpatient clinics of the Mubarak Al-Kabeer Hospital, Kuwait, during 2005–2010. The SLE and RA patients were selected when they had at least four of the Systemic Lupus International Collaboration Clinics/American College of Rheumatology (SLICC/ACR) criteria. Patients with overlapping (OP) autoimmune disease had coexistence of at least two of the following autoimmune diseases, such as SLE, RA, Hashimoto's thyroiditis (HT), diabetes mellitus, and alopecia areata (AA) (Table 1). Healthy volunteers within the age range 19 to 68 years (median 46 years) were randomly

selected from five provinces of Kuwait. Inclusion criteria were general good health and no first degree relatives with autoimmune diseases. Exclusion criteria were any recent history of acute or chronic debilitating illness. Ethnic bias within the population studied was minimized by excluding patients who were not of Arab origin. The conduct of the study was approved by the institution's ethics committee and all the participants provided written informed consent.

Each participant donated peripheral blood for DNA and RNA extraction and serum isolation. Patient and control sera were stored at -20°C for up to 1 month or for long-term storage at -80°C . DNA was isolated from peripheral nucleated blood cells using the Gentra kit and RNA using QIAamp RNA blood kits (QIAGEN). cDNA was synthesized using the Hi Capacity Reverse Transcription kit purchased from Applied Biosystems. Reactions were performed according to the manufacturer's instructions.

DNA Analysis/Genotyping of *CTLA-4* 3'UTR (AT)_n

CTLA-4 3'UTR (AT)_n repeat was genotyped using polymerase chain reaction (PCR) followed by fragment analysis. A 10 μL reaction mix containing 5 pmol of each labeled forward primer: FAM 5'-GCCAGTGAT GCTTAAAGGTTG-3' and reverse primer: 5'-AACATACGTGGCTCTATGC A-3' was prepared. The thermal amplification program consisted of an initial denaturation at 95°C (5 min), followed by thirty cycles at 94°C (30 s), 55°C (45 s), and 72°C (1 min), and a final extension at 72°C (8 min). One μL of the PCR amplicon was mixed with 10 μL of Hi Di formamide and 0.3 μL genetic marker ROX400HD and subjected to fragment analysis using the ABI3100 (Applied Biosystem). Genotyping results were further confirmed by sequencing randomly selected samples.

Exon1 + 49 A/G genotyping

Allelic determinations of the *CTLA-4* Exon1 + 49A/G SNP (rs231775) were studied using a modified PCR- restriction digestion, followed by fragment analysis. The PCR reaction was performed in a total volume of 10 μL , including 5 pmol primers (NED) labeled F-5'-GCTCTACTTCCTGAAGA CCT-3' and R-5'-AGTCTCACTCACCTTGCAG-3'. Five μL of the amplified product was subjected to restriction digestion in a total volume of 10 μL having 5% of Bbv1. Two μL of the restricted fragment was then mixed with 10 μL of Hi Di formamide and 0.3 μL of the genetic marker ROX 400HD, and subjected to ABI3100 (Applied Biosystem). Allele A results in an undigested PCR product of 162 bp, and allele G results in a digested PCR product of 90 and 72 bp; the 72-bp fragment contains the fluorescence-labeled forward primer and could be visualized on the ABI-3100. Genotyping results were further confirmed by sequencing randomly selected samples.

Real-time PCR

Quantitative Real-time PCR (TaqMan probe-based detection) was performed to study the expression of the human sCTLA-4 and mCTLA-4 gene, relative to human GAPDH as the endogenous control. Gene-specific primers and probes for *CTLA-4* mRNA were designed using the NCBI server and purchased from Applied Biosystem, USA. The quantitative real time PCR reactions were carried out according to the manufacturer's instructions.

TABLE 1. STRATIFICATION OF OVERLAPPING AUTOIMMUNE DISEASES

Overlapping autoimmune diseases	Number of cases
SLE + RA	10
SLE + AA	2
SLE + HT	8
RA + HT	10
RA + HT + Diabetes Mellitus type 1	3
HT + Diabetes Mellitus type 1	5
Total	38

SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; AA, alopecia areata; HT, hashimoto's thyroiditis.

Detection of serum sCTLA-4 by enzyme-linked immunosorbent assay

Specific enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical Company) were used for measuring serum sCTLA-4 levels in patients with RA, SLE, OP, and control subjects, according to the manufacturer's protocol. The concentration of serum sCTLA-4 in a total of 134 patients and 68 healthy subjects were evaluated. The results are expressed in ng/mL.

Statistical analysis

The fit to the Hardy-Weinberg equilibrium was verified. The distribution of allelic and genotype frequencies were analyzed using the Pearson's χ^2 test. Statistical significance was defined as $p < 0.05$. The p -value, odds ratio (OR), and 95% confidence interval (CI) were calculated using online statistical programs (<http://faculty.vassar.edu/lowry/odds2x2.html>). Relative expression of mCTLA-4 and sCTLA-4 mRNA were analyzed using the Student's t -test (Graph Pad Prism). The Mann-Whitney test was used to analyze the differences in the serum sCTLA-4 concentration between patient groups and healthy subjects (www.socr.ucla.edu).

Results

Allelic and genotypic frequencies of the Exon 1+49A/G polymorphism in 680 RA, SLE, and OP autoimmune patients and healthy subjects were detected (Table 2). The 1+49A/G allele G (20%) was less available compared to allele A (80%), which dominated the population. The frequencies of CTLA-4 1+49A/G genotypes AA, AG, and GG in autoimmune patients were not significantly different from the healthy control. The GG genotype was less frequent in all three disease groups and healthy subjects (<10%). Lack of association of the 1+49A/G SNP with the risk of having any of the tested au-

toimmune diseases might indicate that, there is no effect of this SNP on disease susceptibility in the Kuwaiti population.

Distribution of the allelic and genotypic frequency of CTLA-4 3'UTR (AT)_n is shown in Table 3. Only two alleles of (AT)_n were detected in the Kuwaiti population, (AT)₁₅ and (AT)₆. The allele with (AT)₆ repeats (>87%) was more frequent in cases and controls than the (AT)₁₅ repeat. Allelic frequencies of (AT)_n failed to show any significant difference in the tested autoimmune cases compared to healthy control ($p > 0.05$). Among the three genotypes of (AT)_n repeat detected in this study (6/6, 15/15, and 15/6), homozygous (AT)_{15/15} represents the rare genotype (<13%). The heterozygous (AT)_{15/6} genotype showed a significant association that confers protectivity rather than susceptibility to SLE ($p = 0.01$, OR = 0.4283, 95% CI = 0.2133–0.8598), whereas the other tested cohorts failed to show any significant association.

Expression of CTLA-4 isoforms

A total of 94 autoimmune patients (32 SLE, 42 RA, and 20 OP) and 28 healthy donors were tested for the expression of mCTLA-4 and sCTLA-4 isoforms of CTLA-4 mRNA. A significant increase in the expression of sCTLA-4 mRNA was observed in SLE ($p = 0.047$) and a suggestive significance in OP patients ($p = 0.05$) compared to healthy controls. A fold difference of 0.84–2.8 and 0.69–4.6 were observed in the expression of target mRNA in SLE and OP patients, respectively, relative to healthy control. Similarly, analysis of mCTLA-4 mRNA revealed a significant difference ($p = 0.01$) only between OP patients and healthy subjects, where a 0.01–5.7-fold increase in the expression of mCTLA-4 mRNA was observed. No significant difference in the expression of sCTLA-4 or mCTLA-4 mRNA was observed between RA patients and healthy control ($p > 0.05$). Analysis of (delta threshold cycle) the Δ CT sCTLA-4/mCTLA-4 ratio revealed no significant differences between patients and control

TABLE 2. DISTRIBUTION OF ALLELIC AND GENOTYPIC FREQUENCIES OF CTLA 4 EXON 1+49 A/G SNP IN FOUR TESTED COHORTS

Case (n)	Allele	Case no. (f)	Control no. (f)	Chi square	p-Value
SLE (472)	A	382 (0.81)		0.21	0.65
	G	90 (0.19)			
RA (228)	A	178 (0.78)	A-450 (0.8)	0.29	0.59
	G	50 (0.22)	G-114 (0.2)		
OP (76)	A	60 (0.79)		0.03	0.86
	G	16 (0.21)			
Case (n)	Genotype	Case no. (f)	Control no. (f)	Chi square	p-Value
SLE (236)	AA	152 (0.64)		0	1
RA (114)		74 (0.65)	182 (0.65)	0	1
OP (38)		24 (0.63)		0.03	0.86
SLE (236)		78 (0.33)		0.39	0.53
RA (114)	AG	30 (0.26)	86 (0.31)	0.69	0.4
OP (38)		12 (0.32)		0.02	0.89
SLE (236)		6 (0.03)		2.03	0.15
RA (114)	GG	10 (0.09)	14 (0.05)	2.07	0.15
OP (38)		2 (0.05)		0.006	0.94

OP, overlapping.

TABLE 3. DISTRIBUTION OF ALLELIC AND GENOTYPIC FREQUENCIES OF CTLA 4 3'UTR (AT)_n IN FOUR TESTED COHORTS

Case (n)	Allele	Case no. (f)	Control no. (f)	Chi square	p-Value
SLE (420)	6	376 (0.90)		1.18	0.28
	15	44 (0.10)			
RA (224)	6	200 (0.89)	6–422 (0.87)	0.63	0.43
	15	24 (0.11)	15–62 (0.13)		
OP (76)	6	66 (0.87)		0.01	0.92
	15	10 (0.13)			
Case (n)	Genotype	Case no. (f)	Control no. (f)	Chi square	p-Value
SLE (210)		182 (0.87)		2.64	0.1
RA (112)	6/6	94 (0.84)	196 (0.81)	0.45	0.5
OP (38)		30 (0.79)		0.09	0.76
SLE (210)		16 (0.08)		0.17	0.68
RA (112)	15/15	6 (0.05)	16 (0.07)	0.21	0.65
OP (38)		2 (0.13)		0.09	0.75
SLE (210)		12 (0.06)		5.9	0.01
RA (112)	15/6	12 (0.11)	30 (0.12)	0.52	0.47
OP (38)		6 (0.16)		0.34	0.56

Bold value indicates significance.

groups ($p > 0.05$). Furthermore, differences concerning mCTLA-4 and sCTLA-4 expressions were not statistically significant between any of the tested cohorts (RA vs. SLE, SLE vs. OP, and OP vs. RA, $p > 0.05$).

Serum sCTLA-4

The range of serum sCTLA-4 detected in autoimmune patients and healthy subjects are presented in Figure 1. Only 36/68 healthy subjects (52.9%) had detectable serum sCTLA-4 with a detection limit of 0.1–9 ng/mL. However, 26/28 OP patients (92%) had serum sCTLA-4 in the range of 0.4–43.3 ng/mL. All the patients with SLE ($n = 41$) and RA ($n = 65$) had a detectable level of sCTLA-4 in the range of (0.1–43.3 ng/mL) and (0.1–37.4 ng/mL), respectively. The average serum concentration of sCTLA-4 was found to be higher in OP patients (8.75 ng/mL) compared to RA (6.8 ng/mL) and SLE (6.34 ng/mL). However, no significant differences in serum sCTLA-4 levels were observed between the patient groups ($p > 0.05$). While the serum sCTLA-4 concentration was found to be significantly higher in autoimmune patients, SLE (6.34 ± 7.8 ng/mL, $p = 0.007$), RA (6.8 ± 7.5 ng/mL, $p = 0.005$), and OP (8.75 ± 11.19 ng/mL, $p = 0.012$) compared to healthy control (2.6–2.7 ng/mL).

Discussion

The allelic and genotypic frequencies of CTLA-4 Exon1+49A/G in all tested autoimmune patients failed to show any significant association when compared to healthy control. Lack of association of the A-G polymorphism with autoimmune diseases could be due to the predominance of the A allele (80%) in the Kuwaiti population. The G allele suspected for susceptibility in other populations (Ban *et al.*, 2003) was less (20%) available compared to the A allele in the Kuwaiti population. Several studies have previously shown the association of the G allele with the occurrence of endocrine autoimmune diseases, such as the Grave's disease (Donner *et al.*, 1997b; Kouki *et al.*, 2000), Addison's disease (Donner *et al.*,

1997a), and insulin-dependent diabetes mellitus (Donner *et al.*, 1997b). Only 6 out of 22 population studies have so far reported the predominance of the G allele (>65%). However, noninvolvement of the G allele in the function and expression of the CTLA-4 gene have also been reported (Xu *et al.*, 2002). This could be explained by the fact that another polymorphism in the linkage disequilibrium may be responsible for the disease; a possibility as the threonine to alanine substitution does not affect the function of the leader peptide or merely because the CTLA-4 A/G polymorphism alone is not sufficient to influence disease expression.

A comparative analysis of our experimental data with other ethnicities were attempted, 9 out of 18 studies on the exon 1+49A/G polymorphism in SLE patients did not show any significant association with SLE, which includes the Spanish (D'Alfonso *et al.*, 2000; Aguilar *et al.*, 2003), English (Heward *et al.*, 1999), Japanese (Takeuchi *et al.*, 2003), Korean (Hudson *et al.*, 2002), Chinese (Liu *et al.*, 2001), Mexican (Mehrian *et al.*, 1998), African Americans (Parks *et al.*, 2004), and Malaysian (Chua *et al.*, 2010). Recently, Chang *et al.* (2012) have reported a detailed meta-analysis on the CTLA-4 exon-1+49A/G SNP suggesting the significance of this polymorphism as a risk factor for SLE susceptibility at least in Asians.

Likewise, 5 out of 10 RA studies failed to show any significant association (Seidl *et al.*, 1998; Gonzalez-Escribano *et al.*, 1999; Barton *et al.*, 2000; Yanagawa *et al.*, 2000; Hadj-Kacem *et al.*, 2001; Milicic *et al.*, 2001; Lee *et al.*, 2002, 2003; Barton *et al.*, 2004; Takeuchi *et al.*, 2006) and this group comprised the Caucasians mostly.

The genotyping of CTLA-4 3'UTR (AT)_n repeats revealed a significant association only with SLE patients. The heterozygous genotype of (AT)_{15/6} showed a significant association with SLE ($p = 0.01$), while RA and OP patients showed a pattern similar to healthy subjects. The allele with (AT)₆ repeats was found to be prominent (>85%) in both cases and controls. Three studies were found on CTLA-4 3'UTR (AT)_n using SLE samples, of which the Japanese (Ahmed *et al.*, 2001) and Portuguese (Baretto *et al.*, 2004) found an association between the disease and the 106-bp (6 repeats) allele, while another study on Japanese (Matsushita *et al.*, 1999) itself failed to show any significant association. A single study was conducted using RA patients as subjects on CTLA-4 3'UTR (AT)_n in a Tunisian population, which even failed to produce any significant association with the disease (Hadj-Kacem *et al.*, 2001). These contradictory results could be explained on the basis of genetic divergence between populations. Several other factors, such as clinical heterogeneity, genotyping and diagnostic errors, differences in statistical strategies, and limitations in the sample size, could also contribute to misleading results. The Kuwaiti population has its roots in the Caucasians, which explains the similarity between their results.

Our study is one of the few to explore the role of CTLA-4 isoforms at the mRNA level. A significant increase in the expression of sCTLA-4 mRNA was observed in SLE patients ($p = 0.04$) and OP ($p = 0.05$) compared to healthy controls. Expression of sCTLA-4 mRNA in SLE and OP were found to be similar probably suggesting the predominance of SLE in OP patients. The majority of OP patients tested in our study were primarily diagnosed with SLE and later with one or more additional autoimmune disorders, such as RA, HT, AA, and diabetes mellitus. It is difficult to classify OP patients to a single disease category since they exhibit a continuous spectrum of

FIG. 1. Serum soluble form CTLA-4 (sCTLA-4) found in autoimmune patients, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), overlapping (OP), and healthy control (HC). The concentration of serum sCTLA-4 in a total of 134 autoimmune patients and 68 healthy controls were analyzed using the ELISA test. Results are expressed in ng/mL.

clinical features ranging from organ-specific to systemic autoimmune diseases. Additionally, we observed a significant increase in the expression of mCTLA-4 ($p=0.01$) in OP patients. Of note, higher levels of mCTLA-4 could not restore homeostasis in OP patients. A recent work by Budarf *et al.* (2011) confirms the existence of multiple risk factors for SLE and supports the notion that some risk factors for SLE are shared with other inflammatory disorders. Hence, it is questionable whether it is adequate to study the *CTLA-4* gene alone to depict the autoimmune etiology.

Several studies have previously reported the increased concentration of serum sCTLA-4 in autoimmune patients. Our experimental data further confirm and extend these findings; consistently, 132 out of 134 autoimmune patients had detectable sCTLA-4 in their serum. A significant increase in the serum sCTLA-4 concentration was observed in SLE ($p=0.007$), RA ($p=0.005$), and OP ($p=0.012$) patients compared to healthy controls. The present study is the first to report a statistically significant association between OP patients and serum sCTLA-4. Furthermore, our data confirms the findings of Liu *et al.* (2003) who showed increased serum levels of sCTLA-4 in SLE patients. Nevertheless, the role played by sCTLA-4 in the pathogenesis of RA is not clearly understood. Since RA is a chronic joint disease characterized by infiltration of synovium by T cells, it is speculated that higher levels of sCTLA may sustain T cell activation leading to autoreactivity. Despite these findings, no correlation was observed between the levels of sCTLA-4 mRNA transcripts and serum proteins in RA patients. The sCTLA-4 mRNA levels ($p=0.56$) failed to show any significant association, while serum proteins showed a significant difference ($p=0.005$) between RA patients and healthy controls. This conflicting result could be due to a shorter half-life of mRNA. The precise mechanisms that control the level sCTLA-4 mRNA are not known. The fact that half-lives of mRNA might change over time and the chemical half-life might not reflect its functional form makes it further difficult to reach a decision.

In short, the potential of our study was to explore the role of the *CTLA-4* gene at the genetic, mRNA, and protein levels in various autoimmune diseases. Our study indicates that *CTLA-4* probably plays a significant role in the pathogenesis of autoimmune diseases, such as SLE, RA, and OP. Increased detection of serum sCTLA-4 in OP and individual autoimmune patients sheds light on the fact that, these autoimmune diseases share a common causative gene or, more probably, polygenes leading to the assumption that a common pathway of etiology leads to the development of autoimmune diseases in genetically susceptible individuals.

An overview of our results suggests that, lack of association at the genetic level does not necessarily indicate the noninvolvement of the candidate gene in the pathogenesis of autoimmune disease. Although no significant association was observed at a genetic level, higher levels of mRNA and protein supports the notion that association analysis at the genomic level alone is not sufficiently informative. Therefore, to explore the pathology of a disease, a deep insight into the mRNA and protein level is necessary. A combined genetic and functional approach is mandatory to depict autoimmune etiology.

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Author Disclosure Statement

No competing financial interests exist.

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