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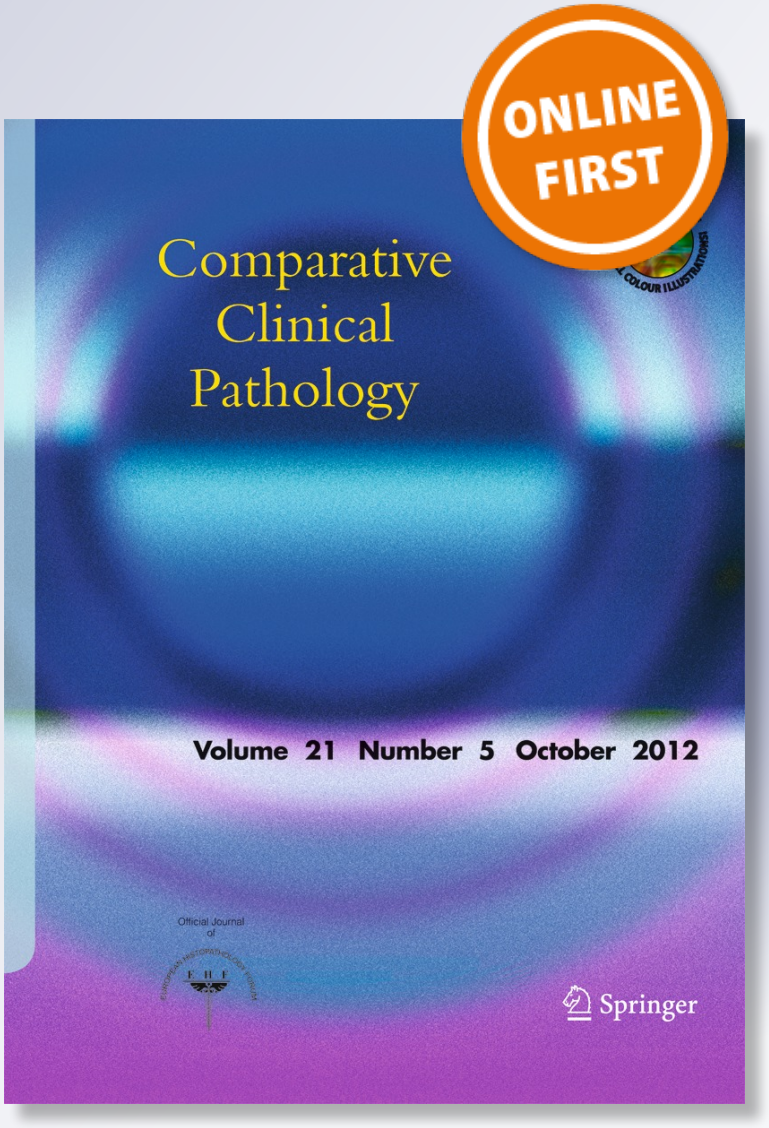

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ORIGINAL ARTICLE

Association analysis of systemic lupus erythematosus and −1514 polymorphism of TBX21 gene in the Egyptian population

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Abstract The T-box21 (TBX21) gene encodes the transcription factor T-bet (T-box expressed in T cells), which influences naïve T lymphocyte development and has been implicated in the pathogenesis of many diseases. We aimed to assess the implication of the TBX21 gene promoter T-1514C polymorphism in susceptibility to systemic lupus erythematosus (SLE) in a cohort of Egyptian population and to study the association between the genetic polymorphism of that gene and the clinical and laboratory data of these patients. The study included 50 SLE patients. Sixty age, sex, and ethnically matched volunteers were included in the current study as a control group. The genotyping of T-1514C single nucleotide polymorphism was performed by using a polymerase chain reaction–restriction fragment length polymorphism assay. There was no statistically significant difference in the distribution of the genotypes between SLE patients and the control group in our study. No association was detected between TBX21 genotypes and the clinical features and laboratory data of the patients apart from an association with the hematologic complications (anemia, leucopenia, thrombocytopenia, or pancytopenia) with increased frequency of hematological complications in the group carrying the wild genotype (TT) (p=0.016).

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by aberrant cytokine milieu and multiple organ involvement. Imbalance in the cytokines produced by the two subsets of T helper cells, Th1 and Th2, probably plays an important role in the pathogenesis of SLE (Lai et al. 2007).

Evidence suggests that the selection of Th1 and Th2 pathways by the immunological system is controlled by the upstream transcription factors rather than by cytokine production downstream (You et al. 2010). T-bet, a nuclear transcription factor belonging to the T-box gene family of DNA-binding proteins, has been found to be the principal transcription factor for the differentiation of type 1 helper T lymphocytes and induction of the hallmark Th1 cytokine, interferon (IFN)-γ (Raby et al. 2006).

T-1514C polymorphism in the T-box21 (*TBX21*) gene (encoding T-bet) promoter can affect transcription activity (Chen et al. 2011). The TBX21 promoter carrying −1514C possessed a significant lower transcriptional activity than that of −1514T. The individuals carrying −1514C allele were determined to have significantly diminished expression of T-bet and IFN-γ and increased IL-4 production in CD4(+) T cells compared with those of −1514T allele (Li et al. 2012). SLE is often considered to be a Th2-mediated disease at the early stage, but the Th1 commitment may replace the Th2 pathway and take over the progression of SLE to active nephritis (Lai et al. 2007). The disease commonly results from the combined effect of variants in a large number of genes (Tsokos 2011). The current study was an attempt to assess the implication of the TBX21 gene promoter T-1514C polymorphism in susceptibility to SLE in a cohort of Egyptian population and to study

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the association between the genetic polymorphism of that gene and the clinical and laboratory data of these patients.

Materials and methods

Subjects

A total of 50 Egyptian SLE patients (46 women, 4 men, mean age 28.88±7.51 years) were enrolled in the study; all were consecutively recruited from Rheumatology and Rehabilitation Department, Kasr El Aini Hospital. The diagnosis was established based on the 1987 American College of Rheumatology criteria for SLE (Tan et al. 1982; Hochberg 1997). A total of 60 age, sex, and ethnically matched healthy subjects were recruited as controls. Peripheral blood samples (5 mL) were obtained from all subjects after they provided a written informed consent. The study was approved by the ethical committee.

SLE patients were subjected to: full history taking, thorough clinical examination, assessment of disease activity using systemic lupus erythematosus activity measure (SLAM), and laboratory investigations which included complete blood count (CBC), erythrocyte sedimentation rate (ESR), serum complement level (C_3 and C_4), serum antinuclear antibodies (ANAs), serum anti-double-stranded deoxyribonucleic acid antibodies (dsDNA), complete urine analysis, and kidney function tests. Clinical and laboratory data of the patients are summarized in Table 1.

Table 1 Clinical and laboratory data of SLE patients (n=50)

Clinical and laboratory data	Patients with SLE n (%)
Malar rash	44 (88)
Photosensitivity	31 (62)
Oral ulcers	30 (60)
Arthritis	42 (84)
Serositis	23 (46)
Lupus nephritis	26 (52)
Lupus cerebritis	19 (38)
Hematological	
Anemia	33 (66)
Leucopenia	5 (10)
Thrombocytopenia	3 (6)
Pancytopenia	1 (2)
ANA	50 (100)
Anti-dsDNA	41 (82)
SLAM	
Mild	44 (88)
Moderate	6 (12)

Genotyping

Total genomic DNA of patients and healthy controls was extracted from about 2 mL anti-coagulated whole blood on EDTA using Qiagen extraction kit (catalog number 51104, USA). The genotyping of T-1514C (rs 17250932) single nucleotide polymorphism was performed by using a polymerase chain reaction (PCR)–restriction fragment length polymorphism assay.

DNA was amplified by PCR using 5′-CAAGACTGTA TAAATCACCC-3′ (*sense*) and 5′-CTATTTGGGAGCA GAGAGTC-3′ (*antisense*) primers spanning the TBX21 promoter region containing T-1514C site. PCR reactions were carried out in a 15-μL reaction mixture containing 1 μL of genomic DNA, 1.5 μL of 10× HotStar Taq PCR buffer (Qiagen, Germany), 3.0 μL of Q solution for PCR (Qiagen), 1.5 mM of $MgCl_2$, 0.033 mM of each dNTP, and 5 pmol of primer, and 1.0 U of HotStar Taq Polymerase (Qiagen) was used. The PCR reaction tubes were then placed in the thermal cycler (Perkin Elmer 9600, Singapore). The PCR cycles were as follows: The reaction was started at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 40 s, annealing at 57.0 and 55.5 °C for 45 s each, extension at 72 °C for 50 s, with final extension carried out at 72 °C for 10 min.

PCR products were digested with ApaLI using the conditions recommended in the manufacturer's instructions (Fermentas, Germany). After digestion, the PCR products were electrophoresed on ultraviolet-transilluminated, ethidium bromide-stained 2 % agarose gel for the identification of TBX21 genotypes, yielding PCR products of 425, 115, and 310 bp for the heterozygotes (T/C), while the wild homozygous genotype (T/T) will appear as a single band of 425 bp and the homomutant (C/C) will appear as two bands of 310 and 115 bp (Cao et al. 2007).

Statistical analysis

The chi-square (χ^2) test was used to analyze the Hardy–Weinberg equilibrium and compare the distribution of the TBX21 genotypes between cases and controls. A p value less than 0.05 was considered significant. Odds ratios (ORs) and 95 % confidence intervals were calculated by multifactorial logistic regression as a measure of association with risk of the development of SLE. All ORs were adjusted for age and sex. Statistical analyses were performed using SPSS version 13.0.

Results

Genotype frequencies of T-1514C in the case–control cohort of patients with SLE were shown in Table 2. These frequencies carry no significant difference as regards the susceptibility to SLE (p=0.5101). Genotype distribution of TBX21 promoter

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Table 2 Genotype frequencies of T-1514C in the case–control cohort of patients with SLE

T-1514C genotype	SLE *n* (%)	Controls *n* (%)	*p* value	OR	95 % CI
TT	42 (84)	55 (91.7)	0.5101	2.095	0.639 – 6.869
TC	8 (16)	5 (8.3)			
CC	0	0			

CI confidence interval

T-1514C polymorphism in controls was in accordance with Hardy–Weinberg equilibrium ($p>0.05$).

As regards to the clinical characteristics of SLE group of patients in relation to TBX21 genotypes, we could not find any statistically significant difference between the group with the wild genotype (TT) and the group carrying the polymorphic genotype (TC) as regards malar rash, photosensitivity, oral ulcers, arthritis, serositis, lupus nephritis, lupus cerebritis, and SLAM.

No statistically significant difference could be detected between the group of SLE patients with the wild genotype (TT) and the group carrying the polymorphic genotype (TC) as regards their laboratory data including anti-dsDNA, ANA, ESR, complement consumption, and serum creatinine (data not shown).

But we found a statistically significant difference between them as regards hematological complications in the form of anemia (66 %), leucopenia (10 %), thrombocytopenia (6 %), or pancytopenia (2 %) with increased frequency of hematological complications in the group carrying the wild genotype (TT) ($p=0.016$; Table 3).

Discussion

Extensive studies focus on cytokines and T helper cells in the peripheral blood of SLE patients in vitro and in vivo. However, reports on the Th1/Th2 imbalance in SLE have been inconsistent (Usui et al. 2006). This variation could reflect the clinical diversity in SLE. Another possibility could be the difference in the genetic background of patients, resulting in distinct alterations in immune balance.

The transcription factor T-bet (T-box expressed in T cells) (*TBX21*) is a key transcriptional activator of Th1 cell differentiation. T-bet plays an essential role in Th1/Th2 balance, where it is the master regulator of Th1 cell fate through promotion of Th1 cytokines and inhibition of Th2 cytokines (Szabo et al. 2000). Because the TBX21 expression in human Th cells correlates with the Th1 profiles, it is not surprising that a growing body of evidence has been accumulated for the pathogenic roles of TBX21 in autoimmune diseases such as rheumatoid arthritis (RA).

Functional data suggested a Th2 cytokine profile in the *TBX21* mutation group, a proinflammatory profile in the *TBX21* wild-type group. In a study by Chae et al. (2009), *TBX21* polymorphisms were shown to be associated with RA, and in previous studies, they were associated with asthma (Th2-mediated disease characterized by overproduction of Th2 cytokines [IL-4, IL-5, and IL-13]) (Ylikoski et al. 2004) and type 1 diabetes mellitus (Sasaki et al. 2004). Finally, the cytokine balance in mice deficient in T-bet is skewed toward Th2 cytokines, and *tbx2*-null mice have displayed increased sensitivity to bleomycin-induced dermal sclerosis (Aliprantis

Table 3 Relation of TBX21 genotypes to clinical and laboratory data of SLE patients

		TC (*n*, %)	TT (*n*, %)	*p*
Malar rash	Yes	8 (100)	36 (85.7)	0.572
	No	0	6 (14.3)	
Photosensitivity	Yes	4 (50)	27 (64.3)	0.459
	No	4 (50)	15 (35.7)	
Oral ulcers	Yes	5 (62.5)	25 (59.5)	0.875
	No	3 (37.5)	17 (40.5)	
Arthritis	Yes	5 (62.5)	37 (88.1)	0.105
	No	3 (37.5)	5 (11.9)	
Serositis	Yes	2 (25)	21 (50)	0.261
	No	6 (75)	21 (50)	
Renal	Yes	5 (62.5)	21 (50)	0.704
	No	3 (37.5)	21 (50)	
CNS	Yes	3 (37.5)	16 (38.1)	0.975
	No	5 (62.5)	26 (61.9)	
Hematological (anemia, leucopenia, thrombocytopenia, or pancytopenia)	Yes	4 (50)	38 (90.5)	0.016
	No	4 (50)	4 (9.5)	
Anti-dsDNA	Yes	7 (87.5)	34 (81)	0.659
	No	1 (12.5)	8 (19)	
SLAM	Moderate	1 (12.5)	5 (11.9)	0.962
	Mild	7 (87.5)	37 (88.1)	

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et al. 2007). These findings indicate that TBX21 might possibly be placed in the category of autoimmune susceptibility gene. Given the potential importance of Th1/Th2 cytokine balance in SLE, we investigated the association of a polymorphism in the *TBX21* with SLE.

In our study, there was a higher frequency of TC genotype (16 %) in SLE patients compared to the control subjects (8.3 %). However, these frequencies carry no significant difference as regards the susceptibility to SLE. This is in contrast to You et al. (2010) whose study revealed that T-1514C polymorphism in TBX21 promoter region influences susceptibility to SLE.

In our study, the SLE patients were classified according to TBX21-1514 genotypes into two groups: TT and TC. The two groups were compared clinically as regards: malar rash, photosensitivity, oral ulcers, arthritis, serositis, lupus nephritis, lupus cerebritis, and SLAM. We could not find any statistically significant difference between the two groups. This is in contrast to Lit et al. (2007) who showed that the relative mRNA expression T-bet/GATA-3 and IFN-γ/IL-4 correlated with lupus disease activity.

Also, the two groups were compared regarding the laboratory data: CBC, anti-dsDNA, ANA, ESR, complement consumption, and serum creatinine. No statistical significant difference could be elicited between the two groups except for hematological complications in the form of anemia, leucopenia, thrombocytopenia, or pancytopenia with increased frequency of hematological complications in the group carrying the wild genotype (TT) (p=0.016). Chan et al. (2007) demonstrated that there was a significant correlation between serum C_3, C_4, and anti-dsDNA antibody level, with glomerular expression of T-bet and IFN-γ in patients with lupus nephritis.

The failure to replicate reported associations is a common event in the search for genetic determinants of complex diseases, due either to genuine population heterogeneity or a different sort of bias. Also, the differences between our results and other studies can be related to the length of disease duration and methodology to assess clinical and outcome variables.

Background factors (genetic and otherwise) differentiating populations can modify the expression of a gene and lead to a different levels of association. The reduced sample size might be a limitation of our study. It is necessary to reproduce allelic association studies in many ethnically diverse populations to evaluate the real importance of this gene in SLE development and outcome.

Conflict of interest The authors have no financial or proprietary interest in any product mentioned in this paper.

References

Aliprantis AO, Wang J, Fathman JW, Lemaire R, Dorfman DM, Lafyatis R et al (2007) Transcription factor T-bet regulates skin sclerosis through its function in innate immunity and via IL-13. Proc Natl Acad Sci USA 104:2827–2830

Cao B, Yang H, Ding H, Qi S, Gao L, Cui H, Dai Y, Xu C (2007) Association analysis of hepatitis virus B infection with haplotypes of the TBX21 gene promoter region in the Chinese population. Clin Chem Lab Med 45(3):333–338

Chae SC, Shim SC, Chung HT (2009) Association of TBX21 polymorphisms in a Korean population with rheumatoid arthritis. Exp Mol Med 41:33–41

Chan RW, Lai FM, Li EK, Tam LS, Chow KM, Lai KB et al (2007) Intrarenal cytokine gene expression in lupus nephritis. Ann Rheum Dis 66:886–892

Chen S, Zhao W, Tan W, Luo X, Dan Y, You Z, Kuang X, Wang Y, Deng G (2011) Association of TBX21 promoter polymorphisms with type 1 autoimmune hepatitis in a Chinese population. Hum Immunol 72(1):69–73

Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 40:1725

Lai FM, Li EK, Chan RW, Tam LS, Chow KM, Li PK, Szeto CC (2007) Expression of T-bet, a type 1 T-helper cell transcription factor, in the urinary sediment of lupus patients predicts disease flare. Rheumatology 46(1):44–48

Li J, Li J, You Y, Chen S (2012) The role of upstream stimulatory factor 1 in the transcriptional regulation of the human TBX21 promoter mediated by the T-1514C polymorphism associated with systemic lupus erythematosus. Immunogenetics. doi:10.1007/s00251-011-0597-6

Lit LC, Wong CK, Li EK, Tam LS, Lam CW, Lo YM (2007) Elevated gene expression of Th1/Th2 associated transcription factors is correlated with disease activity in patients with systemic lupus erythematosus. J Rheumatol 34:89–96

Raby BA, Hwang ES, Steen KV, Tantisira K, Peng S, Litonjua A, Lazarus R, Giallourakis C, Rioux JD, Sparrow D, Silverman EK, Glimcher LH, Weiss ST (2006) T-bet polymorphisms are associated with asthma and airway hyper-responsiveness. Am J Respir Crit Care Med 173(1):64–70

Sasaki Y, Ihara K, Matsuura N, Kohno H, Nagafuchi S, Kuromaru R et al (2004) Identification of a novel type 1 diabetes susceptibility gene, T-bet. Hum Genet 115:177–184

Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100:655–669

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF et al (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25:1271–1277

Tsokos G (2011) Systemic lupus erythematosus. N Engl J Med 365:2110–2121

Usui T, Preiss JC, Kanno Y, Yao ZJ, Bream JH, O'Shea JJ et al (2006) T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. J Exp Med 203:755–766

Ylikoski E, Kinos R, Sirkkanen N, Pykalainen M, Savolainen J, Laitinen LA et al (2004) Association study of 15 novel single-nucleotide polymorphisms of the T-bet locus among Finnish asthma families. Clin Exp Allergy 34:1049–1055

You Y, Zhao W, Chen S, Tan W, Dan Y, Hao F, Deng G (2010) Association of TBX21 gene haplotypes in a Chinese population with systemic lupus erythematosus. Scand J Rheumatol 39:254–258