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Promoter Methylation and Expression Status of Cytotoxic T-Lymphocyte-Associated Antigen-4 Gene in Patients with lupus

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A B S T R A C T

Systemic lupus erythematous (SLE) is an autoimmune disease with both genetic susceptibility and epigenetic modifications. Autoantibodies directly contribute to the destruction of some organs such as kidneys, joints, skin, lungs, central nervous system, and blood-forming (hematopoietic) system. The CTLA4 plays an important role in inhibition of the activity of T cells and preventing autoimmune disorders, for example; the lupus. We analyzed the promoter methylation, polymorphism, and expression status in CTLA4 gene in patients with lupus. Genomic DNA was isolated from 50 individuals' blood samples with SLE and 50 control participants. CTLA4 gene polymorphisms analysis in polymorphic site, -318(CT) and +49(AG), was done by Tetra-ARMS-PCR. Methylation-specific polymerase chain reaction (MS-PCR) was used to estimate promoter hyper methylation of the CTLA4 gene. The present paper also analyzed CTLA4 mRNA levels in 30 blood samples from the intended participants, and healthy control by real-time PCR. Changes in promoter methylation of CTLA4 gene were remarkably different in patients with lupus than healthy controls (OR= 0.48; 95% CI= 0.1959, 1.202; P-value= 0.005). However, gene expression level of CTLA4 was not statistically different in patients than the healthy control group. This epigenetic study gives us an overview of the role of CTLA4 promoter methylation in pathogenesis of SLE, which causes preventing its expression. As we know CTLA4 has the role in immune regulation and down regulates immune responses. In the future a comprehensive understanding of the epigenetic mechanisms contributing to SLE will likely enable development of new therapeutic agents and strategies that target the deregulated genes or correct the aberrant epigenetic modifications (Epigenetic therapies for SLE).

Introduction

Autoimmune disease occurs when the immune system attacks self-molecules. Many autoimmune disorders are strongly associated with genetic, infectious, and/or environmental predisposing factors (Bengtsson & Rönnblom, 2017). Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with a heterogeneous clinical appearance (Cojocaru,

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Cojocaru, Silosi, & Vrabie, 2011). Although the precise etiologic mechanism is unknown, genetic, hormonal, and environmental factors, as well as immune abnormalities, have been identified (Maidhof & Hilas, 2012). The disease mostly targets young people, especially women (Cojocaru et al., 2011). The concordance rate of lupus in monozygotic twins is greater than in the general population, but it is still incomplete, suggests that environmental factors influence the pathogenesis of SLE (Javierre et al., 2009; Liu & Davidson, 2012). Hormones have long been recognized as agents involved in SLE(Achtman & Werth, 2015; Grimaldi, 2006). Women account for 90% of SLE cases (Cojocaru et al., 2011). Estrogen and prolactin enhance immune responses through diverse mechanisms(Cunningham & Gilkeson, 2011; Shelly, Boaz, & Orbach, 2012). So that its prevalence is estimated to be 9 SLE patients per 100,000 people among women in the United States, between 12 and 64 cases per 100,000 in European-derived populations and 40 cases have been reported per 100,000 people in Iran(Ballestar, Esteller, & Richardson, 2006; Davatchi et al., 2008). The risk of developing SLE is, at least in part, genetic, but it is a complex genetic illness with no clear mendelian pattern of inheritance. The disease tends to occur in families. Siblings of SLE patients have a risk of disease of about 2%. However, even identical twins with SLE are concordant for disease in only 25% of cases and are therefore discordant (i.e., where one twin has SLE and one does not) in about 75% of cases (Ghodke-Puranik & Niewold, 2015; Mohan & Putterman, 2015; Niewold, 2015; Relle, Weinmann-Menke, Scorletti, Cavagna, & Schwarting, 2015; Schur, 1995). At present, by genome-wide association studies (GWAS) of this complex autoimmune disease, >100 gene variations/alleles from different ethnicities are known, which are genetic risk factors for lupus (Harley, Kaufman, Langefeld, Harley, & Kelly, 2009). Another study of GWAS of several thousand allelic variants in case-control studies have identified >30 genes involved in SLE (Sestak, Fürnrohr, Harley, Merrill, & Namjou, 2011). Recent studies have emphasized that this disease, like other autoimmune diseases, is a complex genetic trait with contributions from major histocompatibility complex (MHC) genes and multiple non-MHC genes (Vyse & Kotzin, 1998).

Besides the genetic susceptibility to SLE, epigenetic factors are important in the onset of the disease, as even monozygotic twins are usually discordant for the disease. So far, there are 3 widely accepted major epigenetic marks playing an important role in the pathogenesis of SLE, including DNA methylation, histone modifications and altered miRNA profiling. In SLE, global and gene-specific DNA methylation changes have been demonstrated to occur. Moreover, histone deacetylase inhibitors reverse the skewed expression of multiple genes involved in SLE (Ballestar et al., 2006; Zhan, Guo, & Lu, 2016).

The main pathological characteristic of SLE are inflammation and vascular anomalies including vascular injury and sedimentation of immune complexes (Kaul et al., 2016). The damage to the tissues and organs in lupus is due to an abnormal immune response. In healthy subjects, immunologic response is only against infectious and foreign agents that have entered from the environment, while in SLE, response to self-antigens leads to a lack of the balance in immune system results in failure of tolerance and activation of B and T lymphocytes (Foster, 2007). Other factors include self-reactive antibodies and immune system disorders, as well as apoptosis. The amount of apoptosis in SLE has been increased, but its main cause is unknown (Shao & Cohen, 2011). Apoptotic cells must be quickly removed from tissues so that they elicit neither inflammation nor immune responses. Inefficient clearance of apoptotic cells and subsequent accumulation of apoptotic cell debris provoke a chronic inflammatory response and may lead to breakdown of self-tolerance (Muñoz, Lauber, Schiller, Manfredi, & Herrmann, 2010). In normal mode, these apoptotic cells ingest by phagocytic cells, but SLE patients don't have the ability of clearance of the immune complexes. Monocyte and macrophage phagocytosis are dispoiled by FC receptors for IgG FCGR2A (and FCGR3B)(Maidhof & Hilas, 2012).

Protein number 4 associated with Cytotoxic T-Lymphocyte-Associated protein 4, CTLA4, also known as CD152, is a protein receptor that has a controlling function over immunization and its reduction. This receptor is essentially expressed in

the lymphocyte T-regulator, but it also appears in activated T-lymphocytes. After being mixed with CD28 and CD86 at the surface of the antigen processor cells, this protein plays the role of a "silencing key"(Dariavach, Mattéi, Golstein, & Lefranc, 1988). Cytotoxic T-lymphocyte antigen 4 (CTLA4) gene is located in chromosome 2q33(Kordi-Tamandani, Vaziri, Dahmardeh, & Torkamanzehi, 2013). CTLA4 has much higher overall affinities for both CD80 and CD86 ligands compared to CD28. However, CTLA4 also confers 'signaling-independent' T-cell inhibition through sequestration of CD80 and CD86 ligands from CD28 engagement. Furthermore, CTLA4 plays a pivotal role in cancer development and progression(Yang et al., 2007).

It has been reported that epigenetic regulation is one of the mechanisms behind CTLA4 expression in autoimmune disease, so we examined the relationship between CTLA4 gene methylation and expression in Lupus, and because genetic polymorphisms contribute to disease susceptibility, through effects on gene expression and function we also examined the polymorphisms of CTLA4 gene.

2. Materials and Methods

2.1 Patients and tissue samples

The present case–control study comprises 50 samples including patients who had undergone a curative systemic lupus erythematosus. Control samples, also 50 in number, were selected from the unaffected healthy individual; they were confirmed as non-affected by pathologists. All patients and healthy donors enrolled in the present study signed the Consent Forms approved by the Ethics Review Committee.

2.2 DNA modification and methylation-specific PCR (MSP):

Peripheral blood samples were obtained from lupus patients and the healthy subjects and DNA was extracted using a standard procedure. Bisulfite conversion of genomic DNA was performed with the DNA Bisulfite kit according to the manufacturer's protocol. After treatment with sodium bisulfite, unmethylated cytosine residues are converted to uracil whereas 5-methylcytosine (5mC) remains unaffected. Methylation-specific PCR (MSP) was performed using primers specific for the methylated (M) or unmethylated (U) alleles of CTLA4 gene; the primer sequences and annealing temperatures are summarized in Table I. Each PCR reaction contained 1 µL of bisulfite-modified DNA, 0.5 µL of HS-Taq DNA polymerase (2.5 U/µL, Cat. No. 01011, Pishgam Biotechnology), 2.5 µL of 10X buffer, 0.8 mM dNTP mix, 0.4 mM of each primer, and 1.5 mM Mg2+; the reaction had final volume of 25 µL using RNase-free double-distilled water. The MSP amplification conditions was included initial denaturation step at 95°C for 10 min followed by 35 cycles of denaturation at 95°C for 30s, the annealing temperature at 58°C (M) or 57°C (U) for 30s, and extension at 72°C for 40s, which was followed by 1 cycle of final extension at 72°C for 10 minutes. The PCR products were analyzed by electrophoresis using 2% agarose gel followed by ethidium bromide staining and visualization using UV transilluminator.

2.3 RNA isolation and Real Time PCR:

RNA extraction and RT-PCR of total RNA was done using CinnaPure RNA kit (Cat. No. PR891620, Tehran, Iran) according to the manufacturer's protocol. Subsequently, first-strand cDNA was synthesized from 1–10 µg of total RNA using 2-steps RT-PCR kit (Cat. No. RTPL12, Vivantis Technologies) according to the supplier's protocol. Real-time PCR was performed in reactions of 16 µl volume containing Power SYBR Green PCR Master Mix (Applied Biosystems); Applied Biosystems Real-Time PCR instrument was employed, and the reaction conditions included initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 15s, the annealing temperature at 59°C for 30s, and extension at 72°C for 40s, which was followed by 1 cycle of final extension at 72°C for 10 min; finally, the melting curve was obtained over the range 60–95 °C. The sequences of the primers used for expression analysis are listed in Table I. The expression of CTLA4 was normalized to β actin internal control, and analyzed using the 2-ΔΔCT method.

2.4 Tetra_ARMS PCR

Analysis of polymorphism was done by Tetra_ARMS PCR with specific primers, and summarized in Table I. Amplification began with an initial denaturation step: 95°C for 10 min,

followed by 35 cycles (95 °C for 30s, 60°C for 30s (+49AG primer), 58 °C for 30s (-318CT primer), and 72 °C for 30s) which was completed by incubation at 72 °C for 10 min.

Table 1. Methylation, Real-time and T-ARMS–PCR primer sequences and annealing temperatures

Genes	Sequences(5'-3')	Product size	Annealing temp (0C)
CTLA4-M	F:GTTTGTTTTTGGTCGTCGTC R:CGAACGCTATCTTACTCCGAA	131	47
CTLA4-U	F:GTTTGTTTGTTTTTGGTTGTTGTT R:CTTCCCAAACACTATCTTACTCCAA	140	48
CTLA4	F:GGCGCAGCACGTTGAAAAAT R:CACATCATCATGGTCCAGATGG		59
β-actin	F:AGAGCTACGAGCTGCCTGA R:AGCACTGTGTTGGCGTACAG		65
+49CTLA4 (A/G)	Fo: GTGGGTTCAAACACATTTCAAAGCTTCAGG Ro: TCCATCTTCATGCTCCAAAAGTCTCACTC Fi(G): GCACAAGGCTCAGCTGAACCTGGATG Ri(A):ACAGGAGAGTGCAGGGCCAGGTCCTAGT	229 120 162	60
-318CTLA4 (C/T)	Fo: CAATGAAATGAATTGGACTGGATG Ro: TGCACACACAGAAGGCTCTTGAATA Fi(C): CTCCACTTAGTTATCCAGATCTTC Ri(T): ACTGAAGCTTCATGTTCACTCTA	296 201 141	58

2.5 Statistical analysis:

SPSS software Version 20 was applied for statistical analyses. Categorical data was analyzed using chi-squared test ($\chi 2$), and the association between CTLA4 gene methylation and risk of systemic lupus erythematous was examined by estimating odds ratio (OR) and 95% confidence interval (95%CI) using binary logistic regression analysis. Mann–Whitney test was used for comparing expression data between SLE samples and their margins. The level of statistical significance was set as $p \leq 0.05$.

3. Results:

Since aberrant promoter methylation is frequently observed in activation or inactivation of genes, we analyzed the CTLA-4 methylation status in samples including patients who had undergone a curative systemic lupus erythematous and healthy control group. As shown in Table 2, methylation analysis of CTLA4 gene showed that there is a statistically strong association between promoter methylation statuses in patient and control subjects. The frequency of methylation statuses is given in Table 2.

Analysis of gene expression for the CTLA4 gene has not revealed a significant difference between patient subjects and healthy control samples, as shown in Table 3.

The frequencies of -318 C/T and +49 A/G polymorphisms of CTLA4 gene in systemic lupus erythematous patients are shown in Table 4.

Table 2. Methylation status of CTLA4 gene in patient with SLE and healthy controls

Gene	Methylation Status	Controls N=50	Case N=50	pvalue	OR	CI
CTLA4	Present	40(80%)	33(66%)	0.001	0.48	0.1959
	Absent	10(20%)	17(34%)			

Table 3. Comparison of real time gene expression for CTLA4 gene between patients with SLE and healthy controls

Gene	N	Mean±SD	P-value
CTLA4	15	1.240±0.082	0.59
	15	1.07±0.736	

Table 4. Frequencies of cytotoxic T lymphocyte antigen-4 (CTLA-4) -318 C/T and +49 A/G polymorphisms in patients with SLE

Genotypes	Patients (%)	Alleles	Patients (%)	P-value
-318 C/T				
C/C	11(22%)	C	38(76%)	0.034
C/T	16(36%)	T	28(24%)	0.002
T/T	3(6%)			
+49 A/G				
A/A	5(10%)	A	22(44%)	0.08
A/G	11(22%)	G	39(56%)	0.05
G/G	14(28%)			

4. Discussion:

Genes that involved in various pathways are responsible for development of lupus disease. This pathways are include the autoimmune and auto inflammatory and also apoptosis and inflammation. Any pathway causes variation in severity and type of organ affected by the disease.

There are various reports from different countries about the prevalence and characteristics of lupus disease. This is clearly due to the polygenic disorder and the environment effects on disease. Over the years, increasing evidence has demonstrated the important role for aberrant epigenetics factors such as CpG-DNA methylation, histone-tail modifications, and micro-RNAs (miRNAs) as the main epigenetic mechanisms of gene regulation in the pathogenesis of most diseases and also SLE (Altorok & Sawalha, 2013; Ballestar et al., 2006; Iraji & Arish, 2019).

Also, in various studies about the association between the polymorphisms of genes involved in this disease, in various geographical regions, different results suggest the need for a comprehensive study in Iran. All of the results from a few recent years around polymorphism + 49AG show that this contradiction is due to the low sample size and lacks of related literature (Shojaa et al., 2015), and the reason for different results on a single polymorphism should have been because of the influence of the environmental factors involved in the development of the disease, such as age, sex, and the use of different drugs and also the onset of the disease, and even smoking.

The Cytotoxic T lymphocyte associated antigen 4 (CTLA4) plays an important role in the regulation of T cells, Many studies have shown this by stopping the activity of CTLA4 (Kordi-Tamandani et al., 2013).

A growing number of literature supports the concept that impaired CD4+ T cell DNA methylation, caused by environmental influences, contributes to SLE pathogenesis by altering gene expression in genetically predisposed people (Patel & Richardson, 2010), and also T cells from patients with active SLE have a 17% decrease in genomic deoxymethylcytosine content, This suggests that preventing or correcting the altered methylation patterns of T cell DNA, causes auto

reactivity in vitro and a SLE-like disease in vivo and could be therapeutic in SLE (Lu et al., 2002).

In present study, as mentioned above, there is a strong relationship between gene methylation and SLE which cause preventing its expression. Increasing the excessive activity of T cells, and consequently, the incidence of lupus autoimmune disease has been reported through the same study. The analysis of gene expression in this study also did not show a meaningful relationship that may be due to the sample size.

Deciphering the contribution of epigenetic alterations to the pathogenesis of SLE will provide promising insights in this complex autoimmune disease. We hope that in the future, with epigenome-wide studies coupled with functional analysis of the epigenomic changes discovered, will uncover novel pathways important in disease pathogenesis and improve treatment methods. Epigenetic therapies for SLE may be feasible in the future, particularly if they are designed to target specific regions within the genome. Epigenetic alterations are (potentially) reversible and hence candidates for the development of new therapeutics.

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Conflict of Interest Statement

The authors declare no conflict of interest.

References:

Achtman, J. C., & Werth, V. P. (2015). Pathophysiology of cutaneous lupus erythematosus. Arthritis research & therapy, 17(1), 182.

Altorok, N., & Sawalha, A. H. (2013). Epigenetics in the pathogenesis of systemic lupus erythematosus. Current opinion in rheumatology, 25(5), 569-576.

Ballestar, E., Esteller, M., & Richardson, B. C. (2006). The epigenetic face of systemic lupus erythematosus. The Journal of Immunology, 176(12), 7143-7147.

Bengtsson, A., & Rönnblom, L. (2017). Systemic lupus erythematosus: still a challenge for physicians. Journal of internal medicine, 281(1), 52-64.

Cojocaru, M., Cojocaru, I. M., Silosi, I., & Vrabie, C. D. (2011). Manifestations of systemic lupus erythematosus. Maedica, 6(4), 330.

Cunningham, M., & Gilkeson, G. (2011). Estrogen receptors in immunity and autoimmunity. Clinical reviews in allergy & immunology, 40(1), 66-73.

Dariavach, P., Mattéi, M. G., Golstein, P., & Lefranc, M. P. (1988). Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. European journal of immunology, 18(12), 1901-1905.

Davatchi, F., Jamshidi, A.-R., Banihashemi, A. T., Gholami, J., Forouzanfar, M. H., Akhlaghi, M., . . . Salesi, M. (2008). WHO-ILAR COPCORD study (stage 1, urban study) in Iran. The Journal of rheumatology, 35(7), 1384-1390.

Foster, M. H. (2007). T cells and B cells in lupus nephritis. Paper presented at the Seminars in nephrology.

Ghodke-Puranik, Y., & Niewold, T. B. (2015). Immunogenetics of systemic lupus erythematosus: a comprehensive review. Journal of autoimmunity, 64, 125-136.

Grimaldi, C. M. (2006). Sex and systemic lupus erythematosus: the role of the sex hormones estrogen and prolactin on the regulation of autoreactive B cells. Current opinion in rheumatology, 18(5), 456-461.

Harley, I. T., Kaufman, K. M., Langefeld, C. D., Harley, J. B., & Kelly, J. A. (2009). Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. Nature Reviews Genetics, 10(5), 285.

Iraji, R., Arish, M., Aminifard, M., Dianat, T. Expression and promoter-hyper methylation analysis of MGMT gene in patients with pterygium. Journal of Epigenetics, 2019; (): 1-6. doi: 10.22111/jep.2019.28421.1009.

Javierre, B. M., Fernandez, A. F., Richter, J., Al-Shahrour, F., Martin-Subero, J. I., Rodriguez-Ubreva, J., . . . Rider, L. G. (2009). Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. Genome research.

Kaul, A., Gordon, C., Crow, M. K., Touma, Z., Urowitz, M. B., van Vollenhoven, R., . . . Hughes, G. (2016). Systemic lupus erythematosus. Nature Reviews Disease Primers, 2, 16039. doi:10.1038/nrdp.2016.39

Kordi-Tamandani, D. M., Vaziri, S., Dahmardeh, N., & Torkamanzehi, A. (2013). Evaluation of polymorphism, hypermethylation and expression pattern of CTLA4 gene in a sample of Iranian patients with schizophrenia. Molecular biology reports, 40(8), 5123-5128.

Liu, Z., & Davidson, A. (2012). Taming lupus—a new understanding of pathogenesis is leading to clinical advances. Nature medicine, 18(6), 871.

Lu, Q., Kaplan, M., Ray, D., Ray, D., Zacharek, S., Gutsch, D., & Richardson, B. (2002). Demethylation of ITGAL (CD11a) regulatory sequences in systemic lupus erythematosus. Arthritis & Rheumatism, 46(5), 1282-1291.

Maidhof, W., & Hilas, O. (2012). Lupus: an overview of the disease and management options. Pharmacy and Therapeutics, 37(4), 240.

Mohan, C., & Putterman, C. (2015). Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. Nature Reviews Nephrology, 11(6), 329.

Muñoz, L. E., Lauber, K., Schiller, M., Manfredi, A. A., & Herrmann, M. (2010). The role of defective clearance of apoptotic cells in systemic autoimmunity. Nature Reviews Rheumatology, 6(5), 280.

Niewold, T. B. (2015). Advances in lupus genetics. Current opinion in rheumatology, 27(5), 440.

Patel, D. R., & Richardson, B. C. (2010). Epigenetic mechanisms in lupus. Current opinion in rheumatology, 22(5).

Relle, M., Weinmann-Menke, J., Scorletti, E., Cavagna, L., & Schwarting, A. (2015). Genetics and novel aspects of therapies in systemic lupus erythematosus. Autoimmunity Reviews, 14(11), 1005-1018.

Schur, P. (1995). Genetics of systemic lupus erythematosus. Lupus, 4(6), 425-437.

Sestak, A. L., Fürnrohr, B. G., Harley, J. B., Merrill, J. T., & Namjou, B. (2011). The genetics of systemic lupus erythematosus and implications for targeted therapy. Annals of the Rheumatic Diseases, 70(Suppl 1), i37-i43.

Shao, W.-H., & Cohen, P. L. (2011). Disturbances of apoptotic cell clearance in systemic lupus erythematosus. Arthritis research & therapy, 13(1), 202.

Shelly, S., Boaz, M., & Orbach, H. (2012). Prolactin and autoimmunity. Autoimmunity Reviews, 11(6-7), A465-A470.

Shojaa, M., Aghaie, M., Khashayar, P., Amoli, M., Qorbani, M., & Mohammadi, Z. (2015). The survey of association between Polymorphism of CTLA-4 Exon 1 with Systemic Lupus Erythematosus. Armaghane Danesh Bimonthly Journal, 19(10), 893-901.

Vyse, T., & Kotzin, B. (1998). Genetic susceptibility to systemic lupus erythematosus. Annual review of immunology, 16(1), 261-292.

Yang, J. C., Hughes, M., Kammula, U., Royal, R., Sherry, R. M., Topalian, S. L., . . . Mavroukakis, S. (2007). Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. Journal of immunotherapy (Hagerstown, Md.: 1997), 30(8), 825.

Zhan, Y., Guo, Y., & Lu, Q. (2016). Aberrant Epigenetic Regulation in the Pathogenesis of Systemic Lupus Erythematosus and Its Implication in Precision Medicine. Cytogenetic and genome research, 149(3), 141-155.