CTLA-4 Polymorphisms and Systemic Lupus Erythematosus: A Comprehensive Meta-Analysis

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Objective: The aim of this study was to determine whether the SNPs +49A/G and CT60A/G of the CTLA-4 gene are associated with susceptibility to systemic lupus erythematosus (SLE). **Methods:** The comprehensive meta-analysis for +49A/G included 1753 cases and 2279 controls, and for CT60A/G included 676 cases and 576 controls. Allelic and genotypic comparisons between cases and controls were evaluated. For +49A/G, we also subdivided it by population. **Results:** For +49A/G, statistically significant differences were not noted (fixed: odds ratio [OR]: 1.033, 95% confidence interval [95% CI]: 0.937–1.139; random: OR: 1.038, 95% CI: 0.907–1.188). When subdivided into Asia and Europe subgroups, it showed that this polymorphism is still not significantly associated with SLE [for Asia: (fixed: OR: 1.069, 95% CI: 0.932–1.227; random: OR: 1.055, 95% CI: 0.846–1.316); for Europe: (fixed: OR: 0.988, 95% CI: 0.842–1.161; random: OR: 1.015, 95% CI: 0.805–1.281)]. And CT60A/G also did not demonstrate significant differences with SLE (fixed: OR: 1.099, 95% CI: 0.922–1.31; random: OR: 0.918, 95% CI: 0.581–1.448). **Conclusion:** The results suggest that the CTLA-4 gene was not associated with SLE. Further investigations are required to identify whether other at-risk polymorphisms within CTLA-4 confer a risk of SLE and to clarify the role of the CTLA-4 gene.

Introduction

THE CTLA-4 GENE is located on chromosome 2q33 and consists of four exons and three introns (Brunet *et al.*, 1987). Exon 1 encodes a leader peptide sequence, exon 2 codes for an immunoglobulin (Ig) domain, and exon 3 and 4 code for the hydrophobic transmembrane domain and the cytoplasmic domain respectively (Kucharska *et al.*, 2009). CTLA-4 and CD28 (also located on 2q33) are members of the Ig superfamily and bind to the B7 molecule on antigen-presenting cells. This completes the activation initiated when the antigen-specific cell-surface T-cell receptor (CD3 complex) engages the antigen bound to a major histocompatibility complex class II molecule on the surface of an antigen-presenting cell (Vaidya and Pearce, 2004). CTLA-4 has a greater affinity for the B7 molecule than does CD28 and it downregulates T-cell function. Therefore, it may play a crucial role in T-cell-mediated autoimmunity and thus in susceptibility to autoimmune diseases, including systemic lupus erythematosus (SLE).

CTLA-4 Ig suppresses lupus-like illness in the New Zealand Black/New Zealand White F1 mouse model and prolongs life (Finck *et al.*, 1994). CTLA-4 Ig is a chimeric fusion protein consisting of the CTLA-4 extracellular domain and the

Fc portion of human IgG1. Variants in the expression, regulation, and ligand binding for CTLA-4 are, therefore, strong candidates for a role in the pathogenesis of SLE. The characteristics of SLE include the production of autoantibodies directed at nuclear, cytoplasmic, and cell surface autoantigens. These autoantibodies cause end-organ damage via an inflammatory response to immune complexes. Although the etiopathogenesis remains elusive, genetic factors seem to be important in the development of this disease. Twins studies show significantly higher concordance rates in monozygotic than in dizygotic twins (more than 10 times greater) (Deapen *et al.*, 1992; Brix *et al.*, 2001). Loss of actively maintained tolerance to self-antigens and the generation of autoimmunity in the context of immune ignorance are possible mechanisms of autoimmunity. CTLA-4 plays an essential role in peripheral tolerance and any dysregulation of the CTLA-4 may affect the pathogenesis of SLE. Linkage studies in autoimmune diseases, including SLE, have shown a linkage to 2q33-35 in an interval that includes CTLA-4 (Gaffney *et al.*, 1998; Quintero-del-Rio *et al.*, 2002, 2004). And association of CTLA-4 polymorphisms with autoimmune disorders has been demonstrated in several studies (Einarsdottir *et al.*, 2003; Kavvoura and Ioannidis, 2005; Takahashi and Kimura, 2010).

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The functional SNPs of CTLA-4 are believed to be mainly in +49A/G (rs231775) and CT60A/G (rs3087243). The +49A/G (rs231775) SNP causes an amino acid change from threonine to alanine in the peptide leader sequence of the CTLA-4 protein (Chistiakov and Turakulov, 2003), whereas CT60A/G (rs3087243) is important for efficient splicing and production of soluble CTLA-4, and may play a role in mRNA stability of sCTLA-4 (Ueda *et al.*, 2003). A number of case-control studies have been conducted to investigate the association of these polymorphisms with SLE (Heward *et al.*, 1999; Matsushita *et al.*, 1999; Pullmann *et al.*, 1999; D'Alfonso *et al.*, 2000; Ahmed *et al.*, 2001; Lee *et al.*, 2001; Liu *et al.*, 2001; Hudson *et al.*, 2002; Aguilar *et al.*, 2003; Barreto *et al.*, 2004; Parks *et al.*, 2004; Torres *et al.*, 2004; Ulker *et al.*, 2009; Chua *et al.*, 2010; Kimkong *et al.*, 2011). However, these studies have shown inconclusive or contradictory results. This inconsistency may be due to studies with inadequate statistical power, racial and ethnic differences, publication bias, or uncorrected multiple hypothesis testing.

Meta-analysis is a powerful tool for summarizing the results from different studies by producing a single estimate of the major effect with enhanced precision. One of the major advantages of meta-analysis is to increase sample size, which may reduce the probability that random error will produce false-positive or false-negative associations. In this meta-analysis, we explored whether the CTLA-4 polymorphisms {+49A/G (rs231775) and CT60A/G (rs3087243)} contribute to SLE susceptibility.

Materials and Methods

Identification of eligible studies

We performed an exhaustive search for studies that examined the association of the CTLA-4 polymorphisms with SLE. A search of the literature was made using Medline citations to identify available articles in which CTLA-4 polymorphisms were determined in SLE patients and controls through February 2012. References in the Medline-cited studies were reviewed to identify additional reports not indexed by Medline. The

FIG. 1. Meta-analysis of association studies of +49A/G (rs231775) and CT60A/G (rs3087243) polymorphisms and systemic lupus erythematosus (SLE). **(A)** Meta-analysis of association studies of the +49A/G polymorphism (rs231775) and SLE (fixed model). The overall odds ratio (OR) is shown. The OR of each study is marked with a black square. The overall OR is indicated by gray diamond. **(B)** Meta-analysis of association studies of the +49A/G polymorphism (rs231775) and SLE (random model). The overall OR is shown. The OR of each study is marked with a black square. The overall OR is indicated by gray diamond. **(C)** Meta-analysis of association studies of the CT60A/G polymorphism (rs3087243) and SLE (fixed model). The overall OR is shown. The OR of each study is marked with a black square. The overall OR is indicated by gray diamond. **(D)** Meta-analysis of association studies of the CT60A/G polymorphism (rs3087243) and SLE (random model). The overall OR is shown. The OR of each study is marked with a black square. The overall OR is indicated by gray diamond.

following key words and subject terms were searched: "cytotoxic T lymphocyte-associated antigen," "CTLA-4," "systemic lupus erythematosus," "candidate gene," and "SLE." We have only used data from full-published articles, not from any meeting or conference abstract. Two polymorphic sites {+49A/G (rs231775) and CT60A/G (rs3087243)} were identified and used for the meta-analysis. Finally, 15 references were included in this research (Heward *et al.*, 1999; Matsushita *et al.*, 1999; Pullmann *et al.*, 1999; D'Alfonso *et al.*, 2000; Ahmed *et al.*, 2001; Lee *et al.*, 2001; Liu *et al.*, 2001; Hudson *et al.*, 2002; Aguilar *et al.*, 2003; Barreto *et al.*, 2004; Parks *et al.*, 2004; Torres *et al.*, 2004; Ulker *et al.*, 2009; Chua *et al.*, 2010; Kimkong *et al.*, 2011). We subdivided the group of +49A/G (rs231775) into Asia subgroup and Europe subgroup (five studies about Europe and eight studies about Asia).

Quality assessments

All the studies included satisfied all the following criteria: they (1) were association studies between the +49A/G (rs231775) and CT60A/G (rs3087243) polymorphisms in the CTLA-4 gene and SLE; (2) used disease-free people as controls; (3) provided genotypes or alleles distribution in both case and control groups; (4) were independent studies and the subject groups investigated did not overlap with each other; (5) were published in peer-reviewed journals and were indexed by PubMed or cited by articles indexed by PubMed. Authors were contacted where clarification was required.

Data extraction

The following information was independently extracted from the identified studies by two participants in the meta-analysis:

first author, journal, year of publication, ethnicity of the study population, number of cases and controls or odds ratio (OR), country in which the study was conducted, and confirmation of diagnosis. The results were compared and any disagreement was discussed and resolved by consensus.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was tested in controls within each study. Deviation from HWE was tested using the χ^2 test. The effect size was represented by an OR with 95% confidence interval (CI) (Fig.). Sensitivity analysis was conducted by removing each study and analyzing the others to ensure no single study was totally responsible for overall results (Fig. 2). Publication biases were also analyzed (Fig. 3). The significance level was set at 0.05, and all *p*-values were two-tailed.

The meta-analysis was performed using Comprehensive Meta-Analysis software (Version 2.2.046; Biostat, Englewood, NJ).

Results

The eligible studies for analysis included a total of 2148 cases with SLE and 2572 controls (Table 1). Fifteen references met our criteria, of which 14 (Studies No. 1–9, 11–15) studied the +49 A/G (rs231775) variant and 3 (Studies No. 10, 14, and 15) studied the CT60A/G variant (rs3087243) (some references cover more than one variant) (Table 1). The meta-analysis of all the studies about +49A/G (rs231775) or CT60A/G (rs3087243) polymorphisms was not significantly associated with SLE [+49A/G: fixed: OR and (95% CI): 1.033 (0.937–1.139), *p*=0.516; random: OR and (95% CI): 1.038 (0.907–1.188), *p*=0.585; CT60A/G: fixed: OR and (95% CI): 1.099

FIG. 2. The sensitivity analysis of +49A/G (rs231775) and CT60A/G (rs3087243). **(A)** The sensitivity analysis of +49A/G (rs231775). When any one of the studies was removed, the heterogeneity of the population was not changed. The sensitivity of each study is marked with a black square. The overall sensitivity is indicated by gray diamond. **(B)** The sensitivity analysis of CT60A/G (rs3087243). When any one of the studies was removed, the heterogeneity of the population was not changed. The sensitivity of each study is marked with a black square. The overall sensitivity is indicated by gray diamond.

FIG. 3. Egger's funnel plots of publication bias analysis for +49A/G (rs231775) and CT60A/G (rs3087243). **(A)** Egger's funnel plots of publication bias analysis for the +49A/G polymorphism (rs231775). The larger the deviation from the funnel curve of each study, the more pronounced the asymmetry. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies. **(B)** Egger's funnel plots of publication bias analysis for the CT60A/G polymorphism (rs3087243). The larger the deviation from the funnel curve of each study, the more pronounced the asymmetry. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies.

(0.922–1.31), $p=0.293$; random: OR and (95% CI): 0.918 (0.581–1.448), $p=0.712$] (Fig. 1A–D). We subdivided the group of +49A/G (rs231775) into Asia subgroup (eight studies about Asia) and Europe subgroup (five studies about Europe) and found that, still, there is no significant association between the polymorphism and the SLE disease. [for Asia: (fixed: OR:

1.069, 95% CI: 0.932–1.227; random: OR: 1.055, 95% CI: 0.846–1.316); for Europe: (fixed: OR: 0.988, 95% CI: 0.842–1.161; random: OR: 1.015, 95% CI: 0.805–1.281)] (Supplementary Figs. S1–S4; Supplementary Data are available online at www.liebertpub.com/gtmb) A sensitivity analysis was done and the data shown (Fig. 2A, B). The sensitivity analysis

TABLE 1. CHARACTERISTICS OF INDIVIDUAL STUDIES INCLUDED IN META-ANALYSIS

	Study	Year	Country	Ethnicity	Polymorphisms	Case	Control
1	R. Pullmann, Jr.	1999	Slovak	European	+49AG	102	76
2	M. Matsushita	1999	Japan	Asian	+49AG	71	150
3	J. Heward	1999	England	European	+49AG	126	363
4	S. D'alfonso	2000	Italy	European	+49AG	99	99
5	S. Ahmed	2001	Japan	Asian	+49AG	113	200
6	M.-F. Liu	2001	China	Asian	+49AG	81	81
7	Y.H. Lee	2001	Korea	Asian	+49AG	80	86
8	L.L. Hudson	2002	Korea	Asian	+49AG	130	200
9	F. Aguilar	2003	Spain	European	+49AG	276	194
10	B. Torres	2004	Spain	European	CT60	395	293
11	C.G. Parks	2004	America	American	+49AG	229	274
12	M. Barreto	2004	Portugal	European	+49AG	118	173
13	M. Ulker	2009	Turkey	Asian	+49AG	47	100
14	K.-H. Chua	2010	Malaysia	Asian	+49AG, CT60	130	130
15	I. Kimkong	2011	Thailand	Asian	+49AG, CT60	151	153

showed that when any one of the studies was removed, the heterogeneity of the population was not significantly changed (Fig. 2A, B). This indicated that no heterogeneity existed in the population. There was no evidence that the magnitude of the overall OR estimates changed in the same direction over time. And there is also no publication bias in either +49A/G (rs231775) or CT60A/G (rs3087243) (Fig. 3A, B).

Discussion

SLE is described as a systemic, chronic autoimmune disease, which causes the deposition of immune complexes in different organs. Overall, many genes encoding proteins, such as complement components C1q and C4, interleukin-10, and tumor necrosis factors α and β with particular functions in the immune system have been considered important candidates that may be associated with SLE development (Bettinotti *et al.*, 1993; Walport *et al.*, 1998; Pickering *et al.*, 2000; Nath *et al.*, 2005; Lee *et al.*, 2006; Gateva *et al.*, 2009).

Genetic risk factors for SLE are important at many levels. They provide insight into pathogenesis, aid in diagnosis and prognosis, and, perhaps one day, influence therapeutic choices. When multiple polymorphisms at multiple candidate genes are tested for association with complex diseases like SLE, the likelihood of reporting false positive results is increased. Various strategies have been suggested to deal with this problem, including increasing the statistical threshold for reporting a positive association (Colhoun *et al.*, 2003). Alternatively, meta-analysis is a strategy to increase sample size in an attempt to reduce the pernicious influence of the stochastic processes on false-positive and false-negative associations.

CTLA-4 signaling mediates a negative regulator in both the cellular and the humoral immune responses and mediates antigen-specific apoptosis (Gribben *et al.*, 1995). Negative signaling via CTLA-4 plays an active role in regulating autoreactive T cells. Disruption of the normal physiologic control provided by the CTLA-4 can contribute to the pathogenesis of autoimmune diseases, as demonstrated by gene knockout studies in mice (Waterhouse *et al.*, 1995). Autoimmune diseases probably share some genetic background and therefore, CTLA-4 may contribute to multiple autoimmune phenotypes. Consequently, CTLA-4 polymorphisms have been associated with several autoimmune disorders such as type I diabetes, autoimmune thyroid disease, Grave's disease, rheumatoid arthritis, and multiple sclerosis (Kristiansen *et al.*, 2000; Scalapino and Daikh 2008). A number of studies have tested the association of CTLA-4 markers from exon-1 and other polymorphisms with SLE, but the results are conflicting. To better understand the association between this polymorphism and SLE risk, an overall analysis is needed with a large sample analysis performed and heterogeneity explored.

In this meta-analysis, we investigated the association between the CTLA-4 +49A/G (rs231775) and CT60A/G (rs3087243) polymorphisms and SLE risk. For the +49A/G polymorphism (rs231775), our meta-analysis on the available studies showed that the G allele was not significantly associated with increased SLE risk. And also, when subdivided into Asia subgroup and Europe subgroup, the +49A/G site (rs231775) was still not significantly associated with SLE. For the CT60A/G (rs3087243) polymorphism, the result showed that the allele was not significantly associated with SLE.

However, some limitations of this meta-analysis should be discussed. First, this meta-analysis only focused on articles published in English and reported in other languages that might bias the present results, even though the publication bias were not presently detected with Begg's test. Additionally, there might be eligible studies that were not published, not indexed by electronic databases, or published in the journals we did not cover. Second, the lack of individual participants' data has restricted further adjustments by other covariables, such as specific outcome, sex, smoking, drinking, and so on.

Despite these limitations, our meta-analysis suggests that the both CTLA-4 +49A/G polymorphism (rs231775) and CT60A/G polymorphism (rs3087243) are not significantly associated with higher SLE risk.

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Author Disclosure Statement

The authors have declared that no competing interests exist.

References

- Aguilar F, *et al.* (2003) CTLA4 polymorphism in Spanish patients with systemic lupus erythematosus. *Hum Immunol* 64: 936–940.
- Ahmed S, *et al.* (2001) Association of CTLA-4 but not CD28 gene polymorphisms with systemic lupus erythematosus in the Japanese population. *Rheumatology (Oxford)* 40:662–667.
- Barreto M, *et al.* (2004) Evidence for CTLA4 as a susceptibility gene for systemic lupus erythematosus. *Eur J Hum Genet* 12:620–626.
- Bettinotti MP, *et al.* (1993) Polymorphism of the tumor necrosis factor beta gene in systemic lupus erythematosus: TNFB-MHC haplotypes. *Immunogenetics* 37:449–454.
- Brix TH, *et al.* (2001) Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *J Clin Endocrinol Metab* 86:930–934.
- Brunet JF, *et al.* (1987) A new member of the immunoglobulin superfamily-CTLA-4. *Nature* 328:267–270.
- Chistiakov DA, Turakulov RI (2003) CTLA-4 and its role in autoimmune thyroid disease. *J Mol Endocrinol* 31:21–36.
- Chua KH, *et al.* (2010) Study of the CTLA-4 gene polymorphisms in systemic lupus erythematosus (SLE) samples from Malaysia. *Ann Hum Biol* 37:274–280.
- Colhoun HM, *et al.* (2003) Problems of reporting genetic associations with complex outcomes. *Lancet* 361:865–872.
- D'Alfonso S, *et al.* (2000) Systemic lupus erythematosus candidate genes in the Italian population: evidence for a significant association with interleukin-10. *Arthritis Rheum* 43:120–128.
- Deapen D, *et al.* (1992) A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 35:311–318.
- Einarsdottir E, *et al.* (2003) The CTLA4 region as a general autoimmunity factor: an extended pedigree provides evidence for synergy with the HLA locus in the etiology of type 1 diabetes mellitus, Hashimoto's thyroiditis and Graves' disease. *Eur J Hum Genet* 11:81–84.
- Finck BK, *et al.* (1994) Treatment of murine lupus with CTLA4Ig. *Science* 265:1225–1227.

- Gaffney PM, *et al.* (1998) A genome-wide search for susceptibility genes in human systemic lupus erythematosus sibpair families. *Proc Natl Acad Sci U S A* 95:14875–14879.
- Gateva V, *et al.* (2009) A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 41:1228–1233.
- Gribben JG, *et al.* (1995) CTLA4 mediates antigen-specific apoptosis of human T cells. *Proc Natl Acad Sci U S A* 92:811–815.
- Heward J, *et al.* (1999) The A-G polymorphism in exon 1 of the CTLA-4 gene is not associated with systemic lupus erythematosus. *Ann Rheum Dis* 58:193–195.
- Hudson LL, *et al.* (2002) CTLA-4 gene polymorphisms in systemic lupus erythematosus: a highly significant association with a determinant in the promoter region. *Hum Genet* 111:452–455.
- Kavvoura FK, Ioannidis JP (2005) CTLA-4 gene polymorphisms and susceptibility to type 1 diabetes mellitus: a HuGE Review and meta-analysis. *Am J Epidemiol* 162:3–16.
- Kimkong I, *et al.* (2011) Association between CTLA-4 polymorphisms and the susceptibility to systemic lupus erythematosus and Graves' disease in Thai population. *Asian Pac J Allergy Immunol* 29:229–235.
- Kristiansen OP, *et al.* (2000) CTLA-4 in autoimmune diseases: a general susceptibility gene to autoimmunity? *Genes Immun* 1:170–184.
- Kucharska AM, *et al.* (2009) Expression of CD152 (CTLA-4) in children with autoimmune thyroiditis and +49 A/G polymorphism of exon 1 of the CTLA-4 gene. *J Physiol Pharmacol* 60:77–80.
- Lee YH, *et al.* (2001) Polymorphisms of the CTLA-4 exon 1 and promoter gene in systemic lupus erythematosus. *Lupus* 10:601–605.
- Lee YH, *et al.* (2006) Meta-analysis of TNF-alpha promoter –308 A/G polymorphism and SLE susceptibility. *Eur J Hum Genet* 14:364–371.
- Liu MF, *et al.* (2001) CTLA-4 gene polymorphism in promoter and exon-1 regions in Chinese patients with systemic lupus erythematosus. *Lupus* 10:647–649.
- Matsushita M, *et al.* (1999) Lack of a strong association of CTLA-4 exon 1 polymorphism with the susceptibility to rheumatoid arthritis and systemic lupus erythematosus in Japanese: an association study using a novel variation screening method. *Tissue Antigens* 54:578–584.
- Nath SK, *et al.* (2005) Polymorphisms of complement receptor 1 and interleukin-10 genes and systemic lupus erythematosus: a meta-analysis. *Hum Genet* 118:225–234.
- Parks CG, *et al.* (2004) CTLA-4 gene polymorphisms and systemic lupus erythematosus in a population-based study of whites and African-Americans in the southeastern United States. *Lupus* 13:784–791.
- Pickering MC, *et al.* (2000) Systemic lupus erythematosus, complement deficiency, and apoptosis. *Adv Immunol* 76:227–324.
- Pullmann R, Jr., *et al.* (1999) Cytotoxic T lymphocyte antigen 4 (CTLA-4) dimorphism in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 17:725–729.
- Quintero-del-Rio AI, *et al.* (2002) The genetics of systemic lupus erythematosus stratified by renal disease: linkage at 10q22.3 (SLEN1), 2q34-35 (SLEN2), and 11p15.6 (SLEN3). *Genes Immun Suppl* 1:S57–S62.
- Quintero-del-Rio AI, *et al.* (2004) SLEN2 (2q34-35) and SLEN1 (10q22.3) replication in systemic lupus erythematosus stratified by nephritis. *Am J Hum Genet* 75:346–348.
- Scalapino KJ, Daikh DI (2008) CTLA-4: a key regulatory point in the control of autoimmune disease. *Immunol Rev* 223:143–155.
- Takahashi M, Kimura A (2010) HLA and CTLA4 polymorphisms may confer a synergistic risk in the susceptibility to Graves' disease. *J Hum Genet* 55:323–326.
- Torres B, *et al.* (2004) Association of the CT60 marker of the CTLA4 gene with systemic lupus erythematosus. *Arthritis Rheum* 50:2211–2215.
- Ueda H, *et al.* (2003) Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423:506–511.
- Ulker M, *et al.* (2009) CTLA-4 gene polymorphism of exon 1 (+49 A/G) in Turkish systemic lupus erythematosus patients. *Int J Immunogenet* 36:245–250.
- Vaidya B, Pearce S (2004) The emerging role of the CTLA-4 gene in autoimmune endocrinopathies. *Eur J Endocrinol* 150:619–626.
- Walport MJ, *et al.* (1998) C1q and systemic lupus erythematosus. *Immunobiology* 199:265–285.
- Waterhouse P, *et al.* (1995) Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270:985–988.

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