Genetics and Molecular Research

# Association between *PDCD1*, *CTLA4*, and *MECP2* gene polymorphisms and systemic lupus erythematosus in the Chinese Northern Han

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Genet. Mol. Res. 14 (4): 17567-17573 (2015)
Received April 22, 2015
Accepted August 13, 2015
Published December 21, 2015
DOI http://dx.doi.org/10.4238/2015.December.21.29

**ABSTRACT.** Systemic lupus erythematosus (SLE) is an autoimmune disease that results in chronic inflammation of different organ systems. Several susceptibility loci for SLE have been suggested in different populations, but the nature of the susceptibility genes has yet to be determined. The programmed cell death 1 gene (*PDCD1*), the cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*) gene, and the methyl-CpG-binding protein 2 gene (*MECP2*) are considered to be the candidate genes associated with SLE. We analyzed the role of *PDCD1*, *CTLA4*, and *MECP2* gene polymorphisms in Han patients suffering from SLE. Using a case-control study, 263 SLE patients and 263 healthy controls were collected from Chinese Northern Han people. Genomic DNA was prepared from peripheral blood leukocytes and the genotyping was performed using a polymerase chain reaction/ligase detection reaction assay. A statistically

 

significant difference was observed in the rs2239464 and rs2075596 polymorphisms of *MECP2* between SLE subjects and controls. The GG genotype in rs2239464 and the GG genotype in rs2075596 might protect against SLE. In contrast, no such association was found in the *CTLA4* or *PDCD1* polymorphisms. The rs2239464 and rs2075596 polymorphisms of *MECP2* might play a significant role in the development of SLE in the Northern Han of China.

**Key words:** Systemic lupus erythematosus; *PDCD1*; *CTLA4*; *MECP2*; Single nucleotide polymorphism

## INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that is characterized by autoantibody production (Walunas et al., 1996). The disease occurs primarily in women of childbearing age (Utz, 2004). The underlying pathogenic mechanism of SLE remains unclear; the disease is complex and involves multiple genetic and environmental factors (Kyogoku and Tsuchiya, 2007). The genetic component of SLE exerts a strong influence, with familial aggregation studies showing a sibling risk ratio of 20-29 (Hochberg, 1987; Alarcón-Segovia et al., 2005). Recently, several novel susceptibility genes of SLE have been identified. Programmed cell death 1 (*PDCD1*), cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*), and methyl-CpG-binding protein 2 (*MECP2*) genes, among others, have been associated with susceptibility to SLE in several studies. However, more conclusive evidence is required to establish the role of these genes in SLE pathogenesis (Rhodes and Vyse, 2007; Rhodes and Vyse, 2008).

There are many reasons why true susceptibility genes may remain unreplicated in genome-wide association studies, and therefore replication studies of suggested genes continue to be relevant. Our aim was to investigate whether certain susceptibility genes implicated in the recent SLE literature can be replicated in the Chinese Northern Han population.

## MATERIAL AND METHODS

### Patients and controls

The case-control study included 263 SLE patients and 263 age- and sex-matched healthy blood donors (both of Han origin). SLE patients were recruited from the department of Rheumatology at the Affiliated Hospital of Peking Union Medical College, the First Hospital of Hohhot in Inner Mongolia, and the Inner Mongolia Medical University Affiliated Hospital between 2010 and 2012. All patients met the American College of Rheumatology Classification (ACRC) criteria for the diagnosis of SLE (Tan et al., 1982). Unaffected, biologically unrelated family members (spouses and common-law spouses) were asked to participate as control individuals, and an existing collection of unrelated Han individuals was also used. All subjects gave written informed consent for participation in the genetic studies on SLE, and the study protocols were reviewed and approved by local ethical committees.

## DNA extraction and genotyping

Genomic DNA was extracted from the leukocytes in peripheral blood samples using a commercial blood DNA extraction kit (TIANamp Blood DNA kit; TIANGEN BIOTECH, Beijing, China), and was stored at -20°C. All genotyping was performed using a polymerase chain reaction (PCR)/ligase detection reaction assay.

The target DNA sequences were amplified using a multiplex PCR method. PCRs for each subject were carried out in a final volume of 20 µL containing 1X PCR buffer, 3.0 mM $MgCl_2$, 2.0 mM deoxynucleotide triphosphate, 2 µL primers, 0.2 µL Qiagen HotStarTaq Polymerase (QIAGEN, Shenzhen, China), 4 µL 1X Q-solution, and 50 ng genomic DNA. Thermal cycling was performed using a GeneAmp PCR 9600 system (PerkinElmer, Norwalk, Connecticut, USA) with an initial denaturation of 15 min at 95°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 7 min.

The ligation reaction for each subject was carried out in a final volume of 10 µL containing 1X NEB Taq DNA ligase buffer, 2 pmol each probe mix, 0.05 µL Taq DNA ligase (BIOWING, Jiangsu, China), and 4 µL multi-PCR product. A total of 35 cycles for ligase detection reaction were performed at 95°C for 2 min, 94°C for 30 s, and 50°C for 2 min. The fluorescent products of the ligase detection reaction were differentiated using a PRISM 3730 DNA analyzer (ABI).

## Statistical analysis

The Statistical Program for Social Sciences (SPSS version 17.0) was used to carry out statistical analysis. Hardy-Weinberg equilibrium was determined in each group using the chi-square test. Allele and genotype frequencies between groups were completed using SHEsis software.

# RESULTS

## Clinical characteristics of SLE and control subjects

The characteristics of the SLE and control subjects are shown in Table 1. Significant differences in C3, IgA, IgM, white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), lymphocyte count (LYM), neutrophil count (NEUT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and gamma-glutamyl transpeptidase (GGT) were observed between the SLE patients and the controls ($P < 0.05$). There were no significant differences in C4, IgG, and gender between the groups.

## Single-locus association study of genes and SLE

Genotype analysis of all the investigated polymorphisms revealed no significant deviation from Hardy-Weinberg equilibrium in either group ($P > 0.05$). The genotype and allele frequency distributions are shown in Table 2. There were significant differences in the distribution of genotypes frequency in the rs2239464 and rs2075596 polymorphisms of *MECP2* between the SLE subjects and the controls. The GG genotype in rs2239464 and the GG genotype in rs2075596 might protect against SLE. There were no significant differences in the distribution of genotypes and allele frequencies of 318C/T and 1722T/C in *CTLA4*, and no significant differences were found in the distribution of genotypes and allele frequencies in the rs2227981, rs2227982, and rs11568821 polymorphisms of *PDCD1*.

**Table 1.** Characteristics of systemic lupus erythrematosus (SLE) patients and controls.

| | SLE | Controls | P |
|---|---|---|---|
| Male/female | 31/263 | 31/263 | 0.87 |
| Age (years) | 39.40 ± 14.75 | 38.18 ± 13.07 | 0.29 |
| C3 (g/L) | 0.94 ± 0.26 | 1.49 ± 0.25 | 0.00** |
| C4 (g/L) | 0.27 ± 0.88 | 0.27 ± 0.07 | 0.99 |
| IgA (g/L ) | 2.82 ± 1.29 | 4.79 ± 3.35 | 0.00** |
| IgG (g/L) | 13.68 ± 3.83 | 13.39 ± 3.97 | 0.37 |
| IgM (g/L) | 1.13 ± 0.74 | 1.92 ± 0.72 | 0.00** |
| WBC ($10^9$/L) | 7.13 ± 3.43 | 5.75 ± 1.34 | 0.00** |
| RBC ($10^{12}$/L) | 4.45 ± 0.72 | 5.20 ± 0.78 | 0.00** |
| HGB (g/L) | 133.93 ± 19.65 | 157.36 ± 11.67 | 0.00** |
| LYM ($10^9$/L) | 19.76 ± 13.95 | 37.38 ± 5.81 | 0.00** |
| NEUT ($10^9$/L) | 48.98 ± 28.72 | 54.84 ± 7.08 | 0.00** |
| ALT (U/L) | 35.09 ± 36.87 | 25.67 ± 7.79 | 0.00** |
| AST (U/L) | 33.75 ± 34.22 | 28.27 ± 8.09 | 0.01* |
| GGT (U/L) | 57.33 ± 119.12 | 25.91 ± 7.69 | 0.00** |
| ESR (mM) | 20.85 ± 18.24 | 11.59 ± 5.87 | 0.00** |
| CRP (mL/h) | 5.85 ± 6.26 | 1.77 ± 0.82 | 0.00** |

WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; LYM = lymphocyte count; NEUT = neutrophil count; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; GGT = gamma-glutamyl transpeptidase Data are reported as mean ± standard deviation.*P < 0.05, **P < 0.01.

**Table 2.** Frequency distribution of CTLA4, PDCD1, and MECP2 genotypes and alleles.

| Gene polymorphism | Genotype/allele | SLE, N (%) | Controls, N (%) | $\chi^2$ | P |
|---|---|---|---|---|---|
| 318C/T | CC | 209 (0.721) | 224 (0.775) | 4.47 | 0.11 |
| | CT | 79 (0.272) | 60 (0.208) | | |
| | TT | 2 (0.007) | 5 (0.017) | | |
| | C | 497 (0.857) | 510 (0.879) | 1.27 | 0.26 |
| | T | 83 (0.143) | 70 (0.121) | | |
| 1722T/C | CC | 33 (0.114) | 28 (0.097) | 0.46 | 0.79 |
| | CT | 121 (0.417) | 123 (0.424) | | |
| | TT | 136 (0.469) | 139 (0.479) | | |
| | C | 187 (0.322) | 179 (0.309) | 0.26 | 0.61 |
| | T | 393 (0.678) | 401 (0.691) | | |
| rs11568821 | AG | 3 (0.010) | 2 (0.007) | 0.20 | 0.65 |
| | GG | 287 (0.990) | 288 (0.993) | | |
| | A | 3 (0.005) | 2 (0.003) | 0.20 | 0.65 |
| | G | 577 (0.995) | 578 (0.997) | | |
| rs2227981 | CC | 150 (0.517) | 165 (0.569) | 2.76 | 0.25 |
| | CT | 114 (0.393) | 108 (0.372) | | |
| | TT | 26 (0.090) | 17 (0.059) | | |
| | C | 414 (0.714) | 438 (0.755) | 2.55 | 0.11 |
| | T | 166 (0.286) | 142 (0.245) | | |
| rs2227982 | CC | 79 (0.272) | 70 (0.241) | 1.22 | 0.54 |
| | CT | 142 (0.490) | 141 (0.486) | | |
| | TT | 69 (0.238) | 79 (0.272) | | |
| | C | 300 (0.517) | 281 (0.484) | 1.24 | 0.26 |
| | T | 280 (0.483) | 299 (0.516) | | |
| rs2239464 | AA | 189 (0.652) | 201 (0.693) | 14.77 | 0.00 |
| | AG | 87 (0.300) | 56 (0.193) | | |
| | GG | 14 (0.048) | 33 (0.114) | | |
| | A | 465 (0.802) | 458 (0.790) | 0.26 | 0.61 |
| | G | 115 (0.198) | 122 (0.210) | | |
| rs2075596 | AA | 183 (0.631) | 183 (0.631) | 1.75 | 0.00 |
| | AG | 89 (0.307) | 61 (0.210) | | |
| | GG | 18 (0.062) | 46 (0.159) | | |
| | A | 455 (0.784) | 427 (0.736) | 3.71 | 0.054 |
| | G | 125 (0.216) | 153 (0.264) | | |

SLE = systemic lupus erythrematosus *P < 0.05, **P < 0.01

## DISCUSSION

Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) plays a key role in the regulation of T-cell stimulation and inhibits T-cell activation (Chai et al., 2012). Reduced expression or dysfunction of CTLA4 contributes to the pathogenesis of autoimmune diseases. Consequently, *CTLA4* gene polymorphisms are associated with several autoimmune diseases, such as type I diabetes (Ahmadi et al., 2013), rheumatoid arthritis (Davis et al., 2014), and SLE (Kristiansen, 2000). Furthermore, numerous studies have investigated the association between 318C/T and 1722T/C polymorphisms of the *CTLA4* gene and SLE. However, the results are controversial (Parks et al., 2004; Takeuchi et al., 2007; Chua et al., 2010). Shojaa et al. (2014a) conducted a meta-analysis on 1422 SLE cases and 1417 controls from nine published studies. The results suggested that the TC and TT genotypes of the 1722T/C polymorphism are related to an increased risk of SLE in the overall population and especially in Asians. Shojaa et al. (2014b) found that the 318C/T polymorphism of *CTLA4* might play a significant role in the development of SLE in the Iranian population. However, in our study we did not find a relationship between the 318C/T and 1722T/C polymorphisms of *CTLA4* and SLE in the Han population.

PDCD1 is an immunoreceptor that suppresses the autoimmune response and maintains self-tolerance. Reduced expression or dysfunction of PDCD1 might result in the breakdown of peripheral tolerance and the onset of autoimmune diseases, and mice lacking PDCD1 develop an SLE-like disease (Ferreiros-Vidal et al., 2004; Johansson et al., 2005). Many polymorphisms have been found in the *PDCD1* gene, which has the SLE-susceptible chromosomal locus 2q37 (Lindqvist et al., 2000). Moreover, numerous investigations have indicated that *PDCD1* gene polymorphisms contribute to the development of SLE (Ferreiros-Vidal et al., 2004). Prokunina et al (2002) found that a regulatory single-nucleotide polymorphism (SNP) PD1.3G/A located in the programmed cell death 1 (*PDCD1*) gene is involved in susceptibility to SLE in Swedish, European American, and Mexican cases. Meta-analysis has demonstrated a significant association between PD1.3A and SLE among non-Spanish European descendants, while a negative association was observed in a Spanish population (Liu et al., 2009). In this study, we investigated the associations between rs2227981, rs2227982, and rs11568821 polymorphisms of the *PDCD1* gene and SLE, but did not find any.

*MECP2*, with the chromosomal locus Xq28, encodes a 486-amino acid protein that binds methylated DNA and is involved in the transcriptional regulation of methylation-sensitive genes (Miltenberger-Miltenyi and Laccone, 2003). DNA methylation-sensitive genes are overexpressed in SLE (Jones et al., 1998) and MECP2 is critical in the transcriptional suppression of methylation-sensitive genes (Koelsch et al., 2013), which makes *MECP2* an attractive candidate gene for SLE. (Sawalha et al., 2008) confirmed the association between SLE and all eight SNPs within the *MECP2* gene including rs2075596, which has previously been reported in European SLE patients and controls in a Korean population. A meta-analysis of the association between the rs2075596 and rs2239464 polymorphisms of *MECP2* and SLP showed that the SNPs increased the risk of developing SLE (Liu et al., 2013). Our results also revealed an association between the rs2075596 and rs2239464 SNPs of *MECP2* and SLE in Chinese Northern Han.

There are many other factors such as age, gender, age at onset, disease severity, family history, and smoking habits that may explain why a single polymorphism acts differently in dissimilar ethnic groups.

In conclusion, using a candidate gene approach and a case-control genetic association study, we found that the rs2239464 and rs2075596 polymorphisms of *MECP2* might play a significant role in the development of SLE in Chinese Northern Han. To confirm the associations

between *PDCD1*, *CTLA4*, and *MECP2* gene variants and SLE, large-scale studies in other populations are urgently required.

## Conflicts of interest

The authors declare no conflict of interest.

## ACKNOWLEDGMENTS

Research supported by the Inner Mongolia Hohhot Technology Bureau (#2012-Social Development-2-2).

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