

## CONCISE REPORT

## Single nucleotide polymorphisms in the gene encoding the major histocompatibility complex class II transactivator (CIITA) in systemic lupus erythematosus

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**Background:** The major histocompatibility complex (MHC) class II transactivator (CIITA) is a master switch of antigen presentation and activates expression of the MHC II gene. Insufficient up regulation of MHC class II molecules is reported to be one of the major immunological mechanisms in systemic lupus erythematosus (SLE).

**Objective:** To examine the association between single nucleotide polymorphisms (SNPs) in the human CIITA gene (*MHC2TA*) and SLE.

**Methods:** Promoters and coding regions of *MHC2TA* were evaluated for polymorphisms in 100 patients with SLE and 100 healthy donors. Eight oligonucleotide primer sets that covered the coding region and each promoter region were used for genomic analysis of SNPs.

**Results:** Allele frequencies of previously reported SNPs did not differ between healthy donors and patients with SLE. Additionally, a new polymorphism in an intronic region at nt 485 (A→A/G) was identified, which is close to the polymorphism at nt 474 that has been associated with one of the disease causing CIITA cDNA mutations in bare lymphocyte syndrome. This SNP was found in 11% of patients with SLE and in 3% of healthy donors, suggesting it may have a role in the pathogenesis of SLE.

**Conclusions:** A newly identified polymorphism in an intronic region at nt 485 (A→A/G) may have an important role in the pathogenesis of SLE.

The major histocompatibility complex (MHC) class II transactivator (CIITA) gene was originally identified as a defective gene associated with bare lymphocyte syndrome (BLS), a severe combined immunodeficiency syndrome.<sup>1</sup> In addition to activating MHC II expression, CIITA activates the expression of HLA-DM and invariant chain (Ii) genes, which are also involved in antigen processing. Therefore, CIITA is a master switch of antigen presentation in antigen presenting cells (APCs).<sup>1</sup> The human CIITA gene, *MHC2TA*, is 42 kb in length and maps to chromosome 16p13.<sup>1</sup> Transcription of the human CIITA gene is controlled by four alternative promoters that exhibit cell type specific activity.<sup>2</sup> Promoter I (pI) is responsible for constitutive CIITA expression in dendritic cells, promoter III (pIII) is responsible for constitutive CIITA expression in B cells, and promoter IV (pIV) becomes activated by interferon  $\gamma$  (IFN $\gamma$ ) activation in non-professional APCs. Given its pivotal role in MHC class II regulation, *MHC2TA* is also considered to be an important candidate gene in other autoimmune diseases. Indeed, polymorphisms in the CIITA gene have been shown to be weakly associated with multiple sclerosis (MS).<sup>3</sup> In contrast,

analysis of all four promoters disclosed no polymorphic sequence variations associated with rheumatoid arthritis (RA) or insulin dependent diabetes mellitus (IDDM).<sup>4</sup> Thus far, no reports have demonstrated an association between single nucleotide polymorphisms (SNPs) in the CIITA gene and systemic lupus erythematosus (SLE). It has been reported that MHC class II antigen expression is enhanced in lupus nephritis in humans and in mice models.<sup>5–7</sup> Therefore a particular allele of the polymorphic gene which plays a part in the regulation of MHC class II expression might be involved in the predisposition to SLE. In this report, we examine the association between SNPs in the human CIITA gene, *MHC2TA*, and SLE.

## PATIENTS AND METHODS

## Patients

DNA samples were obtained from 100 Japanese patients with SLE (mean (SD) age at onset 25.6 (11.4) years) and 100 Japanese healthy controls. All the patients with SLE fulfilled the American College of Rheumatology criteria for SLE.<sup>8</sup> Informed consent was obtained from each participant in the study.

## Polymerase chain reactions (PCRs)

Genomic DNA was isolated from peripheral blood mononuclear cells obtained from 100 patients with SLE and 100 healthy donors. We used eight oligonucleotide primer sets for genomic analysis of SNPs. The primers used for amplification were as follows: pI: 5'-TGGAGTCTGAATCAACCCAA (forward), 5'-TAGGGTCAAAGAGATCTTCC (reverse); pIII: 5'-AGATATGGCAGCTGGCACC (forward), 5'-TTGGGGCTGACAGGTTAG (reverse); pIV: 5'-GTTGGACTGAGTTGAGAGA (forward), 5'-AGCTCTGGGGCCGCGGC (reverse); C1: 5'-GGCGGCCGATGAGGTTTTTTC (forward), 5'-CCGAGGGAGCAGGGCTC (reverse); C2: 5'-CTCGGTGGACAGGAAGCAG (forward), 5'-CGTGCTGCCAAATTCAGC (reverse); C3: 5'-CCTCCGAGCACTGGCATT (forward), 5'-CTGCCTGAAGTAGCTTGTC (reverse); C4: 5'-CAGTGGGCTTCAGTTAGAC (forward), 5'-AGCTCTGGGGCCGCGGC (reverse); C5: 5'-CTCCACCCCAATGTAGGTG (forward), 5'-CCACCACCCAGGGCAGAGAG (reverse). A PCR was performed with Taq DNA polymerase using the following protocol: 94°C for 5 minutes; 94°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds for 35 cycles (promoter I, promoter III, promoter IV, C1, C2) 94°C

**Abbreviations:** APCs, antigen presenting cells; BLS, bare lymphocyte syndrome; CIITA, class II transactivator; IDDM, insulin dependent diabetes mellitus; IFN, interferon; MHC, major histocompatibility complex; MS, multiple sclerosis; PCR, polymerase chain reaction; RA, rheumatoid arthritis; RT-PCR, reverse transcriptase-polymerase chain reaction; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphisms

for 5 minutes; 95°C 30 seconds, 60°C 30 seconds, 72°C 60 seconds for 35 cycles (C4, C5), and 94°C for 5 minutes; 95°C 30 seconds, 65°C 30 seconds, 72°C 60 seconds for 35 cycles (C3). PCR products were purified from agarose gels and subjected to DNA sequencing using the ABI Prism 3700 DNA Analyzer (Applied Biosystems).

#### Reverse transcriptase-polymerase chain reaction (RT-PCR)

Reverse transcription of RNA isolated from peripheral blood mononuclear cells was performed using the AccessQuick RT-PCR System (Promega, Madison, WI, USA); one cycle: 48°C for 45 minutes followed by 30 cycles PCR amplification using one primer set; SP1: 5'-ACTGGACCAGTATGTCTTCCA (forward), SP2: 5'-CTTTTCTGACTTTTCTGCCCA (reverse). The PCR protocol comprised 94°C 60 seconds; 55°C 30 seconds; 72°C 30 seconds for 30 cycles. PCR products were purified from agarose gels and direct sequencing was performed using dideoxy terminators on an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

#### Estimation of linkage disequilibrium

To test the association between the possession of a haplotype, the genotype data were analysed by QTLHAPLO.<sup>9</sup> The haplotype frequencies were estimated from the genotype data at the six loci within the *C2TA* gene under the assumption of the presence of linkage disequilibrium, and the pairwise linkage disequilibrium between SNP (No 485) and other (No -155, No 1614, No 2509, No 2536, and No 2791) measures.

## RESULTS

### Allele frequencies of previously reported SNPs

To investigate the potential association between *CIITA* polymorphisms and SLE, we first examined the coding regions and promoter elements in 100 Japanese healthy donors and 100 Japanese patients with SLE for polymorphisms and established the allele frequencies of identified SNPs. Eight oligonucleotide primer sets that covered the coding region and each promoter region were used for genomic analysis of SNPs. In addition to one primer set each for coding regions C1, C2, C3, and C4 and one primer set each for pI, pIII, and pIV, which have been reported by Patarroyo *et al.*,<sup>10</sup> we used a primer set for coding region C5, which has recently been recognised for its importance in the subcellular localisation of *CIITA*.<sup>11</sup> Five SNPs that have also been reported by Patarroyo *et al.*<sup>10</sup> were identified and confirmed by genomic DNA sequencing of all samples. One SNP at nucleotide (nt) 1614 (C→G) causes a conservative substitution of alanine to glycine at amino acid 500. This particular productive nt substitution was also observed accompanying disease causing deletions in the *CIITA* cDNA isolated from patients with BLS, a severe immunodeficiency condition that can result from deficient or abnormal expression of *CIITA*. In addition, three silent *CIITA* SNPs (nt 2509 (G→A), nt 2536 (T→G), and nt 2791 (G→A)) and one pIII mutation (nt -155 (A→G)) were identified. There were no differences in the allele frequencies of these polymorphisms between healthy donors and patients with SLE (fig 1). We observed a slightly higher frequency of A at nt -155 in pIII than reported by Patarroyo *et al.*<sup>10</sup> We did not detect any polymorphisms in pI

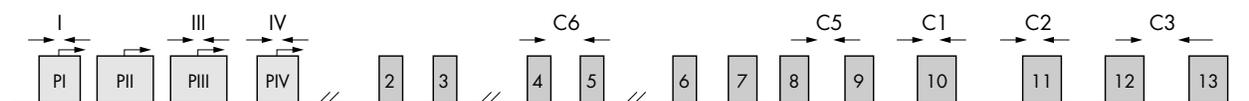
A

Promoter III		Coding sequence				
nt No -155 (III) A→G	nt No 1614 (C1) C→G	nt No 2509 (C2) G→A	nt No 2536 (C2) T→G	nt No 2791 (C3) G→A		

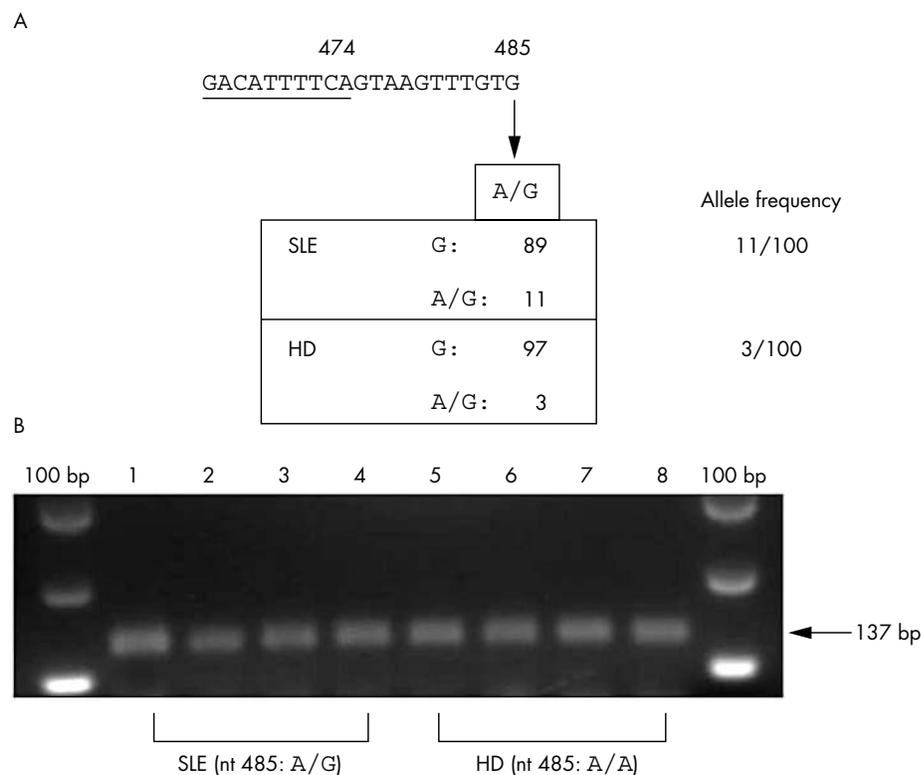
#### Allele frequency

Total	A 184 (92.0%) G 16 (8.0%)	C 64 (32.0%) G 136 (68.0%)	G 139 (69.5%) A 61 (30.5%)	T 64 (32.0%) G 136 (68.0%)	G 69 (34.5%) A 131 (65.5%)
SLE	A 96 (96.0%) G 4 (4.0%)	C 31 (31.0%) G 69 (69.0%)	G 68 (68.0%) A 32 (32.0%)	T 31 (31.0%) G 69 (69.0%)	G 35 (35.0%) A 65 (65.0%)
HD	A 88 (88.0%) G 12 (12.0%)	C 33 (33.0%) G 67 (67.0%)	G 71 (71.0%) A 29 (29.0%)	T 33 (33.0%) G 67 (67.0%)	G 34 (34.0%) A 66 (66.0%)
Patarroyo <i>et al.</i> <sup>10</sup>	A 63% G 37%	C 35% G 65%	G 81% A 19%	T 29% G 71%	G 35% A 65%

B



**Figure 1** SNPs in *MHC2TA* pIII and coding region. (A) Genomic DNA was isolated from peripheral blood mononuclear cells, and PCR and direct sequencing were performed with eight oligonucleotide primer sets for genomic analysis of SNPs (arrows). (B) *MHC2TA* promoter elements and exon organisation. Arrows on the box represent upstream regulatory sequences for each promoter. Promoter pI is used primarily by dendritic cells, pIII by B cells, and pIV for IFN $\gamma$ -inducible *CIITA* expression by non-professional APCs.



**Figure 2** (A) SNP at nt 485 near a splice site (nt 474) of the *MHC2TA* gene and allele frequency in patients with SLE. A  $\chi^2$  test for independence indicated that  $p=0.0266$  between these two groups. (B) mRNA transcripts with and without the SNP at nt 485. An RT-PCR was performed as described in the "Patients and methods" section. PCR products were analysed with 3% agarose gels. Arrow indicates the expected band.

or pIV; therefore, we concluded that pI and IV of the human *CIITA* gene are non-polymorphic.

### New polymorphism in an intronic region

In addition, we identified a previously undiscovered polymorphism in an intron at nt 485 (A→A/G), which is close to the polymorphism at nt 474 that was originally described in association with one of the disease causing *CIITA* cDNA mutations in BLS (fig 2A). This SNP was identified in 11/100 (11%) patients with SLE and 3/100 (3%) healthy donors. In addition, both patient groups satisfied Hardy-Weinberg equilibrium conditions. Each estimated linkage disequilibrium between this SNP and others (No -155 ( $r^2=0.0074$ ), No 1614 ( $r^2=0.0710$ ), No 2509 ( $r^2=0.0147$ ), No 2536 ( $r^2=0.0710$ ), and No 2791 ( $r^2=0.0616$ )) showed weak associations. The newly identified SNP nt 485 is close to the nt 474, which is a splicing donor site of the splicing reaction between exons 4 and 5. Owing to its location, we speculated that this polymorphism might affect the splicing process between exons 4 and 5. To investigate this possibility, we conducted an RT-PCR with a primer located in the 5'-flanking region of the splicing donor site for exon 4 (SP1) and another located in the 3'-flanking region of the splicing acceptor site for exon 5 (SP2). Figure 2B shows that there were no differences in the length of the resultant RT-PCR products between patients with SLE with this SNP (nt 485: A/G) and healthy donors who lack it (nt 485: A/A). We purified these PCR products and performed direct DNA sequencing; no differences in the nucleic acid sequence between groups were found (data not shown).

### DISCUSSION

*CIITA* exhibits cell-specific, cytokine inducible, and differentiation-specific expression that precisely parallels that of MHC class II synthesis in most cases. Class II+ cells, such as B

cells, monocytes, dendritic cells, and human activated T cells, express *CIITA*.<sup>12,13</sup> Additionally, the expression of *CIITA* under inflammatory transplantation conditions parallels the expression of MHC class II.<sup>14</sup> Regulation of *CIITA* expression occurs primarily at the transcriptional level. One promoter, pI, is responsible for constitutive *CIITA* expression in dendritic cells, while pIII is responsible for constitutive *CIITA* expression in B cells. A separate promoter, pIV, directs IFN $\gamma$  inducible *CIITA* expression in non-professional APCs. One SNP (A→G) at nt 168 (-155 relative to transcription initiation) of *CIITA* pIII was found in 29% of patients with MS and 27% of controls.<sup>3</sup> Patarroyo *et al* identified the SNP at *CIITA* pIII nt -155 in 37% of patients with MS, and no SNPs in *CIITA* pI or pIV. In contrast, another group found no polymorphisms within promoters pI, pIII, or pIV in 23 patients with IDDM, 30 patients with RA, or 19 normal subjects.<sup>4</sup> In this study, we have identified a previously unreported polymorphism at nt 485 (A→A/G) that is close to the polymorphism at nt 474, which was originally described in association with one of the disease causing *CIITA* cDNA mutations in BLS.<sup>1</sup> This SNP was found in 11/100 (11%) patients with SLE and 3/100 (3%) healthy donors. Our data presented here have failed to show that this newly identified SNP at nt 485 affects the splicing specificity between exons 4 and 5. Further study is needed to identify the biological roles of this polymorphism.

In conclusion, we suggest that the *MHC2TA* polymorphisms identified in this study will be useful disease associated markers for SLE. However, further study is needed to elucidate the role of this polymorphism in the development of autoimmune disorders such as SLE.

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**CORRECTION**

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**Single nucleotide polymorphisms in the gene encoding the major histocompatibility complex class II transactivator**

**(CIITA) in systemic lupus erythematosus** (Koizumi K, Okamoto H, Iikuni N, Nakamura T, Kawamoto M, Momohara S, Ichikawa N, Furuya T, Kotake S, Taniguchi A, Yamanaka H, Kamatani N. *Ann Rheum Dis* 2005;**64**:947–50.)

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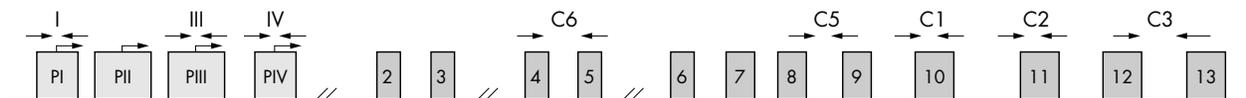
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Allele frequency

Total	G	368 (92.0%)	C	128 (32.0%)	G	278 (69.5%)	T	128 (32.0%)	G	138 (34.5%)
	A	32 (8.0%)	G	272 (68.0%)	A	122 (30.5%)	G	272 (68.0%)	A	262 (65.5%)
SLE	G	192 (96.0%)	C	62 (31.0%)	G	136 (68.0%)	T	62 (31.0%)	G	70 (35.0%)
	A	8 (4.0%)	G	138 (69.0%)	A	64 (32.0%)	G	138 (69.0%)	A	130 (65.0%)
HD	G	176 (88.0%)	C	66 (33.0%)	G	142 (71.0%)	T	66 (33.0%)	G	68 (34.0%)
	A	24 (12.0%)	G	134 (67.0%)	A	58 (29.0%)	G	134 (67.0%)	A	132 (66.0%)
Patarroyo <i>et al</i> <sup>10</sup>	G	37%	C	35%	G	81%	T	29%	G	35%
	A	63%	G	65%	A	19%	G	71%	A	65%

B



**Figure 1** SNPs in *MHC2TA* pIII and coding region. (A) Genomic DNA was isolated from peripheral blood mononuclear cells, and PCR and direct sequencing were performed with eight oligonucleotide primer sets for genomic analysis of SNPs (arrows). (B) *MHC2TA* promoter elements and exon organisation. Arrows on the box represent upstream regulatory sequences for each promoter. Promoter pI is used primarily by dendritic cells, pIII by B cells, and pIV for IFN $\gamma$ -inducible CIITA expression by non-professional APCs.