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BRIEF COMMUNICATION

Single nucleotide polymorphisms in MHC2TA, the gene encoding the MHC class II transactivator (CIITA)

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The MHC class II transactivator (CIITA) is the master regulator for HLA-D (DP, DQ, DR) gene expression. In this report the coding and promoter regions of the CIITA gene, MHC2TA, were evaluated for polymorphisms in 50 normal Caucasian individuals. Allele frequencies were obtained for four separate single nucleotide (nt) polymorphisms (SNPs) identified in the MHC2TA coding region: nt 1614 (C→G), nt 2509 (G→A), nt 2536 (T→G), and nt 2791 (G→A). MHC2TA sequence analysis of 100 chromosomes from these 50 individuals revealed a SNP in MHC2TA promoter (p) III at nt −155 (A→G), but none in CIITA pI or pIV. In addition, we demonstrate the presence of splice variant at a previously undiscovered intron, accounting for a three nt (TAG) insertion at position 474 that was originally described in association with one of the disease-causing CIITA cDNA mutations in bare lymphocyte syndrome.
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The MHC class II transactivator (CIITA), a transcriptional co-activator, is the key intermediate responsible for IFNγ-inducible and constitutive class II expression on antigen presenting cells (APC).[1] CIITA also directs expression of invariant chain (Ii) and HLA-DM,[2] two molecules involved in class II biosynthesis and antigen processing.[3] Thus, CIITA is considered a global regulator of genes involved in class II-restricted Ag presentation.[2] The human CIITA gene, *MHC2TA*, which spans 42 kb,[4] was mapped to chromosome 16p13,[1] a region linked to multiple sclerosis (MS) susceptibility.[5,6] Given its pivotal role in MHC class II regulation, *MHC2TA* is also considered an important candidate gene in other autoimmune diseases.[7] In order to investigate the potential association of *MHC2TA* with autoimmune diseases, we have first examined the coding region and promoter elements in normal individuals for polymorphisms and established the allele frequencies of identified SNPs.

The *MHC2TA* coding region from 50 unrelated Caucasian (non-Hispanic whites) individuals of northern European descent was examined for polymorphisms by bi-directional sequencing of lymphocyte cDNAs. Four SNPs were identified and were confirmed by sequencing genomic DNA of all samples. One SNP at nucleotide (nt) 1614 (C→G) causes a conservative substitution of alanine to glycine at amino acid (aa) 500 (see Table 1). This particular productive nt substitution was also observed accompanying disease-causing deletions in the CIITA cDNA isolated from patients with bare lymphocyte syndrome (BLS),[8,9] a severe immunodeficiency condition that can result from deficient or abnormal expression of CIITA.[1] Three silent CIITA SNPs that have not been previously reported, were identified: nt 2509 (G→A), 2536

Table 1 Single nucleotide polymorphisms in *MHC2TA* promoter III and coding region[a]

	Promoter III	*Coding sequence*			
	nt no. –155 A → G	*nt no. 1614 C → G*	*nt no. 2509 G → A*	*nt no. 2536 T → G*	*nt no. 2791 G → A*
Allele frequency	*A (63%) G (37%)*	*C (35%) G (65%)*	*G (81%) A (19%)*	*T (29%) G (71%)*	*G (35%) A (65%)*

[a]Genomic DNA was isolated from peripheral blood mononuclear cells. Oligonucleotide primers used for genomic analysis of SNP's: pIII nt –155 (–308 to –289) forward: 5′-AGATATTGGCAG CTGGCACC and (+165 to +185) reverse: 5′-CTTGGGGCTCTGA CAGGTAG; CIITA nt 1614 (1549 to 1567) forward: 5′-GGCGGCCGATGAGGTTTTC and (1676 to 1693) reverse: 5′-CCGGAGGGAGCAGGGCTC; CIITA nt 2509 and 2536 (2455 to 2473) forward: 5′-CTCGGTGGACAGGAAGCAG and (2570 to 2588) reverse; 5′-CGTGCTGCCAAATTCCAGC; CIITA nt 2791 (2701 to 2719) forward: 5′-CCTCCGCAGCACTGGCATT and (2827 to 2846) reverse: 5′-CTGCCTGAAGTAGCTTGGTC. PCR amplification of DNA: 96°C for 2 min, 95°C, 15 s; 57°C, 30 s; 72°C, 30 s for 35 cycles. PCR fragments were sequenced using the ABI Prism 3700 DNA Analyzer (Applied Biosystems).

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(T→G), and 2791 (G→A). Allele frequencies for each SNP are shown in Table 1.

The DNA sequence from cDNA amplicons containing nt $^{+}$383–594 could be read in either direction up to nt 474, but not beyond. This observation suggested the presence of multiple mRNA transcripts that diverged at nt 474. Previously, insertion of TAG at CIITA cDNA nt 474, causing the substitution of Ile (I) for Lys (K) at aa 120 and addition of Asp (E) at aa 121 was described in association with one of the disease-causing mutations in certain BLS patients.[8,9] Sequence (Figure 1a) and restriction (*Bsp* 1286I) analysis (Figure 1b and 1c) of the subcloned amplicons ($^{+}$383–594) revealed the presence of CIITA mRNAs with and without the TAG insertion in all 16 individuals examined. In order to further investigate whether the TAG insertion was due to a splicing variation, we examined the genomic DNA sequence in this region. As shown in Figure 2, a 192 base intron (GenBank accession no. 417731) at this location was discovered. No SNPs were identified in this intron in 25 individuals examined. A normal splice donor sequence[10] was found at the 5′ end of this intron. The TAG, which is used in consensus splice acceptor sequences,[10] is located at the 3′ end of this intron. The preceding intronic nt sequence, AAG, can also be used as a splice acceptor site.[10] Interestingly, this TAG was reported in the murine CIITA cDNA sequence.[11] Analysis of genomic DNA from 129SvJ, C57BL/6, B10.PL, and BALB/c mouse strains revealed a 141-base intron (GenBank accession no. 417751) at the same location in the murine CIITA gene, *Mhc2ta*, as in the human *MHC2TA* gene. This intron sequence, which shares 49.8% homology with the corresponding human sequence, contains a similar 3′ acceptor sequence (see Figure 2). Similar to humans, individual mice expressed both CIITA mRNA transcripts with and without the UAG insert (data not shown). Thus, based on our analysis of both human and murine CIITA mRNA transcripts and genomic DNA, the insertion of TAG at nt 474 is a splice variant and not a mutation.

CIITA expression is controlled in a tissue-specific manner by differential activation of multiple nonhomologous promoters (see Figure 3).[12] One promoter (p), pI, is responsible for constitutive CIITA expression in dendritic cells while pIII, is responsible for constitutive CIITA expression in B cells.[12,13] A separate promoter, pIV, directs IFNγ-inducible CIITA expression in non-pro-

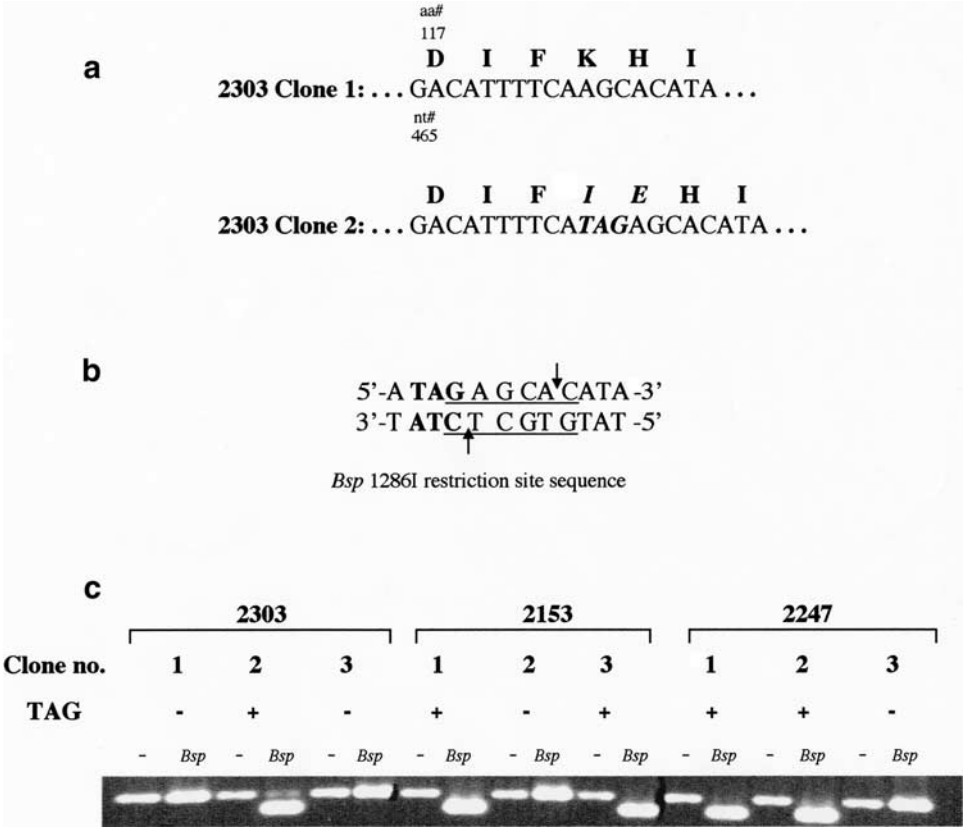

Figure 1 mRNA transcripts with and without the insertion of UAG (TAG) at nt 474 are detected in the same individuals. (**a**) CIITA cDNA sequences from two clones isolated from a representative individual, one which contains the TAG (bold) insertion and one that does not. The predicted aa sequence is shown above. Reverse transcription of RNA isolated from peripheral blood mononuclear cells was performed using the Access RT-PCR System (Promega, Madison, WI, USA); one cycle: 48°C for 45 min followed by 25 cycles PCR amplification using the following CIITA primers (Operon Technologies, Alameda, CA, USA): forward (383–402) 5′-GAGACCAGGGAGGCTTATGC and reverse: (575–594) 5′-GGCTTCCAGTGCTTCAGGTC using the following conditions: 94°C, 15 s; 58°C, 30 s; and 72°C, 30 s. PCR amplicons were subcloned into pCRII-TOPO-TA (Invitrogen, Carlsbad, CA, USA) and sequenced using dideoxy-terminators on a ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). (**b**) The restriction enzyme *Bsp*1286I recognition sequence (underlined). (**c**) *Bsp*1286I digestion of representative CIITA cDNA amplicons with (+) and without (–) TAG sequence. Upper bands represent the undigested 208 or 211 nt (with TAG) fragments. *Bsp*1286I digestion of 211 nt amplicons containing the TAG insertion produced 107 and 104 nt fragments that migrated as a single (lower) band.

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h    GTAAGTTTGTGGTGGGTGGGGAGGTCTTGGCTCAGCCTGCATTTCCTGCCTTGTTCCCTG  60
     ||||||  |  |  | ||      ||| |||||||  ||  ||| | | |
m    GTAAGTCGGGAGAAGTTGA-----TCTAGGCTCAGTCTTTAGTCCTTCCTG---------  46

h    GGGGGTGCCCTAATACCTGACGACCATTCATTGATGGGCAGTCAGACCCCTCTCCCCAAG 120
                  ||||||  ||          |  |||  | |||  | |  ||||  ||  |
m    --------------ACCTGAAGA----------AATGGCTGACAGCCACACCTCCTCATG  82

h    GTGGGTACAATAGAGACTCACCTTGGGCTTTCATTGATTGTGTGAGTTGGTCTCTGGTTT 180
     ||||                |  |||  |||  ||||||||   ||  |||||||||  |
m    GTGGCTTT-------------CCCGGGGATTCTGTGATTGTG-ATGTGAGTCTCTGGTGT 128

h    TTCTC-AAAG TAG 192
     |||||  ||||  |||
m    TTCTCCAAAG TAG 141
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Figure 2 Comparison of human *MHC2TA* and murine *Mhc2ta* intronic DNA sequence identified between coding nts 474 and 475. The human (h) DNA sequence is shown above and murine (m) DNA sequence is shown below. The 5′ consensus donor splice sequence is underlined. A broken vertical line separates the two alternately used trinucleotide sequences (underlined) at the 3′ acceptor splice site.

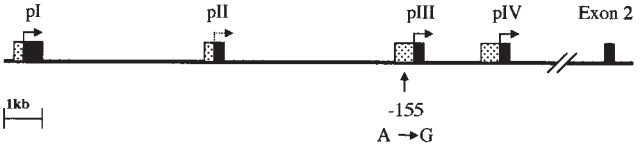

Figure 3 *MHC2TA* promoter elements. pI is used primarily by dendritic cells,[12] pIII by B cells,[12] and pIV for IFNγ-inducible CIITA expression by nonprofessional APC.[12–15] pII is considered inactive.[12] Stippled boxes represent upstream regulatory sequences for each promoter. Arrows represent transcription initiation sites. The SNP located in the DNA binding region of pIII is indicated.

fessional APC, including astrocytes and microglia,[14,15] resident CNS APC that may present antigen to pathogenic $CD4^+$ T cells in central nervous system (CNS) inflammation. In this regard, Rasmussen and colleagues[16] evaluated 111 northern European individuals with either relapsing remitting MS (RRMS) or primary progressive MS (PPMS) and 105 controls and identified one SNP (A→G) at nt 168 ($^-$155 relative to transcription initiation) of CIITA pIII in 29% of individuals with MS and 27% of controls. In contrast, another group[7] did not find any polymorphisms within promoters pI, pIII or pIV in their analysis of 23 individuals with insulin-dependent diabetes mellitus (IDDM), 30 rheumatoid arthritis (RA) patients and 19 normal individuals, all of northern Italian descent. Similar to Rasmussen *et al*.[16] we identified the same SNP at CIITA pIII nt $^-$155 in 37% of tested individuals (Table 1) and no SNPs in CIITA pI or pIV. Population-specific effects, random variation, or low power may account for these discordant findings. The systematic screening of variability in candidate genes for direct association with disease phenotypes is a promising approach for the genetic characterization of complex disorders. The *MHC2TA* polymorphisms identified in this study will be useful for the analysis of autoimmune and other immune related disorders.

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