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Genomic regulation of CTLA4 and Multiple Sclerosis

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Abstract

The cytotoxic T lymphocyte antigen 4 gene (CTLA4) is a critical regulator of T-cell activation and it is an important therapeutic target for cancer and autoimmune diseases. Here, we analyzed the genomic regulation of CTLA4 gene expression in order to identify single nucleotide polymorphisms (SNPs) that affect its expression and splicing, and to assess their association with Multiple Sclerosis (MS). We analyzed 152 healthy subjects and 146 patients with MS, of which 52 controls and 51 patients were used for gene expression analysis. We genotyped 17 SNPs in the CTLA4 gene using the SNaPshotTM Multiplex Kit, and in addition gene expression of the soluble (sCTLA4) and full length (flCTLA4) isoforms was quantified by real-time PCR, while protein levels of sCTLA4 were measured by ELISA. We found that the SNPs at −1577, +6230, +10242, +10717 and +12310 influence CTLA4 expression and the combination of the −1577 GG and +6230 GG genotypes provokes the strongest decrease in CTLA4 gene expression. We found that the SNP at −658 only acted as a regulatory SNP in patients with MS, suggesting the existence of epigenetic changes due to this disease. We also identified a decrease in CTLA4 gene expression levels in patients receiving chemotherapy, although no association was observed between MS and any of the polymorphisms studied. In conclusion, we have identified several SNPs in the CTLA4 gene and studied their influence on its genetic regulation. The involvement of CTLA4 in the pathogenesis of MS may be subtle and related to the functional changes in its pathway rather than predisposing genetic polymorphisms.

Keywords: CTLA4; Regulatory polymorphism; Splicing; Autoimmunity; Multiple Sclerosis

1. Introduction

The cytotoxic T lymphocyte antigen 4 gene (CTLA4) is located on chromosome 2q33 and it is involved in the negative regulation of T-cell proliferation. The CTLA4 gene produces two different isoforms: a full length protein (flCTLA4) encoded by exon 1 (peptide leader), exon 2 (extracellular domain), exon 3 (transmembrane domain) and exon 4 (cytoplasmic domain); and a soluble form (sCTLA4) in which exon 3 is lost (Teft et al., 2006). The soluble form is expressed by resting T cells and its expression disappears within 48 h of activation (Oaks et al., 2000). This soluble form then reappears gradually, whereas the expression of the full length form rapidly increases after cell activation and remains at the same level until the end of the immune response. In activated T cells, the flCTLA4 serves as a transmembrane receptor to inhibit cell proliferation. By contrast, the role of sCTLA4 is not yet known, although it has been shown to act as a functional receptor for B7 antigens, suggesting that sCTLA4 can block the B7–CD28 interaction and interfere with the co-stimulation signal, inhibiting T-cell proliferation (Teft et al., 2006). The lack of CTLA4 in knockout mice produces important lymphoproliferative disorders, multi-organic destruction and premature death (Waterhouse et al., 1995). Moreover, CTLA4 siRNA knock-down in animals leads to the development of autoimmune diabetes (Chen et al., 2006).

The CTLA4 gene is located in a chromosomal region that also contains the ICOS and CD28 genes. There is significant nucleotide homology between these genes, strongly suggesting that they resulted from ancient gene duplications. CTLA4 and

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CD28 homologues are found in all vertebrates whereas ICOS is only found in tetrapods, suggesting that this gene reflects a more recent duplication (Bernard et al., 2007). In humans, long-range linkage disequilibrium of these genes has been identified in worldwide populations, which was explained through demographic and adaptation events in our species (Ramirez-Soriano et al., 2005). Given the evolutionary conservation of CTLA4 and its differential expression in T-cell subsets, transcriptional or translational changes in its expression may have serious immunological consequences (Teft et al., 2006).

In recent years, CTLA4 has become a focus of research interest since it is a useful therapeutic target for immunotherapy in cancer and autoimmune diseases. Abatacept (CTLA4-Ig) is an approved drug to treat Rheumatoid Arthritis and Ipilimumab (anti-CTLA4 antibody) is undergoing clinical trials for the treatment of melanoma and other cancers. In addition, CTLA4 seems to be involved in the pathogenesis of autoimmune diseases (Gough et al., 2005). Several SNPs located in the promoter, the first exon and at the 3′ end of the CTLA4 gene (+6320 SNP aka CT60), as well as a $(AT)_n$ dinucleotide repeat at the 3′ end of the gene, have been associated with autoimmune diseases such as type 1 diabetes (Bouqbis et al., 2003), Grave's disease (Kouki et al., 2002), lupus (Graham et al., 2006) or Multiple Sclerosis (Favorova et al., 2006; Heggarty et al., 2007; Malferrari et al., 2005). However, some of these associations have not been confirmed and there is even some contradictory data (Bagos et al., 2007; Greve et al., 2008; Wray et al., 2008) including the recent whole genome association studies for MS (International Multiple Sclerosis Genetics Consortium et al., 2007). Recently, allelic variation at the 3′ end of the CTLA4 gene was correlated with lower mRNA levels of the soluble alternatively spliced form of the gene (Ueda et al., 2003). Indeed, this polymorphism seems to influence TCR signalling (Maier et al., 2007). While this allelic variation of the CTLA4 region appeared to be associated with several common autoimmune diseases, such as Graves' disease, autoimmune hypothyroidism and type 1 diabetes, some studies have failed to confirm the effect of the +6320 SNP in CTLA4 splicing (Anjos et al., 2005).

Due to the implication of several polymorphisms in the regulation of CTLA4 and the implication of this gene in several autoimmune diseases, we carried out a detailed characterization of the genomic regulation of CTLA4 gene expression in *cis* to improve our understanding of the genomic control of CTLA4 gene expression and to assess its contribution to the pathogenesis of MS.

2. Methods

2.1. Subjects

We studied 152 healthy individuals (HC) with no history of autoimmune diseases, all of southern-European ancestry (Spain and Portugal), and 146 patients with MS (Polman et al., 2005). Significantly, the southern-European population represents a more homogenous genetic group than mixed European populations (Seldin et al., 2006; Tian et al., 2008), with a similar frequency of the common HLA-DR2 allele (Villoslada et al., 2002). Signed informed consent was obtained from all the patients, who were recruited to the study by their neurologist and which was approved by the ethical committee of the University of Navarra. We collected the following clinical information: sex, age, disease duration, disease subtype, disability scores using the Expanded Disability Status Scale (EDSS) and the MS Functional Composite (MSFC). The response to IFN-beta therapy was defined by the presence of two or more relapses, and/or an increase in the EDSS scale, as described previously (Byun et al., 2008; Villoslada et al., 2004).

Patients and controls were recruited from the same geographical area and ethnic group, and the control group was mainly made up of friends or relatives of patients who agreed to participate in the study, but not from familial sufferers of MS. Controls were matched with the patient group by sex (HC: 68 male and 84 female; MS: 51 male and 95 female) and age (HC: 43.7±18.3 years; MS: 40.7±10.1). Patients display low to moderate disability (EDSS: 2.5 (0–8.5); MSFC score: 0.24±0.4) and an intermediate disease duration (5.1±5.3 years). Among the patients, 67 were receiving treatment at the time the blood samples were obtained, 45 with IFN-beta and 22 with chemotherapy. A second dataset was composed of 94 trio American families (MS patient and their healthy parents) from the UCSF MS DNA bank for validation purpose, generously provided by Prof. Jorge Oksenberg.

2.2. RNA and DNA purification and cDNA synthesis

Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) that were isolated from heparinized blood by density gradient centrifugation using Ficoll-Paque (Pharmacia Biotech). PBMCs were immediately submerged in the RNAlater RNA Stabilization Reagent (Qiagen) to preserve their gene expression patterns and total RNA was isolated using the RNeasy Mini Kit (Qiagen). Genomic DNA was removed by DNase treatment using the RNase-Free DNase Set (Qiagen). Alternatively, genomic DNA (gDNA) was isolated from granulocytes obtained by density gradient centrifugation using the QIAamp DNA Mini Kit (Qiagen). Synthesis of cDNA from total RNA was performed using the High-Capacity cDNA Archive Kit (Applied Biosystems).

2.3. Genotyping of SNPs

Seventeen sites of single nucleotide polymorphisms in the region of the CTLA4 gene were analyzed using the SNaPshotTM Multiplex Kit (Applied Biosystems), which consists of a single-base primer extension with fluorescently labeled ddNTPs. The SNPs are located at positions −1765, −1722, −1661, −1577, −658, −319, 49, 6230, 6249, 7092, 7482, 7982, 8173, 10242, 10717, 12131, and 12310 with respect to the initiation codon of the CTLA4 gene (see Fig. 1). The primers for amplification and genotyping are described elsewhere (Ramirez-Soriano et al., 2005). A total of 304 individuals were assayed of which six failed to give reliable genotypes. Thus, a total of 298 genotyped individuals were examined (152 controls and 146 cases).

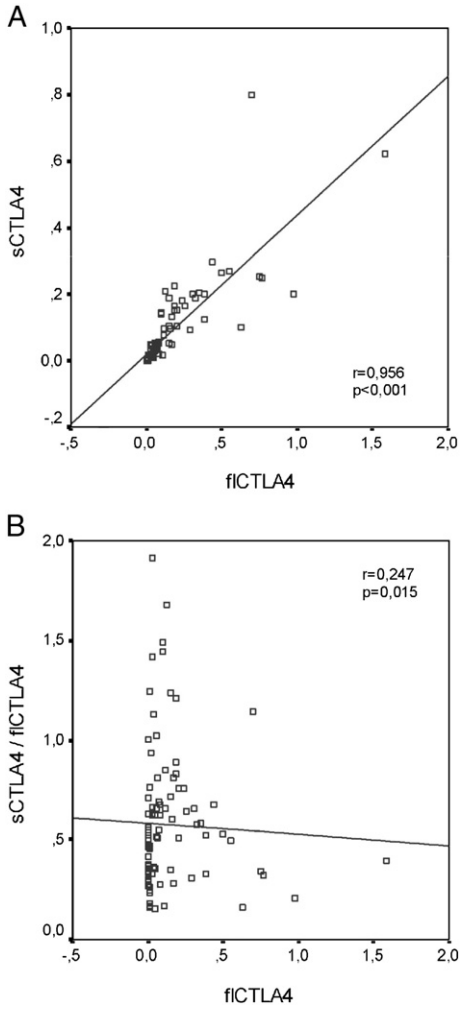

Fig. 1. Correlation between CTLA4 isoforms and the level of gene expression. A) Correlation between flCTLA4 and sCTLA4 gene expression; B) correlation between flCTLA4 and the ratio of sCTLA4 to flCTLA4 mRNA splice forms.

2.4. Quantification of gene expression

Quantitative real-time PCR (rt-PCR) was performed with the DNA Engine Opticon2 (MJ Research). We studied 52 HC and 41 MS patients from the cohort. Each sample was run in triplicate and the target and the endogenous control gene were amplified in different wells of each plate. The primers and probes used were those described previously (Ueda et al., 2003). Normalized gene expression was calculated using the Q-Gene software application (Muller et al., 2002), calculating the mean normalized expression as the average $c(t)$ values of the target and reference genes amplified in triplicate.

2.5. CTLA4 ELISA

The production of sCTLA4 was evaluated by ELISA in the human serum (Oaks and Hallett, 2000). Briefly, 96 well plates (Nunc) were coated with the anti-CTLA4 mAb (clone BNI3; PharMingen, San Diego, CA) and after blocking, 100 µl of a 1:3 dilution of the test samples was applied to the wells. The plates were incubated for 60 min at room temperature and after washing to remove unbound material, a biotinylated anti-CTLA4 mAb (clone AS-33, Antibody Solutions, Palo Alto, CA) was added to the wells for 1 h. The reactions were developed using a streptavidin–peroxidase complex (Zymed, South San Francisco, CA) and 3,3′,5,5′-tetramethyl-benzidine substrate, and the optical density (OD) was read at 450 nm. A standard curve was generated with a serial dilution of a commercially available HuCTLA4-huIgG fusion protein (R&D Systems 325-CT). Each sample was run in triplicate and was corrected for the background by subtraction of the OD obtained when the sample was incubated in wells coated with an isotype-matched mAb of irrelevant specificity (anti-rat CD4).

2.6. Statistical analysis

Genotype, allele frequencies and the Hardy–Weinberg equilibrium were estimated with the Arlequin program (Schneider et al., 2000). Frequencies were compared between patients and controls by means of the chi-square test and through odds ratios. Haplotype frequencies were estimated with the Phase program (Stephens and Donnelly, 2003). Linkage disequilibrium (LD) was assessed through the D' and r^2 values, and their significances were assessed by means of the Haploview program (Barrett et al., 2005). The representation of the LD values throughout the region of the gene was performed using the GOLD program (Abecasis and Cookson, 2000). The normal distribution of each data group was assessed with the Shapiro–Wilk Test. To analyze the relationship between gene expression levels for flCTLA4 and sCTLA4, the Spearman correlation coefficient was calculated. The differences between gene expression levels and of their distribution among the different genotypes were assessed with the Mann–Whitney U test. Bonferroni correction for multiple testing was applied when analyzing the genotype data distribution between patients and controls. Data were analyzed and processed using the SPSS 13.0 statistical package (SPSS Inc. Chicago, USA) and for all tests, p values of <0.05 were considered as significant.

2.7. Bayesian analysis

Gene expression data for flCTLA4 and sCTLA4 were made discrete in two intervals, high and low levels, with the same number of cases associated using an equal frequency discretization algorithm. A Bayesian network for each CTLA4 isoform was built using the Augmented Naïve Bayes algorithm. The functional genotypes were identified using the probability distributions of the inferred Bayesian networks and the Bayesian analysis was processed using the BayesiaLab 3.3 software (Bayesia SA. Laval Cedex, France).

3. Results

3.1. CTLA4 isoforms and regulatory SNPs

The levels of flCTLA4 gene expression were correlated with the levels of sCTLA4 in healthy individuals (Fig. 1A; $r=0.956$; $p<0.001$). To account for the fact that variable numbers of

Table 1
Differential genotypic expression of CTLA4 in healthy individuals

SNP	Genotype	*n*	flCTLA4	*p*	sCTLA4	*p*
−1577	AA	22	0.188±0.380	0.012	0.088±0.139	0.024
	GG	6	0.015±0.024		0.007±0.012	
−658	CC	31	0.103±0.194	ns	0.049±0.069	ns
	CT	6	0.314±0.626		0.149±0.221	
+6230	AA	12	0.200±0.445	0.049	–	
	GG	11	0.054±0.104		–	
+10242	GG	10	0.051±0.107	0.019	–	
	TT	14	0.224±0.416		–	
+8173	CC	20	–		0.099±0.146	0.050
	CT	15	–		0.039±0.061	
+10717	AA	12	0.246±0.447	0.036	–	
	GG	11	0.049±0.101		–	
+12310	CC	15	0.216±0.402	0.015	–	
	TT	10	0.051±0.107		–	

The results are presented as the mean±SD of the normalized gene expression. ns: not significant.

CTLA4-expressing cells were present in the peripheral blood of different individuals, flCTLA4 was used to normalize the values of sCTLA4. Accordingly, we found a negative correlation between the levels of flCTLA4 expression and the ratio of the sCTLA4 to flCTLA4 mRNA splice forms (Fig. 1B; $r=0.247$; $p<0.015$).

There was a decrease in flCTLA4 expression that was associated with the GG genotype in SNP −1577, +6230, +10717, +10242 ($p<0.05$ in all cases, Table 1) and with the TT genotype in the SNP +12310 ($p=0.015$). Alternatively, sCTLA4 expression was lower in the GG genotype when compared with the AA genotype for the SNP −1577 ($p=0.024$). These results indicate that the SNP −1577 is a regulator of sCTLA4 and flCTLA4 expression. By contrast, SNP +6230, +10242, +10717 and +12310 only regulate flCTLA4 expression and these four SNPs are in linkage disequilibrium in our population (Fig. 2; $D'>90$).

3.2. Identification of extended genotypes

In order to identify the different combinations of polymorphisms that regulate the levels of gene expression and the splicing of the CTLA4 gene, we performed a Bayesian network analysis. By analyzing the probabilistic distribution of the expression levels for the CTLA4 isoforms we found that the combination of the GG genotypes for SNPs −1577 and +6230 were associated with weaker CTLA4 expression (up to 95%, Fig. 3). We also assessed whether the presence of these combined genotypes might influence the protein levels of the soluble CTLA4 isoform. When, we measured the levels of sCTLA4 in the serum of 104 HC by ELISA (36 subjects with the genotype GG/GG and 71 with the most common AA/AA genotype), no differences were detected in the level of sCTLA4 in this group irrespective of their genotypes (data not shown).

3.3. Association between CTLA4 SNPs and susceptibility for MS

The 17 SNPs analyzed include 6 SNPs in the promoter region, 1 coding SNP (+49), and 9 SNPs at the 3′ end of the gene (Fig. 2).

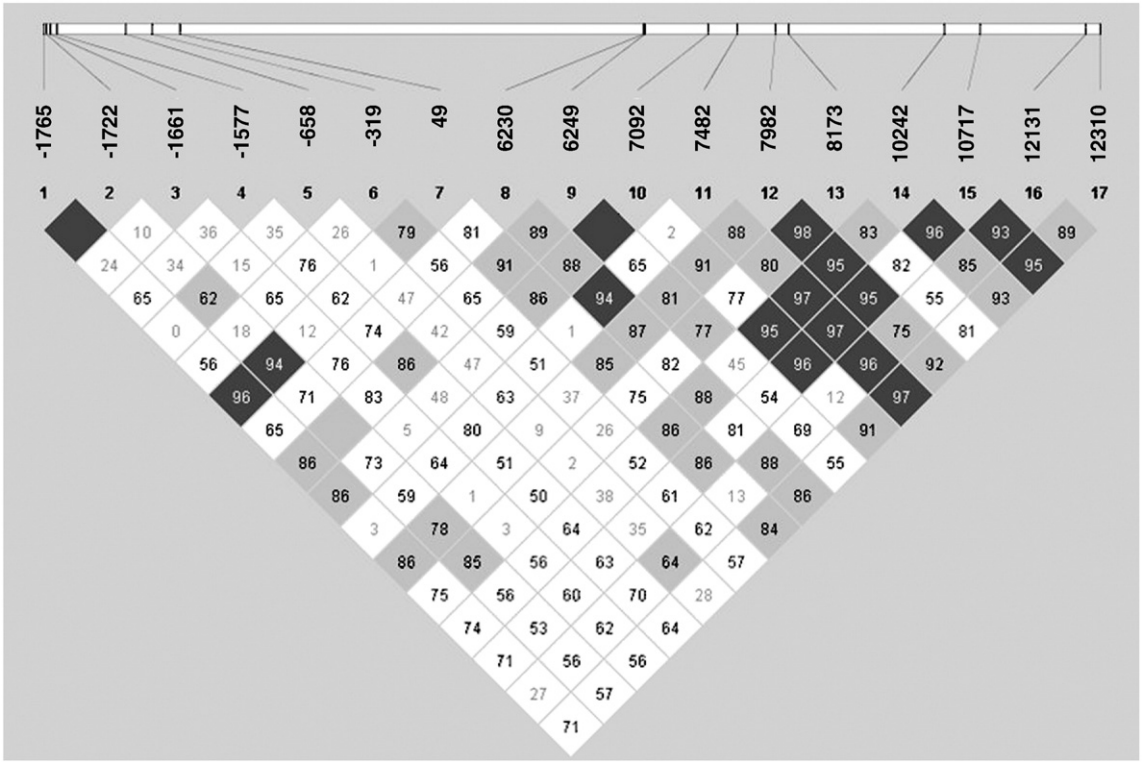

Fig. 2. Linkage disequilibrium D' values between pairs of SNPs along the CTLA4 gene region. The physical distance between markers is shown in the upper part of the chart and the D' values are shown within the squares linking the pairs of markers. Empty squares represent a D' value of 1 and black squares indicate strong evidence of LD, while white squares indicate low LD and grey squares are uninformative.

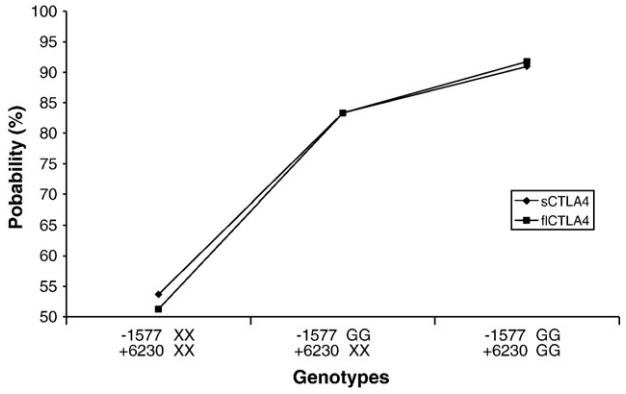

Fig. 3. Bayesian analysis of the influence of CTLA4 SNPs in CTLA4 gene expression. The presence of the GG genotype in the −1577 SNP increases the probability of producing low levels of CTLA4 by up to 80% as compared with the 50% chance when the genotype was unknown. The combination of a GG genotype in the −1577 SNP and in the +6230 SNPs increases the probability of producing low levels of either sCTLA4 or flCTLA4 by up to 95%.

These SNPs also include the 5 haplotype-tagged SNPs defined for the CTLA4 gene in a European sample by Johnson et al. (2001). The allele frequencies of the CTLA4 SNPs are shown in Table 2, and no departure from a Hardy–Weinberg equilibrium ($p>0.05$) was observed either in HC or MS patients after a correction for multiple tests.

The distribution of the genotypes did not differ significantly between MS patients and HC for any of the SNPs analyzed (Table 2). This situation persisted even after stratification according to the DR2 status (data not shown). When we evaluated the effect of CTLA genotypes on the course of the disease, no significant differences were evident in the genotype distribution between patients and controls. This was the case even after stratifying by sex, age, disease duration and subtype, as well as by the presence of other autoimmune diseases or response to IFN-beta therapy ($p>0.05$ in all cases).

Table 2
Allele frequencies and odds ratios in controls and cases for the 17 SNPs analyzed in the CTLA4 gene region

SNP	Controls ($N=152$)	MS cases ($N=146$)	Odds ratio	Confidence interval	p
−1765 T	0.313	0.295	0.918	0.648–1.303	0.633
−1722 T	0.954	0.938	0.735	0.359–1.506	0.399
−1661 A	0.793	0.767	0.861	0.584–1.269	0.450
−1577 G	0.398	0.411	1.055	0.761–1.464	0.748
−658 C	0.878	0.873	0.955	0.587–1.554	0.853
−319 C	0.918	0.904	0.845	0.480–1.486	0.558
49 G	0.273	0.271	0.988	0.688–1.417	0.946
6230 G	0.444	0.442	0.991	0.717–1.369	0.955
6249 G	0.855	0.818	0.763	0.493–1.181	0.224
7092 G	0.247	0.267	1.113	0.770–1.607	0.569
7482 A	0.868	0.839	0.790	0.501–1.246	0.310
7982 A	0.230	0.223	0.957	0.652–1.405	0.823
8173 T	0.260	0.267	1.038	0.721–1.495	0.841
10242 G	0.391	0.404	1.054	0.759–1.464	0.752
10717 G	0.385	0.449	1.300	0.938–1.802	0.114
12131 C	0.849	0.808	0.751	0.490–1.153	0.190
12310 T	0.365	0.418	1.248	0.897–1.735	0.188

Table 3
Haplotype frequencies for the blocks defined in the CTLA4 gene region

	SNPs	Control $N=152$	MS $N=146$	p
Block 1 positions 7982–8173	GC	186	172	ns
	AT	95	90	ns
	GT	15	19	ns
	AC	8	11	ns
Block 2 positions	ACC	154	131	ns
10717–12131–12310	GCT	55	54	ns
	GGT	39	55	0.04430
	GCC	7	13	ns
	ACT	11	30	0.00133
	AGC	8	2	ns
	AGT	29	7	0.00025
	GGC	1	–	ns

ns: not significant.

When the haplotype structure was considered, 84 and 89 haplotypes were found in controls and patients, respectively. Moreover, there were no significant differences in haplotype frequencies between patients and controls. We also analyzed the frequencies of the haplotypic blocks in both groups (Fig. 2, Table 3). The GGT and ACT haplotypes in block 2 were overrepresented in patients ($p=0.004$; $p=0.001$), whereas the AGT haplotype was underrepresented in patients ($p<0.001$). However, this association could not be confirmed in a second dataset from a different population (94 trios from the US). Finally, we found no differences in the distribution of the extended GG −1577 and GG +6230 genotypes, which have a high probability of producing low levels of CTLA4 isoforms in patients and controls (data not shown).

3.4. CTLA4 isoforms and MS

A correlation between the levels of sCTLA4 and flCTLA4 expression was detected independently in the HC and MS patients, either untreated or treated with IFN-beta or immunosuppressive agents ($r\geq0.943$; $p<0.001$ in all cases). When we compared the levels of expression from the different CTLA4 isoforms and the sCTLA4/flCTLA4 ratio between MS patients and HC (Table 4), we were unable to find any significant differences. When analyzing the influence of the treatment on the expression of the different isoforms of CTLA4 (Table 4), there was a significant decrease in the sCTLA4/flCTLA4 ratio

Table 4
Levels of CTLA4 gene expression in HC and MS patients with or without therapy

	N	flCTLA4	sCTLA4	sCTLA4/ flCTLA4	p
HC	52	0.146±0.040	0.067±0.015	0.566±0.044	ns
MS					
Untreated	26	0.116±0.036	0.072±0.018	0.609±0.067	ns
IFN-beta	17	0.166±0.246	0.118±0.214	0.671±0.538	ns
Immunosupressors	8	0.098±0.194	0.049±0.106	0.339±0.147	0.019*

The results are presented as the mean±SD of the normalized gene expression values.
*p value between untreated and immunosupressors treated patient.

between MS patients treated with immunosupressors and MS patients that remained untreated ($p=0.019$). Indeed, when we compared the levels of gene expression in MS patients, we found a decrease in the expression of sCTLA4, flCTLA4 and sCTLA4/flCTLA4 in the CT genotype when compared with the CC genotype in the SNP −658 ($p<0.05$ in all cases).

4. Discussion

In this study, we have identified several regulatory SNPs in the CTLA4 gene, and our data indicate that several SNPs located at both the 5′ and 3′ region strongly influence the levels of expression of both known CTLA4 isoforms, in agreement with previous reports (Gough et al., 2005; Teft et al., 2006; Ueda et al., 2003). In addition, the same regulatory SNPs that are functionally implicated in the activation of T cells are also associated with a predisposition to suffer autoimmune diseases (Gough et al., 2005). Moreover, we identified a cooperative effect of the SNPs −1577 and +6230 in regulating CTLA4 mRNA levels, although we were unable to reproduce the effect of the SNP +49 on the expression of the two isoforms (Gough et al., 2005). Nevertheless, since we have used a smaller dataset for the functional studies when compared to the genetic association analysis, we cannot rule out the possibility that bigger cohorts might confirm such an association. Our data will be helpful to design genomic studies aimed at identifying the contribution of CTLA4 to immunological diseases, as well as for pharmacogenomic studies aimed at identifying responders to CTLA4 targeted therapy.

We did not find any significant differences in the genotype or allele frequencies of any of the CTLA4 SNPs tested in the MS and HC groups, or for the presence of the HLA-DR2 genotype or the response to therapy in any subgroup of patients. There were no significant differences between MS patients and HC in the polymorphisms previously associated to MS (−319 and +49: Kantarci et al., 2000; Maurer et al., 2002), although they were more frequent in healthy controls when compared to MS cases. Conformity to a Hardy–Weinberg equilibrium is evidence that genotyping errors did not cause a significant bias. Indeed, this result is also in accordance with several whole genome association studies and with a recent meta-analysis of CTLA4 and MS that failed to show an association with CTLA4, despite the very large sample size (International Multiple Sclerosis Genetics Consortium et al., 2007; Lorentzen et al., 2005; The Games Collaborative, 2006; Walsh et al., 2003). When considering the genomic block structure, we identified several differences in block 2 (SNPs 10717–12131–12310) between patients and controls, but such differences were not evident in a second cohort.

We also assessed the differences in gene expression between patients and controls. Although we found no major differences in the levels of the two CTLA4 isoforms expressed in patients and controls, chemotherapy did decrease the sCTLA4/flCTLA4 ratio. Such findings suggest that in addition to decreasing the effector T-cell populations, chemotherapy also targets regulatory T cells, which might predispose individuals to future relapses of the disease. By contrast, IFN-beta did not influence CTLA4 levels. We also found a decrease in the expression of CTLA4 isoforms associated with certain alleles of SNP −658 in MS patients but not in controls. This result may suggest either epigenetic changes or changes in *trans* that are generated by the disease process and that might also impair regulatory T-cell function in MS.

Our study may suffer from certain limitations. Although we fine mapped the entire gene and examined the expression data, our sample size was small. However, our failure to confirm an association between CTLA4 and MS is strongly supported by many recent studies that generally reject the involvement of CTLA4 in the genetic susceptibility of MS. Thus, both our results and those of previous studies rule out a strong association between CTLA4 polymorphisms and MS, although weak associations between the CTLA4 polymorphisms and MS cannot be ruled out, or a role of this genomic region in MS susceptibility.

The results of this work show the importance of several SNPs in the regulation of CTLA4 gene expression, which might contribute to the pathogenesis of autoimmune diseases and to the regulation of the immune response. Although the expression of CTLA4 isoforms and their relative proportions do not seem to be implicated in the pathogenesis of MS, we cannot rule out a downstream effect in the regulation of peripheral immune tolerance. For example, we identified a regulatory SNP that modifies the expression and splicing of CTLA4 in MS patients, perhaps through epigenetic modifications or in combination with polymorphisms in other genes. On the other hand, we also identified a regulatory role for several SNPs of the CTLA4 gene previously associated with several autoimmune diseases, suggesting that genetic regulation of CTLA4 might contribute to the development of autoimmune diseases.

CTLA4 is critical for the activity of regulatory T cells. Recent studies have shown that the function of regulatory T cells is impaired in MS (Astier et al., 2006; Correale and Villa, 2008; Martinez-Forero et al., 2008; Viglietta et al., 2004). Thus, even if polymorphisms in the CTLA4 gene do not directly contribute to the susceptibility to MS, CTLA4 function may contribute to the failure of regulatory T-cell function in individuals at risk of suffering MS. Recent whole genome association studies (Gregory et al., 2007; International Multiple Sclerosis Genetics Consortium et al., 2007) point to T-cell biology as an important pathway in the pathogenesis of MS, particularly through the function of IL2, IL7R and perhaps some other molecules as well. The regulation of peripheral immune tolerance is a delicate balance between pro-inflammatory and immunosuppressive factors. In this scenario, the role of CTLA4 or other regulatory molecules is critical and they may become the target for new immunomodulatory therapies for MS.

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