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Sequence variation in the transforming growth factor-β1 (*TGFB1*) gene and multiple sclerosis susceptibility

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Abstract

Genome screenings in multiple sclerosis (MS) have identified multiple susceptibility regions supporting a polygenic model for this disease. Evidence for linkage was consistently observed at ch.19q13 suggesting the presence of an MS gene(s) in this region. Several interesting candidate genes are encoded within this region, including transforming growth factor-beta 1 (*TGFB1*) and interleukin-11 (*IL11*). Both are multifunctional cytokines with significant and well-characterized immunomodulatory properties. We performed a comprehensive evaluation of common polymorphisms within the *TGFB1* and *IL11* loci and three closely flanking microsatellite markers (*D19S421*, *CEA*, *D19S908*) in 161 stringently ascertained and clinically characterized MS multiplex families using tests of both linkage (lod score, sib-pair analysis) and association (pedigree disequilibrium test or PDT). Patients and families were stratified by HLA-DR2 status to search for two-locus interactions. Suggestive evidence for linkage and association to *CEA* (lod score = 1.25, $\theta = 0.20$, $p = 0.015$, respectively), located 0.4 cM from *TGFB1*, was observed in DR2 positive families only. Distinct clinical phenotypes were also examined and an association between a *TGFB1* haplotype and a mild disease course was present ($p = 0.008$), raising the possibility that *TGFB1* or a nearby locus may influence disease expression.

Keywords: Multiple sclerosis; Transforming growth factor-beta 1 (*TGFB1*); Interleukin-11 (*IL11*)

1. Introduction

Multiple sclerosis (MS) is a common inflammatory disease of the central nervous system (CNS) characterized by myelin loss, varying degrees of axonal pathology, and progressive neurological dysfunction (Hauser and Goodkin, 2001). A large body of research supports a multifactorial etiology, with an underlying genetic susceptibility likely acting in concert with undefined environmental exposures (Ebers and Sadovnick, 1994; Oksenberg and Barcellos, 2000). Full genome screenings (Ebers et al., 1996; Multiple Sclerosis Genetics Group, 1996; Kuokkanen et al., 1997; Sawcer et al., 1996) and follow-up studies (Chataway et al., 1998; Multiple Sclerosis Genetics Group, 1998) identified a number of genomic regions in linkage with the disease, supporting a polygenic model for MS, and significantly focusing the list of potential candidate susceptibility genes.

One region identified by all four screens resides within chromosome 19q13 near the *APOC2* and *APOE* genes. Allelic associations within ch.19q13 have also been reported (Barcellos et al., 1997), and a more recent detailed examination of this region by our group has provided additional evidence to support the presence of a susceptibility locus here (Multiple Sclerosis Genetics Group, 2000). This region contains many potential candidate disease genes including transforming growth factor-β1 (*TGFB1*) and interleukin-11 (*IL11*). TGF-β1, a pleiotropic cytokine

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with a significant role in the regulation of immune homeostasis, serves as a potent chemoattractant in the early stages of inflammatory response, and as an immunosuppressant during resolution of such an event (Letterio and Roberts, 1997). Selective disruption of TGF-β1 signaling in T cells leads to loss of self-tolerance with concomitant inflammatory infiltration in several organs and the presence of circulating autoantibodies (Gorelik and Flavell, 2000), and TGF-β1 null (−/−) mice suffer uncontrolled inflammation and die within 3–4 weeks of birth (Kulkarni et al., 1993). In MS patients, high levels of TGF-β1 mRNA expression in mononuclear peripheral cells have been associated with reduced disability (Link et al., 1994), disease duration (Soderstrom et al., 1995) and disease brain activity (Bertolotto et al., 1999). Seven *TGFB1* polymorphisms have been identified (Cambien et al., 1996), including three in the upstream region, one in the nontranslated region, and three located at codons 10 and 25 of exon 1 and codon 263 of exon 5 (Fig. 1). Interindividual variations in the production of TGF-β1 appear to be, at least in part, under the genetic control of some of these polymorphisms (Awad et al., 1998; Grainger et al., 1999).

Interleukin (IL)-11 is also a multipotential cytokine with profound effects on the immune response (Trepicchio et al., 1996; Trepicchio and Dorner, 1998). IL-11 was first isolated from a primate bone marrow cell line based on its ability to stimulate the proliferation of an IL-6-dependent mouse plasmacytoma cell line. IL-11 enhances T-cell-dependent development of immunoglobulin-producing B cells and stimulates the production of tissue inhibitors of metalloproteinases (Maier et al., 1993). Antibody responses and activation of metalloproteinases are necessary for the full development of demyelination (Genain et al., 1999; Leppert et al., 1998). A common variant within the 5′ flanking region of the *IL11* locus has been reported (Bellingham et al., 1998).

To further clarify the contribution of *TGFB1* and *IL11* to MS pathogenesis, we analyzed in detail the distribution of common genomic variants in these loci in a well-characterized dataset of multiple affected member families with the relapsing form of MS. Our findings were largely negative. However, there was suggestive evidence for linkage and association to this region in HLA-DR2 positive families, and an association between the "low-producer" *TGFB1* haplotype and mild disease course.

2. Materials and methods

2.1. Families

Rigorous clinical criteria were employed to identify and collect 161 families with multiple cases of MS within the United States. In keeping with the variety of family structures seen in the general population, ascertainment was not restricted to a single-family type and collection was extended through all affected first-degree relatives if possible, generating more than one sib-pair per family. All affected members were interviewed and parental and ancestral information was recorded by countries of origin. All known ancestors were Caucasian and European in origin. Diagnostic criteria, ascertainment protocols, and clinical and demographic characteristics of the population are summarized elsewhere (Goodkin et al., 1991). In order to limit possible confounding effects of disease heterogeneity and misdiagnosis, families in which a primary progressive course was present in two or more members were excluded.

2.2. Definition of clinical features

Age of onset was defined as the first episode of neurological dysfunction suggestive of demyelinating disease. In determination of the age of onset, the patient was asked to recall his or her initial neurological symptom, including visual blurring due to optic neuritis, vertigo, Bell's palsy, tic doloureux, diplopia, Lhermitte's symptom, focal weakness, sensory symptoms, or paroxymal symptoms. In the majority of patients with visual blurring, a distinction between optic neuritis and brainstem diplopia was made with confidence, but in uncertain cases no designation was made. The designation of spinal cord onset was restricted to cases in which: (a) bilateral sensory and or motor signs appeared acutely with evidence of a sensory level on the trunk, or (b) the site of the attack was confirmed by MRI scanning of the spinal cord. The site of disease onset was

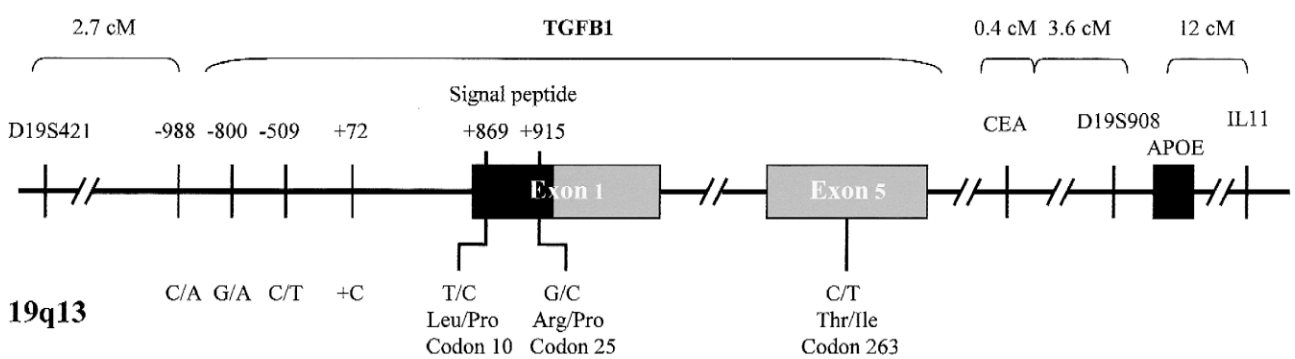

Fig. 1. Genomic organization of the *TGFB1* locus and associated polymorphisms.

scored on the basis of first two MS attacks experienced; each patient was categorized as having 'opticospinal' manifestations (optic neuritis (ON) only and/or spinal cord (SC) involvement without other sites of involvement) or other initial symptoms (e.g. unilateral sensory or motor symptoms, brainstem dysfunction, incoordination, etc.). This phenotypic variable aggregates in our family cohort suggesting an underlying genetic basis for clinical expression (Barcellos et al., 2000a,b). Disability was assessed at entry with the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). A 'mild' course of MS was defined as an EDSS score of < 3 after 10 years of symptoms. In general, patients in this group can walk normally or have mild gait disability only. A 'severe' course of MS was designated in patients who became wheelchair dependent (EDSS = 7 or greater) within 10 years of onset. Complete clinical data was available for over 90% of patients.

2.3. Genotyping

White blood cells are routinely transformed to establish lymphoblastoid cell lines on all ascertained family members. High molecular weight DNA was isolated using a standard desalting procedure. DNA samples were organized into genotyping keys and 10 ng aliquoted into 96-well plates for 25–30 cycles of PCR amplification. *TGFB1* polymorphisms were detected by hybridization with biotinylated sequence-specific oligonucleotide probes (SSOP) in a 96-well dot-blot format assay following development with streptavidin/horse radish peroxidase. Primer and probe sequences have been previously reported (Cambien et al., 1996) and are publicly available (http://www.genecanvas.idf.inserm.fr/), with the exception of codon 10/25 reverse primer sequence used in this study (GGCCAGCCGCAGCTTGGACA). All PCR amplification, hybridization and detection conditions are available upon request. Probe specificity was confirmed by direct sequencing of DNA samples. For analysis of *IL11* and microsatellite polymorphisms (*D19S421*, *CEA* and *D19S908*), fluorescently labeled oligonucleotide PCR products were resolved by electrophoresis in 6% acrylamide denaturing gels on the ABI PRISM 377 Automated DNA Sequencer loaded with the ABI PRISM 672 GENESCAN 2.1 software for fluorescent scanning. Genotyper 2.0 is then used for peak calling and allele binning. HLA typing for DRB1 and DQB1 loci was performed using a non-radioactive PCR-based sequence specific oligonucleotide probe reverse line-blot assay (PCR-SSOP) (Dynal, Norway). Complete HLA data (all affecteds) was available for 87% of the families. Generation of all genotypes was performed blind to pedigree structure and to the clinical status of the family members. Data was formatted on MS-Excel and transferred to pedigrees files drawn with Cyrillic 2.1 software, where Mendelian inheritance was automatically checked and confirmed. Genotype data was exported back into Excel and saved as ASCII files for downloading into the LAPIS data management system and storage in the PEDIGENE® database (Haynes et al., 1995).

2.4. Statistical methods

Linkage analysis included model dependent and model independent methods. Since the mode of MS inheritance is not known with certainty, both an autosomal dominant model assuming an MS disease allele frequency of 0.05, and an autosomal recessive model with an MS disease allele frequency of 0.20, were used for LOD score analysis. Both of these models use phenotypic information only on affected individuals, thus eliminating the phenotypic information on all individuals not clinically definite for MS. Two-point calculations utilized FASTLINK (Schaffer et al., 1994). The two-point LOD scores were examined for any evidence of locus heterogeneity using HOMOG (Ott, 1999). Sib-pair analysis (assuming a locus specific $\lambda_s = 30$) was completed using the sib-phase option of ASPEX computer package (Hinds and Risch, 1998). Marker allele frequencies were estimated from genotypic information derived from all unrelated married individuals in the dataset. These allele frequencies were compared to available data from a Caucasian control dataset and published frequencies. No significant differences were observed. Family-based association studies were performed using the pedigree disequilibrium test (PDT) (Martin et al., 2000). The PDT is a powerful analytical method that utilizes genetic data from related nuclear families and discordant sibships within extended pedigrees. Haplotypes comprised of the five closely linked *TGFB1* polymorphisms were assigned in family members using SimWALK (Weeks and Lathrop, 1995). All initial analyses utilized *TGFB1* haplotypes to increase informativeness, and to avoid multiple testing of correlated markers. Families were stratified for linkage and association analyses by HLA-DR2 status, and were divided into three groups as previously described (Barcellos et al., 2000a,b): (1) 'DR2 all', in

Table 1
Clinical summary of MS patients

Clinical/demographic information	All MS patients
Total no. of individuals with MS (n)	412
Females	297
Males	115
Mean age of onset (years) (SD)	29.6 ($\pm$8.6)
Mean disease duration (years) (SD)	17.0 ($\pm$10.5)
Early clinical manifestations (n, %)	
Optic nerve and/or spinal cord only[a]	97 (26.7)
Other	266 (73.3)
Disease course (n, %)	
Mild MS[b]	51 (12.4)
Severe MS[c]	18 (4.4)

[a] Location of first two attacks.
[b] EDSS < 3 in ≥ 10 years.
[c] EDSS ≥ 7 in ≤ 10 years.

Table 2
Allele frequencies and pairwise linkage disequilibrium coefficients (D') for *TGFB1* polymorphisms in Caucasian controls

Polymorphism	Allele frequencies	*TGFB1 − 800*	*TGFB1 − 509*	*TGFB1Cod10*	*TGFB1Cod25*	*TGFB1Cod263*
TGFB1 − 800	0.94/0.06	–	−0.91**	−0.98***	−1.00 ns	0.07 ns
TGFB1 − 509	0.66/0.34	–	–	0.97*****	−0.79**	1.00***
TGFB1Cod10	0.62/0.38	–	–	–	0.94***	1.00***
TGFB1Cod25	0.94/0.06	–	–	–	–	−0.65 ns
TGFB1Cod263	0.97/0.03	–	–	–	–	–

Analysis based on $2N = 350$ unrelated control chromosomes. Haplotypes were estimated using maximum likelihood methods (Slatkin and Excoffier, 1996). Number of asterisks denotes level of significance (* < 0.05, ** < 0.01, *** < 0.001, ***** $< 10^{-4}$).

which every affected individual carried at least one DR2 allele, (2) 'DR2 some', in which some but not all affected individuals carried at least one DR2 allele, and (3) 'DR2 none' in which all members were DR2 negative.

Pairwise disequilibrium coefficients for *TGFB1* polymorphisms were estimated in the control individuals and were reported as the ratio of the unstandardized coefficients to their minimal/maximal values, i.e. the normalized disequilibrium parameter, D' (Hartl, 1998). The sign in front of the coefficients indicates whether linkage disequilibrium is positive or negative. Significance was measured using the Fisher's exact test.

The effects of *TGFB1* and *IL11* genotypes on clinical variables in MS were examined as follows: age of disease onset and years to EDSS ≥ 7, both continuous outcomes, were analyzed in the multiplex families using linear mixed models (models with both fixed and random effects) as implemented in Proc Mixed in SAS Version 6.12, (SAS Institute, Cary, NC) (Jennrich and Schluchter, 1986). These models are fit by restricted maximum likelihood and use random effects to take into account any correlation between members of the same family, which would violate the independence assumption of ordinary linear regression models. Categorical clinical outcomes such as site of onset (opticospinal manifestations compared to all others) and disease course (mild and severe forms) were analyzed using Generalized Estimating Equations (GEE) (Zeger and Liang, 1986), which also take into account any correlation between family members, as implemented in Proc Genmod in SAS. In order to account for multiple analyses of clinical variables ($n = 5$: age of onset, first two attacks, mild course, severe course, and years to EDSS ≥ 7) a significant criteria of $p = 0.01$ was used. In addition, due to the strong linkage disequilibrium present between the five *TGFB1* polymorphisms and to avoid multiple testing of each *TGFB1* genotype, patients were stratified into two groups according to whether or not they were homozygous for the extended wildtype genotype. Similarly for the bi-allelic *IL11* marker, patients were stratified by the presence or absence of the common *IL11* allele.

3. Results

The familial dataset utilized in this study consists of a total of 1300 genotyped individuals, including 230 affected sib-pairs. Two hundred ninety seven of the affected were females, and 115 males (sex ratio = 2.6:1) (Table 1). To take full advantage of the power of this dataset, a multi-analytical strategy was applied including parametric LOD

Table 3
TGFB1 haplotype frequencies in the familial MS dataset

Haplotype[a]	Controls[b]		All affected individuals		Index cases only	
	N	Frequency	*N*	Frequency	*N*	Frequency
GCTGC	364	0.514	437	0.530	176	0.553
GTCGC	173	0.244	205	0.249	72	0.226
GCCCC	52	0.073	57	0.069	22	0.069
ACTGC	47	0.066	54	0.066	19	0.060
GCCGC	22	0.031	24	0.029	8	0.025
GTTGC	18	0.025	16	0.019	7	0.022
GTCGT	13	0.018	15	0.018	7	0.022
Rare[c]	19	< 0.01	16	< 0.01	7	< 0.01
Total	708		824		318	

[a] *TGFB1* haplotypes were comprised of five biallelic polymorphisms at the following sites, in order : −800, −509, cod10, cod25, cod263. Haplotypes were assigned using SimWALK (Weeks and Lathrop, 1995).
[b] Results based on chromosomes derived from unrelated spouses and founders within the 161 MS pedigrees.
[c] Rare refers to those haplotypes present in < 1.0% of individuals: controls ($n = 9$), all affected individuals ($n = 8$), probands ($n = 7$).

Table 4
Two-point model-dependent linkage results for *TGFB1*, *IL-11* and ch.19q13 microsatellite markers in familial dataset

Loci	All patients ($N = 161$ families)		All affected individuals DR2 + ($N = 79$ families)		Some affected individuals DR2 + ($N = 31$ families)		No affected individuals DR2 + ($N = 30$ families)	
	AD[a]	AR[b]	AD	AR	AD	AR	AD	AR
D19S421	0.92	0.88	0.56	0.85	0.30	0.14	0.10	0.10
TGFB1[c]	0.11	0.21	0.32	0.40	−0.08	−0.05	0.51	0.31
CEA	0.67	1.09	0.92	1.25[d]	−0.06	0.57	−0.01	−0.02
D19S908	0.20	0.64	0.06	0.51	0.06	0.09	0.00	0.00
IL11	−0.22	−0.24	−0.13	−0.18	−0.14	−0.10	−0.04	−0.04

[a]AD = affected dominant.
[b]AR = affected recessive.
[c]The five closely linked single nucleotide polymorphisms within the *TGFB1* locus were analyzed as a single haplotype. Individual haplotypes were assigned using SimWALK (Weeks and Lathrop, 1995). Significant results were not observed for individual *TGFB1* polymorphisms (data not shown).
[d]$\theta = 0.20$.

scores, sib-pair and PDT analyses as described in the previous section. Individual polymorphisms (Fig. 1) and *TGFB1* haplotypes were utilized for all linkage and association analyses. Strong linkage disequilibrium exists between the five *TGFB1* polymorphisms. This is shown in Table 2 which lists the coefficients (D') of linkage disequilibrium between pairs of polymorphisms analyzed in a sample of 175 unrelated US Caucasians. A negative D' value indicates that the less frequent allele at one site is associated with the more frequent allele at the other site, whereas a positive D' value indicates that the less or more frequent alleles at both sites are preferentially associated. The strongest association was observed between wild-type alleles at position −509 (C) and codon 10 (T) ($D' = 0.97$, $p < 0.0001$). Similar results were observed by Cambien et al. (1996). *TGFB1* haplotype frequencies for all affecteds, index cases only, and controls were very similar and are shown in Table 3. The most common *TGFB1* haplotype present was comprised of wild-type alleles at all five polymorphic sites with a frequency of approximately 53.0%. This haplotype consists of −800/G, −509/C, Codon 10/T, Codon 25/G and Codon 263/C, and includes polymorphisms specifically associated with reduced transcriptional activity (Awad et al., 1998; Grainger et al., 1999).

Two-point linkage results are shown in Table 4. To further test for interaction between the candidate loci and the *MHC* at chromosome 6p21.3, the MS dataset was divided into three groups based on *HLA-DR2* status (Barcellos et al., 2000a,b). While potentially entailing some loss of statistical power due to the reduced family sample size in each category, the implementation of this stratification scheme may detect either interaction or independence between the loci under study and the *MHC*. The highest total LOD score of 1.25 ($\theta = 0.20$) was present in the DR2 + subset of families for the *CEA* marker using an affected recessive model of inheritance. Allowing for locus heterogeneity did not affect LOD scores significantly (data not shown). The results of sib-pair and association analyses are summarized in Table 5. Evidence for excess sharing of alleles or haplotypes among affected sib pairs was not observed. Similarly, distortion in allele or haplotype transmission was not observed overall, with the exception of *CEA* in DR2 + families only (global p-value = 0.015). Overall, none of the tested markers appear to be associated or linked to disease susceptibility.

To determine whether polymorphisms at the *TGFB1* and *IL11* loci might influence clinical presentation in MS, all patients from the multiplex families were stratified according to genotypes for analyses of each locus. Clinical variables included age of onset, years to EDSS ≥ 7, early clinical manifestations and disease course (see subjects section). Patients homozygous for the *TGFB1* wild-type haplotype (GCTGC) were compared to patients carrying other haplotypes to look for effects on clinical presentation. A significant association was observed in mild MS patients carrying the wild-type *TGFB1* genotype (OR = 2.31, $p = 0.008$, 95% CI = 1.25–4.27) when compared to all other patients (Table 6). Testing of individual *TGFB1* polymorphisms revealed significant associations between mild MS and wild type genotypes at both −509 and codon 10 positions only (OR = 2.46, $p = 0.007$, 95% CI = 1.27–4.74, and OR = 2.23, $p = 0.02$, 95% CI = 1.14–4.37, respectively). Significant associations with *TGFB1* variation and other clinical variables were not present in this familial data set. For the *IL11* bi-allelic polymorphism, three

Table 5
Model-free linkage and association test results

Loci	ASPEX maximum LOD score	PDT p-value
D19S421	0.16	0.44
TGFB1[a]	0.55	0.51
CEA	0.01	0.60[b]
D19S908	0.38	0.19
IL11	0.00	0.99

[a]The five closely linked single nucleotide polymorphisms within the *TGFB1* locus were analyzed as a single haplotype. Significant results were not observed for individual *TGFB1* polymorphisms (data not shown).
[b]$p = 0.015$ for CEA in DR2+ families (Martin et al., 2000).

Table 6
Effect of *TGFB1* genotype on clinical outcome in MS

Clinical outcome	OR	95% CI	*p*-value
First two attacks			
Restricted to optic nerve and/or spinal cord only	1.31	(0.79–2.19)	0.29
Mild course			
EDSS < 3 after 10 years of onset	2.31	(1.25–4.27)	0.008[a]
Severe course			
EDSS ≥ 7 within 10 years of onset	0.99	(0.29–3.34)	0.98

All affected individuals were stratified into two categories according to whether or not they were homozygous for "wildtype" extended haplotype GCTGC. Homozygous patients: mild course ($n = 22$), other course ($n = 90$); other genotypes combined: mild course ($n = 29$); other course ($n = 240$).

[a]Significant after correction for multiple tests ($n = 5$); significance criteria set at $p = 0.01$.

genotypes were present and were also used to categorize patients. No effects on presentation of any clinical variables examined in this study were observed for this locus (data not shown).

4. Discussion

A comprehensive investigation of the MS-candidate genes *TGFB1* and *IL11*, and flanking microsatellites within 19q13 was performed using a rigorously ascertained US-Caucasian familial MS dataset. Both candidate genes were selected for study due to their suggestive genomic location and important immunomodulatory functions considered relevant to MS pathogenesis. *TGFB1* gene polymorphisms causing increased production have been linked to fibrosis, hypertension and Alzheimer's disease (Awad et al., 1998; Luedecking et al., 2000; Suthanthiran et al., 2000), and polymorphisms potentially leading to decreased production have been linked to low bone mass and increased bone turnover (Langdahal et al., 1997; Yamada et al., 1998). Previous family and population-based studies in MS using microsatellites markers near the *TGFB1* locus failed to detect significant linkage or association (He et al., 1997; McDonnell et al., 1999; Mertens et al., 1998). We observed weak evidence of linkage an association in HLA-DR2 positive families with *CEA,* a microsatellite marker located 0.4 cM telomeric to the *TGFB1* locus, providing additional support for a susceptibility locus in 19q13. A suggestive association with the extended wild-type *TGFB1* ("low-producer") genotype was also observed in patients with a clinical history of mild disease, and underscores the importance of considering clinical information in efforts to identify genes in complex diseases such as MS, where common alleles may modulate clinical presentation and disease progression.

In the animal model of MS, experimental autoimmune encephalomyelitis (EAE), exogenous administration of TGF-β1 reduces the incidence and severity of inflammation and demyelination in the CNS (Jin et al., 2000; Johns et al., 1991; Racke et al., 1991), while the administration of TGF-β1 antibodies potentiates the inflammatory response (Swanborg, 1995). In MS patients, high levels of TGF-β1 mRNA expression in mononuclear peripheral cells have been associated with reduced disability (Link et al., 1994), disease duration (Soderstrom et al., 1995) and disease brain activity (Bertolotto et al., 1999). Furthermore, TGF-β1 was found to be elevated in MS cerebral spinal fluid, and correlated positively with the duration of the acute relapse (Rollnik et al., 1997). Finally, elevated serum levels of TGF-β1 have been detected in response to therapeutic immunomodulation (Aharoni et al., 2000; Hafler et al., 1997; Nicoletti et al., 1998). These observations suggest that protective effects against MS may be achieved through increases in serum TGF-β1 levels, but a counter-intuitive association was observed in our cohort between the "low-producer" genotype and a more benign expression of disease. Interestingly, TGF-β1 is expressed in most CNS cell types and is upregulated in astrocytes and microglia after neural injury. Using a transgenic mouse model in which the bioactive form of TGF-β1 was expressed in astrocytes, Wyss-Coray et al. (1997) demonstrated an increased susceptibility to challenge with spinal cord plus adjuvant, manifested as early onset of clinical symptoms, more severe disease and increased mononuclear cell infiltration, compared with non-tg littermates (Wyss-Coray et al., 1997). It is possible that the EAE-enhancing effect of local TGF-β1 relates to direct effects on blood–brain-barrier integrity, the chemo-attractant properties of this cytokine or to an interference with the clearance of toxic substances facilitating the development of sclerotic lesions (de Groot et al., 1999; Wyss-Coray et al., 1997). In chronic disease, TGF-β1 may also induce the development of fibrotic plaques resistant to the restorative remyelinating process. In any event, higher expression of TGF-β1 in the CNS parenchyma may be harmful to the host rather than protective.

Another potential non-exclusive explanation for the milder disease course observed in the low-producers relates to the effect of TGF-β1 on naïve CD4 + T cells. While TGF-β1 suppresses both Th1 and Th2 T cell maturation, the effect on Th2 T cell development appears to be much more profound than on Th1 development (Heath et al., 2000; Ludviksson et al., 2000). TGF-β1 may preferentially interfere with IL-4 induced signaling resulting in the development of IFN-γ-producing cells (Heath et al., 2000; Nagelkerken et al., 1993; Swain et al., 1991). Since such inhibition does not affect mature Th2 cells, the ability of TGF-β1 to limit the Th2 response requires its presence during T cell priming. Although the dogmatic application of the Th1/Th2 paradigm to human demyelination is considered somehow simplistic, patterns of local and sys-

temic pro-inflammatory cytokine production correlate fairly well with disease (Baranzini et al., 2000; Cannella and Raine, 1995; Schrijver et al., 1999). Hence, a genetically encoded tendency for higher TGF-β1 production in the context of autoimmunity may result in a biased pro-inflammatory response in response to external exacerbating insults, provoking a more severe form of MS. In conclusion, our findings suggest that *TGFB1* or a nearby locus affects disease expression. This observation, which may have prognostic significance for the management of MS, needs to be prospectively confirmed in an independent dataset and further explored by high-density SNP genotyping of the extended chromosomal region.

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