JOURNAL OF INTERFERON & CYTOKINE RESEARCH 24:536–542 (2004)

Gender-Related Association Between the *TGFB1*+869 Polymorphism and Multiple Sclerosis

HANS M. SCHRIJVER,[1] J. BART A. CRUSIUS,[2] M. ASUNÇION GARCÍA-GONZÁLEZ,[2,6] CHRIS H. POLMAN,[1] A. SALVADOR PEÑA,[2,3] FREDERIK BARKHOF,[4] and BERNARD M.J. UITDEHAAG[1,5]

ABSTRACT

Our objective was to investigate whether polymorphisms and haplotypes in the *TGFB1* gene are associated with susceptibility or disease characteristics of multiple sclerosis (MS). In 247 MS patients and 194 controls, single nucleotide polymorphisms (SNPs) at position +869 (Leu10Pro) and position +915 (Arg25Pro) in the signaling sequence of the *TGFB1* gene were determined, and the distribution of alleles, genotypes, and haplotypes was related to clinical data. In addition, magnetic resonance imaging (MRI) data were studied in a subgroup of patients ($n = 96$). The allele distribution of the two polymorphisms studied was in Hardy-Weinberg equilibrium in patients and in controls. No association was found with any of the three haplotypes found in the Dutch population, denoted as haplotype 1 (*TGFB1*+869T–*TGFB1*+915G), haplotype 2 (*TGFB1*+869C–*TGFB1*+915G), and haplotype 3 (*TGFB1*+869C–*TGFB1*+915C). However, the *TGFB1*+869 genotype CC was significantly more frequent in patients ($p = 0.031$, χ^2 test). The highest frequency of the *TGFB1*+869 genotype CC was observed in male patients (25.2% vs. 10.0% in controls, $p = 0.004$, χ^2 test), and carriership of *TGFB1*+869 allele C was correspondingly increased in male patients (74.8% vs. 56.7%, $p = 0.008$, χ^2 test, OR 2.27, 95% CI 1.23–4.17). Although there was no association with clinical markers of disease progression, patients homozygous for *TGFB1*+869 allele C showed a significantly higher annual increase in two MRI parameters: ventricular fraction (central atrophy) and T1-hypointense lesion load (matrix destruction). The *TGFB1* T+869C (Leu10Pro) gene polymorphism is associated with MS susceptibility, especially in males, and with a more destructive course of the disease as illustrated by MRI.

INTRODUCTION

Transforming growth factor-β (TGF-β) is a multifunctional cytokine involved in the regulation and proliferation of cells. TGF-β promotes differentiation of leukocytes but has inhibitory effects on proliferation and activation, suggesting a regulatory role in inflammatory states.[1] The three isoforms of TGF-β (TGF-β1, 2, and 3) are encoded by different genes. In mammalian species, TGF-β1 is the most abundant isoform.[2] The potent immunomodulatory effects of TGF-β make it a reasonable candidate gene for autoimmune diseases, such as multiple sclerosis (MS). Support for a role in MS susceptibility is provided by the location of the human *TGFB1* gene on chromosome 19q13,[3] a region of possible linkage with MS in several genomewide screens.[4–6] Furthermore, TGF-β levels in serum or cerebrospinal fluid (CSF) of MS patients differ significantly from those of controls and between disease stages, suggesting a possible influence of TGF-β on disease course.[7–12] It is disappointing that attempts to link *TGFB1* gene polymorphisms to MS have yielded mainly negative results.[13–16] Recently, though, associations with *TGFB1* gene polymorphisms have been reported in breast cancer,[17] diabetic nephropathy,[18] and Peyronie's disease.[19] The reported associations concern polymorphisms at positions +869 and +915 in the signaling sequence of the *TGFB1* gene that cause changes of codon 10 from leucine to proline (Leu10Pro) and of codon

[1]Department of Neurology, [2]Laboratory of Immunogenetics, [3]Department of Gastroenterology, [4]Department of Radiology, and [5]Department of Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, The Netherlands.
[6]Present address: Aragonés Institute of Health Sciences and Department of Gastroenterology, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain.

25 from arginine to proline (Arg25Pro). These single nucleotide polymorphisms (SNPs) have been shown to influence *TGFB1* production *in vitro*[20] and *in vivo*,[21,22] underscoring that *TGFB1* production is at least partly genetically controlled. In view of these recent observations and the putative role of *TGFB1* in MS pathophysiology, we attempted to identify possible associations between the *TGFB1* gene codon 10 and codon 25 polymorphisms and susceptibility to or disease characteristics of MS.

MATERIALS AND METHODS

Subjects

A total of 247 MS patients, recruited from the outpatient Department of Neurology of the VU Medical Centre and 194 healthy controls participated in the study. All participants were unrelated Dutch Caucasians. The study was carried out with the approval of the Medical Ethics Committee of the VU medical centre, and informed consent was obtained from all participants.

Magnetic resonance imaging

For 96 patients, serial data on T1-hypointense and T2-weighted lesion load and parenchymal and ventricular volumes were available. MRI examinations were performed as described previously.[23] For measuring T2-weighted lesion load, hyperintense lesions (compared with the surrounding white matter) were marked, whereas for measuring T1-hypointense lesion load, lesions hypointense compared with gray matter were marked. Subsequently, T2 (T2LV) and T1 lesion volumes (T1LV) were calculated. To assess the rate of lesion development, ΔT1LV and ΔT2LV were calculated by dividing the difference in lesion volume by the time between the scans. Further, severity of the lesions was assessed by the black hole ratio (BHR), a baseline descriptive defined as T1LV/T2LV. Parenchymal and ventricular volumes were measured on T1-weighted images, and intracranial volume was measured on the corresponding slices of the heavily T2-weighted images. Two ratios were calculated: (1) the parenchymal fraction (PF), defined as whole brain parenchyma/intracranial volume as a measure of global brain atrophy, and (2) the ventricular fraction (VF), defined as ventricular volume/intracranial volume to assess central atrophy. The progression of atrophy was assessed by dividing the difference in PF and VF by the time between the scans.

Genotyping

The method was described recently by García González et al.[24] In their study, the alleles of the codon 10 and codon 25 polymorphisms were shown to exist in a Dutch population as three haplotypes, denoted as haplotype 1 (*TGFB1*+869T–*TGFB1*+915G), haplotype 2 (*TGFB1*+869C–*TGFB1*+915G), and haplotype 3 (*TGFB1*+869C–*TGFB1*+915C).

The region containing the codon 10 T>C polymorphism at position +869 (Leu10Pro) (NCBI SNP CLUSTER ID: rs1982073) and the G>C polymorphism at position +915 (Arg25Pro) in codon 25 of the *TGFB1* gene (NCBI SNP CLUSTER ID: rs1800471) was amplified by the polymerase chain reaction (PCR). Amplified fragments spanning sequences from position +798 to position +1004 were generated using the oligonucleotides 5′-CCTGTTCGCGCTCTCGGCAGTG-3′ and 5′-GACAGGATCTGGCCGCGGATGG-3′. PCR reaction mixtures (25 μl) containing 500 ng genomic DNA extracted from EDTA-anticoagulated blood in 1 × Tsp XI buffer (MRC Holland, Amsterdam, The Netherlands) (50 mmol/L KCl, 19 mol/L Trizma, pH 8.5, 1.6 mmol/L $MgCl_2$, 0.5% Nonidet P-40, and 0.5% Tween 20), 200 μmol/L each of dNTP (GIBCO-BRL Life Technologies, Gaithersburg, MD), 0.2 μmol/L of each primer (Amersham Pharmacia Biotech, Uppsala Sweden), and 0.2 U Tsp XI DNA polymerase (MRC Holland). Amplification was performed in a thermal cycler Perkin-Elmer 9600 (Perkin-Elmer, Norwalk, CT) according to the following parameters: 97°C for 90 sec, 61°C for 90 sec, and 72°C for 60 sec for three cycles, followed by 32 cycles of 97°C for 30 sec, 61°C for 60 sec, and 72°C for 60 sec, and a final elongation at 72°C for 10 min. After amplification, the reaction products were electrophoresed on 2% agarose gels and visualized after ethidium bromide staining under UV transillumination.

Single-stranded conformation polymorphism analysis

A single-stranded conformation polymorphism (SSCP) method was optimized for the simultaneous detection of the biallelic polymorphisms at positions +869 and +915 in the *TGFB1* gene. PCR products diluted 2-fold in a denaturing loading buffer (99% formamide, 0.05% bromophenol blue) were heated at 95°C for 3 min and placed on ice. Electrophoresis on a precast nondenaturing 20% polyacrylamide PhastGel at 20°C and silver staining were performed semiautomatically on the PhastSystem™ (Amersham Pharmacia, LKB Biotechnology AB). Both strands of the PCR products, each representing one of the six different SSCP migration patterns observed, were sequenced with the same primers as used in the PCR reaction using a Big Dye Deoxy Terminator Cycle Sequencing RR Kit (Perkin-Elmer) with an automated sequencer (ABI 310) (Perkin-Elmer) according to the instructions of the manufacturer. The migration of the single strands in the SSCP patterns did coincide with the nucleotides present at the variant positions in codon 10 and in codon 25.

Statistical analysis

Allele, genotype, and haplotype frequencies between patients and controls and associations with gender or disease course (relapsing-remitting or primary progressive) were assessed using the χ^2 test. Kaplan-Meier survival analysis was performed for each gene polymorphism and haplotype using time to reach an EDSS score of 6.0 as a clinical marker of disease progression. Associations of both gene polymorphisms and haplotypes with MRI data were analyzed using nonparametric testing (Mann-Whitney, Kruskal-Wallis). In addition, linear regression analysis was performed with carriership of either allele and haplotype as independent variable and each of the MRI parameters as dependent variable. In the regression analysis, we corrected for disease duration, age at onset, gender, disease course, and use of interferon (IFN). Significance levels were set at 5% ($p < 0.05$).

Table 1. Demographic Data of Patients and Controls

	Controls	*MS patients*
Female/male	104/90	144/103
Age ± SD,[a] years	37.7 ± 11.5	46.9 ± 11.4
Mean EDSS ± SD	—	5.1 ± 2.1
Age at onset ± SD	—	31.8 ± 10.0
Disease course	—	191 RR, 56 PP

[a]SD, standard deviation; EDSS, Expanded Disability Status Scale; RR, relapsing-remitting; PP, primary progressive.

RESULTS

Demographic data of patients and controls are summarized in Table 1.

MS patients vs. healthy controls

The distribution of the *TGFB1* codon 10 and codon 25 gene polymorphisms and their haplotypes in controls is given in Tables 2 and 3. The allele distribution was in Hardy-Weinberg equilibrium in patients and in controls. The distribution of codon 10 genotypes differed significantly ($p = 0.031$), with a higher frequency of the *TGFB1*+869 genotype CC in patients (19.4% vs. 10.3%). The highest frequency of this genotype was observed in male patients (25.2% vs. 10.0% in male controls, $p = 0.004$), and carriership of *TGFB1*+869 allele C was correspondingly increased in males (Table 4) (74.8% vs. 56.7%, $p = 0.008$, OR 2.27, 95% CI 1.23–4.17).

Carriership of haplotype 1 was lower in patients (Table 3) (80.2% vs. 89.7%, $p = 0.006$, OR 0.46, 95% CI 0.27–0.81) compared with controls. Subdividing for gender showed that in male patients, carrier frequencies of all three haplotypes differed from male controls, in concordance with the absence (haplotype 1, $p = 0.004$) or presence (haplotype 2, $p = 0.016$; haplotype 3, $p = 0.046$) of *TGFB1*+869 allele C in the respective haplotype. For the *TGFB1*+915 SNP, no significant differences were observed.

Disease course

No differences were observed in allele, genotype, or haplotype frequencies in patients with a relapse onset or primary progressive onset of disease.

Disease severity

Kaplan-Meier survival analysis with time to reach EDSS score 6.0 as a clinical marker of disease progression showed no association with either of the studied polymorphisms or haplotypes.

MRI data

Significant regression coefficients were observed for *TGFB1*+869 genotype CC, with a higher annual change in VF

Table 2. *TGFB1* Genotype and Allele Frequencies in Controls and Patients

Genotypes	*Controls* n = *194 (%)*		*MS patients* n = *247 (%)*		p *value*
+869 Codon 10[a]					
Allele frequency T	65.5%		59.1%		
Allele frequency C	34.5%		40.9%		
T/T	80 (41.2)		93 (37.7)		0.031[b]
	Female	Male	Female	Male	
	41 (39.4)	39 (43.3)	67 (46.5)	26 (25.2)	
T/C	94 (48.5)		106 (42.9)		
	Female	Male	Female	Male	
	52 (50.0)	42 (46.7)	55 (38.2)	51 (49.5)	
C/C	20 (10.3)		48 (19.4)		
	Female	Male	Female	Male	
	11 (10.6)	9 (10.0)	22 (15.3)	26 (25.2)	0.004[c]
+915 Codon 25[d]					
Allele frequency G	93.3%		89.3%		
Allele frequency C	6.7%		10.7%		
G/G	168 (86.6)		198 (80.2)		0.072[b]
	Female	Male	Female	Male	
	89 (85.6)	79 (87.8)	119 (82.6)	79 (76.7)	
G/C	26 (13.4)		45 (18.1)		
	Female	Male	Female	Male	
	15 (14.4)	11 (12.2)	23 (16.0)	22 (21.4)	
C/C	0 (0)		4 (1.6)		
	Female	Male	Female	Male	
	0 (0)	0 (0)	2 (1.4)	2 (1.9)	

[a]Codon 10: T = *TGFB1*+869 T (Leu10), C = *TGFB1*+869 C (Pro10).
[b]Overall *p* values for genotype distribution of codons 10 and 25.
[c]*p* value (χ^2) for genotype frequency in male patients vs. male controls.
[d]Codon 25: C = *TGFB1*+915 C (Pro25), G = *TGFB1*+915 G (Arg25).

Table 3. *TGFB1* Genotype, Phenotype, and Haplotype Frequencies in Controls and Patients

TGFB1 *Haplotype*	*Controls* (n = *194*) n *(%)*	*HF (%)*[a]	*MS patients* (n = *247*) n *(%)*	*HF (%)*	p *value*[b]
1.1	80 (41.2)	65.5	93 (37.7)	58.9	
1.2	76 (39.2)		83 (33.6)		
1.3	18 (9.3)		22 (8.9)		
2.2	12 (6.2)	27.8	22 (8.9)	30.4	
2.3	8 (4.1)		23 (9.3)		
3.3	0	6.7	4 (1.6)	10.7	
PF haplotype 1[c]	174 (89.7)		198 (80.2)		$p = 0.006$
PF haplotype 2	96 (49.5)		128 (51.8)		n.s.
PF haplotype 3	26 (13.4)		49 (19.8)		n.s.

[a]HF, haplotype frequency; PF, phenotype frequency.
[b]Overall p values for carriership of haplotype (=PF) 1–3.
[c]Haplotype 1: T(Leu10)-G(Arg25); haplotype 2: C(Pro10)-G(Arg25); haplotype 3: C(Pro10)-C(Pro25).

($p = 0.001$) and T1-hypointense lesion load ($p = 0.011$) in patients with genotype CC compared with other *TGFB1*+869 genotypes (Table 5 and Fig. 1).

DISCUSSION

In the present study, we observed a higher frequency of individuals homozygous for *TGFB1*+869 allele C in MS patients. Subdividing for gender revealed that this association was caused mainly by a higher frequency of the *TGFB1*+869 CC genotype in male MS patients, whereas the difference between female patients and controls did not reach significance. Accordingly, male patients were significantly more frequently carriers of *TGFB1*+869 allele C. However, as the heterozygous *TGFB1*+869 allele C genotype was evenly distributed between male patients and controls, the higher carrier frequency of *TGFB1*+869 allele C was due only to the contribution of the homozygous genotype. In addition, in patients homozygous for *TGFB1*+869 C, associations with two of five studied MRI parameters of progression were observed, although these results must be regarded with caution given the limited number of patients for whom such data were available.

Table 4. Carriership of *TGFB1*+869 Allele C

	Carrier allele C[a] n *(%)*	*Noncarrier allele C* n *(%)*
Controls		
Male	51 (56.7)*	39 (43.3)
Female	63 (60.6)	41 (39.4)
MS		
Male	77 (74.8)*	26 (25.2)
Female	77 (53.5)	67 (46.5)

[a]OR for MS in male carriers of allele C 2.27 (95% CI: 1.23–4.17).
*$p = 0.008$ (Pearson χ^2 7.038).

In interpreting the relevance of our findings, the following is of note. First, the involvement of TGF-β in the disease process in MS is widely accepted. Several studies have demonstrated convincingly that TGF-β levels in serum and CSF from MS patients differ from those of controls.[7–11] Whether these observations reflect primary or secondary disease phenomena, however, remains to be answered. Second, as with most cytokines involved in MS pathogenesis, the specific role of TGF-β in MS can be highly variable. Polymorphisms in the TGF-β genes may influence susceptibility or may shape clinical determinants. It is conceivable that the disease course, progression rate, and lesion predilection sites (such as optic nerve or spinal cord) may be influenced by genetically controlled differences in TGF-β production. Also, it has been suggested that the stage of the disease process is important for the net result of the diverse TGF-β-mediated effects: from mainly anti-inflammatory in the beginning to predominantly promoting gliosis and scarring in later stages.[25] Following this hypothesis, our observed associations of the *TGFB1*+869 CC genotype with MRI data might be explained by a promoting effect of *TGFB1* gene polymorphisms on the formation of scar tissue and the overproduction of matrix components, leading to more destructive MS lesions.

The number of studies that have addressed *TGFB* gene polymorphisms in MS is limited. McDonnell et al.,[14] using microsatellite markers, found no association of the *TGFB1* and *TGFB2* genes with MS in Irish patients. Two earlier linkage studies proposed the *TGFB2* locus[13] and the *TGFB3* locus[26] as respective regions of interest, whereas in a recent large study using a combined association and linkage approach, no associations of *TGFB1* with MS were observed.[16] Finally, Green et al.[15] reported an association of a *TGFB1* haplotype comprising five alleles (including the *TGFB1*+869 allele T and *TGFB1*+915 allele G that constitute our haplotype 1) with mild disease course. In other autoimmune diseases, both negative and positive results have been presented. No convincing evidence for a role of *TGFB1* gene polymorphisms in inflammatory bowel disease was found,[24,27] but recently, an association of *TGFB1*+869 allele T with rheumatoid arthritis was reported.[28]

TABLE 5. *TGFB1*+869 GENOTYPE IN RELATION TO MRI PARAMETERS

	Annual change in MRI parameters			
TGFB1+*869 genotype*	*Increase in VF*[a] *(%)*	*Decrease in PF (%)*	*T1-hypointense lesion load (cm^3)*	*T2-weighted lesion load (cm^3)*
CC $n = 18$	8.1[b]	1.1	0.7[c]	0.8
Other genotypes $n = 78$	3.0	0.7	0.3	1.2

[a]VF, ventricular fraction = ventricular volume/intracranial volume; PF, parenchymal fraction = whole brain parenchyma/intracranial volume.

Regression coefficients (standardized beta): [b]VF 0.324 ($p = 0.001$); [c]T1-hypointense lesion load 0.254 ($p = 0.011$).

A puzzling aspect of polymorphisms in cytokine genes in general is the translation of genetic differences into susceptibility or clinical features, but impact on cytokine production is assumed. To date, a number of *TGFB1* gene polymorphisms have been linked to functional or clinical aspects. Grainger et al.[29] demonstrated in a classic twin study that *TGFB1* gene SNPs in the promoter region (−800, −509) influence plasma TGF-β1 levels. Moreover, increased lung fibrosis has been reported in carriers of *TGFB1*+915 allele G,[20] and accelerated decline in lung function has been reported in patients with the *TGFB1*+869 TT genotype.[30] Densem et al.[31] found earlier coronary vasculopathy after cardiac transplantation in conjunction with higher TGF-β production in *TGFB1*+915 allele G homozygotes. In a large French study, a positive association of *TGFB1*+915 allele C with myocardial infarction was observed, as was a negative association of the same allele with hypertension, leading the authors to caution the interpretation of their results.[32] Finally, both earlier renal dysfunction[33] and accelerated graft vascular disease have been shown in patients carrying *TGFB1*+869 allele C.[34] In our view, a general conclusion from this variety of studies is that *TGFB1* gene polymorphisms (or nearby loci) influence the course of several diseases. With regard to MS pathogenesis, current concepts favor a disease-limiting role of TGF-β, as high TGF-β mRNA levels in mononuclear cells from blood and CSF have been associated with low disability.[10]

Both polymorphisms investigated in this study are part of the signal peptide sequence and result in amino acid substitutions in the protein sequence. The signal peptide is involved in the export of newly synthesised TGF-β1 across the endoplasmatic reticular membrane. It is difficult to predict the effects that substitutions of amino acids may have, but it is speculated that they influence the polarity of the peptide, leading to different export rates.

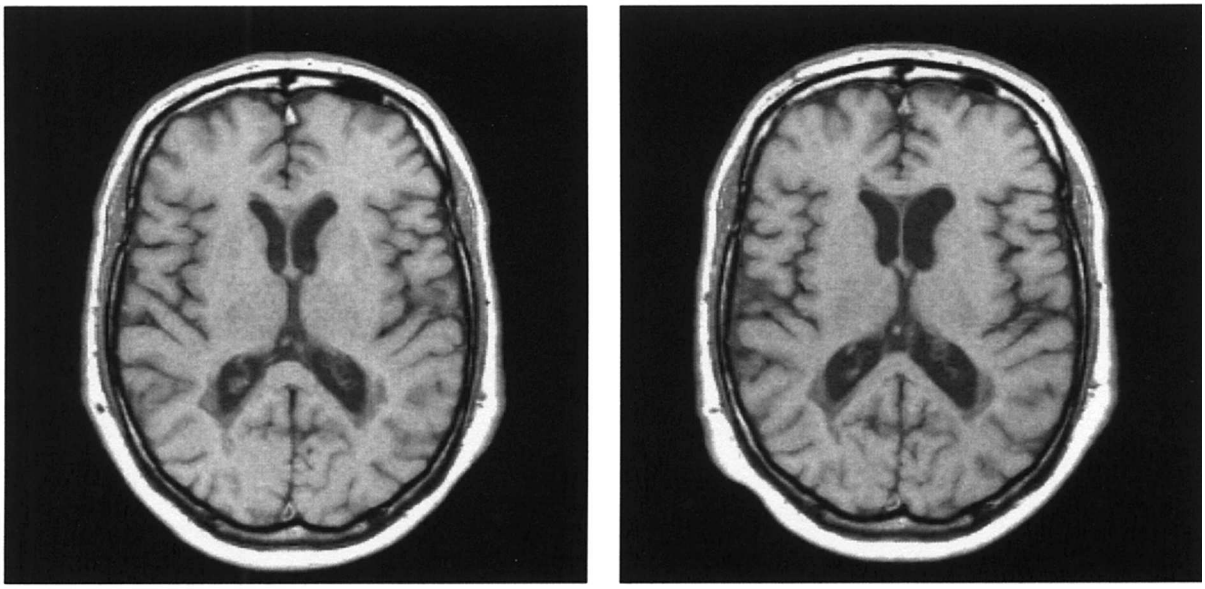

FIG. 1. MRI images at baseline and follow-up of a patient with *TGFB1*+869 genotype CC. Increase in ventricular fraction: 27% (interval of 40 months).

There is some evidence that the *TGFB1*+869 polymorphism is associated with differences in TGF-β1 production, at least *in vivo*. In healthy Japanese individuals, TGF-β1 serum levels correlated with the number of *TGFB1*+869 allele C.[21] Concordantly, higher serum levels of TGF-β1 were observed both in patients with myocardial infarction and in controls who were homozygous for *TGFB1*+869 allele C.[22] However, no differences in *in vitro* TGF-β1 production were observed with respect to the *TGFB1*+869 polymorphism.[20] Obviously, it would be of great interest to see if the association that we observed is reflected in differences in TGF-β1 production, either *in vivo* or *in vitro*. As this study focused primarily on associations with susceptibility and disease characteristics, cytokine production was not studied. Thus, together with the limited evidence in the literature, attempts to translate our observations directly into differences in TGF-β1 production must be considered cautiously.

In conclusion, we found an association of the *TGFB1*+869 CC genotype with MS susceptibility, especially in males. In addition, preliminary MRI data suggest an association of this genotype with a more destructive disease course. Obviously, these findings need confirmation in an independent dataset, ideally combining clinical, functional, and MRI parameters.

REFERENCES

1. BLOBE, G.C., SCHIEMANN, W.P., and LODISH, H.F. (2000). Role of transforming growth factor beta in human disease. N. Engl. J. Med. **342,** 1350–1358.
2. ROBERTS, A.B., and SPORN, M.B. (1993). Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). Growth Factors **8,** 1–9.
3. MASSAGUE, J. (1990). The transforming growth factor-beta family. Annu. Rev. Cell. Biol. **6,** 597–641.
4. KUOKKANEN, S., GSCHWEND, M., RIOUX, J.D., DALY, M.J., TERWILLIGER, J.D., TIENARI, P.J., WIKSTROM, J., PALO, J., STEIN, L.D., HUDSON, T.J., LANDER, E.S., and PELTONEN, L. (1997). Genomewide scan of multiple sclerosis in Finnish multiplex families. Am. J. Hum. Genet. **61,** 1379–1387.
5. SAWCER, S., JONES, H.B., FEAKES, R., GRAY, J., SMALDON, N., CHATAWAY, J., ROBERTSON, N., CLAYTON, D., GOODFELLOW, P.N., and COMPSTON, A. (1996). A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. Nat. Genet. **13,** 464–468.
6. HAINES, J.L., BRADFORD, Y., GARCIA, M.E., REED, A.D., NEUMEISTER, E., PERICAK-VANCE, M.A., RIMMLER, J.B., MENOLD, M.M., MARTIN, E.R., OKSENBERG, J.R., BARCELLOS, L.F., LINCOLN, R., and HAUSER, S.L. (2002). Multiple susceptibility loci for multiple sclerosis. Hum. Mol. Genet. **11,** 2251–2256.
7. BERTOLOTTO, A., CAPOBIANCO, M., MALUCCHI, S., MANZARDO, E., AUDANO, L., BERGUI, M., BRADAC, G.B., and MUTANI, R. (1999). Transforming growth factor beta1 (TGF-beta1) mRNA level correlates with magnetic resonance imaging disease activity in multiple sclerosis patients. Neurosci. Lett. **263,** 21–24.
8. CARRIERI, P.B., PROVITERA, V., BRUNO, R., PERRELLA, M., TARTAGLIA, G., BUSTO, A., and PERRELLA, O. (1997). Possible role of transforming growth factor-beta in relapsing-remitting multiple sclerosis. Neurol. Res. **19,** 599–600.
9. CARRIERI, P.B., PROVITERA, V., DE ROSA, T., TARTAGLIA, G., GORGA, F., and PERRELLA, O. (1998). Profile of cerebrospinal fluid and serum cytokines in patients with relapsing-remitting multiple sclerosis: a correlation with clinical activity. Immunopharmacol. Immunotoxicol. **20,** 373–382.
10. LINK, J., SODERSTROM, M., OLSSON, T., HOJEBERG, B., LJUNGDAHL, A., and LINK, H. (1994). Increased transforming growth factor-beta, interleukin-4, and interferon-gamma in multiple sclerosis. Ann. Neurol. **36,** 379–386.
11. NICOLETTI, F., DI MARCO, R., PATTI, F., REGGIO, E., NICOLETTI, A., ZACCONE, P., STIVALA, F., MERONI, P.L., and REGGIO, A. (1998). Blood levels of transforming growth factor-beta 1 (TGF-beta1) are elevated in both relapsing-remitting and chronic progressive multiple sclerosis (MS) patients and are further augmented by treatment with interferon-beta 1b (IFN-beta1b). Clin. Exp. Immunol. **113,** 96–99.
12. ROLLNIK, J.D., SINDERN, E., SCHWEPPE, C., and MALIN, J.P. (1997). Biologically active TGF-beta 1 is increased in cerebrospinal fluid while it is reduced in serum in multiple sclerosis patients. Acta Neurol. Scand. **96,** 101–105.
13. HE, B., XU, C., YANG, B., LANDTBLOM, A.M., FREDRIKSON, S., and HILLERT, J. (1998). Linkage and association analysis of genes encoding cytokines and myelin proteins in multiple sclerosis. J. Neuroimmunol. **86,** 13–19.
14. McDONNELL, G.V., KIRK, C.W., HAWKINS, S.A., and GRAHAM, C.A. (1999). Lack of association of transforming growth factor (TGF)-beta 1 and beta 2 gene polymorphisms with multiple sclerosis (MS) in Northern Ireland. Mult. Scler. **5,** 105–109.
15. GREEN, A.J., BARCELLOS, L.F., RIMMLER, J.B., GARCIA, M.E., CAILLIER, S., LINCOLN, R.R., BUCHER, P., PERICAK-VANCE, M.A., HAINES, J.L., HAUSER, S.L., and OKSENBERG, J.R. (2001). Sequence variation in the transforming growth factor-beta1 (TGFB1) gene and multiple sclerosis susceptibility. J. Neuroimmunol. **116,** 116–124.
16. WEINSHENKER, B.G., HEBRINK, D., KANTARCI, O.H., SCHAEFER-KLEIN, J., ATKINSON, E., SCHAID, D., and McMURRAY, C.M. (2001). Genetic variation in the transforming growth factor beta1 gene in multiple sclerosis. J. Neuroimmunol. **120,** 138–145.
17. ZIV, E., CAULEY, J., MORIN, P.A., SAIZ, R., and BROWNER, W.S. (2001). Association between the T29 → C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The Study of Osteoporotic Fractures. JAMA **285,** 2859–2863.
18. WONG, T.Y., POON, P., CHOW, K.M., SZETO, C.C., CHEUNG, M.K., and LI, P.K. (2003). Association of transforming growth factor-beta (TGF-beta) T869C (Leu 10Pro) gene polymorphisms with type 2 diabetic nephropathy in Chinese. Kidney Int. **63,** 1831–1835.
19. HAUCK, E.W., HAUPTMANN, A., SCHMELZ, H.U., BEIN, G., WEIDNER, W., and HACKSTEIN, H. (2003). Prospective analysis of single nucleotide polymorphisms of the transforming growth factor beta-1 gene in Peyronie's disease. J. Urol. **169,** 369–372.
20. AWAD, M.R., EL-GAMEL, A., HASLETON, P., TURNER, D.M., SINNOTT, P.J., and HUTCHINSON, I.V. (1998). Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation **66,** 1014–1020.
21. YAMADA, Y., MIYAUCHI, A., GOTO, J., TAKAGI, Y., OKUIZUMI, H., KANEMATSU, M., HASE, M., TAKAI, H., HARADA, A., and IKEDA, K. (1998). Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J. Bone Miner. Res. **13,** 1569–1576.
22. YOKOTA, M., ICHIHARA, S., LIN, T.L., NAKASHIMA, N., and YAMADA, Y. (2000). Association of a T29 → C polymorphism of the transforming growth factor-beta1 gene with genetic suscep-

tibility to myocardial infarction in Japanese. Circulation **101,** 2783–2787.
23. KALKERS, N.F., BERGERS, E., CASTELIJNS, J.A., VAN WALDERVEEN, M.A.A., BOT, J.C.J., ADER, H.J., POLMAN, C.H., and BARKHOF, F. (2001). Optimizing the association between disability and biological markers in MS. Neurology **57,** 1253–1258.
24. GARCIA-GONZALEZ, M.A., CRUSIUS, J.B., STRUNK, M.H., BOUMA, G., PEREZ-CENTENO, C.M., PALS, G., MEUWISSEN, S.G., and PENA, A.S. (2000). TGFB1 gene polymorphisms and inflammatory bowel disease. Immunogenetics **51,** 869–872.
25. DE GROOT, C.J., MONTAGNE, L., BARTEN, A.D., SMINIA, P., and VAN DER VALK, P. (1999). Expression of transforming growth factor (TGF)-beta1, -beta2, and -beta3 isoforms and TGF-beta type I and type II receptors in multiple sclerosis lesions and human adult astrocyte cultures. J. Neuropathol. Exp. Neurol. **58,** 174–187.
26. MERTENS, C., BRASSAT, D., REBOUL, J., EICHENBAUM-VOLINE, S., VUILLEMIN-AZAIS, C., COURNU, I., BABRON, M.C., SEMANA, G., EDAN, G., CLANET, M., CLERGET-DARPOUX, F., BARON-VAN EVERCOOREN, A., LYON-CAEN, O., LIBLAU, R., and FONTAINE, B. (1998). A systematic study of oligodendrocyte growth factors as candidates for genetic susceptibility to MS. French Multiple Sclerosis Genetics Group. Neurology **51,** 748–753.
27. SCHULTE, C.M., GOEBELL, H., ROHER, H.D., and SCHULTE, K.M. (2001). C-509T polymorphism in the TGFB1 gene promoter: impact on Crohn's disease susceptibility and clinical course? Immunogenetics **53,** 178–182.
28. SUGIURA, Y., NIIMI, T., SATO, S., YOSHINOUCHI, T., BANNO, S., NANIWA, T., MAEDA, H., SHIMIZU, S., and UEDA, R. (2002). Transforming growth factor beta1 gene polymorphism in rheumatoid arthritis. Ann. Rheum. Dis. **61,** 826–828.
29. GRAINGER, D.J., HEATHCOTE, K., CHIANO, M., SNIEDER, H., KEMP, P.R., METCALFE, J.C., CARTER, N.D., and SPECTOR, T.D. (1999). Genetic control of the circulating concentration of transforming growth factor type beta1. Hum. Mol. Genet. **8,** 93–97.
30. ARKWRIGHT, P.D., LAURIE, S., SUPER, M., PRAVICA, V., SCHWARZ, M.J., WEBB, A.K., and HUTCHINSON, I.V. (2000). TGF-beta(1) genotype and accelerated decline in lung function of patients with cystic fibrosis. Thorax **55,** 459–462.
31. DENSEM, C.G., HUTCHINSON, I.V., COOPER, A., YONAN, N., and BROOKS, N.H. (2000). Polymorphism of the transforming growth factor-beta 1 gene correlates with the development of coronary vasculopathy following cardiac transplantation. J. Heart Lung Transplant. **19,** 551–556.
32. CAMBIEN, F., RICARD, S., TROESCH, A., MALLET, C., GENERENAZ, L., EVANS, A., ARVEILER, D., LUC, G., RUIDAVETS, J.B., and POIRIER, O. (1996). Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. The Etude Cas-Temoin de l'Infarctus du Myocarde (ECTIM) Study. Hypertension **28,** 881–887.
33. BAAN, C.C., BALK, A.H., HOLWEG, C.T., VAN RIEMSDIJK, I.C., MAAT, L.P., VANTRIMPONT, P.J., NIESTERS, H.G., and WEIMAR, W. (2000). Renal failure after clinical heart transplantation is associated with the TGF-beta 1 codon 10 gene polymorphism. J. Heart Lung Transplant. **19,** 866–872.
34. HOLWEG, C.T., BAAN, C.C., BALK, A.H., NIESTERS, H.G., MAAT, A.P., MULDER, P.M., and WEIMAR, W. (2001). The transforming growth factor-beta1 codon 10 gene polymorphism and accelerated graft vascular disease after clinical heart transplantation. Transplantation **71,** 1463–1467.

Address reprint requests or correspondence to:
Dr. H.M. Schrijver
Department of Neurology
VU University Medical Centre
P.O.Box 7057
1007 MB, Amsterdam
The Netherlands

Tel: 0031-20-4440718.
Fax: 0031-20-4442800.
E-mail: HM.Schrijver@vumc.nl

Received 18 March 2004/Accepted 17 May 2004