TNF neutralization in MS

Results of a randomized, placebo-controlled multicenter study

The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group*

Article abstract—*Objective:* A double-blind, placebo-controlled phase II study was conducted in 168 patients, most with relapsing-remitting MS, to evaluate whether lenercept would reduce new lesions on MRI. *Background:* Tumor necrosis factor (TNF) has been implicated in MS pathogenesis, has been identified in active MS lesions, is toxic to oligodendrocytes in vitro, and worsens the severity of experimental allergic encephalomyelitis (EAE) in animals. Lenercept, a recombinant TNF receptor p55 immunoglobulin fusion protein (sTNFR-IgG p55), protects against EAE. *Methods:* Patients received 10, 50, or 100 mg of lenercept or placebo IV every 4 weeks for up to 48 weeks. MRI scans and clinical evaluations were performed at screening, at baseline, and then every 4 weeks (immediately before dosing) through study week 24. *Results:* There were no significant differences between groups on any MRI study measure, but the number of lenercept-treated patients experiencing exacerbations was significantly increased compared with patients receiving placebo ($p = 0.007$) and their exacerbations occurred earlier ($p = 0.006$). Neurologic deficits tended to be more severe in the lenercept treatment groups, although this did not affect Expanded Disability Status Scale scores. Anti-lenercept antibodies were present in a substantial number of treated patients; serum lenercept trough concentrations were detectable in only a third. Adverse events that increased in frequency in treated patients included headache, nausea, abdominal pain, and hot flushes. *Conclusions:* Lenercept failed to be beneficial, but insight into the role of TNF in MS exacerbations was gained.

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MS is believed to be an inflammatory autoimmune disorder of the CNS with unknown myelin components as target. A number of findings have suggested that tumor necrosis factor (TNF) contributes to propagating the inflammatory response and to tissue injury in MS. In autopsy specimens, TNF has been demonstrated within active MS foci.[1] TNF has been shown to have a direct toxic effect against oligodendrocytes and a proliferation-inducing effect on astrocytes in in vitro studies.[2,3] In patients with MS, elevated TNF levels in the serum and CSF have been correlated in some studies with disease progression.[4,5] Blood mononuclear cells from MS patients, studied just before an exacerbation, secrete greater amounts of TNF in response to mitogen stimulation than at other times.[6] Blood mononuclear cells from MS patients with active disease express higher levels of TNF mRNA than do cells from MS patients with stable disease or healthy controls.[7,8]

Studies of experimental autoimmune encephalomyelitis (EAE) have profoundly shaped views of MS pathogenesis. EAE is an autoimmune disease with pathologic features reminiscent of those seen in MS. TNF treatment worsens EAE,[9] and TNF neutralization by anti-TNF antibody treatment consistently protects animals from EAE.[10-12] Similarly, TNF capture by lenercept, a TNFα receptor–immunoglobulin G (IgG)1 fusion protein, protects in EAE.[13] The above indicates that TNF functions in EAE as a proinflammatory mediator and suggests that TNF depletion might be protective in MS. The hypothesis that neutralization of TNF may reduce or halt MS progression was evaluated in a phase II randomized, multicenter, placebo-controlled study of three doses of lenercept (sTNFR-IgG p55). Lenercept is a dimeric recombinant protein molecule built from two copies of the 55 kDa TNF receptor extracellular domain fused to a fragment of the human immunoglobulin

See also pages 444 and 466

*See the Appendix on page 464 for a listing of members of The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group.

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IgG1 heavy chain.[14,15] In accordance with recently published recommendations, efficacy was assessed by means of MRI.[16]

Methods. *Patients.* A total of 168 patients with clinically definite or laboratory supported definite MS were enrolled in a double-blind, placebo-controlled study. The study was approved by the institutional review boards of the participating centers, and all subjects gave informed consent. At enrollment, patients were between the ages of 18 and 55 years and had an Expanded Disability Status Scale (EDSS) score ≤5.5. For patients with an EDSS score ≤3, the history of MS was limited to a maximum duration of 10 years. All patients had at least two exacerbations within the previous 2 years, but were clinically stable for 4 weeks before the screening MRI and during the 4 weeks between screening and study entry. With the exception of glucocorticoids, any prior administration of agents with a putative effect on MS (including interferons, cyclophosphamide, or azathioprine) led to exclusion. Treatment with glucocorticoids was not permitted within a 4-week period before the screening visit or between screening and baseline. Other exclusion criteria included the diagnosis of primary progressive MS and inability to undergo MRI scanning. A randomization list with treatment blocks (four patients per block) was computer generated by Hoffmann–La Roche (Basel, Switzerland) for each investigation site. During the conduct of the study, the randomization list was available only to the Safety Review Board (SRB) members (see below). A limited number of Roche staff were unblinded at the time of the first analysis of efficacy as defined in the protocol. On study termination, each investigational site was provided with the site-specific randomization code.

Eligible patients were randomized to 10, 50, or 100 mg of lenercept or to placebo, administered IV every 4 weeks. Study duration was 48 weeks, consisting of a 24-week, double-blind treatment period and a 24-week follow-up period. Of the 168 patients randomized to treatment, one patient (randomized to placebo) was identified as ineligible prior to the baseline visit; this patient did not receive treatment, have a baseline MRI scan, or return for follow-up. For the 167 patients who received treatment, compliance to treatment and study procedures was excellent. During the first 24 weeks, 99% (991/1002) of all planned doses were administered and 98% (1303/1336) of all MRI scan sets were performed.

During the follow up period (study weeks 25–48), patients could continue double-blind treatment on a voluntary basis and 130 elected to do so. Those patients who opted not to continue treatment remained in the study and were followed on an intent-to-treat basis. For the full study duration, 10 doses (median) were administered to each treatment group.

For safety purposes, three cohorts of up to 16 patients were enrolled in an ascending-dose design at approximately 6-week intervals. The first cohort was randomized to placebo or 10 mg of lenercept whereas subsequent cohorts were randomized to placebo or 50 mg and finally to placebo or 100 mg of lenercept. An independent SRB evaluated the unblinded study data before each dose escalation during the ascending dose phase of the study. Following these evaluations, the remaining patients were recruited. The SRB reviewed data at 3-month intervals throughout the study. This review included the MRI safety data but did not include a review of the MRI efficacy data.

Magnetic resonance imaging. MRI scans were performed according to a predefined MRI protocol at screening, baseline, and every 4 weeks (before each dose) throughout the first 24 weeks of the study for a total of eight scanning time points. At each time point, three scans with a slice thickness of 5 mm were obtained: 1) proton density/T2-weighted scan, 2) T1-weighted unenhanced scan, and 3) T1-weighted gadolinium (Gd)-enhanced scan 5 minutes after the administration of Gd-DTPA 0.1 mmol/kg. All scans were analyzed according to a prospectively defined MRI analysis plan by the UBC MS/MRI Analysis Group in Vancouver, Canada. After comparison of each MRI follow-up scan with the prior scan, the number of newly active lesions was ascertained by summing the new, recurrent, or enlarging T2 lesions, and the new or recurrent Gd-enhancing lesions. Newly active lesions identified on both the enhanced T1 scan and the T2 scan were counted as single lesions. The primary efficacy measure was the cumulative number of newly active lesions identified on the six treatment scans. Definitions of new, recurrent, and enlarging lesions have been reported previously.[17,18] Persistently enhancing or enlarging lesions were separately identified as persistently active lesions, a secondary measure of efficacy. In this way, new lesions could easily be separated from other types of activity. Other secondary efficacy measures included the percentage of active scans, defined as the proportion of scans with one or more newly active lesions, and the burden of disease, which was assessed as reported previously, at baseline, and at 24 weeks.[17,18] Burden of disease was determined by outlining each MS lesion identified on the T2-weighted MRI scan. These areas were summed slice by slice for a total lesion area recorded as mm^3. In addition, the total number of Gd-enhancing lesions (a measure of safety) was counted for each patient at each scanning time point on an ongoing basis to allow MRI data to be reviewed by the SRB.

Clinical assessments. At the baseline visit and every 4 weeks thereafter for the first 24 weeks, a history was taken, physical and neurologic examinations performed, and adverse events noted. Study drug was administered at the end of each visit. Patients were encouraged to come for additional visits should exacerbations occur between visits. During the second 24-week period, two formal visits were planned at weeks 36 and 48. Whenever possible, patients who withdrew from treatment were asked to continue all study procedures including all MRI scans.

Clinical endpoints. Exacerbations were defined as the appearance of a new sign or symptom or the worsening of an old sign or symptom attributable to MS, lasting at least 24 hours in the absence of fever, and preceded by a period of stability of 28 days. An exacerbation was deemed to have ended when signs and symptoms had begun to improve. For those patients with permanent deficits, the first day of a 28-day period of stability was taken as the ending of an attack. The Neurological Rating Scale (NRS)[19] was completed each time the neurologic examination was performed. As in other clinical trials in MS, a decline in NRS score of 15 points or more was considered to reflect a severe change in the patient's neurologic condition, whereas declines of 8–14 or 1–7 points were considered to reflect

moderate or mild changes, respectively.[17,20] A difference of 0 points was categorized as no change; an increase in the score as an improvement. The EDSS as recommended by Kurtzke[21] was scored at screening and at weeks 24 and 48 by the study neurologist. In 120/167 (72%) of patients, the EDSS was performed at all time points by the same neurologist.

In accordance with the protocol, a first analysis was undertaken after all patients had completed 24 weeks of double-blind treatment and after all MRI scans had been evaluated. An increase in the exacerbation rate was noted in lenercept-treated patients. This finding resulted in the sponsor's decision to terminate the study and to release the treatment code. All study drug administration was stopped promptly and, after a final visit, data collection was discontinued. For this reason, study data through week 48 are incomplete. The follow-up period through week 48, however, was similar in all treatment groups.

Pharmacokinetic/dynamic parameters. Serum samples were obtained at baseline and before dosing every 4 weeks for 24 weeks and at study weeks 36 and 48. The concentration of lenercept and titers of antibodies to lenercept were determined. All samples were analyzed centrally. Lenercept concentrations were measured using an enzyme-linked immunologic and biologic binding assay (ELIBA) developed by Roche (Hoffmann-LaRoche Ltd., Basel, Switzerland); antibodies to lenercept were identified by means of a double antigen antibody test. Samples with detectable anti-lenercept antibodies were further evaluated to determine the neutralizing potential of the antibodies (Medi-Lab, Medicinsk Laboratorium A/S, Copenhagen, Denmark).

Autoantibodies. Serum samples were obtained at baseline and at study weeks 24 and 48 and assayed for IgM–rheumatoid factor (RF), antinuclear antibodies (ANA) (Hep 2), and antibodies to dsDNA (DAKO; Carpinteria, CA) in a central laboratory (A. Wiik, Statens Seruminstitut Copenhagen, Denmark).

Statistical analyses. The cumulative number of newly active lesions was tested with a closed test procedure based on an analysis of variance (ANOVA) of the $\mathrm{Ln}(x + 1)$ transformed sum of the lesions. The protocol required that the analysis of the primary efficacy criterion be performed after imputation of the median number of lesions at a specific time point so as to compensate for missing values at that time point. Of the 1,008 expected values, 34 were missing, resulting in data imputation as noted above. The results of this analysis showed no differences among the treatment groups ($p = 0.417$) or between the pairs of treatment groups. Data imputation was not performed for the analyses presented herein.

A closed tests procedure, based on an ANOVA with the factor "treatment" of $\mathrm{Ln}(1 + x)$, where x denotes the cumulative number of newly active lesions, was used to compare the cumulative number of newly active lesions between the treatment groups. The closed tests procedure was first used to compare the means among all four treatment groups (global test)[22]; then, to compare the means among all combinations of three of the four treatment groups; and finally, to compare the means of each lenercept treatment group with that of the placebo group. For all comparisons, F tests were performed at the same significance level ($\alpha = 0.05$); however, adjusted p values are provided. The mean of a treatment group is regarded as significantly different from that of the placebo group when all comparisons that include the two relevant treatment groups result in a p value ≤ 0.05; i.e., the adjusted p value is the maximum of the p values of these comparisons. The procedure stops early if the global test is nonsignificant. This procedure guarantees a multiple $\alpha = 0.05$. The closed tests procedure described above was also used to assess the cumulative number of persistently active lesions. Center effects were assessed using descriptive methods.

The Kruskal-Wallis test was used to compare the mean change in EDSS scores, change in the burden of disease, and percent of active scans. To assess the influence of baseline imbalances in MRI activity among the treatment groups, covariance analyses using the corresponding transformed baseline MRI values as covariate were performed.

Survival analysis methods (Kaplan-Meier estimates; i.e., product-limit estimates and logrank tests)[23] were applied to analyze the time to first exacerbation and the duration of exacerbations because of right censoring at the end of the observation period. Logrank tests, with a Bonferroni adjustment of the significance level ($\alpha = 0.017$), were used for the multiple comparisons among the three lenercept treatment and the placebo treatment Kaplan-Meier curves. As an exacerbation duration can only be observed in the presence of an exacerbation, we investigated the conditional distribution of their durations. After inspection of the exacerbation data, we assumed a counting process model according to Anderson and Gill[24] with independent increments because the process is slow. For the same reason, exacerbation durations were assumed to be independently and identically distributed between patients and within patients for those patients who had more than one exacerbation.

A chi-square was used to evaluate the number of patients with no, one, two, three, or four exacerbations in each treatment group after 24 weeks of treatment and at the end of the study (through week 48). Because of small frequencies in some cells, the table was collapsed to counting patients with and without exacerbations to allow a valid chi-square test. For the multiple comparisons the unadjusted p values should be compared with the Bonferroni adjusted α of 0.017 ($0.05 \div 3$). Chi-square tests were also used to evaluate the NRS and the rate of RF or ANA among the treatment groups. Cox regression analysis was performed to assess potential predictive factors for the occurrence of exacerbations. The data and analyses were performed on all data available, i.e., through week 48, unless otherwise stated in the text or tables. Two-tailed analyses were used throughout.

Results. Figure 1 depicts the trial profile. The treatment groups were comparable at entry on all baseline disease characteristics and demographics (table 1). The protocol permitted enrollment of both relapsing-remitting and secondary progressive patients, and from 4 to 10 patients with secondary progression were enrolled in each group (see table 1). Prestudy MRI characteristics were likewise comparable among the treatment groups (table 2) although there was a tendency (nonsignificant) for the higher lenercept dose groups to have more MRI activity (median).

MRI results. The results of the cumulative number of newly active MRI lesions, the percentage of persistently active lesions, the percentage of active scans, and the

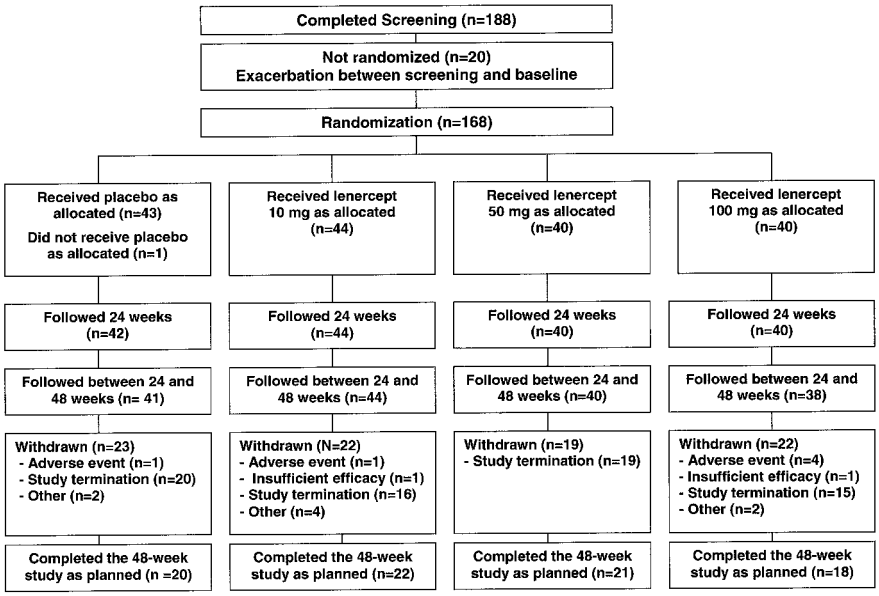

Figure 1. Profile of the lenercept MS clinical trial.

change in burden of disease over 24 weeks of treatment are shown in figure 2 and table 3. There were no significant differences between the treatment groups for any measure. The results of the analyses of the primary efficacy criterion according to the protocol specifications were similar to those presented here. Because of the tendency for higher activity at baseline in the high-dose groups (see table 2), covariance analyses using the corresponding transformed baseline MRI values as covariate were performed, but these, too, failed to show a significant difference between the groups.

Clinical endpoints. Exacerbations. The number of patients who developed exacerbations by week 24 and through study week 48 were both increased in the lenercept groups as compared with the placebo treatment group as shown in table 4. A center effect was not present. Over the entire study period, a total of 36 exacerbations was reported in patients taking placebo as compared with 38,

Table 1 *Demographic and baseline characteristics of patients entered into the lenercept MS trial*

Characteristics	Placebo, n = 44	Lenercept, mg		
		10, n = 44	50, n = 40	100, n = 40
% Female	66	80	78	73
Age, y, mean (range)	36.5 (21–50)	34.6 (23–51)	35.1 (19–47)	34.9 (21–51)
% White	98	100	100	93
No. with SPMS	10	5	10	4
Mean (range) no. exacerbations in prior 2 years	2.7 (2–5)	2.8 (2–8)	2.8 (2–8)	3.0 (2–6)
EDSS, mean (range)	2.45 (0–5.5)	2.52 (0–5.0)	2.83 (1.0–5.5)	2.55 (0–5.5)
NRS, mean (range)	83.2 (51–100)	83.7 (44–100)	81.8 (54–100)	83.0 (57–99)

SPMS = secondary progressive MS; EDSS = Expanded Disability Status Scale; NRS = Neurological Rating Scale.

Table 2 *MRI measurements at baseline of patients entered into the lenercept MS trial*

Variable	Placebo, n = 44	Lenercept, mg		
		10, n = 44	50, n = 40	100, n = 40
Newly active lesions, mean	1.8	1.5	2.1	1.9
Median (range)	0 (0–16)	0 (0–55)	1 (0–11)	1 (0–14)
Persistently active lesions, mean	1.2	0.5	1.6	0.6
Median (range)	0 (0–15)	0 (0–6)	0 (0–22)	0 (0–7)
Burden of disease, mean	2,459.9	2,236.2	3,757.2	2,707.2
Median (range)	1,626.0 (26.2–9,888.7)	1,147.4 (0–14,377.4)	2,142.4 (67.3–16,319.0)	1,365.3 (58.7–10,884.8)

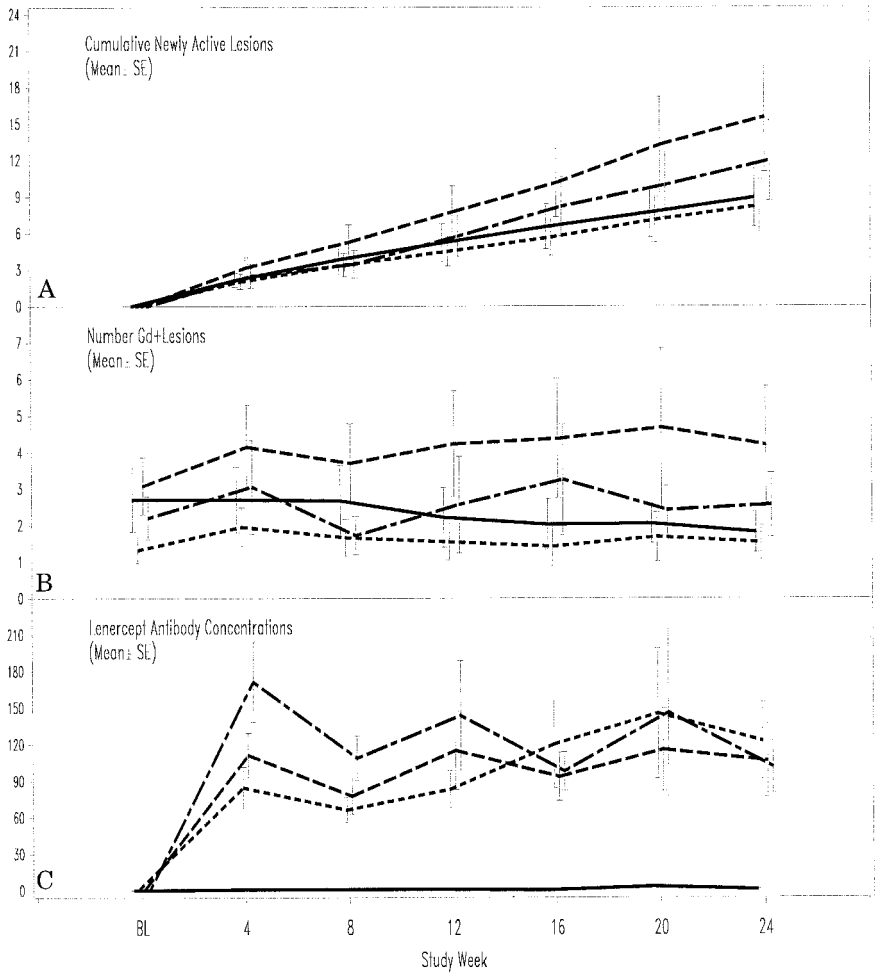

Figure 2. (A) Number of cumulative newly active lesions as determined by MRI (see Methods) over the first 24 weeks of the lenercept MS trial: placebo ——, lenercept 10 mg - - - -, lenercept 50 mg — · — ·, lenercept 100 mg – – – –. The vertical bars give the standard error at the four weekly intervals at which MRI scans were performed. (B) The mean number of gadolinium (Gd)–positive lesions every 4 weeks over the first 24 weeks of the trial. Vertical bars give the standard error. (C) Mean anti-lenercept antibody titers at four weekly intervals.

57, and 49 exacerbations in patients taking 10, 50, and 100 mg of lenercept, respectively. Exacerbation duration showed a tendency to increase with lenercept treatment, but this did not reach statistical significance (see table 4). This assessment was limited to exacerbations with an onset date within the first 24 weeks of the study as exacerbation resolution dates were available in all but four exacerbations (one per treatment group).

Exacerbation rate. The overall exacerbation rate in patients treated with placebo was approximately one exacerbation/patient/year (the expected placebo rate). The exacerbation rate was increased over the placebo rate by 2%, 68%, and 50% in patients treated with lenercept at doses of 10, 50, and 100 mg, respectively (see table 4). To control for a possible effect of unequal follow-up of patients between treatment groups, exacerbation rates were determined for each treatment group by 4-week intervals. The mean 4-week exacerbation rates were then converted to annual rates as presented in table 4.

There was a dose-dependent decrease in the time to first exacerbation as shown in figure 3 (logrank test: global, $p = 0.0006$; 10 mg versus placebo, $p = 0.498$; 50 mg

***Table 3** MRI measurements over the first 24 weeks of the lenercept MS trial*

Variable	Placebo, n = 43	Lenercept, mg			*p* Value
		10, n = 44	50, n = 40	100, n = 40	
Newly active lesions, mean	8.9	8.2	15.5	12.0	0.43*
Median (range)	4.0 (0–92)	3.0 (0–55)	5.5 (0–124)	4.5 (0–102)	
Persistently active lesions, mean	6.2	3.3	9.6	3.3	0.36*
Median (range)	1.0 (0–100)	1.0 (0–49)	1.0 (0–128)	1.0 (0–24)	
Percent of active scans,† mean	45.6	40.6	51.6	49.2	0.58†
Median (range)	50 (0–100)	33 (0–100)	50 (0–100)	55 (0–100)	
Percent change in burden of disease,† mean	6.0	9.9	4.3	4.9	0.74†
Median (range)	−2.2 (−28.8–280.9)	0.0 (−30.5–251.0)	1.4 (−35.2–62.8)	−0.2 (−36.0–81.4)	

* Analysis of variance of Ln(1 + x).
† Kruskal-Wallis test.

Table 4 *Number, duration, and annual rate of exacerbations during the lenercept MS trial*

Exacerbations	Placebo, n = 43	Lenercept, mg			*p* Value
		10, n = 44	50, n = 40	100, n = 40	
Patients with at least one exacerbation through week 24, n	15	21	28	27	0.003*
Patients with at least one exacerbation through week 48, n	22	26	32	32	0.007†
Exacerbations with onset ≤ week 24	22	28	37	33	
Duration (days) of these exacerbations, mean	28.3	38.6	41.6	42.0	0.62‡
Median (range)	28 (1–91)	31 (6–189)	31 (6–201)	25 (4–261)	
Annualized exacerbation rate	0.98	1.00	1.64	1.47	

* Chi-square tests: global.
† Kruskal-Wallis test.
‡ Kaplan-Meier (KM) (means and medians are estimated from the KM curves).

versus placebo, $p = 0.0006$; and 100 mg versus placebo, $p = 0.006$). The comparisons with unadjusted p values for 50 and 100 mg of lenercept as presented above remain significant when Bonferroni adjusted (α of 0.017, i.e., $0.05 \div 3$).

The severity of exacerbations was assessed indirectly by computing the difference between the best pretreatment NRS score (highest value at baseline or screening) and the worst score recorded at any time during the study, i.e., through week 48. Although a trend for increasing severity with lenercept treatment can be perceived (table 5), this difference did not reach statistical significance.

Exacerbations did not appear to occur more frequently immediately before or after study drug administration, but this may reflect no more than difficulties in determining exacerbation onset dates with precision.

Predictors of exacerbations. A Cox regression was performed. None of the following was identified as predictive of exacerbations: age, sex, MS category, number of exacerbations within 2 years before the study, baseline EDSS score, or baseline number of newly active lesions.

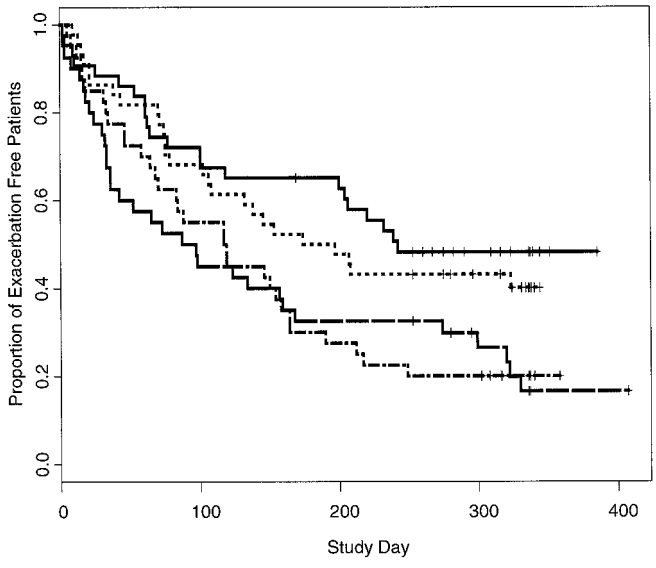

Figure 3. Proportion of patients remaining exacerbation free over the course of the lenercept MS trial: placebo ——, lenercept 10 mg - - - -, lenercept 50 mg - - - -, lenercept 100 mg – – – –. Times of termination for individual patients are shown as vertical bars.

EDSS. There was no difference in EDSS score changes between the treatment groups after 24 weeks of treatment or at the last assessment (table 5). Sixty-seven percent of patients in the placebo group reported new or worsening MS symptoms during the study as compared with 77%, 90%, and 93% of patients treated with lenercept in the 10-, 50-, and 100-mg dose groups, respectively. Symptoms that increased in frequency or severity with lenercept treatment included sensory complaints, limb weakness, visual impairment, fatigue, vertigo, and spasm (table 6).

Safety. Six patients withdrew from treatment during the study as a result of an adverse event. Depression worsened in one patient in the placebo group; development of a rash after the first injection led to the withdrawal of two patients, one in the 10-mg dose group and the other in the 100-mg dose group. Three additional patients withdrew from the 100-mg dose group, one due to the occurrence of a transient episode of flushing, dyspnea, and gastralgia after the fifth dose and two because of exacerbation-related symptoms after the fifth and seventh doses, respectively. Ninety-five percent of patients treated with placebo had at least one adverse event reported as compared with 87%, 90%, and 95% of patients treated with 10, 50, and 100 mg of lenercept, respectively. Adverse events that increased in frequency with active treatment included headache, hot flushes, nausea, and abdominal pain (see table 6). A transient episode of dyspnea associated with the administration of lenercept at doses of 50 and 100 mg occurred in six patients.

Laboratory. RF (IgM) and ANA were present in 1% (2/162) and 20% (33/161) of patients at baseline, respectively. During the study, five patients developed a positive IgM-RF (all in the lenercept treatment groups) and 15 patients developed ANA, 14 of whom were receiving lenercept. One patient on lenercept developed both antibodies. Thus, new occurrences of RF or ANA were more frequent in lenercept-treated than in placebo-treated patients (18/124 versus 1/43; chi-square $p = 0.03$). Clinical manifestations of rheumatoid arthritis or systemic lupus erythematous were not observed, and dsDNA was consistently negative in all patients in whom ANA positivity was detected.

Total serum IgM had increased in a dose-dependent fashion in lenercept-treated patients after 24 weeks of treatment by an average of 0.6, 0.9, and 1.0 g/L in the 10-,

Table 5 *Change in the NRS and the EDSS during the lenercept MS trial*

Rating scale	Placebo, n = 43	Lenercept, mg			*p* Value
		10, n = 44	50, n = 40	100, n = 40	
NRS, weeks 1–48					
No change or improved	14	13	9	9	
Mild deterioration	21	18	14	18	0.37*
Moderate deterioration	3	10	8	7	
Severe deterioration	5	3	9	6	
EDSS, weeks 1–24, n	43	44	40	40	
Mean change (range)	−0.131 (−2.0 to 2.0)	−0.295 (−3.0 to 5.5)	−0.05 (−2.0 to 2.0)	−0.075 (112.0 to 4.0)	0.24†
EDSS, weeks 1–48					
Mean change (range)	0.014 (−1.0 to 2.0)	−0.024 (−3.0 to 5.5)	−0.026 (−2.0 to 2.0)	0.171 (−2.0 to 4.0)	0.71†

* Chi-square.
† Kruskal-Wallis test.

NRS = Neurological Rating Scale; EDSS = Expanded Disability Status Scale.

50-, and 100-mg dose groups. For those patients in the 100 mg dose group who remained on treatment beyond 24 weeks, the mean increase reached 1.2 g/L. Total serum IgG concentrations had increased by an average of 0.5 g/L at 24 weeks on treatment in patients receiving 100 mg of lenercept; for those patients who remained on treatment in the 100-mg dose group, this increase ultimately reached 1.2 g/L. Serum IgG concentrations were not increased in the other groups.

Pharmacokinetics. Trough serum concentrations of lenercept were detectable in only a third of the patients in all dose groups and tended to relate to the anti-lenercept antibody profile. Thus, patients without antibodies had persistently detectable lenercept trough serum concentrations, whereas patients with high antibody concentrations had consistently undetectable trough levels. Antibodies to lenercept at a concentration greater than 20 ng/mL, as determined by the double-antigen screening assay, were detected in the majority of patients on treatment (5% [2/43] of patients on placebo, as compared with 98% [43/44], 88% [35/40], and 100% [40/40] of patients in the 10-, 50-, and 100-mg dose groups, respectively.) Antibody concentrations varied considerably from patient to patient and over time; generally, a dose-dependent peak occurred after the first dose, followed by a relatively stable level that persisted for as long as the patient continued to receive lenercept (see figure 2).

Table 6 *Patient complaints over the course of the lenercept MS trial*

Patient complaints	Placebo, n = 43	Lenercept, mg		
		10, n = 44	50, n = 40	100, n = 40
MS-related				
Any MS symptom	67	77	90	93
Sensory complaints	44	45	58	63
Limb weakness	19	20	33	43
Visual impairment	19	14	28	40
Fatigue	14	14	25	28
Vertigo	2	7	13	15
Spasm	2	5	7	13
Other				
Headache	23	36	35	45
Hot flushes	7	7	8	18
Nausea	5	11	23	15
Abdominal pain	5	18	23	8
Dyspnea	0	0	2	13

Values are %.

Discussion. Lenercept treatment in the phase II study reported here increased MS attack frequency. There was also a suggestion that lenercept increased attack duration and worsened attack severity as judged by the extent of changes noted on the NRS. The lenercept effect was more pronounced at the two higher doses employed (50 and 100 mg) than at the lowest dose (10 mg). An increase in attack frequency was noted in lenercept-treated patients within the first month on drug and persisted throughout the period during which drug was given. The magnitude of the treatment effect on exacerbations could be biased because the trial was stopped early. We believe this is unlikely as the results of the second 24-week study period were consistent with those found during the first 24 weeks.

Despite the seemingly deleterious clinical effects of lenercept, no meaningful difference between treatment groups was noted in terms of worsening of the EDSS score. Whereas MS attack frequency and subsequent development of disability have correlated in large patient series studied over many years, no such correlation was noted in the current study, perhaps because of the relative insensitivity of the EDSS and the prompt cessation of the study following the interim monitoring analysis.

It is important to remember that had the current study shown a positive treatment effect, the results would have had to be validated in a second trial. Prudence dictates that similar caution be applied to the detrimental clinical effect noted in the current trial, particularly in view of the seemingly discordant MRI data. Setting caution aside, it is reasonable to conclude that lenercept is probably contraindicated in MS based on the internal consistency of the clinical results presented here and the untoward results obtained in a preliminary study of two patients administered a humanized mouse monoclonal anti-TNF antibody.[25] The dissociation seen between a significantly increased clinical activity and, at best, a slight trend toward an increase in MRI-measured activity was surprising. It may be due to the lack of a robust correlation between MRI and clinical outcome, although it is generally perceived that MRI changes, in particular newly active lesions, are sensitive measures of disease activity. In the current study, more than 90% of the newly active lesions measured were Gd-enhancing, a reflection of enhanced blood–brain barrier (BBB) permeability. To date, a reduction in Gd-enhancing MRI lesions has been shown to be unequivocally associated with clinical benefit only for the beta interferons.

Could TNF neutralization have enhanced the inflammatory CNS process without affecting BBB permeability? If such occurred, our selection of newly active MRI lesions as the outcome measure for this study may have been ill suited for the assessment of this particular drug. Alternatively, failure to demonstrate an increase in MRI activity may have depended on technical factors. MRI scans over the course of the current trial were obtained immediately before dosing; i.e., 4 weeks after the preceding dose. In two MS patients treated with anti-TNF antibody, increased numbers of Gd-enhancing lesions were observed shortly after drug administration, with return to baseline within 2 to 3 weeks.[25] Had a similar early and transient increase in MRI activity occurred in the current trial, it would not have been detected.

Selection of MRI endpoints for a therapeutic study of any novel agent in MS may have to be individually determined for that agent. This may require a survey of the effects of the drug on MRI dynamics over days or weeks as a prelude to protocol design for a phase II study. Novel therapeutic agents assessed solely by currently recommended MRI methodology could potentially be deemed inert even if associated with deleterious clinical effects, as was seemingly the case in this study. The same could hold for beneficial effects. This study cautions against an overly broad interpretation of MRI results in the absence of corroborating clinical data.

EAE is widely employed as an animal model for MS and is thought to entail at least some of the same pathways of tissue injury as does MS. Lenercept has repeatedly shown potent preventive and therapeutic effects in various EAE protocols[13] and thus the EAE results were not predictive of lenercept's effect in MS. Recently, inordinately severe EAE has been reported in mice lacking TNF following immunization with myelin oligodendrocyte glycoprotein,[26] suggesting that even within EAE models results may differ depending on the model system.

Why lenercept failed is unknown; it could relate to some property unique to the lenercept molecule independent of its TNF neutralizing capacity. Antibodies to lenercept develop promptly in a substantial number of patients who receive it, and although the antibodies do not neutralize TNF binding, they do accelerate elimination of the drug. Furthermore, lenercept contains the Fc-like portion of IgG. It is conceivable that formation of immune complexes and activation of Fc receptors on lymphoid cells may have had a role in enhancing the inflammatory process.

Perhaps lenercept failed because of a flaw in the rationale for TNF neutralization. Consistent with this formulation is the result of a study of two patients with rapidly progressive MS in which increased activity was noted after administration of an anti-TNF antibody.[25] Cytokines are pleiotropic factors and act in a complex network. Certain actions of TNF may be viewed as proinflammatory and others as anti-inflammatory; the latter may contribute to "off" signals in MS. If the on/off balance of TNF-mediated signals is relevant to MS, then removal of TNF could, contrary to widely held perceptions, potentiate disease. Interferon (IFN)–γ administration provokes MS attacks.[27] TNF induces interleukin (IL)-10 and prostaglandin E_2 production and these acting jointly inhibit IL-12 production and, hence, IFN-γ production.[28] Thus, mechanisms may exist by which TNF blockage could augment those immune responses that contribute to MS pathogenesis.

Although lenercept failed in MS, it did reduce signs and symptoms of rheumatoid arthritis in phase II studies.[29] This finding suggests a final caution. An agent that demonstrates a beneficial effect in one autoimmune disease should not be presumed to have beneficial effects in another.

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Appendix

Multiple Sclerosis Study Group: The Lenercept Multiple Sclerosis Study Group comprises the following participating institutions, principal investigator (in italics), and investigative teams (in alphabetical order by principal investigator; team members in alphabetical order).

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The effect of anti-α4 integrin antibody on brain lesion activity in MS

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Article abstract—*Objective:* To determine the effect of humanized monoclonal antibody against α4 integrin (reactive with α4β1 integrin or very-late antigen–4) on MRI lesion activity in MS. *Methods:* A randomized, double-blind, placebo-controlled trial in 72 patients with active relapsing-remitting and secondary progressive MS was performed. Each patient received two IV infusions of anti-α4 integrin antibody (natalizumab; Antegren) or placebo 4 weeks apart and was followed up for 24 weeks with serial MRI and clinical assessment. *Results:* The treated group exhibited significantly fewer new active lesions (mean 1.8 versus 3.6 per patient) and new enhancing lesions (mean 1.6 versus 3.3 per patient) than the placebo group over the first 12 weeks. There was no significant difference in the number of new active or new enhancing lesions in the second 12 weeks of the study. The number of baseline-enhancing lesions (i.e., lesions that enhanced on the baseline scan) that continued to enhance 4 weeks following the first treatment was not significantly different between the two groups. The number of patients with acute MS exacerbations was not significantly different in the two groups during the first 12 weeks (9 in the treated group versus 10 in placebo) but was higher in the treatment group in the second 12 weeks (14 versus 3; $p = 0.005$). The study was not, however, designed to look definitively at the effect of treatment on relapse rate. Treatment was well tolerated. *Conclusions:* Short-term treatment with monoclonal antibody against α4 integrin results in a significant reduction in the number of new active lesions on MRI. Further studies will be required to determine the longer term effect of this treatment on MRI and clinical outcomes.

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Adhesion molecules may play an important role in inflammatory demyelination by focusing systemic immune responses into the target tissue—the CNS. These molecules are upregulated through the actions of cytokines.[1] The process whereby activated T lymphocytes and monocytes gain access to the CNS is governed by an array of adhesion molecules and ligands that are expressed on endothelial cells and leukocytes. Cytokines secreted at the site of inflammation enhance adhesion molecule/ligand expression on vascular endothelial cells, whereas cytokines released at the site promote leukocyte migration across the vascular wall into the CNS.

Integrins are a versatile family of adhesion molecules[2] of which α4β1 integrin (also called very-late antigen-4 or CD49d/CD29) is an important mediator of immune cell migration into the CNS. The interaction of α4β1 integrin on T lymphocytes and monocytes with its counter receptor, vascular cell adhesion molecule-1 (VCAM-1), on endothelial cells is tenacious and will mediate the capture and consequent trafficking of leukocytes across the vascular endothelium.

As a result of successful experiments in experimental autoimmune encephalomyelitis (EAE) models on both clinical and MRI outcomes,[3-5] it was proposed that antibodies against α4β1 integrin may have therapeutic value in MS, both as a potential treatment of acute exacerbations of the disease and to inhibit the occurrence of subsequent attacks. We report an exploratory study of anti-α4 integrin antibody in MS, using MRI activity as the primary outcome measure of efficacy.

Methods. Antegren (natalizumab; Elan Pharmaceuticals, South San Francisco, CA) is a humanized monoclonal antibody (mAb) derived from a murine mAb (called AN100226m) raised against the human α4 integrin. AN100226m was humanized by complementary-determining-region grafting to a human immunoglobulin (Ig)G4

See also pages 444 and 457

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framework to reduce its immunogenicity, increase its half life in vivo, and allow repeated administration, and thus improve the potential therapeutic effect. The humanized form of AN100226m, Antegren, was demonstrated to retain its full inhibitory activity against α4 integrin–mediated cell adhesion both in vitro and in vivo.[6]

Antegren was examined in a randomized, double-blind, placebo-controlled, parallel groups trial in patients with MS. MRI activity, rather than clinical disease activity, was used as the primary measure of outcome because the greater sensitivity of the former makes it a more powerful outcome measure in short-term pilot studies.[7] Treatment of the patients, handling of any side effects and adverse events, and evaluation of most of the secondary efficacy parameters (e.g., clinical relapses) were performed by a single physician at each of the eight study sites, so that for most clinical evaluations there was no distinction between the treating and evaluating physician. This was considered to be acceptable because the primary outcome measure was not a clinical one. An exception to this arrangement was the assessments of neurologic disability, as measured by the Expanded Disability Status Scale (EDSS)[8] and Guy's Neurological Disability Scale (GNDS),[9] which were performed by separate nontreating and blinded observers at each site. The study was approved by the local ethics committees at each of the eight participating centres.

Screening assessment (week −4). Inclusion and exclusion criteria are given in table 1. Patients were enrolled and randomized at eight UK sites with a mean of nine patients entered per site (total 72; range 6 to 13). Patients who fulfilled the eligibility criteria and gave written consent for participation in the study underwent medical history and examination, including a neurologic examination.

A blinded observer then performed EDSS and a GNDS assessment. Vital signs were recorded and an MRI brain study and an electrocardiogram were performed. Blood samples were taken for hematology and biochemistry, and a serum pregnancy test was performed in all women of childbearing potential. A urine sample was also taken. If the patient had an exacerbation of MS (defined as one or more new symptoms, or worsening of existing symptoms, lasting at least 48 hours) between the week −4 visit and the first scheduled treatment date, they were withdrawn from the study.

Treatment dosage and administration (week 0 and +4). Patients were admitted to the hospital for infusion of the study drug at weeks 0 and 4. Only two infusions were given because a preliminary study of a single dose of Antegren in healthy volunteers indicated that this would give serum levels of Antegren expected to fully saturate the α4 integrin receptor for 3 to 4 weeks and because, from a safety perspective, there were no previous studies of repeated dosing. After a brief examination and a repeat EDSS and GNDS examination, an MRI brain study was performed and blood taken as was done previously. The study drug was then infused on two occasions over a period of 30 to 45 minutes. The patients received 3 mg/kg of study drug or placebo diluted to a 100-mL solution with normal saline. Patients were discharged from hospital not less than 6 hours after the study drug was infused. Any adverse events during and after the infusion were noted.

Follow-up visits (weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24). At each visit, any change in the patients' well-being was recorded. MRI brain studies were performed at every visit. There was also a brief clinical examination, and any adverse events, new medication, or MS exacerbations the patients may have had since the previous visit were recorded. On each occasion, blood was taken for hematology, biochemistry, immune function profile (which included IgA, IgG, IgM, C-reactive protein, CD4, and CD8 levels), and Antegren and anti-Antegren antibody levels. A urine pregnancy test was performed before every MRI brain study in all women of childbearing potential. In addition, a full clinical examination was performed at weeks 0, 12, and 24. An EDSS and GNDS were performed at weeks −4, 0, 12, and 24 by a blinded observer. An electrocardiogram was performed at weeks −4, 4, 12, and 24.

MRI protocol. An MRI brain study was performed at each visit according to the following protocol: fast/turbo spin-echo sequence with 3-mm–thick slices (repetition time [TR] 3000 msec, echo time [TE] 20 to 40 msec and 80 to 100 msec, 256 × 256 matrix, one excitation, acquisition time 7 minutes) and, 5 to 7 minutes following 0.1 mmol/kg of IV gadolinium-DTPA (over 1 to 2 minutes), a T1-weighted sequence (TR 600 msec, TE 10 to 20 msec, 3-mm–thick slices, 256 × 256 matrix, one excitation, acquisition time 6 minutes).

Table 1 *Entry criteria*

Criteria
Inclusion
Clinically definite relapsing-remitting or secondary progressive MS[10]
Age 18–55 years at first visit
Weight <90 kg
Expanded Disability Status Scale score 2.0–7.0
Two or more clinical exacerbations in the previous 18 months
>4 Weeks since the onset of the last exacerbation
Fully informed, written consent
Exclusion
Primary progressive MS
Women of child-bearing potential, unless adequate contraception*
Pregnant or breast-feeding mothers
A normal T2-weighted MRI brain study at week −4
Receiving, or had received in last 6 months, immunosuppressive drugs (including azathioprine, cyclophosphamide, and beta-interferon)
Use of methylprednisolone and/or oral prednisone in the 4 weeks before the first (week −4) visit
Previous treatment with anti-CD4 antibodies, other monoclonal antibodies, or total lymphoid irradiation at any time
Previous exposure to any product containing murine protein
Alcohol consumption >21 units (i.e., 315 mg)/week or abuse of other drugs

* Oral or depot contraceptives or intrauterine device were used throughout the study, or the patient was surgically sterile. It was required that a pregnancy test at week −4 was negative. Pregnancy tests were also performed before each dose of study drug was given.

The images from all sites were reviewed by a single neuroradiologist (I.F.M.) blinded to the clinical details. The number of enhancing lesions at the baseline (week −4) T1-weighted study was counted. On the week 0 study, the number of new and persistent enhancing lesions (from week −4) was recorded. Lesions that enhanced on the week 0 study and continued to enhance on subsequent examinations were defined as "baseline-enhancing" (i.e., they existed immediately before the start of treatment). Lesions that first showed contrast enhancement on images from week +1 onward, and that continued to enhance on subsequent studies, were classed as "new enhancing" when first seen and "persistent enhancing" on subsequent studies. A count was also made of new or newly enlarging lesions on the T2-weighted images that did not enhance. "New active" lesions were defined as the sum of new enhancing lesions seen on the T1-weighted images and new or newly enlarging nonenhancing lesions seen on the T2-weighted images.

Outcome measures. Primary outcome. The cumulative number of new active and new enhancing lesions seen on MRI during weeks 1 to 12 from the start of treatment after baseline correction was the primary outcome.

Secondary outcomes. Secondary outcomes included 1) the number of new active and new enhancing lesions during weeks 12 to 24 after the first dose; 2) the number of baseline-enhancing lesions (i.e., those present at week 0) which continued to enhance at 1, 2, and 4 weeks after the first dose; 3) the number of persistent enhancing lesions (i.e., lesions that had first enhanced from week +1) at weeks 2, 4, 6, 8, 12, 16, 20, and 24; 4) the number of MS relapse exacerbations as defined using Poser criteria[10]; and 5) EDSS and GNDS scores.

At each visit, adverse events were recorded for each patient. An independent safety committee was appointed to review adverse and other safety events throughout the trial.

Statistical analysis. Sample size calculations, based on cohorts of untreated patients with relapsing-remitting and secondary progressive MS when employing a parallel groups design with a single pretreatment (run-in) MRI scan, estimated that 2 × 28 patients would need to be imaged monthly for 3 months to detect an 80% reduction in the number of active MRI lesions with a power of 80%.[11,12] To allow for possible drop-outs, it was planned to enroll up to 80 patients, of whom half of those randomized would receive active treatment and half placebo. When it became clear that the drop-out rate was lower than expected, recruitment was stopped when 72 patients had been randomized. The effectiveness of patient blinding was not assessed. All efficacy analyses were carried out using the modified intention-to-treat population. In addition, a confirmatory analysis of the primary efficacy parameter was also performed using the per protocol population. The intention-to-treat population included all randomized patients who received at least one dose of study medication. The modified intention-to-treat population excluded two patients who received study medication before a reformulation necessitated by precipitation of Antegren when diluted.

Patient demographic data (table 2) were summarized for each treatment group by type of variable: categorical data (e.g., sex, race) by counts and percentages, and continuous variables (e.g., age, weight) by means, standard deviations, medians, minima, and maxima, and numbers of patients where appropriate.

Table 2 *Patient demographic data*

Characteristic	Antegren, n = 37	Placebo, n = 35
Mean age, y (range)	39.9 (25–52)	40.8 (25–55)
M/F	12/25	14/21
Mean height, cm (range)	168.9 (154–185)	170.1 (153–195)
Mean weight, kg (range)	65.6 (43–89)	68.7 (46–85)
RRMS, n (%)	25 (68)	28 (80)
SPMS, n (%)	12 (32)	7 (20)
Mean duration of MS, mo (range)	117.2 (11–317)	119.2 (9–356)
Mean EDSS at week −4 (range)	4.9 (2.0–7.0)	4.7 (2.0–6.5)
Mean (%) frequency of relapse*		
<1/y	4 (11)	4 (11)
1–2/y	25 (68)	22 (63)
3–4/y	6 (16)	6 (17)
>4/y	2 (5)	3 (9)

* The mean frequency of relapses is reported for the 18 months before study entry.

RR = relapsing-remitting; SP = secondary progressive; EDSS = Expanded Disability Status Scale Score.

Statistical comparisons of treatment with Antegren and placebo were carried out using two-tailed tests at the 5% level of significance. The difference between treatment regimens was estimated from the analysis and 95% confidence intervals (CI) were constructed around the estimated differences.

The number of new active and new enhancing lesions during the first and second 12 weeks following the first treatment was analyzed using analysis of covariance. The number of new enhancing lesions seen during the 4-week run-in phase was used as a covariate. For the other outcome measures, 95% CI for the differences between the Antegren group and the placebo group were constructed at each time point and p values calculated at appropriate time points.

Results. We originally recruited 72 patients into the trial. However, a problem arose with the original IV formulation that led to a temporary postponement of the trial after two patients had been treated (these two were included in the safety data for the trial and, when the trial was unblinded, were found to have received placebo). Seventy patients received the new formulation and were evaluated for safety and efficacy. In total, 37 patients received Antegren (evaluable for safety and efficacy), 35 received placebo for safety analysis, and 33 received placebo for efficacy analysis. The patients were well matched demo-

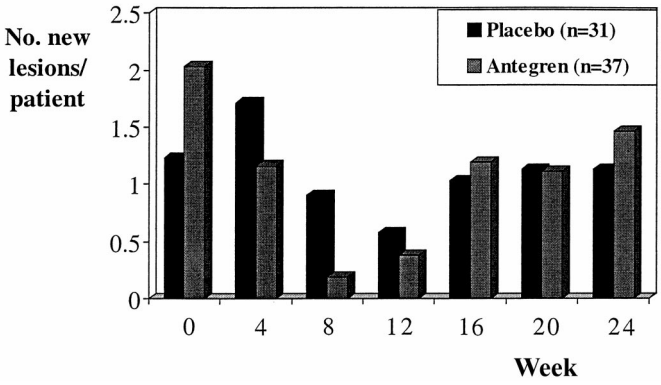

Figure 1. Mean number of new enhancing lesions by 4-week epochs. Figures are means for each 4-week epoch; thus the numbers of new enhancing lesions at weeks 1, 2, and 4, and weeks 6 and 8, have been combined for weeks 4 and 8, respectively. 0 = new enhancing lesions on the week scan.

graphically (see table 2). Two of the placebo patients had to be excluded from the efficacy analysis, having withdrawn from the trial at week 6 (one patient with a fractured hip and another who felt unable to tolerate further imaging). Therefore, there were 68 (94.4%) evaluable patients for the primary endpoint (37 Antegren; 31 placebo). Of a possible 770 MRI studies in 70 patients (i.e., 11 per patient), there were 748 evaluable studies (i.e., 97.5% were performed).

In 70 patients, there was a total of 129 enhancing lesions on the baseline (week −4) studies (59 in the placebo group and 70 in the Antegren group). At week 0, there were 40 new enhancing lesions in the placebo group (mean 1.2 per patient; range 0 to 12; standard deviation [SD] 2.4) and 75 in the treatment group (mean 2 per patient; range 0 to 44; SD 7.3) (figures 1 and 2).

The mean cumulative number of new active lesions in the first 12 weeks posttreatment was lower in the Antegren-treated group than in the placebo group (adjusted mean 1.8 versus 3.6; $p = 0.042$, analysis of covariance [ANCOVA]; see figures 1 and 2). The great majority of new active lesions were areas of new enhancement. The number of new enhancing lesions during the first 12 weeks was also lower in the Antegren-treated group (adjusted mean 1.6 versus 3.3 per patient; $p = 0.017$, ANCOVA).

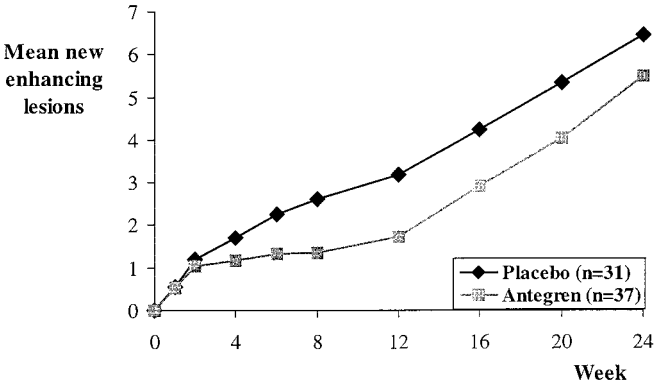

Figure 2. Mean cumulative new enhancing lesions from week 1 to 24.

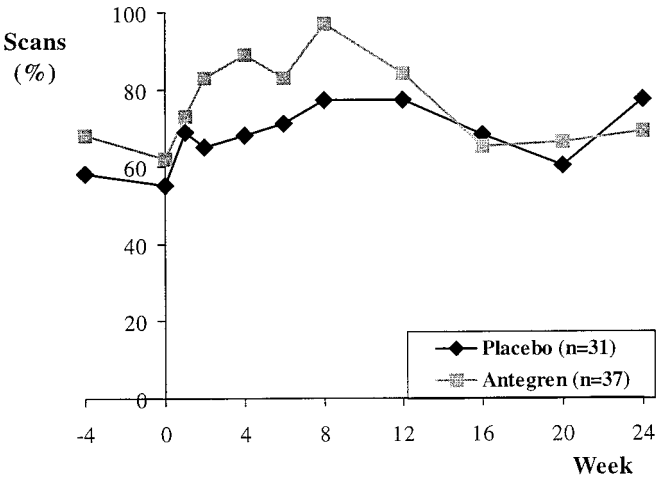

Figure 3. Proportion of imaging studies with no new enhancing lesions, weeks −4 to 24.

The proportion of MRI studies showing no new enhancing lesions during the first 12 weeks posttreatment was higher in the Antegren group than the placebo group (83.6% versus 73.1%, $p = 0.037$, ANCOVA; figure 3). There were no significant differences between the two groups in the mean cumulative number of new enhancing lesions or the proportion of examinations without new enhancing lesions in the second half of the trial (weeks 12 to 24).

There was no difference between the two groups when the proportion of baseline-enhancing lesions that continued to enhance 4 weeks later was compared (figure 4). For patients with higher numbers (≥4) of enhancing lesions at baseline, those receiving placebo had fewer baseline-enhancing lesions at weeks 1 and 2 ($p = 0.01$ and $p = 0.009$, respectively). Estimated treatment differences were 0.72 lesions (95% CI 0.2, 1.3) and 1.04 lesions (95% CI 0.3, 1.8), respectively. Given that enhancement may be expected to cease after 4 to 6 weeks, it is possible that this difference could be attributed to a few patients in the placebo group presenting with "older" lesions at baseline. The mean number of persistently enhancing lesions from weeks 2 to 24 was generally similar in both treatment groups except that they tended to be lower in the Antegren-treated group at weeks 4, 6, and 8.

There were no differences in the biochemical parameters, but in the Antegren-treated group there was a significant lymphocytosis between weeks 1 and 12 (mean

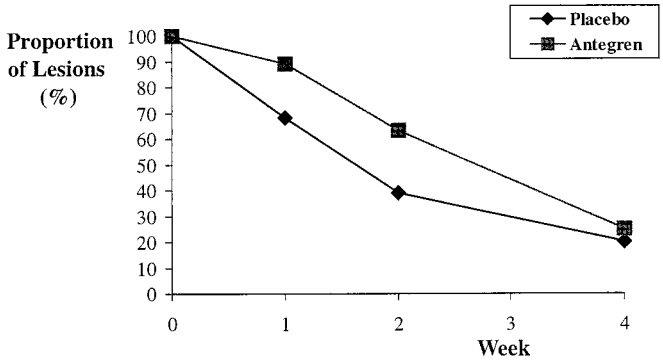

Figure 4. Proportion of week 0 baseline-enhancing lesions that display continued enhancement at weeks 1, 2, and 4.

Table 3 *Comparison of other outcome parameters*

Characteristic	Antegren	Placebo
New T2 lesions		
Week 12, mean	0.8	1.0
Median (SD)	0.0 (1.4)	1.0 (1.0)
Week 24, mean	2.5	1.7
Median (SD)	0.0 (4.8)	1.0 (2.7)
Mean disability scores, EDSS/GNDS		
Week −4	4.85/12.3	4.71/13.0
Week 0	4.88/11.3	4.70/12.6
Week 12	4.82/10.6	4.88/11.6
Week 24	4.86/12.6	4.72/12.3
Adverse events summary, n		
Week 0–12		
Patients	37	35
Patients with AE (%)	32 (86)	34 (97)
AEs	136	137
Mild	86	82
Moderate	44	46
Severe	6	9
Week 12–24		
Patients with AE (%)	19 (51)	24 (69)
AEs	47	51
Mild	20	32
Moderate	23	18
Severe	4	1

EDSS = Expanded Disability Status Scale; GNDS = Guy's Neurological Disability Scale; AE = adverse events.

Table 4 *MS acute exacerbations*

Variable	Antegren	Placebo	*p* Value
Patients with ≥1 acute exacerbation, n (%)	18 (49)	12 (36)	0.30
Patients with exacerbations in first 12 weeks after first dose, n (%)	9 (24)	10 (30)	0.574
Total no. of exacerbations	9	11	
Exacerbations treated with steroids	2	5	
Exacerbations resulting in hospitalization	2	3	
Patients with exacerbations in second 12 weeks after first dose, n (%)	14 (38)	3 (9)	0.005*
Total no. of exacerbations	15	4	
Exacerbations treated with steroids	8	0	
Exacerbations resulting in hospitalization	4	0	

* Significant difference.

increase of 56 to 60% compared to placebo). Lymphocyte counts remained slightly elevated at week 16 in the Antegren-treated group (compared with the placebo group and with baseline levels in the Antegren-treated group), but had returned to baseline levels at weeks 20 and 24. By week 12, 97% of patients no longer had serum Antegren levels above 1 μg/mL (the level at which saturation of α4 integrin is expected to occur). All subjects enrolled into the study were negative for anti-Antegren antibodies before drug administration, and all those randomized to receive placebo remained negative for anti-Antegren antibodies following study drug treatment. A low titer of antibodies to Antegren (ELISA method) was detected in 4 (11%) of the 37 patients treated with Antegren. In these four patients, antibodies were detected at week 4; weeks 6 and 8; weeks 6, 8, 12, and 16; and weeks 16, 20, and 24. Anti-idiotype antibody titers ranged between 1.3 and 5.0 μg/mL. There was no formal analysis assessing whether antibodies to Antegren affected MRI or clinical outcomes in this study.

A variety of adverse effects was seen in both treatment and placebo groups (table 3). There was no significant difference in the incidence of any adverse events reported between treatment groups in the first 3 months of the study when active treatment was given, although there was a trend to more fatigue in the Antegren group (10/37 versus 3/35; $p = 0.065$). Fatigue (12 patients in the Antegren and 4 in the placebo group; $p = 0.047$) and insomnia (4 patients in the placebo group and none in the Antegren group; $p = 0.05$) were the only adverse events to be significantly different in the two groups over the entire study period. The number of acute exacerbations of MS are summarized in table 4. There was no significant difference in the number of patients with at least one MS exacerbation between treatment groups during the whole study period ($p = 0.3$) or in the first 12 weeks ($p = 0.57$). However, during the second 12 weeks after the first dose of study medication, 14 patients (38%) in the Antegren group and 3 (9%) in the placebo group experienced at least one acute relapse. This difference was significant ($p = 0.005$) in favor of the placebo group (see table 4).

The EDSS/GNDS scores over the whole study period are presented in table 3. At week 12, there was a significant difference in the number of patients with an improved (from baseline) EDSS score in favor of Antegren: thus, 31% of patients who received Antegren had an improved EDSS score of 0.5 or better compared to 10% of placebo patients ($p = 0.036$; 95% CI 2.1, 39.0), whereas 28% in the Antegren group and 37% in the placebo group had worsened by 0.5 or more. At week 24, however, there was no difference between the groups.

Discussion. In EAE and MS, the inflammatory cascade has become a prime target for potential new treatments.[13] Transmigration of T cells across the blood–brain barrier (BBB) into the CNS is a multistep process occurring in an ordered sequential fashion initially. In many tissues, leukocytes in blood weakly attach to endothelium via selectins, a family of glycoprotein adhesion molecules. Selectin-mediated adhesion is weak, and allows leukocytes to roll along the vascular endothelial surface. The ini-

tial transient unstable interaction between the T cell and the endothelium is subsequently strengthened by a process of activation and adhesion via the β2 integrins (e.g., leukocyte function-associated antigen–1) and their counterparts, such as the Ig intercellular adhesion molecules (ICAM-1 and ICAM-2). Further binding occurs between α4β1 integrin, which is present on T lymphocytes and monocytes, and its receptor/ligand VCAM-1, present on the vascular endothelium. This binding arrests the rolling process of the T cell and allows the leukocyte to attach firmly to the endothelium. The T cells finally flatten and migrate across the endothelium (diapedesis) in response to chemotactic signals released from the site of inflammation.

mAbs against α4 integrin were examined for inhibitory activity in EAE (induced in Lewis rats[3] and guinea pigs[4]), and were shown to be effective in reducing the clinical and MRI activity in both active and passive transfer EAE models. Early BBB breakdown is associated with leukocyte trafficking and is one of the earliest events in the formation of new lesions in MS, and may well be important in the pathogenesis of demyelination.[14] It was logical, therefore, to determine whether anti-α4 integrin antibodies might inhibit the process of BBB leakage in MS.

Our study is the first to use a humanized mAb to anti-α4 integrin in patients with MS. The results show a significant difference in the number of new active and new enhancing lesions between treatment and placebo groups in the first 12 weeks after commencement of treatment. This suggests that new lesion formation has been suppressed, probably by preventing migration of T lymphocytes and monocytes across the BBB.

There was, however, no shortening of the duration of enhancement of baseline-enhancing lesions, suggesting no apparent effect of therapy on inflammatory lesions that were already established. A possible reason for this is the presence of coexistent processes in established inflammatory lesions that keep the BBB open, such as the continued secretion of cytokines by leukocytes that have already crossed the BBB, or alternative pathways for leukocyte recruitment. The observation that a reduction in the frequency of new enhancing lesions was not apparent during the first 2 weeks following the first treatment (see figure 2) might suggest that an earlier accumulation of lymphocytes and monocytes may have already become established before the appearance of focal enhancing MRI lesions.

Although there were no significant differences in the numbers of patients with acute exacerbations of MS in the first 12 weeks and in the study period overall, it is important to note a significant increase in relapses in the treatment group compared to placebo during the second 12 weeks. However, the study was not powered to examine the effect of treatment on relapses. The number of patients experiencing relapses was relatively small and actually decreased in the placebo group in the second 12 weeks of follow-up, which may have contributed to the significant difference between the groups. Patients enrolling in this study were required to have had at least two exacerbations in the preceding 18 months, so this group would be expected to average approximately one exacerbation per 9 months (i.e., 33% of patients should have had one exacerbation in the study period). This rate is similar to that seen in the Antegren and placebo groups in the first 12 weeks and the Antegren group in the second 12 weeks. Also, we did not observe a rebound of MRI activity in the treatment group. Nevertheless, our findings raise the possibility that there may be a rebound increase in the relapse rate after stopping treatment. One possible explanation for this might be an increase in the expression of VCAM-1 in response to the α4 integrin block. This needs to be investigated along with close monitoring of relapse rate during and after treatment in any further studies involving α4 integrin blockade.

In a short-term study such as this, it is unrealistic to expect a significant change in disability or disease progression, and, indeed, although there was a statistically significant difference in EDSS scores between groups at week 12, this was not the case at week 24. Furthermore, the relatively modest correlation between disability and changes seen on MRI[15] means that any potential new treatment must ultimately be tested in a larger, longer term trial.

Limitations to the current results should be noted. First, the magnitude of the effect of anti-α4 integrin antibody on MRI activity has not been determined. Although the number of new active lesions over 12 weeks was significantly less in the treatment group, the 95% CI (−0.1, −3.6) suggests that there could be as little as a 3%, or as much as a 100%, reduction in new active lesions compared to placebo during that period. Secondly, it is not known how many times Antegren can be given. The preliminary efficacy of Antegren (3 mg/kg) in the treatment of MS has been shown in the current study but the effect was modest and transient. Pharmacokinetic analysis in this study has demonstrated that only a proportion of patients had serum concentrations of Antegren considered likely to be effective 4 weeks after each infusion. To obtain adequate serum concentrations of Antegren between monthly infusions, and to maintain suppression of MRI activity, a higher dose of Antegren administered chronically will need to be evaluated in future studies. It is possible, however, that repeated dosing with a mAb could lead to anti-idiotype antibodies and, if of sufficient magnitude, a loss of efficacy.

Further studies are needed to determine more accurately the magnitude and duration of the effect of Antegren on MRI. These could use the design recently suggested by a US MS Society Task Force, i.e., to show a >50% reduction in new MRI activity over 6 months of monthly MRI, in a parallel groups, placebo-controlled design.[7]

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Optic neuritis as onset manifestation of multiple sclerosis

A nationwide, long-term survey

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Article abstract—*Objective:* To determine the predictive value on survival of optic neuritis (ON) as onset manifestation of MS. *Methods:* We used data obtained from the Danish Multiple Sclerosis Registry, which includes virtually all patients diagnosed with MS in Denmark. From 1949 to 1990, 7,548 unselected patients fulfilling standardized diagnostic criteria of MS were registered. *Results:* The onset manifestation of MS was known in 6,923 patients, and was ON in 1,282 patients (19%). The mean age at onset was 31.1 years for these patients compared with 34.8 years for patients with another or unknown onset manifestation of MS (non-ON) ($p < 0.001$). The mean delay from the first known manifestation of MS to the final diagnosis of MS was 6.1 years (ON) and 4.2 years (non-ON). The median survival time from onset of ON was 30 years in men (compared with 41 years in the matched general male population) and 40 years in women (versus 47 years). The excess death rate increased with age at onset of MS in people of each sex. Excess death rate for women differed significantly between patients with ON as onset manifestation and patients with another or unknown onset manifestation of MS (8.3 versus 13.0). In patients with ON as onset manifestation of MS, the excess death rate was significantly higher in men (14.0) than in women (8.3). *Conclusion:* ON as onset manifestation of MS indicates a more favorable prognosis of survival of MS judged by excess death rate only in women.

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Monosymptomatic optic neuritis (ON), either papillitis or retrobulbar neuritis, may remain isolated, but is often the onset manifestation of MS.[1] Several attempts have been made to clarify the prognostic value of onset manifestations of MS and their relation to the course of the disease. Most studies have suggested that ON and sensory manifestations as onset manifestations of MS, as well as early age at onset, are favorable prognostic factors. On the contrary, motor manifestations are indicative of a more malignant course of MS.[2-8,14] Other reports have argued that patients with ON as onset manifestation only had a more favorable course of MS initially.[9-11] The study of Hartard et al.[12] did not find an association of ON with prognosis, and according to Kurtzke et al., no onset manifestation of MS has any prognostic value in relation to disability.[13]

In the current article, we aimed to clarify whether ON as the onset manifestation of MS is a favorable prognostic manifestation regarding survival compared with other (non-ON) onset manifestations, using the Danish Multiple Sclerosis Registry (DMSR) comprising long-term follow up data from more than 7,000 unselected patients with MS. In Denmark (current population 5.2 million), the prevalence of MS is relatively high, with a crude annual incidence rate of 4.7 per 100,000 population.[15] In addition, the population is homogenous and stable, and a thorough registration system contains vital status for all Danish citizens using a unique personal code (10 digits). This makes Denmark an ideal country for the study of chronic diseases. We also analyzed sex distribution, age at onset, and delay to diagnosis of MS. The study was performed according to the guidelines of the Declaration of Helsinki and was approved by the local Ethics Committee.

Materials and methods. *Description of the DMSR.* The DMSR was established in 1956, after the systematic registration of all Danish patients with MS since 1948 by Hyllested. The purpose of the DMSR was to describe prevalence and incidence rates, mortality, and development of MS over time, as well as whether these were related to sex and age of patients; and to study geographic aspects. The DMSR contains information on calendar year of onset and on onset manifestations (both assessed retrospectively on the basis of case reports and clinical information). Information on family relations and disability status are not available in all cases, and these data were not considered valid for the purpose of this study. Reports of MS cases come from all 22 Danish neurologic departments, private practitioners in neurology, general practitioners, the MS rehabilitation hospitals in Ry and Haslev, The Danish Multiple Sclerosis Society, neuropathologic departments, The National Patient Registry (which contains information on all hospital admissions since 1977), and the National Registry of Causes of Death (in which all direct and underlying causes of death according to death certificate are

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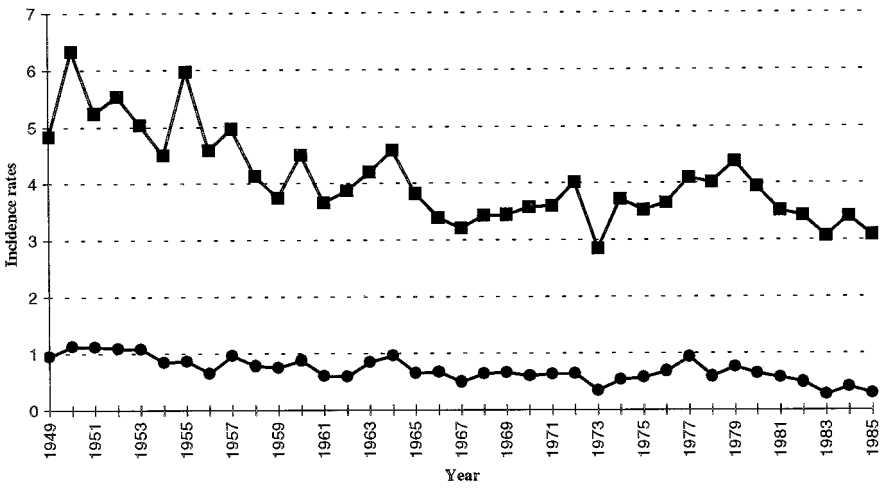

Figure 1. Optic neuritis as onset manifestation: incidence rates. The figure depicts the annual incidence rate per 100,000 of optic neuritis as onset manifestation of MS (●) and MS (■) in Denmark for both genders combined within the period 1949 to 1985.

registered since 1943) to the Danish Institute for Clinical Epidemiology. Based on the incoming reports, a neurologist at the DMSR codes the patients according to an existing profile. The DMSR contains a computer registry with standard information and medical files on every patient. The neurologists Koch-Henriksen and Hyllested have evaluated the DMSR to a completeness of 91.2% (95% confidence interval [CI] 89% to 93%) and a validity of 90%.[15]

Inclusion criteria. The patients were reclassified into the following categories according to diagnostic criteria including laboratory and clinical data[16]: clinically definite MS, clinically probable MS, latent possible MS, possible MS, autopsy verified MS, isolated ON, and observation cases that could represent MS. The two last-mentioned categories were excluded from the current study, which included patients being notified to the DMSR before January 1, 1991, and having initial manifestations within the period January 1, 1949, to December 31, 1990 (time of follow-up). However, some categories of onset manifestations used in the database up to 1964 could not be recorded according to the improved classification used hereafter. Therefore, 625 patients were excluded from analysis.

Statistical analyses. Because the DMSR can be linked to the Central Population Registry and The National Registry of Causes of Death, is it possible to track patients from onset of MS until death, emigration, or end of follow-up (December 31, 1990). Survival analysis was based on this information, by which number of deaths and person-years at risk by sex, age, and calendar year were calculated. The expected number of deaths was estimated using death rates from public vital statistics applied to the Danish population matching the MS population as to sex, age, and calendar year. Finally, the excess death rates (EDR), defined as observed minus expected number of deaths per 1,000 person-years, were calculated. Exact 95% CI of EDR were calculated assuming deaths to follow the Poisson distribution.[17]

Results. *Incidence of ON as onset manifestation of MS.* The number of patients with MS, registered in the DMSR from 1949 to 1990, was 7,548 (3,156 men and 4,392 women). ON was the onset manifestation of MS in 1,282 patients (509 men and 773 women); thus, in 17% (16% men and 18% women) of all MS cases. When including only the 6,923 patients with a known onset manifestation of MS, ON comprised 19% (18% men and 19% women). The incidence rate (per 100,000) of ON as onset manifestation of MS and of MS in both genders is depicted for each calendar-year (1949–1985) in figure 1. It tended to decrease in the last part of the period for both groups and the changes seem to occur simultaneously. It waxed and waned, in parallel for men and women (data not shown). The same pattern was observed in patients with another onset manifestation of MS (data not shown).

The delay of diagnosis of MS. The mean delay from the first manifestation of ON to the final diagnosis of MS was 6.1 years (men 5.8 years and women 6.3 years) with 10%, 50%, and 90% percentiles of 0 years, 4 years, and 15 years, respectively. With another known onset manifestation of MS, the mean delay was 4.2 years (men 4.1 years and women 4.3 years) with 10%, 50%, and 90% percentiles of 0 years, 2 years, and 11 years, respectively.

Age at onset. The age at onset is shown in table 1 for patients with ON as onset manifestation of MS and for patients with another or unknown onset manifestation of MS. The median age at onset was 30 years in the former group compared with 34 years in the latter group ($p <$

Table 1 *Age at onset of MS, 1949–1990*

MS group	n	Mean (SD)	Median
ON			
Men	509	31.2 (8.6)	30
Women	773	31.1 (9.0)	30
All	1,282	31.1 (8.8)	30
Non-ON			
Men	2,647	35.2 (9.9)	35
Women	3,619	34.5 (10.3)	34
All	6,266	34.8 (10.2)	34
All			
Men	3,156	34.6 (9.8)	34
Women	4,392	33.9 (10.2)	33
All	7,548	34.2 (10.0)	33

Mean (SD) and median age at onset of patients with optic neuritis (ON) and patients with another or unknown onset manifestation (non-ON) of MS are shown.

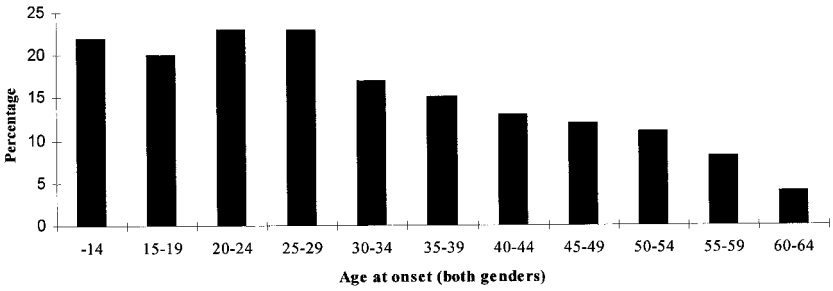

Figure 2. Percentage of patients with optic neuritis as onset manifestation of MS in various age groups within the period from 1949 to 1990.

0.001). The percentage of patients with ON as onset manifestation of MS was above 20% in patients age 30 years or younger, thereafter steadily declining (figure 2).

Survival. EDR (with 95% CI) are shown in table 2 for patients with ON and patients with another or unknown onset manifestation of MS. The data are listed according to age and sex. Generally, the EDR increased (clearest for women) with increasing age at onset. When merging all age and sex groups, the EDR was significantly lower in patients with ON as onset manifestation of MS (EDR 10.4) than in patients with another or unknown onset manifestation of MS (EDR 13.6). Regarding each sex isolated, this finding was only true for women (table 2). In patients with ON as onset manifestation of MS, the EDR was significantly higher in men (EDR 14.0) than in women (EDR 8.3). In patients with another or unknown onset manifestation of MS, the EDR was significantly higher in men than in women only in the subgroup of patients age 20 to 29 years.

As expected because of younger age at onset, both male and female patients with ON as onset manifestation had a higher survival probability than other patients with MS. The median survival time from onset of ON was 30 years in men (compared with 41 years in the matched general male population), and 40 years in women (versus 47 years). In patients with another or unknown onset manifestation of MS the median survival time from onset was 28 years in men (versus 37 years), and 33 years in women (versus 43 years).

Discussion. Several problems should be addressed when studying the epidemiology of MS: sample size, methods of ascertainment, length of follow-up, delay to diagnosis/reporting, geographic clustering, different types of disease course, and loss of benign cases. These factors can explain the discrepancy in results from previous studies, which were either hampered by not being population-based, by comprising fewer patients, or by a short length of follow-up.[2-14] The DMSR provides an excellent source of information, with complete follow-up of virtually all patients with MS since 1948, resulting in the largest unselected cohort prospectively studied so far.

Incidence of ON as onset manifestation of MS. Previous studies indicated that 14% to 45% of the patients with MS had ON as onset manifestation.[6,18-20] We found that ON was the onset manifestation in 19% (18% men and 19% women) of patients with known onset manifestations of MS, with varied frequency according to age, being highest in the younger age groups. Our study seems to show a general decline of ON incidence rates over time. Other reports using the DMSR have concluded that there has been a decline of incidence rates of MS, especially from 1948–1964, with an increase in prevalence rates.[15] A subsequent report based on the data from 1948–1982 confirmed the decline, but a slight increase of incidence rates was observed from 1967, albeit in an irregular pattern.[16] We also see a slight increase from 1973 of cases with ON as onset manifestation of MS, but the downward trend is striking. A previous report from Sweden reported a decline of MS incidence rates from 1974–1988 compared with 1950–1960.[21] The reason for this phenomenon remains unclear. Factors such as better hygiene, better health care systems, and vaccination programs could be a partial explanation, but there are no sure objective indications supporting these theories.

Delay to diagnosis of MS. The mean delay from onset to final diagnosis of MS was 6.1 years for patients with ON as onset manifestation, which is a slightly longer delay when compared with previous reports.[11,22-28] This could be explained in part by the fact that loss of vision is an alarming symptom that prompts patients to seek immediate medical attention, resulting in earlier recording, in contrast to patients with nonspecific or mild sensory symptoms. In addition, patients with ON who are involved in prospective studies evaluating development of MS probably report manifestations compatible with MS earlier. The starting point of the survival analyses is the time of onset, but the DMSR ascertains patients after diagnosis, which may be separated from the onset by several years. The source of bias due to delayed diagnosis was evaluated to be minor in patients with MS.[6]

Age at onset. The age at onset was a median 4 years, mean 3.7 years lower in patients with ON as onset manifestation than in patients with another or unknown onset manifestation. This finding confirms previous reports.[3,4,7,9,11,29] This should be considered when the survival probability is compared for different onset manifestations of MS (see below).

Survival. The largest prospective population-based survey of the survival of patients with MS was based on the DMSR.[6] That article contains a table of the results from previous reports of the survival of patients with MS and its relation to age at onset and sex, and women had a more favorable course of MS than men, judged by a difference in EDR. The crude survival estimates based on the DMSR were in the

Table 2 *Excess death rates (per 1,000 per year) from onset of MS, 1949–1990*

Age at onset, y	Sex	No. observed deaths	No. expected deaths	Person-years	Excess death rate	95% Confidence interval
<20						
ON	Men	9	1.7	841	8.7	2.9–18.3
	Women	7	1.5	1,385	4.0	0.9–9.3
	All	16	3.2	2,226	5.8	2.7–10.2
Non-ON	Men	34	4.9	2,721	10.7	6.9–15.7
	Women	44	4.7	4,669	8.4	5.8–11.6
	All	78	9.6	7,390	9.3	7.0–11.9
20–29						
ON	Men	68	13.8	4,301	12.6	9.1–16.8
	Women	67	16.9	7,161	7.0	4.9–9.5
	All	135	30.7	11,462	9.1	7.2–11.3
Non-ON	Men	227	45.5	13,553	13.4	11.3–15.7
	Women	235	46.2	20,957	9.0	7.6–10.5
	All	462	91.7	34,510	10.7	9.5–12.0
30–39						
ON	Men	73	22.8	3,399	14.8	10.1–20.3
	Women	67	22.8	5,138	8.6	5.7–12.1
	All	140	45.6	8,537	11.1	8.5–14.0
Non-ON	Men	356	113.4	17,203	14.1	12.0–16.4
	Women	402	97.3	22,027	13.8	12.1–15.7
	All	758	210.7	39,230	14.0	12.6–15.4
40–49						
ON	Men	36	13.0	1,172	19.6	10.4–31.4
	Women	48	20.3	2,299	12.0	6.6–18.9
	All	84	33.3	3,471	14.6	9.7–20.4
Non-ON	Men	354	160.3	11,785	16.4	13.4–19.7
	Women	351	112.7	14,039	17.0	14.4–19.7
	All	705	273.0	25,824	16.7	14.8–18.8
≥50						
ON	Men	8	3.6	206	21.4	−0.7–59.0
	Women	11	4.0	296	23.6	5.0–53.0
	All	19	7.6	502	22.7	7.6–44.0
Non-ON	Men	123	73.7	2,806	17.6	10.2–26.0
	Women	142	56.5	4,157	20.6	15.2–26.7
	All	265	130.2	6,963	19.4	14.9–24.2
All ages						
ON	Men	194	54.9	9,919	14.0	11.4–17.0
	Women	200	65.5	16,278	8.3	6.6–10.1
	All	394	120.4	26,197	10.4	9.0–12.0
Non-ON	Men	1,094	397.8	48,068	14.5	13.2–15.9
	Women	1,174	317.4	65,851	13.0	12.0–14.1
	All	2,268	715.2	113,919	13.6	12.8–14.5

Within each age group, the excess death rates for patients with optic neuritis (ON) as onset manifestation and patients with another or unknown onset manifestation (non-ON) of MS for men and women are shown.

same magnitude as in a number of these previous reports. In that report, women tended to have a better prognosis of survival following ON as the onset manifestation of MS, when analyzed by a proportional hazards regression model that included age at onset, initial manifestations, and period of onset.[6] Extension of this model indicated that the effects of onset manifestation and age at onset did not interact statistically significantly (men, $p = 0.37$; women, $p = 0.49$). This analysis was omitted in the current study. Our survey, based on an updated version of the DMSR, has subdivided the material according to onset manifestation of MS. The EDR was significantly lower in patients with ON as onset manifestation than in other patients with MS. In the subgroup of patients with ON as onset manifestation of MS, women had a significantly lower EDR than men. A similar trend was not evident in the subgroup of patients with another or unknown onset manifestation of MS. Why male patients with ON have a worse prognosis for survival than women when they present with ON remains unclear, and no obvious explanation is provided by our data. One can only speculate that hormonal factors could play a role in limiting the immunologic response in women, supported by the apparent decline of relapse rate of MS during pregnancy.[30-31] Interestingly, there are trends indicating that men may also have a worse prognosis than women in related immunologic conditions, such as rheumatoid arthritis and systemic lupus erythematosus.[32-33]

Previous surveys of MS found a better prognosis for young age at onset.[6,9,14,34-37] Poser et al. observed that primarily the clinical course influenced prognosis.[14] Although our material was large, the validity concerning disability data of the DMSR was considered too low to evaluate the influence of clinical course on survival, as this information was not obtained from all patients. A prospective study with regular scoring on a disability scale may better predict the prognosis for disability of MS.

In this large prospective unselected study, ON was the presenting manifestation in 19% of MS cases. As judged by EDR, ON as initial manifestation of MS indicates a shorter life expectancy compared to the general population but a better prognosis of survival of MS for women compared with patients with other initial manifestations of MS. This could in part be attributed to the lower age at onset of patients with ON.

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Multiple sclerosis in children under 6 years of age

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Article abstract—*Objectives:* To characterize MS patients with the earliest onset of disease. *Background*: MS—primarily a disease of young adulthood—begins in childhood in 3 to 5% of cases. However, onset before 10 years of age is considered exceptional. Accordingly, inclusion age at onset is generally between 10 and 59 years. *Methods*: Information was obtained on patients with MS treated at our institution (n = 6) or from reports in Medline or bibliographies. Onset of disease was before 6 years of age, for a total of 49 patients (29 girls, 20 boys). *Results*: All patients had clinically defined MS according to Poser's criteria; 22 were also laboratory supported. The female/male ratio (1.4) was lower than that usually recorded for adult onset MS (2.0) and that of MS with onset between 6 and 15 years (2.2 to 3.0). The group of patients (n = 5) with onset before 24 months of age showed the lowest ratio (0.6) and carried the most unfavorable prognosis. Among initial symptoms, ataxia was preponderant (61%). Optic nerve involvement became more frequent with age. Generalized or partial seizures occurred in 22% of cases. First inter-attack interval was less than 1 year in 63% of the cases. The yearly relapse rate ranged from 1.1 at disease onset to 0.2 after 9 years from disease onset. At follow-up (mean length 6.8 years), the disease was relapsing-remitting in 84% patients and the grade of recovery was complete in 64%. *Conclusions*: Definite MS can be consistently diagnosed by current criteria for adult onset MS in patients with the earliest onset of disease who show peculiar clinical features and natural history. These findings may suggest a reconsideration of current lower limits for MS diagnostic criteria.

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MS, an autoimmune inflammatory disease of the CNS, is primarily a disease of young adulthood with a clinical onset usually occurring between 20 and 40 years of age.[1] However, in 3 to 5% of cases, MS begins before age 15.[2] An onset before 10 years of age is considered exceptional,[3,4] and the reported figures range from 0.2[1,2] to 0.7%[1,5] of total scores. Accordingly, inclusion age at onset (such as for research purposes) is generally between 10 and 59 years.[6] Although a broad spectrum of congenital and acquired diseases may present in childhood with similar symptoms and is often confused with MS,[7,8] current radiologic and immunologic techniques make it possible to diagnose MS at a very early age.[2-4,8-13] Nonetheless, many pediatricians and neurologists are unaware that this disease may occur and manifest itself even during infancy and rarely consider this diagnosis in children at this young age.[14,15]

Although a consistent amount of information has recently been accumulated on MS with onset be-

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tween 6 and 15 years of age,[2-5,8-13,16-20] no attempt has been made to classify MS with onset before 6 years of age.[14,15,21-31] We selected the age of 6 years to be the divider for our research and meta-analysis on the basis of school entrance, considered as the watershed between the initial relatively limited neuromotor, cognitive, and linguistic development in infancy and toddler years and the more sophisticated range of competencies (including unfamiliar immunologic challenges) attained in middle childhood and adolescence.[32] To characterize the group of MS patients with the earliest onset, we investigated the clinical, paraclinical, and neuroimaging features and outcomes of children with MS whose onset was at preschool age.

Methods. *Patients.* According to Poser's criteria,[6] MS was diagnosed in six patients (three boys and three girls) seen at the Department of Pediatrics of the University of Catania (DPUC) whose first symptoms occurred before 6 years of age,[8] with the exception of the first general consideration listed in Poser's criteria (i.e., age at onset between 10 and 59 years inclusive).[6] Cranial CT (HT 8000, Philips machine) and/or MRI (0.5 and 1.0 Tesla; Philips Gyroscan), electrophysiologic studies (visual evoked potentials [VEP], brainstem auditory evoked responses [BAER], and somatosensory evoked potentials [SSEP]), and CSF analysis were performed in all patients. Neurologic disability at follow-up was scored by the Expanded Disability Status Scale[33] (table 1). At present, one patient is still being monitored by a pediatric neurologist; four have moved on to adult neurologists; and one is lost to follow-up.

Literature review. Published reports on MS diagnosed in patients whose first symptoms were before 6 years of age were identified by a Medline search covering the period January 1966 to August 1998. The search reviewed existing articles related to this topic and examined the reference lists of studies identified. We considered only those publications describing at least sex, age at onset, symptoms at onset and relapses, and outcome. Thirty-six articles or chapters of books met our inclusion criteria,[2-5,9-15,21-31,34-46] including a case previously published by ourselves[15] and not included in table 1. Reported cases with insufficient information were excluded.[2,3,5,10,12,34]

Data from the six patients who received follow-up at the DPUC and those from the 43 patients selected from the literature review were grouped and analyzed with the aid of the SAS program.

Results. All of the 49 patients whose data were analyzed[3,4,9,11,13-15,19,21-31,34-46] had clinically definite MS; in 22 the diagnosis was also laboratory supported according to Poser's criteria,[6] which allowed the validation of all reported cases. The main clinical, laboratory, and neuroimaging features are summarized in tables 2 and 3. Other relevant features are discussed below.

Age at onset. The first episode of MS occurred between 10 and 23 months of age (n = 5) or at ages 2 (n = 15), 3 (n = 10), 4 (n = 12), and 5 (n = 7).

Family history, developmental milestones before onset, and associated diseases (number of cases where information was available = 33). Three patients (9.6%) had a first degree relative with MS (one mother, one aunt, and one grandmother). None had remarkable associated diseases.

Initial diagnosis other than MS (number of cases where information was available = 28). Initial diagnosis was acute disseminated encephalomyelitis (ADEM) (n = 10), isolated optic neuritis (n = 9), cerebellar ataxia (n = 7), post-traumatic event (n = 1), and cerebral neoplasm (n = 1).

Fever or illness preceding the episodes (number of cases where information was available = 39). Patients experienced 219 separate episodes of MS. Fever or upper respiratory infections (7.3%) were noted in 16 patients preceding one of their attacks. Two other patients experienced an accidental fall during daytime activity earlier in the day of their first episode.

Symptoms of second episode same as first (number of cases where information was available = 41). Twenty-seven patients (66%) (17 women, 10 men) had the same symptoms during their second bout.

Neurophysiology. EEG (number of cases where information was available = 23). In addition to patients showing an excess of slow waves diffusely (n = 5), in the posterior (n = 4) or frontal (n = 1) areas, with focal spikes (n = 4), the EEG yielded normal results (n = 9).

VEPs (number of cases where information was available = 30). Patients had increased (n = 19), decreased (n = 3), or normal (n = 8) latencies in the affected eye.

BAER (number of cases where information was available = 18). Patients had increased (n = 16) or normal (n = 2) latencies.

Children with ataxia. Of the 30 children (11 boys, 19 girls) who had ataxia at onset, the clinical course was relapsing-remitting (RR) (n = 26), secondary-progressive (n = 3), or primary-progressive (n = 1). Neuroimaging showed cerebellar or brainstem lesions (n = 6), periventricular lesions (n = 10), yielded normal results (n = 5), or was not available (n = 9). MRI of the spine was never obtained during the first episode.

Children with seizures. Of the 11 patients (8 boys, 3 girls) who had seizures[9,14,21,23,24,26,36,39] (table 1), 3 (2 boys, 1 girl) (27.3%) died during the course of the disease,[14,21,24] compared with 1 out of 38 patients (2.6%) who never had seizures. Eight had an RR course of disease. Mean age at onset of seizures was 6.3 years (range 2.6 to 16.2 years). Patients experienced either a single ictal episode (n = 3) or recurrent attacks (n = 8), all occurring in clinical relapses that responded well to conventional anticonvulsants. Seizures were generalized tonic-clonic (GTC) (n = 5), partial simple (PS) (n = 2), PS secondarily generalized (n = 1), PS and GTC (n = 1), or unspecified type (n = 2). The EEG showed focal spikes (n = 4) or diffuse slow waves (n = 4), or data were not given (n = 3). Patients had typical MS lesions at CT or MRI (n = 6), or neuroimaging studies were conducted but results were not provided (n = 4). MRI was not performed in one additional patient.

Treatment (number of cases where information was available = 29). Of the 19 patients treated with high doses of oral prednisone (n = 8), IV methylprednisolone (n = 1), prednisone and methylprednisolone (not in combination) (n = 5), steroids and azathioprine (n = 1), or unspecified steroids (n = 4), a dramatic recovery of symptoms (hours to days from initial dose) was recorded in 11, equivocal bene-

Table 1 *Clinical, laboratory and neuroimaging findings, and outcome in six children with MS*

	Patients					
Characteristics	1	2	3	4	5	6
Sex	M	F	M	F	M	F
Current age, y	20	23	23	11	16	14
Age at presentation, y	5	3.8	3.6	4.7	4	5.1
Age at diagnosis, y	7	6.5	9	6.9	6	9
Initial diagnosis	Cerebellar ataxia	ADEM	ADEM	Cerebellar ataxia	NR	NR
Clinical features and course						
First episode	Fever, ataxia, headache, ON	Fever, ON diplopia, weakness	Ataxia, paresthesia, vertigo, headache	Hypoesthesia, ataxia, poor coordination	Fever, ataxia, headache	Ataxic gait, diplopia
Therapy	Prednisone	Prednisone	None	None	None	None
Duration of bout, wk	3	2	2	4	4	1
Second episode	Headache, ON	Ataxia, ON	Vertigo, tinnitus	Dysesthesia, chorea	Ataxia, ON	Hemiparesis
Time from previous attack	12 mo	12 mo	2 y	11 mo	2 y	4 y
Therapy/duration of bout, wk	Methylpred/3	Methylpred/3	None/5	Prednisone/3	Methylpred/4	Methylpred/3
Third episode	Ataxia, dizziness	Ataxia, vertigo GTCS	Squint, weakness	ON, squint	Headache, ON	Ataxia, diplopia
Time from previous attack, mo	10	12	12	9	6	6
Therapy/duration of bout, wk	Methylpred/4	Prednisone/5	Methylpred/2	Prednisone/6	Methylpred/6	Prednisone/4
Fourth episode	Diplopia, hemiplegia	Paraplegia, hypotonia	Paraplegia, GTCS	Monoplegia, palsy	Headache, ON	Palsy, coma
Time from previous attack	3 y	6 mo	3 mo	2 y	8 mo	4 mo
Therapy/duration of bout, wk	Prednisone/2	Prednisone/5	Methylpred/6	None/3	Prednisone/6	Methylpred/4
Fifth episode	Ataxia, ON, diplopia	—	Gait disturbances	—	—	Ataxia, ON
Time from previous attack, mo	10	NA	10	NA	NA	5
Therapy/duration of bout, wk	Methylpred/4	NA	Prednisone/4	NA	NA	None/6
Sixth episode	Ataxia, ON	—	Vertigo, ON	—	—	—
Time from previous attack	10 mo	NA	3 y	NA	NA	NA
Therapy/duration of bout, wk	Methylpred/7	NA	Prednisone/6	NA	NA	NA
Laboratory and neuroimaging findings						
CSF findings (episode)	High IgG; OB (3)	OB (3)	OB, pleiocytosis (1)	OB, pleiocytosis (1)	High protein; OB (2)	OB (2)
Neurophysiology (altered)	VEP, BAER	VEP	VEP	VEP	VEP	VEP
CT-MRI	MRI = PV, ME, BS	MRI = PV	CT, MRI = PV, BS	MRI = ME, BS	MRI = PV ME	MRI = PV, BS
Current state	Poor coordination, gait disturbances EDSS = 3.5	EDSS = 0	Vertigo, poor vision EDSS = 5.0	EDSS = 0	EDSS = 0	Pyramidal EDSS = 3.0

All patients had a negative family and personal history of MS.

— = absent; ADEM = acute demyelinating encephalopathy; NR = not recorded; NA = not available; GTCS = generalized tonic clonic seizures; c.n. = cranial nerve; ON = optic neuritis; Methylpred = methylprednisolone; OB = oligoclonal bands; (1) = at first attack; (2) = at second attack; (3) = at third attack; nl = normal; IgG = immunoglobulin G; VEP = visual evoked potentials; BAER = brainstem auditory evoked potentials; PV = periventricular; BS = brainstem; ME = mesencephalic; EDSS = Expanded Disability Status Scale (ref 33).

fits in 2, and no effect in 3 patients (including the patient treated with steroids and immunosoppressor); no clear information on steroids efficacy was given in 3 of the 19 patients. Clinical improvement was seen in 2 out of the 3 patients treated with corticotrophin. A spontaneous and rapid recovery was recorded in 7 patients (for a total of 25 episodes) in the absence of any specific treatment. Spontaneous clinical improvement was also recorded during a total of 16 episodes in 15 out of the 19 patients who were previously treated with steroids.

Table 2 *Clinical features, natural history, and outcome in 49 children with MS*

Features	Values
Total no.	49
Sex, F/M (ratio)	29/20 (1.4:1)
Mean age at presentation, y	3.2 (3.2 F, 3.0 M) (range, 10 mo–5.3 y)
Mean age at diagnosis, y (n = 19*)	6.6 (range, 2.1–16)
Symptoms at onset (n = 49*), n (%)	
Trunk and limb ataxia	30 (61); 2 isolated; 28 associated
Optic neuritis	14 (28); 2 isolated; 12 associated
Pyramidal	9 (18)
Muscular weakness	6 (12)
Headache, "impaired gait"	5 (10)
Poor gross coordination; psychiatric	4 (8)
Lethargy-coma; vomiting	3 (6)
Pure sensory deficit, meningeal, diplopia, pain, irritability, paraplegia, cranial nerve palsy, seizure, hypotonia, involuntary movements, nystagmus	2 (4)
Transverse myelitis, vertigo, hemiparesis, hemiplegia, paraparesis, hearing loss, torticollis, dystonia, squint, feeding difficulties, opsoclonus, intellectual deterioration, poor fine coordination, ptosis, ophthalmoplegia, dizziness	1 (2)
Mean time of recovery from 1st attack (weeks) (n = 31*)	4 (3.5 M; 4.3 F) (range, 2 days–5 mo)
Grade of recovery (from 1st attack) (n = 42*), n (%)	
Complete	32 (76)
Subtle neurologic signs	5 (12)
Deterioration	5 (12)
First inter-attack interval (mean, mo) (n = 46*)	11 (range, 1 mo–5 y M; 1 mo–3 y F)
Mean number of attacks (n = 49*)	4 (3.9 M, 5 F) (range, 1–10 M; 2–12 F)
Mean inter-attack interval (after onset), mo (n = 35*)	11.6 (10.4 M; 12.3 F) (range, 1 mo–6 y)
Attack rate per person-year (years after onset)	
1 (40*)	1.1
2 (26*)	0.8
3 (26*)	0.8
4 (15*)	0.6
5 (13*)	0.6
6 (8*)	0.5
7 (7*)	0.3
8 (4*)	0.2
9 (3*)	0.2
Symptoms (in all episodes after 1st attack) (n = 43*), n (%)	
Ataxia or ataxic gait	25 (58); 11 new symptom; 14 had it at onset
Optic neuritis	21 (49); 17 new symptom; 4 had it at onset
Pyramidal	14 (32)
Weakness	11 (25)
Cranial nerve palsy; seizures	10 (23)
"Impaired gait"	9 (21)
Lethargy-coma, diplopia, nystagmus	8 (19)
Feeding difficulties, hyperreflexia, sphincter disturbances, headache, hemiplegia	7 (16)
Pure sensory disturbances	6 (12)
Torticollis, language disturbances, vomiting, hypotonia, squint	5 (10)
Language impairment, torticollis, paraplegia	4 (9)
Tremor, paraparesis	3 (7)
Delayed development, irritability, meningeal, intellectual deterioration, poor coordination, ophthalmoplegia	2 (5)
Tetraplegia, monoplegia, behavioral, chorea, dizziness, vertigo, involuntary movements, pain, dysarthria, aphasia, opsoclonus, ptosis, dysmetria	1 (2)
Mean time of recovery for all episodes (wk) (n = 39*)	4.3 (3.8 M; 4.6 F) (range, 2 days–6 mo)
Grade of recovery (at follow-up) (n = 42*), n (%)	
Complete	27 (64)
Subtle neurologic signs	8 (19)
Deterioration	3 (7)
Death	4 (10)
Disease category (n = 49*)	
Relapsing-remitting	41 (84%) (18 M; 23 F)
Primary-progressive	4 (8%) (2 M; 2 F) (including 2 malignant MS)
Secondary-progressive	4 (8%) (1 M; 3 F)
Mean length of follow-up, y (n = 46*)	6.8 (range, 0.2 [deceased]–22)

* No. of cases where information was available.

Isolated = presented as isolated symptom; associated = associated with other symptoms.

Table 3 *Laboratory findings and neuroimaging data in 49 children with MS*

Laboratory test	1st attack†	2nd attack†	3rd attack†	4th attack†	5th attack†
CSF (n = 42*)	39	20 (17)‡	12 (12)‡	NA	NA
Normal	11	8	2	NA	NA
Pleiocytosis (mean no. of cells)	13 (103/mmc)	6 (39/mmc)	2 (56/mmc)	NA	NA
Protein levels (mean)	12 (46 mg/dL)	2 (45 mg/dL)	1 (40 mg/dL)	NA	NA
OB	3	4 (3)§	7 (7)§	NA	NA
Brain CT (n = 25*)	22	15	8	5	2
Normal	13	7 (3)§	4 (2)§	—	1 (1)§
Nonspecific anomalies	—	1‖	2‖	—	—
Periventricular	5	2 (1)§	1	3 (1)§	—
Subcortical	4	5 (1)§	1	2	1 (1)§
Brain MRI (n = 26*)	26 (13)#	10	9	2	1
Normal	2 (1)§	—	—	—	—
Nonspecific anomalies	1 (1)§	—	—	—	—
Multiple lesions	9 (4)§	1	5	1	—
Periventricular	6	5	2	1	—
Subcortical	2	2	1	—	1
Brainstem	5	2	1	—	—
Spinal cord	1	—	—	—	—

* Total no. of cases where information was available.
† Either at first or following attacks or at first investigation.
‡ In parentheses, no. of cases who had CSF analysis at previous episode.
§ In parentheses, no. of cases who yielded normal results at previous investigation.
‖ These patients had CT scan for the first time in occasion of the current bout.
In parentheses, no. of cases who had CT scan before.

NA = not applicable; OB = oligoclonal bands.

Discussion. We report the largest group of patients with the earliest onset of MS analyzed to date.[1-5,8-31,34-47] Although MS is rare in childhood, we show that no age group is immune to the disease[3,4] and that peculiar clinical features characterize this subgroup of patients.

In the absence of specific guidelines, we successfully adopted the diagnostic criteria devised for adult patients[6] to make a diagnosis of MS in patients at this young age. Poser's criteria[6] actually allowed for an even easier diagnosis of definite MS in our pediatric patients because their disease tends to relapse within shorter intervals than in adults (see table 2), allowing for a faster clinical confirmation of definite MS. However, it should be noted that a significant number of patients in this series were initially diagnosed with isolated optic neuritis, cerebellar ataxia, or ADEM.

The female/male preponderance that we recorded was lower (1.4) than that of adult onset MS (AOMS) (2.0)[1] and that of MS patients with onset between 6 and 15 years (2.2 to 3.0)[1-5,8,16,19,20,47] and was similar to that of MS appearing in the fifth decade, or later in life, which more commonly affects men.[1] Notably, the lowest ratio that we recorded (0.6) was in the group of patients with age at onset younger than 24 months. This flattened ratio might be owing to the absence of early effects of sex hormones on predisposed tissues (e.g., bone marrow, CNS). Other studies also failed to demonstrate a sex prevalence in childhood MS when including MS cases with the earliest onset.[1,18]

The age at onset of our group of patients fits with a Gaussian distribution. Five of our 49 patients (10%) had their first symptoms before the age of 24 months[14,24,27,28,37]: two of them[14,24] died after a rapid and severe course of the disease and a third[37] had a primary progressive form. Extremely early onset of MS, then, appears to carry an unfavorable prognosis.

A positive family history for MS was less frequent (9.6%) than in previous reports of children with MS at older ages (20 to 26%)[1-5,47] and adult MS patients (15%).[1] In 7.3% of our patients, we recorded nonspecific infections, mostly of the upper respiratory tract, before the onset of the disease or a new relapse, thus confirming previous reports of adult patients with MS.[1]

Among initial symptoms ataxia was preponderant (61%) as compared with impairment of other systems (see table 2) and to the prevalence previously reported for MS with onset between 6 and 15 years (5 to 9%)[8,19,47] or AOMS (7.7%) patients.[1] The frequency of ataxia at onset was higher in girls (63%) than in boys. Cerebellar signs and symptoms at presentation

also predominated in other series of childhood MS at older ages, although to a lesser extent.[3,8,18]

The recorded frequency of optic nerve involvement, especially at onset, was in keeping with other studies.[1-5,8-14,16-20,34-47] However, optic neuritis was almost twice as frequent when we considered only symptoms in episodes after the first attack, suggesting that optic pathway involvement may become more common with age.

Overall, the frequency of seizures was higher (22%) than in childhood MS with onset above age 6 years (10%) and in (2 to 5%) patients with AOMS.[1] This significant occurrence may be characteristic of MS patients with the earliest onset of disease or may merely reflect the increased and nonspecific likelihood of seizures in this MS age group as a result of widespread involvement of the CNS. However, 27% of our patients with seizures had a more aggressive clinical course and died. Accordingly, in this age group, seizures represent an unfavorable prognostic factor.

In this age group, the first occurrence of MS appeared to affect the patient's general status much more than in other age groups and was commonly associated with lethargy or coma, vomiting, or seizures. Despite this greater systemic involvement, recovery time was shorter (4 weeks) compared with what has been reported for older children with MS (5 to 6 weeks) and patients with AOMS (6 to 8 weeks).[1-4] Boys recovered faster than girls, an occurrence not previously reported[1-5,8-14,16-20,34-47] and of uncertain meaning. Disability after the initial episode was rare and mild.

Most patients had their second exacerbation within 1 year (63%), differing from that of older age groups. The overall course in this age group was less aggressive than classic adult MS (see table 2). Girls relapsed faster and less frequently than boys. However, a subgroup of patients with the earliest onset of MS might be particularly prone to the pathologic changes of the disease: 4 of the 43 reported infants (9.3%), in fact, died as a result of a relapse, a previously unreported observation in childhood MS.[1,3,13]

Laboratory features were broadly in keeping with previous large childhood MS series,[2-4,8-14,16-20,34-47] showing pleiocytosis and high protein levels in the CSF and an increasing rate of positivity for oligoclonal bands with disease progression (see table 3). Interestingly, epileptic discharges were detected in only 50% of children with seizures where information on EEGs was provided. MRI proved to be the preferred method of investigation on the grounds of sensitivity and noninvasiveness (see table 3). However, MRI is not available worldwide and requires appropriate sedation for younger children, which is generally not necessary for CT scanning. Although CT scan is still valuable in the common clinical evaluation in infants and toddlers with acute neurologic disorders, its value in assessing potential childhood MS is low. In the 43 reported children, CT scan showed a high rate of false-negatives (see table 3) when used as the first neuroimaging tool or during further attacks of the disease.

No controlled studies on the treatment of MS in childhood have been reported so far. Current therapy in children with MS follows the same guidelines used in adult neurology. Notably, some of the reported cases at this young age and most of our patients (see table 1) experienced faster neurologic recovery or a shorter duration of acute relapses by using steroids. Short courses (3 to 5 days) of high doses (500 or 1,000 mg in children over 10 years of age) of IV methylprednisolone were considered in the group of patients seen at DPUC (see table 1) to be most beneficial. Corticotrophin was never used in our experience of treatment of exacerbations. More frequent relapses were recorded on steroids withdrawal, and steroids were ineffective in those children having a chronic progression of disease. However, children with MS in this age group rapidly remitted even without therapy, further suggesting that preschool MS is usually more benign than AOMS, at least in the initial decade after disease onset.

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Does a shift in the T-cell receptor repertoire precede the onset of MS?

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Article abstract—*Background:* Utz et al., in a study of identical twins discordant for MS, showed that antigen-stimulated T cells from the MS twins have a major shift in their T-cell receptor (TCR) repertoires when compared with the healthy twins. We hypothesized that a shift in the TCR repertoire precedes the onset of MS and tested this hypothesis by studying unstimulated naïve T cells because the TCR repertoires of these cells are largely unaffected by disease. *Objective:* To investigate whether unstimulated naïve T cells from MS patients have a detectable shift in their TCR repertoires. *Methods:* We analyzed the TCR J beta (TCRBJ) repertoires of naïve T cells from identical twin pairs discordant for MS, healthy identical twin pairs, healthy unrelated pairs, and unrelated MS patient pairs. The correlation coefficient (*r* value) was used as a measure of similarity of TCRBJ repertoires in each pair of individuals. Fisher's z transformation was then used to test for the significance of the difference between the *r* values from different pairs. *Results:* The TCRBJ repertoires of the discordant MS twin pairs were significantly different from those of the healthy identical twin pairs, whereas MS patient pairs had TCRBJ repertoires similar to those of the healthy unrelated pairs formed from healthy twin pairs and discordant MS twin pairs. *Conclusions:* MS patients have a major shift in their naïve T-cell TCRBJ repertoires compared with healthy individuals, implying that this shift precedes the disease onset. This shift could represent the nongenetic factor that explains MS discordance in genetically identical individuals.

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MS is an inflammatory demyelinating disease of the CNS. Many think that the demyelination process is controlled by a T-cell–mediated autoimmune process directed against various myelin antigens such as myelin basic protein.[1,2] In various experimental allergic encephalomyelitis (EAE) models of MS induced by a myelin antigen, $CD4^+$ T cells can transfer disease from affected animals to healthy syngeneic recipients. Typically, the encephalitogenic T cells from one strain show restricted T-cell receptor (TCR) V beta (BV) and V alpha (TCRAV) usage. EAE induced by the same myelin antigen in a different strain is often associated with different patterns of *TCRV* gene restriction.[3-5] In some MS patients, myelin-reactive T-cell clones and T-cell clones from the CNS show limited expression of particular TCRBV or TCRAV segments.[5-10] However, some patients show heterogeneous TCRV segment usage, and no consensus exists on the extent of preferential *TCRV* gene usage shared by different patients.[5,10-12] This lack of consensus extends to the third TCR complementarity determining (CDR3) region that binds directly to antigenic peptide in the major histocompatibility complex groove.[13] Thus, some investigators report that in different individuals T-cell clones from CNS lesions[14] and myelin-reactive T-cell clones[15,16] share CDR3 sequences, whereas others find evidence of shared CDR3 sequences only within individual patients.[5,10]

In contrast to the numerous reports on TCR usage in MS,[3-16] few investigators have studied the overall TCR distribution profiles, i.e., TCR repertoires, of MS patients. Utz et al.[17] found that in comparison with healthy individuals, MS patients have a major shift in their TCR repertoires. This shift was detectable only after T cells were stimulated by antigen. Our objective was to investigate whether unstimulated T cells from MS patients have a detectable shift in their TCR repertoires. We analyzed the TCR J beta (TCRBJ) repertoire of peripheral blood naïve T cells, which, by definition,[18] have not been stimulated by antigen. We reasoned that a shift in the TCRBJ repertoires of these T cells in MS patients would not be due to the effects of MS on the immune system and, moreover, could precede the onset of MS and also predispose to development of MS.[19]

A basic issue we needed to address was how to determine whether a shift in the TCRBJ repertoires of MS patients is statistically significant. We used the extent of similarity of TCRBJ repertoires of healthy identical twin pairs as our reference point for normality. We then compared the TCRBJ repertoires of identical twin pairs discordant for MS with this reference point.

Methods. We studied six apparently healthy identical twin pairs (ages 15 to 35) who had no significant medical history and six identical twin pairs (ages 35 to 48) discor-

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dant for clinically definite MS by standard criteria.[20] All patients had relapsing-remitting MS.

Lymphocyte preparation. Peripheral blood lymphocytes were isolated over Ficoll-Hypaque, then approximately 30×10^6 cells were incubated for 1 hour at 4 °C with anti-CD4–coated Dynabeads (Dynal A.S., Oslo, Norway). $CD4^+$ T cells were isolated using a magnet, then incubated with 50 μL Detachabead (Dynal A.S.) for 1 hour at room temperature. Dynabeads were removed with a magnet, and 50 μL of mouse IgG2a anti-CD45RO antibody (Becton Dickinson, San Jose, CA) were added to the $CD4^+$ T cells, which were then incubated at 4 °C for 30 minutes. After washing, $CD4^+CD45RO^-$ T cells were isolated by negative selection using Dynabeads precoated with rat anti-mouse IgG2a according to the manufacturer's instructions (Dynal).

Total RNA isolation, first strand cDNA synthesis, and reverse transcribed PCR (RT-PCR). One mL of Ultraspec (Biotecx, Houston, TX) and 0.1 mL of chloroform were added to each preparation of $CD4^+CD45RO^-$ cells and total RNA isolated according to the manufacturer's instructions. Total RNA (1 to 2 μg) was used for oligo d(T)-primed first strand cDNA synthesis as previously reported.[21] We performed semi-quantitative RT-PCR analysis of 13 TCRBJ segments using 13 different BJ primers, derived from published sequences,[22] in separate reaction tubes with a TCRB constant primer[23] and with two TCRAC primers[23] as internal controls. Each 20-μL PCR mixture contained 0.75 U *Taq* polymerase, 240 μM dNTPs, 1.5 mM $MgCl_2$, and 0.165 μM of each primer, including 0.015 μM (10^6 counts per minute [cpm]) of the 3′-BC and 3′-AC primers that were ^{32}P-radiolabeled. PCR amplification was on a DNA thermal cycler 480 (Perkin-Elmer, Norwalk, CT) for 24 cycles consisting of 1 minute at 94 °C, 1 minute at 55 °C, 1 minute at 72 °C, with a final 7-minute extension at 72 °C; preliminary experiments established that 24 cycles are well within the linear phase of the PCR (unpublished result, 1997). After the PCR, the amplified products were electrophoresed in 2% agarose gels and the DNA bands excised after ethidium bromide staining of the gels. Samples were counted in scintillation fluid (Beckman Ready Value, Beckman Instruments, Fullerton, CA) by scintillation spectroscopy as described.[24] We corrected for differences in the efficiencies of PCR priming of each BJ segment,[21,25] then calculated the relative percent expression levels of each BJ segment using cpm obtained for AC segments to normalize cpm obtained for each BJ segment.[21,23,25]

Comparison of TCRBJ repertoires and data analysis. We determined TCRBJ repertoires by analyzing expression of 13 TCRBJ segments in each individual from 12 identical twin pairs. We then used correlation analysis to estimate the extent of similarity of TCRBJ repertoires in each pair of individuals because we found previously that correlation coefficients (*r* values) obtained for TCRBJ repertoires are highly repeatable in duplicate experiments.[25] In any individual the sum of the percent contribution of each TCRBJ segment to the TCRBJ repertoire is 100. Therefore, once we have measured the expression levels of 12 TCRBJ segments, we know the expression level of the 13th segment, i.e., only 12 of the 13 TCRBJ segments have independent levels of expression. To achieve independence of the expression levels of TCRBJ segments, we randomly selected the TCRBJ2S7 segment and dropped its contribution to the total TCRBJ repertoire.

In a previous study of the TCRBV repertoire we reported a statistical approach to test whether *r* values from different pairs of individuals are significantly different, but the number of TCR data points needs to be 25 or higher.[26] To increase the number of independent TCRBJ data points, we combined the TCRBJ repertoire data from three pairs of individuals, thus enabling comparison of 3×12, i.e., 36 TCRBJ data points. We then calculated an *r* value as a measure of the similarity of the three sets of 36 TCRBJ data points. To exclude a possible bias in combining pairs into groups of three pairs we analyzed all possible combinations of similar pairs, e.g., all possible healthy twin pair combinations. Fisher's z transformation was then used to transform each *r* value to a z value:

$$Z_1 = \tfrac{1}{2} \ln (1 + r_1)/(1 - r_1) \text{ and } Z_2 = \tfrac{1}{2} \ln (1 + r_2)/(1 - r_2) \text{ [equations 1 and 2]}$$

A z-test was then used to test for significant differences between *r* values:

$$Z = Z_1 - Z_2/\sqrt{(1/n_1 - 3) + (1/n_2 - 3)} \text{ [equation 3]}$$

where Z_1 and Z_2 are the z transformations of r_1 and r_2, and n_1 and n_2 represent the number of TCRBJ segments in each group, i.e., 33.

Statistical analysis was done using MINITAB Release 10 for Windows (MINITAB Inc., State College, PA).

Results. *Comparison of TCRBJ repertoires between different groups.* We plotted the TCRBJ repertoires of each pair of healthy identical twins (figure 1) and each pair of discordant MS twins (figure 2), then estimated the similarity of TCRBJ repertoires of each pair by calculating *r* values (figure 3). We then tested whether different types of pairs have significantly different TCRBJ repertoires. First, we compared the TCRBJ repertoires of healthy twins and unrelated pairs; each unrelated pair (see figure 3, group a) was formed by combining one individual from each of two healthy twin pairs. All possible comparisons between twins and unrelated pairs were made (see Methods), and of the 40 comparisons 35 gave a statistically significant z-score at 5% level of significance.

Second, we compared the TCRBJ repertoires of the healthy twin pairs and discordant MS twin pairs. Surprisingly, the highest *r* value from the discordant MS twin pairs was lower than the lowest *r* value from the healthy twin pairs (see figure 3). This was an unexpected result. One possibility was that this was due to the age differences between the two groups of twins. However, among the healthy twin pairs the lowest *r* value (0.809) was for the youngest twin pair (see figure 1A). More important, among the discordant MS twin pairs the lowest *r* value (−0.037) was for the youngest pair (see figure 2A), whereas the oldest twin pair had the highest *r* value (0.783) (see figure 2C). Thus, age differences do not explain the differences in *r* values. For statistical purposes, we formed two groups, one group consisting of the six healthy twin pairs and one consisting of the six discordant MS twin pairs. We had, therefore, six pairs of 12 TCRBJ data points per group. After z transformation of each *r* value, a z-score confirms the two groups have significantly different TCRBJ repertoires ($z = 3.69$, $p < 0.001$). In contrast, the *r*

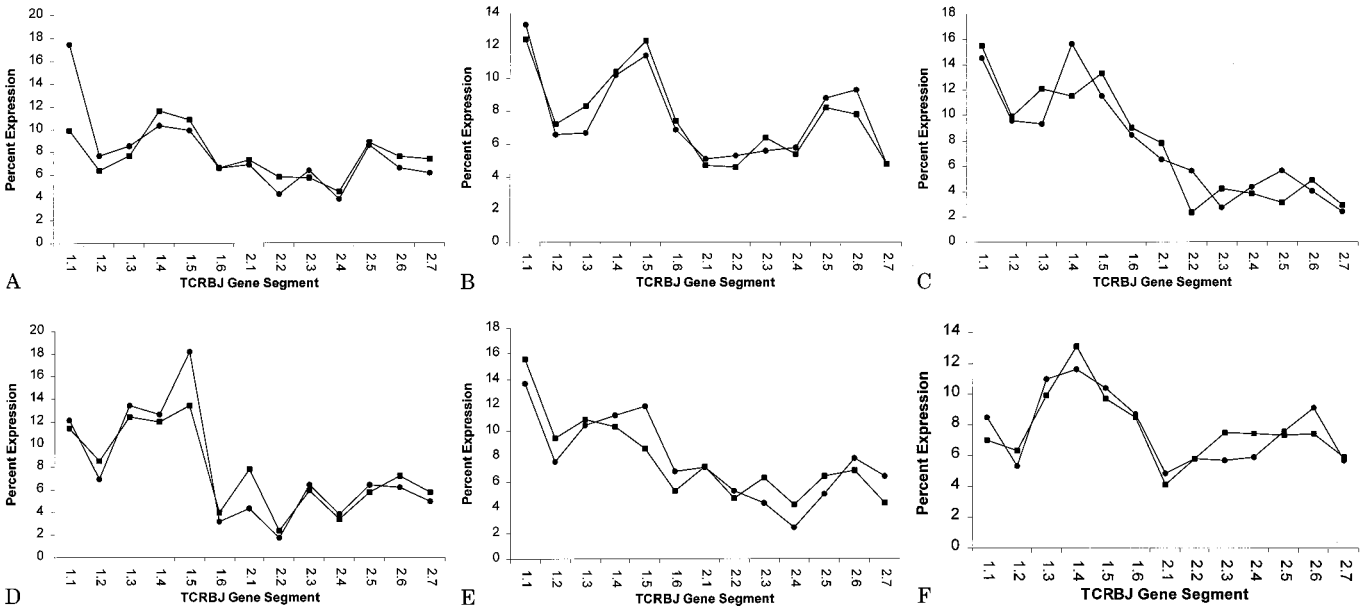

Figure 1. The T-cell receptor J beta (TCRBJ) repertoires (TCRBJ distribution profiles) of naïve T cells from six healthy identical twin pairs. (A) Twin pair 1. (B) Twin pair 2. (C) Twin pair 3. (D) Twin pair 4. (E) Twin pair 5. (F) Twin pair 6.

values for pairs of unrelated MS patients, formed from the discordant twin pairs, and for healthy unrelated pairs overlapped extensively. In fact, all possible comparisons between pairs of MS patients and healthy unrelated pairs gave nonsignificant z values (data not shown).

Third, we addressed the possibility that the significant z-score we obtained for healthy twin pairs versus discordant MS pairs was due to a shift in the TCRBJ repertoires of the healthy members of the discordant MS twins. We formed two groups, namely unrelated pairs from the healthy twins and unrelated healthy pairs from the discordant MS twins. We obtained an r value for each group, and a z-score using Fisher's z transformation was not statistically significant.

Finally, 26 months after the above studies were complete we re-bled one healthy and one discordant MS twin pair and repeated our TCRBJ repertoire analyses. First, we tested whether correlations between twin members are similar at the two time points. We found the healthy twins had remarkably similar TCRBJ repertoires initially (r = 0.946) (see figure 3) and at 26 months (r = 0.844). The discordant MS twin pair had an r value of 0.783 initially, and this was essentially unchanged at 26 months (r = 0.759). Second, we tested whether each individual's TCRBJ repertoires were similar at the initial bleed and at 26 months. One healthy twin had an r value of 0.946, as a measure of similarity of TCRBJ repertoires at the two time points, and the second twin member had an r value of 0.844. One discordant twin had an r value of 0.915, and the second twin member had an r value of 0.55. A t-test[27]

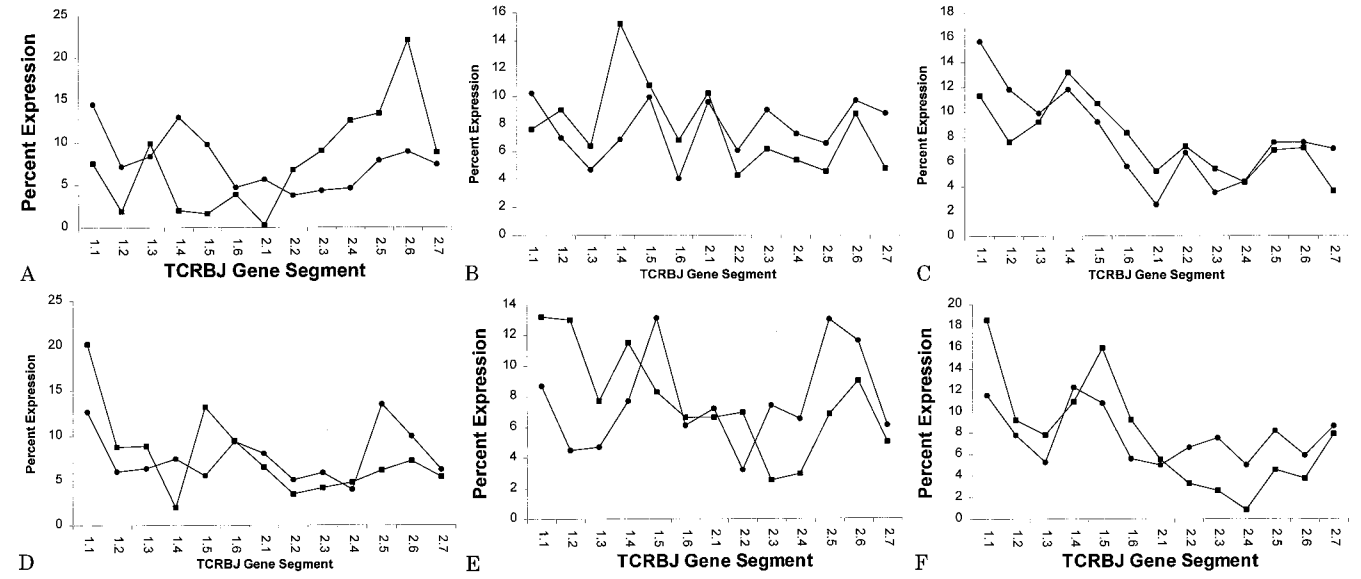

Figure 2. The T-cell receptor J beta (TCRBJ) repertoires (TCRBJ distribution profiles) from six identical twin pairs discordant for MS. (A) Twin pair 1. (B) Twin pair 2. (C) Twin pair 3. (D) Twin pair 4. (E) Twin pair 5. (F) Twin pair 6.

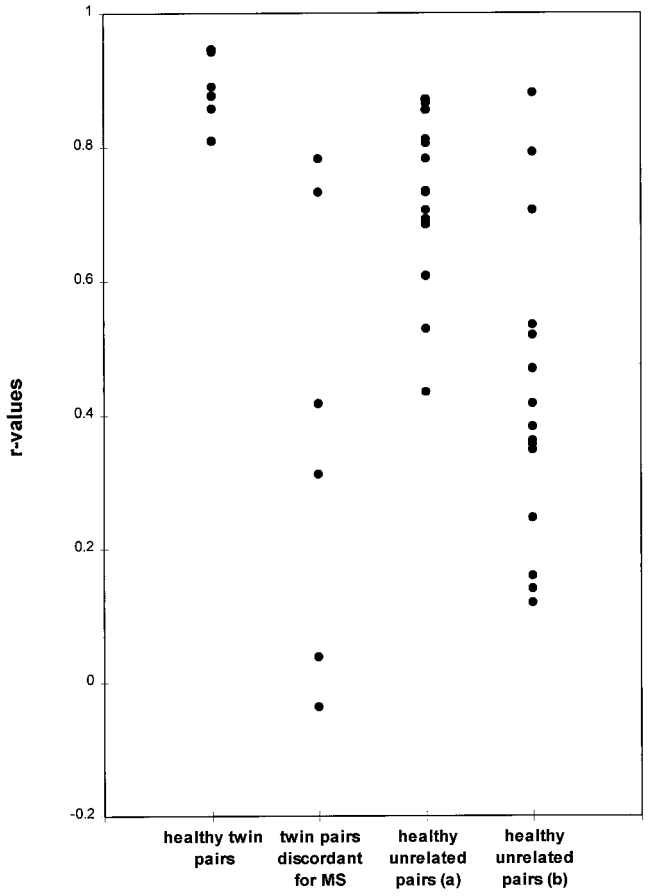

Figure 3. An r *value was calculated as a measure of similarity of the T-cell receptor J beta repertoires in each pair of individuals. Healthy unrelated pairs were formed from healthy twin pairs (a) and from discordant MS twin pairs (b).*

confirmed a statistically significant linear correlation ($p < 0.05$) between TCRBJ repertoires at two time points for each of the four twin members, i.e., the naïve T-cell TCRBJ repertoires are reproducible over time.

Discussion. Studies of the TCR repertoire in disease have several difficulties. One is establishing whether the TCR repertoires of various individuals are actually similar or different. One group of investigators directly compared the TCR repertoires of two individuals by inspection of the TCR distribution profiles, thus detecting obvious differences in expression of particular TCR segments.[17] Another approach analyzes the TCR repertoires in pairs of individuals and then compares the similarities of TCR repertoires in different types of pairs,[23,28-31] e.g., healthy identical twins versus pairs discordant for disease. However, these comparisons are often problematic because they are not amenable to statistical testing to determine whether the TCR repertoires in different pairs are similar or different.[26] In contrast, we recently reported a statistical method based on Fisher's z transformation of *r* values that tested whether the TCRBV repertoires of different pairs are similar or different.[26] We used this method to analyze TCRBJ repertoires. We did not analyze TCRBV repertoires because the PCR-amplification protocol does not easily permit distinction between individual members of a multigene *TCRBV* family, e.g., *TCRBV6*,[32] and individual family members could have widely different expression levels. By contrast, each TCRBJ segment is PCR amplified separately and its expression level determined. Thus, we assumed a priori that we would more easily detect minor differences in TCRBJ than TCRBV repertoires.

An earlier study showed that MS patients have a major shift in their TCR repertoires that is detectable only after T-cell antigen stimulation.[17] An issue for the present study was to develop a method to detect a shift in the TCRBJ repertoire in unstimulated T cells, and we report a three-step approach. First, we compared healthy identical twin pairs with unrelated pairs. Thirty-five of 40 comparisons showed that the genetically identical pairs had more similar TCRBJ repertoires than genetically unrelated pairs, thus implying a genetic influence on the TCRBJ repertoire. This result supports the results of an earlier twin study that claimed genetics influences the TCRBJ repertoires.[33] Second, we compared healthy identical pairs versus discordant MS pairs to determine whether a genetic influence is demonstrable. Clearly, the genetic influence was not demonstrable in the discordant MS pairs. In fact, the TCRBJ repertoires of the discordant MS pairs were not statistically different from those of unrelated pairs. An obvious explanation for the lack of evidence of genetic influence among the discordant MS twin pairs is that patients with known chronic inflammatory disease have a major shift in their TCRBJ repertoires. Third, we compared the TCRBJ repertoires of the two groups of healthy unrelated pairs, formed from the healthy twins (see figure 3, group a) and from the discordant MS twins (see figure 3, group b), to exclude the alternative possibility that the healthy members of the discordant MS pairs had a major shift in their TCRBJ repertoires. The two groups had no significant differences in their TCRBJ repertoires. Therefore, we conclude that the shift in the repertoires occurred among the MS patients. Thus, our twin pair approach seems useful in detecting subtle shifts in the TCR repertoire in MS and should have similar utility in other putative autoimmune diseases. The healthy twin pair analysis predicts the expected level of similarity of TCR repertoires in identical twin pairs who do not have MS. Detection of a TCR repertoire shift in twin pairs discordant for disease, but not in healthy unrelated pairs formed from the discordant pairs, means the patients had a shift in their repertoires away from the "normal" repertoires of their healthy twin counterparts.

Another issue we explored was whether an altered TCRBJ repertoire precedes the onset of MS. We focused on $CD4^+CD45RO^-$ T cells, reasoning that a shift in the repertoire of naïve T cells would not be due to MS because naïve cells should not undergo changes from antigen stimulation.[18] We did not ana-

lyze $CD4^+CD45RO^+$ (memory) T cells because our preliminary study of 20 healthy individuals established that naïve and memory T subsets usually have significantly different TCRBJ repertoires (unpublished result, 1997). We assumed discordant MS twin pairs would have similar or exaggerated differences in naïve and memory TCRBJ repertoires due to the known effects of MS on memory T cells.[1,2] Our finding of an altered TCRBJ repertoire in the discordant twins could mean that the repertoires were altered in patients before disease onset. We considered several other possibilities. Recent work showed that a minority of $CD45RO^+$ memory T cells can revert to the $CD45RO^-$ phenotype.[34,35] Thus, one possibility is that more memory T cells were $CD45RO^-$ in the patients than in healthy members of the discordant twins. However, we know of no mechanism that enhances phenotypic reversion in patients versus controls, nor do we know of data on the total number of revertant T cells in patients versus controls in any disease. Also, the reproducibility of our findings over 26 months does not support the idea that revertant memory cells contribute significantly to the naïve T-cell TCRBJ repertoire. Similarly, twin age differences should be irrelevant because age is not known to influence the naïve T-cell TCRBJ repertoire, and we found no evidence that TCRBJ repertoires become less similar as twin ages increase. In fact, among the discordant twin pairs the oldest twin pair had the most similar TCRBJ repertoires ($r = 0.783$), whereas the youngest twin pair had the least similar repertoires ($r = -0.037$). In summary, the MS disease process probably did not cause the changes in the TCRBJ repertoires in the discordant pairs.

The concordance rate of MS in identical twins is approximately 20 to 30%,[36] and clearly nongenetic factors must play a role in MS susceptibility. The major shift we find in the TCRBJ repertoire of naïve T cells could represent the nongenetic factor that explains discordance.[37] The problem that remains is to identify what factor or factors induced these changes. Stochastic events during thymic selection probably could not have had such profound effects on the TCRBJ repertoires. One explanation is that superantigen is responsible for the altered TCRBJ repertoires of the naïve T cells in MS patients. This seems plausible because TCRBJ segments participate in superantigen binding, and superantigen is a known cause of a shift in TCRBJ segment expression.[38] Finally, we conclude that the altered repertoire we detect likely preceded disease onset. Whether, as previously postulated,[19] an altered TCR repertoire predisposes to MS remains to be investigated.

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Coexistence of temporal lobe and idiopathic generalized epilepsies

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Article abstract—*Objective:* To assess the interrelation of idiopathic generalized epilepsy (IGE) and temporal lobe epilepsy (TLE) when they coexist in the same patient. *Methods:* The authors reviewed the electroclinical features of 350 consecutive patients who had temporal resection between 1975 and 1997 at the Maudsley and King's College Hospitals, London. *Results:* Two patients had the unusual combination of TLE and IGE (0.57%). In the first, the clinical onset of juvenile myoclonic epilepsy followed the surgical resolution of his partial seizures but had been heralded for at least 5 years by subclinical spontaneous and photically induced generalized spike–wave discharges. In the second, TLE and juvenile absence epilepsy had a long parallel course before surgery. After surgery he had no further partial seizures. *Conclusion:* These cases suggest that when an idiopathic absence or myoclonic syndrome manifests in a patient with symptomatic TLE, the phenotype may not be a merged syndrome. Rather, the two conditions can retain their inherent electroclinical profile, responsiveness to treatment, and prognosis.

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In patients with partial epilepsy the appearance of bilateral and synchronous spike–wave discharges (BSSW) may be well documented,[1-3] but occurrence of generalized seizures suggesting the coexistence of idiopathic generalized epilepsy (IGE) is only exceptionally described.[1,4,5] Moreover, there are no reports of patients with symptomatic partial epilepsy and well-defined, concurrent distinct absence syndromes of IGE.

The reasons for this rarity are poorly understood. A degree of misdiagnosis might be hypothesized; for example, "blank spells" on historical evidence alone can be interpreted either as limbic complex partial seizures (CPS) or as typical absence seizures. Alter-

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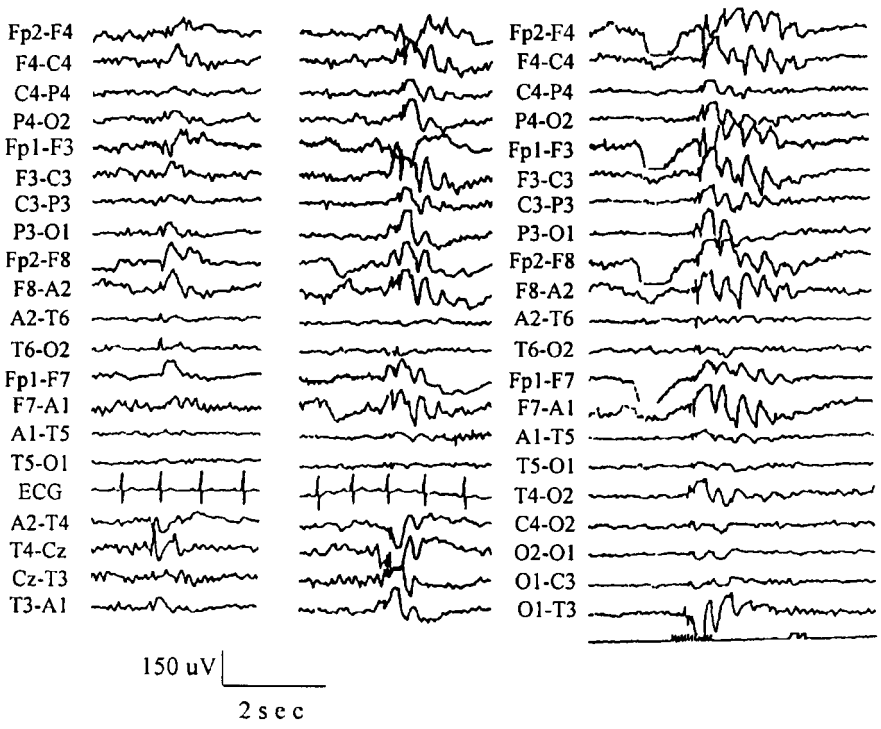

*Figure 1. Preoperative interictal EEG in Patient 1 at 19 years of age showing right temporal sharp- and slow-wave discharges (*left trace*), and brief generalized bursts of polyspike–waves, spontaneous (*middle trace*) and photically induced at 15 Hz (*right trace*). High-frequency filters = 70 Hz; time constant = 0.3 second.*

natively, partial epilepsy might modify the natural history or phenotype of coexistent genetically determined epileptic syndromes, impeding their recognition. Little is known about the possible effects of drugs for partial epilepsies on the natural history of IGE, and a triggering role of adverse antiepileptic drug changes for juvenile myoclonic epilepsy (JME) has been hypothesized.[6]

We present two patients with temporal lobe epilepsy (TLE) and distinct IGE syndromes, focusing on the interrelation of the epilepsies, the electroclinical differential diagnostic criteria and pitfalls, and the implications that early and accurate diagnosis may have for optimal management and anticipation of outcome after resective surgery.

Methods. From a series of 350 consecutive temporal resections performed at the Maudsley and King's College Hospitals in London between 1975 and 1997 for medically intractable partial seizures, we identified patients who had some historical evidence of generalized seizures (such as absences, blank spells, or generalized tonic–clonic seizures (GTCS) without apparent focal onset) or BSSW during presurgical assessment. The preoperative interictal standard and sleep EEGs and prolonged scalp video EEG results were reviewed, and particular attention was given to earlier EEGs, with the actual traces studied when possible. BSSW with clear electrographic evidence of secondary bilateral synchrony (SBS) were excluded. SBS was defined as " . . . bilateral synchronous discharge which can be shown to arise from a unilateral cortical focus"[7] using strict temporospatial criteria.[8,9] Patients with frontal lobe resections not considered as frontal foci are not only the most common triggers of SBS,[7] but may even produce electroclinical absences indistinguishable from the typical absences of IGE,[10] which would blur the relationship studied in this report. Diagnosis of a coexistent IGE required both clinical and EEG evidence according to the criteria of the current classification of epilepsies/epileptic syndromes and seizures of the International League Against Epilepsy.[11,12]

Results. *Case reports.* Two patients (0.57%) had clear electroclinical evidence of coexisting TLE and IGE.

Patient 1. This 23-year-old, right-handed man was referred to the Maudsley and King's College Hospitals epilepsy surgery program at the age of 19 years. He first had a prolonged febrile convulsion at 2 years of age that lasted at least 20 to 30 minutes and was probably repeated several times during that day. The medical history was otherwise unremarkable, and there was no family history of epilepsy. His CPS appeared at 5 years of age and remained stereotyped thereafter. They used to start with a cephalic aura followed by staring, sustained flexion of both arms, and version of the head to the left, and lasted for 2 to 3 minutes. The average frequency was eight per week. He was treated with carbamazepine and sodium valproate from the beginning, remained seizure free between 12 and 13 years of age, but soon after CPS resumed and, once or twice per month, they would evolve into GTCS. Sodium valproate was unsuccessfully replaced by clobazam at age 14 years, and on referral he was on carbamazepine, gabapentin, and lamotrigine but still having daily seizures. Neurologic examination was negative. Previous EEGs, performed between 15 and 18 years of age, were reported as showing right temporal and generalized paroxysmal abnormalities and photoparoxysmal responses.

Preoperative scalp EEG during awake and sleep states showed frequent right anterior temporal sharp- and slow-wave discharges; brief generalized spike/polyspike–wave discharges, especially during hyperventilation; and generalized photoparoxysmal responses at flash frequencies of 10 to 20 Hz (figure 1). MRI showed atrophy of the right

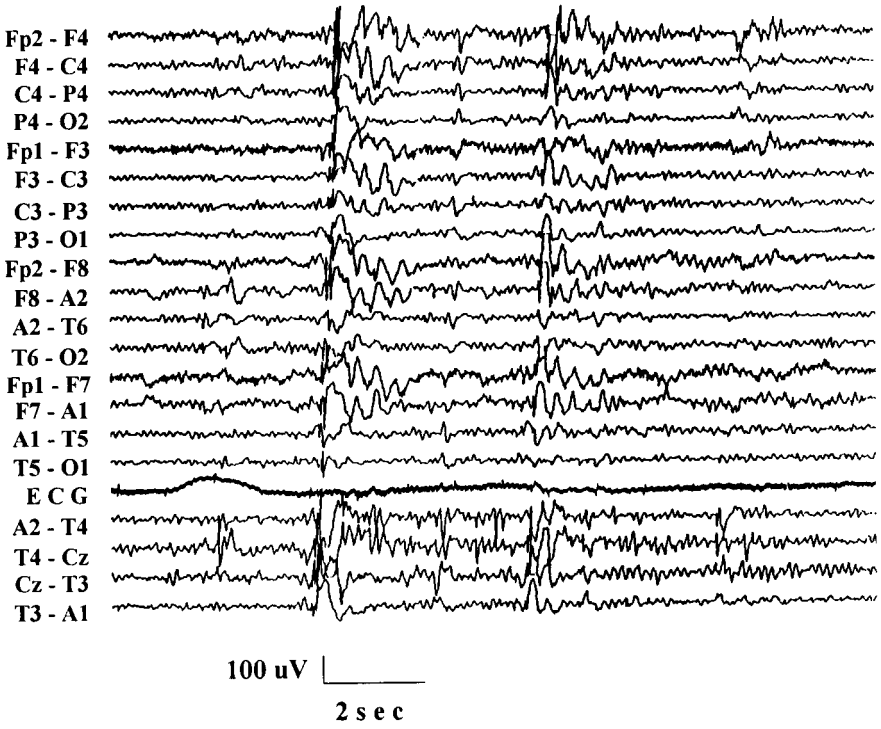

Figure 2. Electroencephalogram in Patient 1 at 21 years of age, 6 months after right temporal lobectomy, showing brief, generalized polyspike–wave discharges associated with myoclonic jerks of both arms. Note the right frontotemporal accentuation of the spike component, and the right temporal enhancement of underlying rhythms, both due to the postoperative skull defect. High-frequency filters = 70 Hz; time constant = 0.3 second.

hippocampus and interictal ^{18}F-deoxyglucose (FDG) PET demonstrated right temporal hypometabolism. Prolonged video telemetry with foramen ovale electrodes recorded three habitual attacks and confirmed a right mesiotemporal onset. An intracarotid amobarbital test showed left-sided dominance for language, and adequate memory on either side without significant interhemispheric difference. The patient underwent right anterior temporal lobectomy at the age 20.5 years, and histopathologic study revealed mesiotemporal sclerosis without features suggesting a neuronal migration disorder. He became entirely free of his habitual CPS and secondary GTCS but, shortly after surgery and for the first time, he experienced clusters of bilateral, arrhythmic clonic jerks of the limbs, mainly on awakening, at times sufficiently violent to cause falls to the ground. A follow-up EEG during awake and sleep states 6 months after surgery again showed photoparoxysmal responses and brief, generalized polyspike–wave discharges during sleep (figure 2), but provided no evidence of focal epileptogenicity. Carbamazepine and gabapentin were phased out, sodium valproate was reintroduced, and currently, 38 months after the temporal lobectomy, the patient has occasional early morning myoclonic jerks, especially when sleep deprived or after alcohol consumption, but no other seizures.

Patient 2. This 23-year-old, right-handed man was referred to our epilepsy surgery program at 20 years of age. Apart from three febrile convulsions between 13 and 23 months of age, one of which lasted for 45 minutes, his medical history was unremarkable. His mother had a single GTCS in childhood. CPS started at the age of 2 years. He had an aura of dizziness followed by impairment of consciousness, restlessness, left-hand automatisms, and sustained posture of the right arm. Attacks lasted for 2 to 3 minutes and were followed by postictal headache and dysphasia. Secondary generalization was frequent. At 13 years of age, a new seizure type became apparent in which he would suddenly become unresponsive for less than 1 minute followed by a prompt, full recovery. Treatment with carbamazepine and sodium valproate did not have substantial therapeutic effect, whereas add-on therapy with vigabatrin at 17 years of age resulted in a clear exacerbation of his absences. Subsequent introduction of lamotrigine reduced the absences but had no effect on his CPS. EEGs at 16 and 17 years of age had been reported as showing spikes and runs of sharp–slow activity over the left temporal area, as well as generalized spike–wave discharges at 3 Hz, varying in duration for up to 45 seconds. A video EEG performed at 18 years of age in another hospital recorded one typical absence seizure during hyperventilation, with the patient stopping the exercise and slowly deviating his eyes from side to side. The seizure lasted 19 seconds and was associated with a generalized regular spike–wave discharge at 4 to 3 Hz. On referral, he was on carbamazepine, sodium valproate, and lamotrigine. Neurologic examination was negative.

A scalp EEG during awake and sleep states showed frequent left anterior to mid-temporal sharp-wave or spike–wave complexes, brief generalized polyspike–wave discharges mainly during sleep, and occasional independent left frontal spikes (figure 3). MRI suggested atrophy of the left hippocampus, and interictal ^{18}FDG PET revealed hypometabolism of the left temporal lobe. Three of his habitual CPS were recorded during prolonged video EEG telemetry with foramen ovale electrodes, with clear onset at the left mesiotemporal structures preceding the first behavioral changes by 20 to 30 seconds. An intracarotid amobarbital test showed mixed cerebral dominance for language and significant memory deficit on the left. The patient underwent a left temporal lobectomy at 21 years of age and histopathologic study revealed mesiotemporal sclerosis without other abnormalities. He remains entirely seizure free 2 years after surgery, and is still on

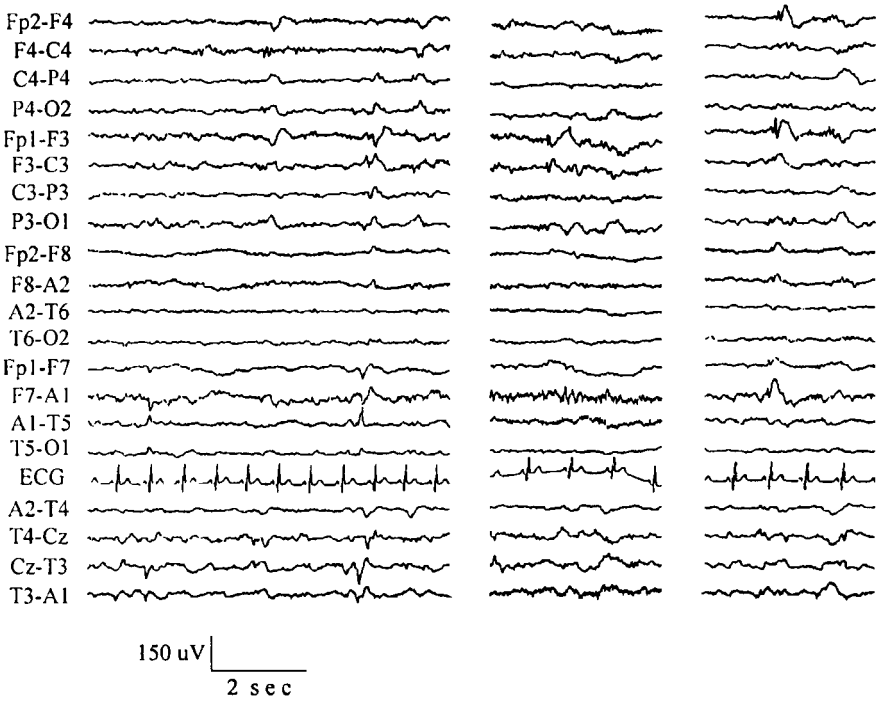

Figure 3. Focal abnormalities in the preoperative interictal EEG in patient 2 at 20 years of age. Left trace*: left temporal slow activity intermixed with sharp- and slow-wave discharges, with phase reversing over the mid-temporal area and corresponding to the symptomatic focus.* Middle trace*: independent left superior frontal low-voltage fast spike–wave complex, producing no disturbance of the physiologic local rhythms.* Right trace*: "abortive generalized" discharge resulting from regional spread of frontal paroxysms (as in middle trace) including the right frontopolar area. These paroxysms do not indicate multifocal symptomatic epilepsy or imply secondary bilateral synchrony, but reflect the diffuse but not uniform cortical hyperexcitability in idiopathic generalized epilepsies. High-frequency filters = 70 Hz; time constant = 0.3 second.*

lamotrigine and sodium valproate. A follow-up EEG 13 months after the operation showed only subclinical, brief generalized polyspike–wave discharges (figure 4).

Discussion. Our patients had chronic, medically intractable TLE associated with hippocampal sclerosis that was cured by resective surgery. In addition, they both had generalized seizures suggesting the existence of IGE, with the relevant electroclinical features providing strong evidence of JME[13] in Patient 1, and juvenile absence epilepsy (JAE)[14] in patient 2. In Patient 1, the appearance of spontaneous and photically induced generalized spike–wave discharges preceded temporal lobectomy by 5 years, with the clinical onset of JME following shortly after the surgical resolution of CPS. In Patient 2, TLE and JAE occurred together for almost 7 years. To our knowledge, coexistence of symptomatic partial (temporal lobe) epilepsy and a distinct absence IGE syndrome (JAE) has not been previously documented.

Diehl et al.[6] described a patient with TLE and well documented JME in whom the idiopathic condition was clinically manifested shortly after her partial seizures were cured by temporal lobectomy. No infor-

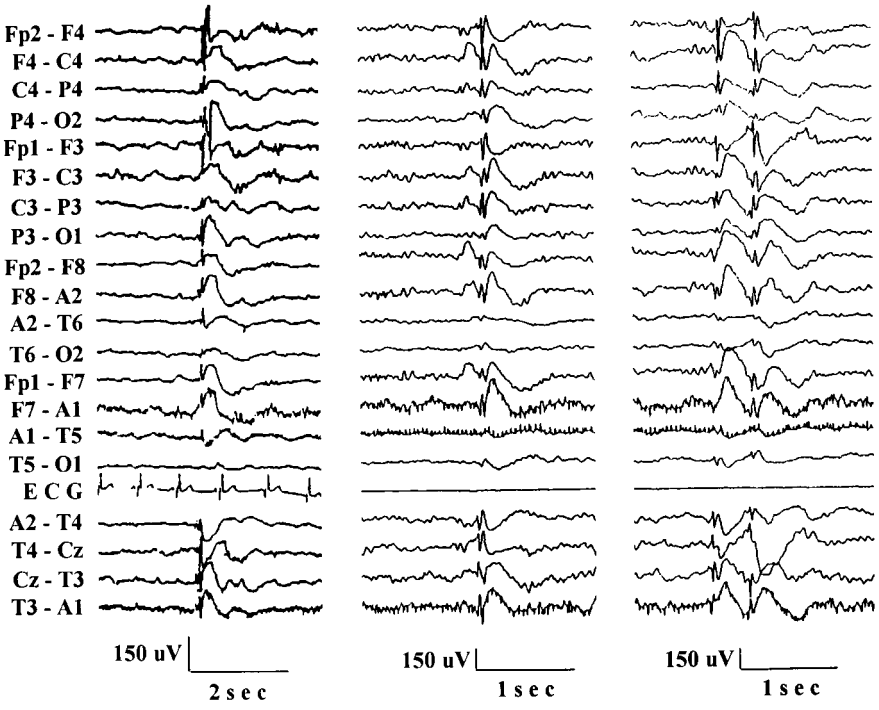

*Figure 4. Brief, generalized polyspike–wave discharges during sleep in Patient 2, before surgery at 20 years of age (*left trace*), and 13 months after left anterior temporal lobectomy at 22 years of age (*middle and right traces*). Note the single phase reversal of the spike component of the discharge over the midline on the left and middle traces, and the apparent double phase reversal over the right and left central/sylvian areas in the right-hand trace (four lower channels). Both patterns appear to be compatible with "primary" generalized epileptogenesis. High-frequency filters = 70 Hz; time constant = 0.3 second.*

Table *Patients with electroclinical evidence of coexisting idiopathic generalized and partial epilepsies*

Reports	Patients	Seizure types
Niedermeyer, 1968[1]	1 (Patient 6 from Group 1)*	"Petit mal," "psychomotor attacks," GTCS
Geller et al., 1995[4]	3	PS, ABS (1 patient); PS, MS (2 patients)
Li et al., 1996[5]	3	PS, ABS (1 patient); PS, GTCS (no warning), MS (2 patients)

* Focal discharges only in depth leads (left amygdala).

GTCS = generalized tonic–clonic seizures; PS = partial seizures; ABS = absence seizures; MS = myoclonic seizures.

mation on EEG findings before presurgical assessment was available. In this woman, sodium valproate had been discontinued just before the operation, and when it was restarted after the onset of JME, all generalized seizures ceased. Because of this timing, the authors hypothesized a combination of inherent age-related factors and adverse drug effects, and also mentioned the possibility that the onset of JME could have been facilitated by the surgical resolution of TLE. This case resembles our first patient, in whom specific questioning failed to elicit a convincing history of absences or myoclonic jerks before the operation. However, a prominent role of antiepileptic medication in the postoperative appearance of JME is not supported in our patient. He had stopped taking sodium valproate almost 7 years before the operation (and well within the age range of onset of JME), and remained consistently on carbamazepine combined with various drugs for partial seizures. Similar evidence is also lacking in our second patient in whom JAE manifested and was diagnosed on solid clinical and ictal video EEG grounds 8 and 3 years before the operation, respectively, while he was consistently on sodium valproate. The parallel and full clinical expression of JAE and TLE indicates the absence of any significant mutual influence, and further implies that any sequential appearance of IGE may not result from facilitation by the surgical resolution of the partial seizures or the brain surgery itself, but may simply be fortuitous.

Review of the literature shows that patients with concurrent symptomatic partial and idiopathic generalized epilepsies are very rarely reported (table). The 0.57% incidence in our series of temporal resections may not reveal the actual incidence of IGE among patients with partial seizures in general, but suggests that such coexistence may occur more frequently than appreciated. It appears that the apparent rarity of similar cases may reflect a degree of underdiagnosis of a concurrent IGE even in specialized tertiary epilepsy surgery centers, where patients are thoroughly investigated but attention is understandably focused on partial epilepsies. Ictal symptomatology in partial seizures is frequently complex and dominates the clinical picture. A history of GTCS without apparent preceding focal features is commonly elicited in patients with partial epilepsies and does not necessarily imply the coexistence of IGE. Myoclonic jerks, especially when asymmetric, may be mistaken for focal motor seizures, whereas typical absences, particularly when associated with mild automatisms or when adults are considered,[15] may be misdiagnosed as limbic CPS.

EEG aspects. In both our patients, preoperative interictal scalp EEG recordings showed unilateral temporal foci and brief subclinical BSSW discharges without a demonstrable lead-in from focal abnormalities. Such bisynchronous bursts are regarded as "age-related phenomena whose clinical significance is limited to a greater tendency for GTCS,"[3] but may not necessarily constitute a marker of IGE. Electrographic evidence of SBS may escape detection by scalp EEG because of rapid spread or deep site of discharge origin, and the limited coverage of the cortex.[16] BSSW at 3 Hz may occur in patients with cerebral cortical dysgenesis,[17] and can even amount to absences mimicking IGE in patients with seizures arising from the medial intermediate frontal area.[10] In the same context, a double phase reversal of the spike component over the right and left frontal regions is not the only topographic pattern of a "primary" generalized discharge,[7] and a single phase reversal over the midline may be also acceptable[18] (figure 4). Therefore, in such equivocal cases and particularly when preoperative assessment is concerned, earlier recordings can provide critical information. This is best illustrated in Patient 2, in whom, without the benefit of past EEG/video EEG recordings, his typical absences might have been misinterpreted as (a second type of) CPS.

On the other hand, caution may be needed when interpreting focal discharges. Focal abnormalities are encountered in more than 30% of patients with IGE,[19] presumably reflecting a widespread nonuniform cortical hyperexcitability. In our experience, they are usually random, frontotemporal, fast sharp waves or spike-and-wave complexes of moderate amplitude, tend to switch sides, and produce no disturbance to the regional physiologic background rhythms (figure 3). Failure to recognize their nonlocalizing nature could lead to misinterpretation of the EEG picture as suggestive of multifocal symptomatic epilepsy with secondary generalization, and possibly discourage further preoperative assessment.

Therapeutic/prognostic considerations. Case 2 illustrates the difficulties in controlling patients with dual epilepsy syndromes where considerations determining choice of medical therapy may be contradictory. In this man, absence seizures were clearly exacerbated by vigabatrin,[20] responded favorably when lamotrigine was added, and apparently stopped when carbamazepine was discontinued after surgery. Drugs that may have adverse effects on "primary" generalized seizures such as carbamazepine, vigabatrin, and possibly phenytoin[21] should be

used with caution and under close monitoring if necessary, whereas those with a broad spectrum of antiepileptic effects such as sodium valproate[22] or lamotrigine may be more appropriate.

Finally, our patients and other reports[4,5] strongly suggest that the decision for epilepsy surgery should not be affected by a coexistent IGE. However, generalized seizures may not necessarily cease after successful epilepsy surgery for partial seizures. Given the natural history of IGE, up to 20% of patients with JAE may continue having brief absences or occasional GTCS and mild myoclonic jerks, although seizures become less severe with age.[14,23] JME, in turn, is usually a lifelong disorder with the patients prone to myoclonic jerks or GTCS if exposed to certain precipitating factors.[13] Therefore, in such cases, treatment should continue with the patients appropriately informed that any further GTCS, jerking, or "blackout" episodes do not necessarily imply recurrence of their partial epilepsy until proven as such on convincing EEG/video EEG grounds.

Note added in proof. Since the acceptance of this article for publication, we have documented in a 19-year-old woman the coexistence of left temporal CPS (left hippocampal atrophy on MRI, left temporal hypometabolism on FDG PET, left temporal ictal onset on scalp EEG) with the syndrome of eyelid myoclonia with absences (absences with eyelid myoclonia, GTCS, photosensitivity). This patient currently awaits left anterior temporal lobectomy for medically intractable CPS.

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Quantitative MRI in temporal lobe epilepsy

Evidence for fornix atrophy

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Article abstract—*Objective:* To investigate whether the fornix and mamillary bodies, being part of the limbic system, are abnormal in patients with mesial temporal lobe epilepsy (MTLE). *Background:* The limbic system comprises the hippocampal formation, fornix, mamillary bodies, thalamus, and other integrated structures. This system is implicated in complex functions, including memory and emotion, and in diseases such as MTLE. *Methods:* The authors performed volumetric measurements of hippocampus, amygdala, fornix, and mamillary bodies in 50 patients with MTLE and compared the results with normal controls and patients with extratemporal lobe epilepsy. *Results:* Control (n = 17) measurements of the amygdala, hippocampus, and fornix revealed larger volumes of the right hemisphere structures ($p < 0.001$). Normalized fornix volumes revealed atrophy in 86% of studies concordant with hippocampal atrophy in all cases but one. Similarly, the mean hippocampal and fornix volumes for the group discriminated the epileptogenic temporal lobe ($p < 0.001$). Limbic volumes were normal in all patients with extratemporal lobe epilepsy. *Conclusions:* Quantitative MRI findings support the concept that MTLE is not a process limited to the hippocampus but also involves other interrelated limbic system structures, in particular, the fornix.

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Mesial temporal lobe epilepsy (MTLE) is associated with a clinically well-defined epileptic syndrome: localized anterior temporal lobe EEG abnormalities, memory dysfunction, and hippocampal atrophy on MRI.[1,2] Commonly, there is underlying mesial temporal sclerosis (MTS), which is pathologically characterized by neuronal cell loss and gliosis primarily involving the CA1, CA3, and CA4 sections of the hippocampus.

Although the cornu ammonis and dentate gyrus are the major site of disease in MTS, studies demonstrate the presence of histologic changes in other temporal structures such as the amygdala, entorhinal cortex, subiculum, and parahippocampal gyrus.[3,4] Furthermore, neuronal loss and gliosis also have been found in the thalamus and cerebellum.[5] The pathologic abnormalities beyond the cornu ammonis often are less severe, suggesting that MTS is a peripherad (worse in the center and less abnormal toward the periphery) process that involves other interconnected limbic and extralimbic structures.

The relevance of the limbic system as a neural circuitry to MTLE is beginning to be recognized in multiple dimensions. Part of this circuit is known as the circuit of Papez.[6] The limbic system comprises the hippocampal formation and additional structures[7] such as the fornix, mamillary bodies, thalamus, cingulum, amygdala, orbitofrontal cortex, and thalamus. Attention has been given to hippocampal and amygdalar volumes in patients with MTS. However, recent qualitative MRI studies suggest that certain limbic system structures, such as the fornix and mamillary bodies, may be atrophic in patients with MTS.[8-11] Although of interest, these studies yield conflicting findings and are of limited use in view of the considerable normal anatomic variation of these extrahippocampal limbic structures.

We therefore performed a quantitative MRI analysis of the limbic structures, including the fornix and mamillary bodies, in patients with partial onset epilepsy. We hypothesized 1) that these structures, forming part of the limbic circuit and projecting from the hippocampus, may be affected in patients with MTLE, 2) that because of the anatomic interconnections, the structural changes will be specific to patients with MTLE, and 3) that quantitative MRI methods can demonstrate these changes.

Methods. *Subjects.* We selected 50 consecutive patients (Group 1) (31 women, 19 men; mean age 32, range 17 to 42) who had undergone temporal lobectomy for intractable temporal lobe epilepsy at the UAB Epilepsy Center between 1994 and 1995 and who had a final diagnosis of MTLE. Patients were excluded if the imaging or pathologic examination showed other disease such as tumors or developmental or vascular lesions in conjunction with MTS (dual disease). The epileptogenic temporal lobe was identified by interictal scalp EEG, prolonged EEG–video monitoring with sphenoidal electrodes for ictal analysis, and

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neuropsychological studies in all patients. In 25% of patients, data were divergent, and intracranial EEG studies (epidural or subdural strip electrodes) were used to confirm the location of the epileptogenic focus. All patients received a standard anterior temporal lobe resection according to previously reported techniques.[12]

To test our second hypothesis, we studied a group consisting of 10 patients (7 men, 3 women, mean age 30 years) with a definitive diagnosis of intractable extratemporal lobe epilepsy (Group 2). These patients underwent the same investigative diagnostic procedures as the group with MTLE, but in addition, ictal SPECT and interictal fluorodeoxyglucose (FDG)-PET were performed for seizure localization. Five of them underwent extratemporal surgical procedures, and the remaining are under pharmacologic treatment. None had evidence of temporal lobe epilepsy per clinical criteria and by the testing described earlier.

The MR control population consisted of 17 normal controls who gave informed consent for the MRI procedure. All controls were free of neurologic or psychiatric symptoms and were not taking any medication at the time of the study. One subject was scanned twice because of motion artifact in the first study. The mean age of the 17 subjects was 35 years (range 24 to 41). There were nine women and eight men.

MR acquisition. The MR studies were performed on a 1.5-T Phillips ACS unit (Best, Netherlands). A fast scout scan (axial and coronal images, 90 seconds) was obtained for proper positioning of the subject. Correction for angulation was done by modifying the angle of acquisition to be parallel to the interhemispheric fissure. After correction, a series of sagittal T1-weighted spin echo images were obtained with 5-mm sections. After these sagittal localizing images were taken, a three-dimensional acquired image through the entire brain was obtained in the coronal plane. This sequence was obtained within an angulation perpendicular to the long axis of the hippocampus (repetition time, 20 milliseconds; echo time, 6.1 milliseconds; matrix size, 218 $\times$ 256; field of view, 23 cm). In most patients, approximately 110 to 130 slices were obtained in the coronal plane. Image slice thickness was 1.5 mm with no gaps. If any set of images had motion artifact, it was repeated.

MR image analysis. The images were transferred to a Phillips Gyroview workstation and analyzed using commercially available software (ISG Technologies Inc., Malton, Canada). Volumetric measurements were performed with an interactive hand contouring device. Each image was enlarged by zooming the image by power of 2 and optimizing contrast to facilitate differentiation between tissues. The amygdala, hippocampus, fornix, and mamillary bodies were measured. The slice volume was calculated by multiplying the area outlined by slice thickness. The total volume of the structure then was calculated by adding each slice volume for the structure. Individual variance of the volume of structure of interest was normalized by the subject's total intracranial volume using validated methods.[13] This permits obtaining measurements of normalized volumes of these structures. The mean, SD, and 95% confidence interval (CI) were determined for the normal control subjects.

To minimize partial volume effect, the three-dimensional MRI data were resampled as 1-mm thick images (no gap) using multiple planes of reconstruction to follow the anatomic structure of the fornix. At the level of the rostral crux, the fornix position usually is perpendicular to the scan plane used to study the hippocampus. However, as it moves caudally, it may acquire a 45- to 60-degree angulation, and, therefore, reformatted images at an angle perpendicular to the hippocampal axis are needed for accurate assessment of the fornix crux. To avoid overlapping regions in the curve areas, we used parallel slices. In contrast, the fornix body is almost parallel to the plane of the hippocampus, and the raw images can be used for volume measurements. Because of anatomic variability, the range of slices measured and obtained for volumetric measurements of the fornix was 10 to 18, with a mean of 15.

Intrarater and interrater reliability of volume measurement was assessed by repeated measurements performed by two observers and repeated twice in the same control by the same observer. Assessment of interrater reliability was performed using the intraclass correlation coefficient (ICC) as described previously.[14] ICC values were calculated on all volumes, but we report only those calculated for the absolute volumes. ICC calculations are more accurate measurements of reliability between observers.

Statistical analysis included a Student's *t*-test to assess for differences between mean volumes for right and left hippocampus, amygdala, fornix, and mamillary body in controls and patients. In addition, an analysis of variance (ANOVA) was performed to correct for other variables, including total volume effects. We used a Pearson's correlation to investigate the relationship between hippocampal and fornix volumes.

Anatomic guidelines. Anatomic guidelines for the structures of interest were established using Duvernoy's anatomic sections.[15] Anatomic guidelines for measurements of the amygdala and hippocampus formation were described previously.[16] The evaluation of each slice excluded the parahippocampal gyrus. Volumetric measurements of the amygdala and hippocampus were directed out from anterior to posterior.

The methodology and anatomic boundaries for measuring the fornix and mamillary bodies have been described by our group in detail previously.[17] In summary, delineation of the fornix boundaries is relatively simple because it usually is surrounded by either gray matter structures or CSF. The posterior boundary of the fornix was established at the most posterior image, where clear visualization and separation of the fornix from the fasciola cinerea was observed (figure 1). The fornix takes a caudal course, curving upward and medially to the posterior end of the callosum, and then runs forward and anterior to the hippocampal commissure, where it becomes the body of the fornix in which both fornices join together to form a bundle (figure 2). Separation from the surrounding structures still is possible anteriorly, but the body becomes difficult to separate into two structures immediately anterior to the foramen of Monro. Although in some individuals this separation into two bundles is possible, we elected to measure the fornix up to the foramen of Monro.

Anatomic demarcation of the mamillary bodies used similar principles. Inferiorly, the CSF signal from the interpeduncular system and, laterally, the substantia nigra, which can be seen on the coronal images, demarcate its boundaries. It also is easy to identify, since the mamillothalamic tract can be well demarcated from the surround-

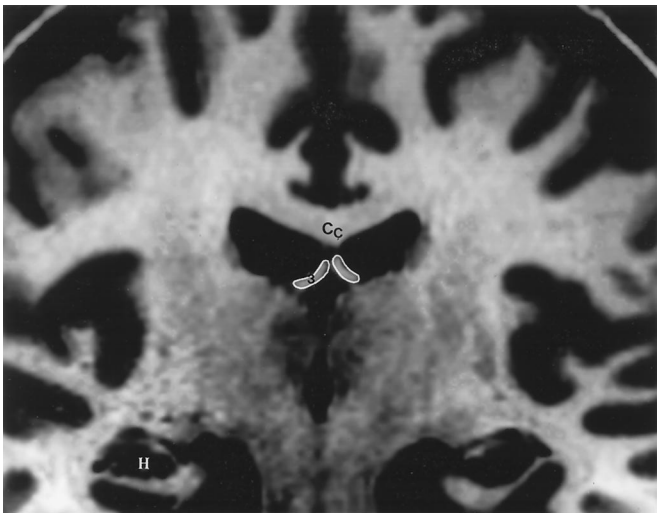

Figure 1. Coronal anatomic section at the level of the third ventricle showing the fornix bodies.[3] The anatomic image was used as reference for the volumetric measurements. Cc= corpus callosum; H = hippocampus.

ing structures medially by the dorsal thalamic nucleus and laterally by the subthalamic nucleus.[15] Because the mamillary bodies are small structures, the measurements were carried out in both coronal and axial planes using three coronal slices and one axial slice. We added the areas of all slices to obtain a single volume correcting by intracranial volume.

Results. *Controls*. Table 1 presents the mean volumetric data in the control population, 2 SD (95% CI), and lower limit of range defined as 2 SD below the mean control values corrected for intracranial volume. The mean scores for the hippocampal and amygdala formation revealed a larger mean volume of the right hemisphere structures ($p < 0.001$). Similarly, volume measurements of the fornix and mamillary bodies revealed a right hemisphere structures asymmetry ($p < 0.001$). This asymmetry has been reported for the hippocampus previously.[13] ANOVA was performed to assess possible variance and effect of whole-brain volumes. ANOVA showed no significant differences (F = 0.9).

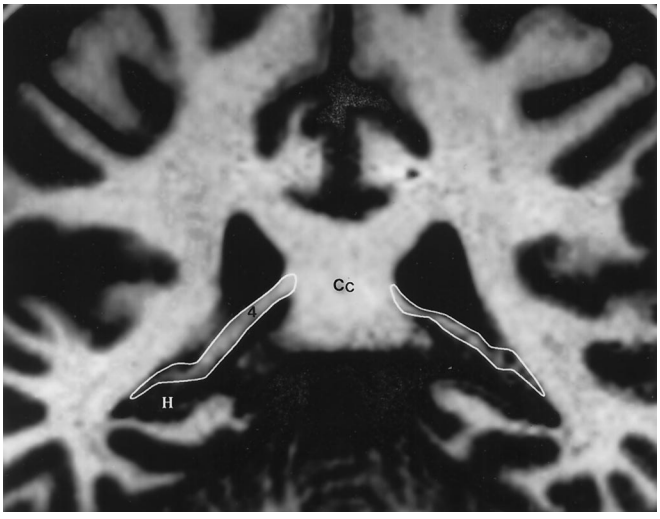

Figure 2. Coronal section at the level of the crus of the fornix and hippocampal tail. The anatomic image was used as reference in the demarcation of the fornix crus[4] for volumetric measurements. Cc= corpus callosum; H = hippocampus.

***Table 1** Mean volumes, SD, and −2 SD for controls (n = 17)*

Structure	Volume*	Mean SD	−2 SD
Left fornix	46	7.6	32
Right fornix	47	6.9	35
Left mamill	18.6	4.2	13
Right mamill	20	3.2	14
Left hippocampus	3,575	292	3,062
Right hippocampus	3,692	293	3,178
Left amygdala	2,390	265	1,924
Right amygdala	2,510	255	2,062

* Normalized volumes are in mm^3.

Mamill = mamillary body.

The intraindividual coefficient of variation was 3.5%. The reliability of measurements and observers for left and right structures was significant at the $p < 0.05$ level except for the mamillary bodies. The ICC for measurements for hippocampus was 0.86, amygdala, 0.78, and fornix, 0.82. The ICC for mamillary body was 0.57.

Patients (Group 1). There were no statistically significant difference between patients and control groups with respect to mean age at time of the imaging study. To avoid possible effects of age on the patient group, only adults (17 to 42 years) were included in this analysis.

Fornix atrophy. Figure 3 demonstrates the subdivision of patients according to the presence or absence of hippocampal atrophy (HA). Forty-eight (96%) patients had evidence of hippocampal volume loss below 2 SD of control values. The hippocampal volumes were normal in two patients. Pathologic examination demonstrated end-folium sclerosis in these two patients.

Patients with unilateral HA. Thirty-seven (74%) of the population studied had evidence of unilateral hippocampal volume loss. The frequency of unilateral HA in the group with hippocampal abnormalities was 77% (37 of 48).

There was evidence of fornix volume loss (mean − 2 SD from normal values for respective side) in 32 (86%) patients. In all patients in this group, the fornix atrophy was ipsilateral to the atrophic hippocampus (figure 4). Five patients had no evidence of fornix atrophy on either side.

Table 2 shows the group comparisons for left and right hippocampus and fornix structures. As demonstrated, statistically significant mean group differences were found for left and right temporal lobe patients for the hippocampus and fornix. In addition, there were no differences between control data and contralateral temporal lobe structures. Pearson's analysis showed no significant correlation between the fornix and hippocampal volume in this group.

Patients with bilateral HA. Bilateral HA was present in 11 (22%) patients. Ten patients had evidence of asymmetric volume loss with statistically significant volume differences for lateralization to the side of surgery. Nine of these patients had evidence of unilateral fornix atrophy.

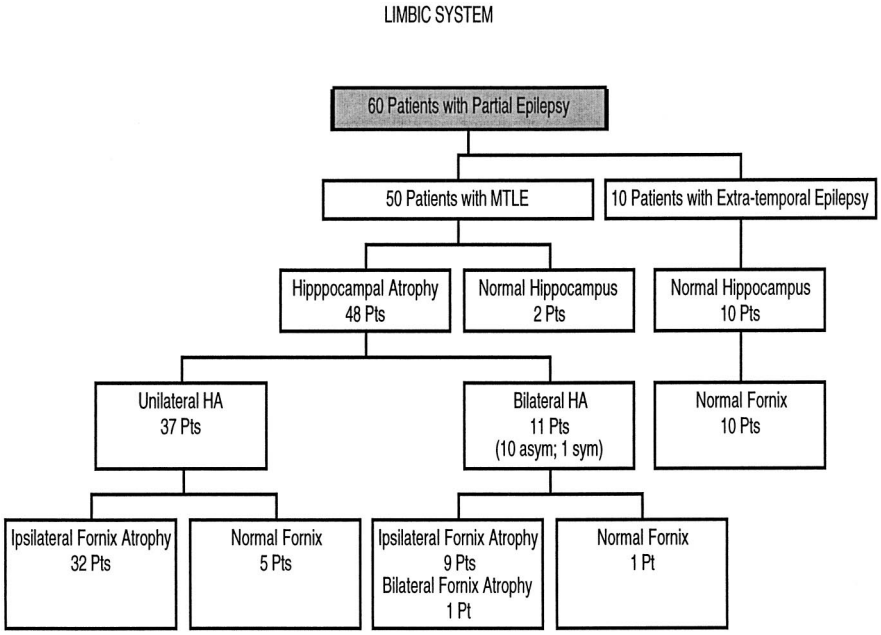

Figure 3. Subdivision of 60 patients with intractable partial onset epilepsy (50 with mesial temporal lobe epilepsy [MTLE]) on the basis of quantitative MRI.

The fornix atrophy was on the same side as the smaller hippocampus. One patient had bilateral fornix volume loss.

The one patient with symmetric bilateral HA did not show fornix atrophy.

Patients with normal hippocampus. Two patients had normal hippocampal volumes. Fornix volumes were within normal range in both individuals.

Patients with unilateral amygdala atrophy. Eleven (22%) patients had evidence of unilateral amygdalar atrophy. Concordant fornix atrophy was observed in eight patients. Three others had evidence of contralateral fornix atrophy. Interestingly, hippocampal atrophy in these three patients correlated with the side of fornix rather than the side of amygdalar volume loss.

Patients with bilateral amygdala atrophy. One patient had evidence of bilateral asymmetric amygdala atrophy with discordant unilateral fornix atrophy. This patient exhibited bilateral hippocampal atrophy.

Patients with extratemporal lobe epilepsy (Group 2). To test the second hypothesis and to test the specificity of the quantitative findings, we studied 10 patients with well-defined extratemporal lobe epilepsy. None of the patients in this group had evidence of hippocampal or amygdala atrophy. Similarly, none demonstrated statistically significant evidence of asymmetric or symmetric fornix atrophy.

Mamillary bodies (Groups 1 and 2). Volumetric measurements indicated that 34 (41%) patients with MTLE (Group 1) had evidence of mamillary body atrophy. Conversely, no patient with extratemporal lobe epilepsy (Group 2) had atrophy of these structures. However, in view of the suboptimal ICC of these structures, the measurements are not valid for the purpose of analysis.

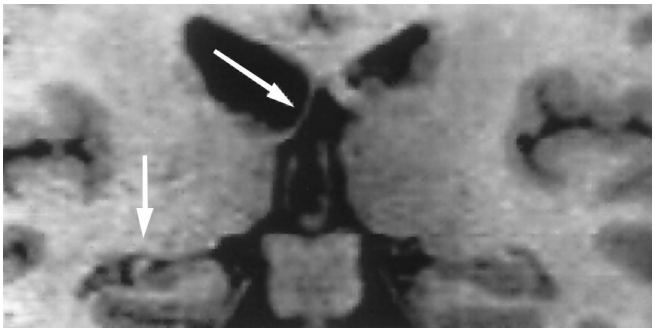

Figure 4. Representative example of MRI showing severe right hippocampal and fornix atrophy in a patient with right MTLE onset and pathologically proven MTS. The volumes of the right hippocampus (arrow) and fornix (arrow) in this patient were 2 SD below normal range, whereas the left-sided structures are normal.

Discussion. In agreement with previous quantitative MRI studies, most of the selected patients demonstrated evidence of unilateral hippocampal atrophy with bilateral hippocampal volume loss observed in 22%. In addition, 25% of our patients had evidence of unilateral amygdalar atrophy, which is consistent with other studies.[16] These results are in agreement with previous studies,[16] but the higher frequency of HA in our population reflects the homogenous nature of the selected group; all patients were selected from a surgical series and had evi-

Table 2 *Volume structural differences from control data (mean and SD in mm^3)*

	Patients		
Structure	R TLE	L TLE	*p* Value
Right hippo	2461 (40)*	3523 (70)	<0.001
Left hippo	3270 (40)	2520 (41)*	<0.001
Right fornix	32 (5.1)*	44 (5.7)	<0.001
Left fornix	41 (7.5)	31 (6.1)*	<0.001

* Statistically significant difference from control values.

Hippo = hippocampus; TLE = temporal lobe epilepsy.

dence of MTS. This group is well suited for the investigation of our hypotheses.

The results of the quantitative fornix measurements showed that 86% of patients with unilateral hippocampal atrophy had evidence of fornix volume loss. Furthermore, fornix atrophy was present in all patients but one of those with bilateral asymmetric hippocampal atrophy. In addition, the two patients without hippocampal atrophy by MRI but MTLE had normal fornix volumes. Our results confirm pathologic data and buttress previous qualitative MRI observations suggesting volume loss in extrahippocampal limbic system structures.[8,9] In addition, they parallel a recent quantitative MRI study suggesting widespread volume loss in subcortical structures of patients with MTLE.[18] This suggests that the findings of ipsilateral fornix atrophy in most patients with MTS are not coincidental but reflect analogous pathologic changes to limbic circuit interconnected structures.

Technical limitations. Although our hippocampal and amygdalar measurements are in close agreement with some groups, they differ from others.[19-21] It is well established that the variability across centers is a problem that may be dependent on the anatomic boundaries and may result from the different methods of volumetric assessment. This also is in agreement with histologic studies in which wide variability between hippocampal formation volumes has been reported.[22] Our study is the first to quantify the volumes of the fornix in controls and patients; hence, no comparative data are available for the fornix in patients with MTLE. However, one study[23] using three-dimensional CT reconstruction of the limbic structures found that the right fornix was larger than the left. The mamillary bodies were found to be symmetric. Since the ICCs for the MB measurements were unacceptably low, we can conclude that the results are unreliable for volumetric analysis.

Another potential problem pertains to partial volume effect. This issue requires particular attention since recent data indicate that errors from volume averaging or interpolated voxel values may result in volumetric differences of up to 15%.[24] Although the fornix fibers at the level of the crus can be well resolved using reconstructed images, partial volume effect remains a potential problem. To reduce this limitation, we used reformatted images (1 mm) obtained in planes perpendicular to the axis of the fornix to improve the accuracy of our measurements. Another limitation is the operator-dependent volume determination. Thresholding techniques can overcome some of these problems and can facilitate the volumetric measurements. However, thresholding techniques can be unreliable when structures are extensively convoluted, such as in the limbic system. A combination of segmentation techniques that perform volume averaging correction for CSF, gray and white matter, and outlining may be optimal for the measurement of small structures.[25]

Significance of findings to epilepsy. Our study strongly supports qualitative MRI observations suggesting that the fornix is atrophic in patients with MTLE.[8,10] It also supports previous anatomophatologic and MRI- based studies showing neuronal cell loss and atrophy in extrahippocampal structures such as the thalamus and amygdala of patients with MTLE.[5] The presence of fornix volume loss is associated with the well-known anatomic pathways identifying the fornix as the major efferent pathway of the hippocampus involving both precommissural and postcommissural fibers.[26] Although we elected not to use the mamillary body analysis in this study, 40% of patients had evidence of atrophy. The findings underscore the differential effect of specific pathway involvement.

Although this study establishes a relationship between hippocampal and fornix volume loss, the mechanism for the current findings is not known. Hippocampal atrophy in MTS is primarily associated with CA1, CA3, and CA4 neuronal cell loss. Although anecdotal, autopsy specimens have shown fornix myelin loss and gliosis in a few patients with MTS.[27] There are two possible explanations for the fornix findings. The first is that fornix atrophy is simply secondary to wallerian degeneration from hippocampal cell damage, resulting in deafferentiation cell loss. If this was the mechanism, we would have expected to find a direct correlation between the hippocampal and fornix volumes (Pearson's = 0.54); that is, the more atrophic a hippocampus, the more atrophy will be present in the fornix. However, such a volume correlation may not be apparent, although a relationship between the two structures may be present. Alternatively, limitations in the accuracy of the fornix measurements from small volumes may prevent a clear correlation. Conclusive evidence for this hypothesis could derive from longitudinally studying children with recent-onset epilepsy and hippocampal injury. A second plausible hypothesis is that the structural changes are the end result of recurrent abnormal excitotoxic damage to axonal flow. Evidence for increased excitability and inhibition may be associated with the sprouting of new excitatory and inhibitory hippocampal connections in the well-documented synaptic reorganization that is associated with hippocampal sclerosis. This mechanism is proposed for the pathophysiologic mechanism of MTS.[28] Animal studies demonstrate that neuronal injury may be produced by abnormal epileptogenic activity along physiologic pathways resembling the changes observed in the hippocampal model.[29] These changes are induced by release of excitatory amino acids such as glutamate. These metabolic abnormalities have been recently reported using in vivo MR spectroscopy in focal status epilepticus.[30] Although the end result may be structural atrophy from myelin loss and gliosis, and, thus, it may be indistinguishable from pure hippocampal deafferentiation secondary to hippocampal cell loss, these mechanisms are pathophysiologically distinct.

Further studies are needed to unravel the mechanism of these changes.

This study demonstrates that fornix atrophy often is present in patients with MTLE from hippocampal sclerosis. Furthermore, these changes are not observed in patients with extralimbic onset epilepsy. These quantitative MRI findings support the concept that MTS is not a process limited to the hippocampus but also involves other interrelated limbic system circuit structures. How these structural changes relate to clinical variables such as febrile convulsions, seizure duration, and surgical outcome are topics currently under investigation. Future studies should answer whether these structural changes represent an epiphenomenon or are intrinsically associated with the development of epilepsy.

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Cognitive and magnetic resonance imaging aspects of corticobasal degeneration and progressive supranuclear palsy

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Article abstract—*Objective:* To identify cognitive and MRI features important for the clinical diagnosis of corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP); these diseases share several clinical features and are often difficult to distinguish on clinical grounds. *Methods:* Cognitive functions and MRI characteristics were examined in 16 patients with CBD and 28 patients with PSP, all diagnosed according to current clinical criteria (none was examined by autopsy). *Results:* MRI findings differed significantly between the two groups: 87.5% of patients with CBD but none with PSP had asymmetric frontoparietal atrophy, whereas 89.3% of patients with PSP but only 6.3% of those with CBD had midbrain atrophy. Cognitive examination showed that ideomotor apraxia (De Renzi's test) was significantly more frequent in CBD, and executive functions (Nelson's test) were significantly more impaired in patients with PSP. *Conclusions:* MRI findings of asymmetric frontoparietal atrophy in CBD and midbrain atrophy in PSP are the most consistent and useful aids to careful clinical evaluation for differentiating between the two diseases.

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Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) have distinctive clinical pictures when fully expressed, but diagnosis is often uncertain at disease onset because many neurologic signs are shared and atypical presentations are not unusual.[1-3] In addition, autopsy series have shown incorrect clinical diagnoses in both PSP and CBD, as well as pathologic overlap between them, and between them and other neurodegenerative diseases.[4-6] A study of the accuracy of the clinical diagnosis of CBD noted that approximately 40% of CBD cases were erroneously diagnosed as PSP.[7]

It is now clear that definite diagnosis of both diseases depends on the presence of a series of clinical and pathologic characteristics.[7,8] Criteria for the probable clinical diagnosis of PSP have been elaborated by Litvan et al.[8] based on pathologically proven cases. A similar set of clinical criteria has not been established for CBD. However, recent clinical papers emphasize that specific patterns of cognitive impairment and distinctive neuroradiologic findings can assist the clinical diagnosis of these parkinsonisms in life.[9,10]

We studied neurologic, neuropsychological, and radiologic features in patients with putative PSP and CBD consecutively presenting at our institute, with the aim of identifying traits most useful for diagnosis in life.

Patients and methods. All patients with clinically probable PSP or CBD presenting at the outpatient clinic or admitted between January 1994 and August 1997 were included in the study. Twenty-eight had probable PSP (19 men, 9 women) and 16 had probable CBD (10 men, 6 women). Probable PSP was diagnosed according to the criteria of Litvan et al.[8]: age at onset greater than 40 years, supranuclear vertical gaze palsy, and postural instability in the first year of illness, with poor or absent levodopa response in the absence of other diseases that could explain the symptoms and signs.

We used the following criteria to diagnose probable CBD[7,11]: presence of slowly progressive asymmetric akinetic-rigid syndrome with one or more of the following signs of cortical impairment: ideomotor apraxia, myoclonus, sensory deficit of cortical origin, and alien limb syndrome, associated with late-onset walking and stability disorders. Again, lack of response to levodopa and exclusion of other diseases that could explain the symptoms and signs were essential for the diagnosis.

All patients underwent neurologic examination when first seen, when neurologic symptoms at disease onset were elicited. Motor disability was quantified using the Schwab and England disability scale[12]; the presence of major depression and dementia were determined according to *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) criteria.[13] The clinical diagnosis of dementia was also supported by a Mini-Mental State Examination (MMSE) score of less than 24.[14]

Mean age was 64.5 (SD 7.1) years in PSP and 67.1 (6.6)

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years in CBD; mean duration of education was 7.1 (4.5) years in PSP and 9.4 (5.2) years in CBD; mean illness duration was 3.9 (3.5) years in PSP and 2.9 (1.6) years in CBD; mean Schwab and England disability scale score was 55.0 (22.5) in PSP and 45.0 (26.6) in CBD; and mean MMSE score was 24.8 (2.9) in PSP and 23.8 (4.5) in CBD.

Patients underwent a comprehensive neuropsychological examination that included the following tests. Raven's Colored Progressive Matrices[15] measures logical reasoning; it consists of a series of visual pattern analogy and matching problems and requires understanding of spatial, numeric, and shape relationships between the elements of the designs presented. The Short Tale test,[16] a long-term verbal memory test, requires free recall of as many units of information as possible from a brief story read aloud. Benton's Visual Orientation Line test[17] examines the ability to estimate angular relationships between different lines by visually matching pairs of lines at different angles to a display of 11 numbered lines forming a semicircle. The Nelson test is a modified version of the Wisconsin Card Sorting test[18] designed to examine set-shifting and categorization abilities; it uses fewer cards than the original test. The Phonemic Verbal Fluency test[19] examines word-searching strategy and requires the subject to generate as many words as possible beginning with a given letter, in a given time. The Visual Search test[19] requires sustained attention, visual scanning, and speed of motor response and requires crossing out all target numbers randomly interspersed in rows of numbers, in a given time. Patients also performed the De Renzi Ideomotor Apraxia test with both arms[20]: the subject is asked to imitate single and in-sequence gestures as well as meaningful and nonmeaningful motor acts.

Adjustments for age and education were performed on Raven test scores,[21] on MMSE scores,[22] and on other cognitive test scores.[23] Exceptions were the Nelson and Ideomotor Apraxia tests, for which no adjustments are available. Based on the Edinburgh Inventory,[24] 27 patients with PSP and 13 with CBD were right handed, and 1 patient with PSP and 3 with CBD were left handed. Patients' performance was also compared with that of a group of normal control subjects matched for age and education.

All patients underwent brain MRI (0.5 or 1.5 T) and the findings were assessed by two experienced neuroradiologists blinded to each other's assessment and to the clinical diagnosis. T1-weighted images were acquired to examine the distribution of atrophy, and proton-density and T2-weighted images to detect signal abnormalities. Pathologic reports show that PSP is associated with midbrain atrophy,[25] whereas CBD is associated with cortical atrophy of the superior frontoparietal region on the side contralateral to greatest clinical impairment.[26] The most frequent MRI abnormalities present in other degenerative parkinsonisms were also considered in the differential evaluation of the images.[10] Particular attention was paid to the presence of the following:

1. Atrophy of midbrain and superior colliculi.
2. Location and distribution (symmetric or otherwise) of cortical atrophy.
3. Signal abnormalities in the basal ganglia and the periaqueductal region or in other locations.

The extent of cortical and midbrain atrophy was not measured quantitatively but graded as absent, slight, moderate, or marked by means of a post hoc, between-patient comparison on homologous slices. When judgments differed, the MRI films were reviewed to reach a consensus.

Table 1 *Symptoms at disease onset in 28 patients with clinically diagnosed progressive supranuclear palsy (PSP) and 16 patients with clinically diagnosed corticobasal degeneration*

Symptoms at disease onset	PSP, n (%)	Corticobasal degeneration, n (%)
Postural instability	9 (32)	
Behavioral alterations	8 (29)	
Visual disturbances	7 (25)	
Walking problems	5 (18)	
Motor slowness	5 (18)	3 (19)
Lateralized sensory disturbance		5 (31)
Lateralized motor clumsiness	2 (7)	6 (37)
Cognitive impairment	1 (4)	
Speech disorders	1 (4)	3 (19)
Tremor		1 (6)
Alien limb		1 (6)
Hallucinations		1 (6)

We used the Mann-Whitney *U* test to analyze nonparametric nonpaired data, and Fisher's exact test to compare proportions. To identify variables that best differentiated PSP from CBD, we performed a logistic regression analysis on the cognitive and radiologic variables considered prognostic for PSP or CBD.

Results. The two patient groups did not differ in age, education, illness duration, or Schwab and England disability score. Mean MMSE scores were low in both groups and did not differ statistically: nine patients with PSP (32%) and six with CBD (37.5%) had MMSE scores $\leq$24; these patients were also demented according to DSM IV criteria. No patients had major depressive illness.

Among the patients with probable CBD, the most frequent symptoms at disease onset were lateralized motor and sensory disturbances, motor slowness, and speech difficulties (table 1). Speech alterations are considered uncommon at disease onset in CBD,[27] but were relatively frequent in our series and consisted of dysarthria, hypophonia, and tachyphemia. Postural instability, behavioral modifications (apathy, abulia), and visual disturbances (blurred vision) were the most frequent onset symptoms in the patients with PSP[28] (see table 1).

Table 2 lists the various neurologic signs presented by the patients at diagnosis, in relation to illness duration: postural instability, eye movement alterations (vertical paralysis, slow horizontal saccades), and walking disorders were frequent early signs in PSP[28]; behavioral and cognitive disturbances (dementia or isolated cognitive impairments) were also common in the disease's early years. In CBD, motor slowness and other motor signs (motor neglect, apraxia, postural tremor and dystonic postures usually affecting one upper limb distally) were frequent in the early stages of the disease, although, unlike in PSP, walking disorders manifested only later. Eye movement alterations were present in 60% of patients with CBD in the early years of the disease and usually consisted of reduced

Table 2 *Clinical signs at diagnosis in 28 patients with clinically diagnosed progressive supranuclear palsy (PSP) and 16 patients with clinically diagnosed corticobasal degeneration (CBD)**

	1–3 Years, n (%)		>3 Years, n (%)	
Clinical signs	PSP, n = 19	CBD, n = 10	PSP, n = 9	CBD, n = 6
Postural instability	19 (100)	3 (30)	9 (100)	5 (83)
Gaze disturbances	19 (100)	6 (60)	9 (100)	6 (100)
Gait disorders	18 (95)	6 (60)	9 (100)	6 (100)
Bradykinesia	18 (95)	10 (100)	9 (100)	6 (100)
Dysarthria	15 (79)	6 (60)	9 (100)	4 (67)
Behavioral alterations	13 (68)	3 (30)	4 (44)	2 (33)
Cognitive impairment	12 (63)	3 (30)	6 (67)	4 (67)
Dystonia	3 (16)	7 (70)	6 (67)	6 (100)
Axial rigidity	14 (74)	2 (20)	9 (100)	3 (50)
Tremor	1 (5)	7 (70)	1 (11)	4 (67)
Ideomotor apraxia		7 (70)	1 (11)	6 (100)
Alien limb		7 (70)		6 (100)
Myoclonus		6 (60)		5 (83)
Cortical sensory deficit		3 (39)		5 (83)

* The two disease groups are subdivided according to illness duration.

excursion of vertical eye movements, jerky pursuit movements, and slow saccades. Only one patient with CBD had vertical and horizontal eye movement palsy.

Cognitive test findings. Cognitive test performance in the two patient groups was significantly worse than that of the normal control group in all tests. The two patient groups did not differ in their performance of most cognitive tests. Exceptions were the Nelson test, where patients with PSP were significantly worse than patients with CBD, and the ideomotor apraxia test, were patients with CBD did significantly worse than patients with PSP (table 3).

Ideomotor apraxia scores were divided according to most and least compromised upper limb. Seven patients with CBD had greater motor impairment in the right arm and nine on the left. In patients with PSP, the arm with the worst performance on the ideomotor apraxia test was considered the most compromised arm, although the difference in performance was minimal. Patients with CBD did significantly worse than those with PSP in both arms (see table 3).

MRI. The cerebral atrophy findings demonstrateded by MRI are summarized in table 4 and show that cortical atrophy was significantly worse in the CBD than PSP group, whereas midbrain atrophy significantly predominated in patients with PSP. In addition, asymmetric cortical atrophy was present in 14 patients with CBD (87.5%) but in no patients with PSP ($p < 0.0001$, Fisher's exact test). Asymmetric atrophy was particularly evident in coronal sections, which showed decreased white matter bulk with widening of the ventricles and sulci on the most affected side, whereas sagittal images best demonstrated atrophy in the posterior frontal and parietal regions[29] (figure

Table 3 *Cognitive and ideomotor apraxia test results in patients with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD)*

Cognitive test	PSP, mean (SD)*	CBD, mean (SD)†	*p* Value‡	Control subjects, n (%)§
Raven test	21.5 (5.8)	24.8 (6.5)	NS	33.1 (2.7)
Short tale test	9.7 (3.4)	9.6 (4.0)	NS	15.4 (4.4)
Verbal fluency	14.7 (6.4)	16.4 (9.1)	NS	35.4 (8.2)
Visual search	28.9 (15.8)	21.4 (9.6)	NS	52.7 (4.3)
Benton test	14.2 (6.9)	15.6 (6.9)	NS	26.1 (3.8)
Nelson test	2.3 (1.3)	3.6 (1.8)	0.02	6.1 (2.0)
Ideomotor apraxia (more compromised arm)	55.6 (10.7)	20.9 (25.9)	0.0005	71.2 (1.6)
Ideomotor apraxia (less compromised arm)	60.2 (7.0)	40.0 (25.9)	0.04	70.6 (2.0)

* n = 28; 19 men, 9 women.
† n = 16; 6 men, 10 women.
‡ Comparison between patients with PSP and those with CBD (Mann-Whitney *U* test). NS = not significant.
§ n = 16; 9 men, 7 women.

Table 4 *Cerebral atrophy shown by MRI in 28 patients with clinically diagnosed progressive supranuclear palsy (PSP) and 16 patients with clinically diagnosed corticobasal degeneration (CBD)*

Degree of atrophy	Midbrain atrophy*		Cortical atrophy†	
	PSP	CBD	PSP	CBD
Marked	4 (14.3)			1 (6.3)
Moderate	11 (39.3)		8 (28.6)	11 (68.7)
Slight	10 (35.7)	1 (6.3)	13 (46.4)	4 (25.0)
Absent	3 (10.7)	15 (93.7)	7 (25.0)	

Values are n (%).

* $p < 0.0001$ (Fisher's exact test).
† $p < 0.01$ (Fisher's exact test).

1). Signal abnormalities consisting of slight hyperintensity in proton-density and T2-weighted images in the atrophic cortex and underlying white matter were seen in six patients with CBD. When midbrain atrophy was present, sagittal sections were particularly useful in demonstrating thinning of the quadrigeminal plate, which was more marked superiorly. Another atrophic feature in patients with PSP was dilatation of the third ventricle, which became wider than the lateral ventricles; this, in association with midbrain atrophy, often resulted in a concave aspect of the posterior part of the floor of the third ventricle on midline sagittal sections (figure 2A).

Putaminal hyperintensity in T2-weighted images (well seen on 0.5 T), suggesting gliosis, and striatal hypointensity (on 1.5 T), suggesting deposition of paramagnetic material, were absent in most patients of both groups. In one patient with PSP, loss of signal intensity in the putamen equaled that in the pallidum; however, in this elderly (79 years of age) patient, putaminal iron accumulation may equal that in the pallidum. Two other patients with PSP had well-defined areas of signal hyperintensity in T2-weighted images in the basal ganglia consistent with ischemic lesions. The most common signal abnormality in our patients with PSP was a slight increase in signal intensity in intermediate or proton-density images in the periaqueductal region (where gliosis is found in pathologic specimens[10]; see figure 2B). This was found in 17 patients with PSP (60.7%), but in no patients with CBD ($p = 0.0005$, Fisher's exact test).

Regression analysis. We could not include the variables midbrain atrophy and asymmetry of cortical atrophy in the logistic regression analysis because some cells remained empty owing to the small sample size; calculation of the odds ratios was therefore impossible. Regression analysis of the remaining neuroradiologic and cognitive variables showed that the set of variables that best predicted the two diseases was bilateral cortical atrophy, the Nelson test, and the ideomotor apraxia test. By examining the β parameters and odds ratios, it was evident that greater cortical atrophy (β parameter = −4.46; 95% confidence interval [CI] = −7.82 to −1.09; odds ratio 0.01; 95% CI = 0.00 to 0.17; $p = 0.01$) and better performance in the

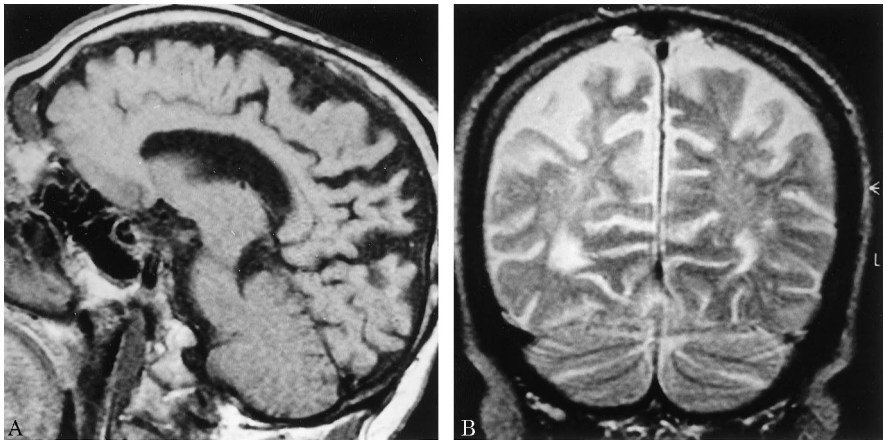

Figure 1. MRI of 66-year-old patient with corticobasal degeneration signs more prominent on left. T1-weighted sagittal section of right hemisphere (A) shows atrophy in posterior frontal and parietal regions. T2-weighted coronal section (B) shows cerebral atrophy, more severe on the right.

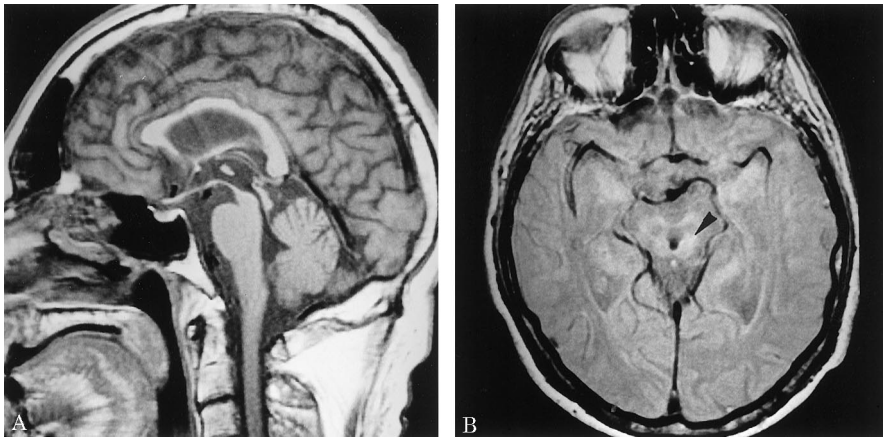

Figure 2. MRI of 59-year-old patient with progressive supranuclear palsy. T1-weighted midline sagittal section (A) shows midbrain atrophy. Axial proton-density image (B) shows slight hyperintensity in dorsal midbrain (arrowhead).

Nelson test (β parameter = −1.29; 95% CI = −2.55 to −0.03; odds ratio 0.28; 95% CI = 0.05 to 0.72; $p = 0.04$) were significantly associated with CBD, whereas better ideomotor apraxia score (β parameter = 0.11; 95% CI = −0.02 to 0.23; odds ratio 1.11; 95% CI = 1.02 to 1.36; $p = 0.10$) was associated with PSP, although not significantly so.

Discussion. Our unselected series of patients with CBD and PSP did not differ in terms of overall motor or cognitive disability, as assessed by the Schwab and England disability scale and MMSE, respectively. Cognitive ability was decidedly compromised in both groups, with 32.1% of patients with PSP and 37.5% of those with CBD scoring 24 or less on MMSE; these patients were also demented according to DSM IV criteria. The frequency of dementia in our PSP group is consistent with other reports,[30] whereas the frequency of dementia in CBD was slightly higher than reported in a large series of patients with CBD who did not undergo comprehensive neuropsychological assessment.[27]

Detailed examination of cognitive performance did not reveal distinct patterns of impairment in the two groups. Only ideomotor apraxia (CBD worse than PSP) and the Nelson test scores (PSP worse than CBD) differed significantly. These results are in agreement with previous reports comparing patients PSP and CBD,[30] and patients with CBD and AD[31] on cognitive performance. According to the De Renzi test, in which a score of ≤53 indicates ideomotor apraxia, 12 patients with CBD (75%) had ideomotor apraxia, which is close to the 80% cited in current literature,[30-32] whereas only 10 patients with PSP (35.7%) were apraxic.

Two of our patients with CBD had apraxia in the left arm only, which was the one with greater motor impairment. The remaining 10 apraxic patients with CBD had bilateral apraxia that was asymmetric in severity, being greater in the arm with greater motor compromise (7 of these patients had greater motor impairment on the right and 3 on the left).

All our apraxic patients could recognize meaningful gestures and discriminate between erroneous and correct gestures, although they were often unable to imitate them, suggesting that space-time movement representations, located in the left inferior parietal lobe, were intact.[33] Furthermore, none of our apraxic patients was able correctly to execute gestures in everyday life (outside the laboratory) and thus did not show the voluntary-automatic dissociation characteristic of patients with ideomotor apraxia. A similar lack of dissociation was found by Denes et al.[34] in five patients with degenerative diseases, four of whom had probable CBD.

Although no patients with PSP had pyramidal or sensory defects on neurologic examination, eight (50%) patients with CBD had cortical sensory deficit consisting of double simultaneous tactile extinction, astereognosis, or reduced graphesthesia, and some who underwent magnetic stimulation had prolonged central motor conduction times. According to the definition of ideomotor apraxia,[35] which requires that motor and sensory pathways be intact, these patients with CBD should not be considered apraxic; minor motor deficits can explain their difficulties in executing single-finger movements when the ability to execute global hand movements is retained. However, the severe impairment of gesture execution observed in our patients with CBD with a De Renzi score in the apraxia range cannot be explained by minimal motor or sensory defects, but points to higher-order motor dysfunction.

Overall these findings indicate the presence of anterior apraxia as proposed by Freund,[36] which includes limb-kinetic apraxia, due to a dysfunction in the premotor cortex or the sensorimotor cortex.

Analysis of apraxic error types showed that they differed quantitatively between patients with PSP and CBD, with patients with PSP making more sequence errors and patients with CBD more clumsiness and orientation errors. A sequence error was defined as one in which the sequence of components in a complex gesture was changed or there was a tendency toward perseveration; a motor error was posited if the gesture was awkward, incomplete, or contaminated by extraneous movements. Slow movement or low amplitude were not considered errors because these are implied in the definition of akinesia.

This difference may be because PSP characteristically involves the motor and premotor cortex, whereas in CBD there is parietal involvement as well. Greater prefrontal dysfunction in PSP, supported by pathologic findings[37] and PET studies,[38] would also explain the greater impairment we found in the Nelson test. This test assesses attentional shifting and categorization abilities, which are known to be associated with the prefrontal areas.

Although we found no other significant differences between the patient groups in the neuropsychological examinations, it is possible that impairment of different cognitive processes underlies equally poor performance in some tests. Thus, in the visual search test, which requires visuomotor coordination and the ability to shift attention between designs, performance was similarly poor in both groups of patients. Although defective attention and motor slowness have been described in both diseases,[30] in PSP impaired ocular scanning due to supranuclear palsy and prominent frontal dysfunction may also have affected performance. Conversely, because most patients with CBD were right handed and had greater motor impairment in their right hand, the disadvantage in using this hand in the visual search test (which requires the motor response of canceling out numbers) could have adversely affected their performance. Again, poor performance in Benton's test could be due either to impairment in visual scanning secondary to alterations in the superior colliculi and periaqueductal regions in PSP, or to defective visuospatial organization due to parietal dysfunctions in CBD.

Our finding that MRI alterations differ substantially in clinically diagnosed patients with PSP and CBD is of great utility in the differential diagnosis. None of our patients, however, had a pathologically confirmed diagnosis. Midbrain atrophy and periaqueductal signal alterations were found with greater frequency in patients with PSP, whereas asymmetric frontoparietal atrophy was present only in CBD. These findings are consistent with the pathologic characteristics of these diseases.[39] Although recent pathologic studies show overlap of histologic alterations between these diseases and other cytoskeletal degenerative diseases,[4,5] semiquantitative analyses show qualitative and regional differences in the distribution of the histopathologic damage between PSP and CBD.[6] This indicates that histologic characteristics alone cannot always distinguish these diseases, and that the concordance of lesion topography and a consistent clinical symptomatology is required.[40]

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