Sagittal T2 weighted MRI showing a spindle shaped area of increased signal extending from C4 to T1.

be included in the irradiated area; the main neurological lesion should be within segments of the cord exposed to radiation; and that other causes for the neurological disorder have been excluded.

Despite the almost syrinx-like appearance of radiation myelopathy on MRI, the clinical presentation of DRM is usually one of progressive rostral spread of sensorimotor symptoms, where the final clinical level usually corresponds to a level well within the boundaries of the length of abnormal signal seen on MRI, typically within 1 year of symptomatic onset.^{3,4} All but one of the reported cases of MRI features of DRM in the literature have shown ring-like enhancement after gadolinium administration, suggesting breakdown of the blood-brain barrier.^{3,4} As yet, there are insufficient data on precise clinical sensorimotor cord levels in relation to MRI appearances (and possible gadolinium enhancement) in the studies of DRM currently published to enable prediction of clinical outcome based on radiological findings at presentation. Our patient's symptoms and signs plateaued at a level which would indicate an upper level of spinal cord damage at C8/T1, whereas MRI showed extensive cord changes from C4 to the upper end of T1. There was no gadolinium enhancement, but T2 weighted images disclosed evidence of marrow change in the vertebral bodies of C1 to T3, suggesting that the lesion was within the irradiated area. Pathological studies in DRM have shown white matter changes of demyelination and malacia, often affecting the lateral and posterior aspects of the cord, with associated vascular changes of fibrinoid necrosis, thrombosis, perivascular oedema, and haemorrhage.¹

The high signal T2 weighted MRI findings with cord swelling resemble those of syringo-

myelia. However, almost all patients with cervical syrinx formation develop marked sensorimotor symptoms or signs in the upper limbs, and a clinical picture of isolated spastic paraparesis is unusual.⁵ The main areas of cord damage after radiotherapy would seem to be the lateral corticospinal tracts and posterior columns from radiological-pathological studies.⁶ Peripheral ring enhancement of the cord after gadolinium administration suggests that the major focus of cord damage or at least blood-brain barrier breakdown does not lie within the central cord regions. Consequently, the central cord swelling seen on T2 weighted images may well represent oedema, produced in response to altered vascular permeability after radiation damage to the vascular endothelium. In direct intrinsic cord compression, this spindle shaped region of cord oedema has limited correlation with clinical signs, as would be suggested by our case. The presence of gadolinium ring enhancement of the cord, missing in our case, serves as a more reliable marker of structural cord damage, but even in its absence, the diagnosis of DRM can be made in a patient with ascending sensorimotor symptoms within months of radiation therapy, where the only clue to radiation damage to the cord is central cord swelling and vertebral body marrow change with increased signal on T2 weighted images. Follow up MRI shows cord atrophy only in the region of previous gadolinium enhancement, correlating with clinical findings.⁷ Our case has shown that the ultimate level of disability cannot be judged from the extent of the T2 weighted central cord changes seen on initial MRI.

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Lack of association between *IL-1A* and *IL-1B* promoter polymorphisms and multiple sclerosis

Multiple sclerosis is an autoimmune and inflammatory disease of the CNS characterised by recurrent destruction of myelin which results in heterogeneous and unpredictable clinical manifestations. Although the factors able to foster the initial changes leading to CNS demyelination in patients with multiple sclerosis are still unknown, a discrete number of genes are likely to predispose to the occurrence of multiple sclerosis and to influence its clinical variables.

Due to the inflammatory nature of the pathogenic mechanisms mediating CNS damage during multiple sclerosis, association studies have been focused on candidate genes coding for immunorelevant molecules. The interleukin (IL)-1 gene cluster (including *IL-1A*, *IL-1B*, *IL-1RN*), located on the long arm of chromosome 2 (2q12-22; OMIM Database of National Center for Biotechnology Information; www3.ncbi.nlm.nih.gov:80/Omim) and polymorphic in different sites, represents a good target for association studies in multiple sclerosis. *IL-1*, in fact, has been detected within multiple sclerosis lesions.¹ Moreover, lipopolysaccharide stimulated peripheral blood monocytes of patients with multiple sclerosis produce more *IL-1α* and *IL-1β* than controls.² Due to the potential involvement of *IL-1α* and *IL-1β* in multiple sclerosis, a genetically determined polymorphic variation in their production may contribute to the occurrence of multiple sclerosis, or modulate its clinical features. Furthermore, we have recently reported the association between the variable number of tandem repeat polymorphism (VNTR) of *IL-1RN* with multiple sclerosis susceptibility and prognosis.³ *IL-1A*-889 and *IL-1B*-511 polymorphic loci are located 375 and 320 kb respectively from *IL-1RN* VNTR. To determine whether a particular allele or genotype of *IL-1A* or *IL-1B* affects occurrence or clinical variables of multiple sclerosis, we performed a case-control association study in a large cohort of patients with multiple sclerosis and age and ethnicity matched healthy controls.

Patients were clinically diagnosed at the multiple sclerosis centre of the San Raffaele Hospital in Milano as affected by clinically definite multiple sclerosis according to Poser's criteria. All patients had a relapsing-remitting course. Genotype frequencies of the *IL-1A*-889 polymorphism were calculated in a cohort of 399 patients with sporadic relapsing-remitting multiple sclerosis (247 women, 152 men; age 37.3 (SD 12); mean age at disease onset 27.3 (SD 9.3)) and 439 healthy controls (151 women, 288 men; mean age 37.6 (SD 10.6)). Gene frequencies of the *IL-1B*-511 polymorphism were calculated in a cohort of 321 patients with sporadic relapsing-remitting multiple sclerosis (203 women, 118 men; mean age 38 (SD 12); mean age at disease onset 27.4 (SD 9.2)) and 403 HC (146 women, 257 men; mean age 38 (SD 10.9)).

IL-1A-889 and *IL-1B*-511 C/T polymorphisms were characterised by polymerase chain reaction (95°C 30 seconds, 55°C 30 seconds, and 72°C 40 seconds; 40 cycles) using genomic DNA and following primers: *IL-1α* FW 5'-AAGCTTGTCTACACCTGAAGTAGGC-3', *IL-1α* RV 5'-TTAATATGAGCCTTCCATG-3'; *IL-1β* FW 5'-TGGCATTGATCTGGTTCATC-3', *IL-1β* RV 5'-GTTTAGGAATCTTCCACTT-3'). Amplified products were digested with restriction endonuclease (NcoI and Aval).

Statistical analyses were performed with the SAS statistical package (SAS Institute Inc, Cary, NC, USA). The p values were considered not significant (NS) when greater than 0.05. Gene frequencies were compared by χ^2 test with two degrees of freedom. The homogeneity of Kaplan-Meier disease free curves over strata was tested using the rank statistics (log-rank and Wilcoxon tests).

The *IL-1A* and *IL-1B* alleles were in Hardy-Weinberg equilibrium in all populations and their distributions were not affected

Table 1 *IL-1A and IL-1B genotype frequencies in healthy controls (HC) and in patients with multiple sclerosis (MS) as well as in patients with different disease courses (benign and non-benign)*

	MS	HC	Benign MS	Non-benign MS
<i>IL-1A</i> genotype (% (n)):				
CC	47 (189)	45 (198)	43 (39)	52 (56)
CT	45 (177)	46 (203)	43 (39)	43 (46)
TT	8 (33)	9 (38)	14 (13)	5 (5)
<i>IL-1B</i> genotype (% (n)):				
CC	38 (123)	39 (159)	34 (25)	33 (27)
CT	48 (155)	50 (201)	44 (32)	56 (46)
TT	14 (43)	11 (43)	22 (16)	11 (9)

by sex or age (data not shown). We found a similar gene free distribution between patients with multiple sclerosis and healthy controls for both *IL-1A* and *IL-1B* polymorphisms (table) (*IL-1A*: multiple sclerosis *v* healthy controls, $\chi^2=0.5$, NS; *IL-1B*: multiple sclerosis *v* healthy controls, $\chi^2=1.3$, NS).

The possible association between a given *IL-1A* or *IL-1B* genotype and accumulation of clinical burden over time was assessed by comparing two groups of patients with multiple sclerosis differing for their disease outcome and classified as "benign" (patients with a stabilised expanded disability status scale (EDSS) score ≤ 3 after at least 10 years from disease onset) and "non-benign" (patients with an EDSS score > 3 within 10 years from disease onset, table).³ In the case of the *IL-1A* polymorphism, the gene frequency of 91 benign was not statistically different from those of the 107 non-benign patients with multiple sclerosis ($\chi^2=5.9$, NS). As for the *IL-1B* polymorphism, the gene frequency of the 73 benign patients was also similar to those of the 82 non-benign patients with multiple sclerosis ($\chi^2=4.0$, NS).

To assess whether *IL-1A* and/or *IL-1B* genotypes had an influence on the age at onset of multiple sclerosis, Kaplan-Meier disease free survival curves were calculated, plotting the age at disease onset of each patient versus the respective *IL-1* genotype (data not shown). Again, neither *IL-1A* (log rank $\chi^2=3.9$, NS) nor *IL-1B* (log rank $\chi^2=0.72$, NS) genotypes seemed to affect the distribution of the age at multiple sclerosis onset.

A handful of inflammation associated polymorphisms have been shown to positively associate with the occurrence of multiple sclerosis or to influence its clinical variables, including polymorphisms located within or nearby the HLA locus (important for multiple sclerosis susceptibility), or the *IL-1RN*, *IL-4* and *IL-1B* genes¹ (possibly important in modulating the course of disease).

Our analysis could not demonstrate any association between these two previously untested *IL-1A* and *IL-1B* promoter polymorphisms and the occurrence of multiple sclerosis, the age at disease onset, or the accumulation of clinical burden over time in affected patients.

Given the inflammatory nature of multiple sclerosis pathological lesions and the positive association of the related *IL-1RN* VNTR with some of the clinical features of multiple sclerosis, these results are somewhat unexpected and contrast with data coming from similar genetic studies in Alzheimer's disease, a degenerative disease of the CNS where the role of inflammatory mechanisms is not as established as in multiple sclerosis.⁴

These data contribute to the debate over the role of inflammation in multiple sclerosis,

especially as recent reports have surprisingly suggested that inflammation may even be protective (rather than having a pathogenic role) in experimental animal models of multiple sclerosis.⁵

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Hyperhomocysteinaemia in Alzheimer's disease and expression of cell cycle markers in the brain

We report that moderately increased concentrations of serum total homocysteine in Alzheimer's disease are associated with the expression of cyclin E (a marker of entry into the cell division cycle) in neurons of the hippocampus. Aberrant entry of neurons into the cell cycle might be an early step in the pathological process.

Expression of proteins related to the control of the cell division cycle has been found in the nuclei of neurons in the cerebral cortex in Alzheimer's disease and it has been proposed that the expression of such proteins, in the absence of cell division, might lead to death of neurons by apoptosis or to the development of neurofibrillary pathology.¹ If the aberrant expression of cell cycle markers is part of the pathological process, then it is important to identify possible factors that might trigger the entry of neurons into the cell cycle. Moderately increased concentrations of plasma total homocysteine, an established risk factor for vascular disease,² are also associated with histopathologically confirmed Alzheimer's disease.³ As *in vitro* studies have shown that homocysteine can influence the cell cycle in vascular smooth muscle cells and in endothelial cells,⁴ we wondered whether the expres-

Serum total homocysteine concentrations (mean $\mu\text{M/l}$ (SD)) in relation to neuronal expression of cyclin B and cyclin E

	Absent	Present	p Value (ANOVA, Kruskal-Wallis)
Cyclin B	17.1 (8.7)	16.5 (4.5)	0.75
Cyclin E	15.35 (4.2)	18.1 (6.2)	0.045

sion of cell cycle markers in neurons in Alzheimer's disease might be associated with hyperhomocysteinaemia.

We studied the first 60 patients in the Oxford Project to Investigate Memory and Aging (OPTIMA) where both serum homocysteine and postmortem hippocampal tissue were available. Forty eight patients had a histopathological diagnosis of pure Alzheimer's disease, nine had Alzheimer's disease mixed with vascular pathology and three were controls, without CNS pathology. Total serum homocysteine concentrations were determined in blood samples taken at entry of each patient into the study, on average 29.7 (SD 19) months before death.³ The expression of cyclins B and E was studied by immunohistochemical methods⁵ in the nuclei of neurons in the hippocampi by an examiner blind to the diagnosis and to the value of serum homocysteine.

Cyclin B expression in neuronal cell nuclei was found in 50 patients with Alzheimer's disease, and cyclin E expression was detected in 27 patients. The concentration of total serum homocysteine in those patients who expressed cyclin B was no different from that in patients who did not express this cyclin. However, the mean concentration of homocysteine in patients who expressed cyclin E in the brain was 18% higher ($p<0.045$) than that in those who did not express this cyclin (table). Serum vitamin B12 and serum and red blood cell folate concentrations were not significantly different in patients who expressed the cyclins compared with those who did not. To control for the possible influence of other known determinants of serum homocysteine, logistic regression was carried out to see if age, sex, serum creatinine, and serum homocysteine were related to the expression of cyclins B or E. The only significant effect was of homocysteine on expression of cyclin E ($p=0.04$), which was independent of the other factors.

The cell division cycle is a tightly controlled sequence of events that is driven by the sequential nuclear expression and activation of cyclin and cyclin dependent kinase complexes. The nuclear expression of cyclin E is evidence that the neurons have re-entered the cell division cycle whereas expression of cyclin B indicates progression of the cycle to the G2 phase. We suggest that the association of hyperhomocysteinaemia with cyclin E expression, but not with cyclin B expression, may indicate a mitogenic role for homocysteine. The mechanism could be indirect, through the action of homocysteine on the cerebral vasculature, or it might reflect a direct mitogenic action of homocysteine on neurons. Further work is required to test these hypotheses. The rate of atrophy of the hippocampus in Alzheimer's disease is more rapid in patients with increased concentrations of serum homocysteine.³ Thus, if entry into the cell cycle is part of the pathogenic process, then lowering homocysteine concentrations in the blood might slow the progression of the disease.