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The prevalence of multiple sclerosis in the Hungarian city of Szeged

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Objectives – The aim of this study was to determine the prevalence of multiple sclerosis in a population in South Hungary. *Methods* – The diagnosis was established with the aid of the Poser diagnostic criteria and the degree of physical disability was determined on the Kurtzke expanded disability status scale (EDSS). The present medical state (EDSS score) was determined from outpatient clinical control tests. The prevalence, the average age at onset of the disease and the proportions of the various clinical forms were calculated, and the patients' disability status was estimated. *Results* – In 1996, the prevalence was 65/100,000, and the incidence from January 1, 1995 through December 31, 1996 was 7/100,000/year. *Discussion* – During a period of 2 years, the number of diagnosed patients has almost doubled. The disease can be recognized in an early stage with a minimal neurological deficit. The development of the diagnostics necessitates re-examinations with modern diagnostic procedures. During the last 3 years, the general practitioner system has been reorganized, and the working relationships between the clinic and family doctors have developed considerably. A comparison of the present findings with those in other countries with a similar climate revealed very similar prevalence data.

Key words: multiple sclerosis; prevalence; incidence

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The diagnostic criteria of multiple sclerosis have recently improved, and the prevalence of this disease can now be determined more precisely. Epidemiologists in many countries worldwide are reanalyzing the prevalence of multiple sclerosis (1–13). The better diagnostic methods permit an improved differentiation of neurological diseases and shed light on earlier stages of the disease. Multiple sclerosis with a later onset is easier to differentiate (by means of MRI imaging and up-to-date-CSF diagnostic methods) from vascular diseases that are more frequent at this age and may cause similar complaints. In 1996, a uniform nomenclature was introduced for the clinical course of this disease, and this necessitates a reclassification of the patients (14). In Hungary, Pálffy et al. (15) calculated the prevalence in Baranya County in 1983, which was 37/100,000. The prevalence in Fejér County in Hungary in 1992 was found by Guseo et al. (16) to be 69/100,000. Unfortunately, some of those patients did not undergo MRI examinations. The aim of the present study was to determine the prevalence in a relatively stable population with the Poser diagnostic criteria (17). The proportions of the various clinical forms were determined in accordance with the new nomenclature; such data have not been published from Hungary previously.

Methods

The Department of Neurology at Albert Szent-Györgyi Medical University in Szeged, Hungary, has a Multiple Sclerosis Outpatient Unit. The Unit is the only organization that deals with the medical care of multiple sclerosis in the city; the prevalence data can therefore be regarded as reliable. According to the files of the Hungarian Central Statistical Office (Központi Statisztikai Hivatal), the number of inhabitants in Szeged on January 7, 1997 was 198,682. The population in this area in the remaining time was fairly stable. The diagnoses were established with the diagnostic criteria of Poser et al. (17), and the degree of physical disability was determined on the Kurtzke (18) expanded disability status scale (EDSS), the most widely used scoring method (19, 20). The first examinations in Szeged were carried out between 1990 and 1994, partly retrospectively and partly prospectively.

From 1990 to 1993, the history of the multiple sclerosis patients (the age at onset, the clinical course of the disease, etc.) was taken from inpatient and outpatient medical records. The current medical state (EDSS score) was determined from the results of outpatient clinical control tests.

At the end of 1993, we had 77 patients with sclerosis multiplex in Szeged (21). By the end of 1996, the number of patients had almost doubled. We chose December 31, 1996 as the prevalence day. At that specific time the prevalence was 65/100,000. In 1995 and 1996, the incidence was determined, the patients were classified according to the nomenclature of the clinical course of the disease, and the prevalence was calculated. In 1995, 30 new patients were added to the records. Seventeen of these were first-attack patients and the other 13 were referred by general practitioners. Five patients moved from Szeged and are now cared for by other multiple sclerosis outpatient units. These 5 patients have therefore been lost from our study. None of the remaining patients died before the end of 1996. In 1996, 28 new patients were diagnosed, 12 of whom were first-attack patients, the remaining 16 being referred by general practitioners. The male:female ratio was determined, and the average age at onset was calculated. The condition of the patients was assessed on the basis of the EDSS score and they were recategorized according to the new clinical course nomenclature. The proportions of the clinical forms were determined.

Table 1. Criteria for the diagnosis of multiple sclerosis

	Number of attacks	Evidence of more than one lesion		CSF, OGP or IgG
		Clinical	Laboratory	
A. Clinically definitive				
A1	2	2		
A2	2	1 and	1	
B. Laboratory-supported definitive				
B1	2	1 or	1	+
B2	1	2		+
B3	1	1 and	1	+
C. Clinically probable				
C1	2	1		
C2	1	2		
C3	1	1 and	1	
D. Laboratory-supported probable				
D1	2	0	0	+

From Poser C, Paty DW, Scheinberg L, McDonald WI, Ebers GC. The Diagnosis of Multiple Sclerosis. New York: Thieme-Stratton, 1984.

Results

With the Poser diagnostic criteria (17, Table 1), the majority of the patients fell into the definitive category of multiple sclerosis, with a significant majority in the subgroup who were diagnosed both clinically and in the laboratory. The criteria for this subgroup are at least 2 lesions, proven clinically, positive MRI imaging, positive oligoclonal bands in the CSF with isoelectric focusing, and a positive Link index. Laser nephelometry analysis was used for the quantitative determination of proteins (Fig. 1). The calculations revealed a multiple sclerosis prevalence in Szeged of 65/100,000 inhabitants. The male:female ratio was 1:3. The average age at the onset of the disease was 35 years (Fig. 2).

As regards the clinical course, 80% of the patients had the relapsing–remitting form, 11% the primary chronic progressive form, 4% the benign form, 4% the secondary chronic progressive form and 1% the relapsing–progressive form (Fig. 3). In accordance with the EDSS scores the majority of the patients are able to live a normal life: 28 (21%) have no symptoms and 74 (57%) have only mild symptoms enabling them to perform everyday activities, 21 (16%) can carry out restricted activity, 2 (2%) are confined to a wheelchair and 5 (4%) are bed-ridden (Fig. 4). At the end of 1993, 77 of the approximately 200,000 inhabitants of Szeged suffered from multiple sclerosis, whereas in 1996 the prevalence was 65/100,000 (Table 2). The incidence from January 1, 1995 through December 31, 1996 was 7/100,000/year (Table 3).

All of the 17 new first-attack patients diagnosed in 1995, and all of the 12 new first-attack patients diagnosed in 1996, had the relapsing–remitting form. Six patients (3 males and 3 females) displayed a late age at onset, the first attack occurring after the age of 50.

Discussion

Dean (22) determined the prevalence of multiple sclerosis within the continental zone as 30–80/100,000. The occurrence was found to be related to geographical distribution, migration and genetic contribution (23–31). In research on the etiologic factors of multiple sclerosis, the occurrence and clinical forms of the disease have been examined in different population groups, such as mestizos, Indians, etc. (1, 29). A distinction between low, medium and high-risk factor areas can be made on the basis of geographical lines of latitude. However, the new prevalence tests reveal that in both low and medium-risk factor areas there can in fact be an enhanced occurrence (5, 6, 29). There are both pro and contra arguments for a north–south gradient distribution (11, 22). In 1961, Lehoczky and Halasi

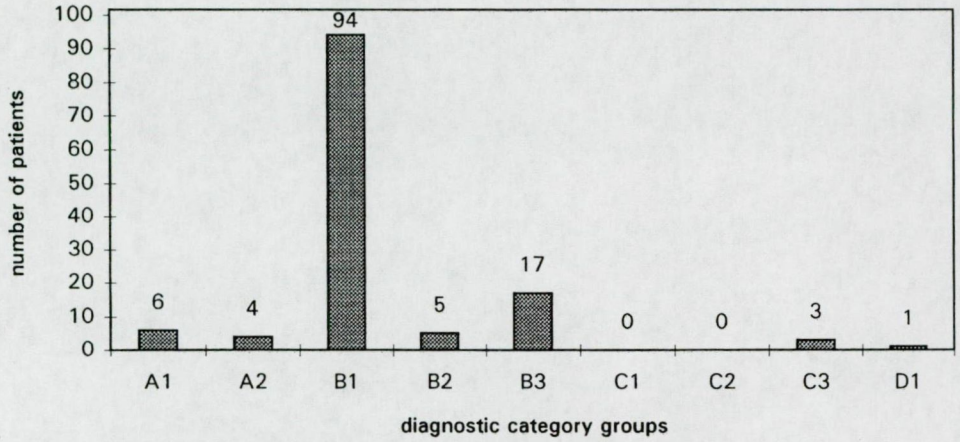

Fig. 1. Patients diagnosed with the Poser diagnostic criteria (total number of patients: 130)

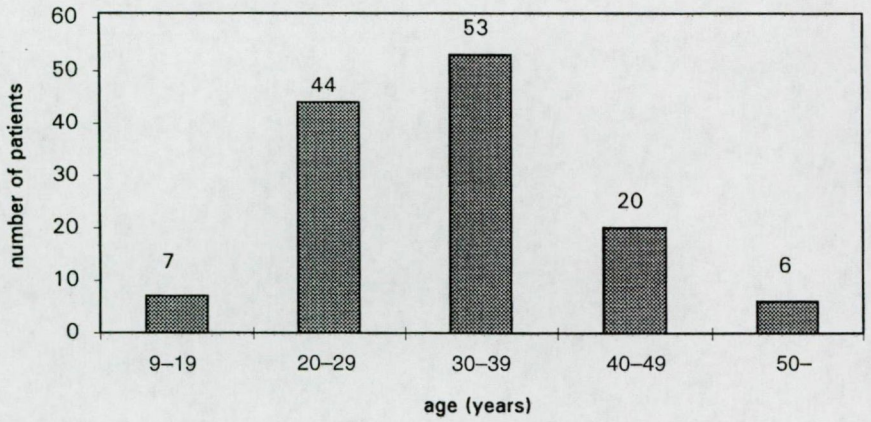

Fig. 2. Age at onset in multiple sclerosis patients (total number of patients: 130)

first determined the Hungarian prevalence of multiple sclerosis, which was found to be 20/100,000 (see in review by Pálffy et al. (15)). In 1983, Pálffy et al. (15) reported the prevalence in Baranya County as 37/100,000, and in 1992, Guseo et al. (16) found the prevalence in Fejér County to be 69/100,000. Hungary is a country lying on the Pannon plateau, situated in a continental temperate zone. Hungarians are of Asian ancestral descent, belonging in the Caucasian race. Through gene analysis, Hungarians with multiple sclerosis were found to be 32.3% HLA-B7-positive and 42.4% HLA-DRW2 histocompatibility-positive (15). In 1983, Pálffy described a male Gypsy with definitive multiple sclerosis and also examined his HLA structure (15). The Gypsy minority race, just like the Lapps of Scandinavia, the North American Indians of Canada, the Afro-Americans, and the Maoris of New Zealand, have been found to be resistant against multiple sclerosis (15). Gyódi et al. (30) compared the HLA types of the Gypsies in Baranya County with those of the Hungarian non-Gypsy population. There was a lack of HLA-B7 histocompatibility positivity in comparison with the Hungarian population, and also an increased frequency of HLA-DR2 antigen.

Our unit currently cares for 380 patients with multiple sclerosis, 130 of whom live in the city of Szeged. Since 1990, the occurrence has been calculated twice in this area; there has been a rise in the number of patients during this period, but no growth in incidence. The reason is that, even though all known multiple sclerosis patients in Szeged are treated by the unit, the patients examined prior to 1990 were not necessarily appropriately diagnosed because of the inadequacy of the diagnostic equipment. Re-examinations in the past 3 years led to an increased number of patients being taken into clinical care. A few patients were admitted to the clinic after several years of complaints that were inadequately diagnosed elsewhere. Our results relating to both prevalence and incidence do not differ significantly from the nationwide data.

The majority of the multiple sclerosis patients in Szeged can be classified into the definitive clinical and laboratory group, which means that the clinical

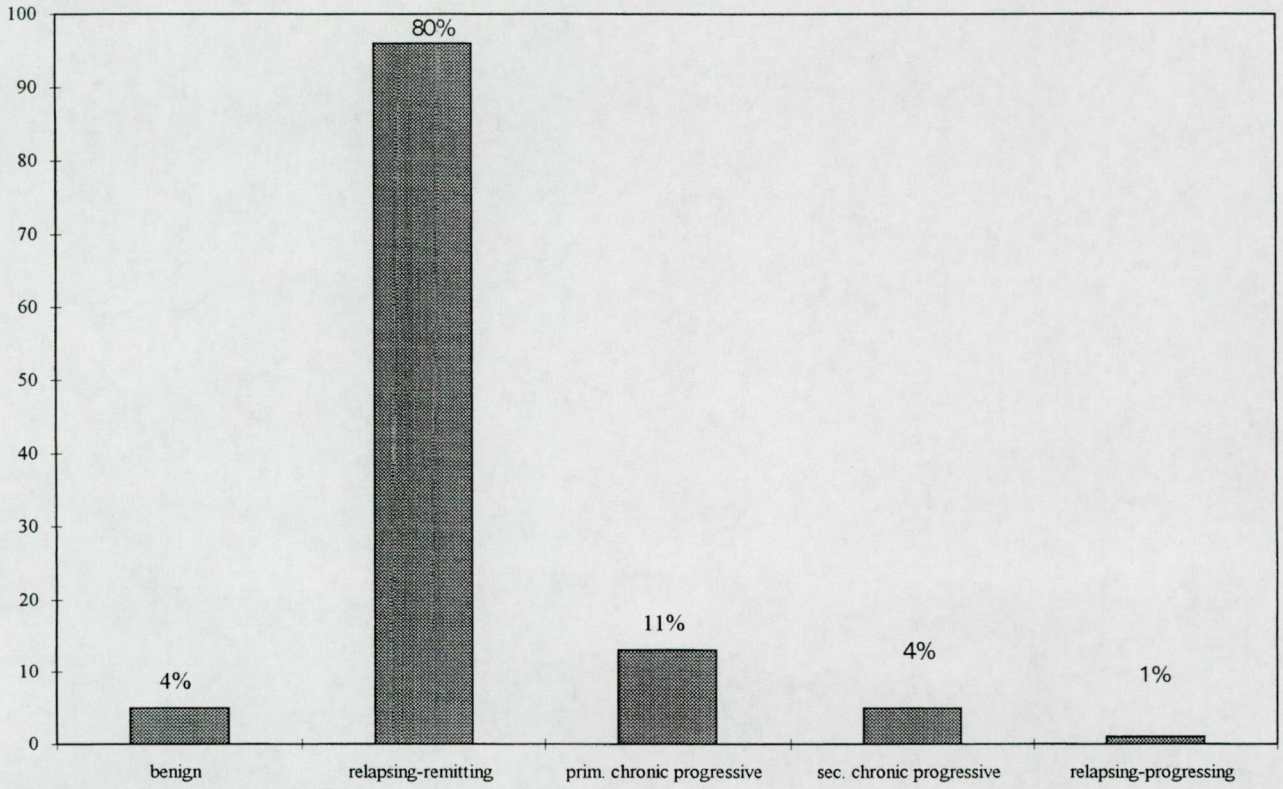

Fig. 3. Clinical forms of multiple sclerosis (total number of patients: 100%=130)

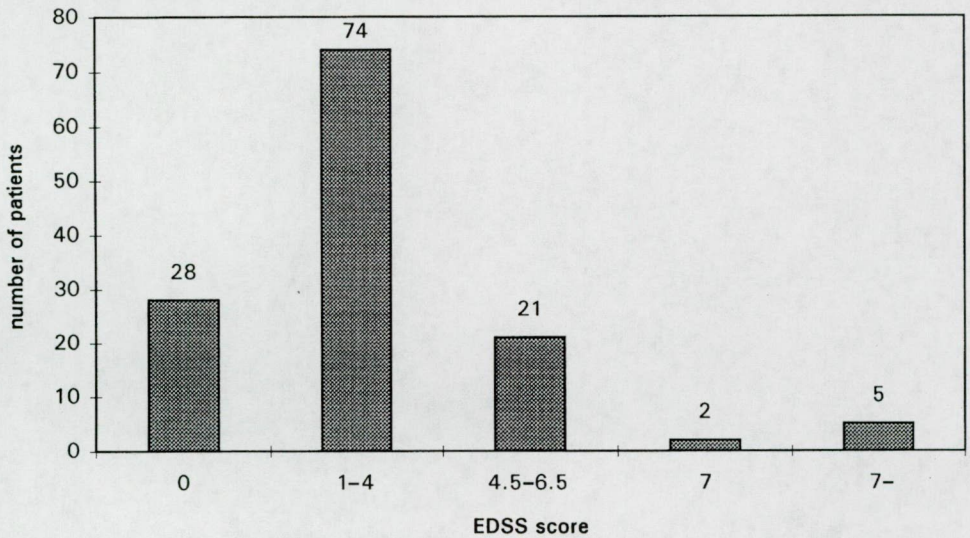

Fig. 4. EDSS scores in multiple sclerosis patients (total number of patients: 130)

outcome, the MRI results and the CSF data all indicate multiple sclerosis. Of the multiple sclerosis patients in Szeged, 21% are asymptomatic and a further 57% are capable of normal activities. This means that at present 78% of the patients are not yet impaired, 16% are not capable of full daily activity, 2% are confined to a wheelchair and 4% require help. Our population contains few 50–60-year-old multiple sclerosis patients, which suggests that some patients are presumably treated in non-neurological departments, or perhaps by family physicians, with other diagnoses.

Throughout the period of 2 years, the number of diagnosed cases has almost doubled. During the last 3 years, the general practitioner system has been reorganized, and the working relationships between

Table 2

	1996
Prevalence	65/100,000
Male:female ratio	1:3
	32 males, 98 females
Mean age at onset	35 years
	(range: 11–64 years)

Table 3

	1995–1996
Incidence	7/100,000/year male:female ratio 1:5 mean age at onset: 37 years (range: 18–64 years)

the clinic and family doctors have developed considerably. This fact, together with the young age of the patients, and their generally good functional condition led us to conclude that the diagnostic developments necessitated re-examinations with modern diagnostic procedures. Thus, the disease can be recognized in an early stage with a minimal neurological deficit. A comparison of our findings with those from countries with a similar climate revealed similar prevalence data.

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The Prevalence of Multiple Sclerosis, Distribution of Clinical Forms of the Disease and Functional Status of Patients in Csongrád County, Hungary

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Key Words
Multiple sclerosis · Prevalence · Incidence · Clinical forms · Functional status

Abstract
Objective: The aim of this study was to determine the prevalence of multiple sclerosis (MS) in the population of Csongrád County, Hungary (400,128 inhabitants) and to determine the functional status (based on the Expanded Disability Status Scale; EDSS) of the patients according to the clinical forms of the disease. ***Methods:*** The diagnosis was established with the aid of the Poser diagnostic criteria, and the degree of physical disability was determined using the Kurtzke EDSS. ***Results:*** In Csongrád County, the prevalence of MS is 62/100,000. The distribution of patients according to the clinical forms of MS was as follows: 15% had the benign form, 54% had relapsing-remitting MS, 20% had secondary chronic progressive MS and 11% had the primary chronic progressive form of MS. Sixty percent of relapsing-remitting MS patients had an EDSS score of 0–4 points and 33% had an EDSS score of 4.5–6.5 points. ***Conclusion:*** The distribution of patients according to the clinical forms of the disease in this representative population is comparable to results in other regions of the world.

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Introduction

The aim of the present study was to determine the prevalence of multiple sclerosis (MS), the distribution of clinical forms of the disease and the Expanded Disability Status Scale (EDSS) scores [1] of MS patients in a large population to obtain exact data about the prevalence of the disease in Hungary. In order to provide new therapies for MS (beta-interferons, glatiramer acetate) the functional status (based on the EDSS score) of patients has to be determined in the different clinical forms. There are no published data on this topic in the population of 400,000 which we investigated in the present study, which comprises 4% of the residents of Hungary.

Pálffy et al. [2] calculated a prevalence of 37/100,000 in Baranya County, Hungary, in 1983, based on Bauer criteria.

In 1997, the prevalence of MS was 65/100,000 in the city of Szeged in Hungary, based on a population of 200,000 residents [3]. In this population, there was a relatively low proportion of patients with the secondary chronic progressive (4%) and benign forms (5%) of MS compared to the international data [4, 5].

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Subjects and Methods

The current study was carried out in the Department of Neurology at Szeged University, Hungary (January 1, 1997 to July 1, 1999). The Multiple Sclerosis Outpatient Unit is the only facility that has dealt with the medical care of MS in the city for the past 35 years. This outpatient unit of the Neurological Department as a university center is specialized in caring for and nursing the MS patients of the south Hungarian region, which comprises 4 counties. With the background of the university, it has a wide range of tools to diagnose MS patients.

In our first epidemiological study in the city of Szeged [3], we checked the medical records of all general practitioners, neurological and ophthalmological departments and homes for the aged in the city. Based on these medical records (onset of disease and the clinical course), patients with probable MS were registered and examined by our outpatient unit. The diagnoses were established using the diagnostic criteria of Poser et al. [6]. Brain MRI was performed in all patients, and a proportion of the patients had optic nerve and/or spinal cord MRI and analysis of CSF. Oligoclonal bands were determined by isoelectric focusing, and laser nephelometry analysis was used for the quantitative determination of proteins to calculate the Link index. In all cases of CSF analysis, we had the informed consent of the patients. The criteria for A subgroups are 2 attacks and 2 lesions proven clinically (A1) or 1 clinical and 1 paraclinical sign of lesion (A2). The criteria for B subgroups are at least 1 lesion (B1) or 2 lesions (B2) proven clinically and positive MRI imaging, positive oligoclonal bands in the CSF and a positive Link index [7]. The degree of physical disability was determined using the Kurtzke [1] EDSS.

The outpatient unit has had an MS register since 1996, with up-to-date patient records of the Szeged patient population. We examine patients every 3 months to determine their neurological status and EDSS score. In the case of relapses, we perform an extra neurological examination and, if necessary, admit the patients to hospital. After discharge, a follow-up visit is scheduled for 2–4 weeks later, at which we check the neurological status and the EDSS score.

If a patient moved away from the city or died, we updated the register. At the end of the year, we calculate the yearly incidence of the disease.

Based on the methods used in this register, we performed a wider survey in Csongrád County as a whole.

According to the files of the Hungarian Central Statistical Office, Szeged City (the county town of Csongrád County) has 198,686 inhabitants, and Csongrád County has an additional 222,506 inhabitants. In the present study, 400,128 inhabitants were the representative sample; the population of a small city with 21,064 residents was excluded because there were no available data for these persons.

We used the same method described above to record and diagnose MS patients in the case of 201,442 inhabitants of Csongrád County.

We chose July 1, 1999 as the day on which we measured prevalence. We determined the prevalence of the disease and the male/female ratio. We estimated the following parameters for the different clinical forms of the disease: distribution of patients; average age at the onset of the disease; average duration of the disease; average age on the prevalence day, and the EDSS score based on the most recent examination (April 1, 1999 to July 1, 1999).

Results

We found 248 MS patients alive on the prevalence day in Csongrád County. Two hundred and eighteen patients had clinically definite MS. According to the criteria of Poser et al. [6], 208 patients were in the A1 and 10 patients in the A2 (with negative CSF oligoclonal bands and Link index) category. Thirty patients were in the category of laboratory-supported definite MS (Poser B2). Although it is not essential for the A1 category, our patients belonging to this subgroup had positive CSF findings, that is, oligoclonal bands and a positive Link index. Based on our study, the prevalence of MS in Csongrád County is 62/100,000. On the prevalence day, 130 patients out of 248 lived in Szeged. Based on our MS register, we diagnosed 26 new MS patients in the city of Szeged between January 1, 1997 and July 1, 1999. In this period, 5 patients died, 12 patients moved from Szeged to elsewhere within the county and 9 moved away from the county. The incidence of MS in the city of Szeged was 5/100,000 in 1997 and 6/100,000 in 1998. Up to July 1, 1999, we diagnosed 4 new cases.

There were 66 (27%) male and 182 (73%) female MS patients found, giving a male/female ratio of 1:2.75.

The distribution of the patients according to the clinical forms of MS was as follows: 15% had the benign form, 54% had relapsing-remitting MS, 20% had secondary chronic progressive MS and 11% had the primary chronic progressive form of MS (table 1).

The EDSS score in patients with the benign form of MS was 0–3 points; the average duration of the disease was 27 years. Sixty percent of relapsing-remitting MS patients had an EDSS score of 0–4 points and 33% had an EDSS score of 4.5–6.5 points. Fifty-six percent of secondary chronic progressive MS patients had an EDSS score of 4–6.5 points; 44% were confined to a wheelchair or bedridden (EDSS score ≥ 7) (table 2).

Discussion

Dean [8] determined the prevalence of MS within continental Europe as 30–80/100,000. The occurrence of MS has been found to be related to geographical distribution, migration and genetic contribution [6, 9–12]. A distinction between low-, medium- and high-risk factor areas can be made on the basis of geographical lines of latitude. However, the new prevalence tests reveal that in both low- and medium-risk factor areas, there can be, in fact, a higher rate [13–20]. There are both pro and con arguments for

Table 1. Patient characteristics according to clinical forms of MS

	Benign MS	Relapsing-remitting MS	Secondary chronic progressive MS	Primary chronic progressive MS
Number of patients	37 (15%)	137 (54%)	48 (20%)	26 (11%)
Mean age at the onset of disease, years	28 (16–41)	28 (16–40)	30 (13–47)	52 (42–62)
Mean age on the prevalence day, years	55 (38–72)	36 (18–54)	59 (39–69)	59 (49–68)
Average duration of disease, years	27 (10–34)	8 (0–15)	29 (5–47)	7 (2–13)

Figures in parentheses represent ranges, except where otherwise indicated.

Table 2. Distribution of patients according to clinical forms of MS and EDSS score

Benign MS			Relapsing-remitting MS			Secondary chronic progressive MS			Primary chronic progressive MS		
EDSS	n	%	EDSS	n	%	EDSS	n	%	EDSS	n	%
0	10	27	0–4	82	60	4–6.5	27	56	3.5	1	4
1	5	13	4.5–6.5	45	33	≥7	21	44	4	2	8
1.5	1	4	≥7	10	7				4.5–6.5	12	46
2	11	30							≥7	11	42
2.5	5	13									
3	5	13									

a north-south gradient distribution [21]. Rothwell and Charlton [22] found a high prevalence rate for MS in southeast Scotland, suggesting that the Scottish population as a whole has a genetic susceptibility to the disease.

In 1983, Pálffy et al. [2] reported the prevalence of MS in Baranya County, Hungary, as 37/100,000. In 1997, the prevalence in Szeged City was found to be 65/100,000 [3]; in 1999, the prevalence was 62/100,000 in Csongrád County, including the population of Szeged City. The prevalence of MS found in the 400,128 persons of Csongrád County in the present study is nearly the same as the previous prevalence data from Szeged City and the prevalence of MS in other communities of the same geographical regions.

The male/female ratio of 1:3 found in Szeged City differs from the international data [4, 5], because in that city, the ratio of females is higher by 8.5% than males. The mean age is lower by 5.5 years in the city than the rest of the county. In Csongrád County, the male/female ratio is 1:2.75, which is comparable to the international data.

In Szeged City, the distribution of patients according to the clinical forms of the disease was as follows: 5% had the benign form, 80% had relapsing-remitting MS, 4% had secondary chronic progressive MS and 11% had the primary chronic progressive form of MS.

In Csongrád County, we found that 15% of patients had the benign form of MS, 54% had relapsing-remitting MS, 20% had secondary chronic progressive MS and 11% had the primary chronic progressive form.

The proportion of secondary chronic progressive MS patients in the county as a whole is higher (20%) than in the city of Szeged (4%). Most of these MS patients were found in homes for the aged in the county. Due to progression of the disease and worsening social status, these chronic progressive MS patients moved to smaller communities away from the city. Therefore, the population of the city is younger than in the rest of the county (difference in average age is 5.5 years). In the current and previous epidemiological study [3] of this region, the proportion of patients with primary chronic progressive MS was 11%, which is equal to international data.

The average duration of the disease in the benign form of MS was 27 years, and the EDSS score for these patients was between 0 and 3. These data fit the criteria of benign MS.

Sixty percent of relapsing-remitting MS patients in this study were capable of normal activities; only 7% were confined to a wheelchair and required help. Of the relapsing-remitting MS patients, 21% were being treated with interferon-β-1b or glatiramer acetate, thus modifying the EDSS score.

Of the secondary chronic progressive MS patients, 56% had an EDSS score between 4 and 6.5; 44% were confined to a wheelchair and required help.

In the larger population of Csongrád County (more representative population than Szeged City), the distribution of patients according to the clinical forms of the disease was comparable to international results [23].

In order to adopt and finance the new therapeutic approaches (interferons or glatiramer acetate), the Central-Eastern European healthcare systems need the most exact data possible on the prevalence of MS. The EDSS classification of the disease by clinical forms is necessary to estimate healthcare expenditures for these new, expensive therapies.

The sample investigated in the current epidemiological study represents 4% of the Hungarian population, which could be quite sufficient to estimate national expenditure.

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Short Communication

A pilot study on the antibodies to HHV-6 variants and HHV-7 in CSF of MS patients

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In the possible role for human herpesviruses (HHV) in the pathogenesis of multiple sclerosis (MS) neither clear distinction between the two variants of HHV-6, nor the involvement of HHV-7 have been described. Therefore, we quantitated HHV-6 variant specific and HHV-7 reacting antibodies in the CSF of 13 patients with MS or other neurological disorders by ELISA. Predominance in the positivity of IgG (67%) and IgM (44%) to HHV-6B over that of IgG (44%) with no detectable IgM to HHV-6A, and no antibodies to HHV-7 were found in the CSF of MS patients. None of these antibodies were found in the CSF of controls. This suggests that, intrathecal chronic active or primary HHV-6B infection might contribute to MS progression, while the local effects of HHV-6A and HHV-7 seem to be less important.

Keywords: MS; CSF; antibodies; ELISA; HHV-6B; HHV-7

Multiple sclerosis is one of the most common neurological disorders in Europe and North America (Bencsik *et al*, 1998; Perron *et al*, 1997). There is serological and molecular evidence that, human herpesvirus type 6 (HHV-6) might have a role in the pathogenesis of MS (Sola *et al*, 1993), but studies on the involvement of HHV-7 in MS, which is closely related to HHV-6 have not been published. Isolates of HHV-6 are grouped as variants A and B, and the contradictory results using different serological and molecular assays (Fillet *et al*, 1998; Soldan *et al*, 1997) raises the possibility that HHV-6A and HHV-6B might exert different effects in disease progression. Recent reports indicate that, HHV-6A and HHV-6B have distinct biological properties and pathogenic potentials. In an individual, no cross-immunity exists between HHV-6A and HHV-6B despite their genomic similarity (Hall *et al*, 1998). In spite of the integrity of the blood–brain barrier (BBB) HHV-7 (Portolani *et al*, 1998), both HHV-6A (Hall *et al*, 1998) and HHV-6B (Challoner *et al*, 1995) may reach the central nervous system (CNS). In children and adults with dual infections, only HHV-6A persists in CSF, which suggests that HHV-6A had greater neurotropism, while HHV-6B tends to be more prominent in other tissues (Hall *et al*, 1998; Luppi *et al*, 1994). In contrast, the presence of HHV-6B sequences is found to be common in the brains from MS patients and controls, and the high degree expression of HHV-6B in the plaque-associated oligodendrocytes (which are destroyed throughout disease course) suggests that rather local than general effects of viruses contribute to MS pathogenesis (Challoner *et al*, 1995). Detection by polymerase chain reaction (PCR) without HHV-6 variant specificity in peripheral blood mononuclear cells did not show any significant difference in frequency and quantity between MS patients and controls. It is suggested that brain cells are the reservoir for the virus (Mayne *et al*, 1998). Both HHV-6 (Patnaik *et al*, 1995) and HHV-7 (Torigoe *et al*, 1996) induce intrathecal synthesis of IgM and IgG. Until the breakdown of BBB in the later stage of disease, variant specific antibodies primarily in the cerebrospinal fluid (CSF) (Patnaik *et al*, 1995) and secondarily in the serum (Ablashi *et al*, 1998; Nielsen *et al*, 1997; Sola *et al*, 1993; Soldan *et al*, 1997; Wilborn *et al*, 1994) might correlate with disease progression.

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For our present studies patients with MS and other neurological disorders (OND) were diagnosed by standard clinical and laboratory criteria (Bencsik *et al*, 1998; Ungurean *et al*, 1996). Cerebrospinal fluid (CSF) and serum of patients obtained with prior informed consent were subjected to low speed centrifugation to establish leukocyte and erythrocyte numbers. Protein content, albumin and immunoglobulin (Ig) fractions were quantitated by laser nephelometry (Dosatec GmbH, Munich, Germany) and isoelectric focusing in an agarose gel as described earlier (Bencsik *et al*, 1998; Seres *et al*, 1998; Ungurean *et al*, 1996). The mean of CSF/serum albumin index was 4.95×10^{-3}. This was within the normal range showing no BBB damage. Two exceptions were Patients 7 and 11, who also had erythrocytes in the CSF. We used a sensitive ELISA to quantitate variant specific IgG and IgM, as described (Maródi *et al*, 1998). Briefly, JJHAN cells infected with HHV-6A GS, MOLT-3 cells infected with HHV-6B Z29, Sup-T1 cells infected with HHV-7 RK strain served as antigens. When OHV-1 monoclonal antibody (MAb; Advanced Biotechnologies Inc., Columbia, MD, USA) to both HHV-6 variants and RK-4 MAb to HHV-7 in indirect immunofluorescent assays detected equal ratio (38–40%) of cells containing viral antigens (data not shown), these cells were fixed to 96 well polystyrene plates. HHV-6 variant specificity was verified by competitive binding of anti-HHV-6B 101k MAb (PE Pellett, Atlanta, GA, USA). Competing IgG was removed from aliquots of each serum and CSF by mixing them with Protein-A Sepharose 4B (Sigma, St. Louis, MO, USA) in 10 : 1 ratio for 1 h with subsequent low speed centrifugation. Twofold dilutions of sera and CSF (1 : 100–1 : 6400) were incubated with the antigens in 96 well polystyrene plates in quadruplicate. After vigorous washings, peroxidase conjugated anti-human IgG and IgM (Sigma), respectively, were adsorbed to human polypeptides in parallel wells, finally orthophenyldiamine (OPD) substrate was introduced in each well to detect enzyme in the immune complexes. The optical density, read at 492 nm, >150% higher than that of the standard deviation of established seronegative subjects were regarded as positive. Sera of known seropositive individuals (kindly provided by L Ceccherini-Nelli, Pisa, D DiLuca, Ferrara, Italy) served as controls. Serum and CSF dilutions at 1 : 100 were tested in quadruplicate for non-specific binding to uninfected cells, but no reactions were detected (data not shown).

In MS patients, oligoclonal bands, protein content, nephelometry and presence of variant specific antibodies showed a significant correlation (Table 1). Predominance in the positivity of IgG (6/9=67%) and IgM (4/9=44%) to HHV-6B over IgG (3/9=33%) with no detectable IgM to HHV-6A was found. This raises the possibility that a previous intrathecal HHV-6A infection silent at time of the test, and chronic active or primary HHV-6B infection (especially in Cases 2, 5, 6 and 7) might contribute to MS. On the contrary, lack of HHV-7 specific antibodies in CSF argues against its direct role in MS. As seven of nine patients had antibodies to HHV-7 in their sera (data not shown), this also demonstrated integrity of BBB. Presence in CSF, but absence in serum of IgM antibodies to HHV-6B found in Patient 7 with damaged BBB suggests its intrathecal synthesis. No CSF antibodies to HHV-6 variants or HHV-7 were found in the youngest MS patients (Patients 1 and 5), which might suggest involvement of another agent(s) in triggering disease. In patients with OND, CSF contained no detectable antibodies

Table 1 Antibodies to HHV-6 and HHV-7 in CSF of patients with MS and other neurological disorders (ELISA)

Number of patients	*Age (years)*	*Sex (M/F)*	*Diagnosis*	*Leukocytes (10^6/L)*	*Protein (g/L)*	*Nephelometry*	*Oligoclonal bands*	*HHV-6A*		*HHV-6B*		*HHV-7*	
								IgG	*IgM*	*IgG*	*IgM*	*IgG*	*IgM*
								antibody titres+					
Multiple sclerosis													
1	25	M	RR	1	0.26	–	+	Ø	Ø	Ø	Ø	Ø	Ø
2	29	M	RR	45	0.38	IgG, IgM	+	Ø	Ø	800	400	Ø	Ø
3	34	M	RR	0	0.22	IgA, IgG, IgM	+	400	Ø	100	Ø	Ø	Ø
4	53	M	RR	1	0.54	IgG (traces)	+	Ø	Ø	100	Ø	Ø	Ø
5	34	F	RR	0	0.26	IgG	+	Ø	Ø	200	200	Ø	Ø
6	36	F	FA	0	0.41	IgA, IgM	–	200	Ø	200	100	Ø	Ø
7	41	F	RR	5*	0.20	IgG	+	400	Ø	800	200	Ø	Ø
Other neurological disorders													
8	42	M	IDR	0	0.40	IgM	–	Ø	Ø	Ø	Ø	Ø	Ø
9	43	M	CHA	0	0.39	ND	–	Ø	Ø	Ø	Ø	Ø	Ø
10	50	M	CHA	1	0.30	–	–	Ø	Ø	Ø	Ø	Ø	Ø
11	1	F	AHE	100**	0.86	IgA, IgM, IgG (traces)	–	Ø	Ø	Ø	Ø	Ø	Ø
12	25	F	PNP	0	0.17	IgG, IgM	+	Ø	Ø	Ø	Ø	Ø	Ø
13	22	F	PNP	21	0.47	IgG	+	400	Ø	Ø	Ø	Ø	Ø

+Reciprocal values of serum dilutions; RR=relapsing-remitting; FA=first attack; IDR=intervertebral disk rupture; CHA=chronic headache; AHE=acute HSV-1 encephalitis; PNP=polyneuropathy; ND=not done; *64 and **1024 erythocytes (10^6/L), Ø=<100

to HHV-6 or HHV-7. The only exception was Patient 13 without BBB damage, who also had low level serum IgG to HHV-6A. The mean titers of the two patient groups were not significantly different. Patient 11 was seronegative for HHV-6 variants and HHV-7, and herpes simplex virus type 1 (HSV-1) was identified as a causative agent of encephalitis.

In the serum of patients with MS and OND, as well as 12 healthy subjects, IgM antibody titers to HHV-6A were not different, and a slight, statistically non-significant increase in IgG titers of both patients groups as compared to the normal individuals was observed. The mean anti-HHV-6B IgG and IgM levels were significantly higher in the MS group than in OND and healthy subjects. This difference was less than that found in the CSF of MS patients compared to OND patients. No IgM to HHV-7 was found in the serum of any persons studied, and even the mean IgG titer of healthy controls was slightly higher than that of the two patients groups. Details will be described elsewhere.

Presence of CSF antibodies in different titers suggests different roles of HHV-6 variants, while the absence of CSF antibodies to HHV-7 seems to exclude its intrathecal contribution to the pathogenesis of MS. In few cases, detection of low level IgG without IgM to HHV-6A indicates past infection without recent expression of viral genes. On the contrary, in nearly two thirds of patients the simultaneous presence of IgG and IgM to HHV-6B suggests ongoing or preceding intrathecal replication of HHV-6B. So far, CSF antibodies have been studied occasionally, but the comparison of serum antibodies with variant specific PCR might support our observations. Recently, in the sera of patients with exacerbation of MS, increased IgM response to the common early polypeptides p38/41 of HHV-6 variants in the absence of increased IgG levels has been found (Soldan *et al*, 1997), which indicates persistent active infection by any of the variants. Primers unable to differentiate between two variants detected HHV-6 in the peripheral blood mononuclear cells (PBMC) of 30% of the same patients (Soldan *et al*, 1997). In another study, IgG and IgM to p38/41 were found in the serum of 68 and 56% of MS patients by ELISA, respectively. Although this also suggests active infection without variant definition, PCR identified HHV-6B DNA sequences in the PBMC of the same patients (Ablashi *et al*, 1998). If HHV-6A GS was used in an ELISA, no difference between IgG titers in MS patients and controls were found (Nielsen *et al*, 1997), that also excludes recent infection by this variant. Using a HHV-6A-like strain, ELISA revealed a significantly higher total serum level of IgG+IgM than in controls or OND patients, but neither IgG+IgM nor HHV-6A DNA were found in any of their CSF samples (Wilborn *et al*, 1994). Significantly higher anti-HHV-6A (GS) IgG titers in MS patients in comparison with the blood donors were found, but only 7% of MS patients without BBB damage had high IgG level in their CSF. In one of 31 MS patients high, in one of 24 controls low copy number of HHV-6A DNA in their PBMC were found, both were negative for serum IgG. These patients might have been positive for IgM, which was not tested, or their IgG level was under the limit of detection as the less sensitive IFA was used (Sola *et al*, 1993). Similarly, different pathogenetic mechanisms can be assigned to HHV-6 variants in HIV-1 infected patients, where DNA of HHV-6A is detected in PBMC, while that of HHV-6B is shown in different organs (Emery *et al*, 1999).

More recently, an MS-associated retrovirus (MSRV) has been isolated, characterized and suggested as another etiological agent (Perron *et al*, 1997). HHV-6, EBV or HSV-1 might behave as cofactors, and exert their effects in MS through transactivating MSRV (Perron *et al*, 1993). This mechanism is similar to the HIV transactivation by HHV-6A in AIDS progression (Ongrádi *et al*, 1990) or enhancing lymphomagenesis by HHV-6B (DiLuca *et al*, 1994) via altering cytokine profile. Up to date, no such role has been found for HHV-7 in these clinical phenomena. HHV-6B might, HHV-6A and HHV-7 might not induce local abnormalities in the intrathecal synthesis of cytokines and therefore affect MSRV and autoimmune demyelination. As the clinical outcome seems to be variant specific, in further reports, therefore, a more clear distinction of HHV-6 variants studied must be declared.

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A genome-wide screen for association in Hungarian multiple sclerosis

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Abstract

Although the pathogenesis of multiple sclerosis (MS) is not fully understood, substantial evidence points to the involvement of genetic factors. We report on a genome-wide screen for disease association in the Hungarian population using 5532 microsatellite markers. These markers were typed in DNA pools that consisted of 88 MS patients (cases), and 128 unrelated controls. Based on a stringent selection criterion, we obtained 33 markers suggesting association with the disease.
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Keywords: Multiple sclerosis; Genome screen; Linkage disequilibrium

1. Introduction

Multiple sclerosis (MS) affects about 65 people per 100,000 in the Hungarian population. The prevalence in Baranya County, Hungary, was estimated in the early 1980s to be 37/100,000, although the prevalence was lower in the Gypsy population (Gyódi et al., 1981; Pálffy et al., 1994). According to recent epidemiological studies (Csongrád county and the city of Szeged), the number of the affected is 62–65/100,000 (Bencsik et al., 1998, 2001).

In the present study, we have typed the 5532 microsatellite markers provided through the GAMES collaboration in two DNA pools derived from MS patients and controls. Using these pools, we have completed an indirect whole genome screen for association.

2. Materials and methods

2.1. Samples

The 88 unrelated MS patients were recruited by the Multiple Sclerosis Outpatient Unit, Department of Neurology, University of Szeged. The 128 healthy blood donors fulfilled the criteria of the regulations of the local ethical committee of the University of Szeged, for whole blood donation and were randomly selected. This study was approved by the local ethical committee and all patients from the cases group meet Poser's criteria (Poser et al., 1983) for the diagnosis of multiple sclerosis.

The mean age was 35 years in the cases group and the sex was consistent with previous population based series 3.6 female:1 male. The ratio was 2.6/1 in the unrelated control group. Considering disease course, 90% of the patients had a relapsing remitting disease course (RRMS) and 10% of patients had secondary progressive disease (SPMS).

2.2. DNA pooling

Genomic DNA was extracted manually using a standard phenol-chloroform method. The concentration was estab-

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lished by measuring the OD twice, only samples with less than 10% difference in these measures were included. Each sample was then diluted to a concentration of 50 ng/μl. Pools (patients and controls) were constructed by combining equal volumes from contributing samples.

2.3. Genotyping

Detailed information about the markers and methods employed are available via the *G*enetic *A*nalyses of *M*ultiple sclerosis in *E*uropean*S* website (http://www-gene.cimr.cam.ac.uk/MSgenetics/GAMES).

All markers were amplified in each of the two pools using standard polymerase chain reaction (PCR) protocol according to the manufactures recommended conditions. PCR products were electrophoresed twice (see Sawcer et al., 2002) using an Applied Biosystems 3700 DNA Analyser (Applied Biosystems) and sized using Applied Biosystems GENESCAN software (version 3.5). The allele image patterns (AIPs) generated were analysed using the perl program ALLELPICKER which was specifically written (by KB) for the GAMES project. This program avoids the need to inspect the AIPs in GENOTYPER and is thus considerably more efficient. On the other hand, the program is critically dependent on the sizing algorithm, such that any error in this can result in erroneous data being collected. In order to identify such errors, all AIPs from markers showing significant results were inspected manually with GENOTYPER.

2.4. Statistical analysis

Since each microsatellite marker was amplified once by PCR and electrophoresed twice, two replicate allele image patterns were generated from each of the two pooled samples. Best-fit allele image patterns were calculated for both the cases and controls, normalised according to the total number of alleles in the respective pools and compared statistically using a χ^2 test. Alleles with a frequency of $<5\%$ were considered together. Given that the majority of typed markers are not expected to be associated with multiple sclerosis, we were able to use the observed distribution of χ^2 results in order to correct for the additional sources of variance introduced by pooling. This process was completed using the software package specifically developed for GAMES (by ES). The empirical p-values thus calculated enable the typed markers to be ranked according to their evidence for association. Data was analysed incorporating the adapting factors suggested by Yeo et al. (2003). More detail about the statistical analysis can be seen in the accompanying paper from Setakis (2003).

3. Results

By typing 5532 microsatellite markers in two pooled DNA samples, we conducted a genome-wide screen for association with MS in the Hungarian population. This screen employed case-control methodology with 88 unrelated multiple sclerosis patients and 128 controls without any neurological diseases.

Table 1
Empirical p-values for the top 20 markers

Locus	Chr	p-value
D16S3097	16	0.006
D7S630[a]	7	0.013
D19S921[a]	19	0.015
DXS6807	X	0.017
D6S275	6	0.019
D9S1853	9	0.02
D2S338	2	0.022
D3S1283	3	0.029
D14S281	14	0.029
D20S892	20	0.03
D18S1127[a]	18	0.032
D6S424	6	0.035
D3S3571[a]	3	0.036
D7S2516	7	0.037
D8S1762	8	0.038
D1S2677	X	0.038
D20S891	20	0.04
D10S1669	10	0.042
D5S502	5	0.043
D5S2045	5	0.044

[a] These markers were included in the 529 considered by Yeo et al.

Usable AIP data was obtained for 3476 markers in this screen. After ranking the markers according to their evidence for association, we examined the raw AIPs from the 150 most promising markers (all with empirical p-values <0.05). The raw AIPs from these markers were examined using GENOTYPER, in order to identify and exclude those markers showing apparent association due to the effects of sizing errors in ALLELEPICKER, manual genotyping errors, or poor quality data. A total of 117 markers were excluded, most due to poor quality data, which left us with a total of 33 associated markers. The empirical p-values for the top 20 markers are listed in Table 1. Six of the markers are from regions previously identified by linkage analyses: D3S3571, D5S2102, D7S2516, D7S630 (Haines et al., 2001), DXS6807 (Akesson et al., 2002) and DXS1223 (Coraddu et al., 2001) and the rest are from novel regions not previously identified.

4. Discussion

Multiple sclerosis (MS) is an autoimmune demyelinating disease of unclarified etiology, the disease is characterized pathologically by multiple plaques of inflammation and scarring in the central nervous system (CNS), and clinically by variable relapses and progressive disability. Several data suggest the involvement of both genetic and environmental factors in the pathogenesis of the disorder. The environmental hypothesis is supported by studies pointing to higher

prevalence rates in temperate as compared to tropical latitudes (Davenport, 1922; Kurtzke and Hyllested, 1979; Dean, 1984) and higher antiviral antibodies levels in affected individuals (Ongrádi et al., 1999). Similarly, the involvement of genetic factors is supported by studies in twins and adopted individuals (Sadovnick et al., 1993; Ebers, 1994; Ebers et al., 1995; Mumford et al., 1994), as well as by observations in multiplex families (Sadovnick et al., 1988, 1993; Poser, 1994; Gaudet et al., 1995; Robertson et al., 1996, 1997; Compston, 1997; Bencsik et al., 2002). Data from the animal model of MS supports the involvement of genes in the pathogenesis of the disease (Teuscher et al., 1999). Previous candidate gene-based studies have concentrated on the HLA region, myelin associated and cytokines genes in their search for the genes determining susceptibility to the disease (Fog et al., 1973; Dupont et al., 1977; Takács et al., 1990; Olerup and Hillert, 1991; Hillert, 1994; Kwon et al., 1999; Seboun et al., 1999; Encinas and Kuchroo, 2000; Masterman et al., 2000; Reboul et al., 2000). While genome-wide linkage studies have failed to identify any major locus determining susceptibility to MS (Sawcer and Goodfellow, 1998).

The absence of a reliable diagnostic test, the variability in disease course, and the probably silent onset, months or years before clinical symptoms are occurring, are the major difficulties in the pathogenesis of MS.

Here, we present a genome-wide screen for association in multiple sclerosis in which we have identified 33 potentially associated markers. Further work on these potentially associated markers will be necessary in order to confirm or refute these observations.

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Case report

Familial multiple sclerosis: case study of three affected siblings

Bencsik K, Rajda C, Seres E, Vörös E, Janáky M, Dibó Gy, Járdánházy T, Vécsei L. Familial multiple sclerosis: case study of three affected siblings.
Acta Neurol Scand 2002: 106: 392–395. © Blackwell Munksgaard 2002.

We report on three sisters with new-onset multiple sclerosis (MS). The symptoms of the eldest sister began in 1993 with lower-limb weakness and paraesthesia. In 1998, she had limb weakness, nystagmus and ataxia. Magnetic resonance imaging (MRI) of the brain, the cerebrospinal fluid (CSF) examinations, and evoked potentials verified MS. The middle sister exhibited left-side optic neuritis in 1998. All findings pointed to MS. The third sister had subjective complaints such as paraesthesias and vertigo. MRI and CSF results supported the diagnosis. Both parents and all four grandparents are without neurological signs; the brain MRI examinations on the parents were negative. The prevalence of familial MS in first-degree relatives is 5–10%, while that in twins is 20–30%. In this case, environmental factors seem to play the crucial role. Although the anamnesis as concerns MS proved negative in the other family members examined here, further genetic examination of the sisters is needed.

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Key words: familial; multiple sclerosis; new-onset

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Objectives

As a consequence of the improved genetic epidemiological tools and statistical methodology, it has become clear that multiple sclerosis (MS) is a complex trait with genetic epidemiology very similar to that of a number of other organ-specific autoimmune diseases. The susceptibility to the illness is determined by a number of largely uncharacterized genes and environmental factors. However, it is not easy to see any analogy or to postulate any particular mechanism whereby these factors exert their effects. It is, therefore, useful to make a careful study of the over- and under-expression of the responses to environmental exposures (1–5). It has become evident that the parents or siblings of the MS patients are often carrying the trait of the disease, however, without any neurological symptom. Shared childhood and adolescence seems to have little impact on the risk of development of MS in siblings; the sequence of birth of affected individuals is more important than year of onset (1, 3, 6, 7). We came across an unusual occurrence where three sisters were suffering from MS without any MS trait regarding the parents and grandparents.

We report here on three sisters with new-onset MS. The family history (four grandparents and two parents) did not have MS or any other autoimmune or neurological disease. Relatives of MS patients are at greater risk for developing the disease than the general population, although this risk is still relatively low in absolute terms. Children of affected parents are reported, but we have not found any other report on affected siblings without any familial MS background.

Case study

The symptoms of Patient I (G.J., born on 30 January 1973) began in 1993 with lower-limbs weakness and paraesthesia. The neurological condition showed the following symptoms: paraparesis, muscle strength 4/5, tendon reflexes in the lower limbs +3 and paraesthesia. The computed tomography of the brain and the evoked potentials (electromyography

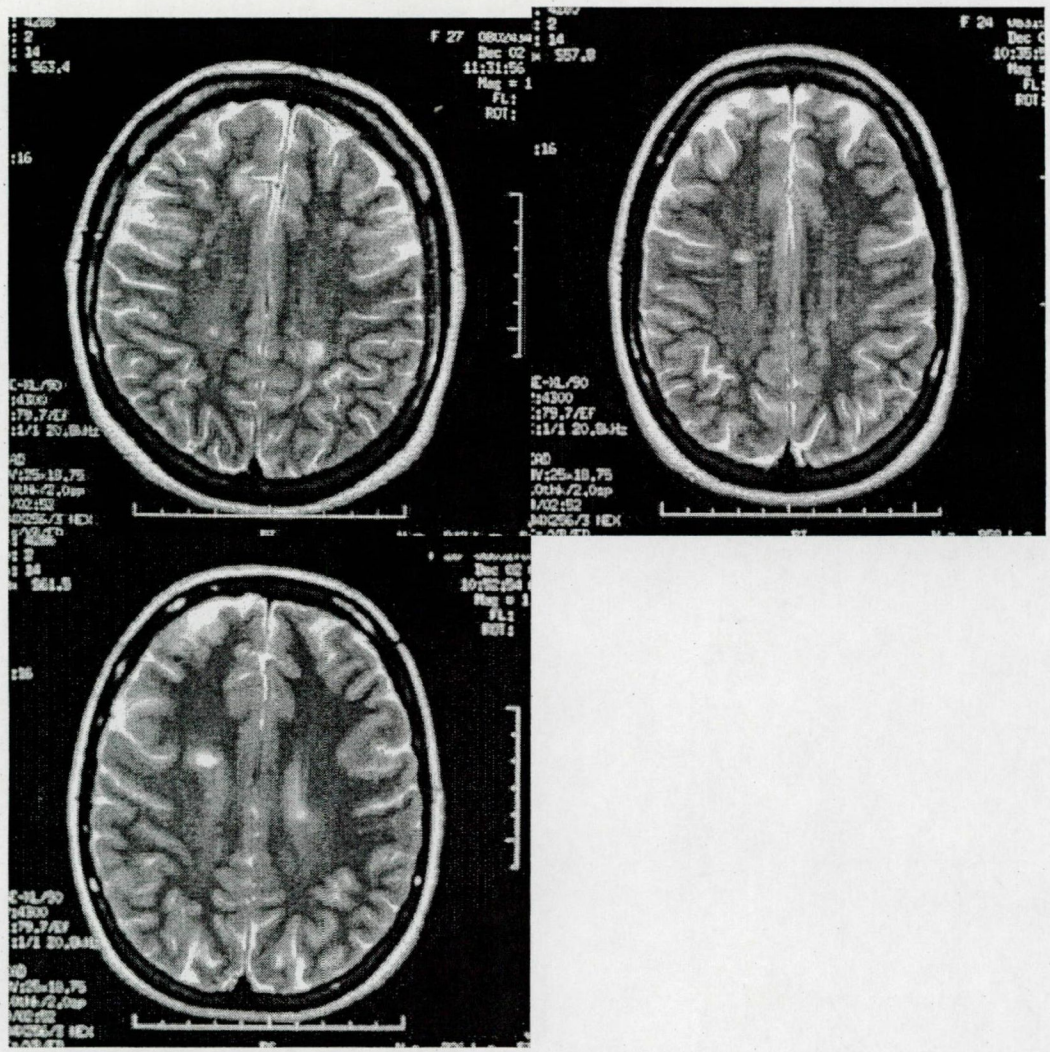

Figure 1. Magnetic resonance imaging of the brain – corresponding to demyelination (Patients I, II & III).

[EMG], electroneurography [ENG] and somatosensory evoked potential [SSEP]) were negative. The condition of the patient improved spontaneously in 2 weeks, and accordingly no further examinations were performed. Two months after her second labour in March 1998, she was admitted because of limb weakness, nystagmus and ataxia. The neurological symptoms were grade I horizontal nystagmus, mild trunk ataxia, hemiparesis on the left side, muscle strength 4/5, tendon reflexes +4, and a positive Babinski reflex on both sides. Expanded disability status scale (EDSS) score: 3 point (8). The MRI of the brain corresponded to demyelination (Figure 1). The cerebrospinal fluid (CSF) findings pointed to local intrathecal synthesis (52%), IgG index: 1.31, cytology: 5/μl lymphocytes, total protein: 208 mg/l, albumin quotient: 3.9×10^{-3}. Oligoclonal bands (OCBs) were detected with electrophoresis followed by IgG immunoblot (Figure 2).

For quantitative analysis laser nephelometry and for qualitative analysis isoelectric focusing and immunoblotting were used. The degree of local intrathecal synthesis was calculated according to the Reiber formula (5). The somatosensory evoked potentials (n. medianus, n. ulnaris, n. tibialis and n. peroneus) revealed central myelin damage. The visual evoked potential showed extended latency on both sides. After the examination, the patient received megadose methylprednisolone (MP) therapy: 1 g MP intravenously for 3 days, followed by 1 mg/bodyweight/day MP orally for 11 days. Remission was achieved after the MP therapy. The patient was symptom-free (EDSS score: 0 point). In July, after her second relapse, she received

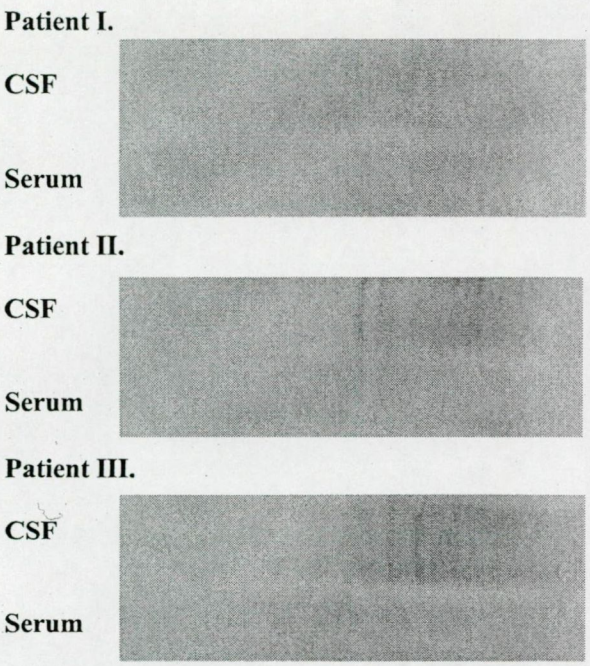

Figure 2. Oligoclonal bands in cerebrospinal fluid detected by IgG immunoblot – order of appearance: Patient II, Patient I, Patient III.

megadose MP therapy and achieved total remission.

Patient II (G.O., born on 22 January 1974) exhibited left-side optic neuritis at the initial examinations in February 1998. The visus at admission was 0.1 on the left side. The neurological condition was negative. The MRI findings on the head and optic nerve pointed to demyelination (Figure 1). CSF findings pointed to local intrathecal synthesis (46%), IgG index: 1.25, cytology 5/μl lymphocytes, total protein: 335 mg/l, albumin quotient: 4.5×10^{-3}. OCBs were detected by electrophoresis (Figure 2). Visual evoked potential showed extended latency on the left side P100: 154 and 98 ms on the right side. After the examination, the patient received megadose MP therapy; the visus on the left side improved to 1.

Patient III (G.A., born on 8 September 1976) was examined because of paraesthesia in all the extremities and vertigo. The only symptom in her neurological condition was the paraesthesia. EDSS score: 1 point. MRI of the brain corresponded to demyelination (Figure 1). CSF findings pointed to local intrathecal synthesis (70%), IgG index: 2.20, cytology: 4/μl lymphocytes, total protein: 315 mg/l, albumin quotient: 4.6×10^{-3}. OCBs were detected by electrophoresis (Figure 2). Visual evoked potential showed extended latency on the left side P100: 142 and 138 ms on the right side. As a result of her negative neurological condition despite the subjective complaints, she did not received MP therapy.

On the Poser (9) diagnostic criteria, all three sisters had definitive MS. Patient I has relapsing–remitting form and Patient II and III have first attack MS patient. The neurological condition and the MRI examination of the parents were found to be negative. We excluded previous neurological disease, and systematically taken medication during the last decades. All four living grandparents are healthy with regard to the neurological findings.

Conclusion

Adoption studies suggest that the familial risk of MS susceptibility is influenced rather by genetic than by environmental factors (2, 10, 11). The influence of environmental factors is difficult to exclude, as the twins and siblings share the same environment. For the differentiation between the influence of genetic and environmental factors, the population of adopted children and their parents have been used with success. A Canadian comprehensive study of recurrence has shown a lifetime risk of 0.2% for the entire population, which is increasing to 3% in other first-degree relatives (relative risk 20) and 1% in second- and third-degree relatives (relative risk 5.5) (12). Comparison studies from the United Kingdom confirmed the highest recurrence rate for sisters (4.4%) and brothers (3.2%) compared with parents (2.8%) and offspring (1.8%). The reduction in risk changes from 2.8% in first-degree relatives to 1 and 0.9% in second- and third-degree relatives, respectively, compared with the background age adjusted risk in this population of 0.3% (13). Previously published data indicate that family risk range from 300-fold for monozygotic twins to 20–40-fold for biological first-degree relatives over the general population prevalence of 0.1% (10). The prevalence of familial MS in first-degree relatives is 5–10%, while that in monozygotic twins is 20–30% (6). These findings support the role of genetic factors in MS.

The role of environmental factors is greater in mono- than in dizygotic twins than in the first-degree relatives, which leads to confusion concerning the aetiology (2). Adoption studies suggest that the familial environment play a role in the development of MS, while the frequency of the disease in non-biological relatives is equal to that in the normal population (10).

According to these findings, the familial occurrence of MS is genetically determined. We report on three affected sisters whose parents and grand-

parents' medical history did not show any neurological or autoimmune disease. Taking into consideration that the familial environment does not necessarily lead to the disease, a genetic mutation of the sisters might be possible. Although the parents and grandparents are healthy, we cannot exclude a common viral infection in their childhood or other environmental factors as a triggering factor of the disease. The crude risk for MS in the Northern European population is 1:600. If a sibling is affected, the risk increases to 1:40 (14). The possibility that in an unaffected family, in a medium-risk zone, all three children would suffer form MS is less than 1:600. In the light of these data the case of these affected sisters might be interesting and their further follow-up, the encoding of the nature's over- or under-expression, might lead to a better understanding of MS (14).

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Catecholamine levels in peripheral blood lymphocytes from multiple sclerosis patients

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Abstract

Circumstantial evidence suggests the involvement of sympathoadrenergic mechanisms in the progress of multiple sclerosis (MS). We studied peripheral blood lymphocytes from MS patients. The levels of dopamine (DA), norepinephrine (NE), epinephrine (E) and their metabolites in extracts of lymphocytes from 58 MS patients and 19 healthy controls were measured by using capillary electrophoresis. The MS patients were divided into clinical subgroups: a laboratory-supported definitive (first-attack) MS group, and a relapsing–remitting (RR) group in remission. The peripheral blood lymphocyte level of epinephrine was significantly higher in the first-attack MS patients ($p = 0.028$) than in the controls. However, the norepinephrine levels were significantly ($p = 0.027$) lower in the RR patients in remission. The catecholamines are known to be able to affect the lymphocyte activity, both by stimulation and by immunosuppression. Our results suggest that the catecholamines are important regulators of lymphocyte activation in MS, and of potential importance as concerns new diagnostic and therapeutic methods. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Capillary electrophoresis; Catecholamine; Dopamine; Epinephrine; Lymphocytes; Multiple sclerosis; Norepinephrine; Peripheral blood mononuclear cells

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Current hypotheses on the pathogenesis of MS suggest that the primary peripheral activation of autoreactive T helper-1 lymphocytes precedes the recognition of CNS auto-antigens. These T cells proliferate, secrete cytokines and cross the blood–brain barrier (BBB) to find their antigens in the CNS where they cause further inflammatory damage. It has been hypothesized that relapsing–remitting (RR) MS is driven by a systemic antigen presentation and that chronic progressive MS depends on the CNS presentation of antigens (Hafler, 1999).

Studies involving experimental models of MS demonstrate the importance of lymphocytes and sympathoadrenergic mechanisms (Anderton et al., 1999). Thus, in experimental autoimmune encephalomyelitis (EAE) lymphocytes crossing the BBB undergo a transformation that is involved in the progress of the disease (Wekerle, 1993). Experimentally induced hypercatecholaminemia in rats seems to protect the lymphocytes from the immunosuppressing effects of other endogenous stress hormones, but causes suppression of peripheral blood lymphocyte activation if the β-receptors are blocked at the same time. Beta-adrenergic agonists suppress chronic/relapsing EAE (Wiegmann et al., 1995) and decrease the number of β-adrenergic receptors on splenic lymphocytes in Lewis rats (Muthyala et al., 1995).

As an immune privileged site, the brain is not totally separated from the immune system, as thought earlier. The

Abbreviations: BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dopamine; E, epinephrine; MS, multiple sclerosis; NE, norepinephrine; PBMC, peripheral blood mononuclear cell; RR, relapsing-remitting.

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CNS is connected to the deep cervical lymphatic nodes and shares messengers with the immune system. One group of these common transmitters is the catecholamines. Immunocompetent cells have been shown to contain and produce catecholamines, serotonin (5-HT), melatonin and acetylcholine (Bergquist et al., 1994; Josefsson et al., 1996; Dedenkov et al., 1986; Musso et al., 1996; Rinner et al., 1998), together with many neuropeptides and hormones, and also to express their corresponding receptors (Blalock, 1992; Santambrogio et al., 1993; Felten et al., 1992; Ricci et al., 1995; Felsner et al., 1995; Costa et al., 1995). Catecholamines and their metabolites have been found in the lymphocytes in a number of studies (Bergquist et al., 1994, 1997; Josefsson et al., 1996; Musso et al., 1996, 1998; Bergquist and Silberring, 1998); the amount of intracellular dopamine (DA) is approximately 10^{-18} mol/cell (Bergquist et al., 1994).

Lymphocytes have a cellular uptake mechanism, but are also capable of the endogenous synthesis of DA and norepinephrine (NE). Additionally, they are also able to store and degrade catecholamines and possibly to regulate their own activity via an autocrine loop. Furthermore, catecholamines have been found inside the nuclear envelope (Bergquist et al., 1998), suggesting a possible direct interaction with the transcription machinery or via an interaction with the nuclear factor-κB (NF-κB) regulatory system (Bergquist et al., 2000). Recent results suggest a crucial role of NF-κB1 in the activation and differentiation of autoreactive T cells. Blocking the NF-κB function can be an effective way to prevent autoimmune encephalomyelitis (Hilliard et al., 1999). Elevated regional levels of catecholamines might lead to suppression and finally apoptosis, which would partly explain the immune privilege of the brain (Bergquist et al., 1994, 1997, 1998).

The catecholamines secreted by the sympathetic nervous system predominantly act on human T cells of the $CD8^+$, $CD28^-$ (suppressor) subset (Karaszewski et al., 1991). This subset has the highest β-adrenergic receptor density. NE stimulates, while norepinephric denervation diminishes the Th1 responses (cellular immunity). Humoral immunity is also affected, perhaps via additional signaling to B cells, NE favoring IgM responses and noradrenergic denervation favoring a shift from IgM to IgG responses (Felten and Felten, 1994).

The discovery of catecholamines in lymphocytes and their functional role involving the control of T and B lymphocytes (Bergquist et al., 1994) led to many questions being raised about their role in neuroimmunological interactions. The regulation of lymphocyte functions by catecholamines could prove to be an important part of immune deactivation in the nervous system. Studies on human neutrophils and peripheral blood mononuclear cells (PBMCs) demonstrated a catecholamine lifecycle in these cells, suggesting the presence of autoregulatory adrenergic mechanisms (Bergquist et al., 1998; Cosentino et al., 1999; Marino et al., 1999).

In the present study it was hypothesized that the deactivation of the immune system after a MS relapse (remission) could be mediated by catecholamines. Accordingly, the intracellular levels of catecholamines in relapsing–remitting (RR) MS patients in remission and in first-attack MS patients are described.

2. Subjects and methods

2.1. Patients and controls

A total of 58 patients were examined and were found to have clinically and laboratory-supported definitive MS according to the Poser criteria (Poser et al., 1983); 10 were laboratory-supported definitive (first-attack) patients, and 48 had RR MS. Both the cerebrospinal fluid (CSF) findings (oligoclonal bands on isoelectric focusing electrophoresis) and the MRI findings (several periventricular T2-weighted lesions) of the first-attack patients supported the MS diagnosis. All the RR patients were in remission. None of the patients had received steroid therapy within 30 days and none of them were on tricyclic antidepressants, cardiac drugs or amantadine. The neurological conditions of the patients were expressed on the Kurtzke expanded disability status scale (EDSS) (Kurtzke, 1983). Healthy individuals ($n=19$) served as controls. The study was approved by the ethical committee of Albert Szent-Györgyi Medical School at the University of Szeged (886/1998). For the statistical analysis various MS subgroups were formed, depending on (a) the clinical course of the disease: first-attack (10) or relapsing–remitting (48); (b) the EDSS score: EDSS score <4.0 (49) or >4.0 (9); (c) the duration of the disease: <5 years (30) or >5 years (28); (d) the time to the last relapse: relapse period <6 months (19) or >6 months (39). More data on the patients are provided in Table 1.

Table 1
Patient's data

Group	No. of subjects	Last relapse (months)	No. of relapses/2 years	Onset (year)	EDSS	Age (year)
First-attack MS	10	14.6 ± 3.0	0.9 ± 0.1	0	0.0	38 ± 2
RR MS	48	16.8 ± 1.8	1.0 ± 0.14	2.5 ± 0.07	2.2 ± 0.05	40 ± 1
Relapse within 6 months	19	3.9 ± 0.4	1.5 ± 0.21	2.4 ± 0.11	2.2 ± 0.08	40 ± 2
Relapse-free for >6 months	39	22.5 ± 0.8	0.8 ± 0.12	2.5 ± 0.08	2.1 ± 0.06	40 ± 1
Controls	19	–	–	–	–	30 ± 2

2.2. Preparation of lymphocytes

Peripheral vein blood samples (12 ml) were prepared by centrifugation at 2500 × *g* for 10 min. Lymphocytes were isolated by centrifugation on a Lymphoprep® (Nycomed Pharma, Oslo, Norway) density gradient and, after washing and centrifugation steps, kept at − 80 °C until analysis. The lymphocytes were extracted by adding 25 μl perchloric acid (containing 1 mM NaEDTA and 1 mM Na_2SO_3) to the pellet, followed by ultrasonication on ice for 2 min using a MSE Soniprep 150 probe. After centrifugation (30 min, 4 °C, 35000 × *g*) the supernatant was frozen and stored at − 80 °C until analysis. The pellet was used for spectrophotometric protein quantitation, using bicinchoninic acid protein assay reagent (BCA, Pierce Chemical, Rockford, USA).

2.3. Capillary electrophoresis with electrochemical detection

The capillary electrophoretic system used was described in detail earlier (Bergquist et al., 1994, 1998; Josefsson et al., 1996). Briefly, a buffer-filled fused silica capillary (Polymicro Technologies, Phoenix, USA) measuring 10 μm in i.d. and 65 cm in length was placed between two buffer reservoirs. High voltage was applied at the injection end, and the reservoir containing the detector end was held at ground potential. Electrokinetic injection was used for all sample introductions, 5 s at 30 kV; the sample volume was approximately 600 pl. The easily oxidized analytes were detected in the amperometric mode with a two-electrode configuration, using optimized end-column detection (Bergquist et al., 1997). A carbon-fiber microelectrode was inserted into the end of the electrophoresis capillary and held at 0.8 V versus a sodium-saturated calomel electrode. Reagents: 2-(*N*-morpholino)ethanesulfonic acid (MES), 5-HT, NE, epinephrine (E), DA, L-dihydroxylphenylalanine (L-DOPA), vanilmandelic acid (VMA), methoxyhydroxyphenyl glycol (MHPG), homovanilic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were obtained from Sigma (St. Louis, USA) and used in the form received. The electrophoresis buffer was 25 mM MES adjusted to pH 5.65 with NaOH. Calibration standards were prepared as 10 mM stock solutions in perchloric acid and diluted to the desired concentration in electrophoresis buffer. Hydrofluoric acid was obtained as a 40% aqueous solution from Aldrich, Milwaukee, USA), and was used for the etching of the detector end of the capillary.

The catecholamine levels of the lymphocytes were quantified by direct comparison with the standard electropherograms run before and after the patients' samples. The catecholamine contents of the lymphocytes are given in fmol/μg protein. Detection limits were determined (for DA, NE 0.13 fmol/μg protein, for E 0.37 fmol/μg protein, and for DOPAC 0.11 fmol/μg protein) and estimated at twice the peak-to-peak noise level by extrapolation from plots of peak area versus concentration. Between the series of runs, the capillary was flushed with 0.1 M NaOH to refresh the inner capillary surface and to maintain reproducible separation conditions. For a more detailed description of the method, see Bergquist et al. (1994).

2.4. Statistical analysis

The Kruskall–Wallis test (SPSS 7.5 for Windows) was performed for statistical analysis to compare the catecholamine levels in the healthy controls and the subgroups of

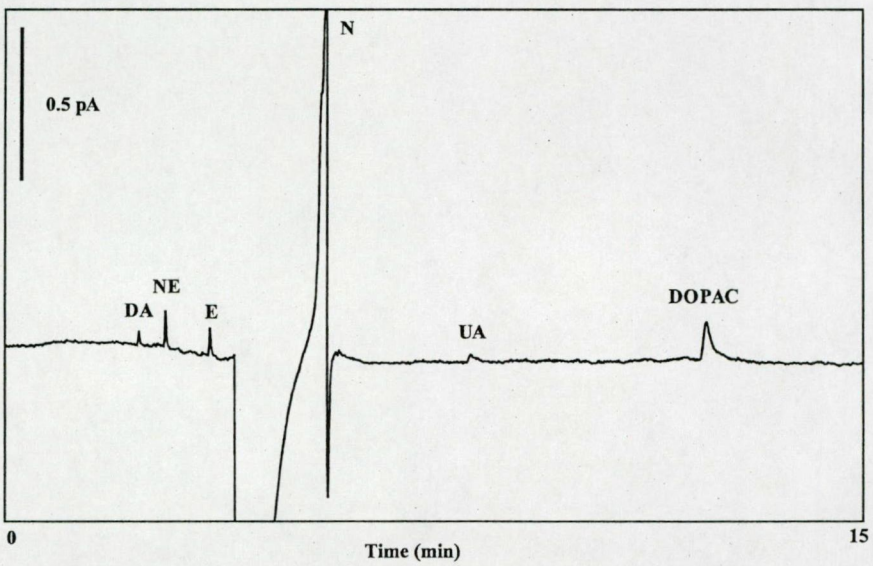

Fig. 1. A representative electropherogram of catecholamines extracted from human peripheral blood lymphocytes, showing the peaks of dopamine (DA), norepinephrine (NE), epinephrine (E), neutral species (N), uric acid (UA) and dihydroxyphenylacetic acid (DOPAC).

MS patients, followed by the Mann–Whitney *U*-test for pairwise comparisons to asses the differences between the patients and the healthy controls. The Kruskal–Wallis test was also used for the statistical analysis of the differences between the healthy controls and the different MS subgroups regarding EDSS score, medication, and duration.

3. Results

The electrophoretic mobilities of the major peaks in the electropherogram corresponded to the calculated electrophoretic mobilities of DA, NE, E, uric acid (UA), and DOPAC (Fig. 1). We excluded the 5-HT, MHPG, VMA and ascorbic acid data because their levels were often under the detection limit (MHPG was detectable in 7/19 controls and in 19/58 MS patients, and VMA in 5/19 controls and 27/58 MS patients). We also excluded L-DOPA since it is a neutral molecule and has the same electrophoretic mobility as all other neutrals, therefore leading to difficulties with the quantification. The levels of the catecholamines are presented in Table 2.

3.1. Healthy controls versus first-attack and RR MS patients

When the MS patient subgroups and the healthy individuals were compared, significantly lower levels of NE (Kruskal–Wallis test, $p=0.027$) and higher levels of E (Kruskal–Wallis test, $p=0.028$) were found in the lymphocytes. Pairwise comparisons with the Mann–Whitney *U*-test showed that the RR MS patients ($p=0.017$) and the first-attack MS patients ($p=0.035$) had lower levels of intracellular NE than healthy controls (Table 2). The E content of the lymphocytes in first-attack MS patients was higher as compared to either the RR MS group ($p=0.008$) or the controls ($p=0.056$).

3.2. Differences between healthy controls and MS subgroups regarding EDSS scores, duration of disease and medication

Both the MS patients with shorter disease duration ($n=30$, mean ± SEM: 378 ± 90 fmol/μg) and those with longer disease duration ($n=28$, mean ± SEM: 453 ± 154 fmol/μg) displayed lower intracellular NE levels (Mann–Whitney *U*-test, $p=0.033$) as compared with the control group ($n=19$, mean ± SEM: 1594 ± 599 fmol/μg). The lymphocytes of both the patients in a better neurological condition ($n=49$, mean ± SEM: 368 ± 64 fmol/μg) and those with an EDSS score >4 ($n=9$, mean ± SEM: 807 ± 516 fmol/μg) contained less NE ($p=0.036$) than the cells of the controls ($n=19$, mean ± SEM: 1594 ± 599 fmol/μg). The administration of anxiolytics did not exert any significant effect on the catecholamine levels of the lymphocytes. Slight, nonsignificant differences in the NE contents of the lymphocytes were found between the group without immunomodulating medication ($n=42$, mean ± SEM: 332 ± 56 fmol/μg), those receiving interferon-β-1b treatment ($n=9$, mean ± SEM: 450 ± 235 fmol/μg), those receiving glatiramer acetate treatment ($n=7$, mean ± SEM: 1039 ± 649 fmol/μg) and the controls ($n=19$, mean ± SEM: 1594 ± 599 fmol/μg).

4. Discussion

Modern analytical tools such as capillary electrophoresis techniques allow the detection of intracellular catecholamine levels and give an insight into their regulation of lymphocyte differentiation, proliferation and apoptosis. The increased beta-adrenergic receptor density on the lymphocytes of MS patients in relapse suggests an involvement of lymphocytes and catecholamines in the pathogenesis of the disease. A general problem in MS research is that the phenomena observed can either be secondary to the disease progress with no causality, or reflect mechanisms of importance for the disease.

Scattered reports suggest a role for low molecular weight neurotransmitters in the pathogenesis of MS. Elevation of the levels of NE by using antidepressants and L-DOPA has been found to affect the symptoms of MS (Berne-Fromell et al., 1987). Maprotilin and lofepramin enhance the levels of NE in the synapses (Baumann and Maitre, 1979). Seventy-five percent of MS patients treated with L-DOPA experienced an improvement after 1–2 months (Berne-Fromell et al., 1987). Numerous

Table 2
Catecholamine contents of peripheral blood lymphocytes in healthy individuals and various subgroups of MS patients

Test group	No. of subjects	DA	NE		E		DOPAC
Healthy individuals	19	1.39 ± 0.32	1.59 ± 0.60	*50.0	0.06 ± 0.02	*43.8	2.02 ± 1.41
RR (first-attack MS)	10	1.89 ± 0.85	0.24 ± 0.10[a]	*30.0[b]	0.18 ± 0.08[c]	*62.9[d]	2.14 ± 2.08
RR (MS in remission)	48	1.68 ± 0.33	0.48 ± 0.11[a]	*36.0[e]	0.23 ± 0.14[c]	*40.0	6.29 ± 2.71

Values given as mean ± SEM fmol/μg protein and as * mean ranks.

RR = relapsing–remitting, DA = dopamine, NE = norepinephrine, E = epinephrine; DOPAC = dihydroxyphenylacetic acid.

[a] Significant difference between the first-attack and RR MS patients and healthy controls with Kruskal–Wallis test, $p=0.027$.

[b] Significant difference between first-attack MS patients and healthy controls with Mann–Whitney *U*-test, $p=0.035$.

[c] Significant difference between the first-attack and RR MS patients and healthy controls with Kruskal–Wallis test, $p=0.028$.

[d] Significant difference between first-attack and RR MS patients with Mann–Whitney *U*-test, $p=0.008$.

[e] Significant difference between RR MS patients and healthy controls with Mann–Whitney *U*-test, $p=0.017$.

studies have revealed that NE may regulate early immune events such as antigen localization, presentation, B cell activation, inhibition of T suppressor cell activation and the functions of both Th1 and Th2 cell function (Sanders, 1998; Madden and Livnat, 1991). NE may also suppress the normal immune response (Bergquist et al., 1998). Elevated levels of NE have been observed in the CSF, but not in the blood of MS patients (Barkhatova et al., 1997). It has been hypothesized that there is a deficiency of NE in the nerve terminals in MS, similar to the DA deficiency in Parkinson disease patients. This hypothesis is supported by the fact that near the fourth ventricle lies the locus ceruleus, a NE-mediated part of the brain regarded as a "stress center". Lower levels of NE in MS could possibly explain the reduced awareness and memory function, the difficulties with micturition and the cerebellar symptoms, which are the opposite of the "fight or flight" reactions (Berne-Fromell et al., 1987). Recent MRI and neuropathological findings suggest early axonal damage in MS that could be prognostic for the further disease progression (Ferguson et al., 1997; Trapp et al., 1998; Raine et al., 1999; Lovas et al., 2000). If the neurons are damaged, there could be an ungoverned release of catecholamines and high local concentrations in the area of the lesion. The lymphocytes in the region may be exposed to these high concentrations causing high intracellular levels by an initial uptake (as may be possible in first-attack).

We found changed intracellular catecholamine levels in the PBMCs of the MS patients. The analyzed changes reflect the whole PBMC population and probably only a small proportion of them are directly involved in the CNS pathogenesis. However, if the effect of immune regulation in MS is more systemic, this could be measured in the periphery. Normally just a few leukocytes are present in the CSF and the collection of these cells would be very difficult and demand single cell analysis. After considering these problems, we concentrated on collecting PBMCs. The inclusion criteria for first-attack patients were several T2-weighted lesions on the brain MRI and positive CSF findings (oligoclonal bands, elevated IgG levels in the CSF, and a positive IgG immune blot). In the city of Szeged, the incidence of MS in 1996 was 7/100.000 (Bencsik et al., 1998). Because of the low number of first-attack MS patients, it was difficult to add more data to this group.

Catecholamines also affect the natural killer cell function through β-adrenergic receptors (Takamoto et al., 1991). Activated lymphocytes have increased numbers of muscarinic and nicotinergic receptors (Besedovsky and DelRey, 1996). A number of reports suggest involvement of the catecholaminergic system in MS. A two-fold increase in β-receptor density was found on the PBMCs during relapse in RR MS patients and in secondary chronic progression MS, while the levels of NE and E in the plasma were similar to the control levels (Zoukos et al., 1992). From patients with

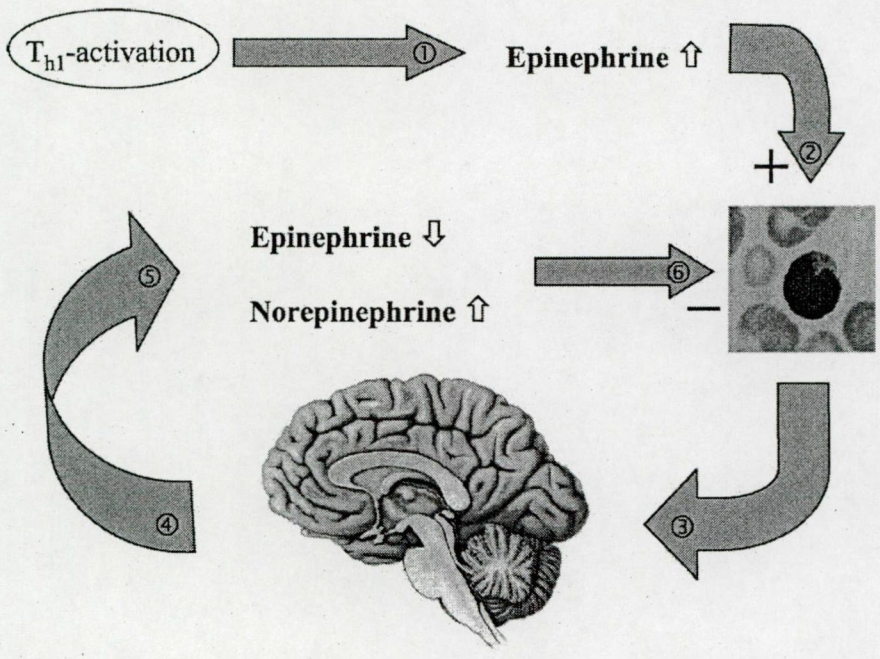

Fig. 2. The hypothesized role of the catecholamines in the pathogenesis of MS. ① Th1 T cell activation takes place in the periphery. ② Increased epinephrine levels activating the lymphocytes augment the entrance of lymphocytes through the BBB. ③ Well inside the CNS, the lymphocytes find their antigens and are activated. ④ After the activation of the lymphocytes, a feedback process is initiated. ⑤ This feedback loop leads to lymphocyte deactivation and the epinephrine content of the lymphocytes is decreased. This epinephrine decrease is then followed by an increase in norepinephrine, causing a down-regulation of the lymphocytes ⑥, and leading to the steady state of remission.

chronic progressive MS, an increased number of β-adrenergic receptors was found on the CD8+ T cells. In contrast, patients with stable MS and those with relapsing–remitting disease before, during or after attacks had unchanged receptor densities (Karaszewski et al., 1991). The plasma E levels in samples drawn from patients in supine and upright positions were similar in chronic progressive MS to those for normal individuals, but the supine plasma NE levels were higher in chronic progressive MS (Karaszewski et al., 1993).

In a recent study, the percentages of T and B cells in the peripheral blood from MS patients in relapse, with viral inflammatory or with noninflammatory neurological disease were similar (Oreja-Guevara et al., 1998). Various cell surface molecules on the peripheral blood CD4+ T cells and the disease activity (by MRI examination) were monitored in relapse and in remission, but no differences and no correlation to disease activity could be found (Stuber et al., 1996).

No differences in plasma dopamine-β-hydroxylase activity have been reported between healthy individuals and MS patients (either in relapse or in remission) (Markianos et al., 1991). The synthesis of catecholamines in lymphocytes is under nicotinic control and acetylcholine might regulate catecholamine synthesis through activation of the rate-limiting enzyme tyrosine hydroxylase (Musso et al., 1997). We have not encountered any other data on differences in enzyme activity related to the catecholamine metabolism in the lymphocytes or peripheral blood of MS patients.

We observed higher intracellular levels of epinephrine in first-attack MS patients, and the lymphocytes express primarily β-adrenergic receptors. Thus, we can propose the following hypothesis, presented schematically in Fig. 2. An increased level of E activates the lymphocytes; they cross the BBB and find their antigens. This process is followed by the production of cytokines, which either result in an inflammatory process or act as the major compartment in the relapse process. A relapse-increased β-receptor density on the lymphocytes has been described, lending support to our hypothesis (Zoukos et al., 1992). It is not clear whether the lymphocytes merely mirror the state of the disease, reflecting the altered hypothalamus–pituitary gland–adrenal medulla (HPA) axis function and drain the catecholamines from the plasma, or are active participants, eliminating the catecholamines by uptake and degradation or releasing them into the MS plaque. The lower level of NE in the peripheral blood lymphocytes of RR MS patients in remission could be due to the β-adrenergic receptor down-regulation after a bout or to the degradation of the catecholamines. Remission may be due to a general down-regulation of the immune response by immunologically nonspecific mechanisms, such as the endogenous secretion of corticosteroids. Later in the disease process, a negative feedback suppresses the production of the catecholamines, resulting in a decreased catecholamine content of the peripheral blood lymphocytes during remission. This may explain why RR MS patients in remission may have lower levels of catecholamines such as NE and also account for the neuroimmunological entity of the relapse.

Higher catecholamine levels in the peripheral blood lymphocytes might prevent relapses. Catecholamines have a relatively short duration of action, which could be triggered by widespread activation, except when the levels are chronically changed. One of the risk factors for autoimmunity is the low NE level in MS patients, which reflects the hypoactivity of the HPA axis.

Relapses can be induced by infection, stress, or an elevated level of E, which activates the lymphocytes, resulting in turn to activation of the disease. After nicotinic activation of the lymphocytes, intracellular NE and L-DOPA production occurs (Musso et al., 1997). The catecholamine levels may play an important regulatory role, especially in RR MS patients, when the β-receptors on the lymphocytes are increased. This needs to be further investigated before any strong conclusions may be drawn.

MS patients have a significantly lower NE content in their peripheral blood lymphocytes than that for healthy individuals, but in the early stage of the disease, and hence in first-attack patients, the E content is higher. With regard to the fact that the lymphocytes in relapse have a higher β-receptor density, new means of early intervention in the pathogenesis of MS at the lymphocyte level may be possible. These data suggest a connection between the peripheral blood lymphocyte catecholamine content and the course of the disease, and may contribute to a better understanding of the pathogenesis of MS. They may also suggest a new therapeutic approach through recognition of the role played by lymphocytes in this disease.

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Peer reviewed article

Experiences with interferon-beta-1b treatment in MS after three year follow-up

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Interferon-beta-1b (IFNβ-1b) was the first drug which has been proven to decrease the number of attacks by 34% in relapsing-remitting multiple sclerosis (MS).

The aim of this open label, observational phase IV study was to evaluate the effect of IFNβ-1b on relapse rate in a three-year follow-up. The data of the enrolled patients in the two years prior to the treatment (1995–96) served as control. The trial was carried out between 1996 and 1999. 31 patients with definite MS received 8M IU IFNβ-1b sc. every other day. The relapse rate, the duration of hospitalisation and the steroid needs for treatment of relapses were calculated. Statistical analysis was made by one-way ANOVA. At baseline the mean age was 37 ± 8 years, the mean EDSS score was 1.8 ± 1.2 and the mean duration of the disease was 4 ± 4 years. Before treatment the annual relapse rate was 1.3, while during the treatment it decreased to 0.3 (Table 1). The relapse rate was reduced by 77% compared to pre-study values ($p < 0.001$). Before starting the therapy the patients spent 16.0 ± 2.5 days in the hospital annually. In the three years of IFNβ-1b the mean time of hospitalisation decreased by 84% ($p < 0.001$). In the two years preceeding IFNβ-1b therapy 5.7 ± 1.9 grams of methylprednisolone (MP) / patient were needed for treatment of relapses. During IFNβ-1b therapy MP needs were reduced by 75% ($p < 0.001$).

Using the patients as their own controls is a methodological problem in MS, taking into account the more or less unpredictable course and also the fact that patients initiating treatment may do so in an active phase of the disease with consecutive spontaneous regression to the mean. Nevertheless, the magnitude of the change observed in this study supports a positive effect of IFNβ-1b on the disease under everyday practice conditions.

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Table 1

Differences observed during the 3 years of IFNβ-1b treatment (mean ± SD).

years	-2	-1	+1	+2	+3
no. of relapses / patient	1.0 ± 0.5	1.6 ± 0.6	0.4 ± 0.5	0.4 ± 0.4	0.2 ± 0.4
days of hospitalisation / patient	14 ± 10	18 ± 11	3.5 ± 6.8	3.1 ± 5.2	0.7 ± 2.1
need of steroid (g) / patient	4.3 ± 2.8	7.0 ± 2.5	1.7 ± 2.9	1.7 ± 2.4	0.6 ± 1.6

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