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Risk factors in multiple sclerosis: a population-based case-control study in Hautes-Pyrénées, France

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ABSTRACT - A population-based study of the prevalence and risk factors of multiple sclerosis (MS) was conducted in the Hautes-Pyrénées, the southwestern region of France. The prevalence rate per 100,000 was equal to 40. Data on the past medical history of 63 MS patients and matched controls were collected. The frequency and age at occurrence of common childhood infections were similar for both the MS cases and controls. There was no difference between the frequency of vaccination for MS patients and for controls. However, the age at which MS patients were immunized against poliomyelitis was significantly higher than the corresponding age for controls (15.8 years versus 8.9 years, $P < 0.01$). Antibody titers for various viruses were measured. The mumps antibody titer was significantly higher in the MS patients than in the controls. Also, MS patients tended to have higher titers for measles antibodies.

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The Hautes-Pyrénées department is situated in southwest France at a latitude of 42° to 43° north. It is a mainly rural area of 4500 square kilometers. At the time of the 1982 census, the population was 227,942 (Figure 1); 22% of which was concentrated in the main town of Tarbes. According to the census, approximately 84% of the resident population over age 20 had been born in the county.

Analysis of the geographical pattern of death rates from multiple sclerosis (MS) in France from 1968 to 1977 showed a risk gradient that increased from south to north (1). However, the Hautes-Pyrénées region recorded significantly higher mortality rates than the neighboring southern counties. We conducted an epidemiologic study in 1983 to investigate this phenomenon.

Methods

General practitioners and neurologists ($n = 450$) were asked to provide the names of individuals whose neurological disease suggested MS. Ninety-five percent of the physicians contacted were willing to cooperate. The criteria used to diagnose MS were those defined by Poser et al. (2). For each MS case, one unrelated control of the same age, gender, and parish of residence was selected. One investigator (J-B R) interviewed the patients and controls in their homes. A summary of questionnaire items is shown in Table 1.

Blood samples were taken from patients and controls. Antibody titers to 7 viruses (measles, rubella, varicella-zoster, mumps, herpes simplex

1, cytomegalovirus, coronavirus) were measured by ELISA. All specimens were tested blindly. Titers were compared by transforming the reciprocal of the dilutions to log 2.

Data were analyzed using statistical tests for matched-pair comparisons (3). A P value of 0.05 or less was considered significant.

Results

Patients

Ninety-one patients (60 women and 31 men) were living in the Hautes-Pyrénées county on prevalence day (1 January 1983). The prevalence rate per 100,000 was equal to 40; 4 patients died during 1983. Sixty-three patients with definite or probable MS for whom both questionnaire and blood samples had been obtained were available for matched-pair comparisons. Seventy-three percent of them were female (F:M ratio = 2.7). The mean age of MS onset was 30.8 years. The mean interval from onset to prevalence day was 13.1 years; the proportion of cases with onset greater than 15 years before prevalence day was 34% (22 of 63).

Thirty-six patients (57%) had a relapsing-remitting form of MS; 22 (35%) had a relapsing-remitting form leading to chronic progressive course; and 5 (8%) had a chronic progressive form with no remission. At the time of the examination, Kurtzke's disability score (4) was greater than 5 in 16 (25%) of the 63 patients.

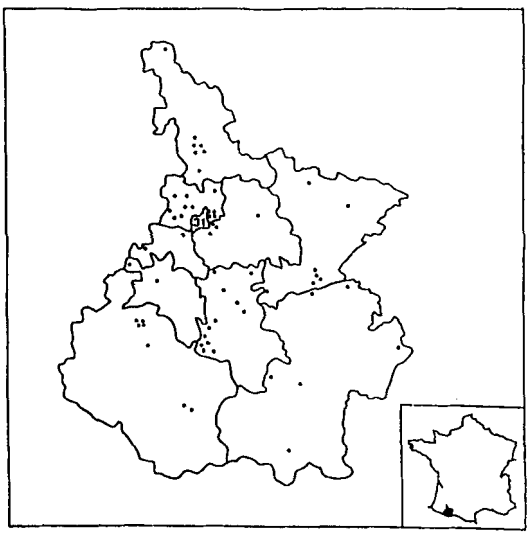

Fig. 1. Distribution of MS cases in the Hautes-Pyrénées county. (Each point represents one case, except for the main town Tarbes, where the total number of cases is noted.)

Medical history

The age of MS onset was noted for the patient in the patient-control pair, and medical events for each member of the pair were considered in relation to this age. Patients and controls were asked whether they had ever contracted the following infectious diseases: rubella, measles, varicella, mumps, viral hepatitis, herpes simplex, zoster, pertussis, chronic sinusitis, and upper airways infections (Table 2). On the whole, more patients than controls gave affirmative answers to these questions; but except for the incidence of zoster, the differences were not significant. Indeed, of the 11 patients who reported having zoster, 10 (17.5%) had been affected before the onset of MS compared with 3 (5.2%) of the controls. The interval between zoster and MS onset ranged from a few months to 17 years; in 5 patients, it was smaller than 5 years. Herpes zoster in varicella cases occurred 2.5 times as frequently in MS patients as in controls. Age at occurrence of common childhood infections was similar for patients and controls, although patients tended towards a higher mean age for rubella and varicella.

Past medical history, including tonsillectomy, operations under anaesthesia, and trauma were similar in patients and controls. Stressful life events before the age at MS onset were significantly more common in patients than in controls (67.2% versus 47.6%, $P < 0.05$). The mean age at menarche was higher in patients than in controls (13.5 years versus 12.7 years, $P < 0.002$). There was no relationship between age at menarche and age at MS onset in MS patients.

There was no difference in the frequency of vaccination against poliomyelitis and tuberculosis in patients and controls (Table 2). No data about other vaccinations had been recorded. There were 15 pairs for whom age at vaccination against poliomyelitis was known for both the MS patient and his or her matched control. Age at the time of immunization was significantly higher for patients than controls (15.8 years versus 8.9 years, $P < 0.001$).

Table 1
Summary of interview items

Items (a)	*P* value
DEMOGRAPHIC	
Month of birth	NS
Birth order	NS
Birth size	NS
Age of mother at birth	NS
Birth place	NS
Number of moves	
within the county	NS
outside the county	NS
SOCIAL CLASS	
Social class by father's occupation	NS
Length of schooling	NS
Academic level	NS
Social class by patient's occupation	NS
HOUSING AND ENVIRONMENT	
Family density	NS
Toilet facilities	NS
Shower/bath facilities	NS
Source of water supply	NS
Animal exposure	
farm	NS
pets	NS
DIET	
Breast-feeding	NS
Meat	NS
Fat	NS
Delicatessen	$P < 0.007$
Milk	NS
Dairy produce	NS
Fish	NS
Fresh fruit and vegetables	NS
Alcoholic beverage	$P < 0.04$

(a) most of these items included several sub-items.

A history of familial MS was reported by 4 patients but by none of the controls ($P < 0.05$).

Viral antibody titers

Table 3 shows the distribution of viral antibody titers in MS patients and controls. Except for mumps, geometric mean titers were similar in both groups. Matched-pair analysis showed that there were 32 pairs in which the mumps antibody titer was higher in the patient than in the control, and 16 pairs in which the reverse was true (chi-square Mc Nemar = 5.3, $P < 0.02$). A similar trend was observed for antibodies against measles (35 pairs versus 21, $P < 0.10$).

Table 2
Past occurrence of various diseases in patients and controls before age of onset of multiple sclerosis

Items	Proportion in		
	MS	Controls	*P* (a)
Rubella	41.2	31.6	NS
Measles	89.5	81.0	NS
Varicella	71.4	54.1	NS (b)
Mumps	49.1	44.3	NS
Pertussis	44.8	42.6	NS
Herpes simplex	9.7	9.5	NS
Herpes zoster	17.5	5.3	$P < 0.05$
Viral hepatitis	14.5	11.1	NS
Tonsillectomy	16.1	15.9	NS
Chronic sinusitis	16.4	12.7	NS
Upper airways infections	33.9	21.0	NS
Important medical or surgical disease	7.4	8.1	NS
Trauma	30.6	27.0	NS
Stressful life events	67.2	27.0	$P < 0.05$
Immunization against			
poliomyelitis	58.6	61.3	NS
tuberculosis	56.7	58.7	NS

(a) Statistical analysis used chi-square test for matched-pairs comparison.
(b) $P < 0.10$

In order to define the complete profile of viral antibody titers, we built an index ranging from 0 (where none of the 7 patients' titers was greater than the corresponding control's titer) to 7 (for the opposite case). The mean value of this index did not differ from the value predicted by the null hypothesis.

We did not find any consistent correlation between antibody levels to the various viruses either in patients, or in controls. High titers against both measles and mumps virus were observed in some patients, but this relationship was not statistically significant ($P = 0.13$). There was no association between antibody levels and clinical features, including sex, age at onset of MS, and the course of the disease.

Agreement between the reporting of viral disease and the detection of corresponding antibodies was assessed by calculating the Kappa coefficient (4). There was very poor agreement: all Kappa values for both patients and controls were smaller than 0.20.

Table 3
Viral antibody titers in MS patients and controls

Virus	Titer (a)		Mean geometric titer
	< 1/400 MS(%)	> 1/2000 MS(%)	
Measles			
MS	7.1	45.6	7.4
Control	10.2	30.5	7.1
	NS		NS
Rubella			
MS	41.8	14.6	6.2
Control	32.8	8.6	6.3
	NS		NS
Mumps			
MS	33.3	10.5	6.3
Control	61.0	3.4	5.7
	$P < 0.01$		$P < 0.003$
Varicella-zoster			
MS	36.8	17.5	6.4
Control	32.2	18.6	6.5
	NS		NS
Herpes simplex			
MS	26.8	30.3	6.6
Control	25.4	27.1	6.6
	NS		NS
Cytomegalovirus			
MS	61.4	15.8	5.7
Control	67.8	8.5	5.4
	NS		NS
Coronavirus			
MS	22.8	28.1	6.8
Control	11.9	25.4	7.0
	NS		NS

(a) Proportion of subjects with titers between 1/400 and 1/2000 complemented the two proportions.

Discussion

With a prevalence of 40 per 100,000 population, the Hautes-Pyrénées can be considered a high-risk zone for MS. In the world-wide distribution of MS, the high frequency areas (including prevalence rates of 30 and above per 100,000) extend from 44° to 64° N latitude (5). The Hautes-Pyrénées region is located at the lower edge of this area.

Most MS studies have been of patients who had attended neurological clinics or who had been referred to neurologists. A possible effect of referral bias on MS research has recently been discussed (6). In contrast to these other studies, our patient sample was pooled from the general population. However, MS clinical features including sex ratio, age at onset, disease severity, and proportion of progressive forms are consistent with other studies (7, 8).

No evidence was found to incriminate demographic characteristics (9, 10), social class and occupation (9, 11–13), level of sanitation, diet (9, 14, 15), exposure to animals (16–18), or travel (19) as possible risk factors for MS. However, the paucity of statistically significant results may be partly due to the fact that controls, who shared an environment similar to that of the patients, were over-matched. It may also be related to study power.

On the whole, analysis of medical history revealed few consistent differences between MS patients and controls. In particular, we did not find associations suggested in previous studies relating MS with sinusitis (20), tonsillectomies, or operations under general anaesthesia (9, 21).

Age at menarche was significantly higher in patients than in controls. There is some evidence that disease course in females may be influenced by hormonal factors, particularly during pregnancy, post-partum, or menopause (22, 23). Also, the role of sex hormones on immunoregulation and autoimmune diseases is well established (24). The possible role of defects in the sex hormone balance in the etiology of MS in females has been recently postulated (25) and the present finding gives some support to this hypothesis.

Of particular interest is the fact that age at vaccination against poliomyelitis was significantly higher in patients than in controls. With respect to this result, it is important to note that in an investigation of MS in the Orkney and Shetland Islands, Poskanser (9) found that 3 of 88 MS patients (and no controls) reported having poliomyelitis.

The present study adds some findings to previous works on clinical viral infections, viral antibody titers, and MS. Unlike many authors (26–28) we did not find any relationship between measles infection and MS. Nevertheless, MS patients tended to have higher measles antibody titers than controls. An important observation is that the mumps antibody titer in MS patients is significantly higher than that of controls, even though there was no difference between the reported frequency of mumps infection in MS patients and controls.

Previous reports have involved mumps infection in the etiology of MS. Alter (29) and Compston (28) found that mumps infections were more frequent in patients than in controls. Several studies (29, 30) demonstrated a trend towards older age at infection by mumps in MS patients than in controls. Similarly, our findings support the hypothesis that mumps infection might be involved in the etiology of MS.

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References

1. Alpérovitch A, Bouvier MH. Geographical pattern of death rates from multiple sclerosis in France. *Acta Neurol Scand* 1982:*66*:454–461.
2. Poser CM, Paty DW, Scheinberg L et al. New diagnostic criteria for multiple sclerosis. Guidelines for research protocols. Ann Neurol 1983:*13*:227–231.
3. Fleiss JL. Statistical methods for rates and proportions. New York: John Wiley, 1981:212–236.
4. Kurtzke JF. On the evaluation of disability in multiple sclerosis. Neurology 1961:*11*:686–694.
5. Kurtzke JF. The geographic distribution of multiple sclerosis: an up-date with special reference to Europe and the Mediterranean region. Acta Neurol Scand 1980:*62*:65–80.
6. Nelson LM, Franklin GM, Hamman RF, Boteler DL, Baum HM, Burks JS. Referral bias in multiple sclerosis research. J Clin Epidemiol 1988:*41*:187–192.
7. Shepherd DI. Clinical features of multiple sclerosis in north east Scotland. Acta Neurol Scand 1979:*60*:218–230.
8. Confavreux C, Aimard G, Devic M. Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. Brain 1980:*103*:281–300.
9. Poskanser DC, Sheridan JL, Prenney LB, Walker AM. Multiple sclerosis in the Orkney and Shetland Islands. II - The search for an exogenous aetiology. J Epid Commun Health 1980:*34*:240–252.
10. Alpérovitch A, Le Canuet P, Marteau R. Birth order and risk of multiple sclerosis: Are they associated and how? Acta Neurol Scand 1981:*63*:136–138.
11. Russel WR. Multiple sclerosis: occupation and social group at onset. Lancet 1971:*2*:832–834.
12. Lauer K, Firnhaber W. Epidemiological investigation into multiple sclerosis in Southern Hesse. III - The possible influence of occupation on the risk of disease. Acta Neurol Scand 1985:*72*:397–402.
13. Antonovsky A, Leibovitz U, Medalie JM, Smith HA, Halpern L, Alter M. Epidemiological study of multiple sclerosis in Israel. II. Multiple sclerosis and socio-economic status. J Neurol Neurosurg Psychiat 1967:*30*:1–6.
14. Alter M, Yamsor M, Harshe M. Multiple sclerosis and nutrition. Arch Neurol 1974:*31*:267–272.
15. Agranoff BW, Goldberg D. Diet and the geographical distribution of multiple sclerosis. Lancet 1974:*2*:1061–1066.
16. Cook SD, Dowling PC. A possible association between house pets and multiple sclerosis. Lancet 1977:*May 7*:980–982.
17. Alter M, Berman M, Kahana E. The year of the dog. Neurology 1979:*29*:1023–1026.
18. Norman JE, Cook SD, Dowling PC. Household pets among veterans with multiple sclerosis and age-matched controls. Arch Neurol 1983:*40*:213–214.
19. Visscher BR, Bunnell DH, Detels R. Multiple sclerosis and multiple moves: an etiologic hypothesis. Am J Epidemiol 1981:*113*:140–143.
20. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. Lancet 1985:*June 8*:1313–1315.
21. Andersen E, Isager H, Hyllested K. Risk factors in multiple sclerosis: tuberculin reactivity, age at measles infection, tonsillectomy and appendicectomy. Acta Neurol Scand 1981:*63*:131–139.
22. Poser S, Raun NE, Wikström J, Poser W. Pregnancy, oral contraceptives and multiple sclerosis. Acta Neurol Scand 1979:*59*:108–118.
23. Poser S, Poser W. Multiple sclerosis and gestation. Neurology 1983:*33*:1422–1427.
24. Talal N, Dauphinee MJ, Ahmed SA, Christados P. Sex factors in immunity and autoimmunity. In: Yamamura Y, Toda D, eds. *5th Int Cong Immunol. New York*: Academic Press, 1983:1589–1600.
25. Van Lambalgen R, Sanders EACM, D'Amaro J. Sex distribution, age of onset and HLA profiles in two types of multiple sclerosis. J Neurol Sci 1986:*76*:13–21.
26. Alter M. Is multiple sclerosis an age-dependent host response to measles? Lancet 1976:*1*:456–457.
27. Fewster ME, Myers LW, Ellison GW, Walford RL. Histocompatibility types and measles antibodies in multiple sclerosis. J Neurol Sci 1977:*34*:287–296.
28. Compston DAS, Vakarelis BN, Paul E, Mc Donald WI, Batchelor JR, Mims CA. Viral infection in patients with multiple sclerosis and HLA-DR matched controls. Brain 1986:*109*:325–344.
29. Alter M, Cendrowski W. Multiple sclerosis and childhood infections. Neurology 1976:*26*:201–204.
30. Poskanser DC, Sever JL, Sheridan JL, Prenney LB. Multiple sclerosis in the Orkney and Shetland Islands. IV: viral antibody titres and viral infections. J Epid Commun Health 1980:*34*:258–264.

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