The increasing incidence and prevalence of MS in a Sardinian province

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Article abstract—*Objective:* To verify incidence rates and their temporal trend in a homogeneous, ethnically, and genetically distinct population of central Sardinia (the Nuoro province). *Background:* Intensive epidemiologic studies carried out in Sardinia since the 1970s have suggested that the prevalence and incidence of MS are much higher in this Mediterranean island compared with those found on mainland Italy. *Methods:* The study area had a population of approximately 274,000 people in the 1991 census. The authors adopted a complete enumerative approach by reviewing all possible sources of case collection available in the investigative area. *Results:* Based on 469 MS patients, the mean annual incidence for 1955 to 1995 was 4.18 per 100,000 (or 4.3 per 100,000 if age- and sex-adjusted to the European population). The incidence, averaging 1.95 per 100,000 during 1955 to 1959, rose progressively over time, reaching rates of 6.6 in the quinquiennium 1985 to 1989 and 6.4 per 100,000 in 1990 to 1995. On December 31, 1994, the crude prevalence, based on 415 MS patients alive in the study area, was 151.9 per 100,000 (156.6 if adjusted to the European population). *Conclusion:* These incidence and prevalence rates are the highest to date that have been estimated for a large community in southern Europe, and they constitute some of the highest rates in the world. Based on other surveys, these results reinforce the position of Sardinia as a higher and rising prevalence area for MS compared with other Mediterranean populations. Genetic and social–historic data strengthen the hypothesis of the environmental role and genetic factors among Sardinians in determining the notable difference in MS frequency between Sardinians and other Mediterraneans.

NEUROLOGY 2000;55:842–847

The epidemiologic studies of the last two decades indicate that the distribution of MS in southern Europe, particularly in Italy and Spain, is more complex than was supposed in the past, when a latitude-related model was commonly accepted.[1-3] In fact, the MS frequency in Europe seems uneven, with substantial variations between areas at the same latitude, and also within countries.[4-7] With regard to the Mediterranean areas, all descriptive studies conducted in Sardinia during the last two decades by our research groups indicate that this Italian island has twice the prevalence and incidence of MS compared with continental Italy.[8-11] The most recent survey performed in northwestern Sardinia indicated a prevalence of 102.6 cases per 100,000 population (in 1991) and a notable increase in MS incidence over time. Although averaging 2 per 100,000 from 1962 to 1971, MS incidence rose to 5 per 100,000 from 1977 to 1991.[12] These results represent striking exceptions to the north–south gradient in Europe and suggest that this island may be considered an area of high and increased risk for MS. We sought to verify the morbidity estimates of MS and its temporal trend in the well-defined areas of central Sardinia in the province of Nuoro, where studies in the 1980s indicated a high risk for the disease. The living population of approximately 270,000 persons is a self-contained and genetically characterized community that was isolated for centuries and excluded from any contact with other ethnically distinct populations occupying the island.

Methods. *Area of investigation.* The province of Nuoro includes 102 towns and villages. In the southern interior part of the province lies Barbagia, an area where MS epidemiologic studies were performed by our research group in the 1970s.[8,9] The mean population during 1955 through 1995 was 273,248 people (135,950 men, 137,298 women). The mean population density was 39 inhabitants/km^2 (range, 8 to 228 inhabitants/km^2). Only 37,527 people inhabit the town of Nuoro, whereas the rest of the population lives in two smaller towns (with a total population of approximately 10,000 people), and rural or sparsely populated areas.

The population is ethnically homogeneous, originating from an early split in the Caucasoid group, and is different from other European populations, Italians included. The origins of the Sardinian people in prehistory are not well known; however, historic, anthropologic, and genetic studies indicate that they are an ethnically distinct, homogeneous group. The original inhabitants of the island,

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Supported by the Associazione Italiana Sclerosi Multipla and by the Istituto Superiore di Sanità.

Presented in part at the European Committee for Treatment and Research in Multiple Sclerosis, Stockholm, Sweden, September 12–14, 1998; and at the 4th American Committee for Treatment and Research in Multiple Sclerosis, Montreal, Canada, October 18, 1998.

Received December 10, 1999. Accepted in final form May 24, 2000.

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particularly those who settled in the mountainous and hilly areas of the interior part of central Sardinia, given the inaccessibility of the territory, had been excluded from any contact with other ethnically different populations who occupied the coastal and flat parts of Sardinia in subsequent centuries. Successive waves of invasions by Phoenicians, Carthaginians, Romans, Vandals, Byzantines, Arabians, Ligurians, Tuscans, Spaniards, and Piedmontese failed to penetrate the wild and barbarous inland, which is represented mostly by the province of Nuoro and particularly by Barbagia. Barbagia, the region of the barbarians, according to the name assigned by the Romans, has preserved an ethnic and genetic peculiarity among aboriginal Sardinians that differs from the remainder of continental Italy.[13] The particular ethnic structure of Sardinians has also been emphasized in a review on MS genetic epidemiology in the world.[14] Comparative studies on human leukocyte antigen (HLA) allelic frequencies have clearly demonstrated the genetic isolation of Sardinians from other Italian and European populations.[15,16] The permanence of certain linguistic relics in the Nuorese languages and the singularity of the family names, which are completely different from those of the rest of Italy, give further support to the previous assumption.[17]

From the end of World War II until 1980, there was mass emigration from the province of Nuoro to the industrialized regions of northern Italy and central northern Europe (based on the official data, the average annual emigration from the province during this period was 520 subjects per 100,000 population). Afterward, the migration flux was unremarkable and did not change substantially over time. Almost all the migrants from and to the province of Nuoro are Sardinians, and the ethnic composition of the population has remained primarily stable over time.

The study area was rural by tradition, most workers having been employed in agriculture and sheep breeding, the latter having been the most important activity in the province in the past. After the last war, the standard of living was low compared with the rest of Italy, where processes of modernization and industrialization were in progress. During the last few decades, the structure of the economy has undergone a slight change and is now also based on the service and construction industries associated with urban development. There is only one minor industrial sector, whereas tourism has more recently been reevaluated, mainly in the coastal zones. According to the data of the 1991 General Census, the active working population is employed as follows: agriculture and breeding, 19%; industry, 26.4%; and tertiary activities, 54.6%.

The health care system has been present in the area for many decades, and the level of medical organization is relatively high. There is a general hospital with a neurologic ward and service, and a motor rehabilitation unit located in the town of Nuoro. Other neurologic outpatient services and rehabilitation facilities are also available in the study area. Some patients are cared for in other hospitals of the bordering provinces of Sardinia, mostly in the university neurologic departments of Sassari and Cagliari, where MS centers have been operative for many years. The level of medical organization has improved during the study period in parallel with the general improvement achieved throughout the rest of Italy as far as diagnostic tools, medical facilities, and neurologic services are concerned. As in other parts of Italy, detailed CSF studies and MRI became available routinely for patients referring to the neurologic services of the Nuoro province in the middle 1980s.

Case collection and ascertainment. Our research group has previously investigated MS scattered in Sardinia and, in particular, some areas of the province of Nuoro, Barbagia, and Macomer. Therefore, MS patients traced during previous studies constituted the starting point for this survey. Other MS patients were drawn from the following sources: archives of discharge diagnoses from the hospital of Nuoro, other hospitals on the island (Lanusei, Olbia, Oristano, Ozieri), the Sassari and Cagliari university hospitals, the neurologic practices serving the community, files from motor rehabilitation and chronic care services, membership rolls of the local branch of the Italian MS Association (AISM), and archives of the National Pension Institute and National Health Insurance scheme. We reviewed the sources from 1950 to the present by manual screening of in- and outpatient records, and patient lists. Cooperation from general practitioners, chemists, and social workers employed in the region was also obtained. Other sources included the archives of national centers where several patients from various parts of Italy go for clinical consultation. The active collaboration of some members of AISM and of other service organizations allowed us to recruit some MS patients who were not identified from other sources.

All patients with diagnoses of MS, demyelinating disease, encephalomyelitis, myelitis, myelopathy, optic neuritis, and ataxia were reviewed by trained neurologists of our team. The inquiry included a detailed interview, clinical and neurologic examination, and further analysis of all clinical documents. For the deceased patients, close relatives were interviewed and available clinical files were examined. The senior neurologists of our team reviewed all data collected for each patient to verify the validity of the diagnosis and to establish the date of clinical onset of the disease, defined as the time of the first symptom referable to MS. Because criteria involving imaging procedures, laboratory, or other diagnostic tests were not applicable to every patient in this survey setting, case definition was based on both the criteria of Poser et al.[18] and on clinical diagnostic criteria.[19,20] Information regarding whether patients were alive and residing in the study area at prevalence day was obtained from register offices of the communes.

From the provisional list of 565 putative cases eligible from the incidence estimate, we excluded 29 patients with onset outside the study area, 41 patients with onset before 1955 or after 1995, 16 patients whose diagnosis of MS was excluded, and 10 patients whose clinical records did not allow us to make a probable or definitive diagnosis and who were not available for further evaluation. From the original list of 564 patients potentially eligible for a prevalence estimate, we excluded 48 patients living outside the province of Nuoro, 72 patients who died before the prevalence point, 16 patients whose final diagnosis was other than MS, 6 patients with disease onset after 1994, and 7 patients with suspected or possible MS. Thus, the final study consisted of 469 patients with MS onset occurring from 1955 through 1995 while they were residing in the study area, and 415 MS patients living in the study area on December 31, 1994.

Table 1 *Age- and sex-specific mean annual incidence rates of MS in the province of Nuoro, 1955 to 1995*

	Mean annual incidence rate per 100,000		
Age group, y	Total	Men	Women
10–14	1.13	0.5	1.8
15–19	8.5	6.1	11
20–24	14.35	8.3	20.6
25–29	13.4	11.5	15.2
30–34	10	6	13.8
35–39	9.2	6.45	11.8
40–44	7.86	5.6	10
45–49	3.8	3	4.5
50–54	1.5	1.1	2
55+	0.5	0	0.98

Table 2 *Incidence trend of MS in the province of Nuoro, 1955 to 1995*

Period	No. of patients	Incidence rate per 100,000	Mean age at onset, y; ± SD
1955–1959	26	1.95	28.6 ± 11
1960–1964	26	1.85	28.3 ± 10
1965–1969	33	2.3	30.8 ± 11
1970–1974	42	3	27.6 ± 9.4
1975–1979	67	4.8	28.4 ± 9.2
1980–1984	80	5.8	28.6 ± 9.8
1985–1989	91	6.6	28.0 ± 8.7
1990–1995	104	6.4	28.4 ± 9
Total	469	4.18	28.5 ± 9.7

Ninety-seven percent of patients included in this study were born within the study area (90%) or elsewhere in Sardinia. Most patients born in mainland Italy or abroad have typical Sardinian surnames.

Statistical analysis. Ninety-five percent CIs were calculated assuming a Poisson's distribution.[21] Crude rates were directly adjusted to the Italian (1981 General Census) and European populations.[22] Student's *t*-test was used to compare two averages, and the z test was used to compare two rates. The comparison between two adjusted rates was performed by calculating the variance of the difference between two directly standardized rates and the z value. The statistical significance of temporal variation in incidence rates was evaluated with the Armitage test for linear trends.[23]

Results. *Incidence.* From 1955 through 1995, 469 subjects (158 men and 311 women) living in the province of Nuoro first showed symptoms that were later determined to represent the clinical onset of MS. They were considered to be incidence cases in the year in which they showed onset of symptoms. The crude mean annual incidence rate was 4.18 per 100,000 people (95% CI, 3.8 to 4.62). The two sexes differed (men: incidence rate, 2.83 per 100,000; 95% CI, 2.41 to 3.3; women: incidence rate, 5.52 per 100,000; 95% CI, 4.9 to 6.18; $z = 6.99$, $p < 0.01$). The female-to-male ratio was 1.95.

The sex- and age-adjusted rate for the Italian population was 4.3 per 100,000 (2.98 for men, and 5.42 for women). The directly adjusted rate for the European population was 4.3 per 100,000 people.

The age at onset (mean ± SD) was 28.5 ± 9.7 years for the total population, 28.5 ± 9.7 years for men, and 27.9 ± 9.5 years for women (a nonsignificant difference). Table 1 shows the age- and sex-specific incidence rates. The highest annual incidence rates were observed between ages 20 and 24 for the total population (12.3 per 100,000) and for women (17.6 per 100,000), and between ages 25 and 29 for men (9.5 per 100,000). The difference in incidence between the two genders was significant for the age groups 15 to 24 years and 30 to 39 years ($p < 0.05$).

Table 2 shows the average annual incidence rates by year of onset for 5-year intervals from 1955 to 1995. The observed incidences increased from 1.95 per 100,000 (95% CI, 1.27 to 2.86) from 1955 through 1959 to 6.6 per 100,000 from 1985 to 1989 (95% CI, 5.3 to 8.1), to 6.4 per 100,000 (95% CI, 5.25 to 7.8) during the last 6-year study period ($\chi^2 = 91.48$, $df = 7$, $p < 0.001$). The increasing trend was confirmed for both sexes ($\chi^2 = 25.75$ for men; $\chi^2 = 66.28$ for women; $df = 7$, $p < 0.001$). During the study period, the mean age at onset of the disease did not change over time (see table 2). The incidence rate did not change significantly (4.1 per 100,000 per year) if the patients were classified according to widely used clinical diagnostic criteria.[19,20]

Prevalence. On the day selected, December 31, 1994, 415 MS patients (128 men and 287 women) were living in the study area. Their mean age was 40.6 ± 12.6 years: 41.6 ± 12.6 for men and 40.2 ± 12.5 for women, a nonsignificant difference. The total population was 273,146 persons (134,905 men and 138,241 women), and the crude prevalence rate was 151.9 cases per 100,000 population (95% CI, 137.6 to 167.7). The crude prevalence rate for men was 94.9 per 100,000 (95% CI, 79.5 to 113,2) and for women was 207.6 per 100,000 (95% CI, 184.7 to 234.4), a significant difference ($z = 7.6$). The directly adjusted rate for the Italian population was 148.8 per 100,000: 93.04 per 100,000 for men and 250.5 per 100,000 for women. The adjusted rate for the European population was 156.64 per 100,000.

The mean duration of the disease from onset to prevalence day was 16.3 ± 9.7 years: 16.1 ± 8.9 years for men and 16.5 ± 6 years for women (not significant).

Table 3 shows age- and sex-specific prevalence rates. They reached a maximum for the age groups 35 to 44 years for the total population (323.7 per 100,000) and for men and women considered separately (219.3 per 100,000 for men and 434 per 100,000 for women). The significant difference in prevalence rates between men and women was confirmed in the age range 15 to 64 years.

The adjusted prevalence in 1994 was significantly higher than in 1985 (102 per 100,000 for men, 94 per 100,000 for women; $z = 4.56$).

The prevalence rate was higher in the urban population of the town of Nuoro than in the other communities and rural areas of the province (200 per 100,000 for Nuoro versus 144.4 per 100,000 for other communities and rural areas; $z = 2.28$, $p < 0.05$).

Table 3 *Prevalence rates (per 100,000) of MS in the Nuoro province (December 31, 1994)*

	Total		Men		Women	
Age group, y	N	Rate	N	Rate	N	Rate
0–14	1	1.96	0	0	1	4
15–24	28	59.1	7	29	21	90.43
25–34	121	284.1	34	157.8	87	413.5
35–44	115	323.7	40	219.3	75	434
45–54	87	284.3	29	194.4	58	370
55–64	48	167.4	11	80.9	37	245.4
65–74	11	53.1	5	53.5	6	52.9
75+	4	23.2	2	27.2	2	20.2

Other features. Disease course at onset was primary progressive in 12.9% of patients and relapsing–remitting in the remaining patients. According to Kurtzke's Expanded Disability Status Scale (EDSS),[24] 56% of prevalence patients showed either no disability or minimal disability (EDSS score, 0 to 3.5 points), 17.5% exhibited relatively severe disability (EDSS score, 4 to 5.5 points) and 26.5% required partial or complete assistance (EDSS score, >5.5 points).

The mean interval between symptom onset and diagnosis was 4.61 years. The average lag time between symptomatic onset and diagnosis shortened over time from 6.3 years for patients with onset before 1974 to 1.9 years for patients with onset after 1984. The average lag time between onset and diagnosis was less than 1 year for patients with onset after 1990.

Pyramidal motor (40.1%) and sensory (42.9%) disorders were the most common presenting symptoms.

The overall MS population included 19 sibling pairs and 8 parent–children pairs.

Discussion. We found a prevalence of 152 per 100,000 and an incidence rate of 4.2 per 100,000 per year in the province of Nuoro. These are the highest rates estimated for a large community in southern Europe, and some of the highest in the world.[3,25] We adopted a complete enumerative approach by reviewing all possible sources of case collection throughout the study area and took advantage of a long-term epidemiologic surveillance in this region implemented by neurologists operating in Sardinia since the 1960s, most of whom belong to this research group.[8,9,26-29] The population size of this study can be considered suitable for accurate case finding because it was manageable within the resources of our research team, and for adequate precision. Conversely, the well-defined community residing in the province of Nuoro is not so small as to be subject to the quirks of clustering. Given the intensive methodological approach of case ascertainment and the repeated surveys in the same area, which usually led to a more careful case collection as a result of greater interest in the disease,[7,30] any underascertainment was expected to be minimal. Moreover, it is unlikely that newly diagnosed patients would go outside the island for hospitalization, because Sardinia is quite far from the mainland and, at the same time, it offers a widespread network of well-equipped public general hospitals, including two departments of neurology at the universities of Sassari and Cagliari, and rehabilitation facilities, providing national services to which general practitioners refer their patients.

On the basis of the previous surveys, our research group cited evidence indicating that the risk for MS is higher in Sardinia than in other parts of Italy,[8-11] disproving the hypothesis that MS distribution follows a latitude-related gradient, at least in Italy and in southern Europe,[2] and prompting the assumption that the frequency of MS in Sardinia is one of the highest in the world.[3] An increasingly high MS incidence was reported from northwestern Sardinia by some researchers of our team using the same methodology.[12] The incidence estimates from our study did not seem to reflect a steady pattern that was also present in previous years and was unrecognized because of different case collection completeness, but rather because of an increasing incidence during the last three decades. Some indications would confirm the latter explanation: The study area has already been investigated intensively by our own research group using the same methodology, giving morbidity figures higher than that of the rest of Italy, but considerably lower than the current estimates.[8,9] Analogous findings have emerged from the other study carried out in the bordering northern area of Sardinia, where an epidemiologic long-term surveillance study has also been implemented.

The possibility that improvement in diagnostic techniques and medical facilities during the past decades could have led to an apparent excess of cases in the last few years, by allowing more complete ascertainment even for mild and benign forms of MS, may be an alternative explanation. However, the percentage of patients showing no disability or minimal disability in our prevalence patients (56%) was close to that (50%) reported in a previous prevalence study in the same area[28] in 1971, suggesting that increased recognition of mild forms could not have played a crucial role in the morbidity estimates over time.

Moreover, substantial stability of incidence rates was demonstrated in an analogous study,[31] which we performed using the same methodology in the province of Ferrara—in northeastern mainland Italy—where, on par with the current study area and in the rest of Italy, new diagnostic tools have become available during the last 15 years (figure). However, differences in case ascertainment as a result of new diagnostic technology were reduced by using clinical inclusion criteria,[19,20] which are strictly clinical and not influenced significantly by time-dependent use of modern laboratory and imaging investigations. It is now accepted that the overall epidemiologic figures remain essentially unchanged when using either clinical criteria or the diagnostic criteria of Poser et al.[32]

Thus, a real change in disease frequency seems to be a more likely explanation for the upward trend

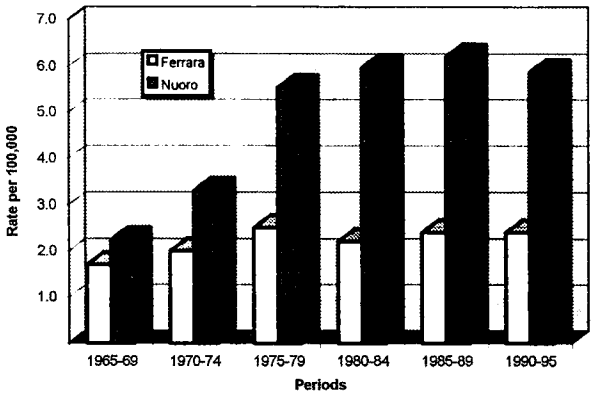

Figure. Incidence trend of MS in the provinces of Ferrara and Nuoro.

rather than methodological issues, which is based on an improvement in medical background because geographic areas where incidence rates of MS exhibit a stable, increasing, or decreasing pattern have been affected by these improvements.

The advances in diagnostic tools, medical facilities, public awareness, and so forth, have certainly led to a significant shortening of diagnostic latency. The lag time between onset and diagnosis, in fact, has been considerably reduced over time in the current population. Some changes in ascertainment factors must be acknowledged. However, our concurrent study with similar ascertainment in Ferrara showed no change in incidence.[31]

Therefore, taken together, the current figures from an ethnically homogeneous community that has not been influenced by substantial migratory flux in the last three decades, leave little doubt that Sardinia is an extremely high risk area for MS in southern Europe. Moreover, this study suggests that the risk is increasing with time.

It is well known that Sardinians are an ethnically homogeneous population, with a genetic structure somewhat different from that of the rest of continental Italian and European people.[13,15] Thus, the differences in MS frequency could reflect differences in genetic patterns, which could determine different susceptibility to the disease in Sardinians compared with other whites.[33-36] In this context, it has been suggested that the higher frequency of MS among Sardinians may depend on the higher frequency of DQB1*0201 and *0302 alleles.[37] The same alleles are related to disease susceptibility in other autoimmune diseases such as juvenile diabetes.[38,39] The distribution of juvenile diabetes in the world is similar to that of MS.[40,41] It is of interest that, among Sardinians, the incidence of insulin-dependent diabetes mellitus shows a temporal pattern that resembles the MS incidence trend: The incidence rates are now at least five times higher than those reported from mainland Italy, and they are the second highest in Europe, after Finland.[42] Sardinians may be genetically prone to insulin-dependent diabetes mellitus because of a particular HLA genotype with a scarcity of protective alleles and a high frequency of susceptible alleles compared with other white populations.[43] A similar hypothesis may be claimed for MS.

Even in populations with a high prevalence of susceptibility haplotypes, in which the supremacy of genetics over the environment seems to be emphasized, nongenetic factors must have a role if a steep increase in incidence is recorded. For insulin-dependent diabetes mellitus and MS, an increasing temporal trend has been detected in Sardinia during the past 20 to 30 years, which is too short a period for substantial changes in the genetic pool. Environmental factors must, therefore, be suspected. In other words, the increase in MS frequency may be related to environmental and socioeconomic changes as a result of the progressive modernization occurring in Sardinia after World War II. Having lost the protection of geographical isolation, the Sardinians were increasingly prone to a growing bulk of new environmental factors, such as increased contact with continental people; improvement in economy and general sanitary conditions with a postponement of childhood infection acquisition; changes in dietary habits, in overall lifestyle, and in industrial development; and growing exposure to toxicants and possibly other exogenous risk factors that, according to the epidemiologic literature, could play a causal role in the etiology of MS.[4,44,45] However, the putative role of unknown environmental factors still remains elusive.[6,46]

The majority of the incidence studies on MS among whites indicate that the distribution of incidence of the disease according to age has a peak in age groups between 25 and 40 years.[31,47,48] In the current study, the highest annual incidence rates are expressed in the age group between 20 and 24 years for both the total population and for female patients, and in the age group between 25 and 29 years for male patients. The mean age of clinical onset did not change during our study period. These findings are in agreement with the fact that, among Sardinians, the proportion of early MS onset is particularly high, perhaps in relation to their peculiar genetic characteristics.[49] These genetic characteristics could exert some influence on the age of clinical onset of the disease.

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