Paper I

Multiple sclerosis distribution in northern Sardinia

Spatial cluster analysis of prevalence

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Abstract—*Background:* A heterogeneous geographic distribution of MS has been reported among different ethnic groups, and also within small communities. Epidemiologic studies conducted over the past two decades using repeated assessments clearly show that Sardinia is at high risk for MS, with a prevalence of 150 per 100,000 in 1997. *Objective:* To present spatial analysis of the disease prevalence to disclose possible "hot" or "cold" spots of disease, further allowing correlations with risk factors. *Methods:* A spatial analysis of the whole province of Sassari, in northern Sardinia, at a microgeographic level (i.e., in the 89 administrative communes and 6 linguistic areas) was conducted. Because of the small number of cases per commune and to overcome random variability, a hierarchical Bayesian approach was adopted. The distribution of prevalent cases by commune of residence on December 31, 1997 and from age 5 to 15 years was analyzed. *Results:* A clustering pattern was found in the southwestern communes of the province based on geographic distribution by both prevalence and residence at age 5 to 15 years. A west-to-east gradient also was observed. *Conclusions:* This study highlights a hot spot of MS in the southwestern part of Sassari province, bordering with the commune of Macomer, where MS was once hypothesized as having occurred as an epidemic. Interestingly, these areas of MS clustering comprise the Common Logudorese linguistic domain. The Catalan area, linguistically and genetically distant from the remaining Sardinian domains, does not show such high estimates. Because MS is not a single-source infectious disease, this study may help test the hypothesis that a widely and evenly spread environmental (infectious?) agent may produce disease in subgroups of genetically more susceptible individuals in areas at higher inbreeding rates, wherein a disease mode of inheritance could be better investigated.

NEUROLOGY 2002;58:277–282

Several investigations have shown a heterogeneous geographic distribution of MS not only among different ethnic groups, but within individual regions and small communities.[1,2] Descriptive epidemiologic studies performed in Sardinia in the past two decades by means of repeated assessments have clearly demonstrated a high risk for MS,[3-10] and a recent survey conducted on a territory of nearly a half-million population in the province of Sassari in northern Sardinia showed a crude total prevalence rate of approximately 150 per 100,000 on December 31, 1997 and increased incidence rates from 2 per 100,000 in the interval 1968 to 1972 to 7 per 100,000 in 1993 to 1997.[11]

The etiology of MS is still obscure and strongly debated, but an interplay between environmental and genetic factors often is hypothesized. Therefore, an analysis of spatial variation of the disease prevalence at a microgeographic level should be performed to disclose possible "hot" or "cold" spots of disease and give clues to its etiology.[12-14]

The aim of the current work was to investigate the distribution of MS prevalence in the province of Sassari in northern Sardinia by means of spatial cluster analysis.

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Methods. *Case ascertainment.* Case ascertainment was based on a MS register created in 1995 at the Institute of Clinical Neurology of the University of Sassari, hosting a MS Center that is relevant at a regional level and the major referral center for patients with MS in northern Sardinia. In addition to the Sassari MS Center inpatient and outpatient medical records and lists of patients who had received interferon or undergone evoked potential testing or MRI, the register comprised data from 1) other Sardinian neurologic institutions (Ozieri, Olbia, and Nuoro State Hospitals, the Neurologic Clinic of the University of Cagliari, Cagliari MS Center), 2) the Institutes of Neurosurgery and Ophthalmology of the University of Sassari, 3) the provincial Centers for Motor Rehabilitation, 4) the files of the National MS Society (AISM) centers, 5) all provin-

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Supported by ISS (Istituto Superiore di Sanità, Rome), FISM (Fondazione Italiana Sclerosi Multipla, grant n. 97/R/69), MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Rome), and RAS (Regione Autonoma della Sardegna).

Received March 26, 2001. Accepted in final form September 29, 2001.

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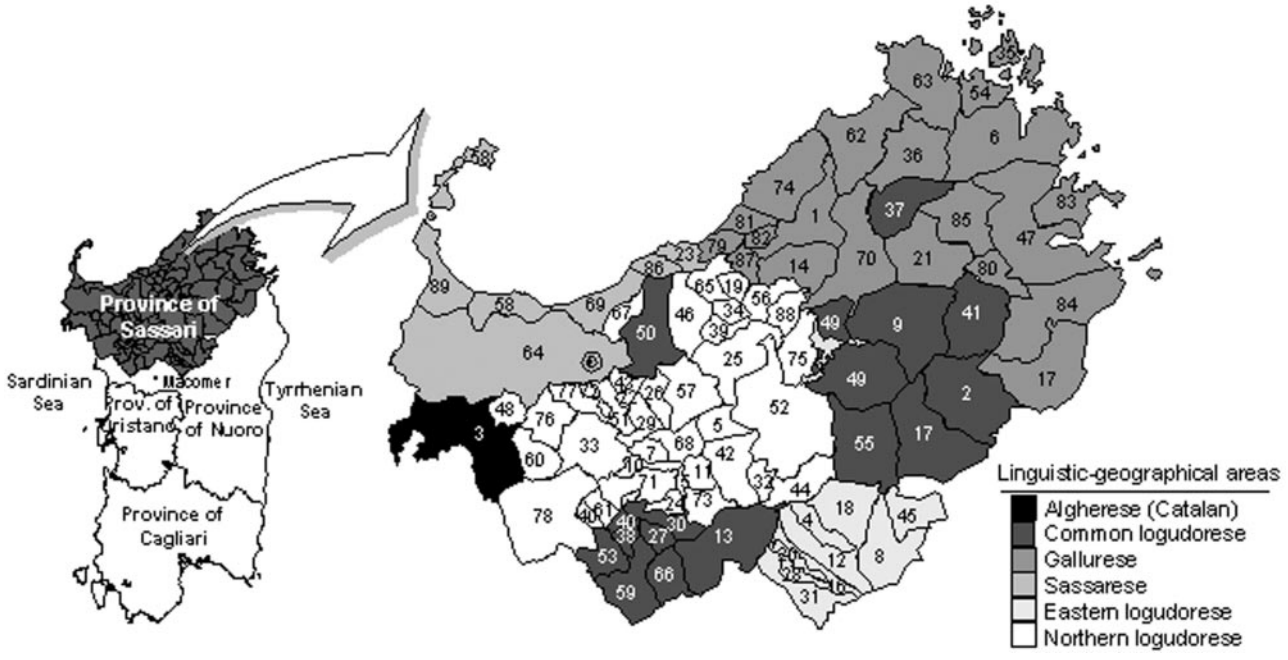

Figure 1. Province of Sassari, Sardinia: commune codes[18] and linguistic areas.[17] Commune codes: 1, Aggius; 2, Alà dei Sardi; 3, Alghero; 4, Anela; 5, Ardara; 6, Arzachena; 7, Banari; 8, Benetutti; 9, Berchidda; 10, Bessude; 11, Bonnanaro; 12, Bono; 13, Bonorva; 14, Bortigiadas; 15, Borutta; 16, Bottidda; 17, Buddusò; 18, Bultei; 19, Bulzi; 20, Burgos; 21, Calangianus; 22, Cargeghe; 23, Castelsardo; 24, Cheremule; 25, Chiaramonti; 26, Codrongianos; 27, Cossoine; 28, Esporlatu; 29, Florinas; 30, Giave; 31, Illorai; 32, Ittireddu; 33, Ittiri; 34, Laerru; 35, La Maddalena; 36, Luogosanto; 37, Luras; 38, Mara; 39, Martis; 40, Monteleone Roccadoria; 41, Monti; 42, Mores; 43, Muros; 44, Nughedu San Nicolò; 45, Nule; 46, Nulvi; 47, Olbia; 48, Olmedo; 49, Oschiri; 50, Osilo; 51, Ossi; 52, Ozieri; 53, Padria; 54, Palau; 55, Pattada; 56, Perfugas; 57, Ploaghe; 58, Porto Torres; 59, Pozzomaggiore; 60, Putifigari; 61, Romana; 62, Aglientu; 63, S. Teresa di Gallura; 64, Sassari; 65, Sedini; 66, Semestene; 67, Sennori; 68, Siligo; 69, Sorso; 70, Tempio Pausania; 71, Thiesi; 72, Tissi; 73, Torralba; 74, Trinità d'Agultu e Vignola; 75, Tula; 76, Uri; 77, Usini; 78, Villanova Monteleone; 79, Valledoria; 80, Telti; 81, Badesi; 82, Viddalba; 83, Golfo Aranci; 84, Loiri Porto S. Paolo; 85, S. Antonio di Gallura; 86, Tergu; 87, S. Maria Coghinas; 88, Erula; 89, Stintino.

cial neurologists in private practice, 6) the most relevant extraregional centers (MRI Section of Gallarate General Hospital, Don Gnocchi's Foundation in Milan) where a proportion of patients may have been diagnosed or undergone MRI, and 7) provincial general practitioners. All neurologists practicing in the province periphery (whether in private or state hospital practice) have been trained at our Clinic and maintained close and constant links with the investigators' team over time, thus providing periodic MS case notification. General practitioners and neurologists from the entire province territory were contacted and their medical records checked for MS cases by the same investigators. Government death certificates also were reviewed for all patients older than 50 years who were not being followed up at the Clinic at the time of the study, to assess possible date of death.

Patients included in the register were diagnosed as having MS according to the Poser Committee criteria[15] for clinical or laboratory-supported definite (CDMS, LSDMS) and probable (CPMS, LSPMS) MS. At the time of the study, 87.6% of the patients were classified as having CDMS, 8.4% CPMS, 2.7% LSDMS, and 1.2% LSPMS. For 92% of patients diagnosed after 1986, MRI was performed that supported the diagnosis of MS, whereas before that date, diagnosis was based on clinical and paraclinical evidence and immunologic study of the CSF (performed in 78% of the cases), with MRI performed subsequently to confirm the diagnosis. Other autoimmune, immune-mediated, and infectious diseases, such as primary and secondary CNS vasculitides, postinfectious leukoencephalopathies, and other demyelinating disorders, were ruled out by means of laboratory tests and neuroimaging, as well as neurologic history and examination.

In particular, information on patients' date and place of birth, residence between 5 and 15 years of age (the putative critical exposure period), residence at clinical onset of disease, and possible exposure to risk factors was recorded, in addition to other clinical data.

Repeated case ascertainments have been carried out in the province of Sassari in the past two decades,[4-6] which increased the accuracy of the register. For the current population-based survey, a "spider" type of epidemiologic approach was adopted,[16] wherein patients with MS from the well defined territory of the province of Sassari have been seen in a network of medical (general and specialized) care institutions and whose medical history has been periodically recorded and updated at the Sassari MS Center and has been easily accessible to the investigators over time.

Study area. The spatial analysis was carried out in the whole province of Sassari, an area of 7,520 km^2 in northern Sardinia that lies between latitudes 40° 30'N and 41°N and encompasses 89 administrative communes and 6 linguistic areas[17] (figure 1). In 1997, the total population was

460,135 (227,215 males and 232,920 females). The 1997 age- and sex-specific populations for each commune, available from demographic data,[18] were used as denominators. Each commune was coded according to the Italian Central Institute of Statistics Coding.[19] Migration flow was modest: in 1997, only 1.8% of the total population registered as resident from other Italian provinces and from foreign countries, whereas 1.6% moved away from the study area.[20] However, with immigration to the study area being mostly from other Sardinian provinces, the study population consisted almost completely of native individuals, and a stable ethnic composition over time could be assumed.

Statistical analysis. The first phase of the study was aimed at calculating the area age- and sex-specific prevalence rates (expressed as the number of cases per 100,000 population) using the 1997 population and at mapping them for each commune of the province of Sassari, the finest geographic grid for which demographic data are available. To remove possible biases due to different age and sex structures for different areas, standardized rates were calculated by the direct method of adjustment using the same standard population and assuming an equal number in each age group.[21] Because the area-specific number of cases is small, traditional statistical methods tend to yield very extreme rates because of the strong influence of random variation. Therefore, the drawback of these maps is that smaller underlying populations are influenced by random variability, and the observers' attention is erroneously drawn to these extremes. To overcome this problem, a hierarchical Bayesian approach was adopted that eliminates extreme values from the map and yields smoothed estimates of disease rates.[22] With this method, the underlying "risks" are assumed to be generated by a probability distribution (i.e., an a priori distribution) that expresses the idea of smoothness. The effect of such an a priori distribution of the prevalence values is to yield, for each area, an estimate that represents a correct compromise between the area-specific risk and the average of the neighboring areas' risks.[23-26] Extreme estimates are thus pulled toward the local mean, the pulling being more substantial when the estimates are unstable, that is, when the corresponding areas have a small number of cases and thus do not provide substantial evidence in favor of the extreme value. The result is that the estimated Bayesian maps best reflect the true geographic risk variation and are epidemiologically more interpretable. Because the data set consists of prevalence rates, a binomial model was assumed to obtain the Bayesian estimates through Gibbs sampling,[27-30] on BUGS software.[31]

In this model, the extrabinomial variation (i.e., the excess in variation with respect to that expected under the binomial model) was assumed as a random area effect through an a priori distribution, including both a spatially unstructured extrabinomial variation (heterogeneity) and a spatially structured variation (clustering).[32,33]

To explain the amount of variation of the true prevalence rates in the map, a combination of degrees of freedom and a scale factor was chosen for the prior distribution of the hyperparameter, as described by Bernardinelli et al.[34,35] A sensitivity analysis allowed us to choose the values of 10 for degrees of freedom and 2 as the scale factor.

In Bayesian terms, the final distribution of prevalence values, which combines the information contained in the a priori model with that contained in the data, is called *posterior distribution*, which is used to produce the posterior probability (PP) of a prevalence rate greater or lower than a given reference value. For the current study, the median value of generated samples was chosen as reference value. The PP is the Bayesian equivalent of the p value[36] and can be mapped to identify those areas wherein the risk is significantly higher or lower. The PP map can be interpreted as follows: $PP > 0.90$ strongly indicates that the area-specific risk is higher than the reference value, whereas $PP < 0.1$ strongly indicates that the prevalence is lower. In those areas where PP falls in the intervals 0.75 to 0.9 and 0.1 to 0.25, only an indication that the risk is respectively higher or lower than the reference value is given. When the PP value falls in the central interval (0.25 to 0.75), no informative evidence is given.

The model was fitted to map the area-specific prevalence rates for patients residing in the study area both on prevalence day and during the putative age of MS acquisition (i.e., between 5 and 15 years of age; henceforth referred to as *5 to 15*).[37] Because of the conspicuous number of areas containing zero cases, the Bayesian approach was not applied for estimates for males only. Three separated chains starting from different initial values were run for each model: total (i.e., both sexes), females, and total 5 to 15. The Bayesian prevalence estimates and tests were obtained after convergence of the hyperparameter using the Gibbs sampler, discarding the first 1,000 iterations of each run as burn-in or preconvergence samples. Convergence at 10,000 iterations was checked by visual examination of sample traces by Geweke's diagnostic[38] implemented in the CODA software.[39]

Results. The results of MS spatial analysis in the province of Sassari for total cases are summarized for each commune in the table (data not shown; additional material related to this article can be found on the *Neurology* Web site; go to www.neurology.org and scroll down the Table of Contents to find the title link for this article), which reports, for each commune on December 31, 1997, 1) the crude rates of MS total prevalence (per 100,000) with the 95% CI obtained by maximum likelihood; and 2) the respective rate estimates (per 100,000) with the 95% credible interval (95% cI) obtained using the Bayesian approach.

On prevalence day, 686 people with MS (492 females and 194 males) were living in the province of Sassari. The number of observed cases to some extent reflects the same variation in the area population sizes. Using as denominator the 1997 population resident in the study area, the total crude prevalence rate was 149.1 (95% CI, 138.3 to 160.7), 211.2 (95% CI, 193.4 to 230.7) for females and 85.4 (95% CI, 74.2 to 98.3) for males.

The overall standardized rate was 142.9, 204.0 for females and 81.8 for males. The crude prevalence rates ranged between 0 to 413.0 (95% CI, 197.0 to 863.7) for both sexes and from 0 to 743.5 (95% CI, 186.0 to 2,922.7) for females.

The overall Bayesian estimates ranged from 119.0 (95% cI, 100.2 to 138.2) to 162.5 (95% cI, 134.5 to 195.1), with a mean value of 142.5 ± 5. Among females, the mean value was 204.9 ± 11.6, ranging from 178.4 (95% cI, 149.9 to 209.5) to 228.0 (95% cI, 184.9 to 277.2).

The total standardized prevalence rates obtained for each of the 89 communes on prevalence day by maximum

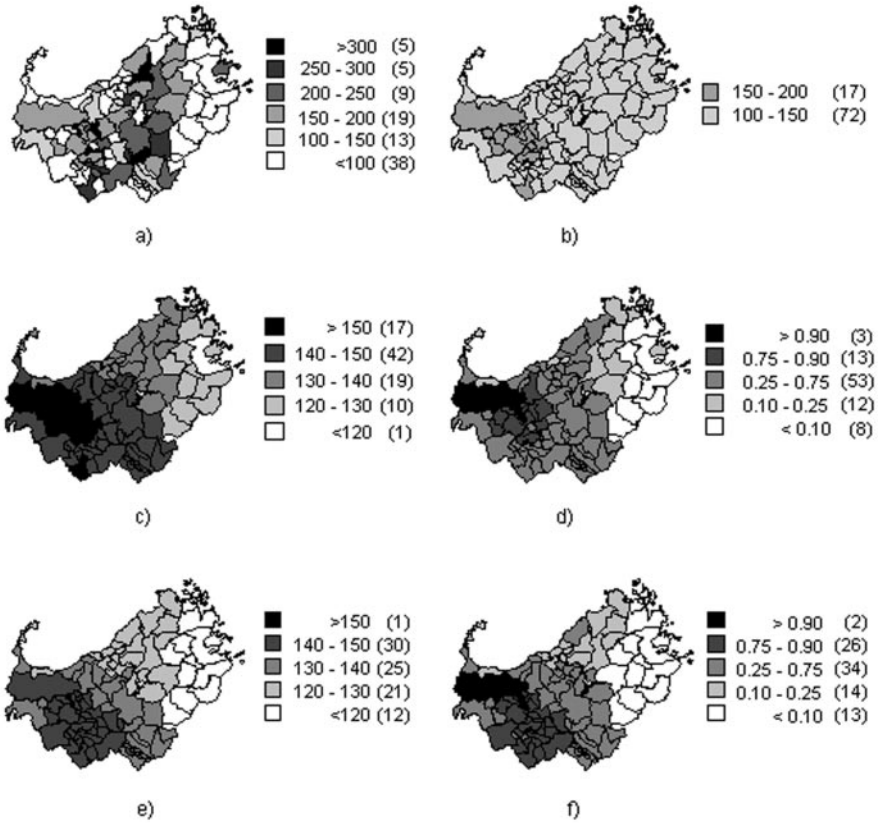

Figure 2. Mapping of MS total prevalence rates (per 100,000) on prevalence day, December 31, 1997 (a–d) and by commune of residence at 5 to 15 years of age (e, f) (see text for explanation).

likelihood and Bayesian approaches are mapped in figure 2, a through c: a specific gray level was linked to each class by using the same set of cutoff points (see figure 2, a and b); the Bayesian estimates represented with a higher resolution of cutoff points, according to their distribution, also were mapped (see figure 2c); figure 2d shows the distribution of PP values for total cases. Bayesian estimates also were calculated based on the commune of residence for patients 5 to 15 years of age and mapped (see figure 2e), and their respective PP values were calculated and mapped (see figure 2f; see Methods). Because of the high variation in total standardized prevalence rates, it is not possible to identify peculiar spatial aggregates from map 2a, and the extreme values tend to occur in the least-populated areas. Such variation does not appear in the Bayesian map (see figure 2b), in which smoothing is considerable owing to the Bayesian tendency to pull toward a collective mean; hence, no black or white areas can be detected. A clustering pattern in the west of the province and a west-to-east gradient appear to be fairly evident in maps 2b and c. In particular, 19.1% of the communes, identified by codes 7, 10, 22, 26, 29, 33, 43, 48, 51, 59, 60, 64, 68, 71, 72, 76, and 77, are dark colored, thus appearing to form a cluster and to be at higher "risk." However, only communes 51, 64, and 72 have prevalence rates higher than the median value, with a high PP (see figure 2d), whereas for the remaining communes of the cluster, only an indication for a higher risk is given. Moreover, map d strongly indicates that in the eastern province, prevalence is lower for eight communes (2, 6, 17, 41, 47, 80, 84, and 85). A similar pattern (not reported in figure 2), but with higher prevalence values, results from the analysis of female's rates, which highlights a high prevalence cluster in the west. The estimates of prevalence rates by commune of residence at 5 to 15 years of age also were mapped (map e), showing a different spatial aggregation of communes with respect to prevalence mapping. A large proportion of the communes (35.9%), located in the western province (areas of Sassarese, western Common, and northern Logudoro), show higher prevalence rates and a tendency to form a cluster, indicating that the "risk" for MS is higher than the median value (map f).

Discussion. Although primarily descriptive, the relevance of disease mapping dates back to 1800,[40] when it began to be used to generate hypotheses about disease etiopathogenesis in comparison with exposure maps, or simply for descriptive purposes. A considerable number of spatial studies reported in the literature consist of post hoc analyses, which are driven by the attempt to rule out an association between disease and one or more possible risk factor(s) in a "hot spot," usually reported by local physicians, patients, or mass media. As opposed to post hoc cluster analysis, an a priori cluster analysis is supported by one hypothesis and is carried out in a population with little previous evidence of clusters, and thus is less subject to bias.[1,12] The current study is a spatial a priori cluster analysis of MS distribution in northern Sardinia conducted at a microgeographic level represented by the 89 communes making up the whole province.

A recent epidemiologic, population-based survey[11] confirmed that Sardinians are at high risk for development of MS. If the reported prevalence rate of approximately 150 per 100,000 reflects the overall provincial mean prevalence rate, no conclusions can

be drawn about the presence of possible true "excesses" or "lacks" of MS cases, at the microgeographic level, which instead represent valid clues for correlating the disease with either risk or protective factors. To explain the spatial variation of MS prevalence and to overcome difficulties with traditional methods of mapping disease risk (see Methods), as already applied to mapping cancer mortality in Sardinia,[41] a Bayesian approach was adopted, which, in our opinion, best reflects the disease's spatial variation in small areas at low population densities. However, as with more traditional epidemiologic methods, maps obtained by means of a Bayesian approach are only a representation of the true disease risks in the area, and therefore can reflect artifacts deriving from potential confounding spatial effects,[42] such as autocorrelation, or from the choice of a prior distribution that will affect posterior inferences. To assign the model the proper prior distribution, the key part of the Bayesian approach, a sensitivity analysis was performed on noninformative, moderately, and highly informative priors.[34,43,44] These results do not actually yield exact and recommended models, but rather allow one to rule out the epidemiologically less informative ones. The "subjective" choice of our prior distribution therefore appeared best to reflect our knowledge of the phenomenon under study. Mapping the distribution of total cases on prevalence day (December 31, 1997) by commune of residence, as well as the data for females, showed a clustering pattern of MS in northwestern areas of the province. In particular, the areas at highest risk are located in the linguistic areas of Sassarese and the western part of Common and northern Logudorese. The communes at low "risk" are instead located in the eastern part of the Gallurese linguistic area, corresponding to the northeastern part of the island.

Although the place of residence on prevalence day may not necessarily reflect the place of putative MS acquisition or exposure to possible risk factors, we believe that mapping the disease distribution of prevalent cases may yield good estimates of possible clusters because of the negligible migratory inflow and outflow between even adjacent communes.[20] Nevertheless, to overcome this possible bias, MS clusters were further searched for by mapping the distribution of all registered cases by commune of residence during their putative age of MS acquisition.[37] This type of analysis is more reliable and informative because the place of residence during 5 to 15 years of age may reflect a possible exposure to environmental risk factors that are particularly concentrated in some geographic areas. Moreover, because of the Sardinian sociocultural context, it gives clues to the patient's genetic background, especially in the interior-most villages and rural communities that account for over two-thirds of the study population. This type of analysis appears strongly to confirm the pattern obtained for prevalence day, highlighting a hot spot of MS in the southwestern province. This area borders with the commune of Macomer (see figure 1), where MS was reported as having occurred in epidemic fashion, appearing in 1952, reaching its highest incidence during 1957 to 1961 (4.8 per 100,000), and slowly decreasing in the subsequent decades.[3] Interestingly, the observed MS cluster and the Macomer communes belong to the same common Logudorese domain. Conversely, the Catalan area, considered linguistically and genetically distant from the remaining Sardinian domains,[45] does not show such high estimates.

Because of the MS case ascertainment methodology adopted for the current study, which fulfilled a "spider" type of approach,[16] we feel reasonably confident in ruling out differences in case ascertainment from different parts of the study area, especially from more as opposed to less urban areas, that might have biased the mapping of MS in the province.

Cluster studies are a powerful tool when looking for disease etiology. When applied to relatively rare and complex diseases such as MS, they may allow one to investigate the variability of the disease risk in relation to the fluctuating concentration of one or more specific environmental factor(s), given the population's genetic homogeneity. Alternatively, because MS clearly is not a single-source infectious disease, cluster studies may help test the hypothesis that a widely and evenly spread environmental (infectious?) agent may produce disease in subgroups of genetically more susceptible individuals. In this perspective, if the identified cluster were on a genetic basis (i.e., located in an area with a high inbreeding rate), a mode of inheritance for the disease could be better investigated. Interestingly, despite evidence based on the geographic distribution of blood groups, HLA gene frequencies,[46-48] and human Y chromosome polymorphisms[49,50] that Sardinians are genetically homogeneous compared with other white groups, a certain degree of genetic heterogeneity, possibly because of different inbreeding rates at the microgeographic level, has been highlighted by analyzing the variability of mitochondrial DNA polymorphisms in two different Sardinian samples.[51]

Spatial and temporal cluster studies based on incidence data for an at-risk population over a defined time span[1] and analytic epidemiologic investigations[52] are needed to shed light on possible associations between MS spatial clusters and etiologic factors in Sardinians.

References

1. Riise T. Cluster studies in multiple sclerosis. Neurology 1997; 49:S27-S32.
2. Kurtzke JF. A Fennoscandian focus of multiple sclerosis. Neurology 1968;18:16–20.
3. Rosati G, Aiello I, Granieri E, et al. Incidence of multiple sclerosis in Macomer, Sardinia, 1912–1981: onset of the disease after 1950. Neurology 1986;36:14–19.
4. Rosati G, Aiello I, Pirastru MI, et al. Sardinia, a high risk area for multiple sclerosis: a prevalence and incidence study in the district of Alghero. Ann Neurol 1987;21:190–194.
5. Rosati G, Aiello I, Mannu L, et al. Incidence of multiple sclerosis in the town of Sassari, Sardinia, 1965 to 1985: evidence

for increasing occurrence of the disease. Neurology 1988;38: 384–388.
6. Rosati G, Aiello I, Pirastru MI, et al. Epidemiology of multiple sclerosis in north-western Sardinia: further evidence for higher frequency in Sardinians compared to other Italians. Neuroepidemiology 1996;15:10–19.
7. Granieri E, Rosati G, Tola R, et al. The frequency of multiple sclerosis in Mediterranean Europe: an incidence and prevalence study in Barbagia, Sardinia, insular Italy. Acta Neurol Scand 1983;68:84–89.
8. Granieri E, Casetta I, Tola MR. Epidemiology of multiple sclerosis in Italy and in southern Europe. Acta Neurol Scand 1995;161:S60-S70.
9. Casetta I, Granieri E, Marchi D, et al. An epidemiological study of multiple sclerosis in central Sardinia, Italy. Acta Neurol Scand 1998;98:391–394.
10. Granieri E, Casetta I, Govoni V, et al. The increasing incidence and prevalence of MS in a Sardinian province. Neurology 2000;55:842–848.
11. Pugliatti M, Sotgiu S, Solinas G, et al. Multiple sclerosis epidemiology in Sardinia: evidence for a true increasing risk. Acta Neurol Scand 2001;103:20–26.
12. Elliott P, Martuzzi M, Shaddick G. Spatial statistical methods in environmental epidemiology: a critique. Stat Methods Med Res 1995;4:137–159.
13. Wakefield J, Elliott P. Issues in the statistical analysis of small area health data. Stat Med 1999;18:2377–2399.
14. Wakefield JC, Kelsall JE, Morris SE. Clustering, cluster detection, and spatial variation in risk. In: Elliott P, Wakefield JC, Best NG, Briggs DJ, eds. Spatial epidemiology: methods and applications. New York: Oxford University Press, 2000: 128–152.
15. Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227–231.
16. Kurtzke JF. The epidemiology of multiple sclerosis. In: Raine CS, McFarland HF, Tourtellotte WW, eds. Multiple sclerosis: clinical and pathogenetic basis. London: Chapman and Hall Medical, 1997:91–139.
17. Pracchi R, Terrosu Asole A. Atlante della Sardegna. Rome: Edizioni Kappa, 1980:2.
18. ISTAT. Regione Sardegna. Popolazione della regione per classi di età. Epidemiologia in Sardegna 1999;3:125–153.
19. ISTAT. Comuni secondo alcune caratteristiche per provincia. In: ISTAT. Elenco dei comuni al 31 maggio 1990. Roma: Abete Grafica, 1991:123–125.
20. ISTAT. Movimento anagrafico e popolazione residente secondo il sesso per Comune capoluogo di provincia. Anno 1997. In: ISTAT. Popolazione e movimento anagrafico dei Comuni. Anno 1997. Pomezio (RM): Stampa Albagraf, 1998:19.
21. Pickle L, White AA. Effects of the choice of age-adjustment method on maps of death rates. Stat Med 1995;14:615–627.
22. Mollié A, Richardson S. Empirical Bayes estimates of cancer mortality rates using spatial models. Stat Med 1991;10:95–112.
23. Besag J. Spatial interaction and the statistical analysis of lattice system. J R Stat Soc B 1974;36:192–236.
24. Gilks WR, Clayton DG, Spiegelhalter DJ, et al. Modelling complexity: applications of Gibbs sampling in Medicine. J R Stat Soc B 1993;55:39–102.
25. Bernardinelli L, Montomoli C. Empirical Bayes versus fully Bayesian analysis of geographical variation in disease risk. Stat Med 1992;11:983–1007.
26. Clayton D, Kaldor L. Empirical Bayes estimates of age-standardized relative risks for use in mapping. Biometrics 1987;43:671–681.
27. Geman S, Geman D. Stochastic relaxation, Gibbs distributions and the Bayesian restoration of images. IEE Trans Pattern Anal Machine Intel 1984;6:721–741.
28. Gelfand AE, Smith AEM. Sampling-based approaches to calculating marginal densities. J Am Stat Assoc 1990;85:398–409.
29. Gilks WR, Richardson S, Spiegelhalter DJ. Markov chain Monte Carlo in practice. London: Chapman & Hall, 1996.
30. Clayton D, Kaldor J. Empirical Bayes estimates of age-standardised relative risks for use in disease mapping. Biometrics 1987;43:671–681.
31. Spiegelhalter DJ, Thomas A, Best NG, Gilks WR. BUGS: Bayesian inference using Gibbs sampling, Version 0.5. Cambridge: Medical Research Council Biostatistics Unit, 1996.
32. Besag J, York JC, Mollié A. Bayesian image restoration with two applications in spatial statistics. Ann Inst Stat Math 1991;43:1–59.
33. Breslow NE, Clayton DG. Approximate inference in generalized linear mixed models. J Am Stat Assoc 1993;88:9–25.
34. Bernardinelli L, Clayton D, Montomoli C. Bayesian estimates of disease maps: how important are priors? Stat Med 1995;14: 2411–2431.
35. Bernardinelli L, Pascutto C, Montomoli C, Gilks W. Investigating the genetic association between diabetes and malaria: an application of Bayesian ecological regression models with errors in covariates. In: Elliott P, Wakefield JC, Best NG, Briggs DJ, eds. Spatial epidemiology: methods and applications. New York: Oxford University Press, 2000:286–301.
36. Meng CYK, Dempster AP. A Bayesian approach to the multiplicity problem for significance testing with binomial data. Biometrics 1987;43:301–311.
37. Dean G, Kurtzke JF. On the risk of multiple sclerosis according to age at immigration to South Africa. BMJ 1971;3:725–729.
38. Geweke J. Evaluating the accuracy of sampling-based approaches to calculating marginal densities. J Am Stat Assoc 1990;85:398–409.
39. Best NG, Cowles MK, Vines SK. CODA: Convergence diagnosis and output analysis software for Gibbs sampling output, Version 0.30. Cambridge: Medical Research Council Biostatistics Unit, 1995.
40. Walter SD. Disease mapping: a historical perspective. In: Elliott P, Wakefield JC, Best NG, Briggs DJ, eds. Spatial epidemiology: methods and applications. New York: Oxford University Press, 2000:223–239.
41. Bernardinelli L, Maida A, Marinoni A, et al. Atlas of cancer mortality in Sardinia 1983–1987. Rome: CNR Unità Operativa P.F. FATMA, 1994.
42. Gelman A, Price PN. All maps of parameter estimates are misleading. Stat Med 1999;18:3221–3234.
43. Pascutto C, Wakefield JC, Best NG, et al. Statistical issues in the analysis of disease mapping data. Stat Med 2000;19:2493–2519.
44. Knorr-Held L, Rasser G. Bayesian detection of clusters and discontinuities in disease maps. Biometrics 2000;56:13–21.
45. Cappello N, Rendine S, Griffo S, et al. Genetic analysis of Sardinia: I. data on 12 polymorphisms in 21 linguistic domains. Ann Hum Genet 1996;60:125–141.
46. Piazza A, Mayr WR, Contu L, et al. Genetic and population structure of four Sardinian villages. Ann Hum Genet 1985;49: 47–63.
47. Olivetti E, Rendine S, Cappello N, et al. The HLA system in Italy. Hum Hered 1986;36:357–372.
48. Cavalli-Sforza LL, Piazza A, Menozzi P, Mountain J. Reconstruction of human evolution: bringing together genetic, archaeological, and linguistic data. Proc Natl Acad Sci USA 1988;85:6002–6006.
49. Semino O, Passarino G, Oefner PJ, et al. The genetic legacy of paleolithic *homo sapiens sapiens* in extant Europeans: a Y chromosome perspective. Science 2000;290:1155–1159.
50. Underhill PA, Shen P, Lin AA, et al. Y chromosome sequence variation and the history of human populations. Nat Genet 2000;26:358–361.
51. Morelli L, Grosso MG, Vona G, et al. Frequency distribution of mitochondrial DNA haplogroups in Corsica and Sardinia. Hum Biol 2000;72:585–595.
52. Lauer K. Ecologic studies of multiple sclerosis. Neurology 1997;49:S18-S26.