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.
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 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, immunemediated, 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 ascertainment has been carried out in the province of Sassari in the past two decades, which increased the accuracy of the register. For the current population-based survey, a “spider” type of epidemiologic approach was adopted, 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 km² in northeastern Sardinia that lies between latitudes 40° 30'N and 41° N and encompasses 89 administrative communes and 6 linguistic areas (figure 1). In 1997, the total population was

![Figure 1. Province of Sassari, Sardinia: commune codes and linguistic areas. Commune codes: 1, Aggius; 2, Alà dei Sardi; 3, Alghero; 4, Anella; 5, Arzachena; 6, Arzachena; 7, Banari; 8, Benetutti; 9, Berchidda; 10, Beside; 11, Bonnanaro; 12, Bono; 13, Bonorva; 14, Bortigadas; 15, Boruta; 16, Bottida; 17, Buddusò; 18, Bulai; 19, Bulzi; 20, Burgos; 21, Calangianus; 22, Cargeghe; 23, Castelsardo; 24, Cheremule; 25, Chiraponti; 26, Codrongianos; 27, Coase; 28, Es浦lato; 29, Florinas; 30, Gave; 31, Illorai; 32, Iltireddu; 33, Ittiri; 34, La Maddalena; 35, La Madalena; 36, Luogosanto; 37, Lucas; 38, Mara; 39, Mars; 40, Monteleone Roccadorio; 41, Monti; 42, Mores; 43, Muros; 44, Nughedu San Nicolò; 45, Nule; 46, Nu; 47, Olbia; 48, Olmedo; 49, Oschi; 50, Osilo; 51, Ossi; 52, Osier; 53, Padria; 54, Palau; 55, Pattada; 56, Perfugas; 57, Ploaghe; 58, Porto Torres; 59, Pozzobonigg; 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, Vididalba; 83, Golfo Aranci; 84, Loiri Porto S. Paolo; 85, S. Antonio di Gallura; 86, Tergu; 87, S. Maria Coghinas; 88, Erula; 89, Stintino.]
460,135 (227,215 males and 232,920 females). The 1997 age- and sex-specific populations for each commune, available from demographic data, were used as denominators. Each commune was coded according to the Italian Central Institute of Statistics Coding. 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. 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. 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. 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. 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 on BUGS software.

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).

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. 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 the 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 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). 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 implemented in the CODA software.

**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
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, 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. 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 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 Sassaresi 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.8 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,16-43 and human Y chromosome polymorphisms49,50 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 span1 and analytic epidemiologic investigations52 are needed to shed light on possible associations between MS spatial clusters and etiologic factors in Sardinians.

References


