Epidemiological studies of multiple sclerosis in a Sardinian population, insular Italy

Cluster studies and health status

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Contents

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
CCPGSMS	Canadian Collaborative Project on Genetic Susceptibility to MS
CDMS	Clinically definite multiple sclerosis
cI	Credible intervals
CI	Confidence intervals
CIS	Clinically isolated syndrome
CP	Chlamydia Pneumoniae
CPMS	Clinically probable multiple sclerosis
CSF	Cerebro-spinal fluid
CT	Computerised tomography
DMSR	Danish Multiple Sclerosis Registry
DSS	Disability Status Scale
EDSS	Expanded Disability Status Scale
EBV	Epstein Barr virus
FS	Functional Systems
HERV-W	Human endogenous retrovirus-family W
HHV-6	Human Herpes virus 6
HLA	Human leukocyte antigen
IQ	Intelligence quotient
IDDM	Insulin-dependent diabetes mellitus

LSDMS	Laboratory-supported definite multiple sclerosis
LSPMS	Laboratory-supported probable multiple sclerosis
MLE	Maximum likelihood estimates
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSRV	Multiple sclerosis associated retrovirus
NNMSR	Norwegian National Multiple Sclerosis Registry
PP	Posterior probability
RCPM	Raven's coloured progressive matrices
RR	Relapsing-remitting
SD	Standard deviation
SF-36	36-Item Short Form Health Survey
SMR	Standardised morbidity/mortality ratio
TDT	Transmission disequilibrium test
UV	Ultraviolet
WHO	World Health Organization

Abstract

MS is a disorder of the central nervous system, manifesting as acute focal inflammatory demyelination and axonal loss, and culminating with chronic multifocal sclerotic plaques. MS involves several nervous functional systems resulting in disability, has a rather unpredictable course, and thus leads to poorer quality of life. It is a disorder of young adults, and the most common cause of non post-traumatic neurological disability. It is believed to be caused by interplay between genes and the environment. Potentially any environmental agent can have a role in determining MS in susceptible populations and yet be neither a necessary nor a sufficient cause. Potential risk factors have been investigated, such as infectious agents, vaccines, stress, occupation, climate and nutrition. At what age in a genetically predisposed individual's life, exogenous factors can interact to initiate MS is rather controversial, although this probably occurs within the first 15 years of life.

The present work involves two broad areas in MS research: (i) epidemiological descriptive studies aimed at disclosing clues to disease etiology and mechanisms of disease induction, and (ii) the study of determinants of health-related quality of life in the MS population at large.

The specific objectives to this work were: to disclose MS variation at a microgeographic level, as a possible expression of the spatial distribution of disease risk factor(s) (Paper I); to update and characterise MS incidence patterns, as a possible expression of temporal distribution of disease risk factor(s) (Paper II); to assess whether individuals that later develop MS have shared the same environment at the same age indicating common exposure to disease factor(s), and if so at what age has this occurred (Paper III); and to compare the self-perceived health status to the general population, with special regards to physical functioning, in MS patients with no or mild disability as objectively measured (Paper IV).

All these studies have been conducted on the province of Sassari, northern Sardinia, insular Italy (450,000 pop. circa) based on a registry system of MS cases.

Epidemiological studies conducted over the past two decades using repeated assessments have shown that Sardinia is at high risk for MS with a prevalence of 150 per 100,000 and an annual mean incidence of 6 per 100,000. To investigate the distribution of MS in Sardinia at microgeographic level a spatial analysis of the disease prevalence in the study area for year 1997 was conducted (Paper I). To overcome random variability due to small numbers of cases per geographic unit, a hierarchical Bayesian approach was adopted. Spatial clustering patterns in the province south-west and a west-to-east gradient were observed.

An incidence study was performed on 689 MS patients with disease onset between 1965 and 1999 in the study area (Paper II). The mean annual incidence rate increased significantly from 1 per 100,000 pop in 1965–69 to 6 in 1995–99, with no differences for gender and province sub-areas. The mean age at onset increased significantly during the same period from 25.7 to 30.6 years, while the proportion of patients with progressive initial course declined over time.

Space-time cluster analysis was performed in the study area to indicate a possible shared exposure to MS risk(s) factors during the disease latent period, and the individuals' age for this susceptibility period (Paper III). Residence changes from birth to clinical onset were recorded for all MS patients with clinical onset between 1965 and 1999 in the study area. Closeness in space and time was defined as living in the same commune, at the same time and at the same age differing only by 1, 2 or 5 years. The analysis was performed from birth until age 25 years or disease clinical onset, and by demographic and clinical subgroups. Clustering was substantial in early childhood. It was most marked in the most recent cases, among women and in patients with relapsing-remitting course. No clustering was found when closeness in time was defined as a fixed number of years before onset, arguing against a fixed latency period of the disease.

MS heterogeneous spatial distribution at microgeographic level, its increased incidence and change of clinico-demographic phenotypes over a relatively short period of time, and evidences of clustering in space and time in early childhood especially

occurring recently, are suggestive for the action of an exogenous factor(s) in determining MS. Ecological and case-control studies need to be designed and implemented to validate and characterise these observations.

The self-perceived health status among MS patients with no or mild disability according to EDSS and the impact of self-rated physical functioning have been compared between a sample of fully ambulatory (EDSS ≤3.5) MS patients and the general population (Paper IV). SF-36 was used to self-rate health status. The 197 MS patients analysed (150 women and 47 men) had significantly lower mean SF-36 scores than the general population, except for bodily pain. Similar results were found for a sub sample of 107 patients (81 women and 26 men) with even lower disability (EDSS ≤2.0). EDSS correlated weakly with the physical functioning subscale, explaining only 2% of the variance in such SF-36 subscale. The regression of the physical functioning subscale on the other seven SF-36 subscales was significantly lower among MS patients than in the general population for all subscales, except for role limitation due to physical health problems and social functioning. Therefore factors other than physical functioning contribute to the low scores for the other dimensions compared with the general population. Neither disease course nor duration correlated significantly with SF-36 subscales. Strategies targeting a broad spectrum of health related issues for MS patients should be implemented starting already in the disease early stage. For cost-of-illness studies these findings provide clues to indicators to the disease socio-economic burden in the early stages, and elements for disease-specific interventions.

List of publications

I. Pugliatti M, Solinas G, Sotgiu S, Castiglia P, Rosati G. Multiple sclerosis distribution in northern Sardinia: spatial cluster analysis of prevalence. *Neurology* 2002;58:277-282.

II. Pugliatti M, Riise T, Sotgiu MA, Sotgiu S, Satta WM, Mannu L, Sanna G, Rosati G. Increasing incidence of multiple sclerosis in the province of Sassari, northern Sardinia. *Neuroepidemiology* 2005;25:129-134.

III. Pugliatti M, Riise T, Sotgiu MA, Satta WM, Sotgiu S, Pirastru MI, Rosati G. Evidence of early childhood as the susceptibility period in multiple sclerosis. Space-time cluster analysis in a Sardinian population. *American Journal of Epidemiology* 2006;164:326-333.

IV. Pugliatti M, Riise T, Nortvedt M, Carpentras G, Sotgiu MA, Sotgiu S, Rosati G. Self-perceived physical functioning and health status in fully ambulatory MS patients. *Submitted revised version, 2006.*

1. INTRODUCTION

1.1. Multiple sclerosis

Multiple sclerosis (MS) took centre stage in the 1860s when clinical neurology began to flourish. By the beginning of the 20th century, MS had become one of the most common reasons for admission to a neurological ward. Now, MS is recognised throughout the world, with around 2.5 million affected individuals,[1] accounting for an estimated annual average expenditure of euro 24,183 per case and euro 1,159 million overall in Europe.[2,3]

From a pathological perspective, MS is a disorder of the central nervous system, manifesting as acute focal inflammatory demyelination and axonal loss with some remyelination. This process culminates with the chronic multifocal sclerotic plaques hence the disease name. From a clinical perspective, MS involves several nervous functional systems and has a rather unpredictable course. It is a disorder of young adults, and the most common cause of non post-traumatic neurological disability. Diagnosis is based on clinical and paraclinical evidence of spatial and temporal dissemination, i.e., for at least two demyelinating lesions, affecting different sites within the brain or spinal cord, separated in time. MS is the prototype inflammatory autoimmune or immuno-mediated disease of the central nervous system for which some knowledge on basic etiopathogenetic mechanisms exists, that has allowed the implementation of strategies for treatment.

The principal target of the immune attack in MS is the oligodendrocyte, the cell responsible for synthesising and maintaining the myelin sheath of about 40 adjacent nerve axons in the central nervous system. Myelin is a membrane wrapped around axons to form the insulating segmented sheath needed for saltatory axonal conduction. Voltage-gated sodium channels are located at the unmyelinated nodes of Ranvier, between myelin segments, from where the action potential is propagated and spreads passively down the myelinated nerve segment to trigger another action

potential at the next node. Demyelination interferes with nerve conduction to different extent from decreased velocity to its block. Paroxysmal symptoms, such as trigeminal neuralgia, ataxia, and dysarthria result from demyelinated axons which can discharge spontaneously and show increased mechanical sensitivity, or ephaptic transmission (cross-talk) between neighbouring demyelinated axons.

The symptoms and signs of MS reflect the functional anatomy of impaired saltatory conduction at affected sites.[4] The cerebrum is almost always involved as shown with magnetic resonance imaging (MRI), even though most white matter lesions cannot be linked to specific events or clinical symptoms. During the disease course, involvement of the visual pathway, with special regards to optic nerves, is encountered in up to 92% of patients. Lesions of the brain stem and cerebellar pathways produce precise clinico-pathological syndromes in over 50% of patients, usually characterised by abnormal oculomotion, incoordination of limbs, bulbar, and axial musculature. The spinal cord is affected in nearly 75% of patients, leading to alterations in motor, sensory, and autonomic functions. More vague symptoms, such as fatigue and mood disorders, are experienced by over 75% of patients, resulting in disability and poorer quality of life.

1.1.1. Etiopathogenesis

MS is almost uninanimously believed to be caused by interplay between genes and the environment. Ethnic groups resistant to MS living in areas at high risk for MS demonstrate that genetics is important in shaping overall population susceptibility.[5,6]

Genetic factors

MS is a genetic complex trait. Few or multiple genes are believed to interplay independently or interactively with non-heritable exogenous agents and start MS. Familial aggregation per se can have both genetic and exogenous causes. Aimed at testing genetic hypotheses, the pattern of familial occurrence has been extensively investigated. The change in the recurrence risk ratio in families of individuals with MS shows that first-, second- and third-degree relatives are more likely to develop

MS than the general population, and according to the degree of biological relatedness.[7,8] The steep drop in rates observed between monozygotic twins (30.8%) and first-degree relatives (3.46%), and the further, yet less dramatic declines between first- and second-degree relatives and second- and third-degree relatives favours oligogenic or polygenic inheritance with epistatic interactions among susceptibility loci.[8]

In a Canadian series of patients with half siblings, the risk among full siblings was 3.11% as compared to 1.89% among half siblings[9] and the rate of MS among first-degree relatives of MS patients that had been adopted in early infancy was similar to that of the background population.[10] Nevertheless, a risk gradient was observed between dizygotic twins (4.7%) and full siblings (3.46%), which cannot be explained fully on a genetic basis, since both groups share the same proportion of genome (50%) with the index case.

Factors contributing to an increased familial risk for MS include gender, age at onset and affected relatives/parents. Based on the Canadian Collaborative Project on Genetic Susceptibility to MS (CCPGSMS) the risk of developing MS is 2-fold higher among the sisters of index cases as among brothers.[11] The risk is nearly 2-fold among maternal than paternal half siblings, suggesting a maternal parent-of-origin effect.[9] Index cases with age at onset lower than 30 years,[11] or with one parent with MS[12] were also associated to higher risk in siblings. A study on consanguineous matings showed that the risk was nearly 4-fold higher in siblings of MS patients with related grandparents versus that in siblings with unrelated ancestors.[13] The CCPGSMS study on conjugal MS showed a risk of 30.5%, similar to that of monozygotic twins in Canada and higher than that of 2.7% among the offspring of matings with only one parent having MS.[14]

The study of conjugal MS can also help determine whether there are factors (exogenous) that may facilitate MS transmission in adulthood. Despite concern about the available small sample sizes, and the role of recall bias, population-based studies on conjugal MS conducted on Canadian and Danish populations have shown that

recurrence rate within these matings does not differ significantly with that in the general population.[14,15] Such evidence, which is used to point to genetically based determinants of MS, argues against an exogenous type of transmission, but only with regards to *adulthood*, and it is not informative to rule out the action of exogenous agents before disease onset.

Population-based studies on twins have been a classical approach for investigating the relative roles of genes and exogenous exposure in determining MS. The studies on twins indicate that genes are involved in the process of susceptibility, but also that they cannot fully explain a partial concordance, for which environmental factors are likely to be responsible.

Environmental risk factors

Reviews on the role of environmental factors[16-21] in MS etiology highlight the complexity in identifying proper specific design approaches and in interpreting the findings obtained. Potentially any environmental agent can have a role in determining MS in susceptible populations and yet be neither a necessary nor a sufficient cause. Potential risk factors investigated have been infectious disorders, vaccines, stress, occupation, climate and nutrition.

Many groups have searched for bacteria and viruses in clinical samples of MS patients, but confirmatory evidence from independent laboratories has not yet been reached.[22,23]

Patients with MS are seropositive for Epstein Barr Virus (EBV), and the titres of virus-specific antibodies are higher in individuals with the disease than in controls.[24] The difference in the seroprevalence rate is even more prominent in children with the disease (83% vs 42% in age-matched healthy individuals).[25] Until now, however, no data unequivocally lend support to a direct role of EBV in the development of the disease, and in which pathways EBV plays a part in the pathogenesis of the disease is not clear. In pediatric MS no differences in seropositivity for most viruses between MS patients and controls has been found except EBV infection, and mumps and

measles after 15 years of age. A nested case–control design within the Nurses Health Study cohort[26] aimed at investigating the serological association between Chlamydia Pneumoniae (CP) infection and the development of MS showed that the presence of CP-specific immunoglobulin G antibodies was associated with both an increased risk of developing the disease and a progressive course. The Human Herpes Virus 6 (HHV-6) has also been hypothesised to be a candidate as infectious agent in MS, based on a significant increase in detected early antigens and on a correlation with exacerbations and onset.

Although modern vaccines do not contain neural tissue derivatives, homologies can exist between microbial and neural epitopes that can stimulate the immune system and activate autoreactive clones.[27] With this respect, it is reasonable to consider a role of vaccination in triggering both the disease *ex novo*, or new relapses. A number of studies, including case-controls designs, have been conducted aimed at finding associations between vaccination and onset of MS, optic neuritis, or relapse. The focus was particularly on influenza, hepatitis B virus, tetanus, measles, mumps and rubella. Confavreux and Compston[27] in a recent review conclude that there is no association with an increased risk of developing MS or optic neuritis, and that vaccinations are not a risk factor for MS.

Among occupational exposures, organic solvents have raised most concern in studies of MS causation. Odds ratios varying from 0.8 to 4.0 are reported from various case–control studies, but statistical significance is almost never reached. Several methodological issues have been raised, such as the cross-sectional nature of such studies based on prevalence, self-reported exposure assessment reflecting recall bias, the lack of adjustment to confounders and the small sample sizes. Few studies have focused on the putative period elapsing between exposure and onset as well as defining ‘exposed’ by the necessary duration of exposure. The results from cohort studies are also controversial.

Sun exposure, ultraviolet (UV) radiation and latitude are inversely correlated with MS. As MS has been reported to vary with latitude, a role of UV radiation has been

hypothesised, also based on the biological effects of UV radiation suppressing T-cell function. Ecological studies have shown a strong inverse correlation between UV radiation and MS and between residential and occupational exposure to sunlight and MS mortality, and showed that the incidence of skin cancer in the MS population was significantly lower than expected. However, other case–control studies found no association. Higher vitamin D intake was associated with a lower risk of MS. Due to confounding related to dietary factors and inconsistent measurements of sunlight exposure, the evidence is too weak to even partly explain the geographical variation in MS risk as an effect of sunlight.

A causative role of traumatic brain injuries due to disruption of the blood-brain barrier has been suggested. Physical trauma (especially head trauma) has, however, not been found to be associated with MS onset, exacerbation or progression. On the other hand, compared with controls, people with MS had more frequently undergone intense mental stress or severely threatening life events a few years before onset. However, these studies have limitations related to inconsistent measurements of exposure, recall bias, small sample sizes and confounding.

As MS is increasingly predominant in women, sex hormones have been hypothesised to play a causative role for their impact on the immune system. Epidemiological studies on sex hormones and MS have focused on four major risk factors: age at menarche, pregnancy, parity and use of oral contraceptives. None has provided sufficient evidence on the role of these hormones in causing MS. Whether pregnancy is associated with fewer relapses and postpartum with more is still debated.

Diet as a risk factor has been of interest in MS studies for over 50 years because it implies potentially toxic agents and because it varies with ethnicity and geographical areas in which MS occurrence also differs. Because most studies are cross-sectional, the role of nutrition in causing MS is inferred from observations on individuals' current nutritional status. Several population-based ecological studies conducted in different areas reported that MS is correlated with the consumption of milk, dairy products, meat and especially animal fat but few case–control studies have confirmed

this. Dietary fat has been reported to correlate with MS and mortality and fatty acids reported to have a role in the MS course. Lower levels of linoleic acid, an omega-6 fatty acid, have been found in the blood, cerebro-spinal fluid (CSF) and brain of MS patients, but the interpretation of such findings is controversial. Other dietary factors such as brain, sweets and confectionery, new potatoes, alcohol, smoked meat products, pasta, bread, horsemeat, coffee, tea and breast milk have been investigated as potential risk factors in MS. Vitamin B12 deficiency is not unusual in MS patients, but no evidence indicates its causative role. No consistent data from analytical studies and clinical trials confirm any relationship between MS and nutrition. Methodological problems in these studies include selection bias, recall bias in patients as compared to controls and within patients before and after diagnosis, the lack of objective measurements of dietary factors and the scarce control for possible confounders, such as socio-economic class.

Results from case–control and cohort studies indicate that cigarette smoking is a risk factor for MS. The risk of developing MS was almost 2-fold among smokers as compared to never-smokers in a Norwegian population.[28]

Lastly, an excess of spring births has been detected for MS patients.[29,30] Pooled analysis of datasets from Canada, Great Britain, Denmark and Sweden (n = 42,045) showed that significantly more people with MS were born in May (9.1%) and significantly fewer were born in November (8.5%). This represents a 19% decreased risk of MS for those born in November compared with those born in May. The association between month of birth and risk of MS,[29] seasonal variation and risk of MS onset, or disease activity[31] has been interpreted as a climate-related interaction between genes and environment during gestation or shortly after birth, at least in northern Caucasian populations. Methodological issues such as random variation, misclassification and statistical methods applied, however, may partly explain such results.[31]

1.2. The latent period

The question arises as to at what age in a genetically predisposed individual's life, exogenous factors can interact to initiate MS.

The manifestation of the first symptom(s) or sign(s) eventually attributed to MS[32] is referred to as the clinical onset of MS, and the age at onset is therefore the age at which such clinical manifestations occur. However, biological initiation of MS does not coincide with the clinical initiation, and the disease process is believed to start earlier in life.

Attempts have been made to conceptualise a disease etiologic process into a sequence of fixed events that could reflect the action of risk factors.[33] The time elapsing between disease initiation (induction) and clinical onset is referred to as the *incubation period*, specifically for infectious processes, and *latent period* otherwise. In disease etiology, however, not only such period simply represents the time lag between disease initiation and detection, but it may reflect part of the etiologic process itself, occurring subsequently to the action of the cause. This appears to apply to putatively multifactorial disorders, in which the initiating cause may not be sufficient, but necessary, and the action of further agents is needed for the disease to develop. The period between causation and disease initiation is defined *induction period*, which includes the time at which the *earliest* component causes influence the etiologic mechanism.[33] Genetics is considered the most common earlier component cause. However, if we referred the induction period to the more *specific* component cause and not the earlier, any attempt at characterising the induction period would be more informative and useful. Therefore, the induction period varies also in relation to what we mean for *cause*. The *latent period* could virtually be null if diagnostic tests were able to detect the presymptomatic disease.[33] Practically as the precise point in time at which disease is initiated is not detectable, the latent period is included in the induction period, and the two cannot be empirically separated. Then, the interval from etiologic action to disease detection is referred to as the *empirical induction period*. A lengthy empirical induction period increases the possibility for

confounding and attenuates the association between cause and disease. Subsequently, in etiological research based on case-control studies, dilution of the effect of a putative factor occurs when a relevant exposure is considered to have acted outside the time span corresponding to the empirical induction period. The attenuation of the effect-estimate leads to nondifferential misclassification, due to a bias which obscures the real effects and generates conflicting results among studies differently designed. In case-control studies, lifetime exposures histories will underestimate an effect which pertains to exposure at a specific time before disease. Methodological approaches aimed at narrowing such interval window using different assumptions (e.g., the induction period varies from 5-10 years, or 10-15 years etc) will help to locate the greater effect in time without misclassification bias. Cases will be considered only individuals with onset after the defined empirical induction period. In cohort studies, using the different exposure assumptions, the highest incidence rate of a disease will reflect the most likely length of the induction period for that disease. In case-control studies, only cases with the defined time of putative exposure will be included.

When does the induction period begin if the exposure is chronic? In this case, it might require years before the exposure becomes relevant in determining the disease. Methodological approaches to obtain estimates of such exposures are based on the assumption that such interval could reflect a time-dependent process, or a cumulative dose effect defined by either time since first exposure, or a function of time and dose rate.[33]

In MS research the term *susceptibility* period is often encountered in literature in relation to the time or age of exposure to the putative risk factor(s) and within the empirical induction period. The susceptibility period can therefore be viewed as an attempt to disclose the disease "induction" period and therefore clues to its etiology.

According to Poser, the susceptibility period consists of the development of the 'MS trait', a systemic, non-pathological condition which does not involve the nervous system parenchyma, and that may develop in some individuals who are genetically

susceptible to MS.[34,35] He defines such condition as the 'premorbid stage of MS'. In principle, this is analogous to the sickle-cell trait, or glucose-6-phosphate dehydrogenase deficiency in that it requires a trigger to develop into the overt disease MS, such as a 'disease waiting to happen', which differs from an asymptomatic condition of MS because no lesions of myelin or axons can be detectable. Poser believes that the MS trait does not necessarily affect all MS-susceptible persons, and that not all persons with the MS trait will eventually develop MS. The MS trait is characterised by an immunological hyperreactivity with an exaggerated response to viral antigens, the presence of oligoclonal bands in the cerebrospinal fluid – a non-specific sign unrelated to activity in the overt disease itself - and an increased vulnerability of the blood-brain barrier, likely the primary event in the disease pathogenesis.[36] As the MS trait is completely asymptomatic and is not even associated to MRI changes, it is not possible to determine at which age it develops. It is hypothesised that it occurs prior to puberty, the age of putative biological onset, as a manifestation of a localized immune response to a variety of non-specific antigenic challenges, most likely viral. In order to develop into MS, it is necessary that immunoactive substances penetrate into the central nervous system after a second and more specific antigenic challenge on the blood vessel wall adhesion molecules. Also, other mechanisms have been suggested such as trauma, electrical injury, and lipid solvents.

Despite the potential of epidemiological studies for investigating MS empirical induction period, and more specifically, for identifying and characterising its susceptibility period, few such studies are reported in literature. Among the designs used are migration and cluster studies, including the analysis of epidemics, and statistical models.[37]

Migration studies are aimed at comparing the incidence of MS in migrants from countries with high risk to those with low risk, or viceversa. Ultimately, they help determine whether the disease initiation is predominantly driven by genetically determined mechanisms, or by the different distribution of environmental factors as

across geographic regions. Relevant studies include those conducted among immigrants from Europe to South Africa and in the population born in South Africa.[38,39] Age-adjusted prevalence and incidence rates were highest in European immigrants, lower in South African English and Afrikaners and lowest among admixed black and Caucasian Africans. The risk of MS was higher among the children of immigrants to the United Kingdom from India, Africa and the Caribbean than among their parents, and it was similar to that among children born in the United Kingdom.[40] In France, the risk of MS was higher among people with Vietnamese mothers who had migrated from Vietnam at age 20 years or younger.[41] These individuals, however, were likely to have admixed genes from French fathers.

The prevalence of MS was 7 among Japanese living in Hawaii, 10.5 among native Hawaiians and 34 among migrant populations from North America[42] largely reflecting the rates of Japanese and Caucasians living in California and in Japan.[43]

Age of migration is crucial in determining the risk. Adult European immigrants to South Africa had a 3-fold higher risk for MS than those migrating at age 15 years or younger.[44] In a study conducted on Ashkenazi (from northern Europe) and Sephardic Jews (from Asia and Africa) in Israel, the risk was higher in the Ashkenazis in relation to the older age at migration (after adolescence).[45] These evidences suggest that age effect is probably related to the first two decades of life.

Conversely, a study on prevalence in a migrant population from the United Kingdom and Ireland to different regions in Australia showed that the risk among individuals who migrated before age 15 years to low-risk areas in Australia did not differ from that among individuals migrating at age 15 years or older, suggesting that environmental factors may operate over a longer period of time after childhood.[46]

The validity of migration studies is undermined by methodological issues, such as small sample sizes, the difficulty of identifying a denominator for the migrant target population, the age of MS onset relatively to that of migration, selection bias and confounding based on socio-demographic features such as age, gender proportion,

health status and reasons for migration.[37,47] Further, too few studies have been conducted on MS incidence among migrants from low- to high-risk areas,[37] and relevant secular changes in the prevalence within the general population over time can mask prevalence in subgroups.

Cluster studies represent a further approach to investigate MS initiation and susceptibility. Epidemics are a special case of clusters of case in space and time with a shorter and better defined latency period and are ideally more successful for characterising the susceptibility period. The most comprehensive report on MS epidemics is Kurtzke's 30-year observational study on the pattern of MS in the Faroe Islands in the North Atlantic, where in 1998 MS prevalence rate was 66 per 100,000.[48] The first MS case among Faroese native residents since 1900 was reported in 1943. Based on the analysis of patterns of MS occurrence and patients' residence history, MS was hypothesised to be acquired at least 2 years after exposure to an exogenous factor and during puberty (age 11 years). After the first case, 21 new cases developed MS in a type 1 epidemic (occurrence due to initial exposure to an exogenous 'virulent' factor in a susceptible population virgin to that specific exposure). The source of such exposure was believed to be a widespread, specific, persistent infectious yet unknown agent introduced by the British troops occupying the Faroe Islands during World War II. The consequent asymptomatic infection, the 'primary MS affection', would convert into clinically detectable MS years later and only in subsets of individuals. Susceptibility to the primary MS affection would be limited to age 11–45 years at the start of exposure, whereas the primary MS affection would be transmitted at age 13–26 years and before clinical onset. The first epidemic was followed by three successive epidemics of 10, 10 and 13 cases, respectively, defined by calendar time and age of exposure, with peaks at 13-year intervals.[48] This led to conclude that MS was the result of a specific transmissible infection from person to person. After reanalysing the data, Cooke[49] critiqued the 'pubertal hypothesis' with a 'protective hypothesis', arguing that the pubertal exposure consisted of a childhood infection with later onset, and that early childhood would instead be the true MS induction period. Methodological issues were raised against

the evidence of MS epidemics due to small sample sizes, multiple assessments over time based on registries, the use of old and more inclusive diagnostic criteria and the plausibility of the role of the British troops in determining the epidemic.[50]

In the Orkney and Shetland Islands, MS prevalence rates steadily increased by almost three-fold to 110 in the Orkneys and from 134 to 184 in the Shetlands between 1954 and 1974. Over the same time period, however, general awareness of MS improved.[51] Incidence rates were stable in 1930–69, but a slight reduction at the end of the period was attributed to underascertainment and to more stringent diagnostic criteria.

In 1979, incidence in Iceland was studied based on the 168 MS cases retrospectively ascertained since 1900.[52] Until 1922, MS cases had been sporadic. The mean annual incidence rate was 1.6 in 1923–44 and 3.2 in 1945–54, followed by plateau and a decline to 1.9 in 1955–74. The age at onset also increased from 1945–49 to 1950–54. This whole incidence pattern was interpreted as a postwar epidemic of MS. This trend was reanalysed[53] and explained by improved diagnostic accuracy due to the increased number of neurologists in the 1930s and in the 1970s and easier access to neurological care.

Space-time cluster analysis is used to test a single source exposure, usually an infectious agent, and the time, space or age of putative susceptibility to such exposure. The idea behind this analysis is that if the number of observed cases that have been close in time and space is significantly higher than expected just by chance, then the pattern suggests an infectious disease spreading from case to case.[54] A detailed description of methodology and limitations is given in Section 1.4.3.

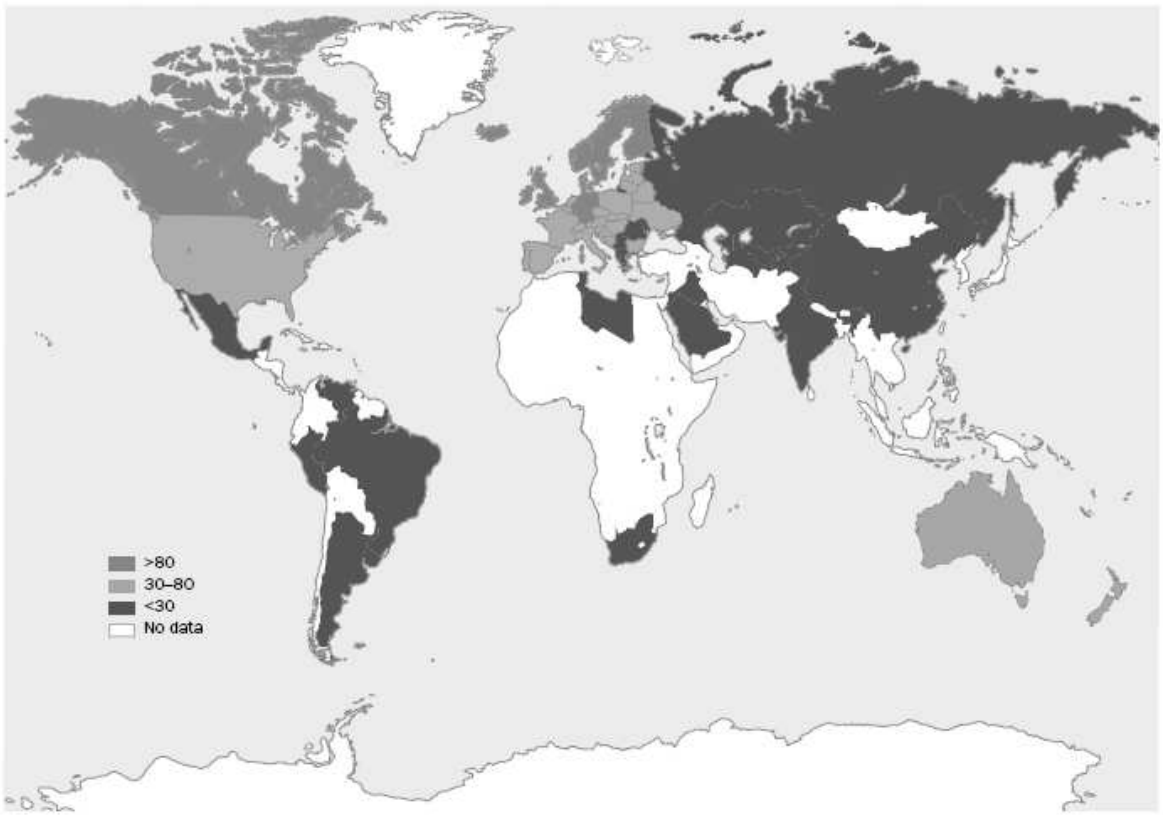
Pre-onset natural history statistical models have been built by Wolfson et al[55] to estimate the distribution of the disease entire latent period. In this model all the patients were assumed to have acquired the disease after a fixed time period, i.e., the susceptibility period. Several such periods previously reported in the literature were accounted for in the model, however the most likely one was found at 10-15 years of

age and the latent period estimated duration was 18 years. Similar findings were obtained in a dataset of French MS patients.[56]

1.3. Descriptive epidemiology of MS: general overview

The geography of MS and its variation over time have been systematically investigated for the past 70 years[6,19] (Figure 1).

Figure 1. The MS prevalence worldwide (per 100 000 population)



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Despite the wealth of publications dealing with occurrence of MS throughout the world, any attempt to define the geography of MS remains as difficult as ever. An increase in the disease occurrence is reported almost worldwide, and exogenous/environmental factors have been hypothesised to account for such change. However, the geographical distribution of absolute rates might depend on the distribution of genetic susceptibility alleles and their interaction with environment. Environmental and genetic determinants are not mutually exclusive, and the *nature-versus-nurture* controversy is a hot debate in MS epidemiology today. Ethnicity and ancestry are often used to refer to populations' genetic origin but they can also refer to cultural habits and lifestyle factors, and confound results.

A summary of MS incidence rates from population-based studies conducted in Europe on samples of 50,000 and larger, is reported in Table 1.

Table 1. Incidence (per 100 000 population per year) of MS in European countries

Country	Time interval	Study pop. size	Rate (95% CIs)
Albania	1968–1987	3 091 000	0.5 (0.4–0.6)
Croatia (northern Adriatic islands)	1956–1998	50 552	1.3 (–)
Croatia (Osijek-Baranya)	1991–1998	298 600	3.5 (–)
Denmark	1980–1989	nationwide	5.0 (4.8–5.2)
Finland (Seinäjoki)	1979–1993	197 000	11.6 (10.1–13.1)[a]
Finland (Uusimaa)	1979–1993	1 278 000	5.1 (4.1–6.3)[a]
Finland (Vaasa)	1979–1993	179 000	5.2 (4.8–5.5)[a]
Finland (central)	1994–1998	263 886	9.2 (7.4–10.9)
France	1993–1997	94 000	4.3 (2.9–7.2)
Germany (Lauer, personal data)	1979–1992	100 000	4.2 (–)
Greece (Evros)	1994–1999	143 000	2.4 (1.4–3.7)
Hungary	1997–1998	400 128	5.5 (–)
Iceland	1991–1995	255 000	0–5 (–)
Ireland (Donegal County)	2001	129 994	5.1 (1.6–11.7)
Ireland (Wexford County)	2001	104 372	4.5 (0.3–8.7)
Italy (Ferrara, north)	1990–1993	368 000	2.4 (1.6–3.4)
Italy (Sicily, insular)	1990–1994	338 000	3.9 (3.0–5.0)
Italy (Sardinia, insular)	1995–1999	454 000	6.1 (5.1–7.2)
Italy (Padua, north)	1995–1999	820 000	4.2 (3.7–4.7)
Malta	1989–1998	400 000	0.8 (–)
Netherlands (Groningen)	1985–1990	560 000	3.0 (–)

Norway (Hordaland County)	1978-1982	405,063	5.0 (-)[b]
Norway (Hordaland County)	1993-1997	441,660	6.0 (5.0–7.2)
Norway (Hordaland County)	1998-2002	441,660	3.0 (2.3–3.8)
Norway (Møre and Romsdal County)	1975-1979	237,278	3.8 (-)
Norway (Vestfold)	1978-1982	188,664	2.4 (-)
Norway (Nord-Trøndelag County)	1974–1998	127 000	5.3 (3.7–7.5)
Norway (Oslo)	1992–1996	484 000	8.7 (6.3–11.9)[a]
Norway (Troms and Finnmark Counties)	1989–1992	225 000	4.3 (3.0–5.9)
Poland (west)	1993–1995	50 000	2.2 (–)
Romania (Mures County)	1976–1986	600 000	0.9 (–)[a]
Russian Federation (Iaroslavl)	1996–2001	–	3.0 (–)
Slovenia	1990s		2.9 (–)
Spain (Mostoles, central)	1994–1998	196 000	3.8 (2.7–5.3)
Spain (Teruel, east)	1992–1996	143 000	2.2 (–)
Sweden (Västerbotten County)	1988–1997	256 000	5.2 (4.4–6.2)
Switzerland (Canton of Berne)	1961–1980	920 000	4.0 (3.7–4.3)
The former Yugoslav Republic of Macedonia	1990s	–	0.2–1.2 (–)
Ukraine (Vinnytsya)	1990–1994	390 000	0.7 (–)
United Kingdom (northern Cambridgeshire)	1990–1995	379 000	4.8 (3.8–6.0)
United Kingdom (southeastern Scotland)	1992–1995	864 000	12.0 (10.6–13.3)

[a] Only Poser Committee diagnostic criteria for definite MS

[b] McAlpine diagnostic criteria

Whether the reported variation in disease rates over time partly reflect a true change in MS risk or merely improved case ascertainment, demographic factors, such as increased survival, or better study methods over time is still debated. The outlining of geographical patterns is undermined by the variation in the size, age structure and ethnicity of the populations surveyed, case ascertainment; the level of healthcare and expertise, degree of public awareness of MS and access to diagnostic procedures; and the methods applied to study designs and statistical analysis. Prevalence rates almost invariably increase with multiple-source repeated assessments over time and when small populations are used.[6,21]

1.4. Investigating variability of disease distribution in space and time: general overview

Epidemiological descriptive studies are of unquestionable importance in investigating the multifactorial etiology of rare diseases like MS. Although they only allow to describe the existing distribution of putative associated variables without regards to causal hypotheses,[57] still they are the first approach to document the health of a population and yield working hypothesis to more rigorous and analytical studies.[58] Traditionally descriptive epidemiological studies focus on person, place and time, but they should also be able to answer five basic "W" questions – *who* has the disease, *what* is the disease under study, *when* is the disease common or rare, *where* does or does not the disease arise, *why* did the disease arise (clues for more sophisticated studies).[58]

Among the descriptive studies are cross-sectional, or prevalence, studies describing the health of populations in terms of the distribution of exposure and outcome which are ascertained at the same time, hence the cross-sectional nature. These study are rather easy to undertake, but, due to their *post-hoc* nature, fail to provide clues on the temporal sequence (and thus to the disease etiology) of exposures and outcomes, with the exception of long-standing exposures, such as gender or blood type.

Surveillance is also a type of descriptive study. Surveillance consists in the "ongoing systematic collection, analysis and interpretation of health data essential to the planning, implementation and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know".[59] Surveillance can be active, i.e. active search for cases, or passive, i.e., the data are already collected in traditional channels, such as death certificates.[58]

1.4.1. Spatial cluster studies

The estimation and presentation of spatial summary of health outcomes, such as prevalence rates of MS, has long characterised the history of epidemiology, and it is referred to as geographical analysis of health data or disease mapping.[60-62] Disease mapping is aimed at providing a description of health outcomes, generating hypotheses, allocating health resources and assessing the variability in underlying disease exposures. Mapping crude estimates is especially difficult for rare diseases and small areas, such as it is the case for MS prevalence by administrative communes. In this scenario, disease mapping is biased by variability due to sampling or chance. Hierarchical models,[61] among which is the Bayesian approach[63,64] can be used to filter out ("smoothing") the random variation from the estimated rates due to the small numbers. The Bayesian ecological model consists of a three-stage hierarchical model within which disease mapping data may be viewed. Health outcomes are usually available as area-level aggregated count data which therefore represent the basis for disease mapping. So we consider a study region A (e.g., a province), which can be divided into N small areas $A_1 \ldots A_N$ (e.g., the communes). For a given set of areas, a set of observed cases $O_1 \ldots O_N$ and corresponding denominators are obtained. The expected number of cases $E_1 \ldots E_N$ in each area is calculated by indirect standardisation, and stratified by age, gender or other confounders, using a set of reference rates, so that the relative risk rather than the crude rate is used.[60] We have thus obtained the standardised morbidity ratios (*SMRs*) expressing the ratio between observed (O) and expected (E) number of counts in an area (A). For rare diseases, the expected values can also be calculated by Poisson regression, and

assuming that O_N are distributed according to Poisson distribution ($\lambda_N E_N$), where λ_N is the relative risk for each area. The *SMR* O_N/E_N is then the maximum likelihood estimate (*MLE*) of λ_N.

Mapping MLEs can however be misleading because of sampling variability that make estimates unstable, and also because of overdispersion or *extra-binomial variation* due to a possible inconstant disease risk distribution in the population.[65]

The Bayesian hierarchical model allows dividing the extra-binomial variation into two components.[66] The first component reflects our *a priori* belief in a simple spatially unstructured (and thus non measurable) extra-binomial variation which we refer to as *heterogeneity*. The second component reflects our a priori belief in a smooth variation across sub-areas and spatially structured, which we refer to as *clustering*. *Explanatory variables* can be also used in the model, which consist of measurable ecological variables known to be related to the disease risk. *Heterogeneity* is a random component whish is assumed to be normally distributed with the mean given by the overall mean, whereas *clustering* is assumed to be normally distributed with the mean given by the means of the neighbouring clustering estimates, i.e., of areas geographically adjacent from which they "borrow strength". The variance distributions of the two extra-binomial variation components are distributed as a *chi-square* variable. The model is hierarchical in that a prior distribution has to be specified at two stages, *heterogeneity* and *clustering*. The true prevalence p_i stems from a combination between the prior distribution including *heterogeneity* and *clustering*, with the information contained in the MLEs. The posterior distribution is so obtained. The analytical form of the desired posterior distribution can be obtained by generating samples using the Markov chain Monte Carlo technique.[67] For each commune, the proportion of the posterior samples with a mean greater than the global posterior mean (i.e., the overall mean prevalence) can be computed, and it is referred to as the posterior probability (PP). The PP is the Bayesian equivalent of the *p*-value[68] and can also 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, while PP<0.10 strongly indicates that the prevalence is lower. In those areas where PP falls in the intervals 0.75-0.90 and 0.10-0.25, 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 (0.25-0.75) no informative evidence is given.

In this model, crucial is the choice of the prior distribution, as it yields an estimate which is a compromise between the area-specific prevalence and the reference value depending on the prior distribution. This allows to study *local* geographical variation of the disease, in contrast to broad-scale (e.g., national) comparisons. Extremes prevalence estimates are pulled towards the reference, the more consistently, the more unstable they are, and so this applies especially for areas with few cases. In this way the empirical map is smoothed and the spatial trends are more interpretable. The choice of the prior distribution (*heterogeneity* versus *clustering*) depends on our belief of how high or low the risk in the cluster will be as compared to the area at large. A *clustering* distribution is chosen if the risk in the cluster is expected to be higher than the area.

Unstructured and structured variability can be combined into one statistical model, the *convolution prior*,[69] to which they independently contribute. The third and final stage of the Bayesian approach is aimed at specifying a hyperprior for the parameters related to the unstructured and structured variability in stage two.[61] This very theoretical statistical step is crucial in that it conditions the final disease mapping. The choice of the hyperprior is usually based on experience and sensitivity analyses.[66,70]

1.4.2. Incidence studies

Incidence is one of the basic measures of disease occurrence.[71] It takes into accounts the number of individuals in a population that develop a disease, and also the length of time experienced by all persons during the risk period in that population. The

incidence rate is the number of new cases of diseases (incident number) divided by the person-time spent in the population.

In chronic diseases with irreversible states, such as MS, the first occurrence of disease onset is the eligible event, and make up the numerator. The number of disease onsets in this case is also the number of people experiencing the event. In epidemiological studies of MS we deal with *open* populations, wherein individual contributions do not begin at the same time and the population is open to new members, who can enter through births, migration, etc., or exit through death, emigration, disease occurrence.[71] These populations are usually at *steady state*, because the number of entering people is balanced by people exiting from the population, in terms of age and gender structure, and distribution of risk factors. There are various levels of definition for incidence, however, because the onset of MS is rare in the short time interval usually considered (within a year), the incidence proportion is what actually we refer to as incidence rate.

Establishing the time (age or year) of onset of MS can be difficult but it is not impossible, as compared to other neurological conditions characterised by a slowlier and insidious onset, such as dementia or Parkinson's disease.[72] By means of collecting the patient's history meticulously, it is possible to allocate in time individuals' signs and symptoms attributable to an onset of MS.[32]

The MS incidence in most high risk areas ranges from 1 to 10 per 100,000/year, and is most often computed retrospectively or by means of cross-sectional surveys, along with prevalence rates. Prospective incidence studies of MS are difficult to undertake as they require following up large population cohorts for long periods of time. However, registry and surveillance systems have now been established that will eventually overcome such difficulties. Among the most relevant such systems in Europe, are the Danish MS Registry (DMSR) and the Norwegian National MS Registry (NNMSR). The DMSR was started in 1948 and since then updated by prospectively and retrospectively recording information on MS incident cases from multiple sources.[73] It has provided unselected patient samples for assessing the

disease natural history, familial risk and risk for comorbidity in both case-control and cohort studies, apart from multiple assessments of incidence, prevalence and survival.[74-82] The NNMSR was established in 1998, and covers at the moment (autumn 2006) over 60% of the whole Norwegian population, with the western region (950,000 pop. ca) being almost thoroughly complete.[83] The NNMSR includes a biobank unit for collection of cerebrospinal fluid and serum, DNA, and tissue samples.

In most other settings, incidence data are obtained from defined geographic areas for which demographic data are available at specific time points (e.g., census data) and cases are actively and more or less systematically searched for in hospitals, outpatient clinics, individual physicians, health insurance organizations, MS societies, etc. Accurate case ascertainment is based on the probability that these patients are diagnosed and identified by the search which also depends on the degree of access to such sources.[5] The diagnosis of MS in turn is influenced by the standards of medical care, level of disease awareness in the community and the sensitivity of the diagnostic criteria applied. Intuitively, if these are lacking, diagnosis is delayed and prevalence underestimated. Incidence can be underestimated only if cases will die before they are diagnosed. However, at least in multiply assessed populations and because of the nature of MS itself, this is quite unlikely and we may conclude that diagnostic delays only marginally affect incidence rates. Furthermore, because of the growing better quality of diagnostic facilities and awareness, the diagnostic delay has dramatically dropped from an average of 10 years to less than 1 year in the past 2 or 3 decades.

To such shortening of time has also contributed the change in the diagnostic criteria. When the two most recent sets of diagnostic criteria, i.e., the Poser et al[84] and the McDonald et al[85] criteria, were applied to 76 patients seeking medical specialist care for suspected MS, MS was diagnosed more often according to the McDonald et al criteria than the Poser et al *clinically definite* criteria for MS (52% versus 38%).[86] Fast diagnoses may lead to a biased increase in the incidence. In fact, because of the retrospective nature of MS epidemiological studies, the need for comparing

epidemiological indices among different settings and the reduced interobserver variability, the Poser et al criteria are still the most commonly encountered classification in epidemiological surveys.[87]

Not only factors biasing the numerator, but the choice of the denominator can obviously change incidence rates. With this respect, and given the possible different age- (most unlikely gender-) structure of the populations under study, age- (or gender-) adjustment to a common standard population is very important as it allows for comparisons and correct interpretations of the data.[88] An example of such importance are the findings from a meta-analysis of population-based incidence and prevalence studies on MS from 1980 through 1998 in which rates were age- and gender-adjusted to the World and the European standard populations.[89] When the mean crude and age- and gender-adjusted prevalence and age-adjusted incidence rates were stratified by latitude, the latitudinal gradient, which was highly significant for the crude rates, became less remarkable for the age- and gender-adjusted prevalence rates and not significant for the age-adjusted incidence rates.

1.4.3. Space-time clustering studies

Cluster studies are among the methodological approaches used when investigating disease initiation and its latent period (see Section 1.2.). A cluster of cases is defined as any departure from the random distribution of cases, either in time or space, or along any other axis.[54] Two types of cluster analysis are most commonly used in epidemiology, i.e., the *post hoc* and the *space-time* cluster analysis. The former is driven by an excess of cases - most frequently only apparent -within small geographic areas, detected by patients, mass media and public opinion.[54,90-92] It represents the basis for disease mapping (see Section 1.4.1.). The problems with *post hoc* cluster studies in determining the validity of such excesses of cases are linked with the cluster report itself, the determination of the number of cases and diagnostic accuracy, the population at risk, the definition of the study area (usually too small), of the study time (usually too short), biological plausibility up to the feasibility of an etiological study with usual small numbers. Ultimately, the crucial issue with *post*

hoc studies is to assess whether the observed cluster represents a real biological event or is simply due to a random increase in incidence.

If *post hoc* cluster studies are motivated by observation, *space-time* cluster analysis is motivated by a hypothesis and should be conducted in populations with no (or little) previous evidence of cluster.[54] This model is based on the study of time and place of residence during the putative acquisition period of a disease, so as for MS, from birth up to clinical onset. Space-time cluster analysis is therefore used to test a single source exposure, usually an infectious agent, and the time, space or age of putative susceptibility to such exposure. Several types of space-time cluster models can be used,[93] all based on comparing the distance in time and space at disease onset or prior to it, among pairs of individuals. The idea behind this analysis is that if the number of observed cases that have been close in time and space is significantly higher than expected just by chance, then the pattern suggests an infectious disease spreading from case to case.[54] Detailed information on patients' changes of residence and relative calendar time are necessary for this analysis, which can only come from population-based registry systems or multiply assessed populations over time.

A space-time clustering effect can also be searched in diseases with long latency such MS as an attempt to disclose "exposure aggregation".[37] Although likely masked by the aspecific multifactorial nature and the long and variable pre-onset history of the disease itself, they can provide relevant clues to exposures, time or age of disease acquisition and more susceptible individuals.

1.5. The Sardinian population

Sardinia lies between latitudes 38° 51' 52" and 41° 15' 42" N and longitudes 8° 8' and 9° 50' E from Greenwich.

The Sardinian population originates from an early split in the Caucasoid group, and features a peculiar genetic asset which differs from that of other Caucasians or even mainland Italians.[94] Sardinians' origins 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 withdrew from contacts with foreign populations who occupied the island coastal and plain areas in subsequent centuries, and settled in the mountainous interior areas of central Sardinia, mostly inaccessible to others. Successive waves of invasions by Phoenicians, Carthaginians, Romans, Vandals, Byzantines, Arabians, Ligurians, Tuscans, Spaniards, and Piedmontese failed to penetrate the wild inland, and especially today's province of Nuoro and its core region *Barbagia*. Barbagia, the "region of the barbarians", as the Romans called it, represents the core of preserved ethnic and genetic peculiarities among aboriginal Sardinians.[94,95] Comparative studies on human leukocyte antigen (HLA) allelic frequencies have clearly demonstrated the genetic isolation of Sardinians from other Italian and European populations.[96,97] The persistence of certain linguistic relics in the Sardinian language and the uniqueness of family names lend further support to their isolation.[98] After World War II until 1980s, there was a consistent emigration from Sardinia to industrialized regions of northern Italy and Europe. Based on official data, the average annual emigration during this period was around 520 per 100,000 population. Later on, the migration flux was negligible (around 1.6%) and did not change substantially over time.

The settlement of the city of Sassari, northern Sardinia, goes back to nearly 2,500 years B.C., i.e., to the Nuragic era. Later, Sassari represented the hinterland to Turris Libyssonis (today's Porto Torres), which was a very active colony and harbour in Roman times and until the seventh century. Sassari became an important urban centre between the seventh and eighth century, when the Saracen incursions gradually depopulated the coastal areas and the aboriginal population concentrated in the hinterland. A series of invasions followed:[99] Sassari was ruled by the Pisans until 1300 ca, Aragonese and Spanish until 1700 ca and Austrians until 1720 ca. In 1720 Sardinia was granted to the King of Piedmont in exchange for Sicily and became part of the Savoy Kingdom. Despite the chain of conquests, Sardinians' population structure did not change substantially. Except for few well identified foreign or mainland settlements on the coast, the gene flow into the aboriginal population had been quite limited.[96] The genetic isolation of the Sardinians has been shown on the

basis of frequencies of genes implicated in determining blood groups,[94] and their cultural isolation on the basis of the preservation of peculiar linguistic relics.[100]

Based on geo-climatic features, four areas can be discerned within the province of Sassari: the *coastal*, the *interior plain*, the *interior hill*, and the *interior mountainous* areas. The *coastal* area is 15 Km broad and less than 200 m high, and has mild climate reflecting the alternating effect of land and sea breeze. The annual mean temperature is 16.5° (range 13.7°-20.5°). The *interior plain* area is over 15 Km distant from the coast with an altitude of 300 m or less, and an annual mean temperature of 15.9° (range 9.5°-23.7°). The *interior hill* area is comprised between 300 and 600 m and can be further distinguished in a part closer to the coast, with milder climate, and an interior one wherein climate is close to continental. The annual mean temperature of these two sub-areas is 15.8° (range 12°-19.1°) and 13.5° (range 9.2°-17.2°), respectively. The *interior mountainous* area, with altitude over 600 m, has mostly continental characteristics, with an annual mean temperature of 12° (range 7.5°-16.7°).

From a socio-economic perspective, the standard of living in Sassari is among the highest in Sardinia. The main occupations, i.e., commerce and tourism, expanded after 1950 as a result of improved air and sea connection with the Italian mainland and Europe. In the 1960s Sardinia became a readily accessible tourist attraction, with an increasing number of foreign tourists, mostly from northern European countries.

According to local statistics on occupation, 27% of the province population is engaged in commerce, 21% in agriculture, 15% in building trade, 13% in services, 11% in manufactory and energy production, 7% in hotel and restaurant industry, 4% in transportation, 1% in fishing and 1% in other occupations.[101] Though nearly 30% lower than the overall European Union level, the employment rate in the province of Sassari is the highest among all the Sardinian provinces, with a rate 56.8% in men and 28.1% in women. Unemployment rate is 10.3% in the male and 20% in the female population.

1.6. Epidemiology of MS in Sardinia

Descriptive epidemiology

MS epidemiology has been investigated in different geographic sub-areas in Sardinia over the past 40 years mostly by means of multiple assessments and common methodology (Figure 2). Prevalence and incidence rates are summarized in Tables 2 and 3.

Figure 2. Sardinian surveyed sub-areas for MS epidemiology

Table 2. MS prevalence rates (per 100,000) in Sardinia for the period 1941-1998

Area	Pop.	Prev. year	N. cases	MS classification	Crude rate (95%CIs)	Adj. rate	F:M ratio
North-central[102]	600,000	1941-61	100	-	16.7 (-)	-	-
Sardinia[103]	1,448,011	1964	239	McAlpine[124] (def and prob)	16.5 (-)	-	-
Sardinia[105]	1,473,800	1970s	288	Schumacher[125] (def)	15-34 (-)	-	1.3
Barbagia[106]	51,611	1975	21	Allison & Millar[126] Schumacher[125] (def and prob)	40.7 (25-62)	48.5 [a]	1.1
Alghero (health distr)[113]	77,981	1980	46	Rose[127] (def and prob)	59 (43.2-78.7)	-	2.1
Barbagia[108]	49,022	1981	32	Allison & Millar[126] Schumacher[125] (def and prob)	65.3 (44-93)	77.9 [b]	1.3
Macomer[111]	11,083	1981	10	Schumacher[125] (def)	90.2 (43-166)	-	1.5
Sassari[115]	124,588	1985	86	Rose[127] (def and prob)	69 (55.2-85.6)	-	2.7
North-west (sub-area)[116]	268,926	1991	276	Poser[84]	102.6 (91.5-115.0)	-	2.5
Nuoro (prov.) [109]	273,768	1993	394	Poser[84]	143.9 (130.4-158.9)	141.4 [b] 149.2 [c]	2.2
Nuoro (prov.) [110]	273,146	1994	415	Poser[84]	151.9 (137.6-167.7)	148.8 [b] 156.6 [c]	2.2
Sassari (prov.) [123]	454,904	1997	686	Poser[84]	144.4 (134-155.6) [d] 150.8 (-) [e] 149.7 (139.1-161.1)[f]	140.9 [g] 153.3 [h]	2.5
Nuoro (prov.)[65]	272,992	1998	428	Poser[84]	157 (143-173)	-	2.3

[a] 1975 Italian population
[b] 1981 Italian census population
[c] European population
[d] 1997 Sassari province population
[e] 1991 Sassari province census population
[f] onset-adjusted prevalence rate
[g] 1996 Italian population
[h] 1991 Italian census population

Table 3. Studies of MS incidence carried out in Sardinia during the past three decades

Area	Mean pop	Inc. interval	N. cases	MS classification	Crude rate (95%CIs)	Adjust. rate	F:M ratio
Barbagia[108]	52,885	1961-80	31	Allison and Millar[126] Schumacher[125] (def and prob)	2.9 (1.9-4.1)	3.2[a]	1.4
		1961-70	*15*		*2.7 (1.5-4.4)*		*1.1*
		1971-80	*16*		*3.1 (1.8-5.0)*		*1.7*
Macomer[111]	9,028	1912-81	13	Schumacher[125] (def)	4.8 (2.5-8.2)	-	1.6
		1957-61			*10.2 (-)*[b]		*3.0*
Alghero (health distr.)[113]	76,450	1971-80	31	Rose[127] (def and prob)	4.1 (2.7-5.7)	-	2.1
		1971-75	*10*		*2.6 (-)*		*-*
		1976-80	*21*		*5.4 (-)* [c]		*-*
Sassari[115]	111,598	1965-85	79	Rose[127]	3.4 (2.7-4.2)	-	2.7
		1965-75	*24*		*2.1 (1.3-3.1)*		
		1976-85	*55*		*4.6 (3.5-6.0)* [d]		
North-west[116]	268,926	1962-91	277	Poser[84]	5.7 (4.5-7.1)	-	2.5
		1962-66	*24*		*2.0 (1.3-3.0)*		*2.0*
		1987-91	*75*		*5.7 (4.5-7.1)*		*3.7*
Nuoro (prov.)[110]	273,248	1955-95	469	Poser[84]	4.2 (3.8-4.6)	4.3[e,f]	2.0
		1955-59	*26*		*2.0 (1.3-2.9)*		
		1985-89	*91*		*6.6 (5.3-8.1)*		
		1990-95	*104*		*6.4 (5.3-7.8)* [g]		
Sassari (prov.)[123]	431,670	1968-97	637	Poser[84]	4.9 (4.6-5.3)	-	2.5
		1968-72	*39*		*2.0 (1.4-2.7)*		*1.6*
		1993-97	*158*		*6.8 (5.8-7.9)* [h]		*2.2*

[a] 1971 Italian census population
[b] peak incidence
[c] Alghero Health District: significant difference between 1971-75 and 1976-80 (chi-square = 3.856, $p<0.05$)
[d] Sassari municipality: significant difference between 1965-75 and 1976-85 (chi-square = 11.25, $p<0.001$)
[e] 1981 Italian census population
[f] European population
[g] Nuoro province: significant difference during the last 6-year period considered (chi-square = 91.48, $p<0.001$)
[h] Sassari province: significant difference during the study period (chi-square, $p=0.02$)

The first epidemiological study of MS in Sardinia was published over 40 years ago.[102] This survey was based on 100 cases observed between 1941 and 1961at the Inst. of Clinical Neurology, University of Sassari, the referral health structure for northern and central Sardinia and covering a population of 600,000 ca. The *morbidity ratio*, i.e., the ratio between observed MS patients and the general population was 16.7 per 100,000, ranging from 13.3 in the coastal areas to 31.1 in mountainous areas (higher than 600 m), though not significant. Based on this study, investigations were extended to the entire island where an overall prevalence of 16.5 per 100,000 in 1964 was estimated[103] with significant differences in the rates among the three provinces (the province of Oristano had not yet been established): 11.6 in the province of Cagliari, 24.8 for Nuoro, and 15.9 for Sassari. These rates were likely to be grossly underestimated due to a long time lag between clinical onset and diagnosis at that time, expected to be 3- to 4-fold greater than the time interval between the prevalence and the study year. Furthermore, case collection was mostly one single source hospital-based. Confidence intervals were not reported so no true comparisons between rates could be made.

The first study on incidence in Sardinia refers to year 1971 and showed a rate of 12.5 per 100,000 per year, with a woman-man ratio of 1.8.[104] Mean age at onset was 27.3 years, lower than that found for other European and northern and central Italian populations at that time.

Of note, this was also the first study conducted by the current main epidemiological research group in Sardinia lead by Prof. G. Rosati and Prof. E. Granieri (University of Sassari and Ferrara, respectively), which incorporates most of this work co-authorship. Epidemiological data of Sardinian MS patients with onset since 1955 have been reviewed by these researchers that have since then shared same survey methodology.

Since early times there has been an interest in investigating spatial variation of MS in Sardinia, with special regards to altitude and climate. Higher rates were tendentially observed for interior mountain and hill areas as compared to coastal hill plain

areas.[105] A positive correlation of the distribution of MS with the distribution of rheumatic heart disease and post-streptococcal nephritis was described, which suggested that MS followed an infection similarly distributed to streptococcal infections.

In 1975 a prevalence of 40.7 per 100,000 and a mean age at onset of 28 years were reported in Barbagia, central Sardinia (50,000 pop. ca.).[106] This rate was higher than any other ever observed in Italy or southern Europe at that time, and was the first epidemiological evidence against the theory of a rate drop in the Mediterranean area.[107] The authors tended to exclude bias due to a change in the population structure between 1960 and 1980, and a reduction of at-risk general population due to emigration for socio-economic purposes. Subsequent surveys conducted in the same area showed that prevalence increased to 65.3 in 1981,[108] to 143.9 in 1993,[109] to 152 in 1994[110] and to 157 in 1998.[65] The latest three updates came from prevalence surveys conducted on the whole province of Nuoro, of which Barbagia is a core sub-area (20% of the province pop. circa). Mean annual incidence rates were stable around 3 per 100,000 for the time interval 1961-80,[108] but significantly increased up to 6.4 in 1990-95 for the whole province.[110] The authors could not explain such increase simply with improved case ascertainment and prolonged survival over time, but suggested that genetic susceptibility might account for absolute higher rates, and environmental factors for the temporal increasing trend.

An incidence study on secular MS trends was conducted in Macomer, central Sardinia in 1980s.[111] Intensive search for all MS cases occurring in this commune since 1912 indicated that MS was absent up to 1952, and that 13 cases had their clinical onset in the commune in the years 1952-81. During this period, the average annual incidence was 4.8 per 100,000, peaking in 1957-61 with 10.2 and slowly decreasing up to 1981. The author suggested an epidemic of MS in this ‘naïve’ population starting after 1945, hypothetically secondary to an influx of a high risk population from the mainland after the end of World War II. At that time similar

evidences were being reported for the Faroe Islands, where MS was hypothesised to have started after the British occupation during World War II.[112]

The health district of Alghero, north-western Sardinia was later investigated by means of population-based design.[113] The prevalence in 1980 was 59 per 100,000, and the mean annual incidence rate for the period 1971-80 was 4.1 per 100,000, with a significant increase from 2.6 in 1971-75 to 5.4 in 1976-80. Mean age at onset was 25.7 years. The study confirmed the high and increasing risk for MS in Sardinia, with special regards to the last 10 year-period during which 72% of the patients had their onset. The action of an exogenous (infectious) agent was hypothesised. A prevalence rate of 100 per 100,000 was also recorded in the town of Tempio in early 1990s.[114]

Same etiological agents were believed to determine MS in a study of the commune of Sassari, north-western Sardinia, where prevalence was 69 per 100,000 in 1985, and an increase in the mean annual incidence from 2.1 to 4.6 per 100,000 in 1965-75 and 1976-85, respectively, was observed.[115]

Later on, a population-based survey was conducted in the island north-western area (270,000 pop. ca) aimed at overcoming biases deriving from small population sizes.[116] The prevalence rate of 102.6 per 100,000 in 1991 was again the highest ever reported for Italy in comparable times. A gradual significant increase in mean annual incidence rates in the study time 1962-91 was observed up to 5.7 per 100,000 in the last quinquennium considered. The attempt to find sub-areas at higher disease occurrence failed to produce any evidence. In this paper, the possibility for case ascertainment bias due to improved diagnostic facilities and higher awareness of the disease was discussed. However, the comparison of Sardinian rates with those from areas with higher diagnostic standards and easier access to facilities pointed, at least partially, to a true rising risk of MS in Sardinians. In the meantime, immunogenetic studies conducted in Sardinia indicated a positive association with HLA-D4 and DQB1*0201 and *0302 alleles possibly conferring a genetically based susceptibility to MS and other immune mediated disorders such as juvenile diabetes (IDDM),[117-121]

which featured, among Sardinians, an epidemiological behaviour surprisingly similar to MS.[122]

The largest population-based survey was conducted in Sardinia in the province of Sassari (455,000 pop. ca.).[123] A prevalence of 144.4 in 1997 and an incidence of 6.8 in 1993-97 with a significant increase from 2.0 in 1968-72 were reported, close to parallel to what registered for the province of Nuoro with independent assessment but same methodology in comparable time.

Across these descriptive epidemiological surveys, different diagnostic criteria for MS were used from time to time, and namely the McAlpine's criteria[124] for definite and probable MS in Caruso et al,[103] the Schumacher Committee criteria[125] for clinically definite MS in Rosati et al,[105] the Schumacher Committee criteria[125] for definite and probable MS and Allison and Millar criteria[126]in Granieri and Rosati,[106] Granieri et al,[108] the Rose criteria[127] for definite and probable MS in Rosati et al,[113,115] and the Poser et al criteria[84] in Rosati et al,[116] Casetta et al,[117] Granieri et al,[110] and Pugliatti et al.[123]

For prevalence studies Poser et al criteria were used since prevalence year 1991. For incidence studies an overlap of different criteria for same time intervals occurred from different studies. In Rosati et al (1996) Poser criteria were used for the incidence interval 1962-91, this means that patients that had been included in previous studies and diagnosed prior to 1983 were reclassified from Rose criteria.

The geographical variation of MS prevalence in the province of Nuoro was investigated by means of Bayesian approach (see Section 1.4.1.) by Montomoli et al.[65] The Bayesian prevalence rates ranged from 143 to 262 per 100,000, with clustering being significant for four communes (Nuoro, Oliena, Fonni and Desulo). Based on the high number of multiplex families and the peculiar genetic asset of this population, the authors interpreted their findings as correlated to a higher susceptibility on a genetic basis.

Genetic epidemiology

The genetic hypothesis has been the focus of a study on the disease recurrence risk in siblings of MS patients in the same population.[128] A risk of 4.7% and a risk ratio of 31 to the general population were reported. MS recurrence risk was positively influenced by age at onset, partly by probands' female gender, and by an interaction between both factors. Based on the number and distribution of affected relatives in the family, the authors proposed a hereditary model for the disease transmission consisting of a single dominant gene with an extremely low penetrance. The same analysis of was then extended in the same population with regards to first-, second-, and third-degree relatives.[129] The study, conducted on 313 MS probands and 12,717 relatives, showed an overall age-adjusted recurrence risk in relatives of 1.90% (95%CIs, 1.57-2.30), 1.26% (95%CIs, 0.60-2.63) in parents, 2.33% (95%CIs, 0.09-5.56) in children, 4.76% (95%CI s, 3.57-6.32) in siblings, 0.72% (95%CIs, 0.42-1.22) in second-degree relatives, and 1.79% (95%CIs, 1.27-2.51) in third-degree relatives. The gender of the probands (male) and of the relatives (female), and the number of affected relatives in the family significantly increased the familial recurrence risk for MS. These observations are in line with what we observed in the population of the province of Sassari, based on 418 probands and 18,799 relatives (Pugliatti et al, unpublished data).

Familial aggregation and the contribution of genetic factors to familial clustering of MS patients were studied in a southern region of the island,[130] which lacks, however, systematic descriptive epidemiological data. Recurrence risk in siblings for 901 Sardinian patients and factors influencing risk (patients' and siblings' gender, patients' age at onset, siblings' birth cohort, and presence of affected relatives other than siblings) were examined. Further, the presence of distant familial relationships among patients was evaluated by tracing the extended pedigrees of all patients with MS born in one Sardinian village. Recurrence risk was 2.3-fold increased in siblings of index patients with onset age less than 30 years and 2.9-fold when having a relative with MS other than a sibling or parent. Pedigree analysis of patients from the village showed that all 11 patients descended from 3 pairs of ancestors, whereas no cases occurred in the remaining village population. The authors argued in favour of

MS familial aggregation in Sardinians being influenced by genetic factors and, by means of independent observations, that isolation and founder effect caused enrichment of "etiologic" MS genes.

The distinct HLA association of MS in Sardinia supports this interpretation. A number of molecular genetic studies have been conducted by different research groups, aimed at disclosing associations between HLA haplotypes or individual susceptibility genes with MS in this population.[22] Initially, association and linkage studies performed in independent datasets using the transmission disequilibrium test (TDT),[131] demonstrated Sardinian MS in linkage disequilibrium with two different DRB1 alleles: DRB1*0301 (DR3) and DRB1*0405 (DR4).[118,132] On the contrary, the DRB1*1601, a DR2 allele different from the DRB1*1501-DR2 which confers MS susceptibility in Caucasian, was associated with a low risk of developing MS.[120] The analysis of allelic variation at candidate loci confirmed MS association with both the Sardinian-specific DRB1*0405-DQA1*0501-DQB1*0301 and DRB1*0301-DQA1*0501-DQB1*0201 haplotypes.[133] However, a comparative analysis of the DRB1, DQA1 and DQB1 could not identify individual loci explaining MS predisposition. Other HLA gene(s), in linkage disequilibrium with the 'Sardinian' HLA-DRB1-DQA1-DQB1 haplotype, such as IFNγ gene polymorphisms, might be primarily or co-responsible for genetic susceptibility to the disease.[134] Subsequent immunogenetic studies indicated that at least part of the HLA-based MS susceptibility in DR3+ Sardinian MS patients was supported by an impaired IL-10 production from mononuclear cells.[135] MS predisposition in DR4+ MS patients might depend on an abnormally high level of TNFα production.[135,136]

More recently, full genome searches on large numbers of affected sibling pairs have been conducted at multiregional level, including Sardinia.[137] Initial results only addressed to regions of common interest in MS susceptibility located on chromosome 6, within the HLA region, chromosomes 3, 18 and 17, but later meta-analyses disproved such results.

Environmental epidemiology

Recent studies have been conducted by our research group aimed at investigating the role on MS-associated retrovirus (MSRV) in Sardinian patients as compared to Sardinian healthy controls.[138,139] MSRV is an exogenous member of the HERV-W family that has been found in plasma and CSF of many patients with MS.[140] The extracellular form of MSRV was detectable in 100% of plasma samples from active, untreated MS patients and in 12% of healthy controls from Sardinia. Over an observational period of 3 years, patients with MSRV-free CSF had a stable MS course, whereas those with MSRV+ CSF disclosed a more severe, treatment-requiring disease, suggesting the prognostic value of MSRV in the CSF at onset.[134,141] However, since titers of MSRV were found to be higher in other inflammatory as compared to other non inflammatory central nervous system diseases, a strict etiologic role in MS can be ruled out.[138]

1.7. Perceived health status and mild MS.

In the past 30 years, the interest in the concept of quality of life has increased significantly, both in research and clinical practice. Based on their application and the main objectives, the studies on MS quality of life may be classified into three categories:[142] (i) evaluating the development and validity of quality of life questionnaires and clinical scales, (ii) evaluating determinants of quality of life or comparing the quality of life among various groups, and (iii) using quality of life as outcome measures in clinical trials and other interventions.[142-144] Quality of life as outcome measure is especially indicated for patients with chronic disorders for whom other more clinical and biological parameters could be less informative to judge interventions efficacy.

The increasing interest in quality of life in health care is explained by (i) the increased life expectancy resulting from improved medical therapies with an impact on health outcomes such as morbidity and mortality, and (ii) the proliferation and advancement of medical and surgical technologies and subsequent necessary consideration of the benefit-burden or -cost ratio of equivalent therapies.[144] Today,

research on quality of life can affect how policy makers allocate health care resources or determine reimbursement policies.

Despite the increasing interest in this topic, consensus is lacking on the definition of quality of life and on whether it is measurable or not, making it an ambiguous concept. Quality of life is used as an *umbrella* term,[145] covering a variety of concepts, such as functioning, health status, perceptions, life conditions, behaviour, happiness, lifestyle, symptoms, etc. Several concept analyses today suggest that quality of life ought to be defined in terms of life satisfaction.[144,146-148] A wide spectrum of quality of life definitions exists in the literature, which can be grouped into the following broad categories: (1) normal life, (2) utility, (3) social utility, (4) happiness/affect, (5) satisfaction with life, (6) satisfaction with specific domains, (7) achievement of personal goals, and (8) natural capacities.[144]

'Quality of life', 'health status' and 'functional status' are often used interchangeably under the assumption that a fully health life is identical to a high quality life.[144] It is not so, and quality of life and health status are two different concepts, as shown with the "disability paradox": physically disabled persons unexpectedly experience a good quality of life, although most observers assume that these people have an undesirable life.[149] The interchangeable use of quality of life, health and functional status is reflected in the measurement of these concepts, such as the 36-Item Short Form Health Survey (SF-36),[150] which is erroneously used to measure quality of life, or 'health-related quality of life', because it truly measures individuals' perceived health status.[151] Furthermore, while measuring health status reflects *objective* dimensions, physical functioning and observable life conditions, quality of life should be a purely subjective experience, determined by one's subjective appraisal of one's life conditions. Objectively measured indicators of living conditions were found to account for 15% of an individuals' quality of life,[152] which therefore has more to do with subjective well-being and life satisfaction.

Another important distinction in quality of life studies is between indicators and determinants.[144] Indicators are events or conditions that typically characterise a

specific situation, whereas determinants are the elements that determine the nature of the situation itself. Some factors, such as employment status, can be both indicators and determinants in quality of life research, depending on *a priori* assumptions. In this respect, 'health status' should be considered one of the determinants of quality of life in that it contributes or influences it, and not an indicator.

Quality of life is not a static concept. It changes due to age-related perspectives, life events, changes in values and priorities, and coping abilities. Personality and "sense of coherence" also influence it.[153] Nevertheless, it is unlikely to be a very highly dynamic concept and its daily fluctuations are probably not relevant within one period of time.

Health is always considered a very important aspect of quality of life. 'Health-related quality of life' has been developed to describe aspects of individual's subjective experience related to his/her health status, disease and disability, within the World Health Organization (WHO) definition of health, as '*a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity*'.[154] Focusing on health-related quality of life, researchers end up with overestimating the impact of the health status in quality of life and underestimating other aspects of life. As already stated, measuring health-related quality of life is often actually measuring patients' perceived health status.[151]

MS patients score lower in health-related quality of life than do patients with other chronic and disabling conditions such as epilepsy, diabetes, rheumatoid arthritis or inflammatory bowel disease.[155-156] Nortvedt et al[143] conducted a study aimed at describing the perceived burden of MS using a structured quality of life instrument in a Norwegian MS population-based sample and comparing these scores with those in a general population. The patients showed markedly and significantly lower mean scores for all perceived health dimensions measured with SF-36 compared with age- and gender-matched general population. This difference was especially high for physical functioning, general health, role limitation of physical functioning, vitality, and social functioning. They also showed that the EDSS score correlated significantly

and negatively with all SF-36 health dimensions except bodily pain, the highest coefficients being observed for physical functioning, social functioning, and general health. The EDSS could explain 73% of the variation in physical functioning, 23% in social functioning, and 21% in general health, and little for the other dimensions.

In clinical practice, the strategies aimed at improving MS patients' health-related quality of life most frequently focus on patients with greater disability. Health professionals perceive more severe physical impairment as a determinant of patients' poorer quality of life,[157] whereas physical functioning among non or mildly disabled MS patients is not usually matter for concern.

The rating scales commonly used to measure physical impairment, such as the Expanded Disability Status Scale (EDSS),[158] almost exclusively reflect health professionals' *objective* assessment. The patients' self-perception of their health status - what ultimately counts in quality of life - can be overlooked, particularly among those with no or little physical impairment.

The studies aimed at investigating health-related quality of life and self-perceived health status among MS patients have mostly been conducted on patients with a wide range of disability (i.e., including severely physically impaired patients).[142] Despite a growing interest in MS early stage, i.e., in the benign disease and clinically isolated syndromes (CIS), little focus has so far been given on health-related quality of life and self-perceived health status in non or mildly disabled MS patients, as defined from objective measurement.

2. AIMS OF THE STUDY

The present work builds up based on two broad questions stemming from the background epidemiological research on MS discussed above.

The first issue regards the relevance to use epidemiological descriptive data to disclose (i) clues to disease etiology and (ii) to interactions between causative agents and the host by attempting at tracing the individuals' age at which these mechanisms (disease induction) occur.

The second area of interest is the characterisation of health-related quality of life for the MS population at large, thus including no or low disabled patients, in order to provide more realistic data for implementing cost-of-illness studies and *ad hoc* health policies.

These two questions have constituted the frame for this work specific objectives, which have been individually dealt with in the articles:

I. To disclose -and characterise -significant prevalence variation at a microgeographic level, possible expression of spatial variation in the distribution of disease risk factor(s);

II. To update incidence trends of MS in northern Sardinia, and disclose different patterns by time periods, gender, initial clinical course, age of onset and sub-areas of residence, as a possible expression of temporal variation in the distribution of disease risk factor(s);

III. To assess whether individuals, that later developed MS, during the disease pre-onset history had been significantly closer to each other in time and space than expected by chance, and shared putative common exposure(s) while residing in the province of Sassari; if so, at what age was such exposure most likely to have occurred;

IV. To investigate the self-perceived health status, with special regards to physical functioning, in Sardinian MS patients with no or mild disability at objective neurological examination, and as compared to the general population.

3. MATERIALS AND METHODS

An overview of the materials and methods follows. Further details are found in each article.

3.1. Epidemiological data (Paper I-III)

3.1.1 Study area

All the four studies were conducted on the population of the province of Sassari, northern Sardinia, insular Italy. A summary of main geo-climatic and demographic features is reported in Table 4.

Table 4. The province of Sassari, northern Sardinia: summary of main geo-climatic, demographic and ethnic main features.

Area	7,520 kmsq
Latitude	Between 40°30'and 41° N
Geo-climatic areas: mean annual temperature (min-max)	Coastal area: 16.5° (13.7°-20.5°)
	Interior plain area: 15.9° (9.5°-23.7°)
	Interior hill area: 15.8° (12°-19.1°)
	Interior mountainous area: 12° (7.5°-16.7°).
N. communes	90
Population	453,628 in 2001 national census
	381,191 in 1971 national census
Migration inflow	1.8% in 1997
Migration outflow	1.6% in 1997
Geographical sub-areas[100]	Sassarese, Gallurese, Northern Logudorese, Eastern Logudorese, Southern Logudorese, Goceano and Algherese (Catalan)

Migration flow in this area is 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.[159] Immigration to the study area is mostly from other Sardinian provinces, so the study population almost completely consists of native individuals and a stable ethnic composition. The low migration flow over time has led to the differentiation and isolation of seven geographic areas with internal homogeneous ethnic composition (peculiar historical, linguistic and cultural patterns).[160,161] A slightly different classification[100] of such sub-areas was used in **Paper II** and **Paper III** as compared to **Paper I**. [162] In order to avoid ambiguity, we shall hereafter refer to the classification by Contini[100] as follows: Sassarese (1), Gallurese (2), Northern Logudorese (3), Eastern Logudorese (4), Southern Logudorese (5), Goceano (6) and Algherese or Catalan (22) sub-areas (Figure 3). The same sub-areas indicated by numbers correspond to the following denomination as from **Paper I**: Sassarese (1), Gallurese (2), Northern Logudorese (3), the eastern area of Common Logudorese and southern Gallurese (4), Common Logudorese (5), Eastern Logudorese (6), and Algherese-Catalan (22).

All these domains share the same historico-cultural Sardinian heritage, with the exception of the Algherese and the Gallurese sub-areas, due to Catalan, and mainland Italian and Corsican influence, respectively.

Figure 3. Historical, linguistic and ethnic domains in the province of Sassari, northern Sardinia

Sub-area	Paper I[162]	Papers II and III[100]
1	Sassarese	Sassarese
2	Gallurese	Gallurese
3	Northern Logudorese	Northern Logudorese
4	Common Logudorese/Gallurese	Eastern Logudorese
5	Common Logudorese	Southern Logudorese
6	Eastern Logudorese	Goceano
22	Algherese (Catalan)	Algherese (Catalan)

NB: the Contini classification[100] has been considered throughout the text

Endogamy has played a role in most inner communities,[163] and at a microgeographic level, the social structure varies from close communities at high inbreeding rate (southern province), to more open communities. A more archaic social structure in the innermost regions of Sardinia has also been demonstrated with population-based studies conducted on the frequency of mtDNA haplotypes.[164]

Consanguinity has involved Sardinians rather heterogeneously over time and space and can be a determinant of clustering of both environmental and genetic factors at a microgeographic level. Geomorphologic, historical and cultural factors have also influenced the frequency and distribution of close communities. Community isolation has been reported to be directly proportional to the altitude above sea level and the distance from the coastline.[163] In northern Sardinia it is higher than in the south, and has especially involved the innermost sub-areas of the province of Sassari

For **Paper I**, the 1997 population of the province of Sassari, consisting of 460,135 individuals (227,215 men and 232,920 women) was computed as an interpolation from the 1991 census data for the province[165] and was used as denominator for prevalence rates. Age- and gender-specific populations for each commune were used as denominators for specific rates. Based on the 1991 census, the study area comprised 89 communes. Each commune was coded according to the Italian Central Institute of Statistics Coding.[166]

For **Papers II** and **III**, the province 2001 census population was used as denominator which consisted of 453,628 (222,191 men and 231,437 women).[167] Based on the 2001 census, the study area comprised 90 communes.

3.1.2. Case ascertainment and data collection

The University of Sassari is the first university institution in Sardinia started in the 16th century. The level of medical organization within the study area public health care system is relatively high, and the University Hospitals of Sassari have long represented the referral health care structure for central and northern Sardinia. The level of health care has improved during the study period parallel to a general

improvement achieved at national level, as far as quality of diagnostics and access to medical facilities and neurological services are concerned. CSF analyses and MRI became available for routinely diagnostics for patients admitted to hospital at the beginning of 1990s. The Regional Centre for the Diagnosis and Treatment of MS (*Centro Regionale per la Diagnosi e Cura della Sclerosi Multipla*) – hereafter referred to as "Sassari MS Centre" - was established by the Sardinian regional government at the University Dept. of Clinical Neurology in 1989. In 1996 the Sassari MS Centre was commissioned to authorise the prescription of immunomodulatory treatments (interferons-beta and glatiramer acetate) for all other regional MS Centres or neurological health care providers.

Given its expertise and diagnostic facilities, virtually all cases of suspected MS refer to the Sassari MS Centre from northern and central Sardinia (800,000 pop. ca. coverage), and undergo standardised diagnostic procedures including CSF analysis, evoked potentials, and MRI.

For research and patients general management purposes, a Register of MS cases was started at the Dept. of Clinical Neurology since 1995. Its characteristics are reported in Table 5.

Table 5. Sassari province MS case Register: summary of main demographic and clinical features*

Beginning of systematic case collection:	1970s for province sub-areas 1995 for the whole province
Survey methodology	Population-based "The spider method"[168]
Location	Sassari MS Centre, Dept. Clinical Neurology, University of Sassari, Sardinia, Italy
Epidemiological sources	- Depts. of Clinical Neurology, University of Sassari (medical records, evoked potentials, CSF and immunomodulatory drugs lists) - Other University Hospitals of Sassari (Ophthalmology, Child Neurology, Radiology) - Other Neurological Depts. (Hospitals of Ozieri, Olbia, province of Nuoro) - Cagliari MS Centre - National MS Society - Centres for Motor Rehabilitation - Neurologists in private practice - General practitioners in the province of Sassari - Extraregional MS Centres (Gallarate General Hospital, Don Gnocchi Foundation in Milan) - Official death certificates
Total n. cases included	1221 (352 men and 869 women)
Distribution	Province of Sassari 1073 (87.9%) Other Sardinian provinces 70 (5.7%) Other 78 (6.4%)
N. deceased	76 (34 men, 42 women) (1938-2004)
Mean age at death (SD)	Men: 53.4 (13.1) years Women: 52.1 (13.5) years
Mean current age (SD) (N=1209)	Men: 49.5 (15.7) years Women: 47.9 (13.9) years
MS classification[84] (N=745)	CDMS 639 (85.8%) LSDMS 21 (2.8%) CPMS 77 (10.3%) LSPMS 8 (1.1%)
Information collected	Population-based: - demographic (gender, place/date of birth, changes of residence since birth) - clinical (date of onset and diagnosis, onset symptoms, disease classification, disease course, degree of disability, immunoprophylactic treatments, comorbidity) Sub-studies: - familial occurrence of MS and other immune mediated

	disorders - environmental exposure history - education - socio-demographic - health-related quality of life

* as to August 2006

The type of survey methodology used to find MS cases has been described as the *spider method*.[168] In this model, MS patients are not sought by investigators, but rather they are caught in a network of local health operators displayed and normally practising throughout the study area who refer them or notify them to one main central health care provider where registry occurs periodically. Our Dept. of Clinical Neurology and the MS case Register represent such referral structure receiving notifications by other peripheral sources in the territory (Table 5). The repeated case ascertainments carried out in the province of Sassari prior to the present work[113,115,116,123] have largely contributed better accuracy to the Register.

Patients were included in the Register with a diagnosis of MS according to the Poser et al. criteria[84] for clinical or laboratory supported definite (CDMS, LSDMS) and probable (CPMS, LSPMS) MS. For **Paper I**, 87.6%, 2.7%, 8.4%, and 1.2% of the patients were classified as having CDMS, LSDMS, CPMS and LSPMS, respectively. In addition to fulfilling the Poser criteria, after 1986 diagnostic MRI was performed in 92% of patients. Prior to that date, diagnosis was almost exclusively based on clinical and paraclinical evidence, and for 78% of cases on the CSF immunological study. Other autoimmune, immunomediated and infectious diseases such as primary and secondary central nervous system vasculitides, post-infectious leukoencephalopathies and other demyelinating disorders were ruled out with support of laboratory tests and neuroimaging.

3.1.3. Study designs

In **Paper I,** to disclose geographical variation of the disease in the province of Sassari, a hierarchical Bayesian approach was applied to a spatial cluster analysis of the distribution of 1997 prevalence rates in each commune. The theory behind this type of analysis has been reported in detail in Section 1.4.1. A prevalent case was defined any individual who resided in the province of Sassari on prevalence day Dec. 31st 1997. Although spatial cluster analysis is not primarily intended for investigating on the disease latent period, an attempt at disclosing spatial clusters of disease at individuals' age of 5-15 years was performed, by mapping the distribution of all registered cases by commune of residence at that age.

The study designs adopted for **Paper II** and **III** were incidence-based. Incidence interval spanned from 1965 and 1999. An incident case was defined as any individual who first experienced symptoms later related to MS[32] while residing in the province of Sassari within the incidence interval considered. With special regards to **Paper II**, incidence rates by time periods, gender, initial clinical course, age of onset and province sub-areas of residence (Figure 3) were analysed. The initial clinical course was retrospectively categorised into relapsing remitting (RR) course or progressive course.

In **Paper III**, to investigate whether individuals that later developed MS had shared exposures to putative causative agents concentrated in the same area and over the same time span, and at which age such exposure might have occurred, a space-time cluster analysis of MS in the province of Sassari, was conducted based on incident cases. The theory behind this method was described in details in Section 1.4.3. Changes in residences by commune from birth to MS onset were collected from the Register. Patients within the same birth cohort were tested for having lived significantly closer to each other than expected[169] and in relation to clinical features, such as disease course and age at onset.[170] Time closeness interval for each pair was arbitrarily chosen at 1, 2 and 5 years. The statistical model applied for communes was also applied to sub-areas (Figure 3).

In order to disclose whether space-time clustering depended on demographic and clinical features, the analysis was run in groups of patients stratified by gender, age at onset (less than versus 30 years or above), initial course of the disease (RR or progressive), cohorts by birth and clinical onset and sub-areas.

3.1.4. Statistical analysis

For **Paper I**, the first step was calculating the area age- and gender-specific prevalence rates (expressed as the number of cases per 100,000 population) using the 1997 population and mapping them for each commune of the province of Sassari. In order to remove possible biases due to different age– and gender-structures for different areas, standardised rates were calculated by the direct method of adjustment using the same standard population and assuming an equal number in each age group.[171] Since the area-specific number of cases is small, traditional statistical methods tend to yield very extreme rates due to the strong influence of random variation. To overcome this problem, a hierarchical Bayesian approach was adopted which eliminates extreme values from the map and yields smoothed estimates of disease rates (see Section 1.4.1). This method best reflects the true geographical risk variation which is epidemiologically more interpretable. Since the data set consists of prevalence rates, the binomial model was assumed to obtain the Bayesian estimates via Gibbs sampling[172-175] on BUGS software.[176] In order to explain the amount of variation of the true prevalence rates in the map, a combination of degree of freedom and scale factor was chosen for the prior distribution of the hyperparameter.[66,70] A sensitivity analysis allowed us to choose the values of ten as degrees of freedom and two as scale factor for this work. The reference value used to produce the PP of a prevalence rate was the median value of generated samples.

The model was fitted in order to map the area specific prevalence rates for patients residing in the study area both on prevalence day and during the age 5 to 15. Because of the conspicuous number of areas containing zero cases among men, the gender-specific Bayesian approach was applied only to women. Three separated chains starting from different initial values were run for each model: total (i.e., both

genders), women, and total 5-15. The Bayesian prevalence estimates and tests were obtained after convergence of the hyperparameter via the Gibbs sampler, discarding the first 1,000 iterations of each run as burn-in or pre-convergence samples. Convergence at 10,000 iterations was checked by visual examination of sample traces by Geweke's diagnostic[177] implemented in the CODA software.[178]

For **Paper II** incidence rates were calculated using data from the census in 1971, 1981, 1991 and 2001. Age standardization was computed by adjusting for the general population of Italy as of the 2001 census.[179] The time period of onset 1965–99 was divided into seven 5-year intervals. A chi-square test was applied to test any difference in incidence over these 5-year intervals between gender, initial clinical course, symptoms and areas of onset.

For **Paper III**, the Knox's method was used,[180] in which all pairs of patients are defined as close or not close in time according to a cut-off point in number of years, and close or not close in space according to some geographical cut-off point. The interval for defining temporal closeness for each pair of patients was arbitrarily chosen at 1, 2 and 5 years: for 1-year closeness, patients were considered to be temporally close if they were born the same year, the year before or the year after. Spatial closeness was defined as residing in the same commune.

The observed number of pairs of patients close in both time and space was compared with the expected number calculated according to a normal two-by-two table (Table 6), where A represents the number of pairs close in both time and space, B represents pairs close in space but not in time and C pairs close in time but not in space.[54] The ratio between observed and expected close pairs was calculated, and values above unity indicated an excess of clustering.

Table 6. Two-by-two table for space-time cluster analysis[180]

		Distance between pairs of patients in ***space***		
Distance between pairs of patients in ***time***		Close	Not close	
	Close	A	C	A+C
	Not close	B	D	B+D
		A+B	C+D	A+B+C+D

Expected (E) number of pairs close in time and space: E = (A+B)*(A+C)/ [n(n-1)]/2
Observed number of pairs close in time and space = A. Observed-to-expected ratio = E/A

The statistical significance of deviation from unity for this ratio is often calculated based on an assumption of a Poisson distribution of the observed number of pairs close, *A*. However, since there is structural dependency in this table caused by each patient participating in (*n-1*) pairs, the assumption of a Poisson distribution does not hold. Because many test statistics do not have a standard asymptotic distribution, or if they do, it may not be reliable in realistic sample sizes and may be too computationally exhaustive to calculate the exact sampling distribution through exhaustive enumeration of all possible samples, the Monte Carlo empirical *p*-values are calculated. These approximate the exact *p*-value without relying on asymptotic distributional theory or exhaustive enumeration. Simulation studies have shown that using the Poisson distribution produces too optimistic *p*-values (Riise, personal communication). Therefore, empirical *p*-values were in this study calculated using 100,000 Monte Carlo simulations by randomly assigning the municipalities of

residence to the patients.[181] The empirical distribution of the ratio between observed and expected close pairs was then used to estimate the *p* value for the actual ratio.

The analysis included 649 MS patients with information on changes of commune of residence until at least age 15 years. This yielded 210,276 pairs [n(n – 1)/2 = 649*648/2] for statistical analysis.

In order to find the age of highest clustering the analyses were repeated using the residing commune each year from year of birth until age 25 years (or onset if before this age). Secondly, to reveal a fixed latency induction period, closeness in time and space was analysed for each year from the year of clinical onset backwards to the year of birth. In this case, a cluster meant an excessive number of pairs of patients had lived in the same commune during a time period corresponding to a fixed number of years prior to the onset for these patients. Cases living outside the province of Sassari at the specific age under study were excluded at this age.

At the age of most clustering, 1 year, we also performed space-time cluster analysis in subgroups according to gender and clinical characteristics: age of clinical onset (≤30 versus >30 years), type of initial clinical course (RR versus progressive), birth year (≤1959 and >1959, where 1959 was the median of the total distribution of years of birth) and geographic sub-areas. Two large sub-areas were defined according to similar environmental and ethnic features: a western sub-area including areas 1, 3 and 22 and an eastern sub-area including areas 2, 4, 5 and 6. The statistical significance for subanalyses was calculated using Monte Carlo simulations as described above.

To examine whether the clustering was related to age at onset, we compared the mean age at onset in the group of clustered cases (patients participating in at least one close pair) and the group of non-cluster cases (patients not participating in any close pair). The difference was tested using a *t*-test.

For **Paper II** and **Paper III**, the SPSS for Windows version 13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for all analyses.

3.2. Self-perceived health status in MS (Paper IV)

3.2.1. Study population

For **Paper IV**, the patients were recruited through the MS Register among those that had consecutively referred to the Centre for clinical follow-up from Jan.1st to Dec. 31st, 2004. Inclusion criteria comprised fully ambulatory status (EDSS score 3.5 or less)[158] and MS clinical onset[32] between 1965 and 2004. Exclusion criteria were comorbidity (chronic disorders) and an exacerbating phase of the disease within the previous 3 months. Clinical information, including EDSS scores and disease course was purposely updated during a neurological examination performed at the time of the study. The disease course was categorized into three classes: RR, relapsing-progressive/secondary progressive and primary progressive.[182]

3.2.2. Measures

The EDSS was used to assess disability. In 1955 Kurtzke described the Disability Status Scale (DSS), a new scale for evaluating disability in MS especially devised as an outcome measure in clinical trials.[183] The DSS had 10 grades from 0 (normal) to status 10 (death due to MS). The scale was intended to measure the maximal function of each patient as limited by neurologic deficits. It was based only on objectively verifiable deficits due to MS assessed with neurologic examination were included, so symptoms were discarded. The final DSS score was based on the scores by Functional Systems (FS) which included the pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral or mental, and other or miscellaneous functions. The FS were mutually exclusive in terms of neuroanatomy, but together comprised all neurologic abnormalities on examination that could be attributed to MS lesions. The FS were not additive, i.e., each FS could be compared only with itself over time. For this reason DSS scores had to be used for overall comparison of the same patient at different examinations.

In 1983 the Expanded DSS (EDSS) (Annex I) replaced the DSS, based on the believed poor sensitivity of the DSS to changes in the middle ranges. The relative weight of FS in EDSS became even greater than for DSS.

The patients' self-perceived mental and physical health status was measured using the SF-36 (Annex II).[150,184] SF-36 is a generic health survey measure assessing the most relevant health domains to both diseased and healthy individuals. SF-36 was purposely chosen as it allows comparisons between MS patients and a reference population by virtue of its construct, psychometric properties and external validity (robustness and generalisability).[184,185] The SF-36 is among the most widely used rating scales for measuring self-perceived health status among MS patients.[142] The SF-36 explores eight main domains: physical functioning, role limitations due to physical health problems (role–physical), bodily pain, general health, vitality, social functioning, role limitations due to emotional health problems (role–emotional) and mental health. The first four subscales primarily measure physical health and the last four primarily measure mental health. The general health and vitality subscales are sensitive to both physical and mental health.

Normative SF-36 data by gender and age are available for the general population of Italy.[186,187] The normative sample was studied in 1995 and consisted of 2031 individuals, 1032 (50.8%) women and 999 (49.2%) men with a mean age of 47.7 years. The sample is representative of the general population, with 37% of respondents residing in southern Italy. Detailed sampling procedures and further sample features are reported in **Paper IV**, and in Apolone and Mosconi.[187]

3.2.3. Procedures

The SF-36 Italian standard version[187] was administered to the patients at the hospital setting. The questionnaire content was outlined to the patients in a standardised way. Cognitive impairment can also be detected in mildly disabled MS patients.[188,189] To overcome potential interference with reliable responses to questionnaires, the Raven Coloured Progressive Matrices (RCPM) test[190] was used to screen for major

intellectual deficits. RCPM is a nonverbal intelligence test based on perceptual ability and visuospatial reasoning. RCPM raw scores were age-adjusted according to normative data (Raven et al, 1998).[190] MS patients with an RCPM score corresponding to an intelligence quotient (IQ) of 69 or higher[190] were included in the study.

3.2.4. Statistical analysis

The SF-36 scores and substitutions for missing values were calculated according to standardised procedures described elsewhere.[184] Scores were transformed to a 0 (minimum) to 100 (maximum) scales. The statistical analysis was performed only for the domains with 50% or more complete items and after substituting missing values. The single subscale scores and not the composite ones were analysed due to their better psychometric properties. The analysis was based on 35 items, as the transitional health status item was not included.

SF-36 subscale scores were then standardised to the general population of Italy, and *z* scores were calculated for each subscale using the mean (SD) of the age and gender-specific reference norms. These scores were then rescaled to a mean (SD) of 50 (10), which was therefore the average score for the general population of Italy on any subscale. These standardised scores were then compared between the MS patients and general population. One-sample *t*-tests were used in comparing the standardised subscale scores for the MS population with the data from the general population.

The association between EDSS and the physical functioning subscale was estimated using Pearson correlation coefficients. The impact of physical functioning on self-perceived health status was assessed by estimating the regression coefficients using physical functioning as an independent variable and each of the other subscales as a dependent variable in separate regression analyses. The regression coefficients between the physical functioning subscale and the other SF-36 subscales were estimated for the MS patients and the general population and also by gender. Significant differences in these regression coefficients between the subgroups were

tested using univariate analysis of covariance (ANCOVA) with each SF-36 subscale score as dependent variable, group (MS patients and the general population) and gender as a fixed factors and the physical functioning subscale as covariate. A significant interaction effect between the physical functioning subscale and group status in this model was interpreted as a significant difference in the corresponding regression coefficients.

Significance was set at $p < 0.05$, two-tailed tests. The SPSS for Windows version 13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for all analyses. For ANCOVA the general linear model procedure was used.

3.3. Ethics – Protection of privacy

For **Papers I-IV**, approval from the appointed local ethics committee was received after reviewing the projects aims, outlining and the *ad hoc* formulated forms to collect patients' informed consent. Special consideration deserved the ethical procedures for the study on self-perceived health status (**Paper IV**). Before entering data into the statistical programs, patients' first and family names were transformed into numeric codes.

4. SUMMARY OF THE RESULTS

The detailed results are presented in each individual paper.

4.1. Evidence of spatial clusters of MS in northern Sardinia (Paper I)

On prevalence day Dec. 31st, 1997 and for each commune we calculated the crude MS prevalence rates (per 100,000) with the 95% confidence interval (95%CI) obtained by MLE, and the respective rate estimates (per 100,000) with the 95% credible interval (95%cI) obtained by Bayesian approach (Table 7).

Table 7. Crude rates of MS total prevalence (P) per 100,000 by maximum likelihood estimate (ML) and rate estimates by Bayesian approaches. 95%CI: confidence interval; 95%cI: credible interval.

	MLE			Bayesian				MLE			Bayesian		
Commune code	P	95%CI		P	95%cI		Commune code	P	95%CI		P	95%cI	
1	342.5	153.9	760.1	139.3	115.4	167.1	46	98.5	31.8	304.8	143.7	122.6	166.5
2	100.6	25.2	401.3	124.9	101.6	150.9	47	98.1	72.8	132.3	119.0	100.2	138.2
3	141.3	109.0	183.1	147.7	127.3	170.2	48	70.9	17.7	283.1	150.5	122.0	181.8
4	109.3	15.4	771.6	145.3	117.2	179.2	49	207.6	103.9	414.6	138.4	117.7	161.7
5	0.0	-	-	147.8	124.9	173.1	50	134.9	56.2	323.7	149.5	126.4	171.0
6	69.0	32.9	144.6	125.2	106.0	148.1	51	186.4	103.3	336.3	156.7	134.4	182.2
7	0.0	-	-	151.3	125.9	180.9	52	231.0	158.5	336.7	147.6	128.4	169.5
8	219.5	91.4	526.2	144.7	116.4	175.6	53	0.0	-	-	148.2	120.4	179.6
9	150.6	62.7	361.3	130.9	107.8	157.8	54	180.7	81.2	401.6	131.1	108.4	158.3
10	403.2	100.9	1597.5	151.4	128.2	176.7	55	270.9	145.8	502.7	143.0	121.2	166.5
11	84.5	11.9	597.5	149.4	124.0	174.3	56	235.6	105.9	523.3	141.0	120.5	164.7
12	126.4	52.6	303.4	144.9	120.3	173.5	57	183.1	95.3	351.5	149.6	126.7	174.3
13	208.3	108.4	399.9	147.9	126.0	171.0	58	79.5	49.4	127.8	132.7	107.1	161.0
14	105.7	14.9	746.4	137.8	115.1	163.0	59	254.5	127.3	508.1	151.4	122.4	187.8
15	0.0	-	-	149.5	122.7	178.6	60	275.9	69.0	1096.1	152.1	127.6	183.8
16	117.1	16.5	826.3	143.6	116.2	173.5	61	151.1	21.3	1064.2	149.4	122.1	181.6
17	15.7	2.2	111.0	126.3	106.2	148.6	62	91.4	12.9	645.9	136.9	116.7	159.6
18	154.7	38.7	616.3	143.5	120.0	169.4	63	72.3	23.3	224.0	132.3	106.9	162.3
19	0.0	-	-	139.0	112.5	168.8	64	199.3	175.7	226.1	158.6	142.4	175.3
20	90.3	12.7	638.4	142.7	108.7	184.6	65	65.8	9.3	465.5	138.5	116.5	162.8
21	190.1	98.9	365.0	129.4	107.4	154.3	66	0.0	-	-	149.7	120.5	186.7
22	154.3	21.7	1087.0	153.4	128.2	182.0	67	134.8	72.5	250.3	146.6	122.1	172.4
23	37.6	9.4	150.3	135.3	110.4	163.9	68	180.5	45.1	718.8	150.4	127.8	174.4
24	193.4	27.2	1359.7	149.7	120.6	181.8	69	91.8	53.3	158.0	140.2	117.0	165.7
25	153.2	49.4	474.0	145.0	124.4	168.0	70	222.2	156.3	315.8	136.9	119.0	156.5
26	304.2	114.2	807.6	152.2	127.5	182.0	71	180.2	81.0	400.6	150.6	128.4	175.1
27	194.7	48.7	775.2	149.4	124.8	175.5	72	413.0	197.0	863.7	162.5	134.5	195.1
28	0.0	-	-	141.9	109.2	180.5	73	92.8	13.1	655.5	148.7	124.5	177.8
29	124.7	31.2	497.1	153.0	127.9	178.8	74	199.3	74.8	529.8	137.8	110.8	167.5
30	273.2	68.3	1085.7	149.5	125.2	178.9	75	59.6	8.4	422.0	140.2	116.8	164.9
31	0.0	-	-	143.1	114.5	179.7	76	129.2	48.5	343.7	153.9	131.7	180.4
32	335.0	83.8	1329.3	149.0	120.3	180.2	77	131.5	54.7	315.5	156.6	131.3	181.5
33	172.5	105.7	281.5	153.6	133.6	176.4	78	37.3	5.3	264.6	148.6	124.1	174.0
34	92.1	13.0	650.6	140.9	116.1	168.4	79	54.1	13.5	215.9	134.4	109.4	160.7
35	117.1	68.0	201.5	126.3	99.7	157.2	80	99.0	24.8	395.0	124.0	100.2	150.5
36	159.4	51.4	493.0	132.9	109.3	161.9	81	54.0	7.6	382.1	134.9	4,5	166.5
37	181.0	75.3	434.0	131.5	108.0	159.8	82	0.0	-	-	135.4	113.1	161.4
38	0.0	-	-	148.1	116.7	184.9	83	191.8	72.0	509.8	123.6	89.1	167.5
39	147.9	20.8	1042.3	143.4	117.0	172.4	84	45.3	6.4	320.6	121.7	96.5	150.3
40	0.0	-	-	148.6	116.4	185.1	85	0.0	-	-	124.6	102.0	149.2
41	0.0	-	-	123.3	102.0	147.8	86	171.8	24.2	1209.2	142.6	120.2	169.3

42	140.7	45.4	435.4	148.4	125.8	173.3
43	128.9	18.1	908.9	154.4	128.2	182.2
44	365.0	137.0	968.3	147.3	124.2	172.9
45	119.0	29.8	474.7	144.1	110.7	187.1
87	0.0	-	-	136.6	114.2	161.7
88	123.6	17.4	872.0	142.2	118.5	168.1
89	76.6	10.8	541.5	144.0	112.6	179.3
total	149.1	138.3	160.7	142.5		

On prevalence day, 686 MS cases (492 women and 194 men) were living in the province of Sassari. The total crude prevalence rate was 149.1 (95%CI, 138.3-160.7), 211.2 (95%CI, 193.4-230.7) for women and 85.4 (95%CI, 74.2-98.3) for men. The overall standardised rate was 142.9, 204.0 for women and 81.8 for men. The crude prevalence rates ranged between 0 and 413.0 (95%CI, 197.0-863.7) for both genders and between 0 and 743.5 (95%CI, 186.0-2922.7) for women.

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

The total standardised prevalence rates obtained for each of the 89 communes on prevalence day by MLE and Bayesian approaches with different cut-off points, and the distribution of PP values for total cases was mapped for the province of Sassari. Bayesian estimates and their respective PP values were also calculated and mapped based on the commune of residence at patients' 5-15 years of age

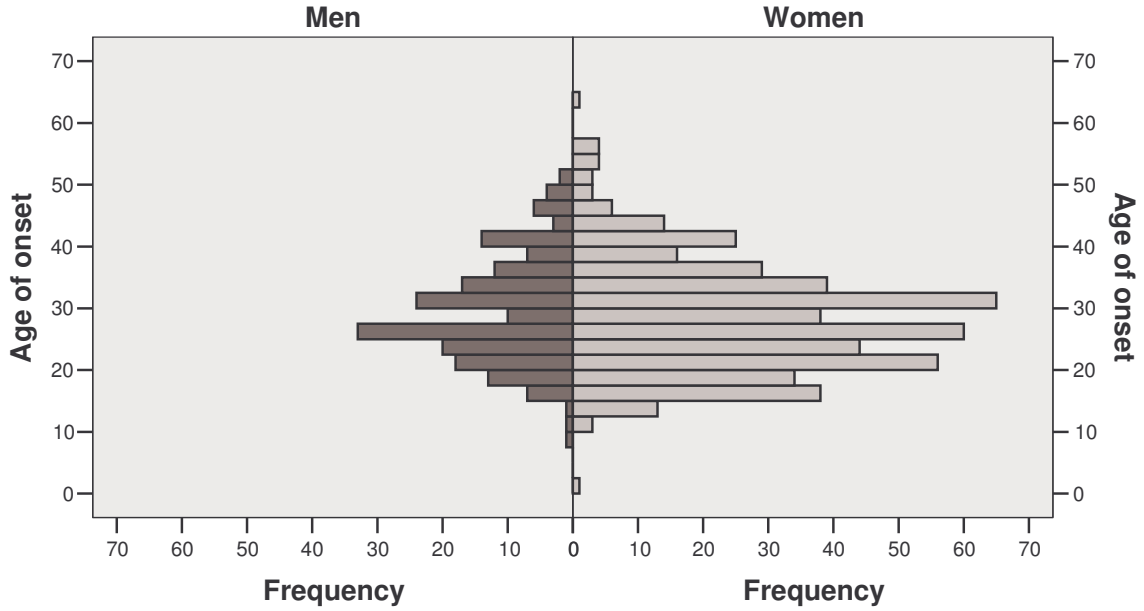
While it is not possible to identify peculiar spatial aggregates by observing the total standardised prevalence rates map due to the high variation, a clustering pattern in the west of the province and a west-to-east gradient appeared to be fairly evident when mapping the Bayesian estimates. In particular, 17 communes out of 89 had a Bayesian estimated prevalence of 150 per 100,000 and above, i.e., higher then the province standardised average rate. However, only 3 communes, i.e., Sassari, Ossi and Tissi (Sassarese sub-area) had a PP higher than 90% and are therefore considered "hot areas", whereas for the remaining 14 communes only an indication for a higher risk could be given. In the eastern province, prevalence was lower for eight

communes. The map estimates of prevalence rates by commune of residence at age 5-15 years showed a different spatial aggregation in the province. A large proportion of the communes located in the western province (Sassarese, Southern and Northern Logudorese, i.e., sub-areas 1, 3, 5) showed a spatial cluster of MS with a probability of 75-90%.

4.2. Incidence trends of MS in northern Sardinia and change of clinical phenotype over time (Paper II)

A total of 689 patients with onset of disease from 1965 to 1999 within the province of Sassari were included in the analyses: 496 women and 193 men, giving a female–male ratio of 2.6 (Figure 4).

Figure 4. Distribution of the MS population by age of onset and gender. Incidence study. (Paper II) (N=689)



The mean annual crude incidence rate for the whole province was 4.6 per 100,000: 6.5 for women and 2.6 for men (woman–man ratio of 2.6). The overall age-adjusted incidence rate was 4.4. The age-adjusted incidence rate increased markedly and significantly from 1.1 per 100,000 population in 1965–69 and 2.2 in 1970–74 to about 6 for the last three 5-year periods, 1985 to 1999. The trend did not differ significantly between genders during the study period.

The crude incidence rate differed significantly between the linguistic areas during the whole study period ($p = 0.02$, chi-square test), with Sassarese showing the highest rate of 5.4 and the Eastern and Southern Logudorese and Goceano areas with the lowest rate of 3.8. The incidence increased over time in all areas, and this time trend did not differ significantly.

The clinical course at onset was retrospectively evaluated for 635 cases, 512 (81%) with RR and 123 patients with a progressive course at onset. There was a significantly higher increasing trend among the group with RR course at onset than among those with progressive one ($p < 0.001$, chi-square test), with an increase for the former from 61% during 1965–79 to 90% during 1985–99.

The most frequent symptoms at onset were sensory (40% of the patients), pyramidal (22%) and visual manifestations (22%). The distribution of manifestations at onset did not differ significantly in the overall incidence interval.

The mean age at onset for the whole study period was 28.6 years (95%CIs: 27.9–29.2), with no significant difference between men and women, and between patients with initial RR course and those with initial progressive course. A late onset (45 years and older) was registered for 4.8% of the whole study population.

The mean age at onset increased steadily and significantly from 25.7 years in 1965–69 to 30.6 years in 1995–99. Since age at onset and clinical course are related and since the distribution of both these clinical variables changed over time, we performed an analysis of variance with age at onset as the dependent variable and

time period (using the seven 5-year intervals) and course of disease as fixed factors. Since the shift towards more cases with initial relapsing course during the last periods is expected to be associated with a lower age at onset, the estimated increase in age at onset over time was even more marked after adjusting for type of initial clinical course. Likewise, the effect of initial course on age at onset was statistically significant in this model, with an estimated difference of 2.6 years as compared with the observed difference of 1.3 years between patients with progressive onset and patients with relapsing onset.

The time lag between clinical onset and diagnosis decreased significantly from 13.0 years in 1965–69 to 0.9 years in 1995–99.

4.3. Early childhood is the age of MS induction period in northern Sardinia (Paper III)

The analysis included 649 MS patients with information on changes in municipality of residence up to at least age 15 years.The analyses using temporal closeness of 1, 2 and 5 years all showed space-time clustering in early childhood. For the 2-year temporal closeness, statistically significant clustering was observed from age 1 to 3 years; clustering peaked at age 1, with a ratio between observed and expected close pairs of 1.08 and an empirical *p* value of 0.039. The strength of the clustering was only slightly less for the other cut points for temporal closeness, with peaks at age 1 year (observed-to-expected ratio = 1.08) for 1-year closeness and age 3 years (observed-to-expected ratio = 1.07) for 5-year closeness.

Subgroups were also analysed according to clinical characteristics (age at onset, initial clinical course and year of clinical onset) and demographic variables (gender, year of birth and geographic sub-areas). All these were analysed using 2-year temporal closeness and clustering at age 1 year. Clustering was significantly increased for women, patients with RR course at onset, patients with recent onset (after year 1982) and patients living in the eastern province (sub-areas 2, 4, 5, 6). Clustering was borderline significant for patients born after 1959 and patients with

age of onset <30 years, while it was increased but not statistically significant for men and for patients living in the western province (sub-areas 1, 3, 22).

The cluster cases had an earlier mean (SD) age of onset of 27.3 (7.8) years versus 30.3 (10.2) years for the non-cluster cases ($p = 0.0005$, *t*-test). The significant difference in the standard deviation ($p < 0.0001$), Levene's test for equality of variance) indicates a more uniform epidemiological pattern in the age of onset among the cluster patients.

Performing the analysis backwards by a fixed period prior to onset failed to show any clustering. The degree of clustering tended to be lower than expected, though non-significantly.

4.4. Poor subjective scoring of health status in patients with non to mild MS at EDSS (Paper IV)

The study was conducted on 203 patients (156 women and 47 men) who gave consent to participation (93% of the total). They underwent clinical neurological examination and the RCPM test. A total of 197 patients (150 women and 47 men, woman-man ratio: 3.2) scored a corresponding IQ > 69 on the RCPM test and were thus administered the SF-36 questionnaire and considered for statistical analysis. The mean age was 41.3 years and the mean EDSS was 2.2.

SF-36 was completed thoroughly by 183 (93%) patients. Of 6,895 items (197 times 35), 66 were missing (1.0%). Substitution could be performed for 41 (0.6%) of these items.

All mean subscale scores for MS patients were significantly reduced, except for bodily pain. The mean score for physical functioning was especially reduced, almost one SD below the mean score for the general population. Mean scores of male and female MS patients did not differ significantly.

Mean standardised SF-36 subscale scores were also analysed for a subgroup of 105 patients (79 women and 26 men) with EDSS ≤2.0: "minimal disability in one

functional system". They were also significantly lower than in the general population, except for bodily pain and mental health.

Mean scores of male and female MS patients did not differ significantly.

The correlation between EDSS and the physical functioning subscale in the total MS sample was rather low (Pearson correlation coefficient = –0.14, $p = 0.05$), with EDSS explaining only 2.0% of the variation in the physical functioning subscale. EDSS also correlated, though poorly, with role–physical, general health and vitality (correlation coefficients of –0.17, –0.19 and –0.17, respectively). EDSS did not correlate significantly with the other SF-36 subscales. Neither disease course nor disease duration correlated significantly with any SF-36 subscale.

Benzodiazepines and antidepressants were assumed by 24 (14.2%) patients as symptomatic treatment. No main effect of such therapies was found on the SF-36 subscales, adjusting for EDSS scores and disease duration (data not shown).

Running regression analyses with the physical functioning subscale as the independent variable and each of the other 7 subscales as the dependent variable showed significantly smaller regression coefficients among the MS patients compared with the general population for all subscales except for role–physical and social functioning. Among men, the regression coefficient was significantly lower for general health among MS patients compared with the general population, whereas the other subscales did not differ. Among women, the MS patients had a significantly lower regression coefficient for all SF-36 subscales except for role–physical and social functioning.

5. GENERAL DISCUSSION

5.1. Main findings

5.1.1. Heterogenous spatial distribution of MS in northern Sardinia

The results of the spatial cluster analysis by means of Bayesian approach reported in **Paper I** have allowed a geographical mapping of the disease prevalence in the province of Sassari, northern Sardinia. Due to a negligible population migration in- and out-flow even between adjacent Sardinian communes, mapping the disease distribution of prevalent cases may yield good estimates of possible spatial clustering of MS.[159]

Mapping the distribution of Bayesian estimates for total cases by commune of residence on prevalence day disclosed a clustering pattern of MS in the western province, with special regards to three communes in the Sassarese sub-area. Further, a west-to-east gradient was observed. In the eastern province, prevalence was lower for eight communes. The map estimates of prevalence rates by commune of residence at age 5-15 years showed that for a large proportion of the communes located in the western province (Sassarese, Southern and Northern Logudorese, i.e., sub-areas 1, 3, 5), prevalence was high but only an indication for a spatial clustering was given.

A considerable number of spatial cluster studies are reported in the literature. However, the great majority consist of *post-hoc* analyses, driven by the attempt to rule out an association between disease and one or more possible risk factor(s) within an observed or believed "hot spot" of disease. **Paper I** is a spatial *a priori* cluster analysis of MS distribution in northern Sardinia. As opposed to *post-hoc* cluster analysis, it is therefore supported by hypothesis, is carried out in a population with no previous evidence of clusters and is thus less subject to bias.[54,60]

A previous epidemiological population-based survey confirmed that Sardinians are at high risk for developing MS.[123] The reported onset-adjusted prevalence rate of 149.7 per 100,000 reflected the overall province mean prevalence rate, and no conclusions could be drawn on the presence of possible true "excesses" or "lacks" of MS cases in the study area, which would instead represent valid clues to either risk or protective factors.

A Bayesian approach was adopted to investigate the spatial variation of MS prevalence and to overcome the difficulties of traditional methods of mapping disease risk.[191] Although the maps obtained by means of Bayesian approach are only a representation of the true disease risks in the area and can therefore reflect artifacts deriving from potential confounding spatial effects,[192] this method best reflects the disease spatial variation in small areas at low population density. Bayesian disease mapping models are conservative with high specificity, but have a low sensitivity, especially in the raised risk-areas having only a moderate excess.[193] Confounders can be due to autocorrelation, or the choice of a prior distribution that will affect posterior inferences. To assign the model the proper prior, the key part of the Bayesian approach (see Section 1.4.1), a sensitivity analysis was performed on non informative, moderately and highly informative priors.[70,194,195] These results do not actually yield exact and recommended models, but rather allow one to rule out those that are less informative for epidemiological purposes. The "subjective" choice of our prior distribution therefore best reflected our knowledge on the phenomenon under study.

The prevalence-based place of residence may not necessarily reflect the place of putative MS acquisition and/or exposure to possible risk factors. However, because of the negligible migration rate into or out of the province, or even between adjacent communes,[159] mapping the distribution of prevalent cases may still yield good estimates of possible clusters. Further, spatial clusters of MS were searched for also by mapping the distribution of cases by commune of residence at age 5 to 15 years, which has been previously indicated as the putative age of MS acquisition.[44] This

analysis was likely to be more reliable and informative, because the place of residence during such age may be associated to exposure to the putative exogenous (environmental) risk factors inducing MS. Further, when searching for etiological clues, and given specific assumptions, spatial aggregates based on residence during adolescence could reflect the exposure to causative exogenous factors particularly concentrated in some sub-areas, or the population's predisposing genetic background secondary to different allelic concentrations at microgeographic level especially found in innermost villages and rural close communities. Interestingly, an indication for spatial clustering for prevalence at age 5-15 was found for parts of sub-areas 1, 3 and 5 which are characterised more by close communities.[163] Of note, this area borders with the commune of Macomer (province of Nuoro), where MS was reported as having occurred as epidemic in the 1950s.[111] This incidence study indicated that no MS cases were detected until early 1950s, and that 13 cases had clinical onset in the years 1952-1981, when the average annual incidence was 4.8 per 100,000. Incidence peaked between 1957 and 1961 with 10.2 per 100,000 and slowly decreased again up to early 1980s. The authors hypothesised an epidemic of MS triggered by exogenous factors introduced by a population from mainland population migrated into a Sardinian 'naïve' population after the end of World War II.

A space clustering study on MS was conducted in the province of Nuoro, central Sardinia, by Montomoli et al.[65] Similarly to our work, prevalence rates and Bayesian estimates were mapped across 100 communes. As compared to the province mean prevalence rate of 157 per 100,000 in 1998, and based on PP mapping, spatial clustering was found for the communes of Nuoro, Oliena, Fonni and Desulo, that is in the Nuorese and Barbagia sub-areas located in the very inner province territory. Interestingly, an indication for a spatial aggregate was found in communes bordering with the province of Sassari and especially with those participating to the spatial cluster for age 5-15 years reported in **Paper I** (southern Logudorese). Based on previously reported evidences on genetic heterogeneity at microgeographic level[161] and on the "high number of multiplex families" assessed, the authors concluded for the genetic nature of such spatial cluster.

Clustering of MS was searched with a large population-based survey in the Tayside region of Scotland for the period 1970-97.[196] The study was conducted by means of spatial scan statistic without a priori specification of the size or location (temporal or spatial) of potential clusters, and capture-recapture methods to test the hypothesis that clusters may be detected due to improved ascertainment either temporally or geographically. The spatial scan analysis identified two clusters. The most likely cluster was exclusively temporal, between 1982 and 1995 with an annual incidence of 8.6 per 100 000 per year. There was also a significant secondary temporal/spatial cluster for the period 1993-95 for a mostly rural area to the south-west of Perth. The cyclical nature and close temporal proximity of the peaks argues against a purely genetic component. Furthermore, the population studied is ancestrally fairly homogeneous, with low migration rate and stable Scottish ethnicity in over 90% of individuals. Similarly to our setting, it was suggested that in the Scottish population where genetically based susceptibility to MS is generally high, other environmental factors become important in modulating the frequency of MS. The spatial cluster was detected in a rural area, at plausible increased genetic susceptibility genes. However, the cluster for this area occurred during a specific two-year period lends support to the relevance of environmental factors. The study design and the statistical methodology used argued against overascertainment of clusters or confounding.

However, whether prevalence-based spatial clustering reflects the predominant role of genetic rather than environmental factors in determining the disease is a difficult question, given the cross-sectional nature of the study design. As MS is clearly not a single source infectious disease, spatial cluster studies may help test the hypothesis that a widely and evenly spread infectious agent may produce disease in subgroups of genetically more susceptible individuals. Therefore, if the identified cluster were genetically determined, i.e., located in an area at high inbreeding rate, a disease mode of inheritance could instead be better investigated. In this perspective, despite evidence based on the geographic distribution of blood groups and HLA gene frequencies[96,97,160] and human Y chromosome polymorphisms[197,198] that Sardinians are genetically homogeneous and distinct, as compared to the rest of Caucasians, a

certain degree of genetic heterogeneity, possibly due to different inbreeding rates at microgeographic level, has been highlighted by analyzing the variability of mitochondrial DNA polymorphisms in two different Sardinian samples.[164]

We can speculate that the distribution of prevalence reflects the distribution of disease risk factors, based on the evidence that Sardinians are a steady population. However, other factors (e.g., socio-economic and health care related) may confound our results and should be adjusted for with further analysis.

Microgeographic heterogeneity of disease spatial distribution may potentially depend on differential case ascertainment. However, because of the methodology adopted for case ascertainment, fulfilling a "spider" type of approach,[168] we were rather confident in ruling out bias due to differential case ascertainment across the province sub-areas.

In **Paper I** we concluded that an indication for the action of an exogenous factor in some sub-areas and during adolescence was given with the spatial analysis of prevalence rates. This evidence could serve for ecological studies purposes,[199] i.e., correlational investigations with the spatial distribution of the disease putative risk factors. Nevertheless, incidence-based space-time clustering studies or an analysis of incidence trends over a defined time interval would be more informative in etiological research.

5.1.2. Incidence temporal trends in northern Sardinia

With **Paper II** we fulfilled one of the implications for further studies underlined in **Paper I**, i.e., a population-based incidence study of MS in the same Sardinian population. We showed a remarkable increase of the disease occurrence since 1965 to 1999 which explained the increased MS prevalence in time previously observed.[116,123] The incidence rate is not in general influenced by improved survival, and if based on the disease clinical onset – as in **Paper II** - and not on the diagnosis, the reduced time lag between clinical onset and diagnosis indicating an intensified case-finding over time, cannot be source of biased increased incidence. The patients diagnosed in more recent periods would still have been assigned their true year of clinical onset.

Improved case-ascertainment can only influence incidence if patients in the early periods had died before being diagnosed. However, this is unlikely to have occurred in our study due to the general low mortality rate of the disease, and to the multiple assessments conducted arguing against inaccurate registry.

Improvement of diagnostic accuracy could be responsible for earlier diagnosis and subsequent registration of a greater number of cases. Nevertheless, when comparing Sardinian data with those from a northern Italian health district with easier access to MS-specific diagnostic facilities (e.g., MRI), the temporal trend of the time lag elapsing from clinical onset to diagnosis and the sensitivity to diagnose mild cases based on EDSS were close to parallel in both settings.[110,123,200] Despite the similar degree of case ascertainment, however, a steady increase in incidence rates was observed only in Sardinians starting in the 1970s, thus pointing to a corresponding change in the distribution of true risk factors.

Incidence rates appeared to be stable on high values during the last three 5-year periods, i.e., 1985-99. Nevertheless, because incidence studies are conducted on patients who have already received a diagnosis, true incidence rates from the more recent period are likely to be underestimated given that some patients have not yet received a diagnosis in relation to features intrinsic to the disease course.[201]

In our study, clinical characteristics at disease onset deviated in two ways from an evenly distributed increase of incidence rates. Firstly, the increase over time mainly applied to patients with a RR course at onset. This can be ascribed to recall bias because patients from the study most remote cohorts may fail to remember, allocate in the correct time and report their first episode(s), and do instead remember the time that the disease grew severe. Some of these patients may therefore potentially be misclassified as having had a progressive onset. Nevertheless, had this bias occurred in our study, age at onset for these cases would have also been affected towards higher estimates. Instead, age at onset was significantly lower for the most remote cases.

Regardless of initial clinical course, and due to the retrospective nature of the survey, a bias toward identifying symptoms as attributable to MS might have been introduced, again especially for the cases in the earliest part of the study period. Should vague symptoms at onset be missed for these patients, then the estimated date of onset would have been later than the true one. Again, the lower age at onset for these cases does not argue for such a bias, and rather points to accurate ascertainment. In support to this statement and in favour of good validity of our data, is also the fact the information on the older cohorts patients were registered at time of these patients' respective diagnosis (possibly 20 or 30 years before the study started) and not at the time of the present study. With this respect, data collection rather followed a prospective design, although a case review was retrospectively performed for the study purposes.

A significant finding was, in fact, the younger age at disease onset for the first time intervals and its increasing trend over the whole study period. As a higher age at onset has been reported for patients with initial progressive course,[202-205] we performed an analysis of variance on age at onset to adjust for initial course.We found that if the distribution of type of initial course had been stable over time, such increase of age at onset would have been even stronger. This further argues against a change of initial course over time due to recall bias and rather points to a change of the MS clinical phenotype over time and maybe to changes in underlying disease determinants.

A higher age at onset in recent cohorts of a population at high risk for the disease such as Sardinians is in contrast with previous evidences of age at onset varying according to the disease prevalence, and being lower in countries with high occurrence.[206]

Despite the wealth of studies on MS incidence temporal patterns, the trend of age of onset is seldom discussed. Observations are not univocal. In a population- and registry-based study on spatial and temporal clustering in the Tayside region of Scotland for the period 1970-97, the mean age of onset was 35.7 years and appeared

to increase from 33.2 in 1970-79 to 38.1 in 1990-97.[196] The mean age at onset remained quite stable over time, ranging from 32.8 years in 1953-62 to 31.7 in 1973-82, in a study conducted in western Norway (Hordaland County) which showed an increase of the annual mean incidence rate from 2 per 100,000 in 1953-62 to 4 in 1968-77.[207] Repeated assessments conducted years later in the same population confirmed increasing incidence rates for the period 1958-87, with 5 per 100,000 in 1978-82, and showed a fluctuation of the age of onset.[208] Its mean was 35.6 years for the period 1953-57, followed by a significant decrease to 30.4 years in 1963-67 and tendency for an increase up to 34.4 years in the period 1983-87. No significant difference in age of onset was instead reported for another population in western Norway (Møre and Romsdal County) (29.7 years in 1961 versus 30.3 in 1985), in which a similar increase to Hordaland County in incidence in comparable time was also observed.[209]

Our results on age of onset were in contrast with a study conducted in another Sardinian MS population which had shown an anticipation of the age at onset, from a mean of 41 years for patients born between 1913 and 1939 to a mean of 22 years for those born after 1970.[210] However, the study was not population-based since it included consecutive patients referred to a clinic from an undefined area of the island. It is therefore likely that a selection bias was introduced by excluding patients with very benign or very severe MS, as it is often observed in hospital-based series. Furthermore, in the absence of a long maintained registry system and multiple assessments over time, the patients' date of onset was estimated only at the time of the mentioned study, thus challenging the validity of these estimates for the oldest cohorts. As already explained, by failing to report the first episode, estimates for age at onset are biased towards older age and obviously especially for most remote cohorts. Lastly, the analysis was performed by decade of birth (<1940, 1940-49, to >1970) leading to an underestimation of age at onset for the youngest cohorts in which a later onset of MS may have not occurred yet. The authors made some kind of adjustment for this, but failed to present more reliable data, e.g. by analysing the data according to time-periods rather than birth cohorts.

In our study, the incidence rates differed significantly between the province sub-areas. The westernmost areas (Sassarese, Northern Logudorese and Algherese) had higher mean rates than the easternmost ones, with some overlapping to what observed with the spatial cluster analysis in **Paper I**. This further strengthens the hypothesis that the heterogeneous distribution of MS cases at microgeographic level reflects a true different risk distribution in these sub-areas. As with **Paper I**, because of the survey methodology adopted for this study study, we feel reasonably confident in ruling out that different rates in urban versus rural areas, as well as in the western versus eastern sub-areas of the province, are due to biased case ascertainment.

Despite such spatial difference in the incidence rate distribution, an almost parallel increasing trend was observed in time for all sub-areas. It can be speculated that differences in incidence absolute rates could reflect a genetically-based predisposition to the disease, or different gene–environment interaction effects at microgeographic level. The action of an exogenous factor whose concentration has increased over time could nevertheless explain the increasing occurrence in disease over time.

Steadily increasing incidence rates in Sardinians over comparable periods of time have also been reported for juvenile diabetes (IDDM).[211,212] Sardinian IDDM has been shown to share specific immunogenetic features with Sardinian MS, such as HLA haplotypes DRB1*0301-DQA1*0501-DQB1*0201.[213] In a study by Songini et al,[211] improved survival rates, reduced stillbirth, perinatal and first-year mortality rate, a more accurate case ascertainment and emigration could not explain IDDM incremental trends in Sardinia. The rather homogeneous geographical distribution of the high IDDM incidence rates throughout the island, coupled with the marked general increase in their temporal trends rather pointed to the action of an environmental factor uniformly distributed in the territory that had rather recently changed its exertion.[212]

5.1.3. Space-time clustering in northern Sardinia

Based on the evidences of a heterogenous spatial distribution of MS at microgeographic level defined by the province sub-areas, and of a significant increase in the disease occurrence in a relatively short time period (little over than a generation time span), with **Paper I** and **Paper II** we argued in favour of the predominant role of exogenous factors in determining MS in Sardinians.

With **Paper III** we meant to use a different methodological approach to explore the presence and action of an exogenous factor(s) inducing MS in the same Sardinian population and over the same time period. Furthermore, we aimed at disclosing the age at which individuals that later developed MS might have shared common exposures, and thus at identifying a putative induction period to Sardinian MS. Lastly, we were interested in investigating whether such exposure(s) was associated with demographic and/or clinical variables, and might account differently in different disease phenotypes.

In order to fulfil this, we used a space-time cluster analysis of incidence data in the province of Sassari (see Section 1.4.3.). The idea behind this type of analysis is that an excess of clustering both in space and in time reflects the role of an exogeneous agent that varies in intensity between areas. The most intuitive such agent would be infectious.

The analysis disclosed patterns of space-time clustering, and these were significant at age 1 to 3 years, suggesting that early childhood is the induction period of MS in Sardinians. Furthermore, clustering was most marked in women, in patients with more recent onset (after 1982), with RR initial course and for sub-areas 2, 4, 5 and 6 of the province of Sassari.

Confounding demographic factors specifically influencing the sub-areas were reasonably ruled out. In fact, if the general population had substantially migrated across the province, it would have been towards the major urban area (Sassarese),

yielding more substantial clustering in the western sub-area and thus biasing the results in the opposite direction as compared to what observed.

With regards to putative age periods for MS susceptibility, two periods of 0 to 5 years and 10 to 15 years were suggested using stochastic models estimating the distribution and thus the length of the latency period for MS.[37,55] Despite the latter of these periods was suggested as the most plausible one, our evidences are in line with the estimated younger period. Wolfson et al. suggested that the mean latency period in their population was 18 years based on 10 to 15 years of age period. According to our data the estimated duration of the latency period could be over 25 years.

That early childhood might comprise the disease induction period is in agreement with other recent evidences obtained with very different study designs and methodological approaches. The relative contribution of ancestry, country of birth and residence in determining MS risk and age of MS onset was studied by Kennedy et al[214] in a population of 44 pediatric and 573 adult MS patients residing in Ontario, Canada. The authors concluded that the place of residence during childhood, irrespective of ancestry, was the major demographic determinant for lifetime risk, suggesting a predominant influence of environment in MS risk. They also found that individuals with ancestors originating from regions at low risk for MS and raised in a region of high MS prevalence, had an earlier age of MS onset. This evidence and ours based on a tendency for space-time clustering in individuals that would later manifest MS at an earlier age, seem to suggest a role of exogenous factors in the disease phenotypic expression and argue in favour of heterogenous etiopathogenetic mechanisms.

A climate-related interaction between genes and environment during gestation or shortly after birth has been hypothesised to trigger MS at least in northern Caucasian populations from the observed excess of spring births in MS patients.[29-31] Pooled analysis of datasets from Canada, Great Britain, Denmark and Sweden (n = 42,045) showed that fewer (8.5%) people with MS were born in November and more (9.1%) were born in May.

A large longitudinal population-based study aimed at investigating incidence changes by analysing women-men gender ratio over time, by year of birth and in a cohort of 27,074 Canadian patients, showed a markedly significant increased of MS in women as compared to men over at least 50 years.[215] According to the authors, this rapid change must have environmental origins acting in individuals' early life, even if it is associated with a gene-environment interaction, and implies that a large proportion of MS cases may be preventable in situ.

In contrast, no space-time clustering around birth was found among 783 patients in Northern Ireland[216] or among 556 patients in the Netherlands.[217] However, residence at birth might not necessarily reflect the actual residence during disease initiation even in apparently stable populations. Another space-time clustering study conducted on two Dutch populations (Groningen and Rotterdam) with three different statistical methods, and based on prevalence at birth failed to disclose clustering patterns in both areas and for methodological approaches used.[217] The authors concluded that perinatal infectious events are unlikely to be relevant factors in determining the disease initiation.

A period of susceptibility to MS between birth and adolescence was indicated with migration studies.[40,45,47,218] These studies, however, can be easily biased by selection of the migrating population, small sample sizes and the difficulty in assessing the time elapsing from migration to disease onset. Further, too few studies have investigated the age at migration among migrants from low- to high-prevalence countries to provide further evidence.[37]

A formal cluster analysis of MS in space and time was carried out in a region of western Norway in a population of nearly 400,000 in the early 1980s when prevalence was 75.5 per 100,000, and the mean annual incidence rate for the previous 20 years was 3.7 per 100,000.[219] No significant space-time clustering was found, but only an indication for clustering according to year of onset the *rural* part of the study area.

A few years later another space-time clustering analysis was performed in the same population, including patients with an onset of disease between 1953 and 1987, and by means of a different statistical model (the generalised regression approach of Mantel).[169] Significant clustering was observed between age 13 and 20 years, with a marked peak at age 18. At this peaking age, an analysis by subgroups of patients according to clinical and demographic variables showed significant clustering in women, in patients with RR initial course and in more recent cases.

In this Norwegian population, space-time clustering was found at different ages as compared to our results suggesting a population-specific difference in the disease putative initiation. Nevertheless, clustering was found in the same subgroups of patients by clinical and demographic variables, which might instead point to a greater susceptibility to the exogeneous factor in these sub-groups.

A further study of this material correlated a high degree of clustering with early age at onset and the female gender.[170] The most clustered patients' age at onset was on average 5 years lower than the least clustered patients'. The authors interpreted this as an evidence of shorter latency for the patients with strong clustering (i.e., different etiological agent), or that these cases were exposed to the agent at an earlier age. Interestingly, as in our study, a greater variation of age at onset was found for the Norwegian non-clustered cases, suggesting greater heterogeneity in the latent period duration for these patients, possibly due to the role of multiple agents of different nature. The more likely infectious nature of MS in clustered patients tending to develop the disease at an earlier age is in agreement with "the pubertal hypothesis" within the Faroes MS epidemics.[220] According to this hypothesis, the putative disease initiation at puberty was believed to be triggered by a viral infection, appeared to be strong candidate for earlier age of disease onset.

Space-time clustering was marked in the eastern province. In this work, this area comprised Southern Logudorese (sub-areas 5) which was part of the spatial clustering based on residence at age 5-15 years reported in **Paper I**.

Our study and others[169] have detected no space-time clustering patterns reflecting fixed latency periods. This argues against the hypothesis that the disease is caused by infection from MS-specific viral agents with fixed incubation time intervals, but rather, by more complex mechanisms and interactions occurring in early childhood.

In a space-time clustering study conducted over 42 and 29 cases in the Orkneys and Shetland Islands respectively,[221] probably due to the small population, no clustering was found using residence at different ages. However, as opposed to the Norwegian study[169] and our work results, a bimodal pattern of clustering was found at a fixed latency period of 21-23 years, and just before clinical onset. The authors hypothesised the action of a "MS-specific" exogenous (viral?) agent with a fixed long two-stage incubation period.

Our findings intuitively lend support to the existence of an infectious agent acting as a risk factor for MS in Sardinia during early childhood. An alternative hypothesis would be that space-time clustering at a certain age reflected instead a *non-exposure* to factors to which individuals are normally exposed, and that would protect the population from developing the disease. The "polio hypothesis" by Poskanzer et al[222] and later supported by Alter et al[223] suggested that the hypothesised agent causing MS is ubiquitously distributed and circulates in populations with low MS incidence, and, conversely, that infections with the same agent later in life may instead increase the risk of MS. Cooke posed a similar hypothesis in a critique to the "pubertal hypothesis" of the Faroe Islands epidemics.[49]

MS epidemics in the Faroe Islands was reported by Kurtzke[112] after World War II and believed to be have been caused by exposure from one source that triggered the onset at about 11 years of age in a population that was virgin to that specific exposure ("pubertal hypothesis"). The clinically overt disease would develop after a fixed latency period. Reanalysing the Faroese data Cooke suggested instead that the increased risk of MS was subsequent to the lack of a highly contagious infection from a widespread (viral) agent that should have taken place before age 3 years conferring protection against MS ("protective hypothesis"). This is in agreement also with the

higher risk of MS found among individuals with late onset of the typical childhood infectious diseases, such as infectious mononucleosis, mumps and measles.[224,225] Furthermore, it has been shown that exposure, within age 6 years, to an infant sibling is associated with a reduced risk of MS, likely in relation to the high rate of childhood infections among infants.[226]

Furthermore, Cooke underlined the importance of the population immunitary background in the disease determination, which can change depending on whether individuals are virgin to newly introduced exogenous (viral) factors, as in the remoteness of Faroe Islands, or on whether they have already been immunised to multiple infections, as for open and populous regions. With respect, the early age of disease initiation within the "protective hypothesis" would not clash with the older one suggested with migration studies.

As for our evidences, the question arises as to what kind of background immunity characterises Sardinians, or at least the eastern subpopulation in which clusters were detected, and whether the "protective hypothesis" could apply to our study population as well. In this scenario, our cluster cases might have been *unexposed* to any of the common infectious diseases in early childhood due to either the absence of the causative agents in those years or areas, or to conditions protecting them. Based on these assumptioms, it is difficult to completely disregard the hypothetical role of newly introduced childhood vaccination programs (e.g., against measles, hepatitis virus B, influenza) in triggering the disease by means of "protecting" individuals from common childhood infections, despite meta-analytical studies have failed to report associations between the disease onset or reexacerbation and vaccines.[27]

Space-time clustering studies have some limitations which are intrinsic to the statistical model and some limitations in relation with the use in MS.[54] In general, these models have usually low statistical power of the tests, with special concern to rare conditions. Also, since observations are pairs and not single individuals, the structural dependence between the observations makes it difficult to apply the correct method to calculate *p* values. Due to a complicated theoretical distribution of the test

statistic based on these observations, only approximate methods can be used. Lastly, these models are depending on assumptions, whose nature can influence the conclusions reached about the characteristics of the latency period, such as its duration or the age of susceptibility, especially in a disease for which there are no or just few concrete leads of its etiology.[227]

As for the use in MS, while the use of space-time cluster analysis is especially indicated for diseases caused by a single-source infectious agent, there is little evidence that MS is caused by an agent with such characteristics. The disease causative agent is rather hypothesised to be commonly widespread and to interplay with other factors, including the genetically based trait and demographic features. This multifactorial etiological pattern might mask a space-time clustering effect, and confer the test a low statistical power. With this regard, it is therefore recommended that inclusion criteria should not be very conservative, and limited, for example, only to definite forms.[54] Furthermore, as the latency period in MS, i.e., the period elapsing between the hypothesised exposure and first clinical manifestation, is long and extremely variable among individuals, the time of and residence at clinical onset might not be informative at all as to etiology or susceptibility.[54]

Nevertheless, despite these potential limitations, we did find significant clustering patterns in our population, which points to the true presence of an infectious-like causative agent. Furthermore, such clustering was more marked when we controlled for gender, initial course, birth cohort and geographic sub-area. It is likely that some forms of Sardinian MS are triggered by infections.

The observed-to-expected ratios in this study were relatively low, but it is difficult to judge what would represent a *large* effect in this type of analysis. This ratio is not comparable with normal values, as for example the odds ratio or relative risk that are based on individual data and not pairs of data. Further, the observed-to-expected ratios were rather similar along with age, due to the strong dependency between the analyses for each year. Only a few individuals included in the analysis were actually

migrating during childhood. Still, there was enough migration such that by the end of adolescence the ratio was reduced to 1.

We cannot exclude the possibility that the clustering was caused by a confounding factor or by a factor(s) unrelated to the disease. However, a specific migration pattern in the general population being responsible for confounding is less likely because of the stability of the population. A rate-based cluster method might nevertheless have given more information on this issue.

5.1.4. Perceived health status and mild MS

In **Paper IV** we showed that MS patients with no to mild disability according to EDSS score ≤3.5 rate their health status as poorer than the general population for all relevant health status domains except for bodily pain. This was true even for a subsample of MS patients with minimal disability in only one FS on EDSS (score ≤2.0). Interestingly, despite patients' low EDSS scores, physical functioning was rated especially poorly compared with the other subscales. These study results appear to be mainly confirmatory; however they have a specific interest, since coming from patients with absent/mild physical disability.

Physical functioning and the role limitation due to physical health problems, i.e., the perception of problems in daily or occupational life as a result of poor physical health, were reduced among MS patients compared with the general population despite no or mild disability. Self-perceived general health, vitality and energy, fear of getting sick or for worsening of health, fatigue and tiredness were also worse in this subset of patients. Their emotional status interfered more with concentration, work productivity and other activities than among the general population. Physical and emotional health status had a greater impact on the quantity and quality of normal social activities compared with the general population in this group of nondisabled to mildly disabled MS patients.

Further, this subset of MS patients also had lower scores than the general population in mental health status, although to lesser degree than for the other scales. Three of

the five questions included in this scale are related to depression and the lower score might indicate that also this group of patients has a slightly higher rate of depression than found in the general population. Fatigue and depression are generally highly frequent among MS patients and probably also affect the other domains of the health-related quality of life.[228] Fatigue might likely be an important component of self-rated poor physical functioning, which is not captured by the more objective disability measurement EDSS in mild MS.

In accordance with other studies[228,229] self-rated bodily pain in our patients did not differ from that of the general population and for both genders. Physical pain is not a common clinical feature of MS, with the exception of pain due to spasticity for EDSS scores higher than those used as inclusion criteria for the study. SF-36 bodily pain subscale has proved to be a reliable measure for painful chronic conditions, such as musculo-scheletal disorders and headache. Given the characteristics of pain in MS and the subscale dependence on physical scores, this subscale might not represent a suitable instrument for self-rating health status in these patients.

In agreement with other studies[228,230] the self-perceived health status among our patients did not depend on the number of years with the disease, nor the disease course significantly affected the patients' self-rated health status. No differences were found between those receiving symptomatic treatment and those did not receive such treatment.

Physical functioning was especially perceived worse among MS patients than among the general population, despite their *fully ambulatory* status on EDSS. This was true even for patients with minimal disability according to EDSS. Because the SF-36 physical functioning subscale measures perceived daily ambulatory functioning, including climbing stairs and walking different distances, the low physical functioning subscale scores in this subset of patients conceptually disagreed with the EDSS definition of *fully ambulatory*. Such discrepancy was further corroborated by the weak statistical correlation (2.0%) found between the EDSS and the SF-36 physical functioning subscale. This evidence raises uncertainty about EDSS

sensitivity in measuring physical impairment at this disease stage and indicates that the SF-36 physical functioning subscale is more sensitive. This is in accordance with other authors reporting on EDSS as an instrument to assess nervous system impairment but not overall mobility.[157,231] Kurtzke's EDSS sometimes considered "the gold standard" for disability scoring in MS,[232-234] is observer-(usually neurologist-) rated and addresses impairment in its lower levels and mobility in the higher ones. The poor inter-rater reliability has lead people to suggest that it is necessary to see quite a large change on the scale (up to one entire point) to believe that the change is meaningful. As the EDSS is not a linear scale, patients spend more time at some levels than at others. This is the reason why a change of 0.5 EDSS points at a level of 5.5 or greater is as clinically important as a change of 1 EDSS point at level of 5.0 and below. Further, it is not clear whether EDSS short-term changes predict long-term disability.[157]

On the other hand, as for SF-36 there is limited information on its application in MS, but there is some concern that it may not be specific enough for this condition, in that the physical functioning subscale has been shown to have reduced sensitivity when applied to patients with severe MS disability, due to marked floor effects.[143,235]

Interestingly, by means of movement analysis technique, subclinical evidence of gait control dysfunction has been reported for MS patients with even "minimal disability in one functional system" (EDSS score of 0 to 2), who therefore had no objective walking restriction, signs of motor involvement or clinical spasticity.[236]

The objective versus subjective measurement of physical functioning in MS may differ in the same patient. The patients may be able to catch their own impaired physical functioning at an earlier stage than neurologists objectively can. Nortvedt et al[237] have shown that self-rated health can predict a change in MS disability measured using EDSS. In this study, high scores for the SF-36 general health subscale and the mental health subscale at baseline were correlated with decreased EDSS after 12 months. The risk of worsening in EDSS after 1 year was two-fold among patients with poor or fair self-rated health status at baseline versus those rating their health as

good, very good or excellent. More objective measures such as EDSS itself had no predictive value at baseline. The authors concluded that self-rated health might partly reflect disease activity and thus represent a risk factor in the natural history of the disease.

The meaningfulness of physical impairment on overall self-rated health status has been reported to differ between patients and evaluators (Rothwell et al, 1997).[238] In this study, both clinicians and patients rated the impact of physical impairment differently compared with the general population and for the most relevant health domains. However, clinicians weighted the physical involvement of the disease more strongly on overall health status than patients did, whereas patients weighted their mental health and vitality as being more important than the clinicians did. The study included patients with moderate to severe disability (EDSS score ranged from 1 to 8).

The SF-36 scores were compared in patients with benign MS (EDSS <3.5 at least 10 years after disease onset), non-benign MS and normal controls in a population-based study conducted in western Norway.[239] Patients with benign MS had a significantly better health status for all eight functional dimensions in the SF-36 compared with patients with non-benign MS. However, as compared to the normal population, they generally perceived a significantly poorer health status for all the dimensions in the SF-36 except for mental health. In addition, 25% of the benign MS patients had been awarded disability pension due to the disease itself. The definition of benign MS has been heavily weighted towards physical disability and in particular ambulation, but although meeting the criteria for a benign course, patients may be heavily disabled due to non-motor symptoms.

Similar conclusions are drawn from our patient sample, in whom, despite the low disability score, the impact of the physical functioning on overall self-perceived health status was generally lower compared with the general population, especially for women. This evidence points to factors other than physical functioning playing a significant role in these patients' self-perceived health status.

Our study on self-perceived health status presents some limitations. It did not comprise all MS patients living in the study area with the specific inclusion criterion of fully ambulatory status. Nevertheless, the hospital-based sample of consecutive MS patients, most of whom were undergoing immune prophylaxis and needed periodic follow-up visits at the Centre, was likely highly representative of a population of MS patients with low disability scores.

Furthermore, cultural differences between Sardinians and mainland Italians[160] might influence self-rated health status. Nevertheless, due to its cross-cultural validity, appropriateness and content comparability in tests on different Caucasian populations,[240-242] the SF-36 was chosen to also avoid capturing the effect of such differences between the study and the reference population. Further, more than one third of the normative SF-36 data for Italy were collected from southern Italy,[187] where Sardinia is located.

5.2. Implications for further research

The evidences based on this work imply further investigations in two relevant areas of MS research in a population at high risk for the disease and characterised by peculiar socio-historical and ethnic features: the search for disease etiology, and the evaluation of the socio-economic burden in the disease early stages.

The evidences stemming from Papers I-III point to the predominant role of exogenous factors in determining the disease and allow us to trace some of the most relevant features of such factor(s): (i) its concentration is particularly elevated in Sardinia as compared to other populations at similar ethnicity or latitude, (ii) its concentration is increasing over time and evenly across the study area, especially starting in the 1980s, (iii) its distribution may vary at microgeographic level (in rural versus urban sub-areas?) or the degree of its interaction with immuno-genetic features may be responsible for such spatial differences observed in the MS distribution (in close versus open communities?), (iv) exposure to such factor(s) is likely to occur in early childhood, (v) its concentration may regard the female more extensively than

the male population, (vi) the previous exposure of such factor should be predominantly searched in patients with RR MS, and (vii) it is probably not a causative agent with fixed-latency incubation period. However, it is worth to underline that such epidemiological characteristics were reported for subsets of patients which lends large support to the recently reappraised pathological heterogeneity of MS.[243] As an example from our study, a smaller variation of age of onset was observed in patients showing space-time clustering as compared to non-clustered patients. Even though our results in general are not compatible with a role of MS-specific infectious agents with fixed incubation period, it is possible that while clustered-patients reflect the occurrence of an infection, the non-clustered patients reflect a multiple heterogeneous nature of causative agents.

On the basis of these clues, it will be possible to design analytical epidemiological studies. Ecological could be aimed at performing correlation analyses of the disease rate with one or more preferably quantified, or dichotomised, exposures distributed by space.[199] These exposures could be environmental, e.g., measurable in air, water, or soil, socio-economic and demographic, e.g., income, social and occupational status, and lifestyle factors, e.g., smoking and diet, in relation to the spatially mapped disease. The obtained MS map could also be correlated with that of other diseases in Sardinia, if available. The correlation of MS with IDDM spatial mapping with MS can be very informative as to disclose common putative causative agents. MS and IDDM share predisposing HLA haplotypes,[117-121] and have shown comparable epidemiological behaviour, with regards to increasing incidence and spatial clustering.[122]

Based on the findings in Papers I-III and on the results from possible ecological studies, case-control studies could be performed. The study subjects should be assessed for the history of the occurrence of exposures (or events) of interest prior to disease onset and especially during childhood. This could be achieved through personal interviews or self-administered questionnaires with which a large number of risk factors could be investigated, including childhood infections and immunisation

history. Relatively to our findings, the following candidate exogenous factors need to be considered: EBV and infectious mononucleosis, CP, vaccinations, occupational exposures and environmental toxicants, sunlight and UV, climate, lifestyle factors, such as diet, macronutrients, saturated fat, linoleic caid, antioxidants, vitamin D, cigarette smoking, physical trauma, pregnancy, oral contraceptives and replacement therapies. Recent-onset patients (incident cases) and matched controls should preferably be included in the study so as to minimise inaccuracy in reporting exposures prior to disease onset or in childhood. Such studies could be conducted according to *ad hoc* guidelines established on the design itself, the questionnaire and database,[244-246] and within a multicentre type of network.

Disease surveillance, i.e., the systematic routine collection and analysis of health outcome data for disease control, can be extended to the adjacent provinces and possibly to the whole region.[247] This could allow for a more sensitive *a priori* identification of clusters in space and time, and enable public health officials to conduct evaluations of situations that may be of public concern, other than the disease etiology itself. An on-going project on e-health supported by the Sardinian regional government will soon allow the surveillance of neurological disorders over the study area, by means of systematic computerised registry.

Lastly, given the disease heterogeneity, efforts should be made to investigate exposures to risk factors by stratifying for clinical variables, like age of onset, type of initial onset and functional systems involved.

With Paper IV we found that MS patients with no to mild disability on an objective measurement of disability rate their health status as being significantly worse than the general population does, and that this is especially true for self-rated physical functioning in both genders. Nevertheless, factors other than physical functioning contribute to the low scores for the other dimensions compared with the general population. These findings should encourage the implementation of strategies targeting a broad spectrum of health issues for MS patients starting in their early stage of the disease. In cost-of-illness studies they can provide clues to indicators to

socio-economic burden in the disease early stages, and elements for disease-specific interventions.

6. CONCLUSIONS

Paper I

Mapping the distribution of prevalence rates by commune of residence disclosed clustering patterns of MS at microgeographic level. In these sub-areas the disease prevalence is significantly higher than the provincial mean rate. Based on 1997 prevalence, spatial clustering was found in the Sassarese sub-area, whereas based on prevalence rates by commune of residence in adolescence, the previous evidence was confirmed and also a tendency for spatial clustering was found in more interior communes (sub-areas 1, 3, 5). This microgeographic variation reflects the spatial distribution of disease risk factors. However, whether the nature of these factors is environmental or genetic, or due to interaction, is impossible to establish given the cross-sectional design of the study.

Paper II

Some heterogeneity in the distribution of incidence rates across sub-areas of the province of Sassari was found. However, regardless of such spatial distribution of incidence, rates steadily increased over time close to parallel in all sub-areas. Because such variation has occurred within a relatively short period of time, a corresponding change in the temporal distribution of an environmental etiological factor(s) in this genetically stable population must be assumed. The observed temporal change in the MS clinical phenotype with an increasing age at onset and a decreasing proportion of patients with progressive initial course, lends further support to this hypothesis.

Paper III

Space-time clustering patterns were found in our MS population, which strongly argues in favour of a common exposure to disease causative exogenous agents. Such clustering was found in early childhood, which is likely to be the age of disease induction of MS in Sardinia, at least for some subgroups of patients. The effect was

most evident in subsets of patients: women, in the most recent cohorts and in patients with RR course. This supports that the disease is heterogeneous and that etiopathogenetic variation may underlie clinical variation. Interestingly, the effect was found in sub-areas partially overlapping with those featuring tendency for spatial clustering in adolescence, which increases the probability for an exogenous factor to act in those areas.

Paper IV

MS patients with no to mild disability on EDSS rate their health status as being significantly worse than the general population does. This is especially true for self-rated physical functioning among both women and men with MS. Nevertheless, factors other than physical functioning contribute to the low scores for the other dimensions compared with the general population. In nondisabled to mildly disabled MS patients, subjective scales aimed at rating physical functioning, such as SF-36 though not disease specific, can detect subclinical physical disability and are thus more sensitive indicators. These findings should encourage the implementation of strategies targeting a broad spectrum of health issues for MS patients also in their early stage of the disease, and provide elements for disease-specific interventions.

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Annexes

I. Functional Systems and Expanded Disability Status Scale[158]

Pyramidal Functions

0. Normal.
1. Abnormal signs without disability.
2. Minimal disability.
3. Mild or moderate paraparesis or hemiparesis; severe monoparesis.
4. Marked paraparesis or hemiparesis; moderate quadriparesis; or monoplegia.
5. Paraplegia, hemiplegia, or marked quadriparesis.
6. Quadriplegia.

V. Unknown.

Cerebellar Functions

0. Normal.
1. Abnormal signs without disability.
2. Mild ataxia.
3. Moderate truncal or limb ataxia.
4. Severe ataxia, all limbs.
5. Unable to perform coordinated movements due to ataxia.

V. Unknown.

X. Is used throughout after each number when weakness (grade 3 or more on pyramidal) interferes with testing.

Brain Stem Functions

0. Normal.
1. Signs only.
2. Moderate nystagmus or other mild disability.
3. Severe nystagmus, marked extraocular weakness, or moderate disability of other cranial nerves.
4. Marked dysarthria or other marked disability.
5. Inability to swallow or speak.

V. Unknown.

Sensory Functions

0. Normal.
1. Vibration or figure-writing decrease only, in one or two limbs.

2. Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration in one or two limbs; or vibratory (e.g., figure writing) decrease alone in three or four limbs.
3. Moderate decrease in touch or pain or position sense, and/or essentially lost vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs.
4. Marked decrease in touch or pain or loss of proprioception, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs.
5. Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head.
6. Sensation essentially lost below the head.

V. Unknown.

Bowel and Bladder Functions

0. Normal.
1. Mild urinary hesitancy, urgency, or retention.
2. Moderate hesitancy, urgency, retention of bowel or bladder, or rare urinary incontinence.
3. Frequent urinary incontinence.
4. In need of almost constant catheterization.
5. Loss of bladder function.
6. Loss of bowel and bladder function.

V. Unknown.

Visual (or Optic) Functions

0. Normal.
1. Scotoma with visual acuity (corrected) better than 20/30.
2. Worse eye with scotoma with maximal visual acuity (corrected) of 20/30 to 20/59.
3. Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual acuity (corrected) of 20/60 to 20/99.
4. Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100 to 20/200; grade 3 plus maximal acuity of better eye of 20/60 or less.
5. Worse eye with maximal visual acuity (corrected) less than 20/200; grade 4 plus maximal acuity of better eye of 20/60 or less.
6. Grade 5 plus maximal visual acuity of better eye of 20/60 or less.

V. Unknown.

X. Is added to grades 0 to 6 for presence of temporal pallor.

Cerebral (or Mental) Functions

0. Normal.
1. Mood alteration only (does not affect DSS score).
2. Mild decrease in mentation.
3. Moderate decrease in mentation.
4. Marked decrease in mentation (chronic brain syndrome-moderate)
5. Dementia or chronic brain syndrome-severe or incompetent.

V. Unknown.

Other Functions

0. None.
1. Any other neurologic findings attributed to MS (specify).

V. Unknown.

Expanded Disability Status Scale (EDSS)

0	Normal neurologic exam (all grade 0 in FS; Cerebral grade 1 acceptable).
1.0	No disability, minimal signs in one FS (i.e., grade 1 excluding Cerebral grade 1).
1.5	No disability minimal signs in more than one FS (more than one grade 1 excluding Cerebral grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 0 or l), or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory.
3.5	Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or l), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters.
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterised by relatively severe disability, usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters.
5.0	Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (eg, to work full day without special provisions). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0.)

5.5 Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding those for step 4.0.)

6.0 Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+)

6.5 Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+)

7.0 Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in w/c some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone.)

7.5 Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+)

8.0 Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems)

8.5 Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally 4+ in several systems)

9.0 Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4+)

9.5 Totally helpless bed patient; unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+)

10 Death due to MS.

QUESTIONARIO
SULLO STATO DI SALUTE

SF-36 (V1) STANDARD

12/94
IQOLA SF-36 Italian Version 1.6

QUESTIONARIO SULLO STATO DI SALUTE SF-36

Data consegna |_|_| |_|_| |_|_| N° codice paziente |_|_|_|_|_|

ISTRUZIONI: Questo questionario intende valutare cosa Lei pensa della Sua salute. Le informazioni raccolte permetteranno di essere sempre aggiornati su come si sente e su come riesce a svolgere le Sue attività consuete.

Risponda a ciascuna domanda del questionario indicando la Sua risposta come mostrato di volta in volta. Se non si sente certo della risposta, effettui la scelta che comunque Le sembra migliore.

1. In generale, direbbe che la Sua salute è:

(Indichi un numero)

Eccellente ……	1
Molto buona ……	2
Buona ……..	3
Passabile ……..	4
Scadente ……...	5

2. Rispetto ad un anno fa, come giudicherebbe, ora, la Sua salute in generale?

(Indichi un numero)

Decisamente migliore adesso rispetto ad un anno fa ……..…	1
Un po' migliore adesso rispetto ad un anno fa ...……	2
Più o meno uguale rispetto ad un anno fa ……...	3
Un po' peggiore adesso rispetto ad un anno fa ……...	4
Decisamente peggiore adesso rispetto ad un anno fa ……..	5

3. Le seguenti domande riguardano alcune attività che potrebbe svolgere nel corso di una qualsiasi giornata. La Sua salute La limita attualmente nello svolgimento di queste attività?

(Indichi per ogni domanda il numero 1, 2, o 3)

	SI, mi limita parecchio	**SI, mi limita parzialmente**	**NO, non mi limita per nulla**
a. **Attività fisicamente impegnative**, come correre, sollevare oggetti pesanti, praticare sport faticosi	1	2	3
b. **Attività di moderato impegno fisico**, come spostare un tavolo, usare l'aspirapolvere, giocare a bocce o fare un giretto in bicicletta	1	2	3
c. Sollevare o portare le borse della spesa	1	2	3
d. Salire **qualche** piano di scale	1	2	3
e. Salire **un** piano di scale	1	2	3
f. Piegarsi, inginocchiarsi o chinarsi	1	2	3
g. Camminare **per un chilometro**	1	2	3
h. Camminare **per qualche centinaia di metri**	1	2	3
i. Camminare **per circa cento metri**	1	2	3
l. Fare il bagno o vestirsi da soli	1	2	3

4. Nelle ultime 4 settimane, ha riscontrato i seguenti problemi sul lavoro o nelle altre attività quotidiane, a causa della Sua salute fisica?

Risponda SI o NO a ciascuna domanda

(Indichi per ogni domanda il numero 1 o 2)

	SI	**NO**
a. Ha ridotto **il tempo** dedicato al lavoro o ad altre attività	1	2
b. Ha **reso** meno di quanto avrebbe voluto	1	2
c. Ha dovuto limitare alcuni **tipi** di lavoro o di altre attività	1	2
d. Ha avuto **difficoltà** nell'eseguire il lavoro o altre attività (ad esempio, ha fatto più fatica)	1	2

5. Nelle ultime 4 settimane, ha riscontrato i seguenti problemi sul lavoro o nelle altre attività, a causa del Suo stato emotivo (quale il sentirsi depresso o ansioso)?

Risponda SI o NO a ciascuna domanda

(Indichi per ogni domanda il numero 1 o 2)

	SI	**NO**
a. Ha ridotto **il tempo** dedicato al lavoro o ad altre attività	1	2
b. Ha **reso** meno di quanto avrebbe voluto	1	2
c. Ha avuto un calo di **concentrazione** sul lavoro o in altre attività	1	2

6. Nelle ultime 4 settimane, in che misura la Sua salute fisica o il Suoi stato emotivo hanno interferito con le normali attività sociali con la famiglia, gli amici, i vicini di casa, i gruppi di cui fa parte?

(Indichi un numero)

Per nulla ..…… 1
Leggermente …… 2
Un po' ……... 3
Molto ….…….. 4
Moltissimo ……... 5

7. Quanto dolore fisico ha provato nelle ultime 4 settimane?

(Indichi un numero)

Nessuno ...…… 1
Molto lieve ..…… 2
Lieve …….. 3
Moderato ….…… 4
Forte ……..……... 5
Molto forte ……... 6

8. Nelle ultime 4 settimane, in che misura il dolore L'ha ostacolata nel lavoro che svolge abitualmente (sia in casa sia fuori casa)?

(Indichi un numero)

Per nulla ..……	1
Molto poco ..……	2
Un po' ……..	3
Molto …..…..……	4
Moltissimo ……...	5

9. Le seguenti domande si riferiscono a come si è sentito nelle ultime 4 settimane. Risponda a ciascuna domanda scegliendo la risposta che più si avvicina al Suo caso. Per quanto tempo nelle ultime 4 settimane si è sentito…

(Indichi un numero per ogni domanda)

	Sempre	**Quasi sempre**	**Molto tempo**	**Una parte del tempo**	**Quasi mai**	**Mai**
a. vivace brillante?	1	2	3	4	5	6
b. molto agitato?	1	2	3	4	5	6
c. così giù di morale che niente avrebbe potuto tirarLa su?	1	2	3	4	5	6
d. calmo e sereno?	1	2	3	4	5	6
e. pieno di energia?	1	2	3	4	5	6
f. scoraggiato e triste?	1	2	3	4	5	6
g. sfinito?	1	2	3	4	5	6
h. felice?	1	2	3	4	5	6
i. stanco?	1	2	3	4	5	6

10. Nelle ultime 4 settimane, per quanto tempo la Sua salute fisica o il Suo stato emotivo hanno interferito nelle Sue attività sociali, in famiglia, con gli amici?

(Indichi un numero)

Sempre .....…… 1

Quasi sempre ...…… 2

Una parte del tempo …..…….. 3

Quasi mai ..…….. 4

Mai ……...……... 5

11. Scelga la risposta che meglio descrive quanto siano **VERE** o **FALSE** le seguenti affermazioni.

(Indichi un numero per ogni affermazione)

	Certamente vero	**In gran parte vero**	**Non so**	**In gran parte falso**	**Certamente falso**
a. Mi pare di ammalarmi un po' più facilmente degli altri	1	2	3	4	5
b. La mia salute è come quella degli altri	1	2	3	4	5
c. Mi aspetto che la mia salute andrà peggiorando	1	2	3	4	5
d. Godo di ottima salute	1	2	3	4	5