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Title: **Seasonal fluctuation of multiple sclerosis births in Sardinia.**

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Abstract

Study results from different geographical areas provide some circumstantial evidences that, when compared to general population, people who later in life develop multiple sclerosis (MS) have a pattern of birth excess in spring and late summer, which may disclose an association with MS-predisposing environmental agents. To identify the presence of season-related cluster of MS birth in Sardinia we have designed a case-control study in the province of Sassari, Northern Sardinia, insular Italy, an area at very-high and increasing risk for MS. Mean birth incidence rate of people with MS (810 cases) on a three- and six-months basis were compared to that of two control populations: the MS unaffected siblings (1069), sharing genetic material with patients, and a representative number of births (247,612) of the general population of the study area. We found that the birth in months peaking in spring significantly represents one risk factor for future MS development. This seasonal deviation of MS births reveals an intriguing epidemiological overlap with common environmental agents, which may open a new scenario of hypothetical explanations for environmental factors perhaps affecting the CNS at the crucial time of myelination or shaping the newborn immune system.

Key words: multiple sclerosis, month of birth, seasonality, risk factors, Sardinia

Introduction

Multiple sclerosis (MS), a chronic demyelinating disease of the central nervous system (CNS), is generally featured by an intermittent clinical course often even in the context of progressive forms [13]. Intensive researches over decades led to the identification of convincingly strong non-Mendelian multigenic interactions in MS susceptibility [6]. However, circumstantially good reasons such as the seasonality of MS-related events [12] also suggest MS to be influenced by environmental factors and the term "multifactorial" best fits with the definition of the enigmatic etiology of the disease [31].

Study results [12] indicate fluctuation of MS-related events (onset and exacerbation) to present at variable frequencies being the highest in spring and the lowest in winter. In theory, identifying seasonal birth clusters of individuals who later in life develop in MS may reveal an overlap with specific environmental agents and create new working ideas on maternal- or newborn-acting predisposing factors. Seasonal birth studies exist for many neurological diseases; in some, perinatal external events unlikely represent major factors in determining MS susceptibility [33] whilst in others MS shows a pattern of birth excess in spring or late summer [4, 10, 25, 32, 36] and a significant nadir in november [36] which, along with a geographical interaction [32], a strong maternal effect [8, 24] and a higher MS rate of dizygotic twins as compared to siblings [35] suggest the presence of environmental factors influencing the CNS during gestation or after birth, at the crucial time of myelination.

Sardinia, an Italian island in the Mediterranean basin, bears a very-high and increasing MS risk over time [20, 21]. Although a genetic influence is thought to have the predominant role [16], past and recent evidences suggest environment to be also influential in determining MS temporal increase in this island [21, 23, 29-31]. To this purpose, we analysed on a case-control basis the seasonality of Sardinian MS births as compared to that of two control populations: the MS siblings, sharing genetic material with patients, and the general population of the Northern province of Sassari (study area). Continuous traits such as the age at MS onset and the rate of conversion toward a progressive course were also quantitated in relation to the period of MS patients' birth.

Methods

Study area

The study population belongs to the province of Sassari, Northern Sardinia, insular Italy (population of 440,517 on 2001 Census with negligible migration inflow and outflow, extension 7,520 Km^2 between latitudes 40°30’ and 41°N). The Sardinian climate is influenced by the sea and the latitude. Summer in Sardinia is hotter but less sultry and much more windy then the continental Tyrrhenean areas with an average maximal temperature around 30°C. During the winter the average maximal temperature is around 15°C and the temperature rarely decreases under 0°C. Precipitations are less abundant than in the centre-northern Thyrrenean areas and the average annual rainfall is much lower. Heavy drought phenomena are a normal characteristic of Sardinian climate, sometimes occurring on unexpected intervals several times during a decade.

Patients

Case ascertainment is based on a MS Register created in 1995 at the Institute of Clinical Neurology, University of Sassari, in addition to several reporting sources from other Sardinian neurological institutions, national MS Society (AISM) local files, relevant extraregional MS centers, and general practitioners in the province [22]. All patients included in the Register have been thoroughly investigated to rule out other immuno-mediated, infectious and post-infectious diseases of the CNS and meet the diagnostic criteria for definite and probable MS [18]. For each index patient, anagraphic, genealogical and clinical informations (symptoms and date of MS onset, date of MS diagnosis, classification, disease course and disability) are available. All MS patients known to the Register were contacted either during a follow-up visit to the Clinic or by telephone to explain the study and request participation.

Controls

<u>MS siblings</u>: MS patients agreeing to participate allowed collection of anagraphical data to enumerate the months of birth of all the sibs of index cases, including male and females. <u>General</u>

population: the second control population was obtained from the Statistical bulletin of the ISTAT, the Italian Statistical Bureau, section of Cagliari (Sardinia) and all available data on the monthly distribution of births in the province of Sassari for the general population, including males and females, were obtained for the 35-year period 1955-1989.

Data analysis

The analysis of the possible different birth distribution during selected month/periods between MS patients, their sibs and the general population, was performed through the use of Epi Info, Version 6, Database and Statistics Software Program, CDC/WHO, Atlanta, GA. Briefly, the number of month/period births of the populations under study were compared by using the 2 x 2 table for χ^2 with Yate's correction, as follows: A: number of MS cases within a given at-risk month/period; B: number of controls (siblings and/or general population) in the same at-risk month/period; C: number of MS patients outside the at-risk period; D: number of controls outside the at-risk period. Statistical significance was established for one-sided P value <0.05.

For the primary purpose of comparing our data with those of other published studies, expecially in the Mediterranean area [25] and in other very high-risk areas such as Scotland [36], initially four consecutive 3-month seasons (winter, spring, summer and autumn) were considered and defined by taking spring as beginning in April; in addition, and with the aim of further verifying the influence of temperature and climate on MS appearance, the analysis of the birth proportion was performed on a six-month basis by considering the first semester from April until September (temperate half-year composed of spring and summer) and the second from October until March (cold half-year, autumn and winter).

As secondary outcomes of the study we were interested in determining whether the distribution of MS births in female was different from that of male MS patients, whether the rate of conversion to progressive MS was influenced by the time of birth and whether the age at MS onset was in relation to season of birth. Given the absence of available data in the literature, the analysis was performed without an *a priori* rationalisation of pre-defined time intervals by using the 2 x 2 table for χ^2 with Yate's correction.

Results

Case and control populations

Cases: Data regarding month of birth, age at onset and rate of conversion toward a secondary progressive course were obtained from our case Register. MS patients who agreed to participate and collect anagraphical data (months of birth) of their siblings were 381, of whom 291 females and 90 males (ratio 3.2). This MS population will be called MS-1 from now onward. The whole population of the Register (called MS-2) comprises 810 cases (587 females and 223 males, ratio 2.6). The remaining 429 MS patients who did not provide sibling information were also separately investigated for their birth distribution rate and compared with MS-1 and MS-2 populations; they are referred as MS-3 from now onward.

With the different composition given as regarding the sex ratio, MS-1 and MS-2 populations were adjusted to the standard population of siblings on a three-month basis as shown in Figure 1. No differences in birth incidence proportion was found between standard and adjusted MS populations after stratification by sex.

FIGURE 1.

Controls: Siblings of MS-1 were 1069 (517 females and 552 males; ratio 0.93; called Sib-1 from now onward), whilst the available general population of the province consisted of 247,612 Sardinian natives born between january 1955 and december 1989, of whom 119,380 females and 128,232 males (ratio 0.93). Since results of the comparative study can be biased by the over-matching of many unaffected siblings to their affected case, we created an *ad hoc* Sib-2 population composed of only one unaffected sibling per matched MS case, by considering only the 381 unaffected control closest in age with the MS-1 case. As for the general population, with the aim of removing the normal seasonality of births derived from the varying number of days in each month/period considered, we calculated for each season the ratio of observed/expected control births, which gave the following results: 1.023 for winter, 0.99 for spring, 1.028 for summer and 0.958 for autumn.

Season of birth

The mean birth incidence rates derived from the analysis of the actual number of the three MS patient populations compared to siblings (Sib-1) and general population (Pop) of the province of Sassari are shown in Figure 2. The seasonal birth proportion clearly shows that the three MS population under study (MS-1, MS-2 and MS-3) have an excess of births in spring (29.4, 27.7 and 29.1% respectively) when compared to both siblings (22.1%; P=0.008, 0.009 and 0.007, respectively) and general population (24.6 %; P=0.036, 0.039 and 0.035, respectively). Not unexpectedly, siblings show a mirror-effect of birth defect in the same period when compared to general population (P= not significant).

FIGURE 2

The slight differences between the MS-1, -2 and -3 populations are not statistically significant (not shown). Therefore, considering that the two MS groups that gave (MS-1) or did not give (MS-3) sibling's birth dates are not significantly different, we continue to report observations taken from the MS dataset of 810 patients (MS-2; case Register of the province). Figure 3 shows the seasonal proportion of births of MS-1 and the two siblings populations Sib-1 and Sib-2, the latter (bold dotted line) being represented by 381 unaffected siblings closest in age with their MS siblings. As compared to the extended sibling population, the Sib-2 population also display a nadir in spring (22.2%) which is significantly different with respect to their matched MS cases (MS-1; P=0.01). Thus, as for the sibling population we continue to report observations taken from the whole dataset of siblings (Sib-1).

FIGURE 3

Three- and six-months period of birth and the influence of sex.

Birth incidence proportions, stratified by sex and analysed on a three- (seasonal, Fig. 4A) and six-month basis (Fig. 4B) are indicated in Figure 5. As already shown in Figures 2 and 3, MS-2 population significantly differ from the Sib-1 population when the seasonal birth rate is considered. When stratifying the MS dataset by sex, although also present in the female MS population, this effect prevails in the male MS subgroup (P=0.01). As for the six-month proportion, there is a significant MS birth excess during the more temperate semester from April to September (referred as spring and summer on a seasonal basis) both in the all MS-2 dataset (54,35% vs. 45,65%, P=0.005) and in the sex subgroups (P=0.006 and 0.01 for male and female groups, respectively). However, when comparing the six-month birth rates with those of Sib-1, this deviation is minor for the female (P= not significant) and significant for the male subgroup of MS-2 population (P=0.03). Nevertheless, a direct comparison of the female and the male birth rates calculated on a season or semester basis showed no significant differences (not shown).

FIGURE 4

Figure 5 shows that, when calculated on a four-month basis, the deviation of the MS birth rate (38,2%) from the Sib-1 birth proportion (31,3%, P=0.001) is significant for both males (P=0.048) and females (P=0.01) MS-2 patients in the hottest four-months interval overlapping spring and summer (from May until August); the percentage of births of the general population in this time interval is 34,3% (not shown in figure 6).

FIGURE 5

MS births and the general Sardinian population.

We have used the general population of the province of Sassari born between 1955 to 1989, the only available at the Italian Statistical Bureau, Sardinian section of Cagliari, though we are aware that many prevalent MS cases of our dataset were born before this time. In such a 35-year period the population structure has, not unexpectedly, remained stable in ethnic composition, sex distribution and month of birth. On the contrary, we asked whether the proportion of months/season of birth of earlier MS population differs from that of more recent MS cases. To answer this question we compared the distribution of MS people born in the three decades from 1955 to 1984 with those of the relative general population in the same years. For reasons related to the low number of annual MS births, the six-month periods were studied and obtained by considering the first temperate semester from April until September (spring and summer) and the second from October until March (cold semester, autumn and winter). Figure 6 shows that, though some fluctuations are present, the matched proportion of births in each selected year has remained rather stable in both populations, being the birth proportion of people who later in life develop MS deviated toward the hotter semester that includes spring and summer.

FIGURE 6

MS birth, age at onset and progression rate

The MS population from the case register (MS-2) was also studied for the age at clinical onset of disease in relation to the period of birth: figure 7A clearly shows that people born in the hotter six-months period from April to September and who will lately develop MS are significantly (P=0.005) more likely of having a higher age at MS onset as compared to those born between October and March. On the contrary, no differences are evident when the conversion rate toward a secondary progression is considered on a three-month interval as shown in figure 7B.

FIGURE 7.

Discussion

Reasonably good data exist supporting an influence of environment in the MS genesis. Common viral infections are documented as being highly correlated with MS exacerbations since 1985 [9, 17, 28]; epidemics of MS have been described in North Atlantic islands and migrants from low to higher MS-risk areas increase their risk starting from age 11 [14]. What is virtually transmitted in such cases is still obscure but clearly not an obvious transmissible factor during adulthood given the similar rate of MS among spouses of MS patients compared to that of the general population [7].

Geographical distribution of MS describes areas at high (northern Europe), medium (Mediterranean basin) and low frequency (Africa) [14, 21]. Not in line with this geo-ethnical gradient is the case of Sardinia. In this genetically distinct population [5, 27, 31] MS incidence has increased in the last 40 years resulting among the highest rates in the world despite the location within the Mediterranean basin [20-22] perhaps as a genetic result of founder effect [16]. However, since a temporal cluster of MS in this island occurred after the breaking of historical isolation [23] and four decades are too short a lag for any change of Sardinians’ genetic pool to occur, an explanatory environmental influence should not be *a priori* rejected [29-31].

In the present study we found strong evidence that the month(s) of birth may represent one of such non-genetic MS-predisposing factors with a significant excess during spring. The use of two distinct, genetically related (siblings) or not-related (general population), control populations has reinforced the finding which is different from that of Salemi and collaborators in Sicily, another Mediterranean island [25]. In this paper, the distribution of births in MS and general populations was not different when tested by the χ^2 test, while the Hewitt's non-parametric test for seasonality showed a controversial excess of MS births in the six-month period between June to November; this finding led the authors concluding that the deviation pattern of MS birth in Sicily is different from that of Northern-Europe perhaps due to the different latitude [25]. On the contrary, our findings are similar to those found in Northern Europeans [36] which may, not surprisingly, indicate that besides different latitude and genetics, environmental MS-risk factors peaking around birth are commonly distributed among different populations. This seasonal cluster reveals an epidemiological overlap with specific environmental agents which may act during the spring season perhaps affecting the CNS myelination or shaping the newborn immune system. Obviously, it cannot be excluded that these unknown agents are operating during gestation at an earlier season (e.g. winter) or, after birth, at a later season (e.g. summer) (Figure 8).

FIGURE 8

This newly-discovered environmental effect in Sardinian MS is associated with a delayed age at clinical onset of MS but not related to the rate of conversion toward a progressive course of the disease. Age at onset is considered among one of the poor prognostic factors for the subsequent MS course [13] and this may open a new scenario of hypotheses on the disabling effect that such environmental factors are able to cause in the long run.

A well-detailed study in pediatric MS recently indicates that while seropositivity for most viruses is similar between MS and controls, a highly significant difference in EBV infection rate is present [1]. Others found a significant relation with mumps and measles after 15 years of age [11]. This may support the idea that individuals who suffered from early infectious mononucleosis and late infection with other common viruses may bear increased MS risk in a infection-reinfection hypothetical model. Thus, if we admit MS having a complex set of initiating factors, then we would not expect randomly chosen MS samples to show consistent positivity for any particular microorganism. In fact, anti-myelin T cells or autoantibodies could be elicited by molecular mimicry based on conformational similarity between epitopes of many infectious agents and self myelin antigens [3, 15, 37].

In conclusion, our data support the rational hypothesis that MS may be induced in genetically susceptible individuals by a first sensitization process occurring early in life (during gestation, at birth, after birth?), and a secondary autoimmune response against self-myelin due to a later (re)exposure [2]. Along with viral agents, cross-reacting pollen-derived allergens also peaking in spring might additionally be influential in the genesis of autoimmunity to self-antigens and potentially implicated in MS onset (Figure 8) [26].

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Legend to the figures

Figure 1. X axis indicates the three-months periods starting from January (Winter= January to March). On left Y axis crude rates of MS population; on right Y axis rates adjusted to standard population.

Figure 2. On Y axis, mean percentage of birth incidence. Three-month (seasonal) rate (winter= January-March). MS-1= MS population participating to data collection (n. 381); MS-2= MS patients from the case Register (810); MS-3= MS patients not providing sibling data; Sib-1= siblings of MS-1 (1,069); Pop= general population of the province of Sassari born between 1955 to 1989 (247,612). Differences are analyzed by χ^2 and significant P values indicated.

Figure 3. On Y axis, proportion (%) of birth. Seasonal (three-month interval starting from winter, i.e. January-March) birth rate in MS-1, Sib-1 (abbreviations and statistics as for Figure 3) and Sib-2 population (n. 381) composed of only one unaffected sibling per matched MS case by considering only the 381 unaffected control closest in birth order with the MS-1 case.

Figure 4. On Y axis, mean rates (%) of birth incidence. A) Three-months, seasonal periods are considered, starting from January-March, i.e. winter. B) Six-months periods (April-September, dark grey; October-March, light grey); Abbreviations and statistics as for Figure 3.

Figure 5. On Y axis, proportion (%) of birth. Four-months periods, starting from january (J-A= January-April) are considered. Abbreviations and statistics as for Figure 3.

Figure 6. On Y axis, percentage of births on a six-month interval basis (in gray the temperate semester from April until September; in blank the second from October until March). The proportion of births in each selected decade remained stable in both the MS and the general population (Pop) and differences are not statistically significant.

Figure 7. A) Patients born in the six-month period April-September have an older age at disease onset as compared to patients born in the semester October-March. B) The percentage of progressive cases shows no seasonal difference.

Figure 8. Pollens and viruses accumulate during spring in Sardinia. Abbreviations: U= urticacee; G: graminacee; O: Oleacee; F: fagacee; Abbreviations for virus: HSV: human herpers simplex; EBV: Epstein-Barr virus; CMV: cytomegalovirus; VZ: varicella-zoster; RSV: respiratory syncitial virus. Adapted from both AIA-Italy; http://www.isao.bo.cnr.it and Costello M, Yungbluth M: Viral Infections. In Henry JB: *“Clinical Diagnosis and Management by Laboratory Methods, 20th Ed”* Saunders, 2001.