Multiple Sclerosis in Twins from Continental Italy and Sardinia: A Nationwide Study

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Knowledge about the balance between heritable and nonheritable risk in multiple sclerosis (MS) is based on twin studies in high-prevalence areas. In a study that avoided ascertainment limitations and directly compared continental Italy (medium-prevalence) and Sardinia (high-prevalence), we ascertained 216 pairs from 34,549 patients. This gives a twinning rate of 0.62% among MS patients, significantly less than that of the general population. In continental Italy, probandwise concordance was 14.5% (95% confidence interval, 5.1–23.8) for monozygotic and 4.0% (95% confidence interval, 0.8–7.1) for dizygotic twins. Results in Sardinia resemble those in northern populations but in limited numbers. Monozygotic concordance was 22.2% (95% confidence interval, 0–49.3) probandwise, but no concordant dizygotic pairs were identified. A questionnaire on 80 items possibly related to disease cause was administered to 70 twin pairs, 135 sporadic patients, and 135 healthy volunteers. Variables positively (7) or negatively (2) associated with predisposition and concordance in twins largely overlapped and were mainly linked to infection. If compared with previous studies, our data demonstrate that penetrance in twins appears to correlate with MS prevalence. They highlight the relevance of nonheritable variables in Mediterranean areas. The apparent underrepresentation of MS among Italian twins draws attention to protective factors, shared by twins, that may influence susceptibility.

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Multiple sclerosis (MS) is a disease of the central nervous system with inflammatory and degenerative components.[1] No causative factor has yet been identified. The variety of clinical and pathological manifestations of MS may reflect causative heterogeneity at the individual level.

Twin studies can assess the relative contribution of genetic and environmental causative factors to the cause of multifactorial diseases.[2] Assuming that twins share similar uterine and early life environments, greater concordance for a disease in monozygotic (MZ) than in dizygotic (DZ) twins would reflect a role of genetic factors in the cause of a disease. By contrast, similar concordance rates between MZ and DZ twins, or discordance within MZ twins, implicate nonheritable factors.

Twin studies in MS have consistently demonstrated a higher disease concordance in MZ twins, highlighting the importance of genetic factors.[3–9] These studies have been performed in high-prevalence areas. However, twin concordance has been found to be lower in one medium-prevalence country.[10] The apparent discrepancy is not explained.[11] Differences in methodology may apply, but it is plausible that in different populations both heritable and nonheritable factors contribute in different proportions and ways to MS

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risk. This hypothesis[9] would extend the concept of causative heterogeneity from individual to population.

In Italy, the world's largest twin registry is available for medical research. (All Italian citizens receive, for fiscal purposes, an alphanumeric code that identifies the individual's surname, first name, and date and place of birth. Individuals with the same family name and place and date of birth are potential twins; this has made it possible to create a nationwide registry of 1,600,000 individuals.[12]) The matching of this registry with the records of the Italian MS clinics and those of the Italian MS Society (Associazione Italiana Sclerosi Multipla [AISM]) has allowed us to conduct a study that ascertained virtually all patients and all twins in a nation with a population of about 57,000,000 inhabitants, where medium- (continental Italy) and high-prevalence (Sardinia) areas can be directly compared. This design reduced the likelihood of selection bias because twin status and presence in the twin registry was used to ascertain twins with MS.

Twin studies in MS, as well as in other multifactorial diseases, have largely focused on factors predisposing to disease. The ascertainment procedure of this study allowed the assessment of the twinning rate in the Italian MS population. The twinning rate was used as a sensor of the influence of factors, predisposing and protective, shared by the cotwins.

Investigations on the effects of environmental risk factors on the development of disease[13] can be performed using a cotwin control design. This provides matching on many known and unknown factors otherwise not possible. We therefore investigated nonheritable risk factors in this population by collecting information about possible causative variables. The results of the cotwin control study were then verified on a population of sporadic cases and matched healthy control subjects.

Materials and Methods

Ascertainment and Characteristics of Twins

The ascertainment period was January 1998 to December 2002, and the following steps were pursued: (1) request of surname, first name, and date and place of birth of patients from Italian MS clinics and AISM; (2) identification of "potential twins" by matching these lists with the Italian Twin Registry (ITR)[12,14]; and (3) communication of the names of "potential twins" to the MS clinics or AISM for twin status confirmation.

Patients were contacted by their own neurologist to obtain relevant information and minimize intrusion. With few exceptions, an MS neurologist confirmed the diagnosis of the index case (probable or definite MS, Poser criteria),[15] visited cotwins reporting neurological symptoms but not already followed, and collected the demographic and clinical data. Zygosity was assessed by means of a validated questionnaire on similarity, a reliable method in large twin populations.[16–18]

We did not deem it justifiable to subject the asymptomatic cotwin of an MS patient to the stress of investigations. These individuals often already perceive a condition of "shared risk," and previous surveys have shown that magnetic resonance imaging data have a low yield in asymptomatic twins, may be difficult to interpret, and can have insurance implications.[7,19,20]

Investigation of Nonheritable Risk Factors

A structured 80-item questionnaire was implemented to investigate environmental causative factors. This included childhood diseases, timing of infections, exposure to putative infectious agents, early life events, medical and surgical history, diet, vaccination schedule, and various other items. Sex was also included as a "positive control." Infections of unknown origin were classified as "perinatal" (occurrence in the first 2 years of life), "early" (from the 3rd year of life until the end of the 11th year), and "late" (from the 12th year of life until the age of onset of the disease).

The questionnaire was sent to the twin pairs who agreed to be interviewed and who had an informant able to provide data on early-life events. A week later, the twins and the informant were interviewed by a neurologist (S.C.). The same procedure was performed in a consecutive series of MS cases and healthy control subjects matched for sex and age.

Statistical Analysis

Comparison between means was performed by Student's t test for independent samples. Statistical analyses of differences between observed and expected frequencies for categorical variables were performed through χ^2 tests; point estimates of odds ratios and corresponding 95% confidence intervals (CIs) were also calculated.

MS concordance was assessed separately for MZ and DZ twin pairs using pairwise and probandwise concordance rates. Pairwise concordance rate is a descriptive statistic and gives the proportion of affected pairs that are concordant for the disease. Probandwise concordance rate is the preferred measure because it represents the probability that a twin in a pair is affected given that his or her cotwin is affected: This probability is estimated by the proportion of all probands that belong to concordant pairs and is informative of the recurrence risk for disease associated with the degree of the relationship of the pair.[21] Probandwise concordance rate can be interpreted as the prevalence of disease in cotwins of probands and can be compared directly with the disease prevalence in the background population and with estimates of recurrence risks in other types of relatives.

We also estimated the tetrachoric correlation that, unlike concordance, uses the information from the unaffected pairs, thus taking into account twin similarity for both disease and health status. The number of concordant unaffected pairs was reconstructed as the difference between the actual number of twin pairs in the ITR and the number of ascertained pairs with at least one affected member. Under the so-called liability threshold model, there exists an underlying continuous variable (liability) that is normally distributed with a threshold that discriminates the unaffected from the affected individuals: The tetrachoric correlation is the correlation in twins for this underlying liability.[22] Within a *structural equation* approach, the total phenotypic variance in the trait of

interest is partitioned into the following: (1) genetic variance, that is, the variance due to genetic factors; (2) common environmental variance, due to exposures shared by the twins; and (3) unique environmental variance, attributable to influences that are not shared by the twins.[23] The proportion of the total phenotypic variance that is explained by the genetic variance is referred to as *heritability*. The tetrachoric correlations in the MZ and DZ groups and the variance components were estimated with the software Mx (University of Richmond, VA).[24]

Logistic regression analyses were performed to evaluate the independent contribution of demographic variables on the risk for disease concordance. The sampling unit was the twin pair and the concordance for MS with the outcome variable. The regression analyses were performed with STATA (release 6.0; STATA, College Station, TX).

The twinning rate in the Italian MS population (number of pairs identified/number of patients screened) was compared with the twin rate in the general Italian population, obtained from the national data on multiple births and mortality rates of the Central Institute of Statistics (ISTAT) in the calendar period from 1935 to 1983 (representative of the age range of our series of twin pairs with MS).[25–27]

The data on risk factors collected from twin pairs, as well as those from MS cases and control subjects, were analyzed separately using logistic regression models with stepwise forward selection procedures adopted to identify only the best subset of covariates for MS prediction. The selection processes were based on formal likelihood ratio test procedures to identify covariables with relevant effect on MS development and on its concordance within twin pairs. Because measures corresponding to individuals from the same twin pair cannot be considered independent of each other, we used a class of statistical techniques that extends the standard logistic approach, by considering each twin pair as an individual cluster. First, we simply adjusted the estimate of covariances for parameter estimates by using a robust "sandwich" estimator; second, the logistic approach was further generalized by adding a Gaussian random effect accounting for potential heterogeneity among twin pairs and dependence between twins in the same pair. Model parameters have been estimated using both a generalized estimating equation and a maximum likelihood approach. No substantial differences between results (for parameter estimates significance) obtained through either method have been observed; thus, this article focuses mainly on the maximum likelihood approach. All analyses were performed with STATA (release 7.0).

Results

Identification of Twins

The database of 73 MS clinics (uniformly distributed throughout Italy) were used together with that of the AISM. The cohort of 33,589 patients identified was matched with the ITR.[12,14] This procedure identified 372 individuals who were expected to be affected twins. In 25 cases, the MS reference clinic was unable to contact the index case to confirm/exclude the twin status. Among 347 patients traced by the MS clinics, 129 individuals denied and 218 confirmed their twin status ("confirmed twins"). The difference between the number of potential twins and the number of confirmed twins was expected, because there is a 25% excess of potential twins in the ITR (individuals who share the same family name and place and date of birth without being twins, a relatively frequent circumstance in individuals with common family names). In addition, we had to consider as potential twins those individuals whose demographic information from the MS clinics was not complete (ie, patients who shared the same family name with no information about place or date of birth, or both). In fact, among 129 patients who denied their twin status, 87 of 347 patients (25%) shared family names and date and place of birth with unrelated individuals in the Italian twin registry, and 42 had been screened as potential twins only because of shared family names, whereas information about place or date of birth, or both, was lacking. The matching procedure proved to be highly accurate because it did not miss any of the 40 twin sets already known before the start of the study (from research files of single MS clinics that used database systems [eg, EDMUS][28] that included the information on twin status). Indeed, this procedure allowed the detection of additional previously unidentified twins in the databases used. Of the 218 individuals who confirmed their twin status, 16 belonged to twin pairs that, owing to disease concordance (see later), had been identified twice. Therefore, 210 twin pairs were ascertained (192 from continental Italy and 18 from Sardinia). Moreover, six discordant twins from continental Italy, not present in the databases of MS clinics or AISM, were reported directly by MS specialists. We eventually identified 216 twin pairs (198 from continental Italy and 18 from Sardinia) in 32,039 patients from continental Italy and 2,510 from Sardinia. The total of 34,549 patients (31,989 from MS clinics, 1,600 from AISM database, and 960 from MS specialists [an estimate based on the twin rate observed: 210/33,589]) corresponds to virtually the entire Italian population of MS patients. In fact, according to the largest studies on disease prevalence in continental Italy, the estimated prevalence is 61.1 (95% CI, 56.7–65.5) per 100,000.[29–37] As the population in continental Italy is 55,958,145 (Central Institute for Statistics [ISTAT], 1999), the expected number of cases is 34,213 (95% CI, 31,603–36,653), which closely approximates the 32,039 patients considered for this study. The reported prevalence of MS in Sardinia is 147.1 (95% CI, 138.4–155.8) per 100,000.[38,39] Considering a population of 1,654,470 (Central Institute for Statistics [ISTAT], 1999), the number of expected cases (2,434; 95% CI, 2,290–2,578) is in keeping with the number of patients we screened (n = 2,510).

Table 1. Demographic Characteristics of Italian Twins with Multiple Sclerosis

	Pairs identified			Mean age ± SD			F/M ratio		
Group	MZ	DZ	DZ/MZ	MZ	DZ	All	MZ	DZ	All
Italian study (total)	59	157	2.6	39.0 ± 11.5	42.3 ± 11.6	41.3 ± 11.7	2.05	1.77	1.84
Continental Italy	51	147	2.8	37.3 ± 9.9[a]	42.4 ± 11.8[a]	41.1 ± 11.5	2.12	1.74	1.82
Sardinia	8	10	1.25	44.2 ± 14.6	42.3 ± 12.3	43.2 ± 13.0	1.66	2.33	2.00

[a]MZ vs DZ (continental Italy) $p = 0.005$

MZ = monozygotic; DZ = dizygotic.

Population Characteristics

Table 1 summarizes the demographic characteristics of the twins. In continental Italy and Sardinia, the DZ/MZ ratios of twins with MS differed and were respectively higher (2.8:1) and lower (1.25:1) than expected in the general population (2:1). The comparison of the mean ages did not indicate any significant differences, except for that between DZ and MZ twins in continental Italy ($p = 0.005$). Overall, the age distribution of twins in our series was similar to that reported for all patients in Sardinia[39] and in continental Italy[35,36] (data not shown). Female/male ratios were within the expected values (1.5–2.6)[40] both in continental Italy and in Sardinia.

Twin Rates

The twin rate in the Italian population of patients with MS was 0.62% (216 pairs from a population of 34,549 patients; 95% CI, 0.55–0.64), which is significantly less ($p < 0.0000001$) than the twin rate of the general population (1.0%; see Statistical Analysis earlier in article). The low Italian twin rate reflected a marked decrease in MZ and, to a lesser extent, in DZ twins in continental Italy and a decrease in DZ twins in Sardinia, where the number of MZ twins nearly approached the expected figure (Table 2). No significant difference emerged when we calculated the twin rates separately for northern, central, and southern Italy and Sardinia (not shown).

Table 2. Twin Rates (grouped for zygosity and total) in Italian Patients with MS, Compared with Twin Rate of the Corresponding General Populations (in parentheses)

Group	MZ	DZ	Total
Continental Italy	0.15 (0.33[a])	0.46 (0.67[a])	0.61 (1.0[a])[b]
Sardinia	0.32 (0.36)	0.40 (0.74)[c]	0.72 (1.1)[d]
Italian study (total)	0.16 (0.33)	0.46 (0.67)	0.62 (1.0)[b]

[a]Italian twin rate calculated from birth and mortality rates of Italian twins collected by the Central Institute of Statistics.[25–27]
[b]$p < 0.0000001$;
[c]$p < 0.003$;
[d]$p < 0.0003$.

Disease Concordance and Heritability

In Italy, taken as a whole, disease concordance was 5 of 59 for MZ twins (pairwise: 8.4%, 95% CI, 1.3–15.4; probandwise: 15.6%, 95% CI, 6.7–24.4) and 3 of 157 for DZ twins (pairwise: 1.9%, 95% CI, 0–4; probandwise: 3.7%, 95% CI, 0.8–6.6). In continental Italy, disease concordance was 4 of 51 for MZ twin pairs (pairwise: 7.8%, 95% CI, 0.4–15.1; probandwise: 14.5%, 95% CI, 5.1–23.8) and 3 of 147 for DZ twin pairs (pairwise: 2.0%, 95% CI, 0–6.6; probandwise: 4.0%, 95% CI, 0.8–7.1). In Sardinia, concordance was 1 of 8 in MZ twins (pairwise: 12.5%, 95% CI, 0–35.4; probandwise: 22.2%, 95% CI, 0–49.3) and 0 of 10 in DZ pairs (Table 3). In continental Italy, the sample size was large enough to apply a structural equation approach, under a liability threshold model: The estimated tetrachoric correlations for MS were 0.77 (95% CI, 0.61–0.88) in MZ and 0.53 (95% CI, 0.34–0.67) in DZ pairs; the heritability estimate was 0.48 (95% CI, 0.06–0.86), whereas the environmental contribution was 0.29 (95% CI, 0–0.60) for shared and 0.23 (95% CI, 0.12–0.39) for unique (individual-specific) environmental factors.

A multivariate analysis was performed to search for associations between demographic characteristics and disease concordance (Table 4). A significant association, adjusted for sex and age, between concordance and zygosity emerged (OR, 5.38; 95% CI, 1.21–23.89). An excess of female concordant pairs, which was not statistically significant (OR, 1.72; 95% CI, 0.33–8.99), was observed among both MZ and DZ twins.

Investigation of Nonheritable Risk Factors

The questionnaire on nonheritable risk factors was administered to 70 twin pairs (36 MZ, 34 DZ [20 pairs were the same sex]), to 135 sporadic MS patients, and to an equal number of healthy control subjects matched for age, sex, and ethnic origin. Four twin pairs were concordant (3 MZ, 1 DZ) and 66 were discordant for MS (33 MZ, 33 DZ). The female/male ratio was 1.8:1 for the twin pairs and 2.1:1 for sporadic MS patients. Mean age was 39.24 (±9.8) years for MZ, 37.42 (±10.8) years for DZ, and 40.6 (±12.6) years for sporadic MS patients.

Table 3. Pairwise and Probandwise Concordance in Italian Twins with Multiple Sclerosis

	Pairwise		Probandwise	
Group	MZ	DZ	MZ	DZ
Italian Study (total) (CI 95%)	8.4% (5/59) (1.3–15.4)	1.9% (3/157) (0–4)	15.6% (10/64) (6.7–24.4)	3.7% (6/160) (0.8–6.6)
Continental Italy (CI 95%)	7.8% (4/51) (0.4–15.1)	2.0% (3/147) (0–6.6)	14.5% (8/55) (5.1–23.8)	4.0% (6/150) (0.8–7.1)
Sardinia (CI 95%)	12.5% (1/8) (0–35.4)	0	22.2% (2/9) (0–49.3)	0

MZ = monozygotic; DZ = dizygotic; CI = confidence interval.

Covariables positively associated with MS development were early infections, breast-feeding, herpes virus infections (type 1, type 2, and zoster), red measles, and allergy (environmental, food, or drugs). Negatively associated variables were mumps and milk consumption (Table 5).

A logistic analysis was also performed considering disease concordance as the primary outcome. It confirmed the positive association of early infections, breast-feeding, herpes virus infections, red measles plus two other variables, late infections and whooping cough, and the negative association of mumps and milk consumption (Table 6). The analysis of sporadic patients and healthy control subjects confirmed the positive association with early life infections and red measles (results not shown).

Discussion

Twin studies conducted in Northern Europe and North America, where MS prevalence is high, consistently agree on an excess of concordant pairs among MZ twins compared with DZ twins.[3–9] Pairwise concordance rates for MZ twins range from 21 to 40%, whereas those for DZ twins are around 5%. In Italy, pairwise concordance is lower than in high-prevalence countries, though the difference between MZ and DZ twins remains. Despite the different recruitment methods, pairwise concordance in MZ twins is similar to that reported in France,[10] where disease prevalence is comparable with that in continental Italy. Probandwise concordance among MZ twins is lower in Italy (15.6% and 14.5% for continental Italy) compared with Canada (25.3%). Our heritability figure and those estimated using prevalence data[41] extend and confirm differences in genetic susceptibility among diverse geographic areas: lower heritability in continental Italy and France (48 and 24%, respectively) compared with United Kingdom and Canada (74 and 86%, respectively). Overall, these findings point to a relation between relative weight of genetic influences and disease prevalence. Concordance figures in Sardinia must be interpreted with caution due to the relatively small sample size. They are compatible with a genetic background that makes for greater risk through the interaction with changes in environment, reflected by a rapid increase in MS incidence and prevalence in recent decades in the island.[39]

Table 4. Multivariate Analysis on Disease Concordance of Italian Twins with Multiple Sclerosis

Variable Category	OR	CI 95%
Sex		
Male	1.00	—
Female	1.72	0.33–8.99
Age		
≤30 yr	1.00	—
31–50 yr	1.99	0.22–18.17
>50 yr	2.74	0.22–33.71
Zygosity		
DZ	1.00	—
MZ	5.38	1.21–23.89

OR = odds ratio; CI = confidence interval; DZ = dizygotic; MZ = monozygotic.

Methodological considerations are unlikely to account for the differences in concordance rates between our study and those conducted in Northern Europe and North America. The concordance rates in this study may be slightly underestimated because we chose to avoid a systematic assessment of the healthy cotwin. However, clinical and magnetic resonance imaging assessment of the 14 unaffected twins who reported symptoms (11 MZ) did not increase concordance, nor did close follow-up of 2 years in the 70 twin pairs (36 MZ) of the cotwin control study. Furthermore, the Canadian studies have shown a strong correlation for age of onset when concordance occurs in MZ twins.[9] Taken together, these observations indicate that clinical and paraclinical examinations,[19,20] as well as longitudinal follow-up,[7,9] have surprisingly little effect on concordance (even when the asymptomatic cotwin has magnetic resonance imaging lesions suggestive of MS). It has been suggested that previous population-based studies may not be free from a bias of overascertainment of concordant pairs and overrecruitment of MZ pairs. This is unlikely given the consistency of the results in all those studies despite different ascertainment methods. A hypothetical source

Table 5. Variables Associated with Multiple Sclerosis Development in Twins (parameter estimates for random effect logistic model with random terms representing twin pairs heterogeneity, maximum likelihood estimation)

Variable	Parameter Estimate	Standard Error	Z[a]	P > |z|	CI 95%
Intercept	−6.05	2.61	−2.27	0.023	−11.32–0.84
Male sex	−1.41	0.71	−1.97	0.049	−2.82–0.00
Mumps	−0.52	0.28	−1.85	0.045	−1.08–0.03
Milk consumption	−0.94	0.48	−1.92	0.054	−1.90–0.02
Herpes	1.52	0.64	2.37	0.018	0.27–2.87
Red measles	0.82	0.43	1.87	0.061	−0.04–1.76
Early infections	1.23	0.39	3.13	0.002	0.47–2.01
Breast feeding	1.93	0.97	1.98	0.047	0.02–3.84
Allergy	1.03	0.56	1.83	0.067	−0.07–2.1

[a]Positive estimates correspond to an increase in the disease risk, whereas negative estimates correspond to a decrease in the disease risk.
CI = confidence interval.

of discrepancy between our own data and those from Northern Europe and North America relies on the possibility that concordance rates increase with the increase of the age of the twins. However, the mean age of MZ and DZ twins in our investigation is within the range of most twin studies. The multivariate analysis points to a possible relation between sex and concordance, suggesting that female twin pairs may share pathogenetically relevant risk factors. This result emerges more clearly from the data of the Canadian Twin Cohort follow-up, where the excess of concordance in MZ twins was primarily due to the female pairs.[9]

Disease concordance in twin studies has been used as an indicator of genetic or environmental factors, shared by twin pairs, that predispose to disease, but they also provide an opportunity to examine shared protective factors. In our Italian sample and in a series of 16,000 pairs of white male twins from the United States,[42] the low concordance and underrepresentation of twins could reflect such shared protection. The underrepresentation of twins is contrary to the expected bias of finding more twins in populations ascertained by the presence of disease because twins are more notable and usually more likely to be referred. It is possible that the underrepresentation of MS among twins in our series reflects a protective influence associated with the phenomena of twinning itself. Indeed, events early in life, perhaps even in gestation, may influence susceptibility of this adult-onset disease.[43,44] Finally, recent data support our findings and provide a third interpretation, not necessarily mutually exclusive, linked to reduced fertility in parents who have offspring with MS.[45] In the Canadian[5,7,9] studies, where the twin pairs identified can be divided by the MS population screened, the twin rates are higher than those of either of the Italian MS populations. This may suggest that the high prevalence of MS in Canada is probably related not only to predisposing factors, but also to a dearth of active protective influences.

Despite the potential of twin cohorts for the identification of nonheritable risk factors in cotwin control investigations, only one study of this kind has been performed in MS in North America.[46] Since its publication in 1982, new statistical approaches have been introduced to optimize the information obtainable from such stud-

Table 6. Variables Associated with Multiple Sclerosis Concordance (parameter estimates for random effect logistic model with random terms representing twin pairs heterogeneity, maximum likelihood estimation)

Variable	Coefficient	Standard Error	Z[a]	P > |z|	CI 95%
Intercept	−2.81	1.52	−1.84	0.065	−5.8 to 0.18
Male sex	−1.90	0.87	−2.18	0.030	−3.6 to −0.18
Mumps	−2.39	1.53	−2.03	0.043	−6.11 to −0.10
Milk consumption	−2.39	0.79	−3.03	0.002	−3.94 to −0.84
Herpes	1.95	1.03	1.89	0.059	−0.07 to 3.98
Red measles	−2.81	1.52	1.84	0.065	−5.81 to 0.17
Early infections	3.0	1.26	2.37	0.018	0.52 to 5.49
Late infections	1.75	0.41	4.19	0.000	0.93 to 2.56
Breast feeding	2.05	0.91	2.25	0.024	0.02 to 3.84
Whooping cough	2.04	0.86	2.38	0.017	0.36 to 3.72

[a]Positive estimates correspond to an increase in the disease risk, whereas negative estimates correspond to a decrease in the disease risk.
CI = confidence interval.

ies.[47,48] In principle, a population such as the Italian one, where environmental risk may be more influential, represents a convenient setting for the identification of nonheritable variables, compensating, at least in part, for the methodological limitations that inevitably accompany such studies. The most important of these is recall bias, which will require additional careful consideration. The cotwin control random-effect logistic analysis of candidate environmental factors in this population of twins detected numerous associations, indirectly supporting the influence of nonheritable factors highlighted by the concordance analysis. Further work is required to determine whether these associations are specific or represent markers for other variables more directly involved in the pathogenesis of the disease (ie, infections may be directly relevant for MS pathogenesis or reflect a defective immune response in these patients; the decision about breast-feeding one or the other twin may be influenced by factors more directly relevant to the pathogenesis of the disease).

In summary, in the Italian population, we find that MZ concordance exceeds DZ, consistent with other MS twin studies. However, the lower concordance levels highlight a correlation between changes in penetrance in twins and MS prevalence. Sardinian data within Italy, although limited in size, once again appear to be distinct, resembling those from northern countries. Surprisingly, a reduced risk for MS is found in twins, suggesting that the reduced population risk, which characterizes much of Southern Europe, may be connected with protective factors that are shared by the cotwins and are particularly active in this geographic area. These results demonstrate components of heterogeneity at population levels in the cause of MS.

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Appendix

The following is a listing of the other participants in the Italian Study Group on Multiple Sclerosis in Twins: Alessandria—M. Melato; Ancona—R. Dellantonio; Aosta—L. Sironi, E. Bottacchi; Ascoli Piceno—M. Signorino, R. Angeloni (Ospedale Murri); L. Curatola, C. Paci (Ospedale Madonna del Soccorso); M. Ragno, G. Sirocchi (Ospedale Civile "C&G Mazzoni"); Asti—A. M. Vurchio, E. Duc; Avellino—D. Spitaleri; Bari—M. Trojano, M. Liguori; Belluno—N. Cimini, G. Moretto; Bergamo—M. Porta (Policlinico S. Marco), M. R. Rottoli, A. Mamoli, M. Camerlingo; Biella—E. Nardozza; Bologna—T. Sacquegna (Ospedale Maggiore), S. Stecchi, C. Scandellari (ASL, Città di Bologna); Brescia—L. Callea, R. Capra, M. Codella; Cagliari—M. G. Marrosu, E. Cocco (Dipartimento di Neuroscienze, Università di Cagliari); A. Spissu, G. Cossu (Azienda Ospedaliera "G. Brotzu"; Ospedale S. Giovanni di Dio, Università di Cagliari); S. Tronci (Ospedale S. Giovanni di Dio, Università di Cagliari); Caserta—A. Di Lauro, E. Lombardi; Catania—A. Reggio, F. Patti; Catanzaro—P. Valentino (Clinica Neurologica); Cosenza—A. Quattrone; Chieti—D. Farina, M. E. Nives, A. Lugaresi; Cuneo—F. Perla, M. G. Rosso; Ferrara—M. R. Tola, E. Granieri; Firenze—M. P. Amato (Prima Clinica Neurologica), L. Massacesi (Dipartimento di Scienze Neurologiche e Psichiatriche); Frosinone—E. Millefiorini, V. Durastanti; Genova—G. L. Mancardi, A. Murialdo (Dipartimento di Scienze Neurologiche, Università degli studi di Genova); N. R. Pizio (Ospedali Galliera di Genova); Isernia—P. Bellantonio, R. Fantozzi; L'Aquila—R. Totaro, A. Carolei; Latina—F. Giramma, A. T. Lazzaro; Lucca—C. Giraldi, M. Mazzoni; Macerata—G. Giuliani, E. Pucci; Mantova—P. Previdi; Messina—M. C. Fazio, M. Buccafusca, P. Girlanda, C. Messina (Dipartimento di Neuroscienze, Scienze Psichiatriche e Anestesiologiche dell'Università di Messina), G. D'Aleo (Centro Studi Neurolesi, ASL5, Università degli Studi); Milano—C. Milanese, L. Lamantia (Istituto Neurologico C. Besta); D. Caputo (Fondazione Don C. Gnocchi); E. Scarpini, R. Clerici (IRCCS Ospedale Maggiore); L. Moiola, M. Gironi (Ospedale S. Raffaele); Modena—E. Merelli, F. Casoni; Napoli—S. Bonavita, G. Tedeschi; Novara—M. Leone, D. Mittino; Nuoro—S. B. Murgia, L. Musu; Padova—P. Gallo, P. Perini (Azienda Ospedaliera di Padova); E. Frasson (Ospedale Civile di Cittadella); Palermo—G. Salemi, G. Cuccia; Parma—E. Montanari, L. Manneschi (Ospedale Civile di Fidenza); D. Saviola, M. Antonelli (Università degli Studi di Parma); Pavia—V. Cosi, R. Bergamaschi; (Universita Dipavia-IRCCS "Mondino") Perugia—V. Gallai, D. Murasecco, P. Sarchielli (Università degli Studi di Perugia); R. Urcioli, G. Perticoni (Azienda Ospedaliera di Perugia); Pisa—G. Meucci, G. Moscato; Pordenone—B. Lucci, E. Covezzi; Potenza—M. G. Coniglio, D. Acquistapace; Reggio Emilia—L. Motti; Rimini—B. Dossi Currò; Roma—M. Frontoni, C. Mainero, P. Giannetti (Prima Clinica Neurologica, Università di degli Studi di Roma "La Sapienza"); I. Pestalozza, S. Di Legge, M. Spadaro (Quinta Clinica Neurologica, Università di degli Studi di Roma "La Sapienza"); C. Pozzilli, S. Romano (II Facoltà di Medicina, Università di degli Studi di Roma "La Sapienza", Azienda Ospedaliera S. Andrea); B. Mercuri, C. Scoppetta (Dipartimento di Fisiologia Umana e Farmacologia, Università degli Studi di Roma "La Sapienza"); C. Gasperini, S. Galgani (Azienda Ospedaliera S. Camillo, Forlanini); M. G. Grasso, S. Paolucci (Fondazione S.Lucia); P. A. Tonali (Università Cattolica del Sacro Cuore, Policlinico Gemelli); Savona—A. Leonardi, A. Oneto; Sassari—G. Rosati, M. A. Sotgiu; Torino—A. Bertolotto, M. Capobianco (Ospedale S. Luigi Gonzaga); L. Durelli, M. Clerico (Università di Torino); L. Sosso, R. Bongioanni (Ospedale Mauriziano); Trento—D. Orrico; Treviso—C. Carbonin, U. Freo; Varese—M. Zaffaroni, A. Ghezzi; Viterbo—N. Falcone.

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