Differential Twin Concordance for Multiple Sclerosis by Latitude of Birthplace

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Objective: To address the inconsistency in the reported concordance of multiple sclerosis (MS) among twins by zygosity, sex, and latitude.

Methods: Four hundred eighteen medically documented monozygotic (MZ) and 380 same-sex dizygotic (DZ) pairs were ascertained from 1980 to 1992 and followed. The study population was representative of twins with multiple sclerosis. Twins from Canada and adjacent US states (at or above 41–42° N) were considered "northern," and ancestry was dichotomized from descent from high-risk populations. Diagnosis before median age 29.3 years was considered "early."

Results: The MZ/DZ concordance ratio was 2.9 (95% confidence interval [CI], 1.0–8.9) among men and 2.6 (95% CI, 1.5–4.5) among women. The average age at northern diagnosis was independent of ancestry and 2 years earlier for both MZ ($p < 0.02$) and DZ ($p < 0.01$) patients. Among DZ twins, concordance was independent of all characteristics. Among MZ twins, concordance was 1.9 times (95% CI, 1.2–3.2) greater among northern twins, 1.9 (95% CI, 1.1–3.6) times greater among twins with high-risk ancestry, and 2.1 (95% CI, 1.2–3.6) times greater if diagnosis was early. Ancestry and early diagnosis made independent significant contributions to the differential concordance by latitude.

Interpretation: Multiple sclerosis is similarly heritable by sex, and the apparent variation in MZ concordance by latitude is influenced by environmental and genetic factors.

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The incidence and prevalence of multiple sclerosis (MS) increases with distance from the equator in both the northern[1] and southern[2] hemispheres. Migrant studies suggest that this gradient can be explained by an environmental determinant acting in childhood.[3] Infectious agents have long been under investigation,[4] and solar radiation has been of recent interest.[5]

Heredity is also a determinant of MS. People of African[6] and Asian[3] heritage have a lower risk, and people of Scandinavian and Celtic origin have a higher risk.[7] The risk to a first-degree relative, cumulatively 3 to 4%, is 7 to 40 times that of the general population,[8,9] and risks to second- and third-degree relatives are correspondingly increased.[10] Cause is believed to be polygenic, but other than the HLA-DR2 haplotype, which is highly prevalent and predictive of disease in people of North European origin, the specific alleles responsible have not been identified.[11]

The pattern of twin concordance for a complex disease may shed light on the nature of both genetic and environmental determinants. Among twins from northern populations (mostly female twins), concordance for MS has been reported to be 20 to 40% among monozygotic (MZ) and 3 to 5% among dizygotic (DZ)[12,13] pairs, but the sole observed rate of concordance in male MZ pairs was even lower than that in male DZ pairs.[14] Especially low rates of MS concordance in MZ twins have been reported from France[15] and Italy.[16] This article addresses these issues of twin concordance by zygosity, sex, and latitude using North American twins.

A prerequisite for the study of MS patterns of occurrence is a system of representative ascertainment. No population-based MS registries exist other than those in the relatively small populations of Scandinavia. The only detailed analysis previously available is based on the assumption that Canadian patients attending urban specialty clinics are uniformly representative of all Canadian patients for age, zygosity, and sex.[17]

The International Twin Study, a continent-wide repository of twins with cancer and other chronic diseases, was designed to attract the participation of twins representative of all cases among newspaper readers,[18] and pairs ascertained in this way are certainly not rep-

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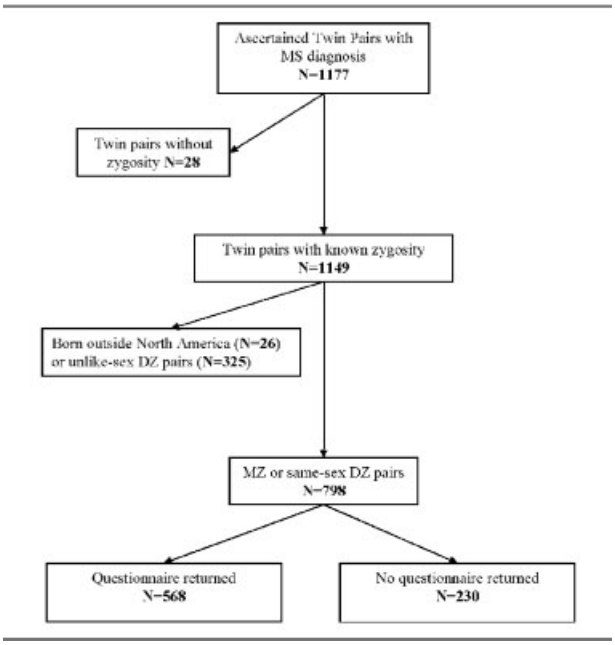

Fig 1. Schematic presentation of the North American twin registry. DZ = dizygotic; MS = multiple sclerosis; MZ = monozygotic.

resentative of all twin cases for age, sex, zygosity, or place of residence. Although the relative participation of concordant pairs is predictably higher than that of singly affected pairs, this gradient does not appear to vary by sex or place of residence, and this twin repository was used in this study.

Subjects and Methods

Ascertainment

Twins with MS (or another chronic disease, such as a cancer) were sought by advertisements in North American newspapers and other periodicals from 1980 through 1992. Ascertainment was designed to capture pairs of twins in whom at least one member had physician-diagnosed MS. No concordant pairs were doubly ascertained. Pairs identified as discordant for the disease were verified based on the neurological health of the unaffected co-twin, most often by direct contact. We have estimated that approximately 27% of the North American twin cases prevalent at any time during the period were identified. The characteristics of the twin respondents have been described previously.[18] Each living member of an affected pair was asked to complete a detailed questionnaire exploring possible predictors of risk, including the national origins of each grandparent.

Of the 1,177 initial respondents with a specific diagnosis of MS, we excluded 28 pairs of uncertain zygosity (Fig 1) and 26 pairs born outside of North America. Although 325 opposite-sex DZ pairs were used for some comparisons, this analysis is largely based on 418 MZ and 380 same-sex DZ pairs. A more detailed analysis with additional variables (described later) is based on the 292 MZ and 276 DZ twin pairs from whom completed questionnaires were received (Table 1).

Latitude

Birthplace was dichotomized into "northern" and "other" categories. Canadian provinces and states adjacent to Canada at or above 41 to 42° N (ie, Alaska, Oregon, Washington, Idaho, Montana, Nebraska, North Dakota, South Dakota, Wyoming, Michigan, Minnesota, Wisconsin, Connecticut, Maine, Massachusetts, New Hampshire, New York, Rhode Island, Vermont) represented the "northern" birthplaces, as defined by Hernan and colleagues,[19] and the more southern US states represented the "other" birthplaces.

Ancestry

If one or more grandparents had been born in Scotland, Ireland, Iceland, Denmark, Norway, or Sweden, the twins were considered to have Celtic or Scandinavian ancestry. Pairs could not be classified in more detail according to the number of such grandparents due to small numbers.

Zygosity

Zygosity was assigned according to the twins' own perception. This method has been shown to be more than 90% accurate,[20] and we have previously used molecular biology to validate the perceptions of about 250 adult twin pairs,[21–23] confirming self-reported zygosity in all but 3 pairs.

Age at Diagnosis

Diagnoses made before the age of 29.3 years (the median age of diagnosis among MZ cases) were considered to be "early" (Table 2).

Diagnostic Validation

After receiving written consent from each case, the most recent medical provider was contacted, and medical records were requested to validate the diagnosis. Academic MS neurologists (L. Weiner, W. Weiderholt) reviewed the records of 145 cases who were reported early in study but, like most twin cases, long after diagnosis. Applying the Schumacher clinical criteria,[24] we confirmed the perceived diagnosis (probable or definite MS) in 141 (97.2%) of these 145 cases. Of the four pairs with misdiagnosed cases, one single case pair was excluded from the registry, and the other three pairs were redefined as discordant pairs.

Follow-up

Periodically, each subject was located (most recently in 2005) and queried about the basis for the diagnosis and the level of certainty of the current clinician. Specific questions were asked about the neurological health of the originally healthy co-twin. As practitioners were identified, with the permission of each subject, follow-up records, including magnetic resonance imaging reports, have been gathered.

As of 2005, 81.2% of the 798 same-sex pairs (83.6% of male, 80.4% of female pairs) had been followed for at least 10 years subsequent to the initial diagnosis (see Table 2). The overall median and interquartile range of follow-up was 26 and 18 to 35 years, respectively. The length of follow-up was similar by zygosity, sex, age at diagnosis, birthplace, and ancestry. Of the 568 pairs who completed questionnaires, 90.8% were followed for at least 10 years. At least 85% of the pairs in each subgroup (MZ or DZ, male or female, northern or other, early or late initial diagnosis) were fol-

Table 1. Descriptive Characteristics of Study Population

Category	Questionnaire Available[a] (N = 568) n	%	No Questionnaire Available[a] (N = 230) n	%	*p*
Sex-pair					0.66
Male–male	138	24.3	59	25.7	
Female–female	430	75.7	170	73.9	
Missing	NA		1	0.4	
Twinship					0.33
Monozygotic	292	51.4	126	55.2	
Dizygotic	276	48.6	104	44.8	
Birthplace					0.003
Northern states and Canada	276	48.6	85	37.0	
Other US states	292	51.4	145	63.0	
Age at first MS case diagnosis, yr					0.08
1-15	5	0.9	2	0.9	
16-20	52	9.2	11	4.8	
21-30	239	42.1	88	38.3	
31-40	187	32.9	81	35.2	
41-50	65	11.4	32	13.9	
>50	20	3.5	16	7.0	

[a]Registry data include information on all twin pairs first interviewed by telephone. Questionnaire data include information on the subset for which at least one twin completed a questionnaire.

NA = not applicable; MS = multiple sclerosis.

lowed for at least 10 years after the diagnosis. More than 93% of the initially unaffected co-twins were followed beyond the age of 50 years, and all but one was followed beyond the age of 40 years.

Adjustments

Over the course of follow-up, 17 originally unaffected twins were diagnosed with MS, and the physicians of 7 cases originally diagnosed as MS spontaneously revised that initial diagnosis. In addition, we reviewed the medical records of all cases from the 54 eligible pairs lost to follow-up within 5 years of ascertainment and identified 6 patients for whom no truly objective diagnostic evidence was available. Five of the 13 questionable cases thus eliminated were in single-case pairs deleted from the registry; the remaining 8 cases were in concordant pairs recategorized as single-case pairs.

Table 2. Median and Interquartile Range of Age at Diagnosis and Duration of Follow-up Since Initial Diagnosis according to Zygosity and Baseline Characteristics

Characteristics	Age at Diagnosis (yr) MZ Median	MZ IQR	DZ Median	DZ IQR	Duration of Follow-up (yr) MZ Median	MZ IQR	DZ Median	DZ IQR
All twins	29.3	24.4–36.4	30.8	24.4–37.9	26.0	21.0–35.0	25.0	16.0–34.5
Sex								
Male–male	32.0	25.4–40.0	30.4	23.5–35.6	25.0	21.0–36.0	26.0	16.0–33.0
Female–female	28.8	24.3–35.0	31.1	24.5–38.7	26.5	21.0–35.0	25.0	16.0–35.0
Age at diagnosis, yr								
≤29.3	24.2	21.0–26.8	23.5	20.8–26.1	29.0	23.0–37.0	26.0	17.0–37.0
>29.3	36.4	33.0–41.1	36.7	32.2–42.0	24.0	19.0–31.0	24.0	14.0–32.0
Birthplace[a]								
North	27.9	25.3–37.8	29.5	23.5–36.3	27.0	21.0–35.0	26.0	16.0–36.0
Other states	30.6	23.5–34.0	31.8	25.2–38.5	26.0	21.0–35.0	24.0	16.0–33.0
Ancestry[b]								
Celtic/Scandinavian	28.7	24.4–34.5	29.1	23.4–35.6	23.0	18.0–33.0	28.5	21.5–36.5
Other	29.1	23.9–36.2	30.1	23.5–37.7	27.0	21.0–35.0	25.0	15.5–33.0

[a]Canada and the adjacent US states represent North.
[b]Twin pairs with at least one grandparent with Celtic or Scandinavian origin.

MZ = monozygotic; DZ = dizygotic; IQR = interquartile range.

Primary clinical records of all but two of the second diagnoses in the remaining concordant pairs were available. Each of these patients lived separately from the co-twin and consulted a different practitioner after the onset of novel, specific, distressing, and objectively verifiable symptoms. Review of the available physicians' notes indicates that each subsequent diagnosis of MS was made independent of, and almost always in ignorance of, the diagnosis in the first twin.

Statistical Analysis

Comparison of the baseline characteristics of pairs with and without questionnaire data was performed using χ^2 tests. Student's *t* tests were performed to compare the mean age at diagnosis in different subgroups.

The primary outcome of interest in this analysis was MS concordance assessed separately for MZ and DZ pairs. Because we preferred a simple comparison between demographic subgroups, without estimation of heritability, we measured pairwise concordance (although comparisons based on pairwise and casewise concordance give similar results). To identify the factors that determine twin concordance, we modeled the probability that a twin pair would become concordant separately in relation to zygosity, sex, birthplace, ancestry, and age at first diagnosis. χ^2 statistics were used to assess the significance of these links, to detect differences between all registry volunteers and the subset of questionnaire respondents, and to compare the various group-specific estimates of concordance and the proportions of those diagnosed "early" according to ancestry and region of birth.

Factors modifying the association between concordance and birthplace were evaluated using an adjusted logistic regression model with concordance as the dependent variable. The strength of the associations between different covariates also was tested using regression models. The final model used to assess the strength of these associations was selected by adding covariates by the magnitude of each effect according to univariate analysis; this model also was used to search for effect modification and confounding. Confounders were confirmed if the main (unadjusted) effect varied by at least 10% after the addition of the putative confounder to the model. The change in odds ratio (OR) after adjustment for each factor in a single regression model provided an estimate of the effect of that covariate in explaining the strength of the association. Appropriate interaction terms were added to the model to assess effect modification.

To evaluate the relative strength of different factors as explanations of MS concordance, we used a stepwise selection method (logistic regression at a significance level of 0.05) to identify the most parsimonious model. Once the parsimonious model was selected, we compared each univariate model with the full model using Pearson's goodness-of-fit statistics. The univariate model giving the highest *p* value was considered to be the model with a better fit, relative to the model with the next highest value. Initially, we combined MZ and same-sex DZ twins to estimate the relative effect of zygosity. To identify the relative effect of different environmental and genetic factors within the set of MZ twins, we then limited our analysis to those pairs. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Results

Representativeness of Cases

Among opposite-sex pair respondents, each potentially reported by a person of either sex, the female-to-male case ratio was 2.1 (104 male and 221 female cases), which was consistent with the ratios found in most North American series.[25] The same ratio calculated for same-sex fraternal and identical twin cases was higher at 3.0 (see Table 1), possibly because more female than male individuals chose to participate, although some case series have produced ratios at least as high.[26] The overall DZ-to-MZ twin ratio of 1.7:1 (709/409) is consistent with the ratio of 2:1 expected from the literature,[27–29] and the DZ-to-MZ ratio among same-sex pairs of 0.9:1 is consistent with the ratio of 1.1 expected from twin prevalence in the population. The population of that region designated "north" contains about 24% of the continental population, but, as expected, contributed a higher proportion (44%) of twin cases. The ratio of northern to other cases was 1.8, comparable with the range of analogous estimates from military cases of 1.4 to 2.4,[30] but less than the only civilian estimate of 3.1.[31] This apparent deficit could reflect a change in that ratio over time,[32] but it is more likely due to the smaller fraction of the northern population served by large-circulation newspapers.

Characteristics of the pairs for whom questionnaire information was available were similar to the other pairs in respect to zygosity, sex, and age at diagnosis (see Table 1). Twins born in the north were slightly more likely to complete the questionnaires than twins born in other states (76.4 vs 66.8%).

Pattern of Pairwise Concordance

ZYGOSITY. The concordance rate for MS was 13.4% (56/418) among MZ, 5.0% (19/380) among same-sex DZ twins and, 3.7% (12/325) among opposite-sex DZ twins. The overall MZ-to-DZ concordance ratio of 3.0 (MZ/same-sex DZ ratio, 2.7) reflects the heritable nature of MS.

SEX. Although concordance rates among male MZ and DZ pairs are somewhat lower than those among female pairs, the MZ-to-DZ ratio among both male (2.9) and female (2.6) pairs is still convincingly high in biological terms (Table 3).

LATITUDE. Concordance among MZ pairs born in the north was nearly twice as high as among those born elsewhere (18.6 vs 9.5%; see Table 3). This latitude effect was reasonably consistent by sex among MZ pairs; concordance rates were 15.5 and 19.3% among northern-born MZ male and female twins, respectively, and 9.5 and 9.5% among male and female twins born elsewhere. No such substantial latitude effect on concor-

Table 3. Variations in Pairwise Concordance for Multiple Sclerosis according to Zygosity and Baseline Characteristics

Strata	PC[a]		Ratio (95% CI)
	MZ Pairs	Same-Sex DZ Pairs	
Sex			
Female–female	13.9% (45/323)	5.4% (15/277)	2.6 (1.5–4.5)[b]
Male–male	11.6% (11/95)	3.9% (4/103)	2.9 (1.0–8.9)[c]
Ratio (95% CI)	1.2 (0.6–2.2)	1.4 (0.5–4.0)	
Age at diagnosis, yr[d]			
≤29.3	18.2% (38/209)	6.1% (10/164)	3.0 (1.5–5.8)[b]
>29.3	8.6% (18/209)	4.2% (9/216)	2.1 (0.9–4.5)[e]
Ratio (95% CI)	2.1(1.2–3.6)[b]	1.5 (0.6–3.5)	
Birthplace[f]			
North	18.6% (33/177)	5.4% (10/184)	3.4 (1.7–6.7)[b]
Other states	9.5% (23/241)	4.6% (9/196)	2.1 (1.0–4.4)[c]
Ratio (95% CI)	1.9 (1.2–3.2)[b]	1.2 (0.5–2.8)	
Ancestry[g]			
Celtic/Scandinavian	24.4% (11/45)	7.1% (4/56)	3.4 (1.2–10.0)[c]
Others	12.5% (31/247)	5.9% (13/220)	2.1 (1.1–3.9)[c]
Ratio (95% CI)	1.9 (1.1–3.6)[c]	1.2 (0.4–3.6)	

[a]The pairwise concordance (PC) was calculated as C/(C+D), where C is total number of concordant pairs and D is the total number of discordant pairs.
[b]$p < 0.01$; [c]$p < 0.05$; [e]$p < 0.1$.
[d]The median age of diagnosis for the first case of all twins, 29.3 years, was used to distinguish between early and late diagnosis.
[f]Canada and the adjacent US states represent North.
[g]Twin pairs with at least one grandparent with Celtic or Scandinavian origin. Information on ancestry was available from 568 twin pairs with completed questionnaire.

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

dance appeared among either same-sex DZ pairs (5.4 vs 4.6%) or opposite-sex DZ pairs (4.0 vs 3.3%). Thus, the overall impact of northern birthplace among MZ was 18.6/9.5 or 1.96, whereas the impact among all DZ twins was 4.4/3.8 or 1.17. Considering that the a priori established increased risk in the north, we calculated the one-tailed probability that these two ratios differ at 0.1, indicating that, although chance could account for the observed difference by the conventional criterion, the odds are still 9 to 1 that the difference is real.

The overall same-sex MZ/DZ pairwise concordance ratio was therefore 3.4 (95% confidence interval [CI], 1.7–6.7) among twins born in the north and 2.1 (95% CI, 1.0–4.4) among those born elsewhere (see Table 3).

ANCESTRY. MZ pairs with Celtic or Scandinavian ancestry were 1.9 (95% CI, 1.1–3.6) times more likely to become concordant than twins without such ancestry (see Table 3). Again, the ratio was much lower (1.2) among same-sex DZ twins.

AGE AT DIAGNOSIS. The diagnoses of 94.6% of the twin cases occurred between ages 15 and 50 with a range of 8.5 to 65.5 years. The median age at diagnosis did not differ by zygosity (see Table 2). Relative to those born elsewhere, the mean age at diagnosis of northern-born cases from discordant pairs was 2.3 years earlier for MZ twins and 3.1 years earlier for DZ twins (Table 4). The co-twin of an MZ case diagnosed before age 29.3 years was 2.1 times (95% CI, 1.2–3.6) more likely to become affected than the co-twin of a case diagnosed at a later age (Table 3). A lesser such tendency (1.5 times) was seen when DZ twins were diagnosed early.

The median (mean) lag between the first and second diagnosis was 7.7 (9.3) years and was shorter among MZ twins, male twins, and twins diagnosed in later calendar years (data not shown). Neither the average age at MZ or DZ case diagnosis (see Table 4) nor the length of the interval between MZ concordant cases varied by ancestry (data not shown). The age at first diagnosis from concordant pairs did not vary appreciably by latitude, and no statistically significant difference in the mean interval between concordant case diagnoses was observed by latitude (data not shown).

FACTORS IN COMBINATION. The three "significant" predictors of MZ concordance (latitude of birth, ancestry, and age at first diagnosis) were mutually associated, and their relative contributions to the gradient of concordance by latitude were therefore assessed by the calculation of adjusted ORs. Northern cases were more often diagnosed early (OR, 1.8; 95% CI, 1.2–2.6) and were more often of high-risk ancestry (OR, 2.9; 95% CI, 1.5–5.6), but early age at diagnosis was not associated with ancestry. Among twins reporting Celtic or

Table 4. Mean Age of Multiple Sclerosis Diagnosis according to Zygosity and Concordant Status

Zygosity and Disease Status	Stratified by Birthplace			Stratified by Celtic or Scandinavian Ancestry		
	North, mean (SD)	Other States, mean (SD)	p[a]	Celtic/Scandinavian, mean (SD)	Other, mean (SD)	p[b]
All MZ cases	29.7 (8.9)	32.0 (8.5)	0.030	30.9 (8.0)	31.0 (8.9)	0.98
All same-sex DZ cases	29.9 (8.8)	33.0 (9.7)	0.006	31.1 (9.2)	31.4 (9.4)	0.81
Discordant MZ cases	29.6 (9.5)	32.0 (8.8)	0.035	31.4 (8.6)	30.9 (9.2)	0.77
Discordant DZ cases	29.7 (8.9)	32.8 (8.9)	0.008	31.3 (7.04)	34.6 (5.4)	0.44

[a]Based on Student's t test for the difference in mean age of diagnosis between twins born in "North" (northern states and Canada) and others states.
[b]Based on Student's t test for the difference in mean age of diagnosis between twins with or without Celtic/Scandinavian ancestry.
SD = standard deviation; MZ = monozygotic; DZ = dizygotic.

Scandinavian ancestry, concordance differences by both latitude (northern: 30%; other: 13.3%) and early diagnosis (early: 36%; later 10%) were exaggerated.

The variables predictive of MZ concordance by univariate analysis were then included in multivariate models. The effects of ancestry and age at diagnosis on concordance were neither mutually confounded (Table 5) nor multiplicative (data not shown). Together, they explained most of the link between pairwise concordance and northern birthplace. No statistically significant two- or three-way effect modification was detected among early diagnosis, ancestry, and birthplace. Although those born in northern states appeared more likely to be female (OR, 1.6; 95% CI, 1.0–2.6), female status was neither a confounder nor an effect modifier for associations between concordance and ancestry, early diagnosis, and birthplace (data not shown).

RELATIVE IMPORTANCE OF FACTORS. For MZ and same-sex DZ twins combined, the most parsimonious model selected by the stepwise procedure included both zygosity and early age at diagnosis. Pearson's goodness-of-fit p value was 0.005 for a univariate model with early age of diagnosis and 0.01 for one with zygosity, each compared with the full model. Among MZ twins, the most parsimonious model included no factors other than early age at diagnosis.

Validity of the Observations

Potential sources of error include differential ascertainment, follow-up, or diagnosis. As addressed later, none of these errors could produce the observed differences in twin concordance with respect to sex, age at diagnosis, or latitude.

DIFFERENTIAL ASCERTAINMENT. Responses to newspaper advertisements are certainly likely to vary by zygosity, sex, and location, but because concordance is calculated within each specific subgroup or by logistic regression, control for these variables is automatic. An additional difference in ascertainment may depend on whether one or both twins are affected, but the psychological factors responsible for this difference should not be influenced by latitude of birth.

This method of ascertainment simultaneously targeted twins with malignancy and those with MS, permitting ascertainment bias to be assessed by comparison with population-based cancer rates.[18] We ascertained 52 same-sex twin pairs concordant for colon cancer, the most common malignancy affecting both sex. By rela-

Table 5. Odds Ratio and Confidence Limits of Monozygotic Concordance Estimates for Multiple Sclerosis according to Birthplace, High-Risk Ancestry, and Early Diagnosis of First Case

Characteristics	Univariate Analysis[a]	Multivariate Analysis	
	OR (95% CI)	Model 1,[b] OR (95% CI)	Model 2,[c] OR (95% CI)
Celtic/Scandinavian ancestry	2.2 (1.0–4.9)[d]	2.0 (1.0–5.2)[d]	2.2 (1.0–4.9)[d]
Age at diagnosis ≤ 29.3 years	2.6 (1.3–5.4)[e]	2.5 (1.2–5.2)[e]	2.6 (1.3–5.4)[e]
Born in Northern states or Canada	1.8 (0.9–3.4)[f]	1.4 (0.7–2.8)	—

Analysis was restricted to 292 monozygotic (MZ) twin pairs with 40 concordant pairs with no missing data for either of the 3 analysis variables.
[a]Conditional logistic regression model was fitted to assess the effect of ancestry, age at diagnosis, and birthplace on multiple sclerosis (MS) concordance univariately.
[b]Odds ratio (OR)and 95% confidence interval (CI) from multivariate model where all three variables are adjusted for each other.
[c]OR and 95% CI from the multivariate model measuring the effect of "ancestry" and "early case diagnosis" on MS concordance, with simultaneous adjustment for each other but not place of birth.
[d]$p \leq 0.05$; [e]$p \leq 0.01$; [f]$p \leq 0.10$.

Table 6. Odds Ratio and Confidence Limit of Pairwise Concordance Estimates of Ancestry, Birthplace, and Age at Diagnosis for Monozygotic Twins Pairs with Available Objective Medical Data

Covariate[a]	Strata	Pairwise Concordance	Univariate Analysis, OR (95% CI)	Multivariate Analysis, OR (95% CI)
Ancestry	Celtic or Scandinavian	28.6% (10/35)	2.1 (0.9–4.9)[b]	1.7 (0.9–3.4)[b]
	Other	16.1% (25/155)		
Age at diagnosis	≤29.3	26.8% (34/127)	3.5 (1.7–7.3)[c]	3.7 (1.8–7.6)[c]
	>29.3	9.4% (11/117)		
Birthplace	Northern states/Canada	25.7% (27/105)	2.3 (1.2–4.5)[d]	1.3 (0.7–2.3)
	Other states	12.9% (18/139)		

This analysis was restricted to the 244 monozygotic (MZ) twin pairs for whom objective multiple sclerosis (MS) diagnostic (magnetic resonance imaging, computed tomography, and/or lumbar puncture) data were available.
[a]Conditional logistic regression model was used to assess the effect of ancestry, age at diagnosis, and birthplace in univariate and multivariate models.
[b]$p < 0.1$; [c]$p \leq 0.01$; [d]$p \leq 0.05$.

tive incidence and population prevalence, one would expect 38 of these pairs to be female. In fact, 35 were female, giving no indication of bias in ascertainment by sex. Among the 681 pairs with breast cancer born in the north, 17.5% of MZ and 7.9% of same-sex DZ pairs ultimately became concordant, whereas among the 2,341 pairs born elsewhere, 18.2% of MZ and 8.0% of same-sex DZ pairs did so. Thus, no evidence of differential ascertainment of concordant pairs by either sex or latitude was evident.

We tested the validity and robustness of our findings by using alternative study samples in a sensitivity analysis. Since reports have been published on Canadian twins since 1986, this might have created awareness among the Canadian twins and their attending physicians. However, when the principle analysis was performed after the exclusion of the 70 pairs born in Canada, as well as those cases ascertained after 1986 (the year of the first Canadian report of concordance), the results were unchanged (data not shown). Concern might be raised about differential follow-up and the validity of diagnosis before age 15 or after age 60 years. Exclusion of pairs followed less than 20 years or of cases diagnosed before 15 or after 60 years old did not change the findings (data not shown). When we restricted the analysis to the 244 MZ pairs for whom objective diagnostic data (magnetic resonance imaging, computed tomography, or lumbar puncture) had already become available, each concordance association was strengthened (Table 6). The effect of northern birthplace was strengthened by restricting the analysis to twins who continued to reside at their place of birth at least until age 15 years (OR, 2.14; 95% CI, 1.1–4.3). Finally, we took the "intent-to-treat" approach for MS diagnosis by conducting the analysis on all cases ascertained without the exclusion of questionable diagnoses or the inclusion of cases who experienced development of MS during the follow-up period. Again, the conclusions were unchanged (data not shown).

INCOMPLETE FOLLOW-UP. Most second twins were followed for more than 10 years after the first diagnosis (well after the majority of second cases would be expected), and the members of each study subgroup (sex, latitude, ancestry, age at diagnosis) were followed for a roughly identical period (see Table 2).

DIFFERENTIAL MISDIAGNOSIS. Because no comprehensive diagnostic review of a thousand cases of MS scattered throughout North America is feasible, some errors in diagnosis are to be expected. This is especially true for cases diagnosed by strictly clinical means, and most of those cases were diagnosed before 1990. However, such a serious diagnosis, if false, is unlikely to be retained through decades of clinic visitation, and we could find reason to question no more than 1% of the diagnoses. Although biased overdiagnosis of the identical twin of an MS case might especially be expected from informed diagnosticians, it also occurred in no more than 1% of cases. Empirically, nearly all follow-up records have supported the original diagnosis.

Moreover, there is little reason to expect any residual diagnostic misclassification to be linked to sex, ancestry, age at first diagnosis, or even latitude. Those few misdiagnoses that have been identified represent both men and women and people from all regions. Finally, when a subset of identical twin pairs with objective diagnostic evidence was analyzed separately, conclusions were unaltered.

Discussion

Sex

The significant difference in concordance detected between fraternal and identical twin pairs confirms previous evidence supporting a heritable susceptibility to MS. Despite the clear evidence of a much higher incidence of MS among women, we found high MZ/DZ concordance ratios among both female (2.6) and male

(2.9) individuals, implying that mechanisms of inheritance are probably identical by sex. The analogous ratios that had been obtained by screening at Canadian clinics were 9.8 and 0.8,[17] results that are biologically implausible and suggestive of differential ascertainment.

Latitude

The latitudinal gradient in concordance among identical twins within this uniformly ascertained and documented sample of affected twin pairs reflects links between concordance and both ancestry and early diagnosis. The estimated concordance levels among these MZ (17.0%) and DZ (4.4%) female twins from northern regions are consistent with those from other northern populations.[13,33,34] Our estimates derived from twins born farther south conform to estimates based on French twins[15] and from the large Italian twin registry.[16]

The gradient in concordance according to latitude among MZ pairs is parallel to and consistent with the geographic gradient in North American MS incidence. No such gradient was apparent among the 31 concordant DZ pairs, and although chance might have accounted for this discrepancy, the odds are strongly against it. Such a zygosity-specific discrepancy probably precludes an explanation based on variable MS prevalence alone.

Genotype

Scandinavian/Celtic ancestry (ie, ethnic evidence of a susceptibility genotype) is an independent and significant predictor of concordance in these MZ twins and might explain some of the gradient in twin concordance. The substantial overall difference between the concordance rate in MZ and same-sex DZ twins is consistent with the literature in suggesting a polygenic mode of inheritance,[35] and if the entire susceptibility genotype is represented in proxy by ancestry, it also should do so equally according to zygosity. However, if only one of several required genetic components is represented by the ancestry variable, it would be strongly linked to concordance among MZ twins, who share the entire susceptibility genotype, but much less strongly linked to DZ twins, who are unlikely to share all polygenic components.

Environment

Early onset of MS is a characteristic of all northern cases, as well as an independent predictor of MZ concordance, unrelated to ancestry. Early onset previously has been linked to an increased sibling recurrence risk,[36] to the HLA-DR15 haplotype among British[37]and Swedish[38] MS cases, and to the chemokine receptor 5.[39] However, in this study, it predicted concordance equally well among those with and without high-risk ancestry, and does so especially among the northern-born pairs. It is therefore more likely to reflect an environmental correlate of latitude than a genetic one, as has been concluded from an earlier study.[40] Early onset could represent either early exposure to a causal factor, such as a virus, or an early environmental deficit in protection, such as by exposure to solar flux.

If any environmental factor was to be the sole explanation for the latitudinal gradient, it also would produce a parallel concordance gradient among DZ twins. Only in interaction with genetic susceptibility would the gradient appear solely among concordantly susceptible MZ twins.

Comment

Environmental determinants of twin concordance imply differences in the commonality of familial environment, ie, "microenvironmental" variation. Failure to demonstrate statistical significance between the different recurrence rates of DZ co-twins and ordinary sibs[17] has led some to categorically deny the existence of such intrafamilial or microenvironmental exposure variations. On the contrary, an observed variation in MZ concordance due to environment directly implies that environmental exposure varies, at least in intensity, between family members. Contact with a virus is made more or less likely by the degree of intimacy with the source. Alternatively, protection by an environmental agent, such as solar flux, depends on not only the residential history, but also the degree to which a person, intentionally or otherwise, adopts or rejects pertinent behavior, such avoidance of the sun.[41]

Conclusions

Concordance is strongly determined by zygosity in both male and female twins. When MZ and same-sex DZ twins are combined, both zygosity and early age at diagnosis are important predictors, but the effect of zygosity is greater than that of early age at diagnosis. Among MZ twins, the environmental factor represented by early age at diagnosis appears to be the strongest predictor of concordance. Thus, this study provides evidence that MS concordance is determined by interplay between environmental and genetic factors. In this circumstance, concordantly susceptible identical twin pairs are of great interest, not only because of the particulars of their genome, but because the timing and intensity of crucial experiences may shed light on the environmental component of the MS puzzle, as it has on the cause of diseases such as breast cancer.[42,43]

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References

1. Kurtzke JF. Geography in multiple sclerosis. J Neurol 1977; 215:1–26.
2. Hammond SR, McLeod JG, Millingen K, et al. The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle, and Hobart. Brain 1988;111(pt 1):1–25.
3. Dean G, Elian M. Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. J Neurol Neurosurg Psychiatry 1997;63:565–568.
4. Levin LI, Munger KL, Rubertone MV, et al. Multiple sclerosis and Epstein-Barr virus. JAMA 2003;289:1533–1536.
5. van der Mei IA, Ponsonby AL, Dwyer T, et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ 2003;327:316.
6. Dean G, Bhigjee AI, Bill PL, et al. Multiple sclerosis in black South Africans and Zimbabweans. J Neurol Neurosurg Psychiatry 1994;57:1064–1069.
7. Ebers GC, Sadovnick AD. The geographic distribution of multiple sclerosis: a review. Neuroepidemiology 1993;12:1–5.
8. Nielsen N, Westergaard T, Rostgaard K, et al. Familial risk of multiple sclerosis: a nationwide cohort study. Am J Epidemiol 2005;162:1–5.
9. Sadovnick AD, Dircks A, Ebers GC. Genetic counselling in multiple sclerosis: risks to sibs and children of affected individuals. Clin Genet 1999;56:118–122.
10. Robertson NP, Fraser M, Deans J, et al. Age-adjusted recurrence risks for relatives of patients with multiple sclerosis. Brain 1996;119(pt 2):449–455.
11. Barcellos LF, Oksenberg JR, Begovich AB, et al. HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. Am J Hum Genet 2003;72:710–716.
12. Sadovnick AD, Armstrong H, Rice GP, et al. A population-based study of multiple sclerosis in twins: update. Ann Neurol 1993;33:281–285.
13. Mumford CJ, Wood NW, Kellar-Wood H, et al. The British Isles survey of multiple sclerosis in twins. Neurology 1994;44: 11–15.
14. Willer CJ, Ebers GC. Susceptibility to multiple sclerosis: interplay between genes and environment. Curr Opin Neurol 2000; 13:241–247.
15. Multiple sclerosis in 54 twinships: concordance rate is independent of zygosity. French Research Group on Multiple Sclerosis. Ann Neurol 1992;32:724–727.
16. Ristori G, Salvetti M, Canonni S, et al. A nationwide study of multiple sclerosis in Italian twins. Twin Res 2004;7:375.
17. Willer CJ, Dyment DA, Risch NJ, et al. Twin concordance and sibling recurrence rates in multiple sclerosis. Proc Natl Acad Sci U S A 2003;100:12877–12882.
18. Mack TM, Deapen D, Hamilton AS. Representativeness of a roster of volunteer North American twins with chronic disease. Twin Res 2000;3:33–42.
19. Hernan MA, Olek MJ, Ascherio A. Geographic variation of MS incidence in two prospective studies of US women. Neurology 1999;53:1711–1718.
20. Torgersen S. The determination of twin zygosity by means of a mailed questionnaire. Acta Genet Med Gemellol (Roma) 1979; 28:225–236.
21. Deapen D, Escalante A, Weinrib L, et al. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 1992;35:311–318.
22. Kumar D, Gemayel NS, Deapen D, et al. North-American twins with IDDM. Genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. Diabetes 1993;42:1351–1363.
23. Cozen W, Gill PS, Ingles SA, et al. IL-6 levels and genotype are associated with risk of young adult Hodgkin lymphoma. Blood 2004;103:3216–3221.
24. Schumacher GA, Beebe G, Kibler RF, et al. Problems of experimental trials of therapy in multiple sclerosis: report by the panel on evaluation of experimental trials therapy in multiple sclerosis. Ann N Y Acad Sci 1965;122:552–568.
25. Sadovnick A, Ebers G. Epidemiology of multiple sclerosis: a critical overview. J Can Sci Neurol 1993;20:17–29.
26. Sloka JS, Pryse-Phillips WE, Stefanelli M. Incidence and prevalence of multiple sclerosis in Newfoundland and Labrador. Can J Neurol Sci 2005;32:37–42.
27. Allen G, Parisi P. Trends in monozygotic and dizygotic twinning rates by maternal age and parity. Further analysis of Italian data, 1949-1985, and rediscussion of US data, 1964-1985. Acta Genet Med Gemellol (Roma) 1990;39:317–328.
28. Sweeney VP, Sadovnick AD, Brandejs V. Prevalence of multiple sclerosis in British Columbia. Can J Neurol Sci 1986;13:47–51.
29. Vogel F, Motulsky A. Human genetics: problems and approaches. New York: Springer, 1997.
30. Kurtzke JF, Beebe GW, Norman JE Jr. Epidemiology of multiple sclerosis in U.S. veterans: 1. race, sex, and geographic distribution. Neurology 1979;29:1228–1235.
32. Visscher B, Detels R, Coulson A, et al. Latitude, migration, and the prevalence of multiple sclerosis. Am J Epidemiol 1977;106: 470–475.
33. Wallin MT, Page WF, Kurtzke JF. Epidemiology of multiple sclerosis in US veterans of the Vietnam era and later military service: race, sex and geography. Ann Neurol 2004;55:65–71.
34. Heltberg A, Holm N. Concordance in twins and recurrence in sibships in multiple sclerosis. Lancet 1982;1:1068.
35. Kinnunen E, Juntunen J, Ketonen L, et al. Genetic susceptibility to multiple sclerosis. A co-twin study of a nationwide series. Arch Neurol 1988;45:1108–1111.
36. Ebers GC, Sadovnick AD, Dyment DA, et al. Parent-of-origin effect in multiple sclerosis: observations in half-siblings. Lancet 2004;363:1773–1774.
37. Sadovnick AD, Yee IM, Ebers GC, Risch NJ. Effect of age at onset and parental disease status on sibling risks for MS. Neurology 1998;50:719–723.
38. Hensiek AE, Sawcer SJ, Feakes R, et al. HLA-DR 15 is associated with female sex and younger age at diagnosis in multiple sclerosis. J Neurol Neurosurg Psychiatry 2002;72:184–187.
39. Masterman T, Hillert J. HLA-DR15 and age at onset in multiple sclerosis. Eur J Neurol 2002;9:179–180.
39. Barcellos LF, Schito AM, Rimmler JB, et al. CC-chemokine receptor 5 polymorphism and age of onset in familial multiple sclerosis. Multiple Sclerosis Genetics Group. Immunogenetics 2000;51:281–288.
40. Kurtzke JF, Page WF, Murphy FM, Norman JE Jr. Epidemiology of multiple sclerosis in US veterans. 4. Age at onset. Neuroepidemiology 1992;11:226–235.
41. Ponsonby AL, van der Mei I, Dwyer T, et al. Exposure to infant siblings during early life and risk of multiple sclerosis. JAMA 2005;293:463–469.
42. Mack TM, Hamilton AS, Press MF, et al. Heritable breast cancer in twins. Br J Cancer 2002;87:294–300.
43. Hamilton AS, Mack TM. Puberty and genetic susceptibility to breast cancer in a case-control study in twins. N Engl J Med 2003;348:2313–2322.