Twin concordance and sibling recurrence rates in multiple sclerosis

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Size and ascertainment constraints often limit twin studies to concordance comparisons between identical and fraternal twins. Here we report the final results of a longitudinal, population-based study of twins with multiple sclerosis (MS) in Canada. Bias was demonstrably minimized, and an estimated 75% of all Canadian MS twin pairs were ascertained, giving a sample sufficiently large (n = 370) to permit additional informative comparisons. Twinning was not found to affect prevalence, and twins with MS did not differ from nontwins for DR15 allele frequency nor for MS risk to their siblings. Probandwise concordance rates of 25.3% (SE ± 4.4) for monozygotic (MZ), 5.4% (±2.8) for dizygotic (DZ), and 2.9% (±0.6) for their nontwin siblings were found. MZ twin concordance was in excess of DZ twin concordance. The excess concordance in MZ was derived primarily from like-sexed female pairs with a probandwise concordance rate of 34 of 100 (34 ± 5.7%) compared with 3 of 79 (3.8 ± 2.8%) for female DZ pairs. We did not demonstrate an MZ/DZ difference in males, although the sample size was small. We observed a 2-fold increase in risk to DZ twins over nontwin siblings of twins, but the difference was not significant.

Multiple sclerosis (MS) is one of the most common neurological diseases affecting young adults (1). In Canada, at least 1 in 1,000 individuals has the disease (2–4), and twice as many women are affected compared with men. It is widely believed that susceptibility to MS is determined by a complex interaction between susceptibility genes and environment. Twin studies have played an important role in current concepts of complex traits including MS and have consistently demonstrated an excess of monozygotic (MZ) over dizygotic (DZ) concordance (refs. 5–19 and Table 1).

The magnitude of the MZ-to-DZ difference has implied non-Mendelian inheritance (20). However, in more recent data, it seems that the absolute level of concordance in MZ twins also reflects the background population prevalence. Whereas in Canada and northern Europe concordance rates are high, in southern Europe twin concordance rates mirror the lower prevalence (ref. 5 and L. Ristori, S. Cannoni, and M. Salvetti, unpublished data). Uncertainty has remained about final concordance rates, because twins in previously reported studies retained considerable residual risk based on corrections for age of onset. Corrections can be readily calculated for the general population, but applying it to twins may be inappropriate because the age onset correlations are so much larger for concordant MZ pairs than for siblings (sibs) (21).

Twin studies in MS and other putative autoimmune diseases have generally been small. Data have been insufficient in any single study to adequately assess the impact of ascertainment bias, a common confounder of twin studies (22). Questions beyond the comparison of MZ with DZ concordance have been left unanswered, and even this comparison shows wide confidence intervals in published data. Furthermore, it has not been possible to study any potential influence of twinning on MS risk, and previous studies have not examined the difference in concordance rate between DZ twins and sibs. This comparison addresses factors related to intrauterine environment and timing as well as other factors more commonly shared between twins than sibs. Although there has been no indication otherwise, it has not been formally shown that twins with MS carry the same susceptibility alleles as the general population of MS patients. This bears on the generalizability of twin concordance data.

With these considerations in mind, we have attempted over the last two decades to collect all twins with MS in Canada. We aimed to assess whether twinning influences MS risk and whether twins are representative of the patient population for family history of MS and presence of susceptible HLA alleles. We compared the concordance rates for MZ and DZ twins. We also asked whether concordance rates are influenced by the HLA-DR*15 allele or by gender. Finally, we determined the recurrence risk to sibs of twin probands to determine whether DZ twins had any excess risk compared with their nontwin sibs.

Materials and Methods

Ascertainment of Twin Subjects. The Canadian Collaborative Project on Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) (23) is a network of specialized Canadian MS clinics that see patients from a wide geographical area. The clinics are located in Vancouver, Prince George, Calgary, Edmonton, Saskatoon, London, Hamilton, Toronto, Ottawa, Kingston, Montreal (2), Quebec City, Halifax, and St. John's.

All MS patients identified through the CCPGSMS (23) were asked whether they are a member of a twin pair or other multiple birth. Each proband who replied "yes" was given specific "twin" questionnaires in addition to the regular information collected as part of the Canadian study. Detailed clinical information re-

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Abbreviations: MS, multiple sclerosis; DZ, dizygotic; MZ, monozygotic; sib, sibling; CCPGSMS, Canadian Collaborative Project on Genetic Susceptibility to Multiple Sclerosis.

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Table 1. Previously published MS twin concordance studies

Studies	Female MZ concordance	Male MZ concordance	Pairwise monozygous twin concordance (%)	Pairwise dizygous twin concordance (%)
Ref.				
French Research Group (5)	1	0	1/17 (5.9)	1/37 (2.7)
Bammer *et al.* (6)			1/6 (16.7)	2/7 (28.6)
Bobowick *et al.* (7)		2*	2/5 (40)	0/4
Cendrowski (8)				0/3
Currier and Eldridge (9)	8	0	8/22 (36.4)	3/29 (10.3)
Gardner-Thorpe and Foster (11)			0/1	
Heltberg and Holm, Danish twin register (12)			4/19 (21.1)	1/28 (3.57)
Kinnunen *et al.*, Finnish twin cohort (13)			1/11 (9.1)	0/10
Kinnunen *et al.* (14)	1	1	2/7 (28.6)	0/6
Mackay and Myrianthopoulos (15)			6/36 (16.7)	3/26 (11.5)
Mumford *et al.*, British Isles twins (16)	9	2	11/44 (25)	2/61 (3.3)
Thums (18)			0/14	1/36 (2.8)
Williams *et al.* (19)	6	0	6/12 (50)	2/12 (16.7)
Total (all studies)	25	3	38/175 (21.7)	14/231 (6.1)
Canadian study	22	2	24/133 (18.0)	9/221 (4.1)
Total combined	47	5	62/308 (20.1)	23/452 (5.1)

*Males-only study.

garding age at onset of symptoms, age at diagnosis, first symptoms, and clinical course were obtained for both the proband and the affected co-twin. Ethics approval was obtained by each clinic.

Clinical Criteria. ***Twin Probands.*** As for all CCPGSMS probands, the twin proband had to meet the diagnostic criteria of Poser (24) for probable or clinically definite MS.

Co-Twins. Co-twins who were reported by the index case to have symptoms of MS, co-twins with a previous diagnosis of MS, or co-twins who were ascertained independently through the CCPGSMS were examined by a neurologist from the Canadian MS Clinic network. Appropriate laboratory tests [e.g., MRI and oligo clonal banding (OCB)] were conducted as indicated for diagnostic clarification. Co-twins who met the diagnostic criteria for probable or clinically definite MS (24) were considered concordant.

Co-twins with a previous diagnosis of "possible" MS or who reported signs and symptoms suggestive of MS were reexamined by a network neurologist and given the appropriate laboratory assessments. If these individuals still did not fulfill criteria for probable or clinically definite MS, the twin pair was called "discordant." Longitudinal follow-up of these pairs, done as part of the CCPGSMS, resulted in some pairs meeting the criteria for concordance. As part of the data collection for the first two reports on Canadian twins (10, 17), it was possible for one neurologist (G.C.E.) to examine any reportedly asymptomatic co-twins and, if appropriate, perform MRI and lumbar puncture for oligo clonal banding (OCB). Again, the Poser committee criteria were the diagnostic standard at this time for being "affected."

Nontwin Sibs of Twin Probands. In families where the index case reported that their nontwin sibs had either symptoms of MS or had previously been given a diagnosis of MS, the sib was assessed in the manner described above for co-twins.

Zygosity Determination. Blood samples were collected from consenting index cases and their co-twin. DNA was isolated by using either a phenol-chloroform or salting-out method. Each twin pair was genotyped at 8–10 polymorphic unlinked microsatellite markers by using Applied Biosystems 373s and 377s. Genotyping was performed blinded with respect to clinical phenotype, reported zygosity, and twin genotype. The likelihood of monozygosity was calculated by using the informativeness of the markers to determine the likelihood of identity by state (IBS) sharing in fraternal twins. After unblinding, any twin pair for whom the reported and molecular zygosities differed was typed again in two additional panels of 6–10 unlinked microsatellite markers by using a separate DNA sample. After this analysis, the molecular zygosity was taken to be accurate. Any twin pair that did not provide a blood sample was asked whether they were identical or fraternal twins and the self-reported zygosity was taken to be true (25).

DRB1 Genotyping. Individual samples were genotyped by using a low-resolution panel of allele-specific PCR primers along with a positive control primer (26). PCR products were visualized on a 1.5% agarose gel and visualized with ethidium bromide and UV light.

Analysis. Probandwise concordance rates were calculated for all independently ascertained index cases (27) in which the co-twin of the index case lived to at least age 30 years. The probandwise concordance rate was used because this sample had incomplete ascertainment and this statistic provides a better estimate of recurrence risks and thus the increased MZ concordance rate over the DZ concordance rate and allows direct comparison to sib recurrence rates (28).

All concordance rates were calculated with 95% confidence intervals (29). A Kaplan–Meier survival curve was used to estimate the proportion of risk remaining for each unaffected individual. Non age-corrected recurrence risks were calculated for sibs to facilitate comparison to non-age-corrected twin concordance rates. The average age of twin sibs was not significantly different from that of nontwin sibs.

Results

Inclusion of Cases. The CCPGSMS database contained 19,938 MS probands who met the diagnostic inclusion criteria for the study and were asked whether they were part of a twin or other multiple birth (triplets). Of the 486 twin pairs originally identified, 96 (19.8%) were excluded from analyses.

1. Forty-three co-twins did not survive to age 30, the vast majority having died shortly after birth ($n = 33$) or just before birth ($n = 6$).

2. Thirty-one index cases did not meet the diagnostic inclusion criteria (19 remained "possible" MS despite longitudinal follow-up; 12 were found to not have MS).
3. Six triplet sets were excluded.
4. Sixteen twin pairs were excluded because of insufficient information (10 co-twins refused to participate; 5 probands refused to participate; 1 proband was adopted out separately from the co-twin and there had been no contact). Nevertheless, it was known that of these 16 excluded pairs, 8 were clearly DZ, 4 were reported by family members to be MZ and 7 were female and 4 were male.

Four co-twins (three MZ) were felt to have possible MS (three female and one male). After longitudinal follow-up and multiple examinations, they did not fulfill the inclusion criteria of probable or clinically definite MS (as of October 2002) and therefore had to be considered "unaffected" for the purpose of calculating concordance.

Zygosity Typing. DNA typing for zygosity was possible for both members of 120 like-sexed twin pairs. Zygosity typing was performed on 3 like-sexed DZ concordant pairs, 41 DZ discordant like-sexed pairs, 15 MZ concordant pairs, and 61 MZ discordant pairs. Typing was performed on 64% of concordant pairs and 47% of discordant pairs. The molecular zygosity differed from self-report in four pairs, three of whom were male and all were discordant. All these pairs were found to be genetically identical at a minimum of 21 microsatellites ($P = 1.3 \times 10^{-9}$), thus confirming monozygosity. The two concordant MZ male pairs were not available for zygosity typing. However, both of the DZ male concordant pairs were typed, and the two members of one pair differed in genotype at 6 microsatellite loci of 10 markers typed, and the other pair differed at 8 of 10 loci. If the rate of zygosity misreport was the same in the nontyped group as was observed in the typed group, we would expect to find no changes in zygosity status of any concordant pairs and a change in zygosity of an additional four discordant pairs.

Rate of MS in Canadian Twins. There are two methods of determining whether twins are at higher risk of developing MS, and both need to consider whether biased ascertainment of twin and nontwin probands has occurred. The first estimates the twinning rate in a sample of individuals with disease, and the second estimates the number of available twins with MS in the country if the twinning rate is not related to MS status.

Here we identified a total of 19,938 MS probands, 19,585 of whom had clinically definite or probable MS (98.2%). From the originally identified 486 possible twin probands, 466 were twins with a diagnosis of clinically definite or probable MS (95.9%). This implies a twinning rate among cases of definite or probable MS of 1/42 individuals or 1/84 births. This frequency is very similar to the reported twin birth rate in Canada during the time the twins in this study were born, and this rate is reportedly between 1 per 80 and 1 per 110 births (30).

We next determined the expected number of twins in Canada with MS by multiplying the number of Canadians by the known rate of twinning (at birth) and MS prevalence. In the 2001 census, there were 30.081 million Canadians (Statistics Canada), and the twinning rate is 1 twin pair (two twin individuals) per 110 births, and the prevalence of MS in Canada was assumed to be 100/100,000. By multiplication of the population by the twinning and MS rates, we expected to have an available pool of 547 twin index cases with MS in Canada. The total number of Canadian-born twin probands identified was 466 including those ascertained through family members, those unable to be followed-up, those in which the co-twin was deceased, the second member of a twin pair, and probands where the co-twin died at birth. However, at the end of the ascertainment period, 12 probands had deceased and were excluded from the calculation of ascertainment probability to correct for the comparison of a longitudinal ascertainment to the expectation that was calculated for a single point in time. By estimating the expected number of twin index cases with MS in Canada, we have estimated the ascertainment success of our scheme to be 83% (454/547). However, by looking at the number of doubly and singly ascertained concordant pairs, we found that 16 concordant pairs were ascertained twice and 17 concordant pairs were ascertained only once out of 49 index cases from concordant pairs, which suggests a rate of ascertainment of 65%.

Sample Characteristics. In the final data set there were 370 twin index cases with MS from 354 pairs. Overall, there were 120 male and 250 female index cases. The twin index cases have a female-to-male ratio of 2.1:1, which given the overall CCPGSMS gender ratio of 2.4:1 (14,081:5,857), shows no ascertainment bias by gender. The male index cases were ascertained from 72 DZ and 45 MZ pairs for a DZ/MZ ratio of 1.6:1, and the female cases were from 149 DZ and 88 MZ pairs for a DZ/MZ ratio of 1.7:1. Of the 221 DZ pairs, 111 were like-sexed and 110 were unlike-sexed.

The twins in this sample had a median age of 51 and an average age of 51.9 (SD = 12.1). MZ twins were slightly younger, the average age of the MZ sample was 50.1, and the DZ twins were an average of 53.0 years of age. The age of onset for all affected index cases was 31.48, and the median was 31 (SD = 12.0) and was not significantly different for MZ vs. DZ or females vs. males. The average age of onset was nonsignificantly lower for concordant index cases than for discordant (concordant mean = 30.0 and SD = 10.9; discordant mean = 31.7 and SD = 9.1). Kaplan–Meier survival analysis based on a survival curve of affected twin ages of onset suggests that the MZ co-twins that were unaffected had passed 90.4% of their lifetime risk of developing MS, and the DZ co-twins had passed 91.9% of their lifetime risk. However, this is a conservative estimate because there is a strong age-of-onset correlation in MZ concordant pairs (7). For this reason, we did not age-correct the probandwise twin concordance rates.

Although some twins from the original two Canadian studies were reascertained through the systematic scheme of the CCPGSMS, we did not consider them separately, nor did we include the twins reported in the previous studies but not reascertained through the scheme ($n = 22$). Within this group, there were 14 DZ discordant pairs, 1 concordant DZ pair, and 7 MZ discordant pairs.

MZ vs. DZ Concordance Rates. The overall probandwise MZ concordance rate of 25.3% (37/146, SE = 4.4%) was significantly greater than the overall DZ probandwise concordance rate of 5.4% (12/224, SE = 1.8%, $P = 3.2 \times 10^{-5}$; Table 2). After segregation by gender, the female MZ probandwise concordance rate of 34.0% (34/100, SE = 5.7%) was significantly and 10-fold greater than the female DZ concordance rate of 3.8% (3/79, SE = 2.8%, $P = 2.1 \times 10^{-6}$). These pairs represent the two largest twin subgroups. The male probandwise concordance rates are 6.5% for MZ (3/46, SE = 4.6%) and 11.4% for DZ (4/35, SE = 7.4%). This was derived from a total of only four concordant male–male pairs, of which two were MZ and two were DZ.

The rate in unlike-sexed DZ pairs is given in Table 2. The unlike-sexed DZ concordance rate for female DZ twins of male probands was 7.7% (3/39, SE = 4.3%), which is not significantly different from 3.8% for female twin probands in like-sexed pairs (3/79, SE = 2.8%). Correspondingly, the rate for male DZ twins of female probands was 2.8% (2/71, SE = 2.0%) and for male DZ co-twins in like-sexed pairs 11.4% (4/35, SE = 7.4%). This difference was not significant. Combining across sex of proband,

Table 2. Probandwise concordance rates in 367 Canadian twins

Sex of co-twin	Proband type	Concordant pairs	Concordant probands	Discordant pairs	Probandwise concordance rates (±SE)
Female	Female MZ	22	34	66	34.0 (±5.7)
Female	Female DZ	2	3	76	3.8 (±2.8)
Female	Male DZ	3	3	36	7.7 (±4.3)
Female	All DZ	5	6	112	5.1 (±2.3)
Male	Male MZ	2	3	43	6.5 (±4.6)
Male	Male DZ	2	4	31	11.4 (±7.4)
Male	Female DZ	2	2	69	2.8 (±2.0)
Male	All DZ	4	6	100	5.7 (±2.9)
Both	MZ	24	37	109	25.3 (±4.4)
Both	DZ	9	12	212	5.4 (±1.8)

the risk to DZ twin females is 5.1% (SE = 2.3%) and to DZ twin males 5.7% (SE = 2.9%).

DZ vs. Sib Rates. There were 238 twin index cases from which complete family history information was available. Only full sibs that had been raised together with the index case were included in recurrence risk calculations. The sib recurrence risks in these families are presented in Table 3. For sibs ascertained through a twin index case, this recurrence risk was 2.9% (SE = 0.6%). The risk to sibs or co-twins did not differ by gender of proband, thus the risks for female and male probands were not considered separately. The risk to sisters was 3.9% (SE = 1.1%), which was not significantly different from the risk to DZ twin sisters of 5.1% (SE = 2.3%, $P = 0.50$). The risk to brothers of twin probands was 1.9% (SE = 0.7%), which was not significantly different from the risk to DZ twin brothers of 5.7% (SE = 2.9%, $P = 0.26$).

HLA Status in Twins. HLA-DR15 was found to be present in 94 of 195 twin probands tested (48.2%), which is significantly increased over the population rate of 30% ($P < 10^{-5}$) but not significantly different from the rate reported in singleton MS cases. The rate in unaffected DZ co-twins was 34 of 92 (37%), which is increased over the rate in population controls as expected (nonsignificantly), because parents would be expected to bear this allele more often than the general population. The carrier frequency in concordant (9/20, 45%) compared with discordant (23/45, 51.1%) female MZ pairs was not different. In males, there were only data for discordant MZ pairs but only 8/28 (28.6%) were HLA-DR15-positive, which was a lower carrier frequency than that observed in discordant female MZ pairs ($P = 0.055$). Among affected male DZ discordant pairs, the rate of DR15-positive cases was 16/33 or 48.5%.

Discussion

The size and longitudinal nature of these studies made it possible to address questions that often cannot be addressed in twin samples but are nevertheless important in interpreting results. Because twin studies are difficult to execute, they tend to attract less critique than they might otherwise deserve. Small samples make difficult any assessment of potential ascertainment bias and exacerbate vulnerability to this common confounder. The tendency toward overascertainment of females, MZ pairs, and concordant pairs can make the interpretation of concordance results difficult and in particular can obscure gender effects. On the other hand, the use of strict ascertainment criteria entails weighing the relative merits of reduction in sample size and power against the chance of invalid conclusions caused by sample bias.

Table 3. Gender-specific sib recurrence risks in twin families

Proband gender	Affected sibs/total sibs	Recurrence risk (±SE)
Sister of female twin	9/223	4.0 (±1.3)
Sister of male twin	4/108	3.7 (±1.8)
Brother of female twin	5/241	2.1 (±0.9)
Brother of male twin	2/120	1.7 (±1.2)
Total sib of twin	20/692	2.9 (±0.6)

By ascertaining twins from a very large population of patients, we obtained a sample size sufficient to address ascertainment issues and have shown any bias to be minimized or absent. The sex ratio of twin probands was not different from the sex ratio present in the population of MS patients, and the MZ/DZ ratio was very similar to that observed in the Canadian population. The usual excess of female or MZ pairs was demonstrably absent. The twinning rate in MS patients was very similar to the twinning rate observed in the Canadian population and suggests that biased ascertainment toward twins was not present.

Four observations related to ascertainment increase the reliability of the results. The twin population ascertained likely represents ≈3/4 of the MS twin population of Canada. Observations of other patient subgroups in the Canadian population indicate that those not ascertained are mainly derived from geographic areas not covered by the MS clinics. Second, we observed the twinning rate in MS patients to be similar to the rate observed in the Canadian population, which suggests that twinning itself is unrelated to MS risk. Furthermore, the recurrence risks for sibs of twin probands did not differ from those derived from nontwin probands (Table 4). This indicates that twins are representative of the general population of MS patients and are probably influenced by the same susceptibility genes and environment as nontwin cases. Further supporting this conclusion is the increased allele frequency of HLA-DR15 found in twin probands, which was not different overall, compared with nontwin MS patients. Having demonstrated no ascertainment bias and presented evidence that the twin population is representative of the general MS population, we can now consider the results of the study with greater assurance.

For the total twin population of 354 pairs, we obtained a probandwise concordance rate of 25.4% in MZ twin pairs and 5.4% in DZ pairs. These results are very similar to those we reported previously with smaller sample sizes. This study is similar in size to all previously published reports combined that, when summed, have very similar pairwise rates to those presented here. The initial Canadian twin studies (10, 17) reported similar MZ-to-DZ concordance findings in independent samples, and they were reconfirmed in a national study from the United Kingdom (16). The unaffected co-twins in our sample

Table 4. Recurrence risk to Canadian full siblings of twin and nontwin probands

Region	Probands	Affected/total siblings	Recurrence risk (95% confidence interval)	Age-corrected recurrence risk (95% confidence interval)	Ref.
Canada	288	20/692	2.89 (1.64–4.17)	3.23 (1.90–4.48)	Twin probands, this study
Canada	1,243	60/2,124	2.82 (2.12–3.21)	3.40 (2.56–3.86)	31 (update from half-sib series)
Vancouver, British Columbia	815	54/1,886	2.86 (2.11–3.27)	3.91 (2.89–4.46)	32 and 33
Middlesex County, Ontario	203	16/451	3.55 (1.84–4.47)	4.0 (2.1–5.0)	34

had surpassed >90% of their risk for developing MS, and our previous studies with long follow-up information suggest that the concordance rate will change very little in a population of this age (17).

When concordance results are broken down by gender, it was found that the great majority of MZ concordant pairs (22 of 24) were female. *A priori*, the expected concordance rates should be in excess among female pairs because the MS population gender ratio is approximately two females to each male, although this projection assumes that gender ratio is independent of factors leading to concordance in this comparison.

This finding is also seen in the literature-derived data (see Table 1), in which 25 of 28 concordant pairs were female. In the latter, data might be explained partly on the basis of ascertainment bias, because some of the studies had a female excess overall. However, in the study presented here this was not the case, and the proportion of concordant female pairs in the literature data were found to be nearly identical. In the combined samples (Table 1), 47 concordant MZ twin pairs were female compared with 5 concordant male MZ pairs. Although this sex ratio (9.4:1) is high, it is not significantly different from expectations derived from the observed 2.37:1 gender ratio in all MS probands assuming multiplicativity of the sex ratio for two twins vs. singletons (i.e., 5.6:1).

In contrast to the female–female twin pairs, concordance rates in the male MZ and DZ twin pairs were very similar. Although the female bias would expectedly increase the magnitude of the concordance rates in females for both MZ and DZ pairs, the overall MZ/DZ ratio should be similar in both sexes if both sexes have a similar level of polygenicity. The finding that the MZ and DZ concordance rates in males are very similar is unexpected but by itself cannot be taken to mean that genes do not influence susceptibility in males. Although the inability to find a difference is intriguing, this likely reflects small numbers and lower prevalence in men. It is clear that the risk for male relatives as one goes from twins to sibs, half-sibs, and cousins progressively drops (unpublished data), consistent with the serial dilution of susceptibility genes.

The rate of twin concordance seems to reflect background prevalence as shown by MZ concordance rates in the order of 6–11% in France and Italy, which have a prevalence approximately half that of Canada (ref. 6 and L. Ristori, S. Cannoni, and M. Salvetti, unpublished data). There are differences in gender ratio across different countries, with a tendency for those regions with the highest prevalence having the largest female/male ratio. There seems to be a relationship between the gender ratio, disease prevalence, and twin concordance.

We sought to determine the influence of gestational and neonatal factors more commonly shared between twins such as those related to shared gestational or prenatal timing on risk by assessing the rate of MS in DZ twin pairs and comparing this to the optimal control, i.e., the full sibs of the DZ twins. The MS rates in the sibs of twins are nearly identical to those seen by long-term observation in nontwin sibs of MS patients in general (Table 4 and refs. 31–33). The overall DZ rate at 5.4% exceeded that for sibs at 2.8%, although the difference was not significant. The full sib recurrence rate of twin probands we report here is remarkably consistent with other proband groups we have studied (30–32). Accordingly, the modestly increased DZ rate found here may yet prove to reflect environmental effects shared uniquely by twins.

In summary, these conclusions derive from a study in which ascertainment bias could be carefully assessed and in which it was found to be largely absent. This population-based study is of similar magnitude to all previous MS twin studies combined. Twins were not found to differ from nontwin MS patients in terms of sib recurrence risks nor for the allele frequency of the MS-associated DRB1*1501 allele, which points to the generalizability of this twin study. The MZ-to-DZ difference in this study was derived primarily from an excess of concordance in female MZ pairs.

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