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# Space–time clustering of multiple sclerosis cases around birth

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*Objectives* – To investigate whether infectious events around birth and during early infancy are likely to be of relevance in MS pathogenesis. *Subjects and methods* – Data are available from two regions in The Netherlands: Groningen ($n$=320) and Rotterdam ($n$=226). Simultaneous clustering in birth date and birth location of MS cases is tested by the methods of Mantel, Knox and Jacquez. *Results* – No evidence was found for a space–time interaction between place and time of birth. *Conclusion* – Perinatal infectious events are unlikely to be a major factor in determining MS susceptibility.

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Key words: multiple sclerosis; space–time clustering; infectious pregnancy complications; puerperal infection; computerized medical records system

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A long standing issue in the study of multiple sclerosis is the question to what extent environmental factors contribute to the development of the disease, and if so, at what ages the relevant events occur. It has been suggested that early viral infections play a role in the disease etiology, and that the disease is acquired through infection by a "slow virus" during infancy and early childhood. This may be inferred from the association between MS and communicable diseases like measles, poliomyelitis, varicella, mumps and whooping-cough (1–5), and from the association between MS and ubiquitous infectious agents like herpes virus type 1 and 2 (6, 7) and human herpes type 6 (8). MS might also be initiated as a fierce immunological reaction to a late infection of a common virus. Some have argued the existence of a late susceptibility interval located somewhere between 10–15 years of age (9, 10). One study identified an age interval of 13–20 years, with a peak occurring at 18 years of age (11). Migrant studies show fairly consistently that the probability of acquiring the disease is determined before adolescence. This implies that environmental factors would play no role anymore after the age of, say, 15 years. Migrant studies are, however, not very informative with regard to the question as to what might be the most critical age periods before adolescence, that is, between conception and the 15th birthday. It could be that the "window of opportunity" for infection is quite narrow, similar to the relation between measles and Crohn's disease (12), but a long window is also possible. Knowing the period of maximal susceptibility helps to appropriately focus epidemiological and etiologic research, and may assist in the timely implementation of preventive measures.

The notion that viral infections may contribute to susceptibility for MS has recently been supported also by the finding that a small heat shock protein in the central nervous system, viz. alpha B-crystallin, acts as an immunodominant myelin antigen to the human T-cell repertoire (13). Since heat shock proteins may be induced also by viral infections, it is well conceivable that in this way, viral infection may contribute to MS pathogenesis.

If an infectious component is part of the etiology, it is to be expected that persons of susceptible age within the proximity of already infected persons have an increased probability of becoming infected and, therefore, have an increased disease susceptibility. In that case, geographical location and age are likely to be related for persons who developed MS, i.e., they are closer within time and space than would be expected by chance. Various statistical procedures exist for testing space–time interaction. Such tests do not directly compare cases to controls, but determine to what extent the observed time–space pattern in the case group can be created by chance. The usefulness of such procedures relies on the idea that the causes of space–time clustering should vary in the same pattern as the disease. Viruses and toxic chemicals are 2 examples of type of causes that vary in time and space.

The main question dealt with in this paper is whether the disease initiation of MS is associated with exposure to an environmental agent that varies in time and place, e.g., an epidemic virus, during pregnancy, infancy and early childhood. The perinatal period is interesting because it is here that clues could be found as to where the start of a susceptibility window is located. The time and location at birth are used as indicators of the location of each case during during pregnancy, infancy and early childhood.

Previous studies have failed to produce evidence for MS clustering around birth (11, 14–18). However, many of these studies used crude measures of closeness in space and/or time, such as being born within the same city or year, or had small sample sizes. Clustering on a smaller scale may be hidden by an inadequate resolution. Our study has a higher resolution of the distance in both space and time, so infectious processes on a smaller scale will be better detectable. The high population density of The Netherlands increases the probability that viral infections spread over the population, thereby enhancing the possibility to detect disease clusters.

## Subjects and methods

### Subjects

Data on time and place of birth for 2 groups of MS patients, registered at the neurological departments of the academic hospitals of Rotterdam and Groningen respectively, were available for this study. The city of Groningen is the capital of the province of Groningen located in the north of The Netherlands. Rotterdam is one of the major cities in the densely populated western part of the country.

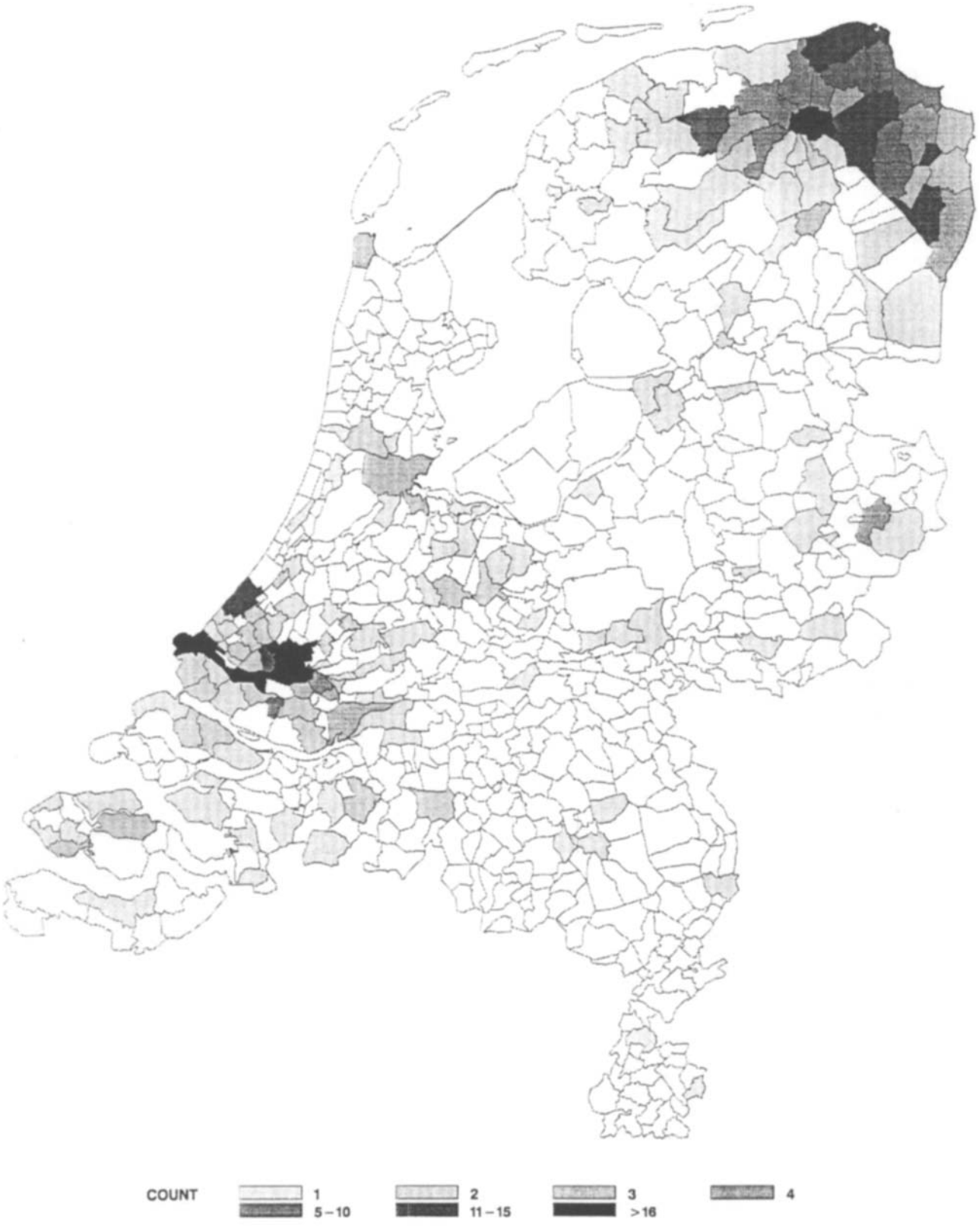
The data from Groningen were obtained in 3 previous prevalence studies in the province of Groningen held in 1981, 1985 and 1992 (19). The latest survey in 1992 resulted in 423 cases of MS living in the province of Groningen. Data concerning birth location are present for a subset of 320 cases. These cases have been classified according to Poser's criteria (20) as definite MS ($n$=297) and probable MS ($n$=23). The Rotterdam data were obtained from the Rotterdam MS Databank of the Department of Neurology of the Academic Hospital Rotterdam. The databank was set up in 1992 with the purpose of storing and retrieving relevant data for the MS patients seen at or admitted to the department. The 226 prevalent cases that are present in this study are classified as definite MS ($n$=145) and probable MS ($n$=81) in accordance with the EDMUS implementation of the Poser guidelines (21).

Table 1 gives a summary of some background information in both data sets. Since the register of Groningen existed before the Rotterdam registration started, the Groningen cases are generally somewhat older. Fig. 1 illustrates the geographic distribution of birth locations of the cases in the data. As expected, cases are grouped around the academic centres of Groningen in the North and Rotterdam in the West. The figure contains the number of cases per municipality in the sample and reflects population density as well as geographic clustering. The black areas in the centre correspond to the cities of Rotterdam and Groningen, respectively.

Distances in space between individual cases were determined by matching the names of the birth locations to a centroid file of topographic names.

Table 1. Summary of the data from Groningen and Rotterdam

| | Groningen | Rotterdam | Both |
|---|---|---|---|
| Sex | | | |
| male | 110 | 89 | 199 |
| female | 210 | 137 | 347 |
| Year of birth | | | |
| before 1930 | 54 | 8 | 62 |
| 1930–1939 | 63 | 35 | 98 |
| 1940–1949 | 71 | 59 | 130 |
| 1950–1959 | 77 | 61 | 138 |
| 1960–1969 | 47 | 52 | 99 |
| after 1970 | 8 | 11 | 19 |
| Year of first presentation of MS | | | |
| before 1950 | 16 | 1 | 17 |
| 1950–1959 | 24 | 1 | 25 |
| 1960–1969 | 44 | 13 | 57 |
| 1970–1979 | 67 | 23 | 90 |
| 1980–1989 | 144 | 101 | 245 |
| 1990–1995 | 25 | 72 | 97 |
| unknown | 0 | 15 | 15 |
| Age at first presentation of MS | | | |
| before 20 | 48 | 20 | 68 |
| 20–29 | 121 | 74 | 195 |
| 30–39 | 86 | 64 | 150 |
| 40–49 | 48 | 33 | 81 |
| above 50 | 17 | 20 | 37 |
| unknown | 0 | 15 | 15 |
| Form of MS | | | |
| relapsing–remitting | 106 | 148 | 254 |
| secondary chronic progressive | 116 | 61 | 177 |
| primary progressive | 98 | 17 | 115 |
| Birth location | | | |
| city of Groningen | 69 | 0 | 69 |
| within 50 km of city of Groningen | 156 | 3 | 159 |
| Rotterdam | 5 | 76 | 81 |
| within 50 km of Rotterdam | 16 | 90 | 106 |
| other regions in The Netherlands | 39 | 21 | 60 |
| outside The Netherlands | 0 | 12 | 12 |
| unknown | 35 | 24 | 59 |
| $n$ | 320 | 226 | 546 |




**Birth locations of 475 cases of Multiple Sclerosis**

*Fig. 1.* Geographic location of MS patients in the combined Groningen and Rotterdam sample ($n$=475). The shading of the figure corresponds to the number of patients per municipality.

A topographic name refers to a part of a municipality. We had access to a digital file that contains the boundary locations for 3941 4-digit postal code areas in The Netherlands in longitude–latitude coordinates. Centroid locations of 2425 different topographic names in The Netherlands were obtained from this file by averaging the $x$–$y$ coordinates belonging to the same topographic name. After matching the patient data and this centroid file on birth location, the coordinate, assigned to each patient, formed the basis for computing the Euclidean distance between pairs of MS cases. The level of contact between cases is thus approximated as the geographic distance between patients, expressed in units of a kilometre (km). Distance in time is simply defined as the difference between birth dates of cases, expressed in units of days.

Cases that have migrated into the catchment areas of the study, that is, cases that are born abroad or in other parts of The Netherlands, will often be classified as being distant from all other cases. If, by coincidence, some of these cases have birth dates close to others, such pairs weaken the sensitivity of the detection methods. Therefore, the analyses are performed separately for all cases born within a radius of 50 km from Groningen ($n$=228) and Rotterdam ($n$=187).

### Statistical tests

Three statistical tests for detecting space–time clustering are used, the classic methods by Mantel (22) and Knox (23), as well as the $k$ nearest neighbour test as recently proposed by Jacquez (24). Mantel's generalized regression approach is a general clustering test, whereas the method of Knox is used here to search for more specific clustering patterns. The $k$ nearest neighbour method requires fewer arbitrary assumptions and provides an alternative for the 2 classic tests. Since the tests of Knox and Mantel are well established, the results of these tests are included here because they could be easily compared to other work. None of the tests requires knowledge of the underlying population density as long as the distribution of the background population is constant with time (25). The probability that a patient enters the register is thus allowed to vary *between* different geographic locations (which is clearly the case in our application). In contrast to this, we assume that this probability is constant *within* every location.

*Test 1* – Suppose $x_{ij}$ is a measure of closeness in space between case $i$ and $j$, and $y_{ij}$ is a measure of closeness in time between case $i$ and $j$. Mantel's method compares the test statistic $Z=\Sigma\Sigma\, x_{ij}y_{ij}$ to a reference distribution that pertains to the null hypothesis of no space–time interaction. Mantel suggests taking the reciprocal of the simple Euclidean distance between two cases as $x_{ij}$ and take the reciprocal of $y_{ij}$ as the closeness measure $y_{ij}$ in time. In cases where this leads to division by zero, we use a constant of 1/2 as a replacement value. Since our measures are in kilometres and days, this corresponds to defining respectively 2 km and 2 days as the minimum distance between close pairs. The reference distribution of $Z$ under the hypothesis of no clustering was obtained by creating random permutations of the time variable, and then recomputing the $Z$ statistic for each replication. Repeating this procedure 100 times yields an estimate of the distribution of $Z$ under the null hypothesis of no clustering. An approximate $P$-value is obtained as the proportion of simulated $Z$-statistics that are equal to or exceed the observed $Z$ statistic. Increasing the number of replications to 500 did not influence the results. A $P$-value$<0.05$ is taken as criterion for statistical significance.

*Test 2* – The test of Knox is a special case of Mantel's method where $x_{ij}=1$ if cases $i$ and $j$ are closer in space than a pre-defined cut point $c_x$ and $x_{ij}=0$ otherwise, and where $y_{ij}=1$ if cases $i$ and $j$ are closer in time than a pre-defined cut point $c_y$ and $y_{ij}=0$ otherwise. For these choices the $Z$-statistic is simply equal to the number of pairs that are both "close in space" and "close in time". If one forms the 2 by 2 contingency table of $x_{ij}$ and $y_{ij}$ the expected (E) number of pairs under independence, that is under the hypothesis of no space–time interaction, is equal to the product of both marginal counts divided by the total number of pairs. Suppose that O denotes the number of observed pairs close in both time and space, then the ratio O/E is a measure of strength of the clustering similar to the Relative Risk. A value larger than one indicates a possible clustering. Knox conjectured that under the null hypothesis O is approximately distributed as a Poisson variate with expectation E. This is appropriate as long as O is relatively small compared to the total number of pairs (26), which is the case here. In order to find "optimal" time and space windows in which clustering might occur, the procedure of Knox is applied under different cut-points. Due to multiple testing and chance capitalization, the $P$-values from this procedure will be smaller than the true chance of making a Type I error (actually, Mantel's method was introduced to repair this deficit by eliminating the need to choose cut points). We therefore do not look so much into the $P$-values *per se*, but rather concentrate on the accompanying effect estimate, that is, on the O/E ratio in Knox's test derived under the assumption of independence.

*Test 3* – The $k$ nearest neighbour (NN) method of Jacquez retains the virtues of the classic methods and corrects some significant weaknesses, in particular the need to specify arbitrary constants. Simulations done by Jacquez suggest that the statistical power of the NN method is superior to the other interaction tests, but this finding still awaits confirmation (24). The idea is to count the number of pairs, $J_k$, that are $k$ nearest neighbours in both space and time. The $k$ nearest neighbours are the set of cases as near or nearer to a case than the $k$-th nearest neighbour. So, the test statistic $J_2$ is the number of case pairs that are second nearest neighbours in both space and time, and includes all pairs that were first space–time nearest neighbours. The difference statistic $\Delta J_k = J_k - J_{k-1}$ gives the pair count beyond that for level $k$–1. The null distributions of $J_k$ and $\Delta J_k$ are derived by simulation in the same manner as in Mantel's method. Since our data contain many cases with identical birth locations, there is often no unique first (or 2nd, 3rd . . .) nearest neighbour in space. We addressed this problem by drawing, without replacement, one case randomly from all potential first (or 2nd, 3rd . . .) nearest neighbours, and then used this case in the computations. The problem with ties is that the proper order of distances that is needed for the computation of $J_k$ is missing. Suppose that the size of the tie block is equal to $p$. Then $p!$ potential orders could be thought of, each of which has probability $1/p!$. The procedure given above will randomly draw just one of those orders with probability $1/p!$. Although we haven't attempted any formal proofs, we expect that, for a given set of ties, this method will be neutral with respect to the estimated clustering intensity.

## Results

*Test 1* – For cases born within 50 km of Groningen, we found $Z=5.43$ as compared to $E(Z)=7.74$ ($P=0.86$). For Rotterdam, we found $Z=11.26$ as compared to $E(Z)=8.46$ ($P=0.08$). Thus, Mantel's test failed to detect any space–time interaction around birth.

*Test 2* – The O/E ratios from Knox's test under various cut points are summarized in Table 2 and 3. Most O/E ratios are located within a range of 10 percent from unity, that is, in the interval (0.91–1.1). Such effects are probably too small to be of any practical relevance and fall well within the bounds of sampling variation. The largest ratio for Groningen is 41/35.2=1.17 (95% c.i.: 0.83–1.49), located at 120 days by 5 kilometres, but it is not significant ($P=0.14$ under the assumption that observed counts follow a Poisson distribution with expectation 35.2). The largest ratio for Rotterdam is 6/3.65=1.64 (95% c.i.: 0.62–2.67). The distribution of the $P$-values corresponding to Tables 2 and 3 is approximately uniform, which is precisely what would be expected if no clustering is present. In fact, the only significant results we found were counter-intuitive in the opposite direction and indicate anti-clustering at cut point distances of beyond 15 km for cases born in Groningen.

Table 2. Knox O/E ratios for MS cases born within 50 km of Groningen ($n=228$)

| | Cut-off distance in km | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| O/E ratio | 1 | 2 | 3 | 5 | 7 | 10 | 15 | 20 |
| Cut-off distance in days | | | | | | | | |
| 10 | 0.35 | 0.35 | 0.34 | 0.33 | 0.83 | 0.77 | 0.66 | 0.65 |
| 30 | 0.63 | 0.63 | 0.62 | 0.59 | 0.80 | 0.84 | 0.62 | 0.73 |
| 60 | 1.08 | 1.07 | 1.06 | 1.01 | 1.05 | 1.02 | 0.83 | 0.82 |
| 90 | 1.13 | 1.12 | 1.11 | 1.13 | 1.12 | 1.09 | 0.92 | 0.92 |
| 120 | 1.13 | 1.12 | 1.14 | 1.17 | 1.11 | 1.13 | 0.95 | 0.92 |
| 150 | 1.08 | 1.07 | 1.09 | 1.10 | 1.11 | 1.07 | 0.96 | 0.91 |
| 200 | 0.92 | 0.92 | 0.93 | 0.93 | 0.94 | 0.95 | 0.89 | 0.86 |
| 250 | 1.02 | 1.01 | 1.02 | 1.02 | 1.00 | 1.00 | 0.91 | 0.88 |
| 365 | 1.03 | 1.02 | 1.02 | 1.04 | 1.02 | 1.00 | 0.90 | 0.90 |
| 500 | 0.95 | 0.95 | 0.96 | 0.98 | 0.95 | 0.94 | 0.88 | 0.91 |

Table 3. Knox O/E ratios for MS cases born within 50 km of Rotterdam ($n=187$)

| | Cut-off distance in km | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| O/E ratio | 1 | 2 | 3 | 5 | 7 | 10 | 15 | 20 |
| Cut-off distance in days | | | | | | | | |
| 10 | 1.64 | 1.64 | 1.64 | 1.59 | 1.25 | 1.16 | 1.06 | 0.95 |
| 30 | 1.07 | 1.07 | 1.07 | 1.04 | 0.94 | 0.90 | 0.95 | 0.94 |
| 60 | 0.91 | 0.91 | 0.91 | 0.89 | 0.78 | 0.82 | 0.92 | 0.87 |
| 90 | 1.08 | 1.08 | 1.08 | 1.07 | 0.99 | 0.96 | 1.00 | 0.94 |
| 120 | 1.04 | 1.04 | 1.04 | 1.03 | 0.95 | 0.97 | 0.99 | 0.94 |
| 150 | 1.03 | 1.03 | 1.03 | 1.02 | 0.95 | 0.95 | 0.98 | 0.96 |
| 200 | 1.04 | 1.04 | 1.04 | 1.03 | 0.95 | 0.95 | 0.99 | 0.97 |
| 250 | 1.09 | 1.09 | 1.09 | 1.08 | 1.00 | 0.98 | 0.99 | 0.98 |
| 365 | 0.98 | 0.98 | 0.97 | 0.96 | 0.96 | 0.95 | 0.95 | 0.95 |
| 500 | 0.95 | 0.95 | 0.95 | 0.94 | 0.93 | 0.93 | 0.95 | 0.97 |

*Test 3* – Table 4 and 5 contain the results of the $k$ nearest neighbour tests for $k=1, \ldots, 10$ for Groningen and Rotterdam. No pair counts were obtained that were significantly higher than the expected value at any level of $k$, so these tests agree with the results given before.

All 3 tests are negative, so our data provide no evidence for rejecting the null hypothesis that multiple sclerosis cases are randomly distributed by date and place of birth simultaneously. Perhaps the most convincing evidence we have is that even after

Table 4. Results of $k$'th Nearest Neighbour test for MS cases born within 50 km of Groningen ($n$=228). The overall test probability is $P$=0.17

| $k$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| $J$ | 0 | 2 | 9 | 19 | 29 | 32 | 45 | 59 | 67 | 81 |
| $E[J]$ | 0.91 | 3.89 | 9.14 | 16.5 | 26.0 | 36.7 | 49.4 | 64.3 | 82.3 | 101 |
| $P$ | 1.00 | 0.90 | 0.56 | 0.33 | 0.27 | 0.79 | 0.77 | 0.73 | 0.98 | 0.99 |
| $\Delta J$ | 0 | 2 | 7 | 10 | 10 | 3 | 13 | 14 | 8 | 14 |
| $E[\Delta J]$ | 0.91 | 2.98 | 5.25 | 7.40 | 9.45 | 10.7 | 12.8 | 14.8 | 18.0 | 18.8 |
| $P$ | 1.00 | 0.83 | 0.30 | 0.24 | 0.50 | 1.00 | 0.52 | 0.65 | 1.00 | 0.92 |

Table 5. Results of $k$'th Nearest Neighbour test for MS cases born within 50 km of Rotterdam ($n$=187). The overall test probability is $P$=0.09

| $k$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| $J$ | 3 | 8 | 14 | 27 | 35 | 41 | 54 | 76 | 93 | 114 |
| $E[J]$ | 1.02 | 3.88 | 8.64 | 15.9 | 25.1 | 36.3 | 49.3 | 64.5 | 80.7 | 100 |
| $P$ | 0.10 | 0.11 | 0.10 | 0.02 | 0.05 | 0.32 | 0.29 | 0.06 | 0.09 | 0.12 |
| $\Delta J$ | 3 | 5 | 6 | 13 | 8 | 6 | 13 | 22 | 17 | 21 |
| $E[\Delta J]$ | 1.02 | 2.86 | 4.76 | 7.31 | 9.16 | 11.2 | 13.0 | 15.2 | 16.2 | 19.3 |
| $P$ | 0.10 | 0.17 | 0.35 | 0.03 | 0.69 | 0.99 | 0.56 | 0.04 | 0.45 | 0.34 |

trying a range of possible cut point for Knox's test we still get no significant results.

## Discussion

Due to various factors, the data comprise of only a part of the population of MS patients in The Netherlands. For example, we estimate that we know birth locations for (320–35)/4.23=67 percent of the entire population of MS cases born in the province of Groningen and alive in 1992. To what extent does incomplete coverage of cases influence the conclusions? With respect to selection bias, we cannot imagine a realistic scenario in which clustered cases would selectively drop out of the registration, thereby dissolving and masking the entire clustering structure. Given the seriousness of the disease, it is unlikely that drop out by economic considerations (e.g., more cases that are missing from low SES locations) is a factor that could mask clustering. A relation between the probability of selection and the existence of a space–time interaction seems implausible. Also, missing cases could lower the sensitivity of the method. Suppose that a virus spreads from case A to C via B. Incomplete ascertainment could break the link between cases A and C if B is not reported. This effect could be accounted for by increasing the critical distances of the Knox test at the expense of a smaller signal/noise ratio. In order to get an idea about the sensitivity of the Mantel's test, we conducted a small experiment in which we created an artificial clustering by randomly duplicating a small number of cases. It turns out that only 3 to 4 duplications out of 478 cases are needed to produce a significant result. Mantel's test is thus quite responsive to a few strong clusters. Note that randomly duplicated cases correspond to highly clustered cases (both time and space are identical) and Mantel's method depends on a constant that is used to deal with just this situation. In a more realistic simulation, we added uniform noise between plus and minus 5 km to each duplicated record (in both $x$- and $y$-direction). Now about 30 pairs are needed. Evidently, the data contain less clustering than the equivalent of 30 pairs born on the same day within $\sqrt{(5^2+5^2)}=7$ km of each other.

Note that our analyses are based on prevalent cases. Since the use of prevalent cases can induce information bias in the exposure measurement, it is generally preferable to study incident cases. However, in present study the exposure factor (time and place of birth) can nearly always be determined without error. The use of prevalent cases can also cause selection bias because people that have recovered from the disease can be included as cases. Since we are studying the determinants of a chronic disease, the event of interest in this study is whether or not a person has ever been diagnosed as having MS. The seriousness of the disease at a given point is not a relevant outcome here. It is therefore unlikely that either type of bias could have affected the results.

The tests assume that the distribution of the background population is constant with time. To check this assumption, we stratified the data by 5 decades of birth and computed Mantel's test *per stratum*. No significant clustering was found in this way, so the assumption of constant probability does not seem to be critically violated in our application.

The spatial resolution of the analysis is related to the size of the birth place. People born in small villages are positioned accurately on the map within 200 to 300 meters of the actual place of birth. Since we only have place of birth of the cases, those born in larger places cannot be distinguished from each other and are all mapped to the centre of the city. The largest cities, Rotterdam (88 4-digit codes) and Groningen (47 4-digit codes), measure approximately 8 by 8 km and 5 by 5 km in size respectively, so here the mapping is considerably less precise. Pairs of cases from the same city will always be counted as close in space. Since this may dilute any clustering effects if clustering occurs at distances below, say, 5 km, the analysis was also applied to reduced samples that excludes the cases born in Rotterdam ($n$=106) and the city of Groningen ($n$=159). The conclusions emanating from these analyses are the same as before. More specific analyses using subgroups of types of MS or sex also did not reveal any apparent space–time clustering.

General interaction tests such as the Knox and Mantel methods have been criticized for their low power. The least powerful test (Knox) has a statistical power over 0.80 for sample sizes larger than 125 (24). Though both our samples are larger than this, several other factors may affect the power of the study. We have seen above that both presence of ties and incomplete case ascertainment decrease statistical power. Power will also be reduced for those time periods for which the birth location is a bad indicator of the actual residence in that period. So the farther one moves from time of birth, the less sure one can be that in fact no interaction exists. We boosted the power by combining the cases of Rotterdam and Groningen ($n=478$) in one analysis. Mantel's statistic then equals $Z=17.44$. This is even lower than the value $E(Z)=19.4$ expected under independence, so the combined analysis also provides no evidence of space-time clustering ($P=0.64$).

In general, absence of space-time interaction is only interesting provided that the presumed infectious agent varies in both space and time. If there is no temporal (spatial) variation, there can be no space–time interaction (if a factor does not vary, it cannot interact with any other factor). Variations in space of MS cases are well documented, e.g., as the global north–south gradient and by occasional epidemics. It has been observed that the frequency of MS relapses correlates with the existence of viral infections (27). This suggests that infection by possibly relevant viruses like influenza does vary in time. In addition, it has been found that season of birth of MS cases deviates from the general population (28), thereby indicating a possible viral component. Others (29) could not confirm this, however.

The results indicate that at least in 2 separate regions in The Netherlands, no evidence can be found in support of the idea that a perinatal infectious event represents a major factor in determining MS susceptibility. Interpretation of the present study must take into account that transmissibility of infection may not always be directly proportional to geographic distance. Also, absence of clustering does not completely rule out a role for perinatal infections. For example, widespread transmission of an agent occurring at birth, with only a small proportion of infections resulting in disease, does not produce space–time clustering and cannot be uncovered by the methods used here. Given these caveats, however, our findings appear to be in agreement with other studies that have suggested puberty and adolescence rather than the earliest childhood as the stage at which distinct infectious events may affect MS susceptibility.

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