American Journal of Epidemiology

Vol. 164, No. 4
DOI: 10.1093/aje/kwj190
Advance Access publication June 5, 2006

Original Contribution

Evidence of Early Childhood as the Susceptibility Period in Multiple Sclerosis: Space-Time Cluster Analysis in a Sardinian Population

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Received for publication October 4, 2005; accepted for publication February 16, 2006.

The authors analyzed the natural history of multiple sclerosis (MS) before onset to identify the period of susceptibility and exogenous factors that might play a role in causing the disease. Space-time cluster analysis was performed among northern Sardinians, a genetically stable Italian population that showed an increasing risk of MS between 1965 and 1999. Residence changes from birth to clinical onset were recorded for all MS patients with clinical onset between 1965 and 1999 in the province of Sassari. Closeness in space and time was defined as living in the same municipality and differing in year of birth by 1, 2, or 5 years. Analyses were performed for the period from birth to age 25 years or MS onset and in demographic and clinical subgroups. Clustering was substantial in early childhood. Clustering was most marked in the most recent cases, among women, and among patients with early age at onset, a relapsing-remitting course, and in the eastern subarea. No clustering was found when closeness in time was defined as a fixed number of years before onset, which argues against a fixed latency period. Early childhood seemed to be a period of increased susceptibility to MS. This evidence and the increasing incidence of MS in northern Sardinia are compatible with a change in environmental exposure.

child; cluster analysis; Italy; multiple sclerosis

Abbreviation: MS, multiple sclerosis.

Multiple sclerosis (MS) is a chronic demyelinating inflammatory and degenerative disorder of the central nervous system (1) and is the most common disabling nervous system disease among young adults. The etiology of MS is unknown but is believed to be multifactorial. The age of clinical onset, when detectable signs and symptoms are manifested, is often uncertain but is usually young adulthood, peaking in the third and fourth decades of life (2). Before this age and probably after birth, the causal mechanisms, comprising interaction between exogenous exposure and immunogenetic makeup (3), trigger biologic onset and later, in some persons, clinically overt disease (4).

Studies on the age of MS clinical onset provide limited information on the causation of MS, whereas natural history before clinical onset is much more relevant. In infectious diseases, the time elapsing between causal mechanisms and onset is referred to as the induction period (5). In chronic diseases with an unknown and probable multifactorial causation and assumed complex genetically based predisposition, such as MS, the susceptibility period refers to the age at which people are exposed to putative risk factors (biologic onset) and is followed by a latency period that lasts until the appearance of clinical manifestations. Allocating the susceptibility period within MS natural history before

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clinical onset, and thus investigating the age of susceptibility and the length of the latency period, is an arduous task for MS; both periods probably vary from a few years to several years. It is, however, critical for identifying patterns of exposures with a role in causing MS.

Various methods have been used to investigate the susceptibility period for MS, such as statistical models, migration studies, and analysis of disease epidemics (6, 7). Although somewhat undermined by methodological limitations, all of these studies have indicated a period of susceptibility in the first 15 years of life. From this perspective, common childhood infectious diseases in genetically prone persons are among the most likely mechanisms initiating MS (8). The overt disease becomes clinically manifest after a latency period of unknown and probably variable duration (9).

Space-time cluster analysis is used in epidemiologic studies of disease causation to test infection hypotheses (10). This model allows researchers to assess whether people with MS have lived closer to one another than would be expected by chance during a specific time period, in which they would thus have been exposed to a common risk factor. In diseases with long latency periods, such as MS, this approach is based on the date and place of residence at clinical onset and before onset. This model is therefore suitable for investigating the susceptibility period for MS, revealing patterns of exposure to possible exogenous factors triggering the disease (10, 11). The potential for detecting space-time clustering in relation to a change in putative exogenous factors is enhanced when investigators study an isolated and well-defined population with a disease risk that is increasing over time as a possible effect of specific environmental changes.

In this study, we aimed to identify and characterize the susceptibility period of MS in Sardinia, Italy, using space-time cluster analysis, regardless of any known, real, or perceived "epidemic" of disease in any specific time interval or geographic area (10, 12–14). Over the past three decades, the population of Sardinia has been found to be at increasing risk of MS, and changing patterns of the clinical phenotype have been observed during that period, suggesting a role of a newly introduced or differently concentrated putative exogenous agent in the area and over time (15, 16).

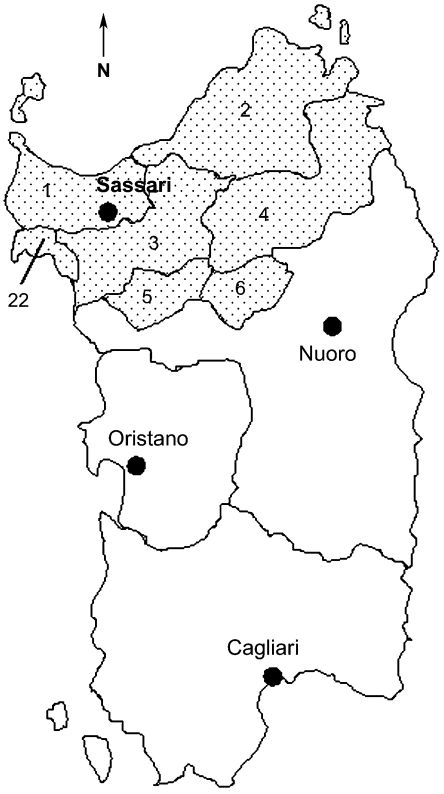

FIGURE 1. Map of Sardinia, Italy, showing the seven geographic subareas included in a study of multiple sclerosis patients with onset between 1965 and 1999.

MATERIALS AND METHODS

Study area

The study area was the province of Sassari in northern Sardinia (figure 1), encompassing 90 municipalities in an area of 7,520 km^2 between latitudes 40°30′N and 41°N. The population increased from 381,191 in 1971 to 453,628 in 2001 (17). Migration has been modest, with only 1.7 percent of the total population registered as moving into the study area from other provinces or countries and only 1.6 percent moving away from the study area in 1995 (18). Since the proportion of residents born outside the province of Sassari is negligible and the inward migrants are mostly from Sardinia, the study population is assumed to consist only of native-born persons. With endogamy playing a role, especially in the most inland communities (19), specific historical, linguistic, and cultural patterns have differentiated seven geographic subareas over time (figure 1) (20): Sassarese (1), Gallurese (2), Northern Logudorese (3), Eastern Logudorese (4), Southern Logudorese (5), Goceano (6), and Algherese (Catalan) (22). The prevalence of MS was 150 per 100,000 population in 1997 (15), and the incidence was 5.8 per 100,000 population per year from 1995 to 1999 (16).

Study population

Cases were identified using the MS case registry established at the Institute of Clinical Neurology, University Hospital of Sassari, the main referral center for MS patients in northern Sardinia. Case ascertainment and registry enrollment have been described in detail previously (16). Patients were diagnosed according to the Poser Committee criteria (21) and by excluding infectious and other immune-mediated diseases of the central nervous system. Information was recorded on patients' date and municipality of birth, changes in municipality of residence prior to onset, date and symptoms of onset, date of diagnosis, and disease classification. For this study, the initial clinical course was retrospectively categorized into relapsing-remitting course at onset and progressive course at onset.

TABLE 1. Two-by-two table for space-time cluster analysis (Knox's method (22)) of multiple sclerosis at age 1 year (n = 556), Sassari, Italy*

	Distance between pairs of patients in *space* (municipalities)		Total
	Close	Not close	
Distance between pairs of patients in *time* (±2 years)			
Close	A = 2,334	C = 17,091	19,425
Not close	B = 14,822	D = 120,043	134,865
Total	17,156	137,134	154,290

* Expected (E) number of pairs close in time and space: $E = (A + B) \times (A + C)/[n(n - 1)]/2 = (2{,}334 + 14{,}822) \times (2{,}334 + 17{,}091)/[556(556 - 1)]/2 = 2{,}159.9$. Observed number of pairs close in time and space (A) = 2,334. Observed:expected ratio = 2,334/2,159.9 = 1.08.

The target population comprised 689 MS patients from the case registry (496 women and 193 men; female:male ratio = 2.6) with onset in the province of Sassari between 1965 and 1999. Mean age at onset was 28.6 years (standard deviation, 9.0), and a relapsing-remitting initial course was reported for 80.6 percent of patients. Information on residence changes from birth to onset was obtained by trained investigators administering ad hoc questionnaires in a standardized way. Complete data were collected from 573 patients, while for 105 persons, only information on residence at birth and at ages 5–15 years was available. Among these persons, 76 had the same municipality of residence at birth and at ages 5–15 years; thus, because of the population features, these persons were assigned the same municipality of residence from birth to age 15 years. These persons were considered eligible for statistical analysis up to age 15 years.

Statistical analysis

The goal of space-time cluster analysis is to determine whether patients with cases that appeared closely in time also tended to live geographically closer to each other at that time than would be expected by chance. We used the simplest and most frequently used model, Knox's method (22), where all pairs of patients are defined as being either close in time or not close in time according to a cutoff point in number of years and close or not close in space according to some geographic cutoff point. The intervals for defining temporal closeness for each pair of patients were arbitrarily chosen as 1, 2, and 5 years; for 1-year closeness, patients were considered to be temporally close if they had been born in the same year or during the year before or the year after each other. Spatial closeness was defined as residing in the same municipality.

The observed number of pairs of patients close in both time and space was compared with the expected number calculated according to a normal two-by-two table as $(A + B) \times (A + C)/[n(n - 1)/2]$, where A represents the number of pairs close in both time and space, B represents pairs close in space but not in time, and C represents pairs close in time but not in space (10) (table 1). The ratio between observed and expected numbers of close pairs was calculated; values above unity indicated an excess of clustering. The data used in this table refer to the analysis at age 1 year (see table 2).

The statistical significance of deviation from unity for this ratio is often calculated based on an assumption of a Poisson distribution of the observed number of close pairs, A. However, since there is structural dependency in this table caused by each patient's participating in $(n - 1)$ pairs, the assumption of a Poisson distribution does not hold. Simulation studies have shown that using the Poisson distribution produces p values that are too optimistic (T. Riise, unpublished data). Therefore, empirical p values in this study were calculated using 100,000 Monte Carlo simulations by randomly assigning the municipalities of residence to the patients (23). The empirical distribution of the ratio between observed and expected numbers of close pairs was then used to estimate the p value for the actual ratio.

The analysis included 649 MS patients with information on changes in municipality of residence up to at least age 15 years. This yielded 210,276 pairs [$n(n - 1)/2 = 649 \times 648/2$] for statistical analysis.

In order to find the age of highest clustering, we repeated the analyses using the residing municipality each year from the year of birth to age 25 years (or the year of onset if onset occurred before age 25). Secondly, to uncover a fixed latency induction period, we analyzed closeness in time and space for each year from the year of clinical onset backwards to the year of birth. In this case, a cluster meant that an excessive number of pairs of patients had lived in the same municipality during a time period corresponding to a fixed number of years prior to onset for these patients. Cases living outside the province of Sassari at the specific age under study were excluded at that age.

At the age of most clustering, 1 year, we also performed space-time cluster analysis in subgroups according to gender and clinical characteristics: age at clinical onset ($\leq$30 years vs. >30 years), type of initial clinical course (relapsing-remitting vs. progressive), birth year ($\leq$1959 vs. >1959, where 1959 was the median of the total distribution of years of birth), and geographic subarea. Two large subareas were defined according to similar environmental and ethnic features: a western subarea including areas 1, 3, and 22 and an eastern subarea including areas 2, 4, 5, and 6 (figure 1).

TABLE 2. Observed and expected numbers of pairs of multiple sclerosis patients in the province of Sassari, Italy, with onset between 1965 and 1999 who were close* in both time and space, categorized by age for determining municipality of residence

Residence at age (years):	No. of subjects	No. of pairs close in both time and space		Observed:expected ratio	*p* value†
		Observed	Expected		
0	559	2,284	2,149.3	1.06	0.068
1	556	2,334	2,159.9	1.08	0.039
2	554	2,329	2,160.5	1.08	0.041
3	558	2,393	2,220.5	1.08	0.042
4	562	2,378	2,228.8	1.07	0.063
5	570	2,454	2,331.9	1.05	0.11
6	571	2,497	2,372.6	1.05	0.11
7	574	2,586	2,450.7	1.06	0.093
8	575	2,647	2,522.7	1.05	0.12
9	576	2,611	2,494.6	1.05	0.14
10	573	2,606	2,504.0	1.04	0.17
11	574	2,717	2,632.9	1.03	0.22
12	572	2,771	2,711.5	1.02	0.30
13	573	2,849	2,791.5	1.02	0.31
14	573	2,899	2,828.5	1.02	0.28
15	569	2,917	2,881.9	1.01	0.37
16	483	2,218	2,180.0	1.02	0.30
17	478	2,138	2,073.9	1.03	0.20
18	466	2,010	2,006.7	1.00	0.45
19	445	2,010	1,995.7	1.01	0.39
20	418	2,011	1,963.0	1.02	0.27
21	414	2,018	1,982.3	1.02	0.32
22	391	1,661	1,686.9	0.98	0.62
23	364	1,444	1,510.7	0.96	0.84
24	343	1,295	1,357.1	0.95	0.85
25	317	1,086	1,122.9	0.97	0.75

* Temporal closeness was defined as having been born within 2 years of each other; spatial closeness was defined as living in the same municipality.

† Empirical *p* values were calculated by means of 100,000 Monte Carlo simulations for each year.

Statistical significance for subanalyses was calculated using Monte Carlo simulations as described above.

To examine whether clustering was related to age at onset, we compared mean ages at onset in cluster cases (patients participating in at least one close pair) and noncluster cases (patients not participating in any close pair), evaluating the difference by *t* test.

RESULTS

The analyses using temporal closeness of 1, 2, and 5 years all showed clustering in early childhood. For the 2-year temporal closeness, statistically significant clustering was observed from age 1 year to age 3 years (table 2); clustering peaked at age 1, with a ratio between observed and expected numbers of close pairs of 1.08 and an empirical *p* value of 0.039. The strength of the clustering was only slightly lower for the other cutpoints for temporal closeness, with peaks at age 1 year (observed:expected ratio = 1.08) for 1-year closeness and age 3 years (observed:expected ratio = 1.07) for 5-year closeness.

Subgroups were also analyzed according to clinical characteristics (age at onset, initial course, and recency of clinical onset) and demographic variables (gender, year of birth, and geographic subarea) (table 3). All of these variables were analyzed using 2-year temporal closeness and clustering at age 1 year. Clustering was significantly increased for women, patients with a relapsing-remitting course at onset, patients with recent onset (after 1982), and patients living in the eastern subarea. Clustering was borderline-significant for patients born after 1959 and patients with age of onset less than 30 years, while it was increased but not statistically

TABLE 3. Observed and expected numbers of pairs of multiple sclerosis patients in the province of Sassari, Italy, with onset between 1965 and 1999 who were close* in time and space at age 1 year and observed:expected ratios, by subgroup

Subgroup	No. of subjects	% of total	No. of observed close pairs	No. of expected close pairs	Observed:expected ratio	*p* value†
Gender						
Male	165	30	166	158.3	1.05	0.28
Female	391	70	1,255	1,133.0	1.11	0.03
Clinical course at onset						
Relapsing-remitting	412‡	78	1,632	1,439.4	1.13	0.006
Progressive	118‡	22	61	62.7	0.97	0.53
Recency of birth						
Recent (birth >1959)	264	47	1,422	1,303.5	1.09	0.06
Less recent (birth ≤1959)	292	53	831	823.8	1.01	0.39
Age (years) at clinical onset						
≤30	348	63	1,290	1,188.5	1.09	0.06
>30	208	37	244	260.6	0.94	0.78
Geographic subarea						
Eastern	197	35	134	107.5	1.25	0.01
Western	359	63	2,200	2,107.4	1.04	0.11
Total	556		2,334	2,159.9	1.08	0.04

* Temporal closeness was defined as having been born within 2 years of each other; spatial closeness was defined as living in the same municipality.

† Empirical *p* values were calculated by means of 100,000 Monte Carlo simulations.

‡ Information on clinical course at onset was missing for 26 patients.

significant for men and for patients living in the western subarea. Confounding demographic factors specifically influencing the subareas were reasonably ruled out. In fact, if the general population had substantially migrated across the province, it would have been towards the major urban area (Sassari), probably revealing more substantial clustering in the western subarea and thus biasing the results in the opposite direction. The observed difference in clustering between the two subareas instead seems to indicate biologic explanations.

The cluster cases had an earlier mean age of onset (27.3 years; standard deviation, 7.8) than the noncluster cases (30.3 years; standard deviation, 10.2) ($p = 0.0005$, t test). The significant difference in the standard deviation ($p < 0.0001$, Levene's test for equality of variance) indicates a more uniform epidemiologic pattern in the age of onset among the cluster patients.

Performing the analysis backwards by a fixed year prior to onset failed to show any clustering. Table 4 shows the observed:expected ratios and their significance for years 1, 2, 5, 10, 15, 20, and 25 from MS clinical onset backwards. The degree of clustering tended to be lower than expected, though nonsignificantly.

DISCUSSION

The analysis revealed a significant space-time clustering pattern at ages 1–3 years. Clustering was most marked in the most recent cases, in patients with early onset, in patients with a relapsing-remitting course, and for the eastern part of the province of Sassari. Thus, early childhood seems to be a period of increased susceptibility to MS in Sardinians.

Two putative age periods for disease susceptibility, 0–5 years and 10–15 years, have been suggested on the basis of stochastic models estimating the distribution and thus the length of the latency period for MS (6, 9). The latter of these periods was suggested to be the most plausible one, while the observations in the present study are consistent with the estimate of the first period. The authors of the previous study suggested that the mean latency period in that population was 18 years based on the age period 10–15 years. According to our data, the estimated duration of the latency period, at least in some subsets of Sardinians, could be more than 25 years.

Migration studies also indicated that the MS susceptibility period lies between birth and adolescence (8, 24–26). These studies can be biased, however, by selection of the migrating population, small sample sizes, and the difficulty of assessing the time elapsing from migration to onset. Furthermore, too few studies have investigated ages at migration among migrants moving from low-prevalence countries to high-prevalence countries to provide further evidence (6).

Space-time cluster analyses on MS have been conducted in other Caucasian populations. No space-time clustering around birth was found among 783 patients in Northern Ireland (27) or among 556 patients in the Netherlands (28), whereas 381 cases were found to be clustered in late adolescence in western Norway (29). The clustering in

TABLE 4. Observed and expected numbers of pairs of multiple sclerosis (MS) patients in the province of Sassari, Italy, with onset between 1965 and 1999 who were close* in both time and space, categorized by number of years from MS clinical onset backwards for determining municipality of residence

Residence from MS clinical onset (*X*) backwards (years)	No. of subjects	No. of pairs close in both time and space		Observed:expected ratio†
		Observed	Expected	
X − 1	530	3,727	3,904.6	0.96
X − 2	526	3,596	3,767.1	0.96
X − 5	528	3,607	3,799.2	0.95
X − 10	538	3,565	3,748.9	0.95
X − 15	531	3,254	3,373.6	0.97
X − 20	454	2,354	2,346.3	1.003
X − 25	339	1,261	1,228.5	1.026

* Temporal closeness was defined as having been born within 2 years of each other; spatial closeness was defined as living in the same municipality.

† All ratios were nonsignificant using the Poisson assumption; therefore, empirical *p* values were not calculated.

western Norway, where the incidence had increased almost threefold during the study period, was most marked among recent cases, women, and patients with a relapsing-remitting course. In further study of this material, Riise and Klauber (30) found a correlation between a high degree of clustering and early onset. The clustering in our study was most marked in the same clinical subgroups, suggesting that MS heterogeneity may not be population-specific but intrinsic to the disease, simply reflecting different mechanisms of causation.

The space-time clustering in childhood we found was most evident during recent time periods (i.e., for clinical onset after 1982), although we cannot preclude that clustering was also present during the previous decades. Still, this finding and the reported increase in incidence in the same time frame (16) are compatible with a change in environmental exposure in a genetically peculiar population or a change in the spatial and temporal concentration of such exposure. Furthermore, the marked clustering in the eastern part of the province of Sassari is in agreement with the results of a pure space cluster analysis of the distribution of MS prevalences (31), which disclosed a ''hot spot'' in subarea 5, included in this study's eastern area.

The hypothesis behind space-time cluster analysis is that an excess of clustering reflects the presence of a causative agent that varies in prevalence over time between subareas. The most intuitive interpretation of the finding in our study is the existence of an infectious agent during early childhood. However, being close in space and time at a certain age could reflect nonexposure to factors that would normally protect the population from developing the disease. The ''polio hypothesis'' of Poskanzer et al. (32), later supported by Alter et al. (33), suggested ubiquitous distribution and transmission of the agent causing MS early in life in populations with low MS incidence and that infections with the same agent later in life may increase the risk of MS. Cooke (34) posed a similar hypothesis in a critique of the ''pubertal hypothesis'' of the epidemics in the Faroe Islands. The MS epidemics Kurtzke and Hyllested (35) reported in the Faroe Islands after World War II were hypothesized to be caused by exposure from one source that triggered onset at approximately 11 years of age in a population that was virgin to that specific exposure (''pubertal hypothesis''). The clinically overt disease would develop after a fixed latency period (36). Cooke reanalyzed the data from the Faroe Islands and concluded that the increased risk of MS was instead subsequent to lack of a highly contagious infection from a widespread (viral) agent that probably occurred before age 3 years.

Our cluster cases might have been unexposed to any of the common infectious diseases in early childhood either because of an absence of the causative agent(s) during those years or areas or because of conditions protecting them. This is in agreement with the ''protective hypothesis'' and consistent with the higher risk of MS found among persons with late onset of the typical childhood infectious diseases, such as infectious mononucleosis, mumps, and measles (37, 38). Exposure, within age 6 years, to an infant sibling has been associated with a reduced risk of MS, probably related to the high rate of childhood infection at that age (39).

Our study and others (29) have detected no space-time clustering patterns reflecting fixed latency periods. This does not support the hypothesis that the disease is caused by infection from MS-specific viral agents with fixed incubation time intervals. As already hypothesized, susceptibility to MS might instead depend on more complex mechanisms and interactions occurring in early childhood.

The observed:expected ratios in this study were relatively low, but it is difficult to judge what would represent a large effect. This ratio is not comparable with normal values (i.e., the odds ratio or relative risk) that are based on individual data and not pairs of data. Furthermore, the observed:expected ratios showed only small changes with increasing age, since there was strong dependency between the analyses for each year. Only a few persons included in the analysis were actually migrating during childhood. Still, there was enough migration that by the end of adolescence, the ratio was reduced to 1.

We cannot exclude the possibility that the significant clustering we observed was caused by a confounding factor or by a factor not related to the disease. However, no specific exogenous etiologic factor has been established for MS, and a specific migration pattern in the general population being responsible for the finding is less likely because of the stability of this population. Nevertheless, a rate-based cluster method might have given us more information on this issue.

Cluster studies also present general limitations, since they are based on samples or populations of patients studied years after diagnosis. Some exposed persons might not be included in a study because they migrated before onset (6). In this case, space-time clustering could be underestimated, though nondifferentially. In addition, the *p* values in cluster studies cannot be interpreted too rigorously, since multiple tests are performed. Nevertheless, since the analyses for each year were highly correlated in our study, adjustment for multiple testing in this study would have been impractical and less important.

The use of space-time cluster analysis is especially indicated for diseases caused by a single-source infectious agent, which might be transmitted by close contacts between patients as reflected in a clustered distribution of the disease. There is little evidence that MS is caused by a specific single-source infectious agent, but such an agent is commonly hypothesized to be widespread and to interact with other factors, such as genetically based traits. This type of etiologic pattern can therefore mask a space-time clustering effect, and the test would have low statistical power. In MS, significant clustering would result from a relevant variation in the intensity of the infectious agent over time and throughout the study area (10). Despite these potential limitations of space-time cluster analysis in studies of MS, we found significant clustering patterns in our population that were more evident for certain subgroups.

Our study revealed space-time clustering of MS onset in early childhood and indicated that, at least for some subgroups of patients, this age is the susceptibility period for MS (i.e., the age of critical exposure) in Sardinia. The effect was most evident among the most recent cases and among patients with early onset and patients with a relapsing-remitting course, supporting the hypothesis that MS clinical heterogeneity depends on different causative mechanisms.

ACKNOWLEDGMENTS

The European Neurological Society (Professors A. Steck and G. Said), the Fondazione Italiana Sclerosi Multipla (grant 97/R/69), and the Regione Autonoma della Sardegna (Assessorato all'Igiene e Sanità Pubblica) supported this study.

Conflict of interest: none declared.

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