Creutzfeldt-Jakob disease (CJD) is the most commonly occurring human transmissible spongiform encephalopathy. The annual incidence of the disease in Europe is approximately 1 per million population. Some 10–15% of CJD cases are associated with mutations in the prion protein (PRNP) gene (inherited cases), while another few percent are iatrogenic, the result of human-to-human transmission during medical procedures. In France, most iatrogenic Creutzfeldt-Jakob disease cases are attributable to human growth hormone treatment. In Japan, dura mater grafts constitute the most frequent reported source of iatrogenic Creutzfeldt-Jakob disease. In 1996, a new variant of CJD (vCJD) was identified in the UK. These cases have been attributed to infection with the agent responsible for bovine spongiform encephalopathy (BSE). Until now, only 4 such cases have been identified outside the UK (3 in France and 1 in Ireland). Thus the great majority of CJD cases, 80–85% of the total, are of unknown origin and are commonly referred to as ‘sporadic’ cases.

One possible origin of sporadic CJD is a spontaneous conformational change in an individual’s prion protein (PrP), from PrPSEN into the disease-related isoform PrPres. Epidemiological data indicate that an individual’s risk of the disease is influenced by their PRNP genotype at codon 129. Individuals who are homozygous at codon 129 (methionine-methionine or valine-valine) are at higher risk of developing sporadic CJD than heterozygous individuals (methionine-valine). Alternatively, the conformational change might be induced by exposure to PrPres from an unknown external source.

Analysis of the geographical distribution of sporadic Creutzfeldt-Jakob disease in France between 1992 and 1998

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Background Creutzfeldt-Jakob disease (CJD) is a rare fatal dementia caused by a transmissible agent. However, the mechanism leading to the disease is unknown in the majority of cases. The presence of geographically clustered cases might indicate a common environmental exposure to the transmissible agent, or case-to-case transmission of the agent. This study sought evidence of clustering of cases of sporadic CJD in France.

Methods A total of 402 individuals who died from definite or probable sporadic CJD in France between 1992 and 1998 were analysed. The geographical distribution of cases was analysed using three different clustering methods. An analysis of the distribution of the distances between pairs was performed to look for evidence of clustering. Then, two methods of cluster detection were used to identify the locations of clusters.

Results Each of our analyses found some evidence of clustering, though the extent of that clustering differed between approaches. The strongest evidence, statistically, related to three cases living in a small rural area in South-West France (P = 0.001). Two of the three cases lived in the same area throughout life. They had also both undergone surgery on several occasions. Little information is available on the third case.

Conclusion Some sporadic CJD cases in France may be aetiologically linked. There was strong evidence that three cases in South-West France formed a cluster but the precise mechanism underlying this cluster of cases remains unclear. The potentially long incubation period of the disease makes the identification of links between such cases difficult.

Keywords Epidemiology, Creutzfeldt-Jakob disease, clustering analysis

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Case-control studies of environmental risk factors for sporadic CJD have investigated place of residence, occupation, exposure to animals or animal products, dietary habits and medical history as potential risk factors.\textsuperscript{10–12} No consistent findings have emerged from these studies, and age and codon 129 genotype remain the only well-established risk factors.

Small, unexplained ‘clusters’ of sporadic CJD have been reported in Italy, Japan, and Canada\textsuperscript{13–15} but rigorous statistical analyses of these clusters have not generally been performed. Geographical clustering of sporadic CJD cases, if it occurs, might reflect case-to-case transmission of the agent, common exposure to an environmental source or, perhaps, a shared genetic susceptibility lying outside the PrP coding region.

Following the epidemic of BSE in the UK, national surveillance of CJD was implemented in France in 1991. Since 1992 it has provided data on the incidence of CJD throughout the country.\textsuperscript{16,17} In the present study, a variety of statistical methods were used to look for objective evidence of clustering in 402 individuals who died from sporadic CJD between 1992 and 1998.

### Data

Case ascertainment is based on direct notification of suspected cases of CJD by neurologists, neuropathologists and laboratories involved in the examination of the prion protein gene or, since 1998, the detection of 14–3–3 protein in cerebrospinal fluid. Suspected cases were examined, when possible, by the study neurologist (JPB) and approximately 60% of patients with suspected CJD underwent post-mortem. The diagnostic criteria for CJD used over the study period have been published.\textsuperscript{18} In this paper, analyses are based on definite and probable cases.

Cases were classified as iatrogenic when the patient had a known history of exposure to pituitary growth hormone or dura mater. They were classified as inherited if they carried a disease-associated mutation of the prion protein gene or if, in the absence of genetic analysis, they had a family history of definite or probable CJD. One case was classified as vCJD on the basis of their neuropathological features.\textsuperscript{19} All other cases were classified as sporadic.

A total of 503 patients with definite or probable CJD died between January 1992 and December 1998, yielding an annual mortality rate of 1.2 per million inhabitants. Among these 503 CJD cases, 60 were classified as iatrogenic (most of them being related to human growth hormone), 40 were classified as inherited and 1 case was a variant CJD. Among the remaining 402 sporadic CJD cases, the median age at death was 67.5 years and ranged from 38 to 88 years (sex ratio: 170 males/232 females). In all, 296 (74%) underwent analysis of the PRNP gene, of whom 121 (40.9%) were methionine homozygotes, 34 (11.5%) were methionine-valine heterozygotes.

Place of residence at the time of disease onset was available for all sporadic cases and place of birth was known for all but two cases. Eight cases were excluded from our analyses because they lived outside France at the time of disease onset. A total of 56 patients were born outside France, mostly in North Africa. Thus, the geographical distribution of places of birth could be analysed for 344 cases and distribution of place of residence at onset could be analysed for 394 of the 402 cases. For 126 (31.3%) of the 402 cases, places of residence throughout life were known because these individuals were included in a separate case-control study.

Central grid co-ordinates of 36,609 districts across France were obtained from the French Institut National de Géographie. Data on the populations of these districts were obtained from the 1995 census of the French population. The smallest occupied district had less than 100 inhabitants, the largest (Paris) 2,175,200. Large districts such as Paris, Lyon (415,487 inhabitants) or Marseille (800,550 inhabitants) were subdivided into arrondissements with populations ranging from several thousands to about 200,000. Thus, in total, 36,654 areas with data on location and population size were identified. The median population size of these areas was about 300 and 95% of the areas had a population of less than 5000.

### Statistical Methods

#### Analysis 1

Initially statistical evidence of clustering of cases of sporadic CJD in France was sought through an analysis of the distribution of the distances between all possible pairs of cases.\textsuperscript{20} We first calculated the observed number of pairs of cases within a particular distance of each other. This observed number of pairs was then compared to distribution of this number under the null hypothesis of no clustering. This null distribution was obtained by Monte-Carlo simulation, by repeated random sampling of the population with the probability of including a person from a specific area being proportional to the population of the area (1,000 simulations performed). This method provides an overall test for presence of clustering but does not aim to give precise locations of possible clusters. Analyses were performed separately for place of residence at onset and place of birth. Analyses stratified by codon 129 genotype were also performed.

#### Analysis 2

Further analyses were performed to identify possible clusters, initially using the method of Besag-Newell. This method was originally developed for the study of the geographical distribution of childhood leukaemia. According to the authors, the method is suitable for rare diseases of unknown aetiology.\textsuperscript{21} The basis of this method is relatively simple: circles are drawn centred on each case that encompasses its k nearest neighbouring cases. Then, for each circle, the Poisson probability of observing k or more cases within this circle is calculated given the population at risk within the circle. Ideally, the age structure of the population at the district level should be used to adjust for age in calculating the expected number of cases, but these data were not available to us. The analysis was repeated for different values of k. A cluster was classified as ‘consistent’ if its statistical significance at the 5% level persisted over three values of k. A case was then defined as clustered if it was included in the largest circle of one of the consistent clusters (which could result in clusters of size possibly larger than k). The value of k specified, together with the total number of cases, largely determines the radii of the circles that are explored by the method. Large values of k focus on large ‘clusters’ and thus tend to investigate circles (and clusters) of large radius. For the purposes of this analysis, k was set successively to 4, 6 and 9 in order to consider cluster radii up to about 70 km.
Analysis 3

A criticism of the Besag-Newell method is that the control of multiple testing through the definition of consistent clusters is arbitrary and informal. The method is therefore said to provide many false positive clusters. To overcome this problem of multiple testing, we applied another method of cluster location according to Kulldorff et al.22,23 This method uses a spatial scan statistic which can detect clusters of any size located anywhere in the study region, whether or not they conform with administrative borders. The maximum cluster size to be investigated was set to 50% of the total population at risk. Essentially the method examines circles of continuously varying size centred on a large number of different locations. For each circle, a likelihood ratio is computed for the alternative hypothesis that there is an increased risk of disease inside the circle against the null hypothesis that the risk inside the circle is the same as the risk outside it. The ‘most likely cluster’ is that with the largest likelihood ratio. The statistical significance of this largest likelihood ratio is assessed by determining its distribution under the null hypothesis through Monte Carlo simulation (1000 simulations performed). This takes account of the multiple testing inherent in the procedure. Each analysis by this method identifies a single ‘most likely cluster’. To look for further clusters, we reapplied the same procedure to datasets from which the cases and the population data for the most likely cluster had been removed.

For Analyses 1 and 2, computer programs were written in C. For Analysis 3, we used the StsWin (SatScan) software developed and provided by the method developer (URL: http://dcp.nci.nih.gov/bb/satscan.html).

Results

Figure 1 shows the standardized mortality ratios of sporadic CJD by Department. Table 1 summarizes the results of the first analysis of clustering and shows the observed and expected numbers of pairs of cases born, or living at onset, within distances of 1, 5, 10, 20 km of each other. For example, among the 394 cases of sporadic CJD, 117 pairs of cases were found to have places of residence at onset less than 1 km apart compared with a median of 218 pairs observed under the null hypothesis. Thus, there is no evidence of an excess of pairs of cases living within 1 km of each other. The same was true for pairs of cases living within 5 km of each other. However, the table does indicate that for sporadic CJD there is some evidence of clustering of places of onset at distances of less than 10 and 20 km.

Analyses using place of birth instead of place of residence at onset did not detect any evidence of clustering. An analysis of the geographical distribution of sporadic cases by codon 129 genotype did not reveal any evidence of clustering (data not shown).

The Besag-Newell method identified five possible clusters of sporadic CJD by place of residence at onset, persisting over the three values of k used in the analyses. These clusters were widely distributed across France (North, West, Centre and South-West). To protect patient confidentiality, the precise location of the clusters is not given. One cluster included 7 cases, three included 10 cases each, and one included 25 cases. In three of the five identified clusters, more than 95% of individuals forming these clusters lived in urban areas. This proportion was about 70% in the two other clusters.

The method of Kulldorff et al. identified only one cluster of three cases located in the South-West ($P = 0.001$, radius 4.5 km). These three cases formed part of one of the larger clusters identified by the Besag-Newell method. The next most likely cluster (three cases, North of France) had a $P$-value of 0.18 (radius

<table>
<thead>
<tr>
<th>Sporadic Creutzfeldt-Jakob disease</th>
<th>No. of observed pairs at a distance of (median/95th/99th percentile of number of pairs under the null hypothesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 km</td>
</tr>
<tr>
<td>At onset</td>
<td>394</td>
</tr>
<tr>
<td></td>
<td>(218/332/381)</td>
</tr>
<tr>
<td>Place of birth</td>
<td>344</td>
</tr>
</tbody>
</table>

$^a$ Indicates that the value exceeds the 95th percentile of the expected distribution (thresholds values are given below in parenthesis).
2.5 km). None of the other clusters identified by the Besag-Newell method approached statistical significance using the spatial scan statistic (P > 0.20 for all three).

We examined the records of the cases involved in the two most likely clusters detected by Kulldorff’s method. Some characteristics of the cases are summarized in Table 2. In the Southwest cluster, three cases aged 60–84 years at onset lived at the time of disease onset in two small rural parishes less than 5 km apart. Two had lived in the same parish throughout life. The third case was born about 100 km from his place of residence at the time of disease onset, but his residential history throughout life was not recorded. Two cases were methionine homozygotes, the third case was not genotyped. Detailed information on medical history and other possible exposures were available for only two cases. One case with onset in 1996 had a history of five surgical procedures between 1959 and 1996 including vertebral column surgery in 1991. The other case, with onset in 1997, had ocular surgery in 1994 and had had other surgical treatments in 1977 and 1993. The two patients had been operated on in the same hospitals, but not within a one-year period of each other. They had both undergone dental treatment by the same dentist who practised in the area. One case was reported to eat brain of veal or lamb at least once per month, while the other reportedly ate brain occasionally. Both ate veal thymus several times per year and venison occasionally and both had lived on farms.

In region 2 (North), three cases, aged 57–72 years at onset, were living less than 3 km apart in an urban area at disease onset. One case, whose lifetime residential history was known, had lived in the same parish throughout life, while the other two cases were born less than 10 km from the place where they lived at disease onset. One had a past history of vertebral column surgery. None were genotyped. In the patients with known past surgical history, the possibility of iatrogenic transmission of the disease through dura mater implants was investigated by examining records and by interviewing a referral expert in neurosurgery. According to this expert, none of the cases reported here were likely to have had dura mater implants.

### Discussion

A major limitation of the present study is that data on lifetime places of residence were not available for all cases. Studies of iatrogenic CJD and kuru indicate that the incubation period of these diseases can be very long. Therefore, evidence of residential proximity 10, 20 or more years before disease onset is likely to be more meaningful than geographical clustering at disease onset. We observed no evidence of clustering of places of birth of sporadic CJD cases. A weakness of this analysis is that the reference population for this analysis is based on the 1995 census data, when we should be using the population distribution at around the time of birth of the cases (60+ years before on average).

Another limitation of the present study is that the age-structure of the reference population used for calculating expected number of cases was not available. In France, the greatest variations in the age distribution are likely to be found between rural and urban areas, the mean age in rural areas being greater than that in urban areas. Because the incidence of sporadic CJD is much higher in older individuals, it is possible that ‘false-positive’ clustering resulting from confounding by age might be observed in rural areas. Such an effect could account for some of the clustering observed in Analysis 1. With respect to Analysis 3, the ‘North’ cluster is centred in an urban area and is unlikely to be explained by such confounding. The ‘South-West’ cluster is located in a rural area. Even if we were to assume that all of the population in that area was aged greater than 55 years, the expected number of cases would increase only to 0.08, compared with three observed cases.

Apparent clustering of sporadic CJD cases might also be observed if diagnostic practices vary across France. In 1998, the 14–3–3 test was added to the diagnostic criteria for probable CJD. However, this was very close to the end of the period considered in this paper and the availability of this test became very rapidly well-known by neurologists following the emergence of vCJD in Great Britain. In the two clusters considered, three cases had a positive 14–3–3 result, all of whom underwent a...
post-mortem. We think it unlikely that regional differences in the utilization of the 14–3–3 test explain the observed clusters.

On the other hand, if clustering of sporadic CJD does occur through common environmental exposure to the disease agent, analyses based solely on place of residence at onset are likely to underestimate the true strength of clustering.25 Interestingly, at least two of the individuals in the ‘most likely cluster’ had lived in the same small area throughout life and thus may have been exposed to common environmental factors many years before disease onset. Alternatively, one of the cases may have transmitted the infectious agent to the others, although the short time delay between the onsets of the cases in the absence of any neurosurgery linking the cases argues against this explanation. Given that a full PrP gene analysis was not performed on these cases, we cannot exclude the possibility that they are familial cases perhaps with unidentified co-paternity.

Given the limitations outlined above, it is perhaps not surprising that, overall, the evidence for geographical clustering of sporadic CJD in France is rather weak, with some inconsistencies apparent between the results obtained by the different methods. The cluster identified by the method of Kulldorff et al. involves only a small proportion of all cases (<1%) and this cluster cannot explain the excess of pairs of cases living close together identified in our initial analysis of clustering (Table 1). While the method of Besag-Newell identifies a higher proportion of cases as occurring in clusters (17%), which could account for the excess of pairs observed in Table 1, this method is known to be susceptible to false positive results.

A case-control study performed in Australia reported an increased risk of sporadic CJD associated with surgery, though the methodological limitations of the study prevent any firm conclusions being drawn with regard to this association.11 In the present study, we found that some of the cases belonging to two possible clusters had undergone several episodes of surgery. One possible explanation for the presence of these clusters is, therefore, that some of these cases were iatrogenically infected, although we could not identify any clear surgical links between any of the cases. We should note that cluster analyses based on place of residence at onset might miss clusters of unrecognized iatrogenic CJD if the surgery took place relatively far away from the place of residence of individuals at onset.

In summary, we used three methods to investigate the geographical distribution of sporadic CJD cases in France. Each of these methods found some evidence of clustering, though the extent of that clustering differed between approaches. The strongest evidence, statistically, related to three cases living in a small rural area in South-West France. Two of the three cases lived in the same area throughout life and could have shared an environmental exposure to the infectious agent. They had also undergone surgery on several occasions. Little information is available on the third case. The precise mechanism underlying this cluster of cases remains, therefore, unclear.

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References
Commentary: Geographical distribution of sporadic Creutzfeldt-Jakob Disease in France

Martin Kulldorff

In this issue of the International Journal of Epidemiology Huillard d’Aignaux et al.1 present a very interesting geographical analysis of sporadic Creutzfeldt-Jakob Disease in France. A number of non-traditional statistical methods are used, and here we offer some comments regarding their purpose and interpretation.

Tests for Spatial Randomness

An important first step in any geographical investigation of disease is to present a descriptive map, such as the one provided by Huillard d’Aignaux et al.1 in Figure 1. Whether or not there are true geographical differences in risk, there will always be some geographical patterns apparent to the naked eye. For example, in the aforementioned map, there are three apparent low-risk regions in the south, north and west, respectively. As in all medical research, it is important to evaluate whether observed patterns/results are likely to be due to chance or not. For geographical data, this is done using a test for spatial randomness, adjusting for the geographical distribution of the population at risk, as well as covariates such as age. Such tests are not a replacement for the maps, but an important complement. If the null hypothesis of spatial randomness is rejected, it means that there is likely to be predictors that are geographically unevenly distributed. If the null hypothesis is not rejected, the geographical pattern observed is less likely to provide important information, and we should watch out not to spend a lot of time trying to interpret random spatial noise.

Different tests for spatial randomness have different purposes, and Huillard-d’Aignaux et al.1 are at the forefront of the field by evaluating their data using more than one test statistic. Mantel-Bailar’s Test,2 used in Analysis 1, is a global clustering test.3 It evaluates whether clustering exists as a global phenomena throughout the map, without pinpointing the location of specific clusters. This may occur through two different types of random processes.4 It could be that initial cases generate other cases close by, as when a disease is infectious. It could also be that there are a number of health hazards, each creating an increased risk for the disease in a limited surrounding area.

Other global clustering tests that have recently been proposed include Cuzick-Edwards’ k-Nearest Neighbor Test5 and Tango’s Maximized Excess Events Test.6 Mantel and Bailar6 use a fixed geographical distance to define the scale of clustering, that is, the maximum distance at which two cases are considered to be close. As a contrast, Cuzick and Edwards’ test defines the scale in terms of the number of neighbours, so that one case is close to another if it is among the k nearest neighbours. This means that two cases 4 km apart may be considered close in a rural area but not in a city. In comparing the two methods, Mantel-Bailar’s test is therefore expected to have higher statistical power if the clustering is stronger in urban areas while Cuzick-Edwards’ test will have higher power if the clustering is stronger in rural areas. Both methods require the user to specify a parameter representing the scale of clustering, and as the scale is typically unknown, it is good to perform the test for multiple parameter values. Tango’s Maximized Excess Events Test deals with this issue directly, evaluating the test statistic for multiple parameter values but providing one single P-value adjusted for multiple testing.

The spatial scan statistic,7 used in Analysis 3, is a cluster detection test, which determines the location and statistical significance of specific clusters. If the null hypothesis is rejected, that rejection is dependent on the number of cases inside versus outside the detected cluster, but independent of their specific locations. This means that no matter how the outside cases are distributed we will still reject the null hypothesis, and hence, we can attribute both the rejection and the P-value to the detected cluster.1,7
Besag-Newell’s method, used in Analysis 2, is also designed for cluster detection, being one of the forerunners to the spatial scan statistic. The main difference is that it provides one P-value for each cluster location and size, not adjusted for multiple testing, while the spatial scan statistic adjusts the P-values for the multiple testing inherent in the many potential cluster locations and sizes.

Data Quality and Interpretation
For the population at risk, Huillard d’Aignaux et al. used data from the 1995 census, and the fact that this lies in the middle of the 1992–1998 study interval makes migration bias unlikely in the ‘place of residence’ analyses.

As pointed out by the authors, a more troubling aspect is the lack of age-specific population numbers, making it impossible to adjust the geographical analyses for age, irrespectively of what statistical method is used. While the significant global clustering results in Analysis 1 could be due to a geographically uneven age distribution, the lack of age adjustment cannot explain the cluster with three cases in the southwest. This is because the P-value is very small (P = 0.001) and a slight change in the population-based denominator will not make a big difference when the relative risk is high. Larger clusters with lower relative risk are more prone to be caused by inadequate age adjustments. The lack of age adjustment may also have the opposite effect, leading to false-negative results where a test fails to find clustering that would have been apparent in an age-adjusted analysis.

The fact that one test statistic provides a significant result, while another does not, is not a cause for concern. Rather, the difference in results should be viewed as information on the type of clustering present in the data. Such information must be used with flexibility, and it is sometimes appropriate to investigate non-significant clusters, as Huillard d’Aignaux et al. have done with respect to the cluster in northern France.

Geographical analyses and tests for spatial randomness can provide important clues about a disease, but rarely any definite answers. That requires detailed case histories, searching for the presence of known or potential risk factors, and often, the design of traditional non-geographical epidemiological studies to evaluate newly generated hypotheses. It is my hope that the thorough and excellent geographical study by Huillard d’Aignaux et al. will result in such further studies, be it in France or other countries.

References