types aggregate in QTL regions, combined with physiological profiling, provides a novel approach to facilitate the positional cloning of genes underlying cardiovascular function and hypertension. While strategies for accomplishing these goals will continue to evolve, this first attempt at developing knowledge at a systems biology level sets the stage for future improvements and provides investigators with a powerful set of tools for discovery.

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Estimation of Epidemic Size and Incubation Time Based on Age Characteristics of vCJD in the United Kingdom

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The size of the variant Creutzfeldt-Jakob Disease (vCJD) epidemic in the United Kingdom is a major public health concern and a subject of speculation. The cases are young (mean age = 28). Assuming that the risk of developing the disease in susceptible exposed subjects decreases exponentially with age after age 15, that all infections occurred between 1980 and 1989, and that the distribution of the incubation period is lognormal, we estimate that the mean duration of the incubation period is 16.7 years [95% confidence interval (CI): 12.4 to 23.2] and that the total number of cases will be 205 (upper limit of the 95% CI: 403).

As of 1 May 2001, there were 97 cases of definite (n = 86) or probable (n = 11) variant Creutzfeldt-Jakob Disease (vCJD) in the United Kingdom. These patients probably contracted the disease by oral ingestion of food contaminated by the agent of bovine spongiform encephalopathy (BSE), before the UK bovine-specified risk materials (SRM) ban in 1989 (*1*). The number of BSE-infected animals is estimated to have been in the range of 900,000 to 1,130,000, with between 460,000 and 482,000 slaughtered for consumption before the introduction of the November 1989 specified offal ban (*2*). The epidemic may have started as early as 1980 (*3*), and the number of people exposed to potentially infective doses through food may be extremely high (*4*). Therefore, one could pessimistically assume that virtually everybody in the population has been in contact with food, or bovine products, originating from BSE-infected animals. The public health response in Europe has been to develop procedures and diagnostic tests that avoid, as far as possible, the entry of any infected animal into the food chain. The consequences of the BSE epidemic in terms of human disease are not yet known: With different assumptions for risk analysis, in 1997, the cumulative cases of vCJD in the United Kingdom were estimated from as few as 75 to as many as 80,000 (*5*) and more recently from 70 to 136,000 cases (*6*). These estimates are markedly dependent on assumptions made about the mean duration of the incubation period. Unfortunately, no studies in animals or of other human spongiform encephalopathies provide precise data for the incubation period. In addition, the observation that only a few cattle, and often only one, from the same age cohort in a herd have developed BSE suggests that additional individual or environmental factors may influence the development of the disease. It is be-

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cause the relation between the risk of the disease and the dose, route of infection, host factors, and environmental factors is unknown that predictions of the possible future of the epidemic rely on mathematical extrapolations based on currently available data.

One striking epidemiological characteristic of vCJD is the young age distribution of the cases (Fig. 1A). The mean age at death of these patients is 28 years. Only 6 of 90 patients who have died were older than 50 years, in comparison with 93% of cases of sporadic CJD (*7*). This observation provides a clue to the incubation period in vCJD, i.e., the "age of the patient at the diagnosis" = "age at infection" + "incubation time." The ages of the patients with vCJD at diagnosis and death are collated by the National Creutzfeldt-Jakob Disease Surveillance Unit in the United Kingdom. The causal link between BSE infection in cattle and human vCJD indicates that the age at infection in people must parallel the course of the BSE epidemic. One can therefore compute the distribution of incubation periods by a deconvolution technique taking into account the censorship of current data, as was done successfully with acquired immune deficiency syndrome (AIDS) (*8*). In vCJD, the analysis of the censored data is more complicated because the possible dates of infection range over an extended period of time. Thus, additional statistical modeling was necessary. The model we propose provides estimates of the incubation period and the future burden of the vCJD epidemic.

To explain that most of the cases are young, two (nonexclusive) hypotheses have to be made: (i) the incubation period is shorter in the young than in the old, and (ii) the young are more susceptible to infection. We do not prejudge the underlying determinants to these hypotheses that may be related to differences in susceptibility or other unknown factors. If the first assumption was true, older cases with longer incubation periods have a greater chance of being identified later than younger cases. Because there is no statistically significant relation between the date of diagnosis and age (Fig. 1B), we discarded this first assumption. We therefore chose the second assumption, namely that the probability per year that a susceptible subject becomes infected [the "force of infection" (*9*)] decreases with age a. For the calculation, we assumed that all susceptible children aged between 0.5 and 15 years, exposed to the infectious agent on year t, experience the same force of infection. After 15 years of age, we assume that the force of infection decreases exponentially (*10*). This hypothesis is in line with the epidemiological data, which show that vCJD rarely occurs in older subjects. The age cutoff of 15 years was an arbitrary choice. However, this parameter has been further studied in a sensitivity analysis. We assume that the whole population may have been exposed to the infectious agent, with the exception of infants aged less than 0.5 years.

We denote by $S(t,a)$ the survival function from all causes of mortality at date t and age a, by $\lambda(t, a)$ the force of infection at date t and age a, and by h the probability density function of the duration of the incubation period. $\lambda(t, a)$ is the product of a function $f(a)$ of age and of a function $g(t)$ of calendar time: $\lambda(t, a) = f(a)g(t)$. The choices of functions f and g express the age-risk relation described above and the variation of the BSE infectious risk, respectively (*11*). The probability distribution of the duration of the incubation period, $h(u)$, was assumed to be lognormal, because this is a widely accepted model in infectious diseases (*12*). A Gamma distribution was also used in the sensitivity analysis.

The full statistical formulation necessary to write the likelihood equation implies that a probabilistic model be chosen to estimate the population at risk of developing the disease, i.e., the exposed/susceptible subjects. According to the formulation we chose, the dates and ages of the observed cases are a realization of a planar Poisson process (*13*). In this model, s is the average number of individuals per generation who can be infected, provided they have been exposed to a sufficient dose of infectious agent and have the appropriate individual characteristics (genetic, environmental, and so forth). In each generation, these s subjects pass through the 1980–89 "window" at different ages, and therefore the final number of persons who will ultimately develop the disease varies accordingly. Finally, the probability $P(t,a)dadt$ that a case aged between a and $a + da$ occurs between times t and $t + dt$ is expressed mathematically, then the maximum likelihood approach is used to estimate the unknown parameters from the available observations, and the future of the epidemic is assessed with these parameter values (*14*).

The distribution of the vCJD incubation period that best fits the data within the framework of our model has a mean of 16.7 years, with a standard deviation of 2.6 years. The 95% upper percentile of this distribution is 21.4 years. The 95% confidence interval (CI) of the estimates of the mean and standard deviation is relatively narrow: The 95% CI for the estimate of the mean incubation period is 12.4 to 23.2 years, and the 95% CI of the standard deviation is 0.9 to 8 years (*10*). The decrease in susceptibility to infection in exposed subjects older than 15 years, as estimated from the parameter α, was found to be very sharp: 16% per year of age (CI: 12 to 23%). This means that, under the best fitting hypothesis, an individual aged 20 years in 1981 had 55% less risk of becoming infected than a child aged 15

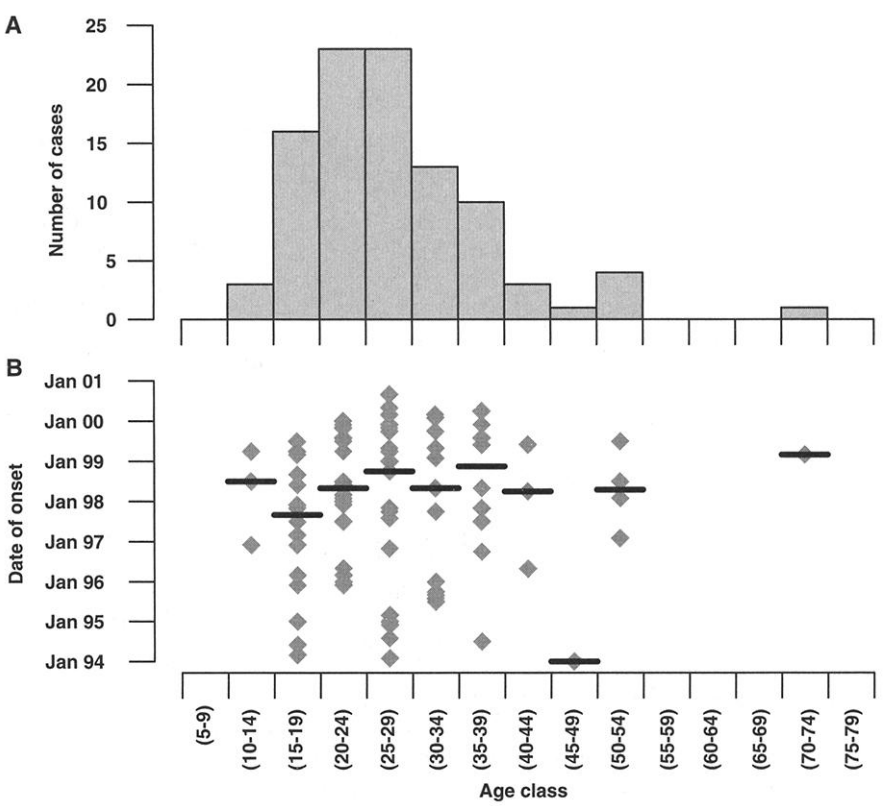

Fig. 1. (**A**) Age distribution of vCJD cases. Data are as of 1 May 2001, including 97 patients. (**B**) The distribution of the date of onset of vCJD by age class. Diamonds indicate observed values; a line is drawn at the median date. There is no apparent relation between age and date of diagnosis (correlation coefficient = 0.03, $P = 0.8$), as would have been expected if the incubation time was strongly age dependent, causing older cases to be detected later than younger cases.

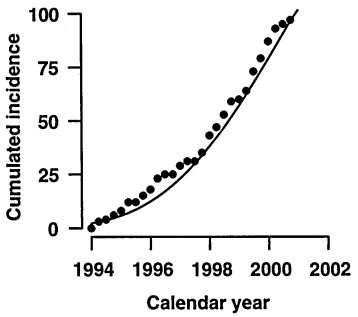

Fig. 2. Observed cumulated incidence of vCJD (dots) and incidence predicted by the model (line).

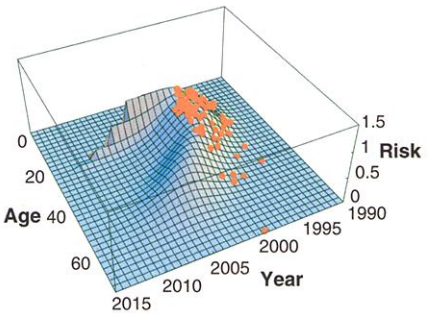

Fig. 3. Risk function for onset of vCJD as a function of age and time. Red dots indicate the 97 observed cases as of 1 May 2001.

years (99.9% for an individual aged 70).

The model predictions fit the past cumulated incidences, as shown in Fig. 2. Similarly, there is a good fit to the observed age distribution (*10*), as the model predicts that 22 out of the first 97 diagnosed cases should be less than 20 years of age (19 in the surveillance data set) and 82 should be less than 40 years of age (87 in the surveillance data set).

Details of the variation in the risk function (*14*) with age and time up to 2012 are shown in Fig. 3: The model predicts that the peak of the epidemic will be in 2000/2001 and that the annual number of cases should gradually decrease after this date. The total number of expected vCJD cases is, according to our model, low at 205 cases (upper limit of the 95% CI: 403). One prediction from the model is that in the next few years, the age distribution will become bimodal and that the proportion of older patients will increase.

The model presented here addresses human infections occurring between 1980 and 1989, as we assume that the likelihood of new human infection with BSE after the bovine SRM ban in 1989 should have been considerably reduced.

The strength of the method used in this report is that it is focused on the most striking epidemiological characteristic of vCJD, namely, the age distribution of the cases. In contrast to other models, which are based primarily on scenarios related to dietary exposures to BSE in the United Kingdom, our method does not make any assumptions about this parameter that cannot currently be estimated.

To express the necessary dependence between age and force of infection, we made one of the most parsimonious choices possible, i.e., a function beginning with a plateau during childhood, followed by an exponential decrease. The sensitivity analysis in which all possible alternatives to the 15-year age limit were tested ruled out an even simpler model with only one exponential function (age limit = 0) and found as the optimal value an age limit of 16. We have no clear physiological explanation for this age limit: Although a relation with puberty may be hypothesized, experimental evidence should be sought to support such a possibility.

The results presented here are based on a lognormal distribution of the incubation period, which is commonly used in the epidemiology of infectious diseases. However, we repeated the estimation process assuming that the incubation period was Gamma distributed and found almost identical values for the incubation parameters, the future epidemic size of the epidemic, and the occurrence of a bimodal age distribution in the future (*10*).

The incubation time we find for vCJD is longer than in human growth hormone–related CJD, which is between 9 and 10 years (*15*) in the sensitive homozygous genotypes. It is shorter than the one estimated in Kuru (*16*), which may exceed three decades, although, a priori, one would have expected a longer value because in Kuru, there is no species barrier and the disease was transmitted orally as in vCJD. The *PRNP* 129 genotype has a crucial importance in determining the risk of developing CJD. Yet to date, all tested vCJD patients have been homozygous for methionine at codon 129, as are about 40% of the total UK population. If there are two subpopulations of vCJD patients, one with "short" incubation times and methionine homozygosity, the other with "long" incubation times and valine homozygosity or heterozygosity, our method would not identify the second distribution parameters, because cases of vCJD with genotypes other than methionine homozygosity have not yet been identified.

Our age-risk model allows an estimate of the future size of the epidemic. If we independently introduce our estimates of the incubation period in the Ghani or Cousens models, we find similar "low" predictions: 80 to 630 cases with the Ghani model and 801 with the Cousens model (*17*). In conclusion, our prediction of the epidemic of vCJD lies in the "optimistic" end of the ranges of previously published figures, and this low value is in favor of a large species barrier between cattle and humans.

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11. The function $f(a)$ was taken in the form $f(a) = 0$ if $a < 0.5$, as infants younger than 0.5 year were assumed to not be exposed to the BSE agent; $f(a) = \lambda_0$ if $0.5 < a < 15$, which corresponds to the constant risk assumed in children before 15; and $f(a) = \lambda_0 e^{-\alpha(a-0.5)}$ if $a > 15$. The function $g(t)$ was chosen to reflect the increasing probability of the presence of contaminated meat products in the human diet, in parallel to the increasing incidence of BSE in animals over the period 1980–89. Possible exposures after 1989 were not considered in this analysis. Mimicking the incidence profile observed in the BSE epidemic during the same period, we set $g(t) = \gamma_0 e^{-\gamma(t-1989)}$. From the knowledge of the doubling time observed in the BSE epidemic (14 months) during the 1980–88 period (*2*), we extrapolate $\gamma = \ln(2)/(14/12) = 0.596$. As only the product $\lambda_0\gamma_0$ was identifiable, because of the form for the force of infection, we fixed γ_0 at 1.
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    The log-likelihood of the observed cases is

    $$\sum_i \log\left[sP(t_i,a_i)\right] - \int\int_D sP(t,a)dadt,$$

    where the first sum is over all observed cases and the integration is made in the domain D corresponding to ages between 0.5 and 100 years and to years between 1980 and 2000. s is the intensity of the Poisson birth process. Numerical maximization of the likelihood was done with a modified Levenberg Marquardt algorithm (SLATEC) after reparameterization of the model A logarithmic transformation was used for all variables but incubation mean time. The maximization was independently performed with a Newton-Raphson method (NAG), and all presented results were in agreement in the two methods, with differences between estimates of less than 10^{-2}. Numerical integrations were carried out to a precision of 10^{-4}.
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18. We thank J. Gagnon for helpful discussions.

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