PRION DISEASE

Variant Creutzfeldt–Jakob disease epidemic looks increasingly unlikely in the UK

A large-scale study looking for the existence of the abnormal prion protein (PrP^{CJD}) associated with variant Creutzfeldt–Jakob disease (vCJD) in discarded human tonsils has found no samples positive for this protein. The study verified the high sensitivity of the assays used and concluded that there are probably few undetected asymptomatic cases of vCJD in people in the UK born before 1995, who could potentially have been exposed to meat or meat products derived from cows infected with bovine spongiform encephalitis (BSE). "It is too early to discount the possibility that no further vCJD cases will occur in the UK; we will need to continue testing appropriate tissues, and use new methods as they become available," explains lead author Jonathan Clewley.

The new findings are part of ongoing efforts to establish the prevalence of PrP^{CJD} in the population of Britain with improved accuracy. The Health Protection Agency set up the National Anonymous Tissue Archive (NATA) in 2004 to look for PrP^{CJD} in extracted tonsils. The results presented in this latest study were obtained from 63,007 discarded tonsils, 12,763 of which were from the 1961–1985 birth cohort, in which most of the 168 definite or probable cases of vCJD have occurred in the UK.

The observed prevalence of PrP^{CJD} in this cohort was zero with an upper 95% confidence limit of 289 per million people. A zero overall prevalence was also reported in the 32,551 samples obtained from people born between 1961 and 1995, which included 19,908 samples from the 1986–1995 birth cohort. The upper 95% confidence limit was 113 per million overall, which is lower than but still in the same range as that observed in a previous survey of appendix tissue (95% CI 60–853 per million). "We will continue to test tonsil samples until we have tested 100,000 and we plan also to test appendix samples," says Clewley.

Validation of the assays to ensure they had sufficient sensitivity to detect PrP^{CJD} at very low concentrations was an important part of the study. The first step was to work with the National Institute for Biological Standards and Control to undertake a blinded study of candidate tests. "This led us to select two enzyme immunoassay (EIA) tests, each of which has a different biochemical basis," reports Clewley. One test complements the other, giving confidence in the overall sensitivity of the approach.

The two EIAs were validated with 250 tonsil samples verified as negative by the Medical Research Council (MRC) Prion Unit in London, UK, which established that neither assay gave false-negative or high background results. The sensitivity of the assays was initially assessed by demonstrating their ability to detect sheep prion protein (which cross-reacts with the antibodies used in both tests) at extremely low concentrations. Confirmation was obtained by follow-up tests using human vCJD tonsil tissue from both the National CJD Surveillance Unit in Edinburgh and the MRC Prion Unit. "From the results obtained, we estimated that the assays we used should have been able to detect amounts of PrP^{CJD} as low as 1,000-fold less than those found in tonsils from some patients with vCJD," adds Clewley. Finally, immunoblotting and immunohistochemistry were used to investigate any samples flagged up by EIA screening—two measures that would have definitely identified a true positive, had one been present.

Continued monitoring of the prevalence of PrP^{CJD} in the apparently healthy population of the UK is necessary because of the extremely long potential incubation period for CJD—some experts suggest this could be as long as 60 years. To date, four cases of vCJD infection have been shown to result from blood transfusion, raising the specter of a possible secondary epidemic that could arise through human-to-human transmission in health-care settings. "Despite years of research, prions and the diseases they cause are still imperfectly understood; it would be wise not to be complacent about the threat of future cases of vCJD," concludes Clewley.

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Image of tonsil specimens provided by Dr Jonathan P. Clewley

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