

Research

Variant Creutzfeldt-Jakob disease: prion protein genotype analysis of positive appendix tissue samples from a retrospective prevalence study

James W Ironside, Matthew T Bishop, Kelly Connolly, Doha Hegazy, Suzanne Lowrie, Margaret Le Grice, Diane L Ritchie, Linda McCauley, David A Hilton

Abstract

Objective To perform prion protein gene (*PRNP*) codon 129 analysis in DNA extracted from appendix tissue samples that had tested positive for disease associated prion protein.

Design Reanalysis of positive cases identified in a retrospective anonymised unlinked prevalence study of variant Creutzfeldt-Jakob disease (vCJD) in the United Kingdom.

Study samples 3 positive appendix tissue samples out of 12 674 samples of appendix and tonsil tested for disease associated prion protein. The patients from whom these samples were obtained were aged 20-29 years at the time of surgery, which took place in 1996-9.

Setting Pathology departments in two tertiary centres in England and Scotland.

Results Adequate DNA was available for analysis in two of the three specimens, both of which were homozygous for valine at codon 129 in the *PRNP*.

Conclusions This is the first indication that the valine homozygous subgroup at codon 129 in the *PRNP* is susceptible to vCJD infection. All tested clinical cases of vCJD have so far occurred in the methionine homozygous subgroup, and a single case of probable iatrogenic vCJD infection has been identified in one patient who was a methionine/valine heterozygote at this genetic locus. People infected with vCJD with a valine homozygous codon 129 *PRNP* genotype may have a prolonged incubation period, during which horizontal spread of the infection could occur either from blood donations or from contaminated surgical instruments used on these individuals during the asymptomatic phase of the illness.

Introduction

In a prevalence study for variant Creutzfeldt-Jakob disease (vCJD), we identified three appendixes that stained positively for disease associated prion protein (PrP). We looked at 12 674 specimens (11 109 appendixes, 1565 tonsils) removed between 1995 and 2000. Most of the patients (83%) were aged 10-30 years at the time of operation.^{1,2} This number of positive results is greater than would be predicted from the number of patients diagnosed with vCJD in United Kingdom (161 to date). Furthermore, the annual incidence of new cases of vCJD has declined from a peak in 1999. As all patients with vCJD belong to the methionine homozygous subgroup, determined by the codon 129 polymorphism in the prion protein gene (*PRNP*),³ one possible explanation for this apparent discrepancy could be a differ-

ent *PRNP* genotype in the three positive cases (the prevalences of *PRNP* codon 129 genotypes in the general UK population are about 40% methionine homozygous, 10% valine homozygous, and 50% heterozygous). This possibility was supported by a slightly different pattern of immunoreactivity in the second and third positive appendix cases in comparison with clinical cases of vCJD.² We recently identified a case of asymptomatic vCJD infection that seemed to have been transmitted by red cell transfusion in a *PRNP* codon 129 heterozygote, demonstrating that the methionine homozygous genotype is not uniquely susceptible to vCJD infection.³

Methods

We analysed the *PRNP* codon 129 polymorphism in the three samples of appendix tissue embedded in paraffin that stained positively for disease associated prion protein in the prevalence study. In the first case, a transmission study is currently under way using material from the remaining unstained sections. This meant that only immunostained sections were available for genotype studies and the extracted DNA was not good enough for further analysis. In the two remaining cases, as there was not sufficient material available for both transmission studies and genotype studies, and in view of possible *PRNP* influences on the staining pattern of disease associated prion protein in these cases, we used the remaining material for DNA analysis. A single 6 µm unstained paraffin section was available from each case, and these were de-paraffinised and scraped into individual microcentrifuge tubes for DNA extraction with the Puregene DNA Purification Kit (Gentra Systems, USA). Pelleted DNA was rehydrated for one hour at 65°C and then used as a template for amplification by the polymerase chain reaction (PCR), along with positive and negative control samples. PCR primers used were specific for a 506 bp region of *PRNP* containing the polymorphic sequence for the codon 129 residue. PCR products were digested at 37°C with the restriction enzyme Nsp1 (New England Biolabs, UK), which specifically recognises changes at the *PRNP* codon 129 polymorphic DNA sequence. Digest products were analysed on 1.5% agarose gels with positive controls for the codon 129 variants (MM, MV, VV).

Results

For both cases the genotype was confirmed as homozygous for the valine allele (VV) (figure). This method has been previously validated^{4,5} and was controlled in our laboratory by studying the

PRNP codon 129 genotype in both paraffin embedded sections and frozen tissues from 25 other cases.

Discussion

These results give the first indication that *PRNP* codon 129 valine homozygotes may be susceptible to vCJD infection. Though the immunohistochemical technique used in our earlier study seems to be specific for disease associated prion protein,⁶ it is unlikely to be 100% sensitive, suggesting that the true prevalence of vCJD infection in the UK population may be even higher than earlier estimated (3/12 674).² Genetic studies of kuru, another orally transmitted human prion disease, found that *PRNP* codon 129 MV and VV genotypes were associated with longer incubation periods than the MM genotype.⁷ As the ethical approval for our study placed restraints on the identification of individual cases, we are not able to state with certainty the age of the patients in the positive cases at the time of surgery. We can, however, state that they were aged 20-29 years at the time of surgery, which took place in 1996-9. No clinical cases of vCJD at any age have yet been identified in *PRNP* codon 129 valine homozygotes, indicating the need for continued surveillance of all cases of vCJD in the UK.

Though it is inadvisable to overinterpret the data from only three positive cases in this study, it is perhaps surprising (given the relative prevalences of *PRNP* codon 129 genotypes in the general population) that both the positive cases analysed here were valine homozygotes. Though this may represent a chance finding, we should consider the possibility of differences in the peripheral pathogenesis of vCJD that depend on the *PRNP* codon 129 genotype. The patient who developed asymptomatic vCJD infection after red blood cell transfusion was a codon 129 heterozygote in whom both tonsil and appendix tissues were negative on staining for disease associated prion protein with methods identical to those used in this study, though the spleen and lymph nodes gave positive results.³ *PRNP* polymorphisms in sheep infected with scrapie also have a major influence on the incubation period and timing and distribution of disease associated prion protein in lymphoid tissues during the incubation period.⁸

A prolonged incubation period after infection with vCJD is likely to result in an asymptomatic carrier state (which cannot yet be identified), which represents a potential risk for horizontal transmission of vCJD infection by blood transfusion, blood products, or contaminated surgical instruments. These uncertainties further underline the need for continued surveillance of vCJD in the UK (including surveillance for subclinical or asymptomatic infection⁹), a requirement to continue to reduce the possibility of secondary iatrogenic transmission, and the inclusion of carrier states and susceptibility to vCJD infection in all *PRNP* codon 129 genotypes in future disease modelling.

Contributors: JWI and DAH were responsible for the prevalence study and the analysis of the results, including the selection of the cases for analysis, and drafted and modified the manuscript. MTB established the methods for DNA extraction and analysis, designed and executed the validation study, and drafted and modified the manuscript. KC and DH performed the DNA extraction on the test materials and in the validation study, and modified the manuscript. MLeG, SL, DLR, and LMCC identified cases for the validation study and prepared the paraffin sections for DNA analysis and modified the manuscript. JWI is guarantor.

Funding: The prevalence study was funded by the Department of Health (1216963 DAH; 1216982 JWI).

Competing interest: None declared.

Ethical approval: The prevalence study received approval from the South and West multi-centre research ethics committee (MREC reference 99/6/32) and for each of the centres included, appropriate local research ethics committee approval.

- Hilton DA, Ghani AC, Conyers L, Edwards P, McCardle L, Penney M, et al. Accumulation of prion protein in tonsil and appendix: review of tissue samples. *BMJ* 2002;325:633-4.
- Hilton DA, Ghani A, Conyers L, Edwards P, McCardle L, Ritchie D, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004;203:733-9.
- Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a *PRNP* codon 129 heterozygous patient. *Lancet* 2004;364:527-9.
- Hainfellner JA, Liberski PP, Guiroy DC, Cervenakova L, Brown P, Gajdusek DC, et al. Pathology and immunohistochemistry of a kuru brain. *Brain Pathol* 1997;7:574-53.
- McLean CA, Ironside JW, Alpers MP, Brown PW, Cervenakova L, Anderson RM, et al. Comparative neuropathology of Kuru with new variant Creutzfeldt-Jakob disease: evidence for strain of agent predominating over genotype of host. *Brain Pathol* 1998;8:429-37.
- Hilton D, Sutak J, Smith MEF, Penney M, Conyers L, Edwards P, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* 2004;57:300-2.
- Goldfarb LG, Cervenakova L, Gajdusek DC. Genetic studies in relation to kuru: an overview. *Curr Mol Med* 2004;4:375-84.
- Ersdal C, Ulvund MJ, Espenes A, Benestad SL, Sarradin P, Landsverk T. Mapping PrPSc propagation in experimental and natural scrapie with different PrP genotypes. *Vet Pathol* 2005;42:258-74.
- Bird SM. Attributable testing for abnormal prion protein, database linkage and blood-borne vCJD risks. *Lancet* 2004;364:1362-4.

(Accepted 7 March 2006)

doi 10.1136/bmj.38804.511644.55

National Creutzfeldt-Jakob Disease Surveillance Unit, School of Molecular and Clinical Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU

James W Ironside *professor of clinical neuropathology*

Matthew T Bishop *geneticist*

Kelly Connolly *genetics technician*

Suzanne Lowrie *biomedical scientist*

Margaret Le Grice *biomedical scientist*

Diane L Ritchie *research assistant*

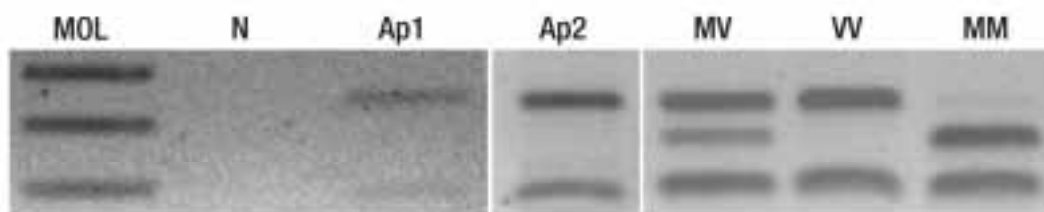
Linda McCardle *biomedical scientist*

Department of Histopathology, Derriford Hospital, Plymouth PL6 8DH

Doha Hegazy *research technician*

David A Hilton *consultant neuropathologist*

Correspondence to: J W Ironside james.ironside@ed.ac.uk



Restriction digest pattern for *PRNP* codon 129 genotype analysis in two paraffin section tissue samples (shown combined). The test sample results clearly show banding patterns equivalent to the VV genotype control (Mol=molecular weight ladder, N=PCR negative control, Ap1=appendix tissue from positive case 2, Ap2=appendix tissue from positive case 3, positive control samples from *PRNP* codon 129 MM, MV, and VV genotypes)

What is already known on this topic

A recent prevalence study of accumulation of prion protein (as a marker for variant Creutzfeldt-Jakob disease) in appendix and tonsil specimens in the UK found three cases in 12 674 samples, which is more than expected from the current number of clinical cases of vCJD

What this study adds

Analysis of DNA from two of the three positive samples found they were valine homozygotes at codon 129 in the prion protein gene, indicating that this genetic subgroup (which is a different subgroup to that in which all cases of vCJD so far have occurred) is susceptible to vCJD infection

Individuals with this genotype may have a prolonged incubation period with subclinical infection and could cause secondary spread of vCJD by blood transfusion or surgery