Edinburgh Research Explorer

Variant Creutzfeldt-Jakob disease

Citation for published version:

Digital Object Identifier (DOI):
10.1136/bmj.38804.511644.55

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
BMJ

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
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 Three 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.

What is already known on this topic

The evidence base for prescribing drugs to children lacks sufficient pharmacokinetic and pharmacodynamic data

Adult doses are often extrapolated to children without taking account of potential differences in drug handling with age or dose requirements for effectiveness

Guidelines on pharmacokinetic and pharmacodynamic data for children often ignore the European recommendations

The evidence base for prescribing drugs to children lacks sufficient pharmacokinetic and pharmacodynamic data

Licensing data for paediatric dosing are often sparse, and subsequent studies may result in important changes to recommended doses

What this study adds

HIV infected UK and Irish children have been underdosed with antiretrovirals in the past nine years

Inadequate dosing also arises through failure to adjust for ongoing growth

UK Department of Health has launched the Medicines for Children Research Network (www.mcri.ac.uk/mcrn), which aims to develop closer links between the drugs industry, regulators, families, and paediatricians, links that will be needed to meet the challenges of developing and manufacturing appropriate paediatric drugs (www.hivforum.org).

The Collaborative HIV Paediatric Study (CHIPS) is a collaboration between the Medical Research Council Clinical Trials Unit, UK, and the National Study of HIV in Pregnancy and Childhood (NSHPC) at the Institute of Child Health, London. Committees and participants are on bmj.com.

Contributors: See bmj.com

Funding: CHIPS is funded by the London HIV Consortium and in the past has received additional support from Bristol-Myers Squibb, Boehringer Ingelheim, GlaxoSmithKline, Roche, Abbott, and Gilead.

Competing interests: None declared.

Ethical approval: UK multicentre research ethics committee and relevant local research ethic committees.

What is already known on this topic

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, 1,565 tonsils) removed from 1995-2000. Most of the patients (83%) were aged 10-30 years at the time of operation. This number of positive results is greater than would be predicted from the numbers of patients diagnosed with vCJD in the 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 (PRNP, one possible explanation for this apparent discrepancy could be a different 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. 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.

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 NspI (New England Biolabs, UK), which specifically recognises a restriction sequence for the codon 129 MV and VV, PRNP codon 129 MV, VV, and MM genotypes.

Results

For both cases the genotype was confirmed as homozygous for the valine allele (VV) (figure). This method has been previously validated and was controlled in our laboratory by studying the PRNP codon 129 polymorphism 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, 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). 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. 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.
A recent prevalence study of accumulation of prion protein (as a marker for vCJD infection) in appendix and tonsil specimens in the UK found 3/12 674 positive cases, 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 them to be valine homozygotes at codon 129 in the prion protein gene, indicating that this genetic subgroup (which is a different subgroup from that in which all cases of vCJD have so far occurred) is susceptible to vCJD infection.

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

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 cell blood transfusion was a codon 129 heterozygote in whom both tonsil and appendix tissues were negative for staining for disease associated prion protein with identical methods as used in this study, though the spleen and lymph nodes gave positive results. 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.

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 infection\(^1\)), 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 (guarantor) and DAI 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 DI performed the DNA extraction on the test materials and in the validation study and modified the manuscript. MLG, SL, DLR, and LMcC identified cases for the validation study and prepared the pathology sections for DNA analysis and modified the manuscript.

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.


doi 10.1136/bmj.38894.511644.55

What is already known on this topic

A recent prevalence study of accumulation of prion protein (as a marker for vCJD infection) in appendix and tonsil specimens in the UK found 3/12 674 positive cases, which is more than expected from the current number of clinical cases of vCJD.

### Prescribing for RITA

And so it ends—a decade in the training grade. The last rites performed with a final RITA (record of in training assessment): I'm finally grown up, the authorities deem. In fact, my consultant job starts tomorrow.

After 10 years with it, do I have positive suggestions for postgraduate training in the NHS? Of course, dozens, most involving workforce, reorganisation, and resources. But, as in life, the best tonics are free. It is remarkable that NHS doctors deliver their high quality service for no immediate tangible gain. More extraordinary is that this work receives not a trace of the positive feedback and moral incentive that would be critical to the health of any comparable organisation.

I worked for some time in a prestigious institution of a more advanced healthcare system. What made their people tick? True, they had impressive buildings, state of the art technology, and good salary prospects; but, really, I think they were primarily driven by an ethos that valued excellence and individuality—initiated, fostered, and rewarded it. Right down to the artwork that lined the corridors—oversized portraits of the previous month's star employees, proud pictures of "graduating" trainees, plaques of senior faculty.

A bit over the top perhaps, but preferable to the anonymous line: competent, yes; extraordinary, no. Tragic for individuals and suspicion of those who seem too good or creative are all too common. The end result? Blunted clones coming off an assembly line: competent, yes; extraordinary, no. Tragic for individuals and undesirable for a healthcare system that confronts extraordinary problems.

There, I've had my shout. Tomorrow I step into a new world, recognising that to change it to myself will. I will not forget my morning dose of free tonic.

Giridhar P Kalamangalam

**Specialist Registrar in Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow**

(g.kalamangalam@hotmail.com)