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

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

Poor pharmacokinetic data at licensing results in incorrect drug dosing until important pharmacokinetic results emerge after licensing and inform revision of dosage recommendations

Guidelines stating alternative dosage strategies (by weight or surface area) for the same drug lead to different and inconsistent doses

Inadequate dosing also arises through failure to adjust for ongoing growth

the United States have recently committed to promoting research specific to children's medicines while protecting children as participants in clinical trials. The

UK Department of Health has launched the Medicines for Children Research Network (www.liv.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

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Competing interests: None declared.

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

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

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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.

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

Editorial by Wilson and Ricketts

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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 MV, VV, and MM genotypes)

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 from 1995–2000. 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 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 in the prion protein gene (*PRNP*),² 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.³ 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 micro-centrifuge 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 (MV, VV, and MM).

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.

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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

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 cause 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 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 identical methods as 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 con-

taminated 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 (guarantor) 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.

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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.

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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. I vote for a fresh culture that values and grows people. 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 passage of generations of juniors through Britain's many worthy hospitals. In addition, attitudes of derision towards the less skilled 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 is to change myself. I will not forget my morning dose of free tonic.

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