Irregular presence of abnormal prion protein in appendix in variant Creutzfeldt-Jakob disease

We have investigated the presence of disease related prion protein (PrP\textsuperscript{d}) in appendix samples obtained at necropsy from four neuropathologically confirmed cases of vCJD. \textit{Creutzfeldt-Jakob disease (vCJD)} was detected in only one vCJD appendix, at a level lower than that found in a diagnostic tonsil biopsy sample obtained from the same patient. The single PrP\textsuperscript{d} positive appendix, but not the other samples, also showed abnormal prion protein immunohistochemistry. The finding that appendix samples from three of four cases of vCJD are devoid of detectable PrP\textsuperscript{d} questions the utility of screening appendectomies to estimate the prevalence of pre-clinical vCJD infection within the UK population.

The appearance of a novel human prion disease, \textit{variant Creutzfeldt-Jakob disease (vCJD)}, in the United Kingdom from 1995 onwards, and the experimental confirmation that this is caused by the same prion strain as that causing BSE in cattle, has raised the possibility that a major epidemic of vCJD will occur in the United Kingdom and other countries as a result of dietary or other exposure to BSE prions.\textsuperscript{1} The pathogenesis of vCJD differs significantly from that of other inherited or sporadic CJD. Disease associated prion protein (PrP\textsuperscript{d}) is readily detectable in lymphoreticular tissues in vCJD and not in classic CJD.\textsuperscript{2,3} High levels of PrP\textsuperscript{d} are uniformly found in the central nervous system and lymphoreticular system of vCJD patients.\textsuperscript{4} The highest levels of PrP\textsuperscript{d} seen outside the central nervous system in vCJD are in tonsil (about 10% of that found in brain)\textsuperscript{5} and tonsil biopsy is used for ante-mortem diagnosis of vCJD.\textsuperscript{6} To date, positive prion protein immunohistochemistry has been reported in only a single appendix sample, although, importantly, this was removed from the patient before the onset of overt features of vCJD.\textsuperscript{7} While the stage at which lymphoreticular infection occurs in vCJD is unknown, PrP\textsuperscript{d} accumulation is detectable in the lymphoreticular system in natural sheep scrapie and in experimental rodent models of scrapie at a very early stage of the incubation period, long before the clinical phase of the disease. Based upon these data it has been suggested that large scale screening of surgical tonsillectomy and appendectomy tissues for PrP\textsuperscript{d} could provide early warning of a high level of vCJD prion infection and several such studies are in progress.\textsuperscript{8}

Recently we reported our concern after finding that PrP\textsuperscript{d} was undetectable in appendix samples obtained at necropsy from two neuropathologically confirmed vCJD cases.\textsuperscript{9} While we were not able to examine these samples using immunohistochemical methods, we have now had the opportunity to investigate appendixes from two further vCJD cases by both high sensitivity western blotting and immunohistochemistry.

Methods

Tissue samples

Tissues were collected at necropsy with consent of relatives from two patients with clinical presentations consistent with vCJD. Define diagnosis of vCJD were confirmed by neuropathological examination and the demonstration of type 4 PrP\textsuperscript{d} in tonsil.\textsuperscript{10} Appendix samples from these vCJD cases, and appendixes from single neuropathologically confirmed cases of either sporadic CJD or inherited prion disease (144 base-pair insertion), were divided and prepared as either 10% homogenates in phosphate buffered saline (PBS) or fixed in 10% formal saline.

Immunohistochemistry

Tonsil tissue was fixed in 10% buffered formal saline or fixed in 10% formal saline. The highest levels of PrP\textsuperscript{d} were seen in tonsil (about 10% of that found in brain)\textsuperscript{5} and tonsil biopsy is used for ante-mortem diagnosis of vCJD.\textsuperscript{6} To date, positive prion protein immunohistochemistry has been reported in only a single appendix sample, although, importantly, this was removed from the patient before the onset of overt features of vCJD.\textsuperscript{7} While the stage at which lymphoreticular infection occurs in vCJD is unknown, PrP\textsuperscript{d} accumulation is detectable in the lymphoreticular system in natural sheep scrapie and in experimental rodent models of scrapie at a very early stage of the incubation period, long before the clinical phase of the disease. Based upon these data it has been suggested that large scale screening of surgical tonsillectomy and appendectomy tissues for PrP\textsuperscript{d} could provide early warning of a high level of vCJD prion infection and several such studies are in progress.\textsuperscript{8}

Recently we reported our concern after finding that PrP\textsuperscript{d} was undetectable in appendix samples obtained at necropsy from two neuropathologically confirmed vCJD cases.\textsuperscript{9} While we were not able to examine these samples using immunohistochemical methods, we have now had the opportunity to investigate appendixes from two further vCJD cases by both high sensitivity western blotting and immunohistochemistry.

Methods

Tissue samples

Tissues were collected at necropsy with consent of relatives from two patients with clinical presentations consistent with vCJD. Define diagnosis of vCJD were confirmed by western blotting using high sensitivity enhanced chemiluminescence was performed as described previously.\textsuperscript{7}

Results

Recently we reported that appendix samples obtained at necropsy from two neuropathologically confirmed vCJD cases contained undetectable levels of PrP\textsuperscript{d}.\textsuperscript{9} We have now examined appendix samples from two further neuropathologically confirmed vCJD cases and have detected PrP\textsuperscript{d} in only one vCJD appendix (fig 1A). The level of PrP\textsuperscript{d} present in this appendix was compared directly with the level of PrP\textsuperscript{d} present in a diagnostic tonsil biopsy sample obtained from the same patient. After proteinase K treatment of equivalent aliquots (20 µl) of 10% appendix homogenate or 10% tonsil biopsy homogenate, we observed clear detection of PrP\textsuperscript{d} in biopsy tonsil homogenate, but not in appendix homogenate (fig 1B). Similarly, PrP\textsuperscript{d} was readily detectable in necropsy tonsil obtained from the vCJD patient with PrP\textsuperscript{d} negative appendix (fig 1C). The background single immunoreactive band seen in appendix is also

Detection of PrP\textsuperscript{d}

Sodium phosphotungstic acid precipitation of PrP\textsuperscript{d} from 0.5 ml 10% tissue homogenates and western blotting with high sensitivity enhanced chemiluminescence was performed as described previously.\textsuperscript{7}

Figure 1

(A–D) Western blots of tissue homogenates with anti-PrP monoclonal antibody 3F4. Western blots were analysed by high sensitivity ECL.\textsuperscript{7} The positions of molecular mass markers are indicated in kilodaltons (kDa). (A) Proteinase K (PK) digestion products from a sodium phosphotungstic acid pellet from 0.5 ml 10% normal human tonsil homogenate spiked with a control level of 10% vCJD brain homogenate (C) is compared with PK digestion products from sodium phosphotungstic acid pellets from 0.5 ml 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 20 µl 10% normal human tonsil spiked with a control level of 10% vCJD brain homogenate (C) is compared with PK digestion products from sodium phosphotungstic acid pellets from 0.5 ml 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (C) Proteinase K digestion products from 20 µl 10% normal human tonsil homogenate (NT) is compared with PK digestion products from 20 µl 10% necropsy tonsil homogenate from the vCJD case with PrP\textsuperscript{d} negative appendix. (D) The western blot shows PK digestion products from 10 µl 10% normal human brain homogenate in the absence of spike (NB) or after spiking with either a control level of 10% vCJD brain homogenate (C) or 0.05, 0.1, 0.25, 0.5, or 1 µl 10% brain homogenate from the vCJD case with PrP\textsuperscript{d} positive appendix. The control level of spike of 10% vCJD brain homogenate (C) shown in panels A, B, D is equivalent to 50 µl 10% brain homogenate from a vCJD case with PrP\textsuperscript{d} positive appendix. (E) Photomicrograph of PrP\textsuperscript{d} positive appendix. Immunoreactivity for PrP\textsuperscript{d} in a lymphatic follicle of the appendix (anti-PrP monoclonal antibody 12F10). The immunostaining pattern is similar to that reported in tonsils in vCJD patients and suggests deposition mainly in dendritic cells.
seen in normal tonsil and is attributable to weaker cross-reactivity of the secondary antibody with an immunoglobulin fragment. While this band is consistently observed after high sensitivity enhanced chemiluminescence of total lymphoreticular homogenate, it is not recovered after sodium phosphate-buffered saline precipitation. We determined that the level of PrPSc present in the brain of the vCJD patient with PrPSc positive appendix patient is approximately 15-fold lower than the maximum level we have observed in vCJD brain (fig 1D). Based upon these findings we estimate that biopsy tonsil and appendix, contain levels of PrPSc of about 4% and about 0.5%, respectively, of that found in the brain of the same vCJD patient (see legend to fig 1).

Importantly, we were able to correlate the detection of PrPSc by western blotting in vCJD appendix with the detection of abnormal prion protein staining by immunohistochemistry. Abnormal prion protein deposits were clearly observed on sections from the PrPSc positive vCJD appendix (fig 1E), while prion protein immunoreactivity was unremarkable on sections from the PrPSc negative vCJD appendix or on sections of appendix from the sporadic CJD or inherited prion disease cases (data not shown).

Discussion

Our findings, together with our previously reported inability to detect PrPSc in two other vCJD appendixes, indicate that appendix does not reliably report vCJD infection even at the end stage of the disease. This observation must be considered when estimating the possible prevalence of vCJD based upon the analysis of archival appendectomy tissues. Although only a minority of appendixes in vCJD may contain detectable levels of PrPSc, surgical instruments used for appendectomy should remain a cause of concern for potential iatrogenic transmission of vCJD prions.

S Joiner, J Linehan, S Brandner, J D F Wadsworth, J Collinge

MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College, Queen Square, London WC1N 3BG, UK

Competing interests: none declared

Correspondence to: Professor John Collinge; j.collinge@prion.ucl.ac.uk

References


Cytokine profiles in HIV seropositive patients with tuberculous meningitis

The immunological response in pulmonary and pleural tuberculosis has been extensively studied. However, the response in tuberculous meningitis has not been well documented. In pulmonary disease, exposure to tuberculous antigens results in a T cell and natural killer cell activation, with elaboration of various cytokines, mainly of T helper type 1 (Th1) origin. Stimulated macrophages elaborate tumour necrosis factor (TNF) α, interleukin (IL) 12, and IL 1, promoting further recruitment and activation of T lymphocytes. TNF α correlates with disease severity and may contribute to tissue necrosis; however, TNFα has also contributed to survival in mouse studies. Transforming growth factor β (Th3 cytokine) suppresses macrophage activation. IL 2 may be beneficial in promoting an immune response in HIV seropositive patients. Th1 and Th2 cytokine responses have been observed in cerebrospinal fluid (CSF) of HIV seronegative patients with tuberculous meningitis. Whether the response is similar in HIV seropositive patients with tuberculosis meningitis is unknown.

We studied the cytokine response and its correlation with disease severity in HIV seropositive and HIV seronegative patients with tuberculous meningitis. Tuberculous meningitis was diagnosed on clinical and CSF analysis excluding various cytokines of viral, acute bacterial, and other causes of aseptic meningitis. Disease severity was assessed according to the Medical Research Council stages 1 to 3. HIV ELISA was done on all patients. CSF specimens were subjected to microscopy, culture, protein and glucose analysis, Venereal Disease Research Laboratory test, fluorescent treponemal antibody analysis, cryptococcal antigen analysis, viral studies, cystercercus ELISA, CD4 counts, and determination of concentrations of adenosine deaminase (ADA), CSF IgG, and albumin.

For cytokine assays, CSF was centrifuged at 3000 g, and supernatant was aliquoted and stored at −70°C. TNF α, interferon (IFN) γ, IL 10 concentrations were measured by ELISA kits (Genzyme Diagnostics, Cambridge, Massachusetts, USA) with detection limits of 3 pg/ml, 3 pg/ml and 5 pg/ml respectively. Data were summarised as medians and ranges. Non-parametric Wilcoxon rank sum tests were used to compare HIV seropositive groups with HIV seronegative groups, tuberculous meningitis severity groups, and groups derived according to the blood brain barrier index for cytokine concentrations. Spearman's rank correlation was used to derive correlations of cytokine concentration, ADA concentrations, and CD4 counts in CSF.

There were 27 patients: 18 (67%) women and 9 (33%) men. Seventeen were HIV seropositive and 10 HIV seronegative. The average interval between onset of symptoms and the first clinical assessment was 17 days (range 5–90 days) in 18 patients where this was recorded. The mean (SD) age was 26.8 (11.6) years. There was one patient aged 10 and one aged 60, and the rest were between 25 and 40. The cytokine concentrations were not analysed according to age, as this would make the categories too small and of little value. The IgG index was calculated for 23 patients. There was no significant difference between the HIV seropositive and HIV seronegative groups for ADA (p = 0.4) and CD4 counts (p = 0.19) in CSF and cytokine concentrations (table 1).

Ten patients (37%) were classified as having grade 1 tuberculosis meningitis (59%) had grade 2 and one (4%) grade 3, which for analysis was considered to be grade 2. Table 1 summarises the cytokine concentrations for patients in stages 1 and 2.

Table 1 Cytokine profiles in HIV seropositive patients with tuberculous meningitis

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>Stage 1</th>
<th>Stage 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN γ</td>
<td>569.9</td>
<td>16.0–2048</td>
<td>890.6</td>
<td>0–2048</td>
</tr>
<tr>
<td>TNF α</td>
<td>1.6</td>
<td>0.6–7.5</td>
<td>9.8</td>
<td>0–309.3</td>
</tr>
<tr>
<td>IL 10</td>
<td>24.6</td>
<td>0–127.9</td>
<td>17.3</td>
<td>0–296.3</td>
</tr>
</tbody>
</table>

IFN, interferon; IL, interleukin; TNF, tumour necrosis factor.
Further studies to confirm our findings would be of value.

The significantly greater TNF α and IFN γ concentrations in the severe group of tuberculous meningitis is confirmed by other studies and suggests that disease severity results mainly from the immune response rather than the organism itself.

The lack of correlation between CD4 and cytokine concentrations may be explained by the fact that there are other sources of cytokines in the CSF, namely macrophages and natural killer cells. Concentrations of ADA, which are derived from lymphocytes, are consistent with other reports, where they were found with cytokine concentrations.

There was no correlation between the IgG index and cytokine concentrations, suggesting that the blood brain barrier did not significantly influence concentrations. Unfortunately, corresponding serum concentrations were not available. This would have been valuable. This is the first study correlating CSF cytokine responses to severity of tuberculous meningitis and comparing HIV positive with HIV negative groups. Further studies should be done to confirm these findings, perhaps to define their relevance to complications and to explore the possibility of II 2 treatment in HIV positive patients.

Acknowledgements
This study was sponsored by the Glaxo TB initiative.

VB Patel, Al Bhigjee, PLA Bill
Division of Neurology, Nelson R Mandela School of Medicine, University of Natal, Durban, South Africa

CA Connolly
Biostatistics, Medical Research Council, Durban, South Africa

Correspondence to: Dr VB Patel, Department of Neurology, Ward A3, Wentworth Hospital, Private Bag Jacobs, Durban 4010, South Africa; patelv@nu.ac.za

References

Festination as the leading symptom of late onset idiopathic aqueductal stenosis
Late onset idiopathic aqueductal stenosis (IAS) may become manifest clinically either by headaches or by hydrocephalic symptoms such as gait disturbance, urinary urge, and cognitive impairment. Rarely, patients with IAS may also present with parkinsonism following repeated episodes of shunt failure. Although the gait disorder of IAS has not been fully characterised, it shares similar features with that of normal pressure hydrocephalus of the elderly. Here, we report on two patients who presented with festination as the leading symptom of IAS.

Case histories
Case 1
A 59 year old man had a seven year history of gait disturbance. During the months before admission, he became more unsteady and he no longer walked with the assistance of a wall because of the unsteadiness of his gait. He fell frequently. For several months, no diagnosis was made and his gait disorder was considered possible organic. Only after imaging studies showed pronounced triventricular hydrocephalus was he referred for further evaluation and treatment. On admission, he also reported occasional nocturnal urinary urgency and incontinence. The most remarkable finding of his physical examination was his gait disorder. He was able to walk without falling only when holding on to a handrail or to the wall. When he walked freely, his stride length became significantly shorter, his step height decreased, while his walking speed increased. He was unable to slow his walking speed or to stop abruptly. He then would bend his upper body forward and forward towards a wall. He could not hold on to a wall or to an object. He could stand without support. On testing of propulsion and retropulsion, he did only have postural instability but he recovered unaired. There was no gait initiation or freezing when passing through obstacles. Arm swing was preserved when walking. He took several extra steps on turning. There was mild bradykinesia of his upper extremities but no tremor or rigidity. Otherwise, the gait examination was unremarkable. He scored 29 of 30 points on the mini-mental state examination. Magnetic resonance imaging including high resolution sagittal constructive interference in steady state (CISS) sequences showed no remarkable stenosis. Lumbar puncture was performed and 40 ml cerebrospinal fluid was drained. One day later, there was mild improvement of the gait disorder. Subsequently, the patient underwent endoscopic third ventriculostomy. The operative procedure and the postoperative course were unremarkable. Within a few days after surgery, further improvement of the gait disturbance was noted. He followed up for four months after surgery, festinating gait had completely resolved and the patient could freely walk without assistance.

Case 2
An 81 year old woman who had previously been well presented with a one year history of weakness and unsteadiness of the legs along with several falls. At the time of admission she was unable to walk or transfer herself independently and had a persistent fear of falling. There had been some urgency of micturition and urinary incontinence, since on occasions she could not reach the toilet in time. The major neurological abnormality was a difficulty in maintaining the erect posture and even walking with the assistance of a Zimmer frame. There was a stooped, flexed posture and festinating gait with short steps. Neurological examination was unremarkable except for diminished light touch sensation in a glove and stocking distribution in the hands and feet. In particular there was no rigidity or tremor, parkes, or impairment of joint position sense. The Middlesex elderly assessment of mental state and Wechsler memory scales showed no significant abnormality. Magnetic resonance imaging showed a lateral and third ventricular hydrocephalus with small aqueduct and normal fourth ventricle. Serial lumbar punctures, which showed pressures from 10 to 14 cm H₂O, had no beneficial or adverse effects. Late onset IAS was diagnosed. The patient underwent ventriculo-peritoneal shunting with a medium pressure valve and an anti-siphon device. Postoperatively she made a slow but steady recovery. Two months after shunting her postural stability and balance had improved considerably. She no longer walked with a stooped posture and her speed of walking had improved significantly. She is now able to walk around the house with the assistance of a cane.

Discussion
Festination was the leading symptom of late onset IAS in both patients reported here. Lack of awareness of this association may cause diagnostic difficulties in the treatment of patients with idiopathic normal pressure hydrocephalus. Festination and parkinsonism symptoms. The occurrence of festination may influence the resolution or resolution of this peculiar gait disturbance. Hydrocephalic gait is usually characterised by a decreased walking speed, stride length, stride frequency, step height and forward floor clearance and a description "magnetic gait" fairly well depicts its typical clinical features. The gait may also adopt a shuffling appearance resembling somewhat a parkinsonian gait disorder. It may be classified as being a middle level and a gait disorder according to its presentation and biomechanical evaluations.1,2 Clinical aspects of hydrocephalic gait vary widely depending on the progression of the underlying condition, and the gait disturbance may occur in as many as 30–50% of patients with idiopathic normal pressure hydrocephalus.2,3 Festination, however, is seen only rarely in hydrocephalic patients but when it occurs it is generally associated with more severe hydrocephalic symptoms and a clinical picture of parkinsonism. It is not fully understood how hydrocephalic disorders induce gait disturbance and parkinsonism symptoms. The underlying pathomechanisms may include mechanical distension of fibres of the corticospinal tract and of dopaminergic pathways but also disturbed supraspinal control mechanisms of gait. Secondary to compromise of cerebrospinal fluid and blood flow in the periventricular and deep white matter. Festination, as well as freezing, cannot be explained by specific neurological abnormalities.4 Festination is seen most frequently in more advanced stages of Parkinson’s disease.5 In patients with Parkinson’s disease, there was no association between significant postural reflex abnormalities and festination but a significant association was found between festination and freezing as reported in the activities of daily living part of the unified Parkinson’s disease rating scale. Festination is considered to be a clinical feature of a subcortical hypodynamic gait disturbance. Festination in the presented cases of IAS may have been related more specifically to distension of the third ventricle. The anatomical substrates of festination have not been elucidated but it is most likely related to function of diencephalic or brainstem locomotor centres. This assumption would also explain the reversibility of festination after third ventriculostomy and shunting. In summary, this is the first report of the occurrence of festination as the leading symptom of IAS. As shown, festination may be misinterpreted and be considered psychogenic in an elderly patient who otherwise is suffering only mild hydrocephalic symptoms.
O Leheta, J Boschert, J K Krauss
Department of Neurosurgery, University Hospital, Klinikum Mannheim, Mannheim, Germany

I R Whittle
Department of Clinical Neurosciences, Western General Hospital, Edinburgh, UK

Correspondence to: Prof Dr J K Krauss, Department of Neurosurgery, University Hospital, Klinikum Mannheim, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; joachim.krauss@nch.ma.uni-heidelberg.de

References
5 Nutt JD, Marsden CD, Thompson PD. Human walking and higher-level gait disorders, particularly in the elderly. Neurology 1983;33:268–79.

Myoclonic movement disorder associated with microdeletion of chromosome 22q11

With a prevalence of approximately 1:4000 interstitial chromosome 22q11 deletion within the DiGeorge syndrome critical region this is the commonest chromosome microdeletion syndrome. The better known clinical features of this disorder are cardiac abnormalities, short stature, palatal abnormalities or velo-pharyngeal insufficiency, renal abnormalities, hypocalcaemia, psychotic symptoms, learning difficulties, and developmental delay. There is wide variability in this clinical spectrum and many case reports drawing attention to new clinical features have been published. Alongside the larger studies of 22q11 cohorts these have proved useful in delineating this particular syndrome.

Case report

We present a family where the proband at 3 years of age exhibited the typical facial features of deletion of chromosome 22q11 (fig 1a) of low set posteriorly rotated ears, small mouth and mandible, short philtrum, and short palpebral fissures, as well as developmental delay. His height was on the fourth centile and he also had pectus carinatum. His mother (fig 1b) also had learning difficulties and delayed motor milestones as a child and subsequently developed an unusual movement disorder, which was first noted as a tremor at age 5 and was brought to medical attention in her teens. The movement disorder consisted of longstanding pronounced myoclonus of the head, trunk, and limbs, which is worsened by intention and exacerbated by startle. She had slow tongue movements. Eye movements were normal. Extensive neurological investigations were normal: caeruloplasmin, molecular testing for Huntington’s disease, myotonic dystrophy, mitochondrial mutations, muscle biopsy, electromyography, and magnetic resonance imaging of the brain. Echocardiography and renal ultrasound in both mother and son were also normal. Although standard cytogenetic testing showed a normal karyotype, fluorescence in situ hybridisation analysis with the probe for the DiGeorge syndrome critical region showed that both mother and son had a deletion in this region.

Discussion

In the European collaborative study presenting data on 558 patients with deletions within the DiGeorge syndrome critical region of chromosome 22q11 neurological details were available for 548 patients and 8% had neurological abnormalities. Three per cent had structural brain abnormalities such as cerebr al atrophy, cerebellar hypoplasia, cerebral vascular abnormality, septum pellucidum cyst, hydrocephalus, hypoplasia of the corpus callosum, and enlarged ventricles. Eleven patients had asymmetrical crying faces, three had seventh cranial nerve palsies, and two had facial asymmetry. Seizure information was available for 290 patients of whom 21% had seizures, and in 42 of these were hypocalcaemic in origin. Nine per cent of children were reported to have either behavioural or psychiatric problems and 18% of adults had a psychiatric disorder.

There has been no previous association with a similar neurological disorder and microdeletion 22q11, although a parkinsonian-like movement disorder has been reported. In that case extrapyramidal symptoms (muscle rigidity, oral-buccal movements, and tremors of his tongue and upper extremities) predated the onset of psychotic symptoms at 15 years of age. As the mother of our proband first exhibited signs of a movement disorder at age 5 it will be interesting to see how he develops. Despite extensive neurological testing for the cause of the myoclonus in the mother, no cause was found. Three mutated genes for myoclonus-dystonia have been described in the literature, the most recent in 2001, and are not available as a diagnostic service. However, the phenotype we describe is different from that described in these cases: alcohol sensitive jerks, dystonia, usually torticollis or writer’s cramp, and often showing prominent psychiatric abnormalities. 22q11 microdeletion syndrome is a variable disorder and even within families signs can be very different. It is possible that many patients with physical and psychiatric abnormalities have an unrecognised genetic disorder. Increased awareness and access to testing may show a widening of the phenotypic spectrum of these disorders. We believe myoclonic movement disorder may be a previously unreported clinical feature of the chromosome 22q11 deletion.

D Baralle, D Trump, C ffrench-Constant
Department of Medical Genetics, Box 134, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QG, UK

D J Dick
Department of Neurology, Norfolk and Norwich Hospital, Colney Lane, Norwich NR4 7UZ, UK

Correspondence to: Dr D Baralle, Department of Medical Genetics, Box 134, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QG, UK; diana.baralle@addenbrookes.nhs.uk

Competing interests: none declared

References
Sodium valproate, nifedipine, and cyclosporin have been implicated in the occurrence of gingival hyperplasia. He had used phenytoin in 1988 to treat his partial seizures. The patient had been on phenytoin for over 20 years, but his dosage had been reduced over time due to the occurrence of gingival overgrowth. Serum phenytoin levels were within the therapeutic range (11.2 μg/ml) while the phenobarbitone level was within normal therapeutic limits, although phenobarbitone had been prescribed at a dosage of 120 mg/day. Both phenytoin and carbamazepine were undetectable. The mechanism of this side effect remains unclear. Accurate drug history, thorough investigations, and punctilious reporting of such cases may help us gain a better understanding of the condition.

Acknowledgements

We acknowledge with thanks the secretarial assistance of Mr M V Srinivas and Mr K Bhashar, Department of Neurology, and the staff of the Department of Medical Illustration and Health Education, NIMHANS.

References


Phenobarbital induced gingival hyperplasia

Among the long term adverse effects of anticonvulsants, gum hyperplasia is cosmetically disturbing and can give rise to complications such as bleaching and gingivitis. Long-term use and high doses of phenytoin sodium (diphenylhydantoin) have been implicated in the occurrence of gingival hyperplasia. Serum phenytoin level in these patients is high. Rarely other drugs such as primidone, sodium valproate, nifdefpine, and cyclosporin have also been implicated. Treatment consists of stopping the offending agent and providing corrective surgery.

Case report

A 26 year old man had been suffering from hot water reflex epilepsy and primary generalised tonic-clonic seizures since he was 12 years of age. The seizures were poorly controlled because of his inability to avoid taking hot baths, inadequate dosage of anticonvulsants, and poor compliance. Since 1987, he had been taking phenobarbital, 60 to 90 mg/day. In 1988, phenytoin (100 mg twice daily) was added for six months but was stopped because there was no benefit. In 1989, carbamazepine (200 mg twice daily) was added but discontinued by the patient in 1996 for unspecified reasons. From 1996 onwards he had been receiving only phenobarbitone 120 mg/day. He was referred to us for progressive gum hyperplasia with bleeding from the gums, both spontaneously and with tooth brushing, since 1996. There was no history suggesting any haematological malignancy, and he was not on any other drug treatment.

On examination, he had gingival hyperplasia involving both the upper and the lower jaws (fig 1). There was occasional bleeding from the hyperplastic gum tissue. General physical examination did not reveal any pallor, hepatosplenomegaly, or lymphadenopathy and neurological evaluation was unremarkable.

Haematological investigations, including haemoglobin, white blood count, erythrocyte sedimentation rate, and peripheral smear, were all normal. Blood glucose, renal and liver function tests, serum electrolytes, and urinary analysis were normal. Scalp electroencephalography and cranial computed tomography were normal. Serum concentrations of phenobarbital, phenytoin, and carbamazepine were measured. Phenobarbitone was within the therapeutic range (11.2 μg/ml) while the other two drugs were not detectable.

Discussion

This patient with longstanding epilepsy probably had phenobarbitone induced gingival hyperplasia. He had used phenytoin in 1988 for only six months without developing this symptom, and it only appeared later in 1996 when he was on phenobarbital alone. After that time it ran a progressive course. Primidone, the metabolites of which contain phenylthethylmalonamide and phenobarbitone, has also been reported to be a rare cause of gingival hyperplasia. However, it is difficult to state which of the two components contributes to the hyperplasia.

Phenytoin sodium is known to be the most common cause of gingival hyperplasia. A minor degree of hyperplasia occurs quite commonly and this generally causes only cosmetic problems, especially for women. However, when phenytoin is used for prolonged periods in high dosage a severe degree of gingival overgrowth occurs. In such cases the hyperplastic gingiva bleed with minimal trauma or spontaneously, and may sometimes become secondarily infected.

The mechanism of anticonvulsant induced gingival hyperplasia is not known. It is believed that these drugs cause tissue collagen proliferation but the reason for this is unknown. It has been speculated that a high phenytoin concentration in the pinitary and adrenal glands may be related to hirsutism and gingival hyperplasia. However, there is at present no evidence that high concentrations of phenytoin in these tissues produce changes in their secretion rate, or indeed that these alterations cause hirsutism and gingival hyperplasia.

Antiepileptic drug levels are generally high in cases of gingival hyperplasia. However, in our case the phenobarbitone level was within normal therapeutic limits, while phenytoin and carbamazepine were undetectable. Buchmann et al reported nine cases of phenytoin induced moderate to severe gingival hyperplasia who underwent corrective gingivectomy. It was found that in eight of these cases the tissue diphenylhydantoin levels were higher than the plasma levels.

Treatment consists of stopping the offending drug and providing supplements of folic acid and ascorbic acid. If regression does not occur, reconstructive surgery of the hyperplastic gingival tissue is advised. Our patient has been switched to sodium valproate, and reconstructive cosmetic surgery is planned.

In conclusion, we report a case of phenobarbitone induced gingival hyperplasia because of its rarity. The mechanism of this side effect remains uncertain. Accurate drug history, thorough investigations, and punctilious reporting of such cases may help us gain a better understanding of the condition.

Hashimoto’s encephalopathy mimicking Creutzfeldt-Jakob disease: brain biopsy findings

A previous report in this journal described seven cases of Hashimoto’s encephalopathy (HE) clinically resembling Creutzfeldt-Jakob disease (CJD). Brain biopsies in such cases are rare and have suggested “vasculitis”. We contribute a report of rapidly progressive dementia in a patient undergoing brain biopsy before the diagnosis of HE was established, showing features suggesting early spongiform change but with inflammation.

A 57 year old woman was taken to a local hospital following a generalised seizure. She was discharged that night after negative cranial computed tomography and cerebrospinal fluid (CSF) analysis. Within a few days she was noted by family members to be acting strangely and hallucinating. Her doctor found her to be somnolent and rigid without focal neurological findings. Magnetic resonance imaging of the brain showed a questionable increase in gadolinium contrast uptake in a 7 mm area of the left medial frontal cortex. An electroencephalogram (EEG) showed bihemispheric slowing without epileptiform activity. Despite an extensive inpatient evaluation (including biochemical, haematological, endocrine, infectious, autoimmune, and toxic analyses), no cause for the encephalopathy could be found. The patient was then referred to our institution for brain biopsy and further care.

Samples of left frontal cortex showed light microscopic evidence of rare vacuoles abutting neurons, suggesting early spongiform
Figure 1 Haematoxylin and eosin stained section of cortex (×400) shows vacuoles within or closely adjacent to neuronal cytoplasm (arrows), giving a spongiform appearance. Elsewhere, microglia were scattered throughout the brain parenchyma and a sparse mononuclear infiltrate was seen in perivascular spaces (not shown). Gliarial fibrillary acidic protein immunostain (not shown) highlighted established gliosis.

change (fig 1). Gliarial fibrillary acid protein staining showed moderate gliosis. A few perivascular lymphoid cells and macrophages were present, with microglia scattered throughout the parenchyma; there was no evidence of true vascuities (inflammation and fibrinoid necrosis of arterial vessels) or of microglial nodules. Because prion disease could not be excluded histologically and because the clinical suspicion of CJD remained high, a tissue block was sent to the National Prion Disease Reference Laboratory (NPDRL) for diagnosis.

Subsequently the patient’s condition deteri- orated. She became wheelchair bound, akinetic, and mute, with startle myoclonus and prominent frontal release signs. CSF analysis showed increased protein (890 mg/dl, reference range < 450 mg/dl). A sample was sent to be tested for the 14–3–3 protein, which is a CNS protein detected in spinal fluid (named after the chemical formula for one of its seven distinct isofoms and two phosphorylated forms) and has been shown recently to have a high sensitivity and specificity (96% and 99%, respectively) for the diagnosis of CJD. Those results that of the EEG has recently been added to the clinical criteria for the “probable” sporadic form of the prion disease.1 Magnetic resonance imaging repeated with gadolinium and diffusion weighted sequences showed only postictal changes. Repeat EEG showed continued bihemispheric slowing with the addition of triphasic waves. The patient had a family history of hypothyroidism but at the time of admission was euthyroid (thyroid stimulating hormone 2.89 mIU/l, reference range 0.5–5 mIU/l; thyroxine 65.79 pmol/l, reference range 64.5–141 pmol/l; triiodothyronine 1.386 pmol/l, reference range 1.078–2.464 pmol/l). Nevertheless, in the absence of a clear cut cause and in view of prior reports of the clinical overlap between CJD and HE, we measured thyroid peroxidase antibodies, which were increased (19 U/ml, reference range 0–20 U/ml). An ultrason of the thyroid showed several hypoechoic areas, fine needle biopsies of which were non-diagnostic. The fact that many euthyroid middle aged women can be shown to have mildly increased thyroid peroxidase antibodies inevitably gave rise to speculation that the finding was incidental. However, in the absence of another obvious cause for the rapidly evolving demen- tia, and giving due weight to the findings in previous studies that the majority of reported cases were euthyroid, we began steroid treatment for a presumptive diagnosis of HE.2

Within 24 hours the patient had visible neurological improvement and within one week she was mobile and conversant, with reversal of most of her cognitive and motor deficits. Shortly thereafter, the NPDRL reported that the tissue did not show evidence of abnormal prion protein. The CSF did not contain the 14–3–3 protein. The patient was discharged on a slow taper of steroids. Six weeks later she was seen in the outpatient clinic and was considered fully recovered by her husband and her doctors.

Rapidly progressive encéphalopathy with myoclonus, extrapyramidal rigidity, and akitic mutism suggest the diagnosis of prion disease such as CJD. However, the differential diagnosis is wide and includes infections of the CNS, toxins, vitamin B12 deficiency, hypothyroidism, autoimmune or idiopathic cerebrovascularities, paraneoplastic encephalitises, epileptic pseudodementias, and other rapidly progressive dementias, especially familial forms of Alzheimer disease. HE, a disorder of presumed autoimmune origin in which patients present with altered levels of consciousness, seizures, and autonomic changes.3,5 The initial laboratory evaluation, the lack of convincing imaging findings, and the EEG were also consistent with early prion disease.6 In fact, according to the World Health Organization criteria, the clinical and EEG features of this case alone already indicated a diagnosis of “probable” CJD.7 Furthermore, the presence of vacuoles on the brain biopsy was very suggestive of the spongiform change (due to swelling of neuritic processes) in early CJD, a diagnostic possibility additionally evoked by the moderate gliosis.8 Although the absence of pathognomonic amyloidial (“kuru”) plaques on a limited biopsy does not exclude a diagnosis of “definite” CJD in a suspected patient, we felt that the features of the case justified immunosassay for prion protein in the tissue and CSF. The presence of perivascular mononuclear cells, though sparse, is not typical for CJD, and may, in the end, be the greatest supportive evidence of encephalitis as the true cause of the symptoms. The changes are reminiscent of those illustrated by Nolte et al., although we strongly disagree with their term “vascularitic”, instead preferring to reserve that term for true necrotising arteritis, which implies a different pathogenesis. Unlike their patient in whom the inflammation was confined to basal leptomeninges in the setting of prior subarachnoid haemorrhage, ours involves parenchymal vessels, with permeation of the cortex by microglia, suggesting a more cred- ible substrate for the seizures and cognitive deficits.

In conclusion, we recommend that patients who, according to World Health Organization criteria, have clinical and EEG features of probable CJD be considered for empirical treatment with steroids for HE, pending labo- ratory results for antithyroid antibodies as well as for 14–3–3 protein. Furthermore, we present additional evidence that HE is an encephalitic process, likely autoimmune given the dramatic response to anti-inflammatory treatment.

C P Doherty, M Schlossmacher, N Torres, E Bromfield, M A Samuels
Department of Neurology, Partners Neurology, Brigham and Women’s Hospital, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA

R Folkert
Department of Pathology (Neuropathology)

Competing interests: none declared

Correspondence to: Dr C P Doherty; cpdoherty@partners.org

References

Long term tolerability of high dose ergoline derived dopamine agonist therapy for the treatment of Parkinson’s disease

During the past decade novel direct acting dopamine agonists (DAs) have become widely deployed for the treatment of the early stages of Parkinson’s disease (PD), because randomised controlled trials (RCT) have demonstrated that their use is associated with a reduced incidence of motor fluctuations and dyskiniesias compared to levodopa therapy.9 However, published randomised controlled trials of DAs with levodopa have tended to focus on the development of motor fluctuations and dyskiniesias as primary end points.10–14 Curiously these studies have also shown that the effect of levodopa on motor function or activities of daily living (measured by Unified Parkinson’s Disease Rating Scale (UPDRS) parts II and III) was superior to that obtained by the DAs, irrespective of whether the latter were administered as monotherapy or with open label levodopa supplemen- tation.15–17

Our understanding of the reasons behind why the DAs were consistently less effective than levodopa on the UPDRS is poor, particularly as these RCTs were performed double blind. Two plausible explanations are: (1) the UPDRS does not capture the whole picture; for example, the DAs and levodopa may have differential effects on mood; or (2) the dose ranges (therapeutic levels) deployed in these studies were set so that the DAs were slightly suboptimal compared to levodopa, but this difference was not apparent to the blinded clinicians assessing the trial patients.

www.jnnp.com
The latter notion encouraged us to examine the dose of the DAs used to treat PD in routine clinical practice. Thus we systematically reviewed the database of all the PD patients under our care in order to identify those receiving DA therapy (cabergoline, pergolide, pramipexole, or ropinirole) above their maximum recommended therapeutic daily dose (respectively 6 mg, 5 mg, 4.5 mg, and 24 mg per day).

We identified 18 patients fulfilling these criteria. All were either on cabergoline (n = 11) or pergolide (n = 7), which are ergoline derived dopamine agonists. This represents about 23% and 27% of PD patients in our practice. In these patients the dose of the appropriate dopamine agonist had been titrated upwards against clinical response (as would be the case with levodopa). There were no patients on “high” doses of the non-ergoline DAs pramipexole or ropinirole, even though the distribution of patients on each DA within the practice was: 45% cabergoline, 21% pergolide, 17% pramipexole, 17% ropinirole. This was probably because of our greater duration of therapeutic experience with the two former drugs.

Table 1 details patients maintained on high dose cabergoline or pergolide therapy.

Table 1: Details of patients maintained on high dose cabergoline or pergolide therapy

<table>
<thead>
<tr>
<th>Receptor affinity</th>
<th>Cabergoline</th>
<th>Pergolide mesylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2 dopamine receptor</td>
<td>2–6 mg/24 h</td>
<td>5 mg/24 h</td>
</tr>
<tr>
<td>D1 and D2 dopamine receptor</td>
<td>2–12 mg/24 h</td>
<td>5 mg/24 h</td>
</tr>
</tbody>
</table>

Demographics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Mean (range)</th>
<th>No. of patients</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>62.6 (40–75)</td>
<td>60.71 (42–71)</td>
<td></td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>8.6 (2–20)</td>
<td>6.9 (2–12)</td>
<td></td>
</tr>
<tr>
<td>Hoehn and Yahr*</td>
<td>1.73 (1–2)</td>
<td>1.14 (1–2)</td>
<td></td>
</tr>
</tbody>
</table>

Dopamine agonist therapy

| DA therapy duration (y) | 2.3 (0–8–4) | 2.5 (2–4) |
| DA therapy duration above recommended dose (y) | 1.4 (0.5–3.0) | 2.1 (1.4–3.6) |
| DA dose (mg/24 h) | 8.8 (7–12) | 9.4 (6–12) |

Additional medication

| L-dopa (mg)/24 h | 750 (300–1000) | 508 (100–950) |
| Apomorphine (mg)/24 h | 15 (15) | 0 |
| Entacapone (mg)/24 h | 1000 (600–1200) | 933 |
| Benztropine (mg)/24 h | 6 (6) | 0 |
| Amantadine (mg)/24 h | 250 (200–300) | 600 (600) |
| Propranolol (mg)/24 h | 0 | 20 (20) |
| Pramipexole (mg)/24 h | 3 (3) | 0 |

Side effects (mild)

| Akathisia | 2 |
| Dyskinesia | 5 |
| Hallucinations | 1 |

*Hoehn and Yahr score at initiation of DA therapy, while on other antiparkinsonian treatment. †Dyskinesia was present prior to the introduction of DA therapy.

We acknowledge the neurologists’ experiences of using these drugs for prolonged periods above their recommended doses. There is a case for further studies involving high dose dopamine agonist treatment for people with PD.

Finally, it should be noted that serosal inflammation is a rare, probably idiosyncratic, potentially reversible complication of ergoline derived dopamine agonist administration, which typically develops about two years after introducing this class of drug.7 However, we have not encountered this problem in our practice.

Acknowledgements

We would like to thank Patrick O’Sullivan (drug information pharmacist, Charing Cross Hospital), Janet Munro (Lilly), and Kevin Bridgman (Pharmacia) for assisting with our search for data on serosal inflammatory disorders associated with pergolide and cabergoline therapy in Parkinson’s disease.

P Navan, P G Bain

Department of Neurosciences, Imperial College, Charing Cross Campus, London, UK

Competing interests: PN was reimbursed by Pharmacia for attending the XIV International Congress on Parkinson’s disease (Finland, 2001). The authors also conducted two double blind trials comparing the antitremor effects of pergolide with pramipexole, to which Pharmacia freely supplied the masked medications.

Correspondence to: Dr P G Bain, Department of Neurosciences, Imperial College, Charing Cross Hospital, London W6 8RF, UK, p.bain@ic.ac.uk

References

7 ABPI. Cabaser (cabergoline), Celance (pergolide mesylate), Mirapexin (pramipexole), and Requip (ropinirole) data sheets. In: ABPI compendium of data sheets and summaries of product characteristics. London: Datapharm Publications, 1999–2000 or ABPI website: http://emc.vhn.net.
Irregular presence of abnormal prion protein in appendix in variant Creutzfeldt-Jakob disease

S Joiner, J Linehan, S Brandner, J D F Wadsworth and J Collinge

*J Neurol Neurosurg Psychiatry* 2002 73: 597-598
doi: 10.1136/jnnp.73.5.597

Updated information and services can be found at:
[http://jnnp.bmj.com/content/73/5/597](http://jnnp.bmj.com/content/73/5/597)

*These include:*

**References**
This article cites 4 articles, 0 of which you can access for free at:
[http://jnnp.bmj.com/content/73/5/597#BIBL](http://jnnp.bmj.com/content/73/5/597#BIBL)

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to:
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to:
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)