LETTERS

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

We have investigated the presence of disease related prion protein (PrP\(^\text{Sc}\)) in appendix samples obtained at necropsy from four neuropathologically confirmed cases of vCJD, Creutzfeldt-Jakob disease (vCJD), PrP\(^\text{Sc}\) was detected in only one vCJD appendix, at a level lower than found in a diagnostic tonsil biopsy sample obtained from the same patient. The single PrP\(^+\) 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\(^+\) questions the utility of screening archival appendicectomy tissue samples to estimate the prevalence of pre-clinical vCJD infection within the UK population.

The appearance of a novel human prion disease, 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. The pathogenesis of vCJD differs significantly from that of other sporadic Creutzfeldt-Jakob disease associated prion protein (PrP\(^\text{Sc}\)) is readily detectable in lymphoreticular tissues in vCJD and not in classic CJD.\(^1,2\) High levels of PrP\(^\text{Sc}\) are uniformly found in the central nervous system and lymphoreticular system of vCJD patients.\(^3\) The highest levels of PrP\(^\text{Sc}\) seen outside the central nervous system in vCJD are in tonsil (about 10% of that found in brain)\(^4\) and tonsil biopsy is used for ante-mortem diagnosis of vCJD.\(^5\) 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.\(^6\) While the stage at which lymphoreticular infection occurs in vCJD is unknown, PrP\(^\text{Sc}\) 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 appendicectomy tissues for PrP\(^\text{Sc}\) could provide early warning of a high level of vCJD prion infection and several such studies are in progress.\(^7\)

Recently we reported our concern after finding that PrP\(^\text{Sc}\) was undetectable in appendix samples obtained at necropsy from two neuropathologically confirmed vCJD cases.\(^8\) 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. Definite diagnoses of vCJD were confirmed by neuropathological examination and the demonstration of type 4 PrP\(^\text{Sc}\) in tonsill.\(^9\) 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 and inactivation of prion infectivity was accomplished by incubation in 98% formic acid for one hour. After further washing for 24 hours in 10% buffered formal saline, tissue samples were processed and paraffin wax embedded. Sections were cut at a nominal thickness of 4 µm, treated with 98% formic acid for five minutes and then boiled in EDTA-TRIS-citrate buffer pH 7.8 for 20 minutes. Immunohistochemical staining was performed with anti-PrP monoclonal antibody 12F10 on a Ventana automated immunohistochemical staining machine using a basic diaminobenzidine detection system according to the manufacturers instructions (Ventana Medical Systems, Tucson, Arizona).

Detection of PrP\(^\text{Sc}\)

Sodium phosphotungstic acid precipitation of PrP\(^\text{Sc}\) from 0.5 ml 10% tissue homogenates and western blotting using high sensitivity enhanced chemiluminescence was performed as described previously.\(^1\)

Results

Recently we reported that appendix samples obtained at necropsy from two neuropathologically confirmed vCJD cases contained undetectable levels of PrP\(^\text{Sc}\).\(^8\) We have now examined appendix samples from two further neuropathologically confirmed vCJD cases and have detected PrP\(^\text{Sc}\) in only one vCJD appendix (fig 1A). The level of PrP\(^\text{Sc}\) present in this appendix was compared directly with the level of PrP\(^\text{Sc}\) present in a diagnostic tonsil biopsy sample obtained from the same patient. As in proteinase K treatment of equivalent aliquots (20 µl) of 10% appendix homogenate or 10% tonsil biopsy homogenate, we observed clear detection of PrP\(^\text{Sc}\) in biopsy tonsil homogenate, but not in appendix homogenate (fig 1B). Similar results were readily detectable in necropsy tonsil obtained from the vCJD patient with PrP\(^\text{Sc}\) negative appendix (fig 1C). The background single immunoreactive band seen in appendix is also present 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.

![Figure 1](http://jnnp.bmj.com/)

(A–D) Western blots of tissue homogenates with anti-PrP monoclonal antibody 3F4. Western blots were analysed by high sensitivity ECL.\(^1\) 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 0.5% vCJD brain homogenate (I) 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 0.5% vCJD brain homogenate (C) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 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 homogenate (C) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 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 homogenate (C) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases. (B) PK digestion products from 0.5 ml 10% normal human tonsil (NT) is compared with PK digestion products from 20 µl 10% appendix homogenates from vCJD (V), sporadic CJD (S) or inherited prion disease (I) cases.
seen in normal tonsil and is attributable to weak 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 phosphotungstic acid 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 the levels 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 seen 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 appendicectomy tissues. Although only a minority of appendixes in vCJD may contain detectable levels of PrPSc, surgical instruments used for appendicectomy should remain a cause of concern for potential iatrogenic transmission of vCJD prions.

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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 cellular response, elaborating various cytokines, mainly of T helper type 1 (Th1) origin. Stimulated macrophages elaborate tumour necrosis factor (TNFα) and interleukin (IL) 12, and IL 1, promoting further recruitment and activation of tuberculous T lymphocytes. TNFα 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 tuberculous 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 examination, after exclusion 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, tonsils and lymph nodes were subjected to microscopy, culture, protein and glucose analysis, Venereal Disease Research Laboratory test, fluorescent treponemal antibody analysis, cryptococcal antigen analysis, viral studies, cysticercus 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. TNFα, interferon (IFN) γ, and 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 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, 1 patient (4%) had grade 2, and 16 (59%) had grade 3 (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.

Patients with stage 2 disease had significantly stronger Th1 responses. There was no difference in the IL 10 concentrations. The two patients with stage 2 disease who died had very high IFN γ concentrations, both greater than 2048 pg/ml.

IL 10 concentrations were moderately positively correlated with IFN γ concentrations (r = 0.53). The correlation coefficients were 0.18 for IFN γ, 0.33 for TNFα, and 0.34 for IL 10. Correlation coefficients between ADA and cytokine concentrations were 0.34 for IFN γ, 0.47 for TNFα, and 0.22 for IL 10. Cytokine concentrations correlated poorly with CD4 counts in CSF.

It is postulated that in HIV infection a predominant Th2 response accounts for extra-pulmonary disease. This study does not favour a predominance of either Th1 or Th2 in the CSF of patients with tuberculosis. It is possible that a Th0 response, which is a non-differentiated response seen early on in immune activation, was seen in our patients, as they were examined untreated and relatively early in the disease. Other investigators have also documented this phenomenon. The positive correlation between IFN γ and IL 10 suggests that these were produced concurrently. This may reflect a control mechanism regulating Th1 and Th2 responses.

There was no difference in cytokine and ADA concentrations and CD4 counts between HIV seropositive and HIV seronegative patients. It is known that the clinical response to antituberculous treatment in both groups is similar. Perhaps this similarity correlates with similar immune responses in both groups. The size of each group is small and a type 1 statistical error has to be considered.

Table 1 Differences between HIV seropositive and HIV seronegative groups and tuberculous meningitis severity

<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 [pg/ml]</td>
<td>Median 559.9 16.0–2048</td>
<td>890.6 0–2048</td>
<td>0.9</td>
<td>184.5 0–1771.0</td>
</tr>
<tr>
<td>TNFα [pg/ml]</td>
<td>1.6 0–67.5</td>
<td>9.8 0–309.3</td>
<td>0.11</td>
<td>0.35 0–92.3</td>
</tr>
<tr>
<td>IL 10 [pg/ml]</td>
<td>24.6 0–127.9</td>
<td>17.3 0–296.3</td>
<td>0.9</td>
<td>3.68 0–53.0</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 increased 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 IL 2 treatment in HIV positive patients.

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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 urgency, 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 characterized, 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 was not able to walk without assistance because of pronounced hastening of his steps. He fell frequently. For several months, no diagnosis was made and his gait disorder was considered possible parkinsonian. Only after imaging studies showed pronounced ventriculoperitoneal hydrocephalus was he referred for further evaluation and treatment. On admission, he also reported occasional nocturnal urinary urge 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 successively shorter, 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 fall if he could not hold on to a wall or to an object. He could stand without support. On testing of propulsion and propulsion he had mild postural instability but he recovered unaids. There was no gait ignition failure or freezing when passing through obstacles. Arm swing was preserved while walking. He took several extra steps on turning. There was mild bilateral bradykinesia of his upper extremities but no tremor or rigidity. Otherwise, the 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 aqueductal 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 evident. About four months after surgery, festinating gait had completely resolved and the patient could walk freely without assistance.

Case 2

An 81 year old woman who had previously been well presented with a one year history of gait disturbance. The gait disorder was noted to worsen as her mental state examination. Magnetic resonance imaging including high resolution sagittal constructive interference in steady state (CISS) sequences showed aqueductal 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 evident. About four months after surgery, festinating gait had completely resolved and the patient could walk freely without assistance.

Discussion

Festination was the leading symptom of late onset IAS in both patients reported here. Lack of awareness of this association may cause diagnostic delay. 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.
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 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, hypothalamic syndrome, psychotic symptoms, learning difficulties, and developmental delay.1 There is widespread 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 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 normal.

Discussion

In the European collaborative study presenting data on 558 patients with deletions within the DiGeorge syndrome critical region of chromosome 22q11 neurocognitive details were available for 548 patients and 8% had neurocognitive abnormalities. Three per cent had structural brain abnormalities such as cerebral 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 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.2 In that case extrapyramidal symptoms (muscle rigidity, oral-buccal movements, and tremors of the 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.

Figure 1 (a) Proband with facial features of 22q11 microdeletion syndrome. (b) Mother of proband.

References

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

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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

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References

Phenobarbitone induced gingival hyperplasia

Among the long term adverse effects of anticonvulsants, gum hyperplasia is cosmetically disturbing and can give rise to complications such as bleeding 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 are high. Rarely other drugs such as primidone, sodium valproate, nifedipine, and cyclosporin have also been implicated. Treatment consists of stopping the offending agent and providing corrective surgery. Phenobarbitone (phenobarbital) has not yet been reported to cause gum hyperplasia. We describe a patient who developed this complication in association with the long term use of phenobarbitone.

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 despite use of allopurinol for his cholesterol levels and since 1996 he had been taking phenobarbitone, 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 1998, carbamazepine (200 mg twice daily) was added but was 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 urine analysis were normal. Scalp electroencephalography and cranial computed tomography were normal. Serum concentrations of phenobarbitone, 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 phenobarbitone alone. After that time it ran a progressive course. Primodine, the metabolites of which contain phenylthylmalonamide 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 phenytion concentration in the pituitary and adrenal glands may be related to hirsutism and gingival hyperplasia. However, there is still 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 diphenylhydrantoin 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 unclear. Accurate drug history, thorough investigations, and punctilious reporting of such cases may help us gain a better understanding of the condition.

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References


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 bilateral hemispheric slowing without epileptiform activity. Despite an extensive inpatient evaluation (including biochemical, haematological, endocrine, infectious, autoimmunue, 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 aborting 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). Glial fibrillary acidic protein immunostain (not shown) highlighted established gliosis.

change (fig 1). Glial 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 vasculitis (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).

Subsequently the patient’s condition deteriorated. She became wheelchair bound, akinetic, and mute, with startle myoclonus and rigidity, has recently been added to this list. 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 daughters. Rapidly progressive encephalopathy with myoclonus, extrapyramidal rigidity, and akinetic 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 cerebrovascularitis, paraneoplastic encephalitis, epileptic pseudodementia, 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 extrapyramidal and extrapyramidal rigidity, has recently been added to this list. Patients with HE are usually euthyroid, with increased antithyroid antibodies often discovered only after the diagnosis of encephalopathy.

Although presentation with a seizure is unusual in CJD, our patient’s other clinical features of rapid cognitive deterioration, myoclonus, and extrapyramidal rigidity were highly suggestive of prion disease. 4 The initial laboratory evaluation, the lack of convincing imaging findings, and the EEG were also consistent with early prion disease. In fact, according to WHO criteria, the clinical and EEG features of this case alone already indicated a diagnosis of “probable” CJD. 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 true necrotising arteritis, which implies a different pathology in CJD (fig 3). Within 24 hours the patient had visible neurological improvement and within one week she was mobile and conversant, with neurological improvement and within one week she was mobile and conversant.

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References

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

During the past decade new 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 dyskinesias compared to levodopa therapy. 1–3 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 supplementation. 4–6 Our understanding of the reasons behind why the DAs were consistently less effective than levodopa on the UPDRS, 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 doses 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.
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 the practice receiving cabergoline or pergolide respectively. 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 illustrates the clinical characteristics of these patients and shows that cabergoline and pergolide are tolerable, on average follow up periods of 2.3 and 2.5 years respectively, at dosages well above those recommended in their data sheets. Indeed it is clear from these data that the therapeutic windows of the ergoline derived dopamine agonists are at least twice that presently acknowledged.

Consequently, we speculate that the ergoline derived dopamine agonists (cabergoline and pergolide) may not have been deployed to their full therapeutic potential, either in the major RCTs comparing DAs with levodopa or in current clinical practice. However, we do not know whether this capacity is confined to the ergoline derived DAs. As the direct acting DAs are now considered to be first line therapy for younger people with Parkinson’s disease, we would be very interested to hear of other 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. However, we have not encountered this problem in our practice.

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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.

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References
7 ABPI. Cabaser (cabergoline), Celence (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://www.mvh.net.

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>
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</tbody>
</table>

Demographics

<table>
<thead>
<tr>
<th>No. of patients (sex)</th>
<th>Mean (range)</th>
<th>No. of patients (sex)</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>62.6 (40–75)</td>
<td>63.7 (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

<table>
<thead>
<tr>
<th>DA therapy duration (y)</th>
<th>Mean (range)</th>
<th>No. of patients (sex)</th>
<th>Mean (range)</th>
<th>No. of patients (sex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA therapy duration above</td>
<td>2.3 (0–8–4)</td>
<td>2.5 (2–4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>recommended dose (y)</td>
<td>1.4 (0.5–3.0)</td>
<td>2.1 (1.4–3.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA dose (mg)/24 h</td>
<td>8.8 (7–12)</td>
<td>9.4 (6–12)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional medication

| L-dopa (mg)/24 h             | 750 (300–1100)| 508 (100–950) | 6 |
| Apomorphine (mg)/24 h        | 15 (5–13)     | 0             | 0 |
| Entacapone (mg)/24 h         | 600 (100–1400)| 3             | 3 |
| Dopamine agonist (mg)/24 h   | 6 (6)         | 1             | 0 |
| Amantadine (mg)/24 h         | 250 (200–300)| 600 (600)     | 1 |
| Pramipexole (mg)/24 h        | 0             | 20 (20)       | 1 |
| Side effects (mild)          |              |              | 0 |
| Dyskinesia†                  | 2             | 1             | 2 |
| Hallucinations               | 1             | 0             | 0 |

*Hoehn and Yahr score at initiation of DA therapy, while on another anti-parkinsonian treatment.  †Dyskinesia was present prior to the introduction of DA therapy.