Hypokinesia and presenile dementia in a Dutch family with a novel insertion in the prion protein gene

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Summary
The clinical features and disease course of six patients from a family with autosomal dominant inheritance of presenile dementia and a hypokinetic syndrome are described. In the past, these patients have carried diagnoses of Pick's disease, Huntington's disease, Parkinson-dementia, and one patient was described as suffering from a 'peculiar type of presenile dementia' in a case report. In the two cases examined, the most distinctive neuropathological features were extensive globular deposits of periodic acid-Schiff plus diastase (PAS)-positive material, having tinctural properties of amyloid only to a limited degree, in the cerebellum and cerebral cortex. These globules stained positively with antibodies against prion protein. Southern blot of MspI-digested genomic DNA showed an abnormal band of ~950 bp in all three patients from which material was available. Direct sequencing of the abnormal allele revealed an insert consisting of eight extra 24-nucleotide repeats in the patients, which was absent in a healthy first degree relative who was considered well beyond the age of onset of symptoms in this family. The nucleotide sequence of the abnormal insert of 192 bp was different from that of a previously described insert of equal length. Adding to previous descriptions of mutations in the prion protein gene, this report emphasizes the clinical, neuropathological and genetic heterogeneity of inherited prion diseases.

Keywords: prion disease; Gerstmann–Sträussler–Scheinker disease; amyloid plaques; presenile dementia; genetic disease

Abbreviations: CJD = Creutzfeldt–Jakob disease; GSS = Gerstmann–Sträussler–Scheinker syndrome; ORF = open reading frame; PAS = periodic acid–Schiff plus diastase; PCR = polymerase chain reaction; PrP = prion protein

Introduction
Prion protein (PrP) is a sialoglycoprotein that is expressed predominantly in neurons (Prusiner, 1993). The normal cellular function of PrP is unknown, although some observations suggest a role in cell signalling or adhesion (Collinge et al., 1994). Mutations in the human PrP gene on chromosome 20 have been found in familial Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome (GSS), fatal familial insomnia and hereditary encephalopathies with other clinical phenotypes (reviewed in Prusiner, 1993). Individuals carrying a point mutation or insert mutation in the PrP gene open reading frame (ORF) usually present with dementia or gait-ataxia, but clinical features and disease progression may show distinctive variation in individual cases, even among members of one pedigree (Collinge et al., 1992).

With fatal familial insomnia as a possible exception, the symptoms, disease course and neuropathological findings in inherited prion disease can generally be described with reference to a spectrum with classical CJD (with dementia, a rapid course and marked spongiform changes) and GSS (with ataxia, a slow course and multicentric amyloid plaques) at the extremes (Collinge et al., 1992; Prusiner, 1993). Stam et al. (1968) published the case history of a patient suffering from what was labelled as 'a peculiar type of presenile dementia' which could not be classified more specifically. Their patient suffered from a rapidly progressive dementia,
Patients and methods

Information on three affected family members (I-1, I-2, I-4, Fig. 1), including the case described by Stam et al. (1968) (I-2), was obtained by studying original hospital case records. The three youngest affected individuals (II-1, I-2 and III-1) were examined recently by us. The neuropathological findings in case I-2 are summarized from the original post-mortem report. Formalin-fixed, paraffin-embedded sections of brain tissue from case II-1 were examined using haematoxylin-eosin, Bodian-silver, and PAS plus diastase stainings. For β-amyloid- and PrP-immunoreactivity, we used anti-β-amyloid (Boehringer, Mannheim, Germany) and IA8 anti-PrP (donated by Dr Farquhar, Edinburgh, UK), respectively.

Patients

Patient I-1
In May 1959, this 50-year-old craftsman was fired because he became increasingly passive and lost his tools at work. He had become short-tempered and his wife noticed crying spells. When first seen, 6 weeks later, he was disoriented in time and place. Recent memory was impaired. He gave only monosyllabic answers, but responded correctly to simple commands. He appeared unaware of his deficits. His mood was described as somewhat euphoric. No abnormalities were noted on examination of the cranial nerves and sensory system. He was described as having an ataxic gait, dysarthria, a positive grasp reflex and a left extensor plantar response. He had hypokinesia with rigidity in both arms without weakness. Investigation showed normal routine biochemistry and haematology, including a Wasserman reaction and examination of CSF. An EEG showed slow background activity. Symmetrically dilated ventricles were noted on pneumoencephalography and it was concluded that atrophy was frontally more pronounced. A diagnosis of Pick’s disease was made and the patient was referred to a residential home. Here his condition deteriorated rapidly, with increasing dementia, hypokinesia and incontinence. He died of pneumonia 5 months after the onset of his illness. Autopsy was not performed.

Patient I-2
In mid 1966, the wife of this 55-year-old man reported that her husband suffered from memory impairment, irritability, sleep disturbances and ‘jerky’ movements with his hands and feet. His employer reported that the patient had already performed poorly for 18 months. Since his son had died of leukaemia in 1960, he had become introverted. When hospitalized in early 1967, he was disoriented, aphasic and apathetic. He had a cautious gait and he was noted to have a tremor, hypokinesia and rigidity. Coordination of voluntary movements was described as normal. During the examination he showed perseverations. An initial diagnosis of ‘Parkinson-dementia-syndrome’ was made on admission. Routine haematology, biochemistry and syphilis serology were normal or negative, as was CSF examination. His third EEG, depicted in the publication of Stam et al. (1968), showed di- and triphasic wave complexes. Pneumencephalography showed diffuse dilatation of the lateral ventricles. Over the next 3 months he became mutistic and he died 12 months after his first referral. A conservative estimate of the time elapsed since the onset of his symptoms is at least 2 years.

On neuropathological examination the brain weighed 1280 g. Stam et al. (1968) described mild spongiform changes in the cortex, gliosis, and disseminated PAS-positive deposits in the cortex, striatum, thalamus and molecular layer of the cerebellum. The deposits were described as ‘bunches’ of eosinophilic globules and they were labelled racemose (‘grape-like’). After studying the histochemical characteristics of the deposits, Stam et al. (1968) concluded that they consisted of a sialomucoprotein. Neurofibrillar degeneration was absent. Unfortunately, the microscopic preparations were lost.

Patient I-4
This patient died in 1983 at the age of 59 years after a myocardial infarction. According to his wife, when interviewed in 1993, he had developed memory problems, behavioural changes and walking problems at least 5 years before his death. On admission for his myocardial infarction, it was noted that he had a dysarthric speech and a nystagmus. He responded slowly to simple commands. His reflexes were described as normal. The background pattern on the EEG was slow and it was periodically interspersed with sharp waves described as triphasic. CT of the brain revealed diffuse atrophy. He died 18 days after admission, autopsy was not performed.
Patient II-1
In approximately 1988, this 45-year-old teacher noticed unsteadiness during walking. When first seen in 1990 he also reported memory difficulties and his wife had noticed troubles with articulation. On examination his speech was mildly dysarthric and his voluntary movements were characterized as 'clumsy'. A positive snout, corneomandibular reflex and a brisk masseter reflex were noted. He had mild rigidity and hypokinesia, without tremor. On neuropsychological examination his performance intelligence quotient was reduced with deficits of concentration, visuoperception, abstract reasoning and eye-hand coordination. The results of the EEG, CSF examination and routine laboratory investigations were all unremarkable. A CT brain scan showed an enlarged occipital horn on the left. A diagnosis of Huntington's disease was considered. He slowly deteriorated and when next seen in early 1993, his spontaneous speech was sparse and dysarthric with perseverations. He cried during the examination and he had a mild action tremor. He was disoriented and he had recent memory disturbances with preserved memory for events of the past. MRI showed enlarged ventricles with normal signal intensities on T_{1}- and T_{2}-weighted images. Over the next few months his condition rapidly worsened. He became incontinent and he was periodically agitated. On a repeated EEG, triphasic waves against a slowed background rhythm were noted. He died in January 1994, ~6 years after the onset of his illness.

Neuropathological examination showed mild gross atrophy. In the molecular layer of the cerebellum many clusters of eosinophilic globular deposits measuring 5–20 μm were found (Fig. 2). There was no cell loss in the Purkinje and granular layers of the cerebellum. The cerebral cortex showed mild spongiform changes and dispersed clusters of globular deposits, most pronounced in the parahippocampal and temporal cortex. There was only mild neuronal loss and minimal gliosis. The caudate nucleus and putamen also contained extensive globular deposits and mild spongiosis. Signs of inflammation, abnormal intraneuronal inclusions (including neurofibrillary tangles) and neuritic plaques were absent.

The globular deposits were PAS positive and only faintly congophilic, with equivocal birefringence on examination with polarized light. Staining of the globules with anti-PrP antibody was positive both in the cerebellar and cerebral cortex.

Patient II-2
On advice of her elder sister, this 38-year-old cashier sought medical advice because she showed symptoms resembling those of her father and uncle. She experienced memory impairment, walking difficulties and emotional lability. When first examined in 1992, she was apathetic, had small amplitude myoclonic jerks of the fingers, and showed mild rigidity. Routine laboratory examinations and an EEG were normal.

CT showed cerebral and cerebellar atrophy. On admission for a second opinion in September 1993, she was depressed and hypokinetic. Cognitive examination revealed deficits on visuomotor tasks and tests of memory with relatively preserved recognition and normal orientation resulting in a CAMCOG-score of 67 (maximum 107, cut-off 80) (Roth et al., 1986). She showed impaired smooth pursuit eye movements, slow tongue movements and a positive snout reflex. Voluntary movements were slow, but not ataxic. Her gait was unsteady; she had a flexed posture, mild rigidity in the arms, and small amplitude myoclonic jerks, mainly distally. The tendon reflexes were brisk with an extensor plantar response on the left. Values for lactate, copper and ceruloplasmin were normal. Syphilis serology was negative and the results of CSF examination were in the normal range. The background activity on the EEG was diffusely slow. MRI confirmed the diffuse cerebral and cerebellar atrophy with normal signal intensities of the white matter and basal ganglia. Technetium-HMPAO single photon CT showed reduced perfusion throughout the cerebral cortex. On follow-
up her depression appeared to have responded well to treatment with fluvoxamine, but the other clinical symptoms had not changed and a repeated CAMCOG-examination resulted in a score of 66 in September 1994.

**Patient III-1**

Four months after her father (II-1) had died, this 21-year-old student was admitted to a psychiatric hospital because of depression, memory problems and gait difficulties. During the last year of her father’s illness, she had given up her studies. On examination she was hypokinetic, with subtle myoclonic movements of the fingers. The eye movements were normal. The glabellar tap was positive and reflexes were brisk with flexor plantar responses. There was dysdiadochokinesis and she had an intention tremor. Her affect was labile, she was easily distracted and she showed perseverations during the examination. Laboratory examinations were unremarkable. The EEG showed diffuse background slowing. On T2-weighted MRI images the basal ganglia were hyperintense.

**Molecular studies**

For molecular genetic studies, DNA was extracted from white blood cells of three patients (II-1, I-2 and III-1) and a healthy, 79-year-old first degree relative (I-3). Seventy-nine years was considered well beyond the age of onset of symptoms in this family. The major part of the PrP gene ORF was amplified using the polymerase chain reaction (PCR). The oligonucleotide sequences of the primers were: 5'-TGCTGGTTCTCTCTTTTG (forward primer F72) and 5'-AAGGGTGTGCAGGTTTGATAC (reverse primer R886). Polymerase chain reaction conditions were: 1.0 mM MgCl2, 0.375 mM dNTPs, 5.5% DMSO, 1X PCR-buffer ( Gibco BRL, USA), 0.4 units Taq-polymerase (Gibco BRL, USA), and 50 ng of each primer in a total volume of 50 μl. Cycling conditions were: 2 min at 93°C, 3 min at 72°C. Finally, there was an 8-min extension at 72°C. Polymerase chain reaction was performed in a MJ Research PTC100 thermal cycler.

Southern blotting of chromosomal DNA was performed after restriction with MspI. As a probe for hybridization we used the PCR product generated with the primers described above and control DNA. The probe was labelled with [32P]dATP using random primed labelling kit (Boehringer Mannheim GmbH, Germany).

Autoradiography was carried out on a Phosphor Imager (Molecular Dynamics, USA).

The length and position of the abnormal insert was determined by restriction of PCR products with BglI and PvuII. After phenol extraction and precipitation, the restricted DNA was endlabelled using T7 DNA polymerase and [32P]dATP, and, combined with a length marker, electrophoresed on an acrylamide gel.

DNA sequencing was performed in both directions on PCR products generated as described above with biotinylated primers. Polymerase chain reaction products were electrophoresed on a 1.0% low melt agarose gel and normal and mutant alleles were excised. DNA was purified out of the agarose using the QIAEX-kit (Qiagen Inc., USA) and was made single stranded and the strands were separated using streptavidine Dynabeads M-280 (Dynal, A.S., USA). For sequencing, [32P]dATP end-labelled primers and the Sequitherm Cycle Sequence kit (Epicentre Technologies, USA) were used. To sequence the entire ORF except the first 50 nucleotides, we used the PCR-primers F72 and R886 (above) and four additional primers; forward primers F179 and F320 (both 18-mers), and reverse primers R352 and R530 (a 21-mer and 19-mer, respectively).

**Results**

**Clinical symptoms and pathological findings**

Personality changes, gait problems and memory difficulties were the first symptoms noted in all patients. When first seen all patients showed hypokinesia and/or rigidity. Limb ataxia of mild degree was noted only in patient III-1 on first examination. Cognitive deficits had features of both cortical and subcortical dementia. Anti-social personality disorder as
described in the family with a 144 bp insertion was not observed (Collinge et al., 1992).

The most distinctive neuropathological features were the extensive globular deposits of PAS-positive material, having tinctural properties of amyloid only to a limited degree (Fig. 2). Staining with anti-PrP antibodies showed that these deposits contain PrP. Astrocytosis and spongiform changes were mild, and neurofibrillary degeneration and β-amyloid plaques were absent. The abnormal deposits of PrP were most extensive in the molecular layer of the cerebellum, in the temporal and parahippocampal cortices and in the striatum. The cerebellum showed no cell loss and only mild neuronal loss was found in the cortex, caudate nucleus and putamen.

Molecular genetic analysis

Southern blot of MspI digested genomic DNA showed an extra band after hybridization with the PCR products in all three patients from which material was available (I-1, I-2 and III-1). In addition to the normal band generated by MspI of ~750 bp, there was a MspI-fragment of ~950 bp in all three patients analysed (Fig. 3). This extra band was absent in the healthy first degree relative (I-3) and in three non-related controls. Restriction-mapping of the PCR products showed an abnormal insertion of ~192 bp between bases 200 and 400. Direct cycle-sequencing of the PCR products of the alleles of normal and increased length revealed eight extra 24-nucleotide repeats in the abnormal allele. For the normal allele, the sequence of the octarepeats was in agreement with previous reports (Goldfarb et al., 1991). The nucleotide sequences flanking the 192 bp insert were identical to that of the wild-type allele. The length of the abnormal insert in this Dutch pedigree, labelled ‘A’, is equal to the insert in a French pedigree with inherited prion disease (‘Che’-family) (Goldfarb et al., 1992). However, the octarepeat sequences are different in both families (Fig. 4). Moreover, in the fourth octarepeat, R3' in Fig. 4, the seventh triplet of the normally appearing R3-octarepeat is changed from GGA into GGG in family ‘A’. Thus, the R3’ sequence in the mutant allele differs from R3 by a single base and has not been observed in the wild-type sequence or in the ‘Che’ family (Goldfarb et al., 1992). This abnormal repeat R3’ is equal to a repeat which was found in families having five, six, seven or nine extra octarepeats (labelled R3g by Goldfarb et al., 1991).

Discussion

We documented the symptoms, clinical course and neuropathological characteristics of an inherited disorder in patients having a 192 bp insertion within the coding region for tandem repeats of the PrP gene ORF. An association between a PrP gene insert mutation and a familial neurodegenerative disease, as described here, does in itself not prove a causal pathogenetic role for the genetic abnormality. However, all insert or point mutations described so far were clearly associated with the occurrence of hereditary encephalopathy, and high lod-scores provided statistical evidence for linkage in some of these pedigrees (Prusiner, 1993). Spontaneous neurodegeneration occurred in transgenic mice carrying a codon 102 point mutation in the PrP gene (Hsiao et al., 1990) and transmission of other forms of inherited prion disease with an insert mutation has been reported (Goldfarb et al., 1991). Brain tissue from a patient with an insert mutation of equal size to the one reported here transmitted a spongiform encephalopathy to an inoculated chimpanzee (Goldfarb et al., 1992). These observations, taken together with the absence of the 192 bp insert in the healthy first degree relative and in non-related controls (this study) or in groups of more than 500 healthy subjects reported in the literature (Goldfarb et al., 1992), strongly support a pathogenic role of the mutation described here.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient allele</th>
<th>Sex</th>
<th>Age at onset</th>
<th>Duration of illness</th>
<th>Mutant allele codon 129</th>
<th>Normal codon 129</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1*</td>
<td>M</td>
<td>50 years</td>
<td>5 months</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>I-2*</td>
<td>M</td>
<td>52 years</td>
<td>2 years</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>I-4*</td>
<td>M</td>
<td>54 years</td>
<td>5 years</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II-1</td>
<td>M</td>
<td>45 years</td>
<td>6 years</td>
<td>Valine</td>
<td>Valine</td>
</tr>
<tr>
<td>II-2</td>
<td>F</td>
<td>38 years</td>
<td>&gt;2 years (alive)</td>
<td>Valine</td>
<td>Valine</td>
</tr>
<tr>
<td>III-1</td>
<td>F</td>
<td>21 years</td>
<td>&gt;1 year (alive)</td>
<td>Valine</td>
<td>Methionine</td>
</tr>
</tbody>
</table>

*No DNA available.

Fig. 4 Comparison of the octarepeat region in the PrP gene in normal individuals and in affected subjects in the ‘A’ and ‘Che’ families, respectively. Diamonds indicate the differences. To indicate the different octarepeats, the abbreviations proposed by Owen et al. (1992) are used. The sequence of the variant octarepeat R3' is specified in the text, the irregular sequence of R2' in the ‘Che’ family is equal to R2a as reported by Goldfarb et al. (1991). Direct sequencing (in both directions) showed the normal allele to code for valine at position 129 in two cases and for methionine in the youngest patient (Table 1). In each case the insertion was in the allele coding for valine at position 129.
The medical history of this family illustrates a consequence of increasing knowledge about the molecular abnormalities underlying neurodegenerative diseases. In the past, Pick's disease, Huntington's disease and Parkinson-dementia had been considered as diagnoses in several family-members, and patient I-2 was diagnosed as a peculiar and unclassifiable form of presenile dementia (Stam et al., 1968). Suspicion of an inherited form of prion disease in the fifth affected patient (II-2) allowed for a correct retrospective diagnosis in the preceding patients and resulted in an early diagnosis in patient III-1. Even in a neurodegenerative disease for which no therapy is available, an early, correct diagnosis has an intrinsic value by preventing unnecessary diagnostic procedures or ineffective therapeutic attempts, and by enabling adequate counseling of patients and care-givers. Moreover, identification of a specific mutation allows for presymptomatic detection and prenatal exclusion of PrP gene defects in families with inherited prion disease (Collinge et al., 1991b).

Apparently, Stam et al. (1968) did not identify the familial nature of the disorder in their patient, otherwise they might have diagnosed GSS on the basis of the multicentric plaques. Interestingly, they gave an accurate description of the prion protein on the basis of their extensive analysis of the histochemical characteristics of the racemose deposits. More than 10 years before the introduction of the term 'prion', and its subsequent biochemical characterization as a protein with glycoinositol phospholipid anchors having sialic acid residues (Prusiner, 1993), Stam et al. (1968) concluded correctly that the cerebral deposits in this family contained sialomucoproteins, which we could now identify immunohistochemically as PrP.

On the basis of the slow progression in five of the six patients, combined with the multicentric plaques, the symptoms in the A-family resemble more the clinical descriptions of GSS than CJD, although the disease course in patient I-1 was consistent with CJD. Another clinical characteristic in the present family is also inconsistent with a straightforward classification as GSS. In their original report Gerstmann et al. (1936) described prominent cerebellar ataxia, whereas parkinsonian signs were not mentioned. Our patients presented with gait difficulties without distinctive limb ataxia or dysmetria on walking. In the past, complaints about walking and symptoms of dysequilibrium in prion diseases, may have been interpreted too easily as reflecting 'cerebellar' dysfunction. Intuitively this may have been attractive because of PrP deposits in the cerebellum, but this neuropathological characteristic may have biased accurate description of clinical signs in some cases. Abnormal deposits in the cerebellum do not necessarily implicate cerebellar dysfunction as is illustrated by the cerebellar β-amyloid deposits in Alzheimer's disease without clinical involvement of the cerebellum in this disease. We suggest that the gait problems in the current family reflect the combined involvement of the extrapyramidal, pyramidal and cerebellar systems. This interpretation is supported by the initial diagnosis of Parkinson-dementia made in patient I-2 and by the fact that several physicians who independently examined various members of this family never expressed suspicion of cerebellar disease. In other pedigrees with inherited prion disease progressive walking difficulties in combination with parkinsonian features or spastic paraparesis have also been described (Farlow et al., 1989; Kitamoto et al., 1993). Thus, the characterization of slowly progressive, inherited prion disease as a mainly 'cerebellar' syndrome may be incomplete at best in some families, despite the original description of Gerstmann et al. (1936).

The patients in the present family had many features in common, but remarkable variability was present in the age of onset and the course of the disease (Table 1). In patient I-4 the characteristics of the dementia were clearly identified by his family at least 5 years before his death, but this patient was never investigated for these complaints and he died because of cardiovascular co-morbidity. In his elder brother, the symptoms started at about the same age, but the course of his dementia was very rapid; he died within 6 months after the onset of his symptoms (Table 1). Susceptibility to prion disease has been linked to homozygosity for the codon 129 polymorphism and the hypothesis is that increased homology of protein sequences may lower the threshold for PrP oligomer formation as a crucial step in the pathogenesis. Increased frequencies of homozygotes among patients suffering from iatrogenic or sporadic prion disease and earlier ages of onset in homozygotes with inherited prion disease support this hypothesis (Collinge et al., 1991a; Palmer et al., 1991; Poulter et al., 1992; Brown et al., 1994). The number of patients in the present family is too small to draw any conclusion with respect to this hypothesis. However, the methionine/valine heterozygosity in patient III-1, one of the youngest patients with inherited prion disease ever reported, suggests that common polymorphisms outside the PrP gene or non-genetic factors may modify the phenotypic expression of the 192 bp insert mutation in this family.

The variability in the A-family is in accordance with the distinctive variations in age-at-onset, disease course, clinical and neuropathological phenotypes that have been described in other pedigrees (Collinge et al., 1992; Prusiner, 1993). Given this degree of variation within and between respective pedigrees it is not useful to redefine GSS or familial CJD to comprise all these different subtypes. Inherited prion disease should rather be classified according to the specific PrP gene mutation accompanied by accurate descriptions of the clinical and neuropathological findings (Collinge et al., 1992).

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References


