Spinocerebellar ataxia type 2 with glial cell cytoplasmic inclusions

S Probst-Cousin, T Acker, J T Epplen, M Bergmann, K H Plate, B Neundörfer, D Heuss

Gliarial cell cytoplasmic inclusions were identified in a case of spinocerebellar ataxia type 2. These have not been reported before. The inclusions were found in low frequency in the dentate nucleus, cerebellar white matter, pontine transverse fibres, and the inferior olivary nucleus. They were of variable size and shape and expressed ubiquitin, thus resembling glial cytoplasmic inclusions in multiple system atrophy. However, their immunohistochemical profile was different as they did not show immunoreactivity for either tau protein or α-synuclein. There was no evidence of expanded polyglutamine tracts in these inclusions, which also failed to label with silver stains. As in many other neurodegenerative diseases, in spinocerebellar ataxia type 2 there may be pathogenic contributions of gliial cells other than the common astrogliotic response to neuronal damage.

Spinocerebellar ataxia type 2 (SCA2) represents a genetically defined neurodegenerative disorder characterised by autosomal dominant inheritance and progressive cerebellar ataxia, combined with slow saccades and sensorimotor neuropathy.1,2 The neuropathology comprises olivo-ponto-cerebellar atrophy (OPCA) with axonopathy of posterior columns, spinocerebellar tracts, and peripheral nerves.3 Aetiologically, SCA2 is caused by a CAG trinucleotide repeat expansion in the coding region of the SCA2 gene on chromosome 12, leading to a polyglutamine tract in the encoded ataxin-2 protein, the function of which is still unclear.4–5 Recently, ubiquitinated neuronal intranuclear inclusions (NIs) of unclear pathogenic significance have been shown in some of the affected neuronal populations.6

Gliarial cell cytoplasmic inclusions have been described in different neurodegenerative diseases, including a case of SCA type 1.7,8 These gliarial cell cytoplasmic inclusions differ according to distribution, immunological composition, and quantity. The only disorder described so far in which gliarial cell cytoplasmic inclusions represent the hallmark lesion is multiple system atrophy (MSA), a sporadic disease with a mixture of autonomic dysfunction, parkinsonism, cerebellar ataxia, and corticospinal symptoms.9–12

Here we describe ubiquitin positive gliarial cell cytoplasmic inclusions which we found haphazardly in the morphological examination of a case of SCA2, and we show that these inclusions are different from gliarial cell cytoplasmic inclusions in MSA.

CASE REPORT

At the age of 18 years, this male patient noticed impaired handwriting because of incoordination of his hands. Afterwards, he very slowly developed dysarthria and progressive walking and balance difficulties. At the age of 35, physical examination showed a moderately severe dysarthria, slow saccades, and severe gait, trunk, and limb ataxia. There were also generalised fasciculations, distal loss of vibration sense, and generally absent deep tendon reflexes. Cranial tomography showed cerebellar and brain stem atrophy. At the age of 40, he was using a walker, and more severe limb ataxia was evident on finger–nose testing and rapidly alternating arm movements. He now also showed hypermetric saccades on lateral gaze, while his dysarthria became more mixed, with ataxic, spastic, and dystonic components. With exception of his lost vibration sense, sensory testing revealed no abnormalities. On the last examination at the age of 44, the patient was wheelchair-bound and he now also complained of frequency of urination, difficulty in emptying his bladder, and difficulty in swallowing. In the proximal muscles there was mild paresis and muscular atrophy. Because of his severe dysarthria, his speech was hardly intelligible; there were, however, no signs of cognitive impairment. Two months later he died of aspiration pneumonia.

A family history revealed a progressive ataxia affecting three generations, and genetic testing on our patient confirmed the diagnosis of SCA2 with 42 CAG repeats in the disease allele of the SCA2-gene. Unfortunately, genetic testing could not be carried out on other family members.

METHODS

Sections of brain and spinal cord were stained with haematoxylin and eosin (H&E), cresyl violet, Luxol-fast-blue, Bodian and Gallyas silver impregnations, and periodic-acid-Schiff (PAS). Paraffin embedded and semi-thin sections of peripheral nerves (peroneal and sural) and muscles (gastrocnemius and quadriceps) were stained with toluidine blue, H&E, and Gomori’s trichrome.

Immunohistochemistry was done using the streptavidin-biotin complex/anti-alkaline phosphatase method, with fast red as chromogen. The primary antibodies applied were as follows: monoclonal mouse anti-human α-synuclein (Transduction Laboratories, 1:250; Lexington, Kentucky, USA), monoclonal mouse-anti-human CD68 (Dako, 1:100; Glostrup, Denmark), monoclonal mouse anti-human tau protein AT8 (Innogenetics, 1:200; Ghent, Belgium), polyclonal rabbit anti-human ubiquitin (Dako, 1:100), monoclonal mouse anti-human gliarial fibrillary acid protein (GFAP) (Dako, 1:25), monoclonal mouse anti-human Leu7/CD57 (Becton Dickinson, undiluted; Oxford, UK), and monoclonal mouse anti-polyglutamine tracts 1C2 (Chemicon, 1:100; Temecula, California, USA). For immunofluorescence staining, primary antibodies were coupled with Cy2 (carboxyfluorescein, 1: 50; Dianova, Hamburg, Germany), excited by a light beam of 490 nm, green signal at emission peak 519 nm, or...
Cy3 (indocarbocyanine, 1: 50, Dianova), excited by a light beam of 550 nm, red signal at emission peak 570 nm; sections were observed under a reflected fluorescence microscope with mercury arc lamp (BX 51, Olympus, Hamburg, Germany). Double stained images were recorded and merged using image analysis software (analysis3.0, SIS, Münster, Germany). Two cases of MSA served as controls.

RESULTS
Macroscopically, there was marked atrophy of both the pons and the cerebellum, whereas histologically, moderate neuronal loss and gliosis in the oculomotor nucleus and the substantia nigra was found. Most severely affected, however, was the basal pontine nucleus. We observed severe degeneration of transverse fibres and pontocerebellar fibre tracts, with distinct pallor of the white matter in Luxol-fast-blue myelin staining. In the medulla, marked degeneration of the inferior olives was seen. Both the cerebellar vermis and the cerebellar hemispheres showed moderate to marked loss of Purkinje cells with accompanying gliosis. The cerebellar white matter showed pallor in myelin staining, whereas in the dentate nucleus, only mild neuronal loss and gliosis were observed. In the spinal cord there was mild neuronal loss and gliosis of the anterior horn, with marked degeneration of the dorsal columns. The peripheral nerves showed severe reduction of large myelinated fibres, whereas in the muscles a neurogenic syndrome with angular atrophic fibres was observed.

GFAP staining highlighted astrogliosis in areas of degeneration, and CD68 positive macrophages were present in cerebellar and pontine white matter areas, commonly in the form of rod cells. Numerous ubiquitin positive non-specific dots and corpora amylacea were observed throughout the central nervous system. We found singular ubiquitin positive inclusions in the nuclei pontis and dentate nucleus in up to 3% of remaining neurons. In some Purkinje cell nuclei, diffuse positive staining for ubiquitin was seen. There was neuronal immunoreactivity for polyglutamine tracts as cytoplasmic granular deposits in Purkinje cells and singular neurones of the dentate nucleus. With decreasing frequency, there were ubiquitin reactive glial cell cytoplasmic inclusions in the dentate nucleus (up to four per high power field), in cerebellar white matter (up to three per high power field), in pontine transverse fibres (up to two per high power field), and in the inferior olivary nucleus (up to one per high power field). These inclusions were of variable size; their shape varying from triangular to sickle, oval, or flame shaped (fig 1A), thus resembling glial cell cytoplasmic inclusions in MSA. However, unlike the controls with MSA, they did not show immunoreactivity for either tau protein or α-synuclein.

There was co-localisation of glial cell cytoplasmic inclusions with Leu7/CD57 but not with GFAP or CD68, suggesting they were harboured in oligodendrocytes (fig 1B). The glial cell cytoplasmic inclusions were lacking immunoreactivity with the IC2 antibody and did not appear with any other stains such as H&E, PAS, Luxol-fast-blue, cresyl violet, or the silver impregnations.

DISCUSSION
The presence of ubiquitinated cellular inclusions is a largely non-specific finding, merely indicating activation of cellular protein degradation. The significance of these inclusions is defined by the identity of the labelled protein. Most of the inclusion bodies in neurodegenerative diseases either contain tau protein or α-synuclein, possibly because of their tendency to self associate and form pathological fibrils. As a key regulator of the cytoskeleton, tau protein is tightly associated with the microtubules whereas α-synuclein is associated with synaptic termini and its primary function remains to be determined. The discovery of glial cell cytoplasmic inclusions in MSA has evoked considerable interest in glial pathology in various neurodegenerative diseases. However, these glial inclusions are not uniform but differ according to cell type, distribution, and composition. Thus the descriptive term “glial cell cytoplasmic inclusions” was somewhat misleading as it covered very different lesions. Because of its nosological and pathognomonic impact, it currently refers to self associate and form pathological fibrils. As a key regulator of the cytoskeleton, tau protein is tightly associated with the microtubules whereas α-synuclein is associated with synaptic termini and its primary function remains to be determined. The discovery of glial cell cytoplasmic inclusions in MSA has evoked considerable interest in glial pathology in various neurodegenerative diseases. However, these glial inclusions are not uniform but differ according to cell type, distribution, and composition. Thus the descriptive term “glial cell cytoplasmic inclusions” was somewhat misleading as it covered very different lesions. Because of its nosological and pathognomonic impact, it currently refers to self associate and form pathological fibrils. As a key regulator of the cytoskeleton, tau protein is tightly associated with the microtubules whereas α-synuclein is associated with synaptic termini and its primary function remains to be determined.
Though the morphological appearance is similar, the lack of immunoreactivity of glial cell cytoplasmic inclusions for both α-synuclein and tau protein in our case discriminates them clearly from glial cell cytoplasmic inclusions in MSA, as α-synuclein immunoreactivity, in particular, is relatively specific for MSA and Lewy body diseases.29 Glial cell cytoplasmic inclusions in other disorders—such as the filamentous oligodendroglial inclusions, called coiled bodies, of Alzheimer’s disease, Pick’s disease, dementia with progressive supranuclear palsy—usually lack α-synuclein immunoreactivity.26–29 In contrast to the glial cell cytoplasmic inclusions described in this paper, coiled bodies share immunoreactivity for tau protein and show argyrophilia in Gallyas silver impregnation, though they usually fail to give a positive reaction to anti-ubiquitin antibodies.31–33 Thus the immunohistochemical profile of the inclusions of our case is different from glial cell cytoplasmic inclusions and other common glial cell cytoplasmic inclusions.

It is not clear why glial cell cytoplasmic inclusions in SCA2 have not been described before. Possibly they might just have been overlooked as they are so few in number. Another reason could be that our SCA2 case had a special phenotype associated with the presence of glial inclusions. In this regard it could be speculated either that the SCA2 gene contains polymorphisms, or that additional modifying genes influence the phenotype. So far, we have only been able to investigate this single individual morphologically. Until our findings are reproduced, glial cell cytoplasmic inclusions in SCA2 will remain a non-specific finding of uncertain significance. The future will show whether SCA2 needs to be added to the growing list of diseases associated with glial pathology.

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