

SHORT REPORT

Paradoxical absence of nuclear inclusion in cerebellar Purkinje cells of hereditary ataxias linked to CAG expansion

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Degeneration of cerebellar cortex is one of the principal features of hereditary ataxias linked to expansion of CAG repeat. In an attempt to clarify possible correlation between neuronal depletion and neuronal intranuclear inclusions, both triggered by the pathological expansion of CAG repeat, cerebellar sections from SCA1, SCA2, SCA3, and DRPLA cases were immunostained with anti-ubiquitin or anti-expanded polyglutamine antibody (1C2) and were screened for the presence of neuronal intranuclear inclusions. Although the degree of cerebellar degeneration varied greatly, cerebellar Purkinje cells were uniformly characterised by the absence of neuronal intranuclear inclusion. Complete absence of neuronal intranuclear inclusion in Purkinje cells is apparently paradoxical and hardly explained if neuronal intranuclear inclusion formation is positively correlated to a mechanism accelerating neuronal death. It may, otherwise, suggest an intrinsic link between neuronal intranuclear inclusion formation and neurodegeneration in opposite directions in human Purkinje cells, more or less affected in these CAG repeat disorders.

At least 10 inherited neurodegenerative diseases, including spinocerebellar ataxias (SCA-1, -2, -3, -6, -7, -12, and -17), Huntington's disease, dentatorubral pallidolysian atrophy (DRPLA), and spinobulbar muscular atrophy (SBMA) are associated with pathologically expanded CAG repeats in the coding region of the disease gene.^{1–3} Because most of them are characterised by the presence of neuronal intranuclear inclusions (NIs), which contain the gene product derived from the expanded allele of the responsible gene, formation of NIs induced by expansion of CAG repeat may play a pivotal part in the pathogenesis in common. Although it has been established that an artificial expression of expanded CAG repeat in cultured cells or in transgenic animals induces formation of NIs, it remains to be clarified how NIs are involved in the pathogenesis and neurodegeneration. Accumulating evidence suggests, however, that NI formation is not necessarily correlated with the severity of neuronal degeneration. Discrepancy between neuronal degeneration and formation of NIs has been observed at necropsy in brains with Huntington's disease⁴ or with neuronal intranuclear hyaline inclusion disease (NIHID),⁵ in mouse transgenic lines carrying mutated ataxin-1,⁶ and in some cellular models of polyglutamine diseases.⁷ Because it is essential to know how NIs are involved in these degenerative processes, we systematically screened cerebellar cortex in a series of hereditary ataxias in search of ubiquitin or polyglutamine-immunopositive NIs, with special attention to cerebellar Purkinje (Pj) cells, more or less affected in these disorders. A possible role of NI in neurodegeneration and its relation to neuronal death will be discussed.

METHODS

Sections were obtained at postmortem examination from the brains of three SCA1, three SCA2, six SCA3, and five DRPLA patients from unrelated families and three control subjects without neurological and psychiatric disorders. Diagnoses were based on clinical and pathological findings and were confirmed by genomic DNA analysis, as reported previously.⁸ Each cerebellum was fixed in 10% formalin for one to three weeks, then cut into 1 cm thick sagittal slices for histopathological and immunohistochemical examination. They were embedded in paraffin wax and four serial slices of 5 µm thick sections were immunostained with an anti-ubiquitin antibody (1:1000, rabbit polyclonal IgG, DAKO, Glostrup, Denmark) or with an anti-polyglutamine antibody, 1C2 (1:9000, mouse monoclonal IgG, Chemicon, Temecula, CA) by using the avidin-biotin-peroxidase complex method (ABC Elite, Vector Burlingame, CA).

RESULTS

Pathological features of these cases were previously summarised.⁸ In SCA1 brains, mild to moderate degeneration of the pontocerebellipetal system and of the Pj cells of the cerebellar cortex was found and neuronal loss with grumose degeneration was consistent in the dentate nucleus. SCA2 brains were characterised by severe gliosis involving all three layers of the cerebellar cortex, and loss of Pj cells was uniformly severe in all cases. The dentate nucleus was spared, while some fibrillary gliosis may be seen there in SCA2 brains. In SCA3 and DRPLA brains, the cerebellar cortex was mostly spared, but a slight loss of Pj cells was recognisable. Grumose degeneration was remarkable in the dentate nucleus.

The anti-ubiquitin antibody, at most, lightly immunostained the nucleoplasm and to a lesser extent the cytoplasm of Pj cells in all cases (fig 1 A–D). Ubiquitin-immunopositive NIs were occasionally found in the dentate nucleus of SCA1, SCA3 (fig 1 E) and DRPLA (fig 1 F) cases and rarely in Golgi cells in SCA1 (fig 1 G), SCA2, and DRPLA cases. These NIs were also immunostained with the antibody against expanded polyglutamine (1C2), as shown in fig 1 H (Golgi cell in SCA1). These two antibodies yielded no significant immunolabelling in control cases. Concerning Pj cells, we counted the number of Pj cells in each four serial slices of cerebellum in every case examined in this study (table 1). Approximate mean number of remaining Pj cells per section traversing the dentate nucleus, each including whole sagittal plane of a hemisphere, was about 420 in SCA1, 150 in SCA2, 2320 in SCA3, and 3130 in DRPLA. Neither anti-ubiquitin antibody nor 1C2 identified

Abbreviations: NI, neuronal intranuclear inclusion; Pj, Purkinje; DRPLA, dentatorubral pallidolysian atrophy; SBMA, spinobulbar muscular atrophy

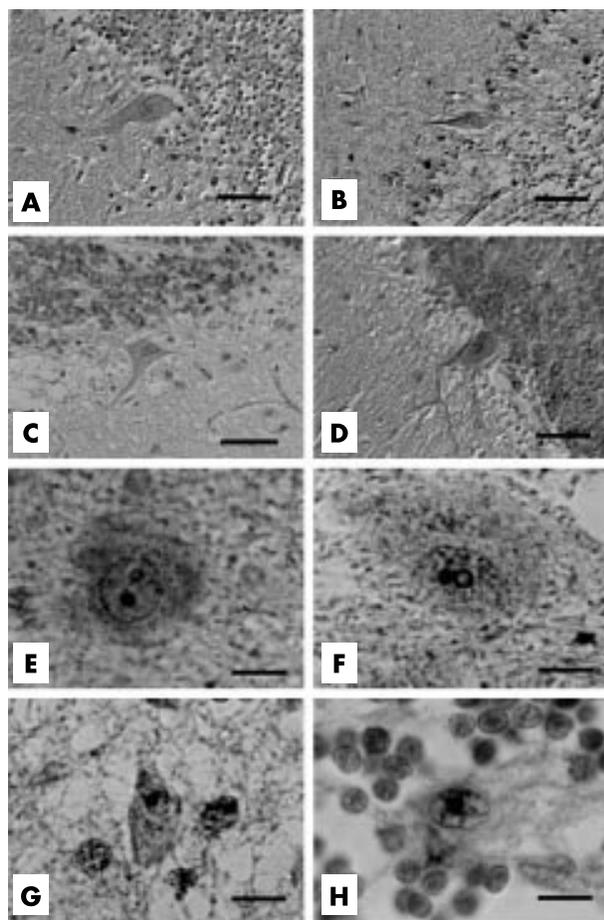


Figure 1 Ubiquitin-like immunoreactivity (A–G) in cerebellar cortex of SCA1 (A), SCA2 (B), SCA3 (C), and DRPLA (D). Neither aggregates nor inclusions were present in nuclei of Purkinje cells in any of cases examined (bars=100 μ m; A–D: the same magnification). NIs were occasionally found in dentate neurons in SCA3 (E), DRPLA (F) and rarely found in Golgi cells, as seen in SCA1 (G). These NIs were also immunostained with the antibody against expanded polyglutamine (1C2), as seen in Golgi cells in SCA1 (H) (bars=10 μ m; E–H: the same magnification).

Table 1 Number of Purkinje cells in hereditary ataxias

	Cases (n)	Range	Average
SCA1	3	310–600	420
SCA2	3	140–160	150
SCA3	5	1290–3720	2320
DRPLA	4	1600–4560	3130

NIs in any of the remaining Pj cells irrespective of the disease examined.

DISCUSSION

Cerebellar ataxia is one of the important clinical features of SCA1, SCA2, SCA3, and DRPLA, all exhibit cerebellar degeneration of different degree. Cerebellar cortex is severely affected in SCA2 and SCA1, while relative preservation of Pj cells is a morphological feature of SCA3 and DRPLA.⁸ Different reports, including ours, confirmed the absence of NIs in Pj cells of SCA2^{9–10} and DRPLA¹¹ brains. In this study, we tried to expand this observation by comparing a variety of cases with

different degree of cerebellar degeneration associated in common with pathological expansion of CAG repeat. Although several animal models were successfully generated to reproduce NI formation and neurodegeneration in the cerebellar cortex,¹² our *in vivo* observation based on a series of human necropsied cases, which includes a variety of hereditary ataxias, failed to identify NIs in any of Pj cells. Because some NIs were identified in dentate neurones or Golgi cells on the same ubiquitin immunolabelled preparations, the absence of NIs in Pj cells was not attributable to a technical failure. Furthermore, we confirmed the absence of NIs in Pj cells by immunohistochemical examination with 1C2, which labels the pathological moiety of abnormal gene product found in NIs.

At least two conflicting interpretations are possible to explain this consistent absence of NIs in Pj cells. Pj cells containing NIs may be more susceptible to neuronal degeneration and degenerate rapidly, leaving only those not containing NIs. If Pj cells develop NIs before degeneration, however, some NIs should have appeared at least once during the degenerative process. The consistent absence of NIs in Pj cells with various severity of degeneration linked to different genetic abnormalities, therefore, is against this hypothesis. On the other hand, accumulating evidence claimed that neurodegeneration and NI formation were not necessarily correlated¹³ or even inversely correlated⁷ with each other, consistent with a possible protective role of NIs against neuronal degeneration. Indeed, inactivation of ubiquitin-proteasome cascade was reported to accelerate cell death and attenuate formation of NIs induced in cultured cell lines transfected with expanded CAG tagged to ataxin-1.⁶ Inversely, inactivation of caspases, which counteracted cell death, was reported to promote NI formation.⁷ This inverse relation between NI formation and cell death has been verified also in necropsied brains with Huntington's disease⁴ or with NIHID,⁵ which exhibit similar ubiquitin-immunopositive inclusions. It supposes, therefore, an intrinsic cellular mechanism that links cell death and NI formation in opposite directions, although both of them are triggered by the expansion of CAG/polyglutamine repeat. As shown in this study, Pj cells without NIs could, therefore, be more vulnerable if NI formation is linked to a mechanism counteracting a cascade that accelerates neuronal death.

It remains to be proved, however, why Pj cells hardly develop NIs in human brains. Because targeted expression of a mutated gene (for example, ataxin-1) to Pj cells induces NIs in murine brains,¹⁴ mechanisms involved after the expression of the mutated gene may be different in human Pj cells. Otherwise, smaller repeat size in the cerebellum, a common phenomenon in several CAG/polyglutamine repeat expansion disorders known as "somatic mosaicism", may locally influence the phenotype by modifying cell death and NI formation. Recent reports demonstrated, however, that transcribed repeat size in Pj cells of DRPLA brains was longer than that in cerebellar granule cells,^{15–16} suggesting that the absence of NI in Pj cells is not related to shorter repeat size. Anyway, this study demonstrated that Pj cells, more or less affected in these hereditary ataxias, were uniformly characterised by the paradoxical absence of NIs in necropsied human brains with different CAG/polyglutamine expansion disorders with ataxia. If NI formation is linked to a protective mechanism against cell death, its complete absence renders Pj cells more susceptible to cell death. Further studies are required to clarify cellular and molecular mechanism to explain these particular characteristics of human Pj cells seen commonly in these hereditary ataxias. It may provide clues to understanding a common mechanistic link, which involves cell death and NI formation both triggered by CAG repeat expansion.

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Addendum

A recent study on knocked-in mice with SCA1 gene flanking expanded CAG repeat demonstrated an extreme scarcity of intranuclear inclusions in degenerated cerebellar Purkinje cells. This corroborated our findings on human necropsied brains (*Neuron* 2002;**34**:905–19).

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