# Expression of Ataxin-2 in Brains from Normal Individuals and Patients with Alzheimer's Disease and Spinocerebellar Ataxia 2

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Spinocerebellar ataxia type 2 (SCA2) is caused by expansion of a CAG trinucleotide repeat located in the coding region of the human SCA2 gene. The SCA2 gene product, ataxin-2, is a basic protein with two domains (Sm1 and Sm2) implicated in RNA splicing and protein interaction. However, the wild-type function of ataxin-2 is yet to be determined. To help clarify the function of ataxin-2, we produced antibodies to three antigenic peptides of ataxin-2 and analyzed the expression pattern of ataxin-2 in normal and SCA2 adult brains and cerebellum at different developmental stages. These studies revealed that (1) both wild-type and mutant forms of ataxin-2 were synthesized; (2) the wild-type ataxin-2 was localized in the cytoplasm in specific neuronal groups with strong labeling of Purkinje cells; (3) the level of ataxin-2 increased with age in Purkinje cells of normal individuals; and (4) ataxin-2-like immunoreactivity in SCA2 brain tissues was more intense than in normal brain tissues, and intranuclear ubiquitinated inclusions were not seen in SCA2 brain tissues.

Huynh DP, Del Bigio MR, Ho DH, Pulst S-M. Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. Ann Neurol 1999;45:232–241

Hereditary ataxias are neurodegenerative diseases leading to abnormalities of balance due to dysfunction of the cerebellum and cerebellar pathways. These symptoms result from degeneration in the cerebellum, basal ganglia, oculomotor structures, and peripheral nerves. The spinocerebellar ataxias (SCAs) are an autosomal dominant, phenotypically heterogeneous group of disorders that include the spinocerebellar ataxia type 1 (SCA1),<sup>1</sup> SCA2,<sup>2-4</sup> Machado-Joseph disease (SCA3 or MJD),<sup>5</sup> SCA4,<sup>6</sup> SCA5,<sup>7</sup> SCA6,<sup>8</sup> and SCA7.<sup>9</sup> The mutational mechanism of these disorders is characterized by an expansion of CAG trinucleotide repeats except for the mutations causing SCA4 and SCA5, which have not yet been identified. Other disorders resulting from the expansion of a translated CAG trinucleotide repeat include Huntington's disease (HD),<sup>10</sup> spinal bulbar muscular atrophy,<sup>11</sup> and dentatorubral pallido-luysian atrophy (DRPLA).<sup>12</sup>

The gene causing SCA2 was recently identified.<sup>2–4</sup> The CAG trinucleotide repeats is located in the 5' coding region of the gene. In contrast to other CAG diseases, the repeat is not highly polymorphic in normal individuals. The SCA2 gene product, designated ataxin-2, is composed of 1,312 amino acid residues with a calculated molecular mass of 140 kd, and is a highly basic protein with an isoelectric point (pI) of 10 due to the high number of glutamine residues. The most common form of wild-type ataxin-2 contains 22 glutamine repeats flanked by a region of proline-rich and serine-rich domains. With expansion of the polyglutamine tract to 35 or more, individuals will develop the SCA2 phenotype. Except for the CAG trinucleotide expansion, the SCA2 gene has no similarity with other proteins containing disease causing polyglutamine tracts or other proteins of known func-tion.<sup>1,2,5,8-12</sup> The protein contains Sm1 and Sm2 motifs<sup>13</sup> and a high number of proline (14%), serine (13.4%), and glutamine (7.7%) residues. The Sm1/ Sm2 motifs are found in proteins that are involved in RNA splicing,<sup>14</sup> suggesting that wild-type ataxin-2 might function as a component of the RNA splicing complex. Furthermore, the mouse SCA2 gene has only

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Received Jun 22, 1998, and in revised form Sep 1. Accepted for publication Sep 4, 1998.

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one CAG codon at the site of the human CAG repeat,<sup>15</sup> suggesting that the normal function of ataxin-2 resides in the regions flanking the CAG repeat.

Other than the predicted biochemical properties based on its primary amino acid sequence, the native function of ataxin-2 is still unknown. At the RNA level, the SCA2 gene was found to have at least three isoforms in the mouse.<sup>15</sup> Type I contains the full cDNA sequence, whereas type II lacks exon 10 and type III lacks both exons 10 and 11. It is interesting that only the type I and II isoforms exist in human brain, spinal cord, cerebellum, T cells, and macrophages.<sup>16</sup> By northern blot analysis of human mRNA, the SCA2 gene was found to be expressed in both neuronal and nonneuronal tissues except the lung and kidney.<sup>2-4</sup> In the nervous system, SCA2 mRNA was found in all regions examined by use of northern blot analyses, which included the amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus, thalamus, cerebellum, medulla, cerebellar cortex, spinal cord, putamen, occipital pole, frontal lobe, and temporal lobe.<sup>2-4</sup> However, these studies did not provide specific cellular distribution of the SCA2 gene product, and it is not known whether ataxin-2 is a nuclear or cytoplasmic protein. We have generated several antibodies to peptides predicted from the SCA2 cDNA sequence and herein describe their characterization as well as the distribution of ataxin-2 in the central nervous system.

### Materials and Methods

### Antibody Production

We raised rabbit antibodies against C-terminal and N-terminal peptides (Quality Control Biochemicals, Hopkinton, MA). SCA2A (amino acids 359–371; numbering according to the human ataxin-2 sequence<sup>3</sup>), AKVNGEH-KEKDLE (in exon 6); SCA2B (amino acids 904–921), A\*A\*EQVRKSTLNPNAKEFN (in exon 16); SCA2D (amino acids 867–879), I\*LSNT\*EHKRGPEV (in exon 15); an asterisk after an amino acid residue signifies differences between the mouse and human amino acid sequence. Each peptide was conjugated to keyhole limpet hemocyanin. Two keyhole limpet hemocyanin–conjugated peptides were injected into each rabbit in duplicate. Collected antisera were affinity purified by using Sepharose-conjugated peptide columns.

#### Protein Extraction and Western Blots

Frozen brain tissues, including frontal cerebrum, frontal white matter, cerebellar lateral lobe, and medulla, were obtained from a 41-year-old SCA2 patient, 70- and 79-year-old Alzheimer's disease (AD) patients, and neurologically normal 32-, 59-, and 79-year-old patients. Frozen tissue samples were obtained from necropsy brains within 24 hours of death and stored at -70°C. Proteins from frozen brain tissues were extracted as follows. Frozen tissues were resuspended in triple detergent buffer (100 mM Tris, pH 7.4, 1% Nonidet P-40, 0.5% sodium dodecyl sulfate, 0.5% deoxycholic acid, 1 mM Pefabloc, 1 mM EGTA, 1 µg/ml pepstatin A, 2 µg/ml aprotinin, and 50 µg/ml leupeptin) and homogenized by using a Polytron homogenizer. The protein extracts were first centrifuged at 1,000 g (3,100 rpm in a JA17 rotor) for 5 minutes. The supernatant was centrifuged at 105,000 g (54,000 rpm in a TLN100 rotor) for 1 hour. The supernatant (S3) was aliquoted and stored at -80°C. Protein concentrations were determined by the Bio-Rad DC-Bradford Protein Assay Kit (Biorad, Hercules, CA). Before loading onto polyacrylamide gels, proteins were concentrated by using a Microcon 10 column (Millipore Corp, Bedford, MA); 100 µg of protein was loaded per lane in a Bio-Rad premade 4 to 20% gradient sodium dodecyl sulfate-polyacrylamide minigel and resolved at 100 V for 1 to 2 hours. Proteins were transferred to an Amersham nitrocellulose filter (Amersham Life Science, Inc, Arlington Heights, IL). The filter was rinsed briefly with Tris-buffered saline (150 mM NaCl, 50 mM Tris, pH 8.0), blocked with 5% nonfat dried milk, and then incubated with the desired dilution of tested antibodies overnight at 4°C. The primary antibody was detected by using the Amersham enhanced chemiluminescence kit.

#### Immunohistochemistry

Human brain tissues obtained at necropsy were fixed with 10% formalin within 24 hours of death and selected samples were embedded in paraffin. All specimens were from the Health Sciences Centre at Manitoba, Winnipeg, Canada. Necropsy permission forms include a clause allowing use of tissue for research purposes. Samples of cerebellum were obtained from a 22-week gestation fetus, a full-term infant, and neurologically normal patients, ages 2, 20, 47, 54, 59, and 99 years. Multiple brain sites were sampled from a 40-yearold neurologically normal male. Cerebellum was obtained from patients with AD, ages 57, 77, and 84 years. Cerebellum and medulla samples were obtained from 3 related female patients with the following ages at death: 41 (daughter), 49 (mother), and 58 (sister of the mother). Sections of 6-µm thickness were cut and mounted onto Superplus microscopic slides (Fisher Scientific, Springfield, NJ). The sections were rehydrated by rinsing twice at 5-minute interval in xylene, 100% ethanol, 95% ethanol, and 70% ethanol. After deparaffinization, sections were treated with a protease cocktail and blocked with avidin/biotin and 3% normal goat serum. Sections were then incubated with 10 to 20 µg/ml of affinity-purified ataxin-2 antibodies overnight at 4°C. Primary antibodies were detected by using the Vector rabbit avidin/biotin-peroxidase complex Elite kit (Vector, CA), enhanced by diaminobenzidine enhancer, and visualized with diaminobenzidine (Fisher Scientific). Sections were counterstained with aqueous hematoxylin (Xymed, South San Francisco, CA). Controls consisted of antibodies preabsorbed with 100 mM of the respective peptide and preimmune sera at comparable concentrations (1:500). All slides for direct comparison were processed in a single batch to minimize variability.

## Results

## Biochemical Analysis of Ataxin-2

For biochemical and immunohistochemical analyses of ataxin-2, we generated rabbit polyclonal antisera to different ataxin-2 peptides.<sup>3</sup> Peptide SCA2A is located at amino acids 359-371, peptide SCA2B at amino acids 904-921, and peptide SCA2D at amino acids 867-879. Enzyme-linked immunosorbent assay tests using the bovine serum albumin-conjugated peptides showed that immune sera had greater than 10<sup>4</sup> higher titer than preimmune sera (data not included). To further test whether these antibodies detected proteins in cell extracts, we generated fusion constructs containing a partial coding region of the SCA2 gene (molecular mass, 124 kd) and green fluorescent protein (GFP; 27 kd) and tested these ataxin-2 immune sera by immunoblotting. As shown previously,15 ataxin-2 antisera detected a 150-kd band representing the GFP-ataxin-2 fusion protein in COS1 cells transfected with the pEGFP-SCA22 construct. The 150-kd band was not present in protein extract from COS1 cells transfected with the GFP vector alone. Both the GFP-ataxin-2 fusion protein and the GFP were detected by the antibody to GFP. To further increase specificity, these immune sera were affinity purified by using Sepharosepeptide columns.

To confirm that these antibodies detected native ataxin-2, we performed western blot analyses of protein extracts from the cerebellum, cerebrum, medulla, and white matter of patients with SCA2 and AD, and control individuals of comparable age, using affinitypurified antibodies SCA2A and SCA2B (Fig 1). Both antibodies detected a 145-kd protein in the cerebellum, cerebrum, and medulla. The 145-kd protein was faintly detected in the white matter. In addition, antibody SCA2B consistently detected a 70-kd protein in all tissues. In some samples, both antibodies also detected a 41- to 50-kd protein. It is interesting that the SCA2D antibody also detected strongly the 70-kd band but very weakly the 145-kd species. The weak binding of the SCA2D antibody to the full-length protein may be due to conformational masking of the native epitope. It should be noted that SCA2D antibody detected the full-length ataxin-2 when fused to the GFP expressed in COS1 cells (data not shown). Both SCA2A and SCA2B antibodies detected an additional 180-kd protein in the cerebrum (see Fig 1B, lane 1) and medulla (see Fig 1C, lane 1) from a patient with SCA2, representing ataxin-2 encoded by the mutant allele with 41 CAG repeats. The expanded ataxin-2 was not detected in the cerebellum of the same patient (see Fig 1A, lane 1). The protein extracts from control (see Fig 1, lane 4) or AD brains (see Fig 1, lanes 2-3) only showed ataxin-2 of the expected molecular mass for normal alleles. Furthermore, a weak 125-kd band was occasionally detected by both SCA2A and SCA2B antibodies (see Fig 1A, lanes 1, 2, and 4, and Fig 1B and D, lanes 3). The faint band larger than 145 kd detected by the SCA2B antibody in the medulla of AD Patient 3 (see Fig 1C, lane 3) was likely an artifact due to aberrant protein mobility in that lane, as that band was not observed in other areas of the same patient (see Fig 1A, B, and D, lanes 3). Antibodies preabsorbed with their respective peptides (see Fig 1A and B, bottom panels) and preimmune sera (data not shown) did not detect these proteins indicating the specificity of the antibodies.

To further study ataxin-2 processing, we isolated proteins from lymphocytes obtained from control and individuals with SCA2. These samples were not from the same patients whose brains were studied. Unexpectedly, the molecular mass of lymphocyte ataxin-2 was higher than central nervous system ataxin-2. Both SCA2A and SCA2B antibodies detected an intense band at 185 kd in all individuals (see Fig 1E). The SCA2B antibody detected two additional bands at 135 and 80 kd. Fainter bands of higher molecular mass (see Fig 1E, arrows) were detected in lymphocytes from SCA2 individuals with 39 (195 and 210 kd), 41 (195 kd), and 51 (very faint 200 kd) CAG repeats, but not from an individual with 22 CAG repeats.

## Regional Distribution of Ataxin-2 in a Neurologically Normal Human Brain

To determine the normal cellular distribution of ataxin-2, we labeled frontal cortex, midbrain, hippocampus, basal ganglia, medulla, and cerebellum from a 40-year-old neurologically normal male, using 10 g/ml IgG affinity-purified SCA2A and SCA2B antibodies (Fig 2). Both antibodies showed identical labeling patterns. In the frontal, temporal, and insular cortex, glial cells in the superficial molecular layer (layer I) were weakly labeled. In layer II, scattered granule neurons were weakly labeled. In layers III, V, and VI, the apical cytoplasm of pyramidal neurons was moderately labeled in a granular pattern with minimal extension into apical dendrites (see Fig 2A). There also tended to be a faint granular label throughout the neuropil. There was no detectable immunoreactivity in the white matter. In the hippocampus, pyramidal neurons (see Fig 2B) were more intensely labeled than the granule neurons of the dentate gyrus (see Fig 1C). In the basal ganglia, large neurons in the putamen labeled weakly, whereas globus pallidus neurons (see Fig 2D) and amygdaloid nuclei (see Fig 2E) were moderately labeled. The neuropil of the globus pallidus also exhibited diffuse granular labeling (see Fig 2D and E). In the midbrain and medulla, large neurons in the substantia nigra (see Fig 2F) and trochlear nuclei (see Fig 2G) were strongly labeled. The levels of ataxin-2 in these neurons were as high as in the Purkinje neurons.





Fig 2. Regional and developmental expression of ataxin-2. All sections were labeled with 10  $\mu$ g/ml SCA2B antibody. (A) Frontal cortex: Layer III pyramidal neurons. (B–C) Hippocampus: Pyramidal neurons (B); dentate gyrus (C); basal ganglia, scattered neurons in the globus pallidus (D); and amygdaloid neurons (E). (F–H) Midbrain: Neurons in the substantia nigra (F); trochlear nucleus (G); and superior colliculus (H). Cerebellar cortex of a 22-week fetus (solid arrow = external granular layer; open arrow = immature Purkinje neurons) (I); 2 years old (J); 20 years old (K); and 59 years old (L). (Original magnification, ×100.)

The labeling pattern was polarized in the cytoplasm. Labeling of neurons in the olivary nuclei and the inferior colliculus (see Fig 2H) was moderate. Some axon bundles in the superior colliculus were labeled, but axons in the trigeminal, tegmental, and corticopontine pathways were not labeled.

To examine the developmental changes in ataxin-2 expression, we stained cerebella from humans ranging from 22 weeks' gestational age to 99 years. Only results from the 22-week fetus and the 2-, 20-, and 59-year-olds are shown (see Fig 2I–L). In the fetus (see Fig 2I) and the term infant (data not shown), weak to moderate labeling was evident in Purkinje neurons and in the external granular layer. Labeling was generally stronger and included the dentate neurons at 2 years (see Fig 2J). By 20 (see Fig 2K) and 59 (see Fig 2L) years of age, a stronger intensity of labeling was evident. In the oldest brain (99 years old) there was subjectively more labeling in glial cells of the white matter (data not shown).

In the cerebellum (Figs 3 and 4; also see Fig 2), Purkinje cells (see Fig 2J–L, and Fig 4A and 4D) were strongly labeled whereas neurons of the dentate nucleus (see Figs 3E and 4G) were moderately labeled. The subcellular distribution was different between Purkinje cells and cerebellar dentate neurons. In Purkinje cells, the labeling was more intense, punctated, and distributed throughout the cells including the dendrites with an intense labeling around the nucleus with a propensity to polarize to one pole of the cell (see Fig 3C and Fig 4A and D). In the dentate nucleus, punctate labeling tended to be restricted to one pole of the cytoplasm (see Figs 3E and 4G). The neuropil of the dentate nuclei exhibited a uniform weak punctate labeling. Similar labeling was evident in scattered foci of the internal granular layer. Scattered glial cells in the white matter were also weakly labeled.

Ependymal cells and choroid plexus epithelium were the most intensely labeled cells, the latter tending to exhibit much larger granules than neurons.

To test for the specificity of the immunostaining, we also labeled corresponding cerebellar sections with affinity-purified SCA2B antibody preabsorbed with 100  $\mu$ M peptide B and preimmune serum at 20  $\mu$ g of



Fig 3. Detection of ataxin-2 in normal human cerebellum with the SCA2B antibody. (A) Low-magnification view of the cerebellum from a control human labeled with 10 µg/ml affinity-purified SCA2B antibody. (B) Adjacent section of A stained with 10 µg/ml SCA2B antibody preabsorbed with 100 µM peptide B overnight. (C) Higher magnification view of the cerebellum showing specific labeling largely confined to cytoplasm of Purkinje neurons. (E) Dentate nucleus neurons exhibiting SCA2B-like immunoreactivity in one pole of the cytoplasm. (D and F) High-magnification view of the Purkinje cells (D) and dentate nuclei (F) labeled with peptide-preabsorbed SCA2B antibody. The dark brown spots in F are artifactual precipitates. (Original magnification, ×13.4 [A and B]; ×100 [C–F].)



Fig 4. Differential levels of ataxin-2 immunoreactivity in the cerebellum from a 49-year-old female control (A, D, and G), a 49year-old female with SCA2 (B, E, and H), and her 41-year-old daughter who also had SCA2 (C, F, and I). All sections were labeled with 10 g/ml SCA2B antibody. Original magnification:  $\times 100$  (cerebellar cortex [A–C]);  $\times 500$  (Purkinje cells, oil immersion [D–F]);  $\times 100$  (dentate neurons [G and I]); and  $\times 500$  (cerebellar granule neurons of the 49-year-old SCA2 patient [H]). The patients' Purkinje and dentate neurons exhibit intense cytoplasmic immunoreactivity. Nuclear immunoreactivity was seen in some granular neurons (H).

IgG/ml. Both the peptide-preabsorbed SCA2B antibody and preimmune serum (data not shown) did not label the Purkinje neurons (see Fig 3B and D) or dentate nuclei (see Fig 3F). The dark brown spots observed in Figure 3F are artifactual precipitates, because they do not localize to any specific cells or structures.

#### Pathological Changes in SCA2 Brains

Three brains from related women, a mother (see Fig 4B, E, and H) and daughter (see Fig 4C, F, and I), and a sister of the mother, were available for both clinical and immunohistochemical examinations. They died at ages 49, 41, and 58, respectively, after a typical

clinical course evolving over 20 to 30 years.<sup>17</sup> The younger subject, the 41-year-old daughter, from whom DNA was available, exhibited 41 and 22 CAG repeats in the SCA2 gene (data provided by Dr T. D. Bird, University of Washington Medical School). Pathological findings in the brains of the 3 patients was fairly uniform. All exhibited moderate atrophy (brain weights, 1,120, 1,130, and 940 g, respectively). Severe neuronal loss was evident in the substantia nigra, basis pontis, and Purkinje cell layer of the cerebellum, with sparing of the flocculonodular lobe. Mild to moderate neuronal loss affected the inferior and accessory olivary nuclei, dentate nucleus, arcuate nucleus, nucleus gracilus, accessory cuneate nucleus, internal granular layer of the cerebellum, and anterior horns of the spinal cord. There was axonal loss from the dorsal roots and posterior columns, dorsal spinocerebellar tract, and in two-thirds of the cases' anterior roots of the spinal cord. In addition, reactive astrogliosis was evident in the globus pallidus, thalamus, subthalamus, and periaqueductal region. These pathological findings are consistent with those that have been previously reported for SCA2 brains.<sup>18–20</sup>

## Expression of Ataxin-2 in SCA2 Brains

To investigate the distribution of ataxin-2 in SCA2 individuals, we labeled cerebella from the 3 related female patients with SCA2 at different ages of death and compared these with neurologically normal controls (see Fig 4A, D, and G) and AD cerebella. Only results from the daughter and mother are shown. In comparison with controls (see Fig 4A, D, and G), labeling of surviving Purkinje neurons was much more diffuse and appeared to engulf the nucleus in the SCA2 Purkinje cells (see Fig 4B, C, E, and F). In the 41-year-old SCA2 patient (see Fig 4C and F), labeling intensity appeared to be stronger in the surviving Purkinje cells than in Purkinje cells of the older SCA2 relative (see Fig 4B and E) and the non-SCA2 cerebella (see Fig 4D); however, in the older SCA2 patient with few surviving Purkinje cells, labeling was variable ranging from very strong to negligible (data not shown). There was no obvious correlation with cell morphology. Labeling in the internal granular layer of the 2 SCA2 cases was abnormal, with strong nuclear staining in up to 30% (estimated) of neurons (see Fig 4H). Glial cell labeling in the white matter was stronger than in controls. Cytoplasmic labeling in neurons of the central nuclei tended to be more diffuse, with stronger intensity in the SCA2 (see Fig 4I) than in the control brains (see Fig 4G), often occupying the entire cytoplasm rather than one pole of the cell. The labeling pattern in the AD cerebella was similar to that in normal controls, although Purkinje cell labeling tended to be less intense (data not shown). In the 3 SCA2 brains examined, intranuclear inclusion bodies that were seen in

DRPLA,<sup>21</sup> HD,<sup>22–24</sup> SCA1,<sup>25,26</sup> MJD/SCA3,<sup>27</sup> and SCA7<sup>28</sup> were not observed (see Fig 4E and F).

## Discussion

There are eight neurodegenerative diseases currently known to result from the expansion of glutamine repeats. These include DRPLA,<sup>12</sup> HD,<sup>10</sup> SCA1,<sup>1</sup> SCA2,<sup>2-4</sup> SCA3,<sup>5</sup> SCA6,<sup>8</sup> SCA7,<sup>9</sup> and SMBA.<sup>11</sup> Several groups have proposed that the mechanism of pathogenesis for these diseases is a gain of a toxic function of mutant proteins.<sup>29–33</sup> The argument for the gain of function theory was based on observations that (1) mutant proteins with expanded glutamines were stably synthesized and sometimes accumulated in cell types involved in the disease phenotype,<sup>29-33</sup> and (2) spinal bulbar muscular atrophy and HD phenotypes were not observed when the androgen receptor and HD genes were deleted.<sup>34–36</sup> In addition, mice lacking the SCA1 gene also showed no signs of neurodegeneration or ataxia.<sup>37</sup> Our findings are consistent with this model. First, we found that ataxin-2 with expanded glutamines repeats was expressed in SCA2 individuals (see Fig 1). Second, the level of immunoreactive ataxin-2 appeared to be higher in SCA2 brains tissues (see Fig 4).

Consistent with RNA expression studies,<sup>2-4</sup> we found a 145-kd ataxin-2 protein in the medulla, cerebral cortex, and cerebellum. The 145-kd ataxin-2 is likely the normal full-length human ataxin-2, based on the following observations: (1) antibodies to different peptides of ataxin-2 recognized the 145-kd species; and (2) antibodies preabsorbed with their respective peptide and preimmune sera failed to detect it. The antiataxin-2 antibodies also detected a 180-kd protein in the cerebrum and medulla of an SCA2 patient (see Fig 1). The 180-kd protein likely represents the mutant ataxin-2 with an expanded glutamine repeat. It is of interest that the apparent molecular masses of wildtype and mutant ataxin-2 did not reflect the small calculated size difference between the two proteins. This phenomenon was also observed with the DRPLA protein<sup>38</sup> and is likely due to conformational changes induced by the great number of glutamine residues. The failure to detect the mutant ataxin-2 (180 kd) in the cerebellum may be due to the severe loss of Purkinje cells, which occurred in the cerebella of SCA2 individuals relative to other brain areas, or may reflect formation of insoluble aggregates as has been observed in mice carrying an SCA1 transgene encoding ataxin 1 with 82 glutamines repeat.39

Additional smaller molecular mass proteins were detected by anti-ataxin-2 peptide antibodies. These proteins were likely enzymatically cleaved products of the full-length ataxin-2, based on three observations. First, the SCA2A antibody occasionally detected an additional band at 41 to 50 kd, whereas the SCA2B detected 41-kd and 70-kd proteins. The combined molecular masses of these proteins is approximately 152 kd, which is close to the observed molecular mass of ataxin-2. Second, the SCA2D antibody, an antibody to a peptide 145 amino acid residues upstream of the SCA2B peptide, also detected the 70-kd protein but not the 41-kd protein. And, third, none of the proteins was detected by anti-ataxin-2 antibodies preabsorbed with their respective peptides. These observations make it unlikely that these proteins were homologous members of the ataxin-2 family, or that they represented products of nonspecific degradation. It is noteworthy that both SCA2A and SCA2B antibodies also detected a faint 125-kd protein, most frequently in the cerebellum samples (see Fig 1A). The size of this protein is close to the calculated molecular mass (133 kd) of type II isoform,<sup>15</sup> suggesting that it is likely the type II isoform. In contrast, the 125-kd may represent another ataxin-2 isoform synthesized by an alternative expression of the SCA2 gene using the immediate downstream ATG codon as the initiation site, as the calculated molecular mass for this gene product is 124 kd.<sup>2-4</sup>

Protein aggregation may play a major role in many neurodegenerative diseases, including the polyglutamine repeat diseases. Recently, inclusion bodies were found in the nucleus of neurons of patients with DRPLA,<sup>21</sup> HD,<sup>22-24</sup> SCA1,<sup>25,26</sup> SCA3,<sup>27</sup> and SCA7.<sup>28</sup> Our analysis of three SCA2 brains has not identified intranuclear inclusions. The lack of abundant inclusions seen in SCA2 may be because the length of the common SCA2 mutant polyglutamine tract in ataxin-2 is significantly shorter than in SCA3/MJD,<sup>5</sup> HD,<sup>10</sup> DRPLA,<sup>12</sup> SCA7,<sup>9</sup> and SCA1.<sup>1</sup> Actually, the longest SCA3, HD, and SCA1 polyglutamine tracts in normal individuals overlap with the most common SCA2 mutant polyglutamine tracts. These data suggested that the lack of intranuclear inclusions in the three SCA2 cerebella was probably due to factors other than the length of the mutant CAG repeats. It is possible that ataxin-2 may have a unique posttranslational processing or modification that prevents its aggregation in the nucleus seen in SCA3/MJD,<sup>5</sup> HD,<sup>10</sup> and SCA1.<sup>1</sup> The lack of intranuclear inclusions in these SCA2 patients raises an interesting possibility that SCA2 pathogenesis is different from other polyglutamine-mediated neurodegeneration.

Cell loss in SCA2 brains did not necessarily correlate with the presence or absence of ataxin-2-like immunoreactivity in a cell population. What mediates the cell type–specific neuronal degeneration? Although SCA2 was expressed in many organs and ataxin-2 had a widespread neuronal distribution, the level of ataxin-2 appeared to be highest in Purkinje cells, the cell population that shows the most severe loss in SCA2. In addition, ataxin-2 immunoreactivity was higher in Purkinje cells than in other cell types and increased immunoreactivity was seen with advancing age (see Fig 4). Although quantification of immunocytochemical labeling was only made by visual observations, these results suggest that ataxin-2 accumulates in Purkinje cells with age and that this process may be greatly enhanced by polyglutamine expansion similar to that found with huntingtin.<sup>23</sup> The different subcellular distribution is also noteworthy. In Purkinje cells, ataxin-2 was localized in a punctate pattern in the cytoplasm. In contrast, ataxin-2 was mostly observed in one pole of the cytoplasm of most other neurons such as the pyramidal neurons of the cortex and hippocampus and dentate neurons (see Fig 2).

In addition, posttranslational modifications or different expression of isoforms may vary between different cell types. For example, the higher molecular mass of normal ataxin-2 in lymphocytes (see Fig 1E) suggested that lymphoid ataxin-2 has more extensive posttranslation modification than brain ataxin-2. Extensive posttranslational modification may decrease the accumulation of mutant ataxin-2 by increasing the turnover rate or by decreasing the aggregation of the mutant protein. This may explain why the level of the mutant ataxin-2 was apparently lower compared with the normal ataxin-2 in lymphoid cells derived from SCA2 patients (see Fig 1E). Finally, interaction with binding proteins that have a cell type-specific expression may accentuate neuronal degeneration. We have recently identified an ataxin-2-binding protein that is predominantly expressed in the cerebellum (Shibata H and Pulst S-M, unpublished data). The detailed comparison of the expression pattern and subcellular localization of polyglutamine proteins will be necessary to understand pathogenesis of these disorders and to delineate shared and divergent paths in polyglutamine pathogenesis.

We thank Trang Ho and Soodabeh Sahba for technical assistance.

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This study was supported by NIH grant NS01428-01A1 (S.-M.P.), the Carmen and Louis Warschaw Endowment Fund (S.-M.P.), and NIH Disabled Scientist Supplement grant NS01428-01A1 (D.P.H.). Dr Del Bigio is a recipient of a scholarship from the Manitoba Health Research Council.

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