

# The mouse *SCA2* gene: cDNA sequence, alternative splicing and protein expression

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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. Sequence analysis revealed that *SCA2* is a novel gene of unknown function. In order to provide insights into the molecular mechanisms of pathogenesis of *SCA2* and to identify conserved domains, we isolated and characterized the mouse homolog of the *SCA2* gene. Sequence and amino acid analysis revealed 89% identity at the nucleotide and 91% identity at the amino acid level. However, there was no extended polyglutamine tract in the mouse *SCA2* cDNA, suggesting that the normal function of *SCA2* is not dependent on this domain. Northern blot analysis of different mouse tissues indicated that the mouse *SCA2* gene was expressed in most tissues, but at varying levels. Alternative splicing seen in human *SCA2* was conserved in the mouse. By northern blot analysis, *SCA2* was expressed during embryogenesis as early as day 8 of gestation (E8). Immunohistochemical staining using affinity-purified antibodies demonstrated that ataxin 2 was expressed in the cytoplasm of Purkinje cells as well as in other neurons of the CNS.**

## INTRODUCTION

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant disorder leading to neuronal degeneration, primarily in the cerebellum, but also in other parts of the central nervous system. The gene causing *SCA2* has recently been identified by three independent groups (1–3). *SCA2* is a member of eight neurodegenerative diseases caused by a CAG/polyglutamine expansion, which include Huntington's disease (HD) (4), spinal bulbar muscular atrophy (SBMA) (5), spinocerebellar ataxia type 1 (SCA1) (6), dentatorubropallidoluysian atrophy (DRPLA) (7), Machado-Joseph disease (SCA3 or MJD) (8), SCA7 (9) and SCA6 (10).

Previous studies (1–3) could not unequivocally identify the *SCA2* initiation codon. Two potential ATG codons are located at

positions 162 and 642 of the *SCA2* cDNA sequence corresponding to Met residues numbers 1 and 161 of the amino acid sequence (1,2). With usage of the first ATG, the human *SCA2* cDNA is predicted to code for a protein of 1312 amino acids and a molecular weight of 140.1 kDa. If translation were initiated at the second ATG, then the resulting protein would contain 1152 amino acids with a molecular weight of 124.3 kDa.

Amino acid sequence analysis of human ataxin 2 revealed no significant homologies to other polyglutamine proteins or other proteins of known function. Significant homologies were only detected with a novel protein designated ataxin 2-related protein (A2RP) (1). Despite the high homology between the two proteins, the polyglutamine tract was not conserved in A2RP (1).

In order to identify the initiation codon and functional domains we isolated the mouse homolog of the *SCA2* gene. Mouse *SCA2* is highly conserved, with 91% identity at the amino acid level between mouse and human proteins. We also provide the first demonstration of protein expression and cellular localization of ataxin 2.

## RESULTS

### Identification of the mouse homolog of ataxin 2

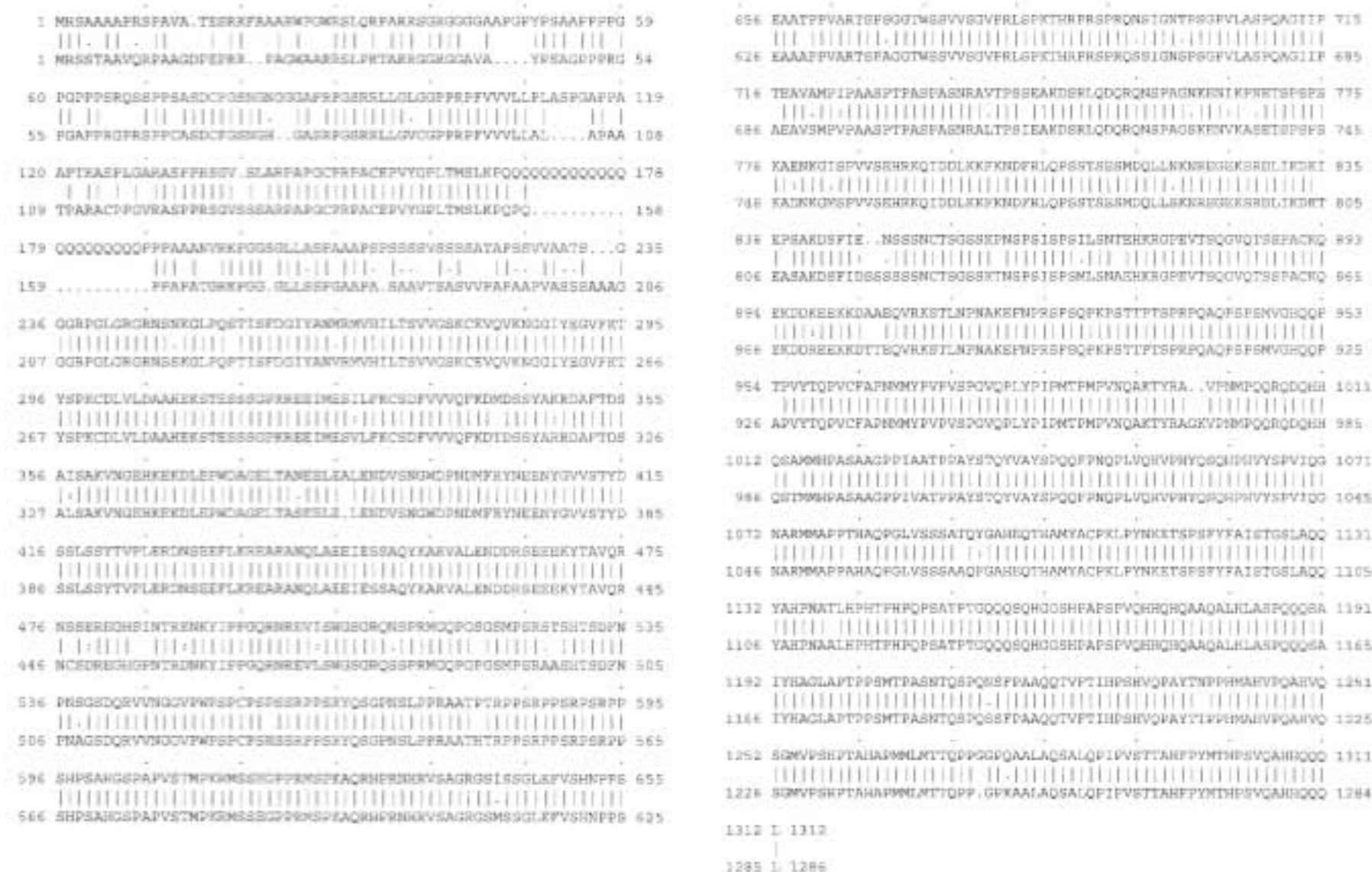
To obtain initial cDNA clones, we screened an adult mouse brain cDNA library (Stratagene) using human cDNA clones S1 and S2 (1). Five cDNA clones, designated M1–M5, were isolated. Subsequently, mouse cDNA clones were used to screen the library further to identify overlapping clones. The screening process was repeated until clones containing the putative termination codon were identified. Mouse clones M2.4.1.1 and M2.4.1.2 contained a termination codon and almost the entire coding region of the gene, including 342 bp of the 3'-untranslated sequence (3'-UTR) (Fig. 1).

The beginning of the most 5'-end mouse cDNA clone corresponded to nt 601 of the human *SCA2* sequence (1). The above cDNA clone contained an in-frame ATG codon at a position corresponding to the second methionine in the human ataxin 2 sequence. Forty two preceding base pairs did not contain an in-frame stop codon. In addition, the sequence was highly conserved between mouse and human, suggesting that it did not represent the 5'-UTR, but represented coding sequence.

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**Figure 1.** Alignment of the predicted amino acid sequence of mouse and human ataxin 2. The 1312 amino acid mouse ataxin 2 exhibits 91% identity and 92% similarity at the amino acid level. Nucleotide sequence of the mouse cDNA composite sequence from partially overlapping cDNA clones was submitted to GenBank. See Materials and Methods for accession number.

To identify upstream sequence we used clones M1 and M2.4.1.1 to screen a mouse genomic 8X bacterial artificial chromosome (BAC) library. Positive BAC clones were digested with *EcoRI* and sequenced after subcloning into the pBluescript plasmid. Sequence analysis of mouse genomic clones revealed a long open reading frame from an ATG codon corresponding to the proposed initiation codon in the human *SCA2* gene (1) to the second downstream ATG codon identified in mouse cDNA clones (corresponding to human codon 161). Twenty six base pairs of sequence upstream of the ATG corresponding to codon 1 of the human sequence did not contain a stop codon. Despite several efforts using manual and automated sequencing protocols, sequencing of the region located further upstream was not possible due to an unusually high GC content.

#### Nucleotide and amino acid sequence analysis

Alignment of mouse and human *SCA2* sequences revealed striking conservation and potentially important differences (Fig. 1). At the nucleotide level, 89% identity was observed, whereas at the amino acid level, 91% identity and 92% similarity were present. The region corresponding to amino acids 1–151 showed only 72% similarity. We observed nine deletions and four insertions of various sizes. All deletions except one were located in the relatively less homologous 5'-end region. Six mouse

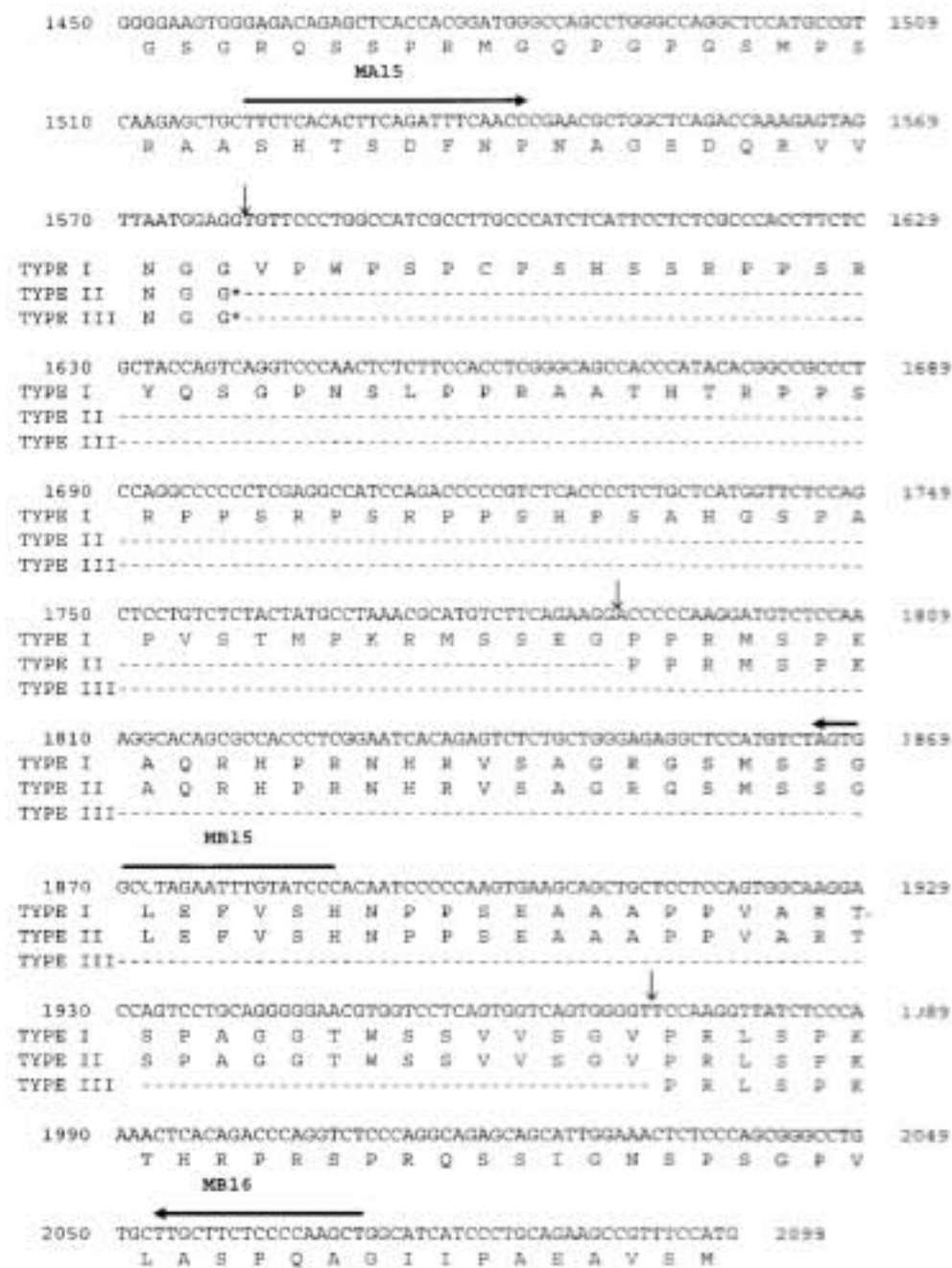
clones, M2.4.1.1, M2.4.1.2, M12, M13, M1 and M4, encompassed the homologous regions flanking the human CAG repeat. All clones had identical sequences with only a single CAG codon. Mouse genomic clones also contained only one CAG codon.

Nucleotide analysis revealed that clones M12, M13, M2.6.1.1, M2.6.2.1 and M2.4.1.1 were deleted of 1580–1789 bp inclusive. The deletion of 210 nt did not interrupt the open reading frame and was not reported earlier in the human sequence. We designated the isoform without the 210 bp deletion as type I, whereas the cDNA sequence with the deletion was designated as type II (Fig. 2).

#### Expression of mouse *SCA2* transcripts

Using northern blot analysis, we detected a 4.4 kb transcript in several mouse tissues, such as brain, heart, lung, liver, kidney, skeletal muscle, spleen and intestine (Fig. 3A). The predominant expression was seen in the brain, whereas less dramatic differences were observed in the distribution in other mouse tissues compared with the human homolog, where no or very little transcript was seen in kidney and lung (1). When total RNAs extracted on mouse embryonic days 8–16 (E8–E16) were probed by northern blot analysis, a transcript of 4.4 kb was detectable at stages E8–E16, with the lowest level of expression at E8. (Fig. 3B).





**Figure 2.** Mouse SCA2 alternative transcripts. cDNA sequence and predicted peptide sequence of the mouse types I, II and III isoforms. The type II transcript lacks 210 bp, the type III transcript lacks these 210 bp and an adjacent 183 bp. Both deletions are indicated by dashed lines. Vertical arrows indicate the beginning of the deletion for the type II and III isoforms. The primer pairs MA15/MB15 and MA15/MB16 were designed to amplify isoforms in RT-PCR experiments. Corresponding to the genomic structure of human ataxin 2 (15), isoform II lacks exon 10 and isoform III lacks exons 10 and 11. The asterisk indicates amino acids in type II and type III isoforms for which the third nucleotide in a three letter codon is shown following the end of the corresponding deletion.

### Expression of alternative transcripts

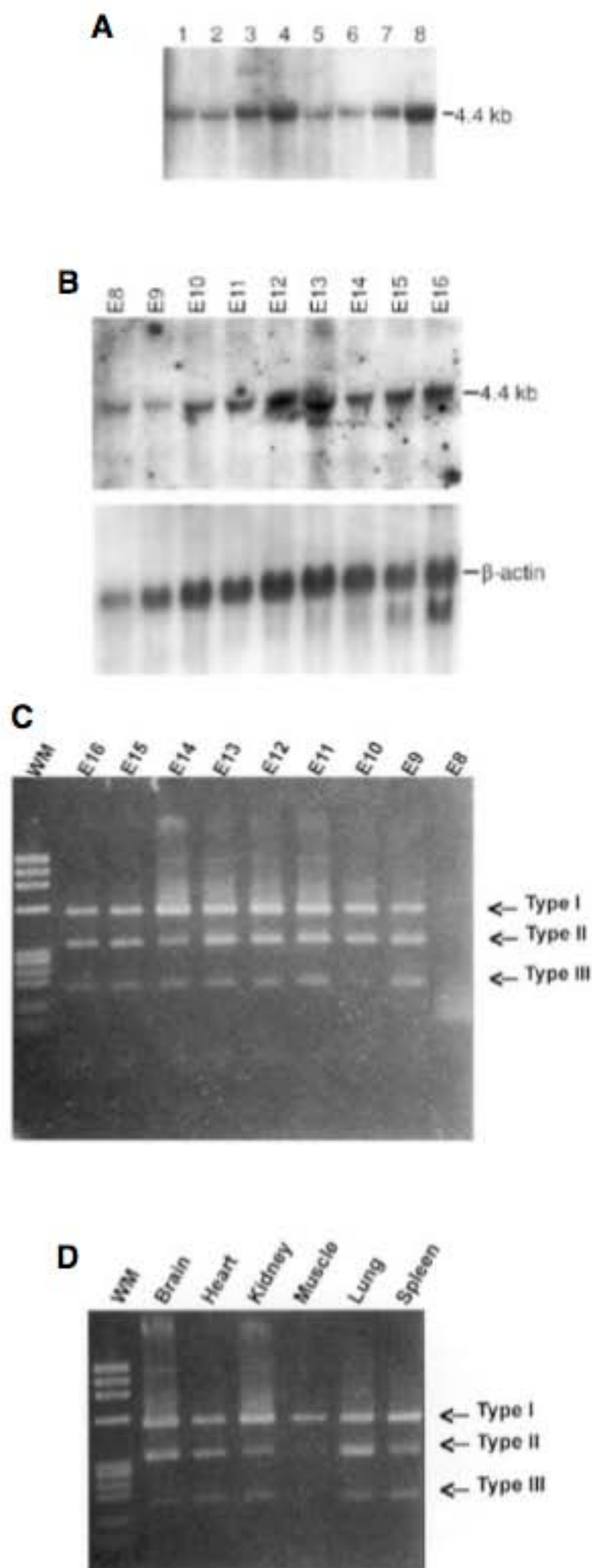
In order to study the expression of alternative transcripts, we used PCR primer MA15, which, in combination with primers MB15 and MB16, flanks the alternative splice site. Corresponding amplicons encompass nt 1520–2069 and 1520–1886 of the mouse cDNA sequence. RT-PCR was performed using 1 µg total RNAs extracted from whole mouse embryos, E8–E16 (Fig. 3C), and adult mouse tissues (Fig. 3D). In addition to two isoforms represented in mouse SCA2 cDNA clones (see above, Nucleotide and amino acid sequence analysis), we detected a third isoform, type III, using primer pair MA15/MB16. Sequence analysis of the third isoform revealed an in-frame deletion of 393 bp, which consisted of the same 210 bp as in type II but also included the adjacent downstream 183 bp (Fig. 2). The expression of all three isoforms was detected in all mouse tissues with the exception of muscle, where only type I was seen (Fig. 3D). Analysis of mRNAs from E8–E16 mouse embryos by RT-PCR showed the existence of three isoforms as early as E8 (Fig. 3C). In

comparison with the type II and type I isoforms, the relative amount of type III transcript was less than for the other isoforms (Fig. 3B). The predicted molecular weights of the three mouse ataxin 2 isoforms encoded by the type I, II and III transcripts are 136.5, 129 and 122.8 kDa respectively.

### Characterization of mouse ataxin 2

To investigate the distribution of the SCA2 gene product, ataxin 2, we produced antibodies to short peptides (designated SCA2A, SCA2B, SCA2C and SCA2D) of ataxin 2. Peptides SCA2A and SCA2C are located 172 and 54 amino acids downstream of the glutamine repeat region respectively. Peptides SCA2B and SCA2D are located 391 and 433 amino acids upstream of the C-terminus. Except for the first two alanine residues on peptide SCA2B, both peptides SCA2A and SCA2B are 100% homologous to the mouse sequence. Therefore, antibodies to these two peptides should be able to recognize mouse ataxin 2. One amino





**Figure 3.** (A and B) Expression of the mouse *SCA2* gene. A 4.4 kb transcript was detected by northern blot analysis in adult (A) and embryonic (B) mouse tissues. (A) Lanes 1–8, intestine, spleen, muscle, liver, kidney, lung, heart, brain. (B) Whole mouse embryos, gestation days 8–16, designated E8–E16. (C and D) Expression of alternative transcripts as determined by RT-PCR in RNAs isolated from whole mouse embryos (C) and in adult mouse tissues. WM,  $\phi$ X174/*Hae*III molecular weight marker.

acid residue is different between human and mouse in the SCA2C peptide sequence and two are substituted in the SCA2D peptide.

For initial evaluation of the antisera, we tested them with ataxin 2–green fluorescent protein (GFP) fusion proteins. On western blots of protein extracts from COS1 cells transfected with plasmid pEGFP containing a partial (3833 bp) sequence of SCA2 cDNA fused with GFP or the pEGFP vector alone, immune sera against ataxin 2 peptides recognized a band of 150 kDa, close to the calculated molecular weight of 151 kDa for this fusion construct. The GFP protein itself was not recognized (Fig. 4A). When the same filter was reacted with an antibody to GFP, both the GFP–ataxin 2 fusion protein (150 kDa) and GFP (27 kDa) bands were detected (Fig. 4A).

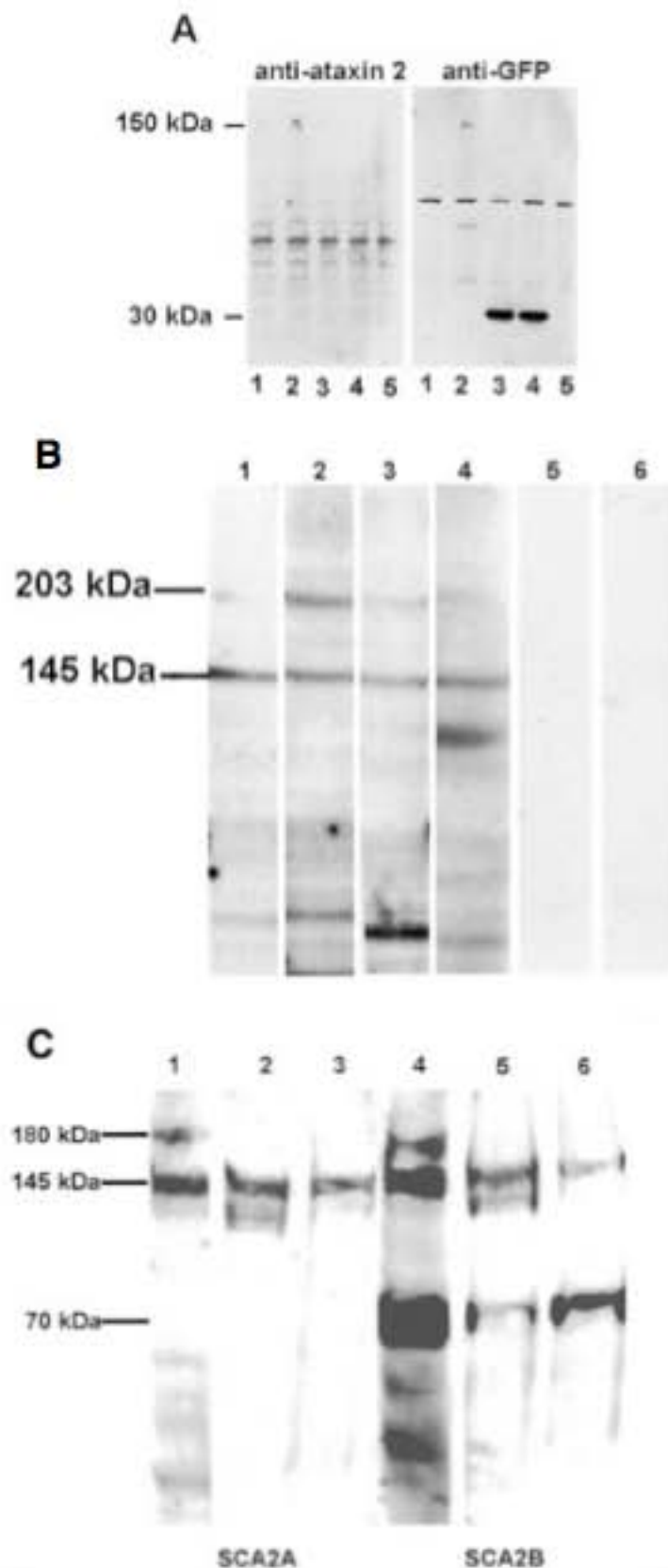
For detection of native ataxin 2, we affinity purified these antibodies and used them to detect ataxin 2 in mouse brain protein extracts. All four antibodies detected bands of 145 kDa in mouse brain (Fig. 4B, lanes 1–4). The 145 kDa protein is close to the predicted molecular weight of 136.5 kDa encoded by usage of the upstream 5' ATG codon. A 203 kDa protein was also detected most strongly by SCA2B antibody. When the SCA2A and SCA2B antibodies were preincubated with their respective peptide, they failed to detect the 145 and 203 kDa bands (Fig. 4B, lanes 5 and 6). The smaller molecular weight bands variably detected by the four antibodies probably represented degraded ataxin 2. When the antibodies were further evaluated in SCA2 brain extracts, both the normal length ataxin 2 as well as a larger protein representing ataxin 2 with an expanded polyglutamine tract were detected (Fig. 4C). This larger protein was absent in an Alzheimer disease brain and in a brain from a normal control.

To determine the distribution of ataxin 2 in adult mouse brain, we stained 7  $\mu$ m sections of paraffin-embedded mouse brain with 20  $\mu$ g/ml affinity-purified SCA2A, SCA2B, SCA2C and SCA2D antibodies. All antibodies resulted in similar staining patterns, but staining was strongest for antibody SCA2B. Figure 5 shows staining of several regions of the CNS with this antibody. Ataxin 2 was widely expressed in neuronal cells of the adult mouse brain. It was found in large pyramidal neurons and selected neurons of the hippocampus, thalamus and hypothalamus. High levels of ataxin 2 were found in Purkinje cells of the cerebellum and gigantocellular neurons of the brain stem. Ataxin 2 was weakly detected in neurons of the granule layer of the cerebellum, dentate gyrus of the hippocampus and the cerebral cortex. Ataxin 2 was undetectable in glial cells. Ataxin 2 staining was localized with a perinuclear and punctated pattern and no nuclear staining was detected. No staining was observed with affinity-purified antibodies pre-absorbed with the corresponding peptides (Fig. 5E and F), indicating the staining specificity of these antibodies. Pre-immune serum at a comparable IgG concentration also failed to stain cells (data not shown).

## DISCUSSION

We isolated and characterized the mouse homolog of the human *SCA2* gene. Mouse ataxin 2 has a very high homology at both the nucleotide and amino acid levels. Like the human gene, mouse *SCA2* exhibits a high percentage of GC base pairs, especially at the 5'-end of the gene, where it reaches 83% for the first 670 bp. This feature may be suggestive of post-transcriptional control of the message (11,12). It is likely that the observed GC-richness and secondary structure contributed to the difficulty of finding





**Figure 4.** (A) Western blot analysis of antibodies to ataxin 2. COS1 cells were transfected with 3 or 5 µg expression vector and extracted proteins were immunoblotted with antiserum to ataxin 2 peptides (left) or with green fluorescent protein (GFP) antibody (right). The ataxin 2 antiserum detected a 150 kDa protein in cells transfected with the pEGFP-SCA2 fusion vector (first two lanes), but not in cells transfected with the pEGFP vector alone nor in untransfected cells. The GFP antibody detected both the 150 kDa fusion protein and the 27 kDa GFP. The low molecular weight proteins represent non-specific proteins stained by the primary polyclonal antibody. (B) Protein extracts of adult mouse brain detected with 1 µg/ml of four affinity-purified antibodies: SCA2A (lane 1), SCA2B (lane 2), SCA2C (lane 3) and SCA2D (lane 4). Lanes 5 and 6 were incubated with SCA2A and SCA2B antibodies pre-absorbed with 100 µM peptides A and B respectively. (C) Western blot analysis of human brain protein extracts from a patient with SCA2 (lanes 1 and 4), Alzheimer's disease (lanes 2 and 5) and a normal control (lanes 3 and 6). Lanes 1-3 were detected with 10 µg/ml SCA2A antibody and lanes 4-6 were detected with 10 µg/ml SCA2B antibody. Both antibodies detected normal 145 kDa ataxin 2 in all samples. In the SCA2 brain an additional protein of 180 kDa, representing ataxin 2 with an expanded polyglutamine tract, was seen. The SCA2B antibody also detected a 70 kDa protein.

cDNA clones containing the 5'-end sequence in reverse transcribed libraries.

Despite the high overall homology, lack of the polyglutamine tract in the mouse suggests that other domains of the protein may represent functionally important domains. A reduction in the length of trinucleotide repeats in the mouse has been observed for other CAG/polyglutamine expansion diseases, such as SCA1 (13) and Huntington's disease (14). This suggests that the expansion of repeats may be a disease of 'primates' and no natural rodent model is likely to exist.

Mouse ataxin 2 was expressed in all tested mouse tissues, including lung and kidney, where virtually no transcript was seen in humans. The observation of conserved alternative transcripts in both mouse (reported here) and human tissues (15) may indicate that different isoforms could potentially represent functional differences. *In situ* hybridization with RNA probes generated from mouse cDNA against particular isoforms will greatly facilitate localization of the particular isoforms in brain and other tissues and provide insights into the functional significance of splicing. The observed alternate splicing can account for the small differences in the size of transcript for mouse tissues detected by northern blot analysis. It is interesting to note the proline- and serine-rich contents of the deleted amino acids in the type II and type III isoforms. The 70 amino acids spliced out in the type II isoform consist of 29% prolines and 23% serines. Similarly, among 131 amino acids spliced out in the type III isoform, prolines and serines contribute 22 and 21% respectively. The proline-richness of the spliced amino acid regions might cause a local but critical structural change in the resulting protein. Proline-rich motifs are responsible for binding Src homology 3 region (SH3) domains, present in a large group of proteins, including cytoskeletal and signaling proteins (16).

Controversy surrounds the putative initiation codon of human ataxin 2. Two methionines, at positions 1 and 161 of the human peptide sequence, were suggested as potential initiators of transcription. Conservation of both methionines at positions 1 and 151 of mouse ataxin 2 with 72% identity in the 151 amino acid region supports the idea that the first ATG likely represents the initiation codon. This is further supported by the finding that antibodies raised against different peptides of human ataxin 2 detected a 145 kDa protein in mouse brain. The failure to isolate a cDNA clone containing the region upstream of the second methionine is likely due to the inability of the reverse transcriptase to transcribe passed the GC-rich tract found in this region. Since the processing of polyglutamine proteins leading to smaller proteins with a tendency to aggregate appears to be a crucial step in the causation of these disorders (17-21), identification of the correct translation start site is of paramount importance for future animal and cellular studies of SCA2 pathogenesis.

We have generated antibodies to four peptides located in different regions of ataxin 2. The specificity of these antibodies was based on the following observations: (i) all four antibodies detected proteins of identical molecular weights (Fig. 4B, lanes 1-4); (ii) antibodies pre-absorbed with the corresponding peptide failed to detect these proteins (Fig. 4B, lanes 5 and 6); (iii) pre-immune sera did not detect the observed bands (data not shown); (iv) the antibodies specifically detected a GFP-ataxin 2 fusion protein (Fig. 4A) and (v) in SCA2 brains, but not in normal brains, an ataxin 2 of larger molecular weight was detected, representing ataxin 2 with an expanded polyglutamine repeat (Fig. 4C).



The 145 kDa protein likely represents full-length mouse ataxin 2, since the size is closest to the predicted molecular weight of mouse ataxin 2. All four antibodies also detected a 203 kDa protein with varying intensity. This makes it unlikely that this band represents a related protein and suggests that the 203 kDa protein is a modified ataxin 2, potentially representing a dimer of truncated ataxin 2. Dimerization has been observed for ataxin 1 (22). Self-interaction of ataxin 2 has also been observed in the yeast two-hybrid system (Shibata and Pulst, unpublished data).

To determine the localization of ataxin 2 in adult mouse brain, we used affinity-purified anti-ataxin 2 antibodies to stain adult mouse brain sections. The specificity of the immunohistochemical staining was determined based on the following observations: (i) all four antibodies produced identical staining patterns (data not shown); (ii) pre-immune sera at identical IgG concentrations gave no signal and (iii) antibodies pre-absorbed with the corresponding peptides gave no detectable staining. Ataxin 2 was expressed in a wide variety of neurons and was not restricted to the cerebellum. However, the most intense staining was seen in pyramidal cortical neurons, in large brain stem neurons and in cerebellar Purkinje cells. It will be of interest to determine if expansion of the polyglutamine tract in ataxin 2 also leads to dysfunction of cortical neurons, since dementia appears to be more prominent in SCA2 than in other SCAs (24,30).

The observation that ataxin 2 has a cytoplasmic location (Fig. 5) is similar to other proteins containing glutamine repeats, such as ataxin 1 (24), atrophin 1 (25) and huntingtin (26). Interestingly, ataxin 1 was also localized in the nuclei of neurons other than Purkinje cells (24) and expanded polyglutamine repeats led to intranuclear localization in HD, MJD and SCA1 (19,27,28). Although the distribution of these proteins in neurons is similar, the exact localization of each protein remains to be determined. It is possible that the subcellular localization of each glutamine repeat-containing protein is different, since no other regional homologies between these proteins are observed and the normal function of each protein is likely different. It will be important to determine whether the localization of ataxin 2 changes to an intranuclear location in mice expressing mutant SCA2 transgenes and whether intranuclear neuronal staining can be detected in patients with SCA2.

## MATERIALS AND METHODS

### Isolation of cDNA clones

Human SCA2 cDNA clones S1 and S2 (1) were random primed (Gibco BRL) with [ $\alpha$ -<sup>32</sup>P]dCTP and used to screen a  $\lambda$ Zap mouse adult brain cDNA library (Stratagene). Phage were plated to an average density of  $10^5$  per plate and plaques were lifted using nylon membranes in duplicate (Duralose-UV). Hybridization and washes were performed according to the manufacturer's protocol (Stratagene). After screening 2 000 000 clones, five mouse cDNA clones, M1–M5, were identified. These clones were sequenced and aligned with the human ataxin 2 sequence to confirm homology and used to re-screen the library. A total of 25 clones were identified in three iterative screens.

### Isolation of mouse genomic clones

An 8X mouse genomic library (Research Genetics) was screened using mouse cDNA clones M1 and M2.4.1.1, labeled by random priming with [ $\alpha$ -<sup>32</sup>P]dCTP (Gibco BRL) following the manufacturer's protocol (Research Genetics). One of the obtained BAC

clones, 176I11, was digested with *Eco*RI and subcloned into the pBluescript plasmid. Clones were picked into 96-well plates, grown overnight and stamped onto nylon membranes lying on top of LB + ampicillin agar plates. Plates were grown overnight and replica sets were denatured and fixed according to the Stratagene protocol. Plasmids positive for the 5'-region of mouse SCA2 cDNA were found by hybridization of oligonucleotides M1 and N1 to the replicates. Oligonucleotides M1 (5'-GGC GGG CGT CCC GGC CTG GG-3') and N1 (5'-GGG CCC ATA CAC CGG CTC GC-3') encompass 645–665 and 452–471 nt of the mouse cDNA sequence respectively. Subsequently, the plasmids were sequenced using primer N1 and sequences aligned with the human SCA2 sequence. A consensus sequence was generated from several overlapping cDNA clones.

### Sequence analysis

Sequence analysis was performed using an ABI 373 sequencer and primer walking. Sequences were assembled into a consensus sequence using the Autoassembler program (Perkin Elmer) and the translated peptide was compared with the human ataxin 2 peptide sequence.

### Northern blot analysis and first strand cDNA synthesis

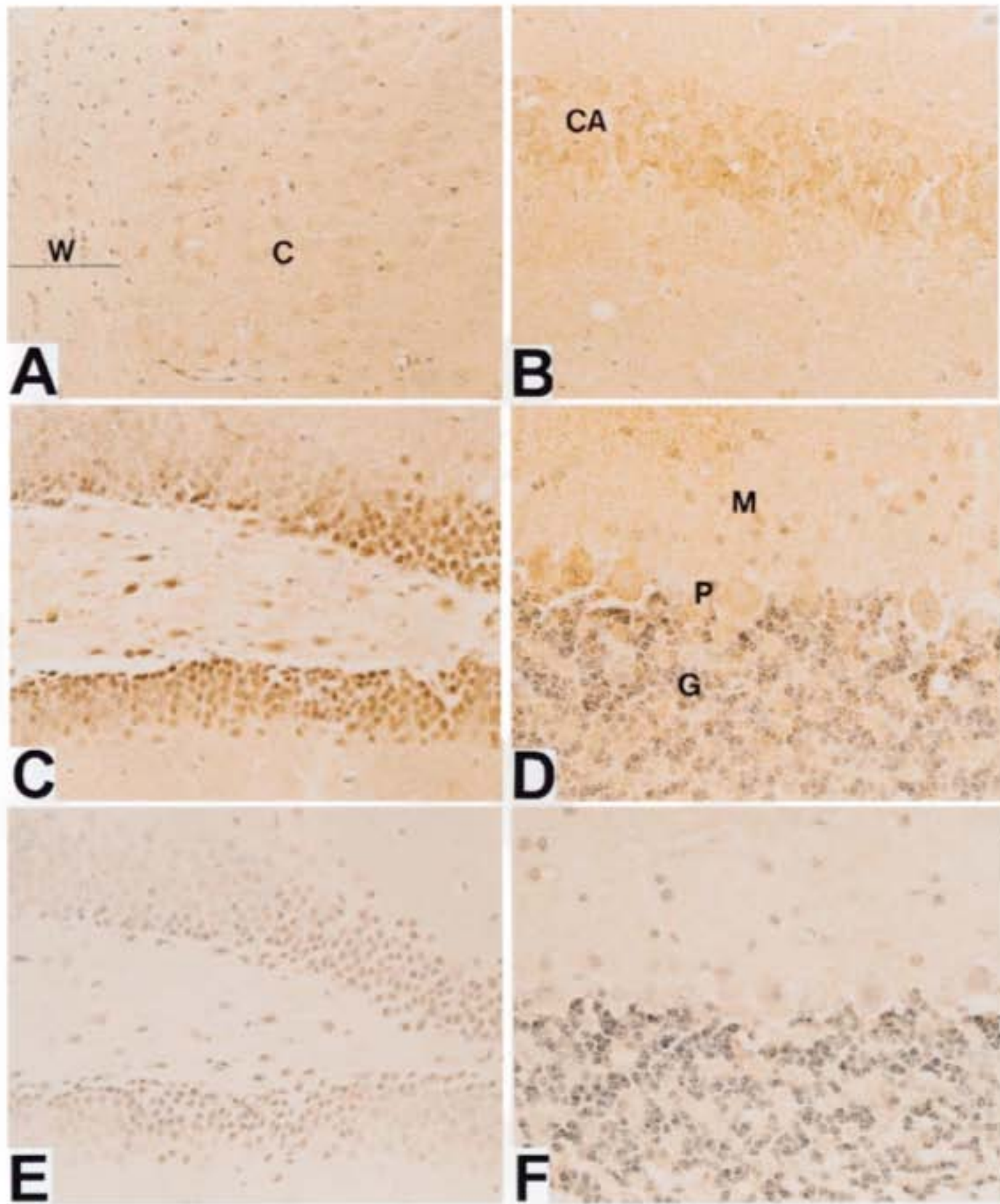
Total RNA from adult mouse tissues and whole mouse embryos (E8–E16) were isolated as described (29) using Trisol reagent (Gibco BRL). Northern blot analysis was performed as described (29). <sup>32</sup>P-labeled mouse cDNA clone M1 was used in hybridization as the probe. Mouse embryos were purchased from Harlan Bio Products for Science (Indiana). One microgram of total RNA was used for first strand cDNA synthesis using a cDNA synthesis kit (Promega).

### Antibody production and western blot analysis

**Antibody production.** We raised rabbit antibodies (Ab) against C- and N-terminal peptides: SCA2A, amino acids 359–371 (numbering according to human ataxin 2; 1), AKVNGEHKEKDLE; SCA2B, amino acids 904–921, A\*A\*EQVRKSTLNPNKEFN; SCA2C, amino acids 241–250, LGRGRNSN\*KG; SCA2D, amino acids 867–879, I\*LSNT\*EHKRGPEV. An asterisk follows an amino acid residue that differs between mouse and human (see Fig. 2). Each peptide was conjugated to keyhole limpet hemocyanin and injected into two rabbits. Collected antisera were affinity purified using Sepharose-conjugated peptide columns.

**Protein extraction and western blots.** Proteins from mouse brain tissues and cell lines were extracted as follows. Fresh or frozen tissues were resuspended in triple detergent buffer (100 mM Tris, pH 7.4, 1% NP40, 0.5% SDS, 0.5% deoxycholic acid, 1 mM Pefabloc, 1 mM EGTA, 1  $\mu$ g/ml pepstatin A, 2  $\mu$ g/ml aprotinin, 50  $\mu$ g/ml leupeptin) and homogenized using a polytron homogenizer. The protein extracts were first spun at 1000 g (3100 r.p.m. in a JA17 rotor) for 5 min. The supernatant was centrifuged at 105 000 g (54 000 r.p.m. in a TLN100 rotor) for 1 h. The supernatant (S3) was aliquoted and stored at  $-80^{\circ}\text{C}$ . Protein concentrations were determined with the Bio-Rad DC-Bradford Protein Assay Kit. Prior to loading onto polyacrylamide gels, proteins were concentrated using a Microcon 10 column (Amicon). Aliquots of 50–100  $\mu$ g protein were loaded per lane in a Bio-Rad premade 4–20% gradient SDS-polyacrylamide mini-gel and resolved at





**Figure 5.** Distribution of ataxin 2 in mouse CNS. All sections were stained with 20  $\mu\text{g/ml}$  affinity-purified SCA2B antibody. (A) A coronal section of mouse cerebral cortex showing layer VI and the white matter. (B) The CA3 region of the hippocampus. (C) The dentate gyrus of the hippocampus. (D) Cerebellum. (E and F) Sections of the dentate gyrus and cerebellum stained with antibody SCA2B pre-absorbed with the SCA2B peptide. Magnification 330 $\times$ . W, white matter; C, cerebral cortex; CA, cornu ammonis; M, molecular layer; P, Purkinje cell layer; G, granule layer.

100 V for 1–2 h. Fractionated proteins were transferred to a nitrocellulose filter. The filter was rinsed briefly with TBS (150 mM NaCl, 50 mM Tris, pH 8.0), blocked with 5% non-fat dried milk and then incubated with the desired dilution of tested antibodies overnight at 4°C. The primary antibody was detected using the Amersham Chemiluminescence ECL Kit.

#### Immunohistochemistry

Mouse brain blocks were fixed with 10% formalin and embedded in paraffin. Seven micrometer sections were cut and mounted onto Superplus microscopic slides (Fisher Scientific). The sections were rehydrated by rinsing twice with a 5 min interval in



xylene, 100% ethanol, 95% ethanol and 70% ethanol. After deparaffinization, sections were treated with a protease cocktail. Sections were incubated with 20 µg/ml affinity-purified anti-ataxin 2 antibodies overnight at 4°C. Primary antibodies were detected using the Vector ABC Elite Peroxidase Kit (Vector, CA), enhanced with diaminobenzidine enhancer and visualized with diaminobenzidine (Biomed, CA). Sections were counterstained with aqueous hematoxylin (Xymed, CA). Controls consisted of antibodies pre-absorbed with 100 µM of the respective peptide and pre-immune sera at comparable concentrations (1/500).

### Construction of the pEGFPC2-SCA2trk construct and COS1 cell transfection

A 3833 bp SCA2 cDNA encompassing the second ATG and the termination codon was ligated in-frame with the pEGFPC2 vector (Clontech) to generate a GFP-SCA2 fusion plasmid, pEGFPC2-SCA2trk. The calculated molecular weight of the fusion protein is 151 kDa, consisting of ataxin 2 truncated at the second ATG site (124 kDa) and GFP (27 kDa).

The expression vector pEGFPC2-SCA2trk was transfected into COS1 cells using SuperFect Transfection Reagent (Qiagen). A sample of 400 000 cells was grown in a 60 mm Petri dish overnight, then transfected according to the manufacturer's method using 2–10 µl/µg DNA. Transfected cells were grown for 48 h. Proteins were extracted using triple detergent and immunoblotted with antibodies to GFP and ataxin 2.

### PCR: conditions and primers

Primers MA15 in combination with MB15 and MB16 were used to amplify alternative transcripts in cDNAs synthesized from adult mouse tissues and mouse embryos. PCR was performed according to standard protocols using annealing temperatures of 55 and 57°C for the MA15/MB15 and MA15/MB16 pairs respectively. Primer sequences were as follows: MA15, 5'-TTCTCACACTTCAGATTCAACC-3', nt 1520–1542; MB15, 5'-GGATACAAATTCTAGGCCACT-3', nt 1866–1886; MB16, 5'-AGCTTGGGGAGAAGCAA-3', nt 2053–2069.

Following separation of RT-PCR products on a 2% agarose gel, bands were excised, purified and sequenced using the corresponding primers.

The GenBank accession no. for the mouse homolog of the SCA2 gene is AF041472.

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