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Mouse Models of Human CAG Repeat Disorders

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Expansions of CAG trinucleotide repeats encoding glutamine have been found to be the causative mutations of seven human neurodegenerative diseases. Similarities in the clinical, genetic, and molecular features of these disorders suggest they share a common mechanism of pathogenesis. Recent progress in the generation and characterization of transgenic mice expressing the genes containing expanded repeats associated with spinal and bulbar muscular atrophy (SBMA), spinocerebellar ataxia type 1 (SCA1), Machado-Joseph disease (MJD/SCA3), and Huntington's disease (HD) is beginning to provide insight into the underlying mechanisms of these neurodegenerative disorders.

Introduction

Over the past decade, the expansion of repeated trinucleotide sequences has been recognized as a significant source of mutation within the human genome. More than a dozen neuromuscular and neurodegenerative diseases have been found to contain repeat expansions within their associated genes (58). One class of mutation, the expansion of a CAG repeat encoding glutamine, has been found in the coding regions of the genes associated with seven different neurodegenerative disorders. This group of diseases includes spinal and bulbar muscular atrophy (SBMA) (43), Huntington's disease (HD) (29), spinocerebellar ataxia type 1 (SCA1) (56), dentatorubral-pallidoluysian atrophy (DRPLA) (8, 40, 52), Machado-Joseph disease / spinocerebellar ataxia type 3 (MJD/SCA3) (37), spinocerebellar ataxia type 2 (SCA2) (34, 59, 63), and most recently, spinocerebellar ataxia type 6 (SCA6) (77). In addition, several other neurodegenerative disorders are believed to be caused CAG repeat tract expansion (47, 73).

Although the genes associated with each of these disorders are unrelated to each other, and have unknown functions (except for the androgen receptor in SBMA), the diseases share a variety of features which suggest they are caused by a common mechanism of pathogenesis. All of these diseases are characterized by autosomal dominant inheritance (except SBMA which is X-linked), genetic anticipation within families, and an inverse correlation between expanded repeat tract length and age of onset and severity of disease. Similarly, the repeat tracts within these genes display common size ranges for both normal as well as mutant alleles.

All of the glutamine-repeat diseases are characterized pathologically by the loss of neurons within the brain, brainstem, and spinal cord. Although studies have indicated that the genes associated with these disorders are widely expressed (4, 13, 50, 54, 70), the neuronal loss seen with each disorder is quite disease-specific. This observation raises the question of how selective populations of neurons are affected in each of the disorders.

The determinants of cell-specificity and death in these diseases have been difficult to study. Pathological examination of patient tissue samples obtained at autopsy has revealed little in regard to the progressive changes occurring during the course of disease. Therefore, the mechanism of pathogenesis remains unknown. An alternative, and perhaps more promising approach for the study of these diseases, is the use of transgenic animal modelling. Transgenic mice expressing the gene with the mutation(s) of interest can be generated by microinjection of genetic material in fertilized embryos (57), or by the manipulation of endogenous genes within embryonic stem cells (11).

In this review, we describe the clinical and pathological features of several of these disorders. The progress in the generation and characterization of transgenic models of these diseases is also discussed. Finally, concluding remarks regarding the results of the transgenic studies are presented.

Spinal and bulbar muscular atrophy

Spinal and bulbar muscular atrophy (SBMA) or Kennedy's disease is an X-linked recessive neurodegenerative disorder characterized clinically by proximal muscle weakness and atrophy, fasciculations, and signs of bulbar dysfunction such as difficulty swallowing (38). In addition, affected males have symptoms result-

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ing from mild androgen insensitivity including gynecomastia, testicular atrophy, and reduced fertility (1). Heterozygous females are usually asymptomatic. The pathological features of the disease include the loss of anterior horn cells in the spinal cord, the depletion of sensory neurons in the dorsal root ganglia, and the degeneration of selective brainstem motor nuclei (68). There is no involvement of upper motor neurons.

In 1991, the SBMA disease-causing mutation was found to be the expansion of a CAG repeat (encoding glutamine) within the coding region of the androgen receptor (AR) gene (43). The CAG repeat within the AR gene is polymorphic on normal chromosomes, ranging in size from 11 to 33 repeat units, and expanded on SBMA chromosomes, ranging in size from 38 to 66 repeat units (43). While the CAG repeats on normal alleles are meiotically stable, approximately 27% of expanded alleles undergo expansion or contraction upon transmission (42). Interestingly, paternally transmitted alleles have been found to undergo alterations in size more frequently than maternally transmitted alleles. In addition, there is an inverse correlation between CAG repeat size and age of onset and severity of clinical symptoms (18, 32, 42). The gain of a novel property by the mutant AR protein containing the expanded polyglutamine tract, rather than its functional inactivation by the repeat expansion, is thought to be the basis of the neuronal loss seen in SBMA. This conclusion is based on the observation that XY males with a deletion of the AR gene present clinically with testicular feminization, but no evidence of motor neuron disease (60).

In an effort to develop a mouse model of SBMA, Bingham and colleagues (7) generated several lines of transgenic mice with human AR cDNA constructs containing either 24 (AR; normal) or 45 (ARexp; expanded) CAG repeats. Two different promoters were used to direct the expression of the transgenes. The murine neuron specific enolase (NSE) (22) was used to achieve constitutive expression within essentially all neuronal cell populations. The second promoter, the murine Mx promoter (2), was used to direct expression of the transgene broadly within both neuronal and non-neuronal cell types. An additional advantage of the Mx promoter is that its timing and level of expression can be regulated by the administration of interferon. Although transgenic animals generated using the NSE promoter produced detectable levels of transgene mRNA in brain and muscle tissue as assayed by RT-PCR, the transgene product was expressed at very low levels, and was not detectable by western blot analysis. Animals in the Mx-AR and Mx-ARexp transgenic lines showed inducible expression of the transgene in the brain, spinal cord, muscle, lung and kidney, with the highest levels (approximately equivalent to endogenous AR protein) being in the lung and kidney.

Morphometric studies of the axons in motor nerves and ventral nerve roots were performed on induced and uninduced Mx-AR and Mx-ARexp transgenic animals, as well as non-transgenic controls at 6 months of age. Phrenic and lumbar ventral root axons of transgenic animals appeared normal with no evidence of degeneration. Furthermore, there were no differences in the mean axon area or total number of lumbar root or phrenic nerve axons between induced and uninduced transgenic and non-transgenic animals. The authors of this study suggest that the inability to produce the phenotypic alterations and neuropathology characteristic of SBMA may have been due to low level transgene expression or reduced susceptibility of murine motor neurons to the detrimental effects of proteins containing polyglutamine repeat expansions.

Machado-Joseph disease

Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3) is one of the most common forms of spinocerebellar degeneration. MJD is an autosomal dominant disorder characterized by a wide range of clinical symptoms including cerebellar ataxia, progressive external ophthalmoplegia, pyramidal and extra pyramidal signs, dystonia with rigidity, and distal muscular atrophies (62). The disease is characterized pathologically by degeneration of the spinocerebellar tracts and the dentate, pontine, and vestibular nuclei. Dorsal columns are also frequently affected. Extrapyramidal structures affected include the substantia nigra, locus ceruleus, and pallidoluysian complex. Neuron loss within the motor cranial nerve nuclei, anterior horn, and posterior root ganglia have also been reported (15, 36). There is only minimal involvement of cerebellar Purkinje cells. In addition, there is a relative sparing of the neurons of the olivary nuclei, allowing pathological distinction of MJD from olivopontocerebellar atrophies (OPCAs).

The MJD locus has been mapped to chromosome 14q32.1 (65, 71). Kawaguchi and colleagues have identified a gene mapping to this region, MJD1, that contains an expanded CAG repeat in MJD patients (37). Like other CAG repeat disorders, the CAG repeat within MJD1 is polymorphic in the normal population, ranging in size between 12 and 37 repeats, and expanded in affected individuals, ranging in size between 62 and 84 repeat units. Mutant alleles of MJD1 also show inter-

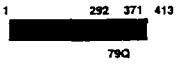
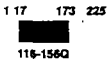
Diseases	Tg line	Promoter	Encoded region	Phenotype	Neuropathology
MJD	MJD79	Pcp-2		normal	not reported
	Q79C	Pcp-2		ataxia	cerebellar atrophy; extensive Purkinje and granule cell atrophy and loss
	Q35C	Pcp-2		normal	not reported
	Q79	Pcp-2		ataxia	not reported
HD	R6[a]	endogenous		chorea seizures resting tremor	reduced CNS structures no focal neurodegeneration
SCA1	A02[b]	Pcp-2		normal	none
	B05[c]	Pcp-2		motor performance alterations; ataxia	Purkinje cell atrophy; mild Purkinje cell loss

Figure 1. *Summary of transgenic animals displaying phenotypic alterations.* The designation of the transgenic (Tg) line, promoter used, region of the gene encoded, animal phenotype, and neuropathology are indicated. Colored boxes represent the coding regions of the MJD (blue), HD (yellow), and SCA1 (green) genes; red boxes indicate the position and length of the polyglutamine tract within each gene. Numbers displayed above the coding region of the genes indicate the amino acids included within the transgenic constucts.

generational instability, particularly upon paternal transmission (65, 71). In addition, there is an inverse correlation between repeat length and clinical severity and age of onset of the disease (10, 48, 51). Interestingly, MJD homozygotes, unlike HD homozygotes (75) , display an additive effect of the mutation with earlier ages of onset and accelerated progression of disease when compared to heterozygotes with similar CAG repeat sizes (46, 69). This finding suggests that level of mutant gene expression influences clinical phenotype of MJD.

Ikeda and colleagues (33) have recently reported the generation of transgenic mice expressing full length and truncated versions of the MJD1 protein, MJD1a (figure 1). The murine Purkinje cell specific promoter Pcp-2 (L7) (55, 74) was used to direct the expression of cDNA transgenes encoding full length and truncated MJD1a proteins with 79 glutamine residues (MJD79, and Q79C, respectively), as well as a truncated MJD1a protein with 35 glutamine residues (Q35C). In addition, transgenic animals expressing only a 79 glutamine residue tract (Q79) were produced. Animals from the Q79C and Q79 lines developed ataxic postures and gait disturbances beginning at 4 weeks of age. None of the animals containing the full length MJD79 or the truncated Q35C constructs were ataxic at 23 and 32 weeks of age, respectively.

Histological examinations of a single 8 week old Q79C transgenic animal and an age-matched non-transgenic littermate were performed. The Q79C animal had significant atrophy of the cerebellum. Although the cerebellum of this animal retained a fundamental structure, it was found to be only about 15% of its normal volume. All three layers of the cerebellum were affected. The molecular layer showed substantial thinning. The Purkinje cells were shrunken, had attenuated dendrites, and showed reduced calbindin immunoreactivity. The granule cell layer showed a significant reduction in granule cell number with many of the remaining cells having an altered, shrunken morphology. Because the promoter used in this study limited the transgene expression to cerebellar Purkinje cells, the extensive granule cell loss represents an effect secondary to the defects of the Purkinje cells. In the absence of a more detailed examination of early postnatal time points in these animals, it seems likely that the extensive granule

cell involvement is a developmental defect occurring shortly after cerebellar foliation. The cerebrum of the Q79C transgenic animal was normal. The histological examinations of animals in the other transgenic lines were not reported.

The authors of this study also used the above constructs to transiently transfect COS cells to examine their effects on the growth of cells in culture. Cells transfected with the Q79C and Q79 constructs were specifically lost from the culture by apoptotic cell death. Conversely, cells expressing the MJD79 and Q35C constructs did not undergo programmed cell death. These results indicate that the expanded polyglutamine tract itself can induce cell death. Interestingly, western blot analysis performed on the transfected cells indicated a covalently modified complex containing the Q79C products. This finding is consistent with a model of pathogenesis proposed by Green (24) in which he speculated that proteins containing extended polyglutamine tracts might serve as aberrant substrates for tissue-specific transglutaminases resulting in their covalent cross-linkage to other non-specific proteins.

The in vitro and in vivo expression studies using the full length and truncated MJD1 constructs led the authors of this study to suggest that the truncated forms of MJD1a, and perhaps other polyglutamine repeat-containing proteins, have increased potency as inducers of cell death relative to their full length counterparts. Furthermore, Ikeda and colleagues have proposed a model in which a cell-specific proteolytic cleavage, possibly followed by the processing of the mutant protein, exposes a subset of cells to the detrimental effects of elongated polyglutamine tracts. It should be noted, however, that a detailed comparison of transgene expression in the Q79C and MJD79 lines was not reported, and that the phenotypic differences between animals in these two lines may simply be due to differences in the levels of transgene expression. In addition, there is currently a lack of evidence for covalent modification of the mutant protein in Q79C and Q79 animals.

Huntington's disease

Huntington's disease (HD) is the most common of the autosomal dominant neurodegenerative disorders caused by CAG repeat expansions, with an incidence of approximately 1 in 10,000 within the North American and Western European populations (27, 28). HD is characterized by chorea, psychiatric disorders, abnormal eye movements, and dementia. The neuropathological findings in HD include the preferential loss of medium spiny neurons of the caudate and putamen along with astrogliosis. The large and small aspiny interneurons of the striatum are spared. Other sites of neuronal loss also include the cerebral cortex and other basal ganglionic structures (27, 28).

Ten years after the original linkage analysis localized the HD defect to chromosome 4 (25), a collaborative team of investigators identified the disease-causing mutation as the expansion of a CAG repeat within the coding region of a novel gene, IT15 (29). The IT15 CAG repeat is polymorphic within the normal population (6 to 40 repeats) and expanded in HD patients (37 to 120 repeats) (19, 41, 67). As in other CAG-repeat disorders, there is an inverse correlation between the repeat size and the age of onset of clinical symptoms. In addition, the CAG repeat displays meiotic instability with a bias for expansion when transmitted through the paternal germline (19, 67). Analyses performed in a variety of species indicate that huntingtin is a ubiquitously expressed protein (44, 72) found in the cytoplasm of cells and associated with microtubules and/or synaptic vesicles (17) There are, however, reports of nuclear localization of huntingtin (16, 31), and the identification of a putative nuclear localization signal within the protein (6).

Substantial clinical and molecular evidence has suggested that the polyglutamine-induced neurodegenerative disorders are caused by a dominant gain-of-function acquired by the mutant protein. Additional indirect support of this hypothesis was recently provided by three independent groups who described the targeted disruption of the murine homologue of the Huntington's disease gene, Hdh, using transgenic technology (20, 53, 76). These groups have created null mutants by inserting a neomycin (neo) gene cassette into various positions within the Hdh gene.

Two of these studies found no phenotypic or neurological abnormalities in animals heterozygous for the Hdh null allele (20, 76) Conversely, transgenic animals heterozygous for a mutation within exon 5 (Hdhex5) that produce a truncated protein of approximately 20 kD display both increased spontaneous activity and cognitive deficits (53). In addition, the heterozygous Hdhex5 animals have an approximately 45% decrease in total neuron number within the subthalamic nucleus, a site of neuronal loss within HD patients (45). While the phenotypic and histopathological alterations found in the Hdhex5 heterozygotes are intriguing because of their similarity to HD, the lack of similar findings in the other heterozygous null mutant animals suggests that the truncated protein in the Hdhex5 mice may simply be acting as a toxic product. The absence of

neuropathology in animals heterozygous for either of the Hdh mutations that completely inactivate an allele suggests that the neuropathology seen in HD is not due to haplo-insufficiency, but more likely results from a gain-of-function aquired by the mutant protein containing the expanded polyglutamine tract.

Animals homozygous for each of the targeted null mutations show early postimplantion embryonic lethality (20, 53, 76). The Hdh nullizygous embryos develop normally up to embryonic day 6.5 (E6.5), at which time they contain the three distinct germ layers organized in an egg cylinder. The mutant embryos then become developmentally retarded and disorganized, and fail to undergo appropriate gastrulation. Virtually all homozygous mutant embryos die and undergo resorption by E10.5. One interpretation of these results is that huntingtin performs an essential function in gastrulation and that lack of this protein leads to embryonic lethality (20, 53). Alternatively, Zeitlin and colleagues (76) argue that huntingtin does not function in gastrulation per se, but rather is involved in counterbalancing apoptotic cell death during this developmental stage. This conclusion is based on their finding of increased regionalized apoptosis within the embryonic ectoderm of the nullizygous mice, a layer normally expressing high levels of Hdh. Although the wildtype function of huntingtin during early development remains a topic of debate, it is generally accepted that the huntingtin protein performs an essential function during early development.

Hodgson and colleagues (30) have recently reported the generation of a number of transgenic lines harboring yeast artificial chromosomes (YACs) containing the entire human HD locus (with a CAG repeat size of 18). Transgenic animals were generated by pronuclear injection of intact YACs, with insert sizes of either 600 or 350 kb, into fertilized mouse embryos. The obvious advantage of this type of strategy is that both the native promoter and genomic organization of the gene are preserved. Independent transgenic lines containing between 1 and 4 tandemly integrated copies of the YAC expressed human huntingtin at levels up to 3 times higher than endogenous murine huntingtin, in essentially a transgene copy number-dependent manner. Western blot analysis, subcellular fractionation, and immunohistochemical staining indicated that the human huntingtin protein had a tissue distribution and subcellular localization identical to that of endogenous murine huntingtin. The ultimate demonstration of appropriate temporal and spatial regulation of the human huntingtin was provided by the ability of the YAC transgene to rescue the homozygous lethal phenotype of Hdhex5 nullizygous animals. This study indicated that human huntingtin is functional in a murine background and that murine gene products neccesary for the appropriate expression, modification, and transport of the endogenous huntingtin are equally effective with the human protein. Therefore, if murine neurons have a similar susceptability to the detrimental effects of glutamine repeat expansion, a YAC-based transgenic approach is likely to be suitable for the generation of an accurate and representative animal model of the human disease.

In order to investigate the role of the expanded CAG tract in the pathogenesis of disease, and to examine the stability of these repeats in mice, Goldberg and colleagues (23) generated transgenic mice using the cytomegalovirus (CMV) promoter to direct the expression of a full length human HD cDNA (10.3 kb) containing an expanded repeat of 44 CAGs. Despite detectable levels of transgenic mRNA by both Northern blot and RT-PCR analysis, there was no evidence of a transgene-encoded protein product. Re-examination of the transgene indicated it contained a single base pair deletion at nucleotide 2349 which introduced a premature stop codon into its coding sequence. Morphometric analyses to determine the individual volumes and cell densities of the caudate, putamen, globus palidus, and subthalamic nucleus indicated no significant differences between transgenic and non-transgenic mice at 9 months of age. The findings of this study, although disappointing in not generating an HD animal model, did allow for important conclusions. The obtained results suggest that the pathogenesis of HD is not mediated via a DNA-protein interaction. Furthermore, abundant mRNA containing an expanded CAG repeat is insufficient to cause disease.

Mangiarini and colleagues (49), in an effort to model the CAG repeat tract instabilty in mice, generated transgenic animals carrying a 1.9 kb genomic fragment of the human HD locus including exon 1 which contained expansions of between 113 and 156 CAG repeats and approximately 1 kb of 5' flanking sequence (figure 1). The variability in the size of the CAG repeats within the construct used to generate these animals was due to repeat instability during its propagation in E. coli. Microinjection of fertilized embryos with this heterogeneous construct mixture resulted in the generation of a single founder animal (R6) with five independent sites of transgene integration. The founder animal and its offspring were bred to establish five independent lines, each with a single integration site but with varying lengths of CAG repeat tracts. Lines R6/0, R6/1, and R6/2 each contained a single copy of the transgene, but

with different CAG repeat lengths (142, 116, and 144 repeats, respectively). A single line, R6/5, contained 4 tandem copies of the transgene with repeat sizes ranging from 128 to 156 CAGs. Line R6/T contained a truncated transgene with no CAG repeat, and therefore, was not analyzed further.

Ubiquitous expression of the transgene was observed in lines R6/1, R6/2, and R6/5; there was no expression in R6/0 animals. Although expressed slightly lower than endogenous Hdh message, transgene mRNA levels varied in a tissue-specific manner comparable to the endogenous Hdh distribution. Western blot analysis performed using an antibody that recognizes expanded polyglutamine tracts in a length-dependent manner (73) detected transgene products in R6/2 and R6/5 cell lysates. The authors speculate that the transgene product is produced, but not detected in R6/1 animals due to its shorter polyglutamine tract length.

Surprisingly, the 1.9 kb transgene fragment was sufficient to generate a progressive neurological phenotype in the transgenic animals with features strikingly similar to HD in humans. Animals in the three lines that expressed the transgene with an intact CAG repeat developed neurological abnormalities in either the hemizygous (R6/1 and R6/2) or homozygous state (R6/5); R6/0 animals, which did not express the transgene, remained phenotypically normal to 14 months of age. Line R6/2 animals had the earliest onset of symptoms, 9-11 weeks, and die suddenly of unknown causes only 2-3 weeks thereafter. These animals had a complex phenotype which included resting tremor, chorea, involuntary movements, and severe handling-induced seizures. In addition, the mice made unusual chirping vocalizations when stressed. At weaning, R6/2 transgenic animals are indistinguishible from non-transgenic littermates. However, coincident with the onset of motor distrubances, animals begin to show weight loss and become emaciated, weighing only 30% to 40% of their normal weight by the time of their death. There is no histological evidence of myopathy. R6/2 females have atrophic ovaries, abnormal uteri, and are sterile; males have reduced fertility. Hepatic abnormalities including polyploid hepatic nuclei and loss of cytoplasmic mass, in the absence of cell death, were noted on autopsy. The other expressing transgenic lines had later ages of onset, milder progression of symptoms, and reduced fertility.

Neuropathological examinations of R6/2 animals displaying severe behavioral abnormalities were performed. All CNS structures were uniformly smaller than those of age-matched control animals. There were no findings of focal areas of malformation or neurodegeneration. Detailed analyses of the cerebral cortex, hippocampus, basal ganglia, cerebellum, and spinal cord revealed no morphological abnormalities or evidence of neurodegeneration.

The findings of Mangiarini and colleagues (49) are consistent with those of Ikeda et al., (33) suggesting that a fragment of an expanded allele encoding the polyglutamine tract is sufficient to generate disease. However, the question remains as to the relevance of these transgenic models to the corresponding human disease. Previous studies do not support the concept that the mutant huntingtin protein is cleaved in tissues from affected individuals (3, 64, 72). It will be critical to determine if the expression of a transgene carrying the entire huntingtin coding sequence retains the ability to induce a neurologic phenotype. In addition, it remains unclear if expression of the HD transgene (49) was driven by promoter sequences present in the 5' flanking region of the transgene, or if endogenous murine sequences at the sites of integration contributed to its expression. Accordingly, the generation of transgenic animals containing larger flanking sequences will assist in addressing this concern. The results of these additional transgenic experiments should help to assess the possiblity of increased potency of truncated proteins containing expanded polyglutamine tracts, and help to identify additional elements that function to establish the cell-specific pathology characteristic of HD.

Spinocerebellar ataxia type 1

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant disorder characterized by progressive gait and limb ataxia, dysarthria, dysmetria, nystagmus, and variable degrees of muscle wasting (78). SCA1 typically is an adult onset disorder that progresses slowly over 2 to 3 decades before resulting in death due to bulbar dysfunction. There are, however, juvenile-onset cases which show a much more rapid progression of symptoms (26, 79). The neuropathological findings of SCA1 include the severe loss of Purkinje cells of the cerebellar cortex, as well as neuronal loss within the deep cerebellar nuclei, pontine nuclei, inferior olives, and selective motor cranial nerve nuclei (78). It should be noted that these findings are variable, even within the same kindred, and numerous neuropathological variants have been recognized.

SCA1 is caused by the expansion of a CAG repeat within a novel gene located on chromosome 6p23 (56). The SCA1 CAG tract, like the repeat tracts found in other CAG disease-associated genes, is highly polymorphic within the normal population ranging in size

between 6 and 39 repeats (12, 35, 56, 61). The mutant CAG tract is expanded, ranging in size between 40 and 83 triplets. While the repeats within the normal size range are meiotically stable, expanded repeat tracts often undergo expansion or contraction when transmitted from parent to offspring. Paternally transmitted alleles display a bias for expansion (12). There also is evidence of an inverse correlation between repeat length and age of onset of disease.

The SCA1 gene encodes a novel protein, ataxin-1, with no homologies to previously identified molecules (5, 56). In situ hybridization analysis performed in the mouse indicates that the murine homologue, Sca1, is ubiquitously expressed. Interestingly, there is a transient burst of Sca1 expression within the cerebellar Purkinje cells on postnatal day 14, a time when the murine cerebellum is beginning to become physiologically functional (4). Immunohistochemical analyses performed with ataxin-1 antisera indicate that ataxin-1 has nuclear localization in most neurons, with the notable exception of Purkinje cells, where it is predominantly nuclear, but also displays some cytoplasmic localization (66). Non-neuronal cell types show cytoplasmic localization of the protein.

As part of an effort to understand the pathogenic basis of SCA1, we have generated transgenic animals expressing either normal (30 CAGs) or expanded (82 CAGs) alleles of SCA1 (figure 1) (9). Transgenic animals were generated using the murine Purkinje cell-specific promoter, Pcp-2 (55, 74), to achieve high levels of transgene expression within the cerebellar Purkinje cells, a primary site of pathology in SCA1-affected individuals. While transgenic lines that expressed the normal, unexpanded SCA1 allele had no neurological disease, animals in 5 independent transgenic lines expressing the expanded allele developed adult-onset ataxia and Purkinje cell degeneration reminiscent of human SCA1. The only 82 repeat-containing transgenic line that did not develop these neurological abnormalities failed to express the SCA1 transgene, thereby demonstrating that expression of the mutant allele is required for pathogenesis. Moreover, this study indicated that the expanded polyglutamine tract, in the context of the full-length ataxin-1 protein, is capable of inducing Purkinje cell pathology and a neurologic phenotype when expressed in transgenic mice.

To gain further insight into the disease progression in the SCA1 transgenic mice, animals from line B05 (containing the SCA1 transgene with 82 CAGs) have been subjected to a battery of behavioral tests which measure motor skill and activity (14). Transgenic animals of the B05 line develop progressive loss of cerebellar function. The behavioral abnormalities first become apparent at five weeks of age when B05 animals display a limited ability to improve their motor performance on the rotating rod apparatus. At this age, the B05 animals perform as well as non-transgenic animals on the first day of trials, but show impaired perfomance improvement on successive days of trials. The absence of abnormalities in gait (footprint analysis), motor activity (open field test), and balance and coordination (bar-cross and full speed rotating rod tests) in five week old B05 animals support the conclusion that the impairment on the rotating rod (using a motor learning paradigm) was due to a decreased ability of the B05 animals to learn the motor task, as opposed to deficits in motor activity, fine motor control, or coordination.

The behavior abnormalities of transgenic animals expressing the mutant SCA1 allele progressively worsen with age. B05 transgenic animals become visibly ataxic, as assessed by home cage behavior at 12 weeks of age. At this age, transgenic animals show more substantial motor learning and coordination deficits, but are able to show moderate improvement with training. By one year of age, B05 animals are profoundly ataxic, and are unable to match the performance of age-matched wildtype animals, even on the first day of rotating rod testing or to improve their performance with training. In addition, one-year-old B05 animals were unable to perform any of the behaviors on the bar-cross apparatus, typically falling off immediately upon being placed on the wide platform (14). These results suggest that the cerebellar dysfunction of the transgenic animals can be divided into two phases; an initial phase which is limited to an impairment of motor skill improvement, and a second phase, which is characterized by deficits in motor activity and coordination.

To correlate the phenotypic abnormalities of transgenic animals expressing the mutant SCA1 allele with the progression of pathologic alterations, we have performed several immunohistochemical and morphometric analyses. These studies indicated that transgenic animals have normal cerebellar development and then develop progressive pathological changes within the cerebellum. The first pathologic changes are the appearance of cytoplasmic vacuoles within the Purkinje cells at approximately postnatal day 25 (P25). By electron microscopic analysis, these vacuoles appear to be aqueous compartments formed by distention of the endoplasmic reticulum. At the time of the onset of the motor learning impairment, there are occasional instances of simplification of the proximal dendrites of

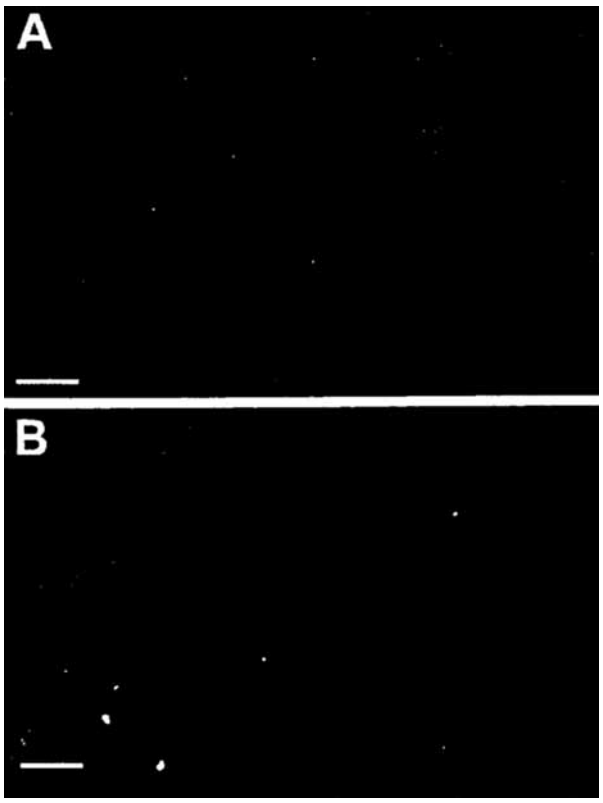


Figure 2. Calbindin immunohistochemistry of the cerebellum of SCA1 (B05/+) transgenic mice at different stages of development. A) At 16 days of age there is normal development of the cerebellar cortex with all Purkinje cells located at the interface of the internal granular and molecular layers. B) At 24 weeks of age there is shrinkage of the molecular layer with atrophy of Purkinje cell dendrites and frequent Purkinje cell perikarya heterotopically located itn the molecular layer. Loss of Purkinje cells is not extensive. Occasional Purkinje cells are hypertrophic. (scale bars = 150 μm)

some Purkinje cells (figure 2). This finding suggests that expression of mutant ataxin-1 affects the maintenance of dendrites and spines. Morphometric analysis of transgenic animals at 12 weeks of age, the time when B05 animals are first beginning to be ataxic, indicates that there is minimal Purkinje cell loss, but substantial alterations in dendritic complexity. Other findings include gliosis and mild shrinkage of the molecular layer. In addition, there is evidence of heterotopic Purkinje cells within the molecular layer, a finding not seen in younger animals. These changes become more widespread and severe as the animals age. Purkinje cell count experiments performed on 24 week old B05 animals indicated an approximately 25-35% reduction in Purkinje cell number, with as many as 10-15% of the remaining Purkinje cells showing heterotopic localizations.

As described above, a striking feature of the B05 transgenic animals was the presence of numerous Purkinje cells with their perikarya heterotopically located in the intermediate levels of the molecular layer . Histological examination of early postnatal time points indicated no evidence of abnormally localized cells, however, by the time of ataxia, heterotopic cells were frequently observed (figure 2) (9, 14). These results indicated that heterotopic positioning of Purkinje cells occurs sometime after completion of cerebellar development. A study of the dendritic changes observed in the transgenic animals has revealed a likely explanation for the occurence of these heterotopic cells. Many Purkinje cells have simplification of the proximal dendrites with loss of branches and dendritic spines as early as six weeks of age. Loss of synaptic input in the proximal parts of the dendritic tree, close to the perykaryon, would make it difficult for the cell to generate an action potential. A compensatory mechanism to maintain the electrical activity in the Purkinje cell might be to retract the non-functional denuded dendritic trunk. Because the morphologically perserved distal dendrites maintain contact with parallel fibers near the pial surface, a retraction of the dendritic trunk involves movement of the perikaryon into the molecular layer concomitant with axon elongation. This hypothesis also provides an explanation for the increased frequency of Purkinje cells with two primary dendrites, and suggests a mechanism that includes the resorption of the denuded primary dendrite to a major branch point. Purkinje cells with this morphology were frequently seen hetertopically located within the molecular layer of older B05 animals (figure 3). Purkinje cell dendritic simplification and loss of spines has been reported in pathologic studies of SCA1 necropsy material (21, 39). In addition, examination of a juvenile-onset case of SCA1, where death occurred prior to extensive Purkinje cell loss, detected rare Purkinje cells heterotopically located in the intermediate levels of the molecular layer (H.B.C., unpublished data). These findings indicate that the SCA1 transgenic animals appropriately model at least some of the pathologic changes seen in the human disease.

The results of this study clearly demonstrate that expression of the mutant form of ataxin-1, in a cell type that is vulnerable in the human disease, can lead to cellular dysfunction sufficient to induce ataxia. An important observation of this study is that the cell dysfunction and phenotypic abnormalities preceded significant Purkinje cell death. Therefore, we conclude that disease in SCA1 transgenic mice is not caused by cell death

per se, rather, the loss of Purkinje cells seen at later stages of disease is likely the result of cell dysfunction induced at an earlier stage.

Conclusions

Transgenic animals displaying abnormal neurologic phenotypes have been generated for SCA1 (9), MJD (33), and HD (49). The ability to induce a phenotype by transfer of the mutant gene, along with a lack of neuropathology seen in Hdh heterozygotes (20, 76), provides strong evidence that CAG repeat disorders are caused by a gain-in-function pathogenic mechanism. A variety of evidence suggests that this gain-in-function, which ultimately results in cell death, is mediated by the mutant protein. Among this evidence is the finding that animals containing transgenes with expanded CAG repeats but failing to express the transgene are phenotypically and histologically normal (9, 23, 49).

The neurons of mice appear to be less vulnerable to the effects of polyglutamine repeat expansion than human neurons. Phenotypic and pathogenic alterations were detected in animals that either over-expressed the mutant protein (9, 33) or expressed a mutant protein with an exaggerated repeat length (49). It is not yet clear whether this is due to reduced sensitivity to the detrimental effects of the extended polyglutamine tracts, or simply to drastic differences in the lifespan of mice and humans. It is possible that there may be cumulative effects of years of exposure to the polyglutamine tracts that result in cell dysfunction and death. For these reasons, the development of transgenic models using endogenous promoters and repeat tract lengths corresponding to the sizes that are pathogenic in humans may be difficult.

The SCA1 and HD transgenic studies suggest that cell dysfunction precedes significant cell death (9, 14, 49). This raises an important issue concerning the way that neurodegenerative diseases are traditionally considered. Neurodegenerative diseases have been defined as such based upon the neuropathological findings observed at the time of the affected patients' deaths. A conclusion drawn from such observations is that the disease itself is the result of the death of certain neuronal populations. A second conclusion, perhaps incorrectly based upon the first, is that the disease can be treated or perhaps even averted by preventing the occurrence of neuronal death. This second inference is probably incorrect for most neurodegenerative diseases. It is likely that neuronal death in many neurodegenerative diseases only occurs after long periods of cellular dysfunction that may or may not have morphological correlates.

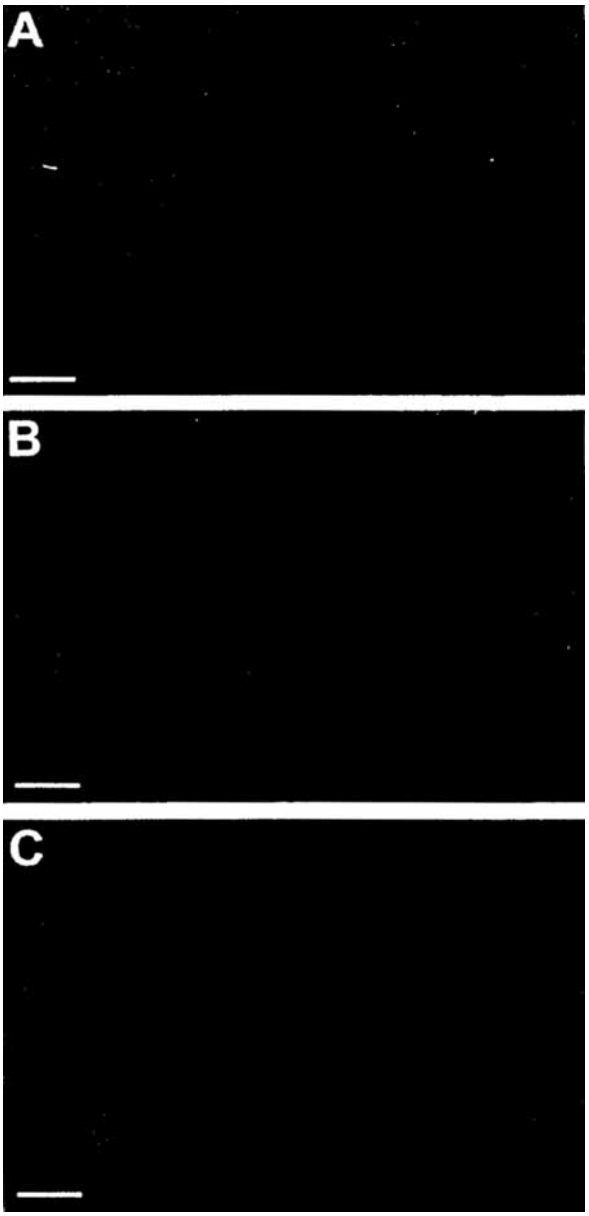


Figure 3. Calbind n immunohistochemistry of the cerebellum of SCA1 (B05/+) transgenic mice at different stages of development showing progressive Purkinje cell changes. A) As early as 6 weeks of age there is evidence of loss of proximal dendritic processes is some Purkinje cells. Distal arborization remains more complex. (scale bar = 25 μm) B) At 15 weeks there is significant alteration of dendritic morphology of Purkinje cells including heterotopic cell somata and loss of dendritic branches and spines. (scale bar = 35 μm) C) At 28 weeks, the loss of dendritic complexity is even more pronounced and there are more heterotopic Purkinje cells. (scale bar = 35 μm)

Certainly in the SCA1 transgenic mice, the conclusion that must be reached is that the affected Purkinje cells

are pathophysiologically and morphologically altered sufficiently to produce clinical disease long before there is any significant loss of the Purkinje cell population. Similar findings are apparent in the HD transgenic mice (49). Therefore, the successful treatment of SCA1, HD, and other neurodegenerative diseases will likely require understanding and correction of the underlying mechanisms of cell dysfunction rather than simply abrogating cell death.

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