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A new humanized ataxin-3 knock-in mouse model combines the genetic features, pathogenesis of neurons and glia and late disease onset of SCA3/MJD

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ABSTRACT

Spinocerebellar ataxia type 3 (SCA3/MJD) is a neurodegenerative disease triggered by the expansion of CAG repeats in the *ATXN3* gene. Here, we report the generation of the first humanized ataxin-3 knock-in mouse model (Ki91), which provides insights into the neuronal and glial pathology of SCA3/MJD. First, mutant ataxin-3 accumulated in cell nuclei across the Ki91 brain, showing diffused immunostaining and forming intranuclear inclusions. The humanized allele revealed expansion and contraction of CAG repeats in intergenerational transmissions. CAG mutation also exhibited age-dependent tissue-specific expansion, which was most prominent in the cerebellum, pons and testes of Ki91 animals. Moreover, Ki91 mice displayed neuroinflammatory processes, showing astrogliosis in the cerebellar white matter and the substantia nigra that paralleled the transcriptional deregulation of Serpina3n, a molecular sign of neurodegeneration and brain damage. Simultaneously, the cerebellar Purkinje cells in Ki91 mice showed neurodegeneration, a pronounced decrease in Calbindin D-28 k immunoreactivity and a mild decrease in cell number, thereby modeling the degeneration of the cerebellum observed in SCA3. Moreover, these molecular and cellular neuropathologies were accompanied by late behavioral deficits in motor coordination observed in rotarod and static rod tests in heterozygous Ki91 animals. In summary, we created an ataxin-3 knock-in mouse model that combines the molecular and behavioral disease phenotypes with the genetic features of SCA3. This model will be very useful for studying the pathogenesis and responses to therapy of SCA3/MJD and other polyQ disorders.

Introduction

Spinocerebellar ataxia 3 (SCA3), also called Machado-Joseph disease (MJD), is a dominantly inherited disease resulting from the expansion of CAG repeats in exon 10 of the *ATXN3* gene (Kawaguchi et al., 1994) (MJD & ATXN3: OMIM 109150 & 607047). Healthy individuals present a non-pathogenic number of repeats, usually between 13 and 41 CAGs (Giunti et al., 1995), whereas SCA3 patients typically express 60–82 CAG repeats in one allele of *ATXN3*. The presence of this mutant allele evokes motor abnormalities, such as ataxia, parkinsonism, sensory loss, spasticity and ocular symptoms, which become evident in the third or fourth decade of life (Riess et al., 2008).

The mechanisms of pathogenesis in SCA3 and other polyglutamine (polyQ) diseases have been thoroughly discussed (Paulson, 2012; Switonski et al., 2012). In brief, the relevant pathogenesis of SCA3 is based on the toxic function of the mutant ataxin-3 protein (Riess et al., 2008), but the exact mechanism of the disease remains elusive. Mouse models of polyQ diseases have been very useful in exploring the pathogenesis and therapies of this disease (for review: (Figiel et al., 2012)). To date, 14 models and variants of SCA3 mouse models have been created, but the majority of them express cDNA driven by unrelated promoters, such as Purkinje-specific L7, PrP, rHTT and CMV promoters (Bichelmeier et al., 2007; Boy et al., 2010; Boy et al., 2009; Cemal et al., 2002; Goti et al., 2004; Ikeda et al., 1996; Silva-Fernandes et al., 2010). In addition, a C-terminal ataxin-3 cDNA genetrap model, without the N-terminal region of the protein and without a CAG repeat tract, has recently demonstrated ataxia-like changes (Hübener et al., 2011). All of these models have reproduced many features of SCA3 pathogenesis. However, the models show an unnatural expression pattern, both in tissues and during development, due to the use of unrelated promoters, the lack of regulatory flanking sequences or the presence of an excessive number of transgene copies. In addition, these models still express mouse wild-type (WT) ataxin-3, so it is difficult to precisely investigate its influence during disease. The only full gene model is the YAC transgenic (Cemal et al., 2002), which contains regulatory sequences of human origin and the mouse ataxin-3 gene. To create a

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truly valid SCA3 mouse, it is necessary to generate a modified mouse knock-in allele. Possible strategies involve the insertion of long CAG repeats into the *Atxn3* gene or creating a modification of mouse *Atxn3* that leads to the full humanization of the allele's coding sequence to produce a human ataxin-3 mutant protein.

Here, we report the successful generation of a knock-in SCA3 mouse that contains a humanized version of the mouse *Atxn3* gene and expresses the human ataxin-3 protein containing 91 glutamines. We reveal that the SCA3 Ki91 knock-in model shows molecular and cellular features of SCA3 pathogenesis. Moreover, Ki91 mice exhibit a late SCA3 disease onset, which manifests as deficits in coordination in the rotarod and static rod tests. These late cellular and behavioral phenotypes are consistent with the human SCA3 condition.

Results

Structure of the targeted Atxn3 gene and the expression of Ataxin-3 in targeted ES cells

To generate SCA3 knock-in mice, we replaced the 3′ fragment of the mouse *Atxn3* gene with the equivalent human coding sequence, which contains a CAG expansion in exon 10 (Fig. 1A). Analysis of the human and mouse ataxin-3 protein sequences showed that the N-terminus of the ataxin-3 protein, which is encoded by exons 1 through 6, is virtually identical in mice and humans (Suppl. Fig. S1). This homology indicates that a possible strategy to obtain a humanized locus could involve exchanging the less homologous 14 kb of the *Atxn3* genomic sequence containing exons 7 through 11 with human cDNA containing human exons 7 through 11 (for a description of the targeting cassette, see Materials and Methods and Fig. 1A).

Human cDNA was derived from the GM06153 line of human fibroblasts (Coriell Cell Repository; Camden, NJ, USA) containing 69 CAG repeats, which expanded to 91 CAG repeats while processing the targeting vector. Furthermore, the modified mouse *Atxn3* allele contained four SNP variants that are present in human GM06153 fibroblasts (Fig. 1A).

Homologous recombination in the *Atxn3* locus changed the PstI and EcoRV restriction fragment lengths, and targeted clones were identified by Southern blotting (Fig. 1B). Prior to blastocyst injection, the positive clone (1H8) was analyzed by RT-PCR and western blotting. These analyses revealed the presence of both WT and mutant ataxin-3 transcripts and WT and mutant ataxin-3 protein expression (Figs. 1C and D). Moreover, antibodies against the expanded polyglutamine stretch (1C2) detected an approximately 67-kDa band corresponding to the mutant ataxin-3 protein (Fig. 1D). The presence of this band indicates that CAG expansion was translated into the polyglutamine domain.

Intergenerational instability of the CAG mutation in Ki91 mice

We established a colony of Ki91 animals originating from four NEOR-free heterozygous animals. These mice were from the F2

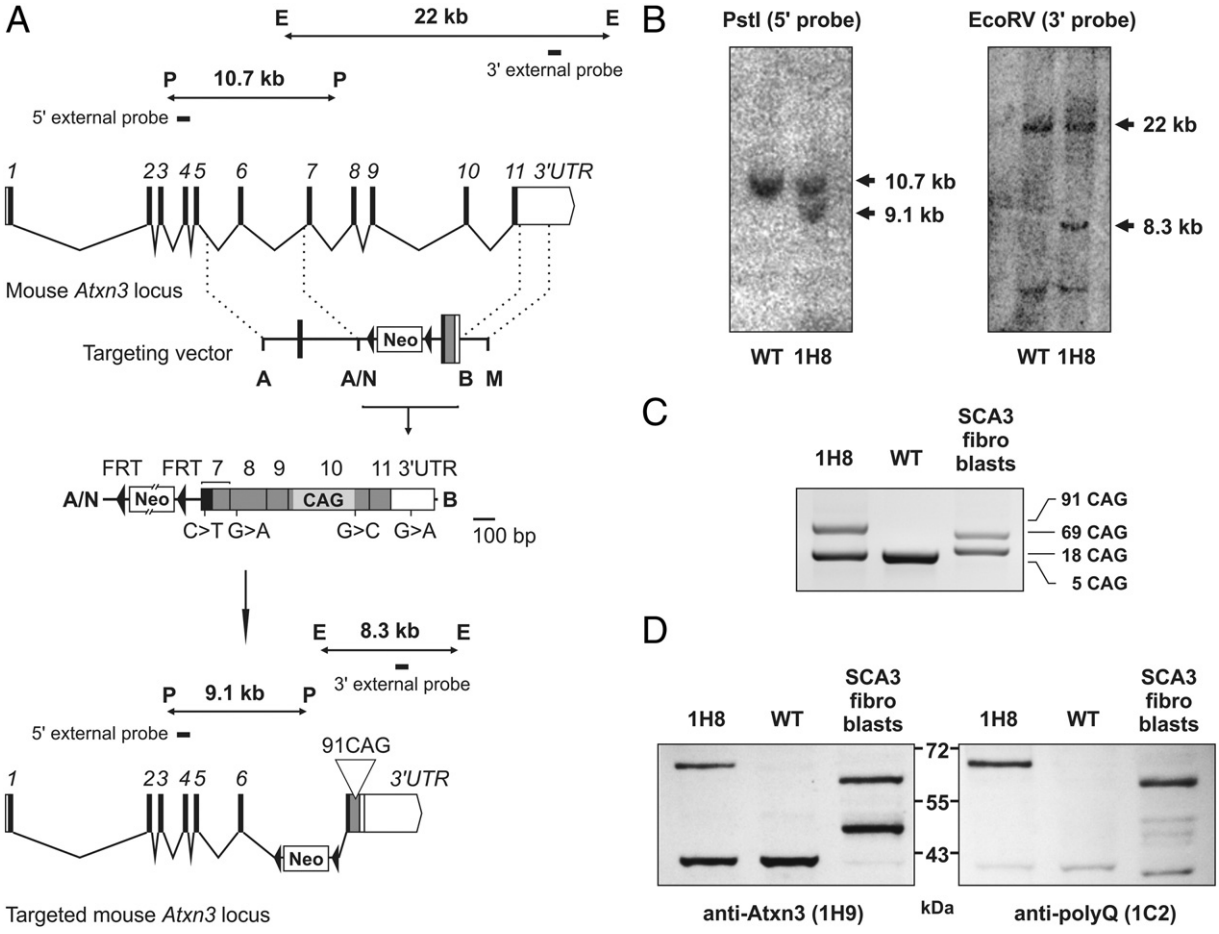

Fig. 1. Targeted modification of the mouse ataxin-3 gene. (A) The targeting vector, containing the hybrid mouse/human exon 7, human exons 8–11 (along with 91 CAG repeats in exon 10) and a fragment of the human 3′ UTR, was used to replace the corresponding mouse sequence. The modified allele contains four SNP variants that are present in human fibroblasts and were used as a source for the cDNA sequence. (B) Modified ES cells showed variable lengths of the PstI and EcoRV restriction genomic fragments, indicating correct homologous recombination (clone 1H8). The clones were identified by Southern blot using two probes located outside the homology arms. (C) The mutant ataxin-3 transcript with 91 CAG repeats along with WT ataxin-3 transcripts was present in an RT-PCR analysis of the 1H8 clone. (D) Modified ES cells expressed both WT and mutant ataxin-3 proteins, which appear as immunoblot bands of 41 and 67 kDa, respectively, using anti-ataxin-3 and anti-polyQ antibodies. Abbreviations: restriction sites EcoRV (E), PstI (P), AvrII (A), BglI (B) and MfeI (M); A/N – sequence generated after AvrII and NheI ligation.