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Abnormal motor function and dopamine neurotransmission in DYT1 ΔGAG transgenic mice

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Abstract

A single GAG deletion in Exon 5 of the *TOR1A* gene is associated with a form of early-onset primary dystonia showing less than 40% penetrance. To provide a framework for cellular and systems study of DYT1 dystonia, we characterized the genetic, behavioral, morphological and neurochemical features of transgenic mice expressing either human wild-type torsinA (hWT) or mutant torsinA (hMT1 and hMT2) and their wild-type (WT) littermates. Relative to human brain, hMT1 mice showed robust neural expression of human torsinA transcript (3.90×). In comparison with WT littermates, hMT1 mice had prolonged traversal times on both square and round raised-beam tasks and more slips on the round raised-beam task. Although there were no effects of genotype on rotarod performance and rope climbing, hMT1 mice exhibited increased hind-base widths in comparison to WT and hWT mice. In contrast to several other mouse models of DYT1 dystonia, we were unable to identify either torsinA- and ubiquitin-positive cytoplasmic inclusion bodies or nuclear bleb formation in hMT1 mice. High-performance liquid chromatography with electrochemical detection was used to determine cerebral cortical, striatal, and cerebellar levels of dopamine (DA), norepinephrine, epinephrine, serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid. Although there were no differences in striatal DA levels between WT and hMT1 mice, DOPAC and HVA concentrations and DA turnover (DOPAC/DA and HVA/DA) were significantly higher in the mutants. Our findings in DYT1 transgenic mice are compatible with previous neuroimaging and postmortem neurochemical studies of human DYT1 dystonia. Increased striatal dopamine turnover in hMT1 mice suggests that the nigrostriatal pathway may be a site of functional neuropathology in DYT1 dystonia.

Keywords: Dystonia; Real-time RT-PCR; *TOR1A*; Nigrostriatal; Dopamine

Introduction

Dystonia is defined as a syndrome of involuntary muscle contractions, frequently causing twisting, repetitive movements and abnormal postures (Fahn, 1988). Dystonia is a common movement disorder, affecting over 300,000 people in the United States alone. Primary dystonia is a designation in which dystonia is the sole presenting disorder, without any underlying disease other than tremor in some cases (Fahn et al., 1998). Early-onset primary dystonia is characterized by twisting of the limbs, typically with onset in the distal leg, which may spread to involve writhing movements and fixed postures in other regions of the body. Symptoms usually appear in childhood; onset before 4 or after 28 years is uncommon (Bressman et al., 1998, 2000). Many cases of early-onset primary dystonia are associated with a GAG deletion in the *TOR1A* gene, which results in a single absent glutamic acid residue near the C-terminus of the encoded protein torsinA. DYT1 dystonia is inherited in an autosomal dominant fashion with less than 40% penetrance.

Postmortem neuropathological studies of brains from subjects with primary dystonia have failed to reveal any consistent evidence of neuronal loss, inflammation, or neurodegeneration. These findings suggest that functional and/or ultrastructural abnormalities, rather than neurodegenerative changes underlie dystonic symptoms (Rostasy et al., 2003). Recent morphological

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and biochemical studies have pointed to the dopaminergic system as a site of potential pathophysiological significance in human DYT1 dystonia. Rostasy et al. (2003) noted an increase in nigral cell density along with somatic enlargement of nigral dopaminergic neurons in humans with DYT1 dystonia. A significant increase in the turnover of dopamine, expressed as the ratio of 3,4-dihydroxyphenylacetic acid to dopamine (DOPAC/DA), as well as a reduction in dopamine D1 and D2 receptor binding has also been reported in DYT1 dystonic striatum (Augood et al., 2002; Asanuma et al., 2005). These findings suggest an imbalance in dopaminergic neurotransmission and lend credence to the idea of a functional disturbance in patients with DYT1 dystonia. Interestingly, *in situ* hybridization and immunocytochemical studies have revealed high-level torsinA protein expression within dopaminergic neurons of the substantia nigra pars compacta and cholinergic interneurons of the striatum (Konakova et al., 2001; Konakova and Pulst, 2001; Walker et al., 2001; Augood et al., 2003; Oberlin et al., 2004; Xiao et al., 2004; Vasudevan et al., 2006). Furthermore, torsinA has been shown to protect dopaminergic neurons from oxidative stress (Kuner et al., 2004; Cao et al., 2005). These results, together with neurochemical findings in dystonic human brain, imply a supportive role for torsinA whereby a mutant and non-functional protein could lead to aberrant dopamine turnover/neurotransmission and the subsequent development of dystonia.

The above noted dopaminergic aberrations in human DYT1 dystonia are somewhat weakened by the limited number of DYT1 brains studied (Augood et al., 2002; Rostasy et al., 2003; Asanuma et al., 2005). Fortunately, animal models of DYT1 dystonia can be used to robustly interrogate findings in humans and open doors to novel avenues of study. In this regard, Sharma et al. (2005) have developed two lines of transgenic mice which harbor mutant (ΔGAG) transgenes (hMT1 and hMT2) along with mice which harbor the wild-type human *TOR1A* transgene (hWT). Transgenic mice which express mutant torsinA show reduced ability to learn motor skills in an accelerating rotarod paradigm at 6 months of age as well as abnormal dopaminergic D2 receptor responses in striatal cholinergic interneurons (Pisani et al., 2006). Furthermore, amphetamine-induced dopamine release is attenuated in this model (Balcioglu et al., 2007). In the work described herein, we rigorously characterize the genetic, behavioral, morphological and neurochemical features of these transgenic models. Specifically, with species-specific primers and probes, quantitative real-time RT-PCR (QRT-PCR) was used to determine the relative expression levels of human and mouse torsinA transcripts in each transgenic line. Next, a comprehensive battery of behavioral tests was used to identify motor abnormalities. Electron and confocal microscopy were employed to evaluate previous reports of neuronal nuclear bleb formation and ubiquitin-positive cytoplasmic inclusions, respectively, in human DYT1 dystonia and other murine models of DYT1 dystonia (McNaught et al., 2004; Dang et al., 2005; Goodchild et al., 2005; Shashidharan et al., 2005; Grundmann et al., 2007). Finally, high-performance liquid chromatography with electrochemical detection (HPLC-EC) was carried out to comprehensively quantify monoaminergic neurotransmitters and their metabolites in multiple neural structures.

Methods

All experiments were approved by the University of Tennessee Health Science Center Animal Care and Use Committee and performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Mice were maintained in a temperature-controlled environment with free access to food and water. Light was controlled on a 12 h light/dark cycle; lights on at 6:00 am.

DYT1 transgenic mouse model and genotyping

Breeding colonies of human wild-type (hWT) and mutant torsinA (hMT1 and hMT2) mice were established at the University of Tennessee Health Science Center (UTHSC) by matings with wild-type (WT) C57BL/6 mice. All mice used in the experiments were C57BL/6 backcrossed at least 8×. Tail DNA from the breeders and their offspring were isolated using the AquaPure Genomic DNA Tissue Kit (Bio-Rad Laboratories, Hercules, CA, USA) for genotyping. Two primers, 5′- CACATTGCACTTTCCACATGCT -3′ and 5′- GTTTTGCAGCCTTTATCTGA- 3′, amplified a 560 bp segment (35 cycles; annealing temperature 60 °C) within the human torsinA coding sequence that was identified via 1% agarose gel electrophoresis (Fig. 1A). The genotype of the breeders was confirmed by restriction digestion with BseRI (New England BioLabs, Ipswich, MA, USA). The human WT torsinA PCR product was digested with BseRI into four fragments (279 bp, 238 bp, 24 bp, and 22 bp). The human mutant torsinA PCR product was digested with BseRI into three fragments (279 bp, 259 bp, and 22 bp); fragment profiles were then identified with 2% agarose gel electrophoresis (Fig. 1B).

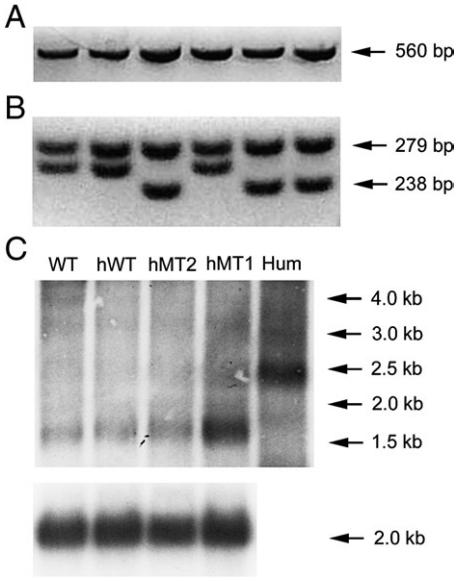

Fig. 1. Genotyping and Northern blot analysis of hWT, hMT1, and hMT2 transgenic mice. Gel images of PCR products before (A) and after (B) digestion are shown. Products from hMT1 or hMT2 mice appear in lanes 1, 2 and 4 while products from hWT mice appear in lanes 3, 5 and 6. (C) Northern blot for human torsinA in WT, hWT, hMT1, and hMT2 mice. Mouse β-actin loading controls appear below each lane. Hum, human mRNA.