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MEMORY DEFICITS, GAIT ATAXIA AND NEURONAL LOSS IN THE HIPPOCAMPUS AND CEREBELLUM IN MICE THAT ARE HETEROZYGOUS FOR PUR-ALPHA

MARY F. BARBE, [a,c*] **JESSICA J. KRUEGER,** [c] **REGINA LOOMIS,** [c] **JESSICA OTTE** [b,c] **AND JENNIFER GORDON** [b,c]

[a] *Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA, USA*

[b] *Department of Neuroscience and Center for Neurovirology, Temple University School of Medicine, Philadelphia, PA, USA*

[c] *Comprehensive NeuroAIDS Center, Temple University School of Medicine, Philadelphia, PA, USA*

Abstract—Pur-alpha is a highly conserved sequence-specific DNA and RNA binding protein with established roles in DNA replication, RNA translation, cell cycle regulation, and maintenance of neuronal differentiation. Prior studies have shown that mice lacking Pur-alpha (−/−) display decreased neurogenesis and impaired neuronal differentiation. We sought to examine for the first time, the behavioral phenotype and brain histopathology of mice that are heterozygous (+/−) for Pur-alpha. Standardized behavioral phenotyping revealed a decreased escape response to touch, limb and abdominal hypotonia, and gait abnormalities in heterozygous Pur-alpha (+/−) mice, compared to wild-type (+/+) littermates. Footprint pattern analyses showed wider-based steps, increased missteps and more outwardly rotated hindpaws in heterozygous Pur-alpha (+/−) mice, suggestive of cerebellar pathology. The Barnes maze and novel object location testing revealed significant memory deficits in heterozygous Pur-alpha mice, suggestive of hippocampal pathology. Quantitative immunohistochemical assays of the vermal region of the cerebellum and CA1-3 regions of the hippocampus revealed reduced numbers of neurons in general, as well as reduced numbers of Pur-alpha+-immunopositive neurons and dendrites in heterozygous Pur-alpha mice, compared to wild-type littermates. Past studies have implicated mutations in Pur-alpha in several diseases of brain development and neurodegeneration. When combined with these new findings, the Pur-alpha heterozygous knockout mice may provide an animal model to study mechanisms of and treatments for Pur-alpha-related cognitive deficiencies and neuropathology.

*Corresponding author. Address: Department of Anatomy and Cell Biology, Comprehensive NeuroAIDS Center, 3500 North Broad Street, Temple University School of Medicine, Philadelphia, PA 19140, USA.

E-mail addresses: mary.barbe@temple.edu, mbarbe@temple.edu (M. F. Barbe).

Abbreviations: FMRP, FMR protein; FXTAS, Fragile X Tremor Ataxia Syndrome; HAND, HIV-1 associated neurological disease.

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INTRODUCTION

The Pur-alpha protein is required for neuronal development and differentiation. Pur-alpha binds to single-stranded DNA and RNA in a sequence-specific manner (binding to GGN repeats within GC rich regions) and is a member of a protein family strongly conserved from bacteria through humans (Gallia et al., 2000). It is a multifunctional protein essential for DNA replication and gene transcription (see Gallia et al., 2000; Johnson et al., 2006, 2013) and is a critical component in the compartmentalized translation of RNA (Ohashi et al., 2002; Johnson et al., 2006). Pur-alpha is a widely expressed ubiquitous protein that exhibits both developmental and tissue-specific regulation (Khalili et al., 2003; Hokkanen et al., 2012; Mishra et al., 2013). For example, while Pur-alpha protein levels are quite low in brain during embryogenesis, its levels quickly rise after the first postnatal week in the mouse and reach their peak levels at 18 days postnatal. Pur-alpha is then maintained at relatively high levels in the adult brain (Khalili et al., 2003). In addition to its nuclear function, Pur-alpha is detected in the cytoplasm of neurons, particularly in dendrites at branch points. Several studies have detected Pur-alpha in complex with polyribosomes and heterogeneous nuclear ribonucleoproteins (hnRNP) (Kanai et al., 2004).

Pur-alpha protein plays an important role in genome maintenance and has specialized functions for regulating gene expression in specific organs. In the CNS, its role in mRNA transport and translation have led to the elucidation that Pur-alpha is a major contributor in this regard, particularly in neurons (Li et al., 2001; Ohashi et al., 2002; Johnson, 2003; Kanai et al., 2004; Johnson et al., 2006; Jin et al., 2007). Mutations of Pur-alpha may be involved in several diseases of brain development and neurodegeneration. For example, Fragile X Tremor Ataxia Syndrome (FXTAS), a neurodegenerative syndrome with progressive intention tremor, gait ataxia and cognitive decline, is characterized by ubiquitin-positive intranuclear inclusions throughout the brain in which Pur-alpha is present, as well as a marked dropout of cerebellar Purkinje cells (Hagerman et al., 2005; Jin et al., 2007). A trinucleotide CGG expansion repeat located in the 5′-untranslated region (UTR) of the Fragile X Mental Retardation gene (FMR1), leading to

chromosomal instability and loss of expression of the encoded FMR protein (FMRP), is the underlying cause of the Fragile X Syndrome (Darnell et al., 2001, 2009). FMRP regulates mRNA transport in neurons and has been found in complex with mRNAs together with Pur-alpha and non-coding RNA molecules in dendrites thus suggesting their role in regulating RNA transport (Kobayashi et al., 2000; Kanai et al., 2004; Johnson et al., 2006). Next a role for Pur-alpha has been demonstrated in the regulation of amyloid precursor protein (APP), which is located on chromosome 21 and implicated in the pathogenesis of Down's syndrome at both transcriptional and post-transcriptional levels (Darbinian et al., 2008; Wiseman et al., 2015). Recent studies have also identified de novo mutations in the PURA gene, which encodes Pur-alpha, that are associated with neonatal hypotonia, encephalopathy with or without seizures, neurodevelopmental delays, learning disabilities, and poor coordination or ataxias in several young human subjects (Hunt et al., 2014; Lalani et al., 2014; Tanaka et al., 2015). Dysregulation of Pur-alpha has also been shown in C9Orf72-related Frontotemporal lobe dementia in association with Amyotrophic Lateral Sclerosis, presumably resulting from accumulation of intracellular inclusion bodies that contain Pur-alpha and RNA complexes, and RNA toxic gain-in-function effects (Daigle et al., 2016). Pur-alpha may even be involved in enhancing HIV-1 transcription, with increasing concentrations of Pur-alpha leading to increased transcription of HIV-1 (Chepenik et al., 1998).

Our lab has developed knockout mice that are either homozygous (−/−) or heterozygous for Pur-alpha (+/−). Pur-alpha deficient (−/−) mice developed in our lab show severe neurological problems, such as severe tremor and spontaneous seizures, and expire by 4 weeks of age (Khalili et al., 2003). Heterozygous mice lacking one allele for the Pur-alpha gene (+/−) produce less Pur-alpha protein in whole-cell brain extracts and live to adulthood, although they display developmental delays and undergo occasional spontaneous seizures during routine handling (Khalili et al., 2003; Mishra et al., 2013). These latter results combined with those described above in association with mutations or dysregulation in Pur-alpha suggest that chronic reduction of Pur-alpha leads to neuropathogenic effects. However, the overall behavioral phenotype and brain histopathology have yet to be examined in Pur-alpha (+/−) mice.

Thus, our goals here were to examine for the first time, whether Pur-alpha (+/−) mice display altered sensorimotor reflexes, locomotive or cognitive dysfunction, and brain neuropathology. Specifically, we examined the hippocampus, cerebellum, amygdala, and prefrontal, frontal and parietal cortices for morphological and neurohistological changes following a comprehensive neurological screen, gait analysis and cognitive memory assays. We hypothesized that mice heterozygous for the Pur-alpha knockout allele would show memory deficits concomitant with neurodegeneration in the hippocampus, as well as locomotor ataxias and other gait abnormalities concomitant with neurodegeneration in the cerebellum.

EXPERIMENTAL PROCEDURES

Animals

The Temple University Institutional Animal Care and Use Committee approved all experiments, and in accordance with the guidelines laid down by the National Institute of Health. Mice that were heterozygous for Pur-alpha (+/−) were bred at Temple University by crossing C57/BL6 females with heterozygous Pur-alpha (+/−) knockout males. Homozygous deletion of Pur-alpha is lethal during late development; therefore mice are maintained as heterozygous (Khalili et al., 2003). Twenty-four wild-type C57/BL6 mice (+/+; 15 females and nine males) and 24 heterozygous Pur-alpha knockout (+/−; 12 females and 12 males) littermates underwent behavioral testing from 7 weeks to 11 months of age. Progeny were screened by PCR analysis using primer sets to distinguish, based on size of the amplicon, between the wild-type gene and knockout which contains a neomycin cassette inserted into the Pur-alpha gene (Khalili et al., 2003). After weaning at day 21, mouse pups were handled for two weeks before onset of experiments. Mice were group-housed with same-sexed siblings on a 12:12 light–dark cycle with food and water *ad libitum*. Mice were allowed at least one hour to acclimate before behavioral testing, after being moved to the animal behavioral testing facility. Experimenters were blind to animal genotype during all analyses.

Primary behavioral phenotype assessment including open field assays

Characterization of the neural behavioral phenotype was performed on 18 mice per group (wild-type: 12 females and six males; heterozygous Pur-alpha (+/−): nine females and nine males) using the Irwin Observation Test Battery (Irwin, 1968) with Paylor's and Crawley's refinements (Crawley and Paylor, 1997; Paylor et al., 2006; Crawley, 2007). This standardized set of assays is also termed the SHIRPA (an acronym of SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment).

General health screen. A general physical examination of the mice was first conducted in home cages. All mice were weighed and presence of any superficial anatomical abnormalities, whisker condition (missing, sparse, short versus normal), coat quality, palpebral closure, presence of nasal and lacrimal discharge, respiration, and head, body, limbs and tail morphology were recorded and scored (0 = normal or absent, and 1 = abnormal or present, as appropriate for the observation) (Crawley and Paylor, 1997; Paylor et al., 2006; Crawley, 2007). Any unusual patterns of locomotion, hyperactivity to handling or fighting in the home cage were also recorded.

Assay of neurologic reflexes. Next, the same 18 mice/group mice underwent evaluation of their neurologic reflexes (Crawley and Paylor, 1997; Paylor et al., 2006; Crawley, 2007). For this, mice were removed