Validation of retinal imaging for characterizing alpha-synuclein-eGFP deposition in a transgenic mouse model of Parkinson's Disease/Dementia with Lewy Bodies

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Abstract

Abnormal accumulation of alpha-synuclein has been hypothesized to underlie neuronal cell death and synaptic dysfunctional leading to motor and cognitive symptoms in synucleinopathies such as Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB), and Multiple Systems Atrophy (MSA). Transgenic mouse models with overexpression of alpha-synuclein have proved useful in characterizing the behavioral, neuropathological, and biochemical consequences of alpha-synuclein aggregation. Nonetheless, the identification of truly translatable biomarkers relevant for therapeutic evaluations has proved difficult and is the focus of large scale collaborative efforts *e.g.,* the Parkinson's Progression Markers Initiative (PPMI). Retinal imaging can provide a non-invasive means to evaluate optical pathology (fundal maps and optical coherence tomography of vasculature and fluorescent-tagged proteins), and there is mounting evidence that ophthalmic pathology mirrors neuropathological processes of the central nervous system. In an effort to evaluate the presence and progression of retinal pathology in transgenic mouse models of PD/DLB, we conducted retinal imaging studies of mice overexpressing fused alpha-synuclein-GFP under the PDGF-beta promoter (PDNG78 line). Retinal imaging revealed perivascular and nerve terminal green fluorescent protein (GFP) labeling in transgenic, but not non-transgenic, PDNG78 mice. These observations correlate with central nervous system (CNS) observations in this same mouse line, which demonstrates the utility and relevance of retinal imaging by bright field and fluorescent studies for in vivo characterization of transgenic mouse model phenotypes as well as therapeutic evaluations.

Introduction

Abnormal accumulation of alpha-synuclein (ASYN) is hypothesized to underlie neuronal cell death and synaptic dysfunction leading to motor and cognitive symptoms in Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). Transgenic mouse models with overexpression of ASYN have proved useful in characterizing specific behavioral, neuropathological, and biochemical consequences of ASYN aggregation (*see* Chesselet and Richter, 2011 *for a comprehensive overview*), though no model reproduces all disease relevant features. A persistent challenge in CNS research has been to find disease relevant features in these transgenic mouse models with translational value for evaluation of clinical trials in patient populations.   Varied degrees of changes in retinal structure and/or functional visual impairment have been observed in Parkinsonian patients and patients with other neurodegenerative diseases (Inzelberg *et al.*, 2004; Altintas *et al.*, 2007; Chiu *et al.*, 2012; Frost *et al.*, 2013; Koronyo-Hamaoui *et al.,* 2012; Park *et al*, 2011; Parnell *et al.*, 2012). The non-availability of imaging ligands targeting alpha-synuclein is a specific challenge to retinal imaging of humans or transgenic mouse models of synucleinopathies. The ideal ligand would be bioavailable, non-toxic, alpha-synuclein specific, and stable for imaging. In the absence of such a ligand, we conducted retinal imaging of mice overexpressing fused ASYN-eGFP under the PDGF-beta promoter (PDNG78 line; Rockenstein *et al.,* 2005), using a retinal imaging microscope (Phoenix Micron III; Phoenix Research Labs, Pleasanton, CA) for non-invasive brightfield and fluorescent retinal imaging. The PDNG78 transgenic mouse has many PD/DLB-relevant biochemical and neuropathological features, and thus presents a unique opportunity for evaluating the presence and progression of retinal pathology in transgenic mouse models of PD/DLB.

Animal subjects

The PDNG78 transgenic mouse expresses fused ASYN-eGFP under the PDGF-beta promoter at levels 2-5 fold greater than non-transgenic control mice (**Figure 1**, *left diagram*). The CNS expression levels are highest in limbic system including the neocortex and hippocampal regions of PDNG78 transgenic mice (**Figure 1**, *middle panel*). Cellular distributions of eGFP-ASYN mirror synucleinopathy-relevant features including accumulations in neuronal cell bodies, diffuse staining of the neuropil, synaptic punctate staining, and perivascular deposits (**Figure 1**, *right panel*).
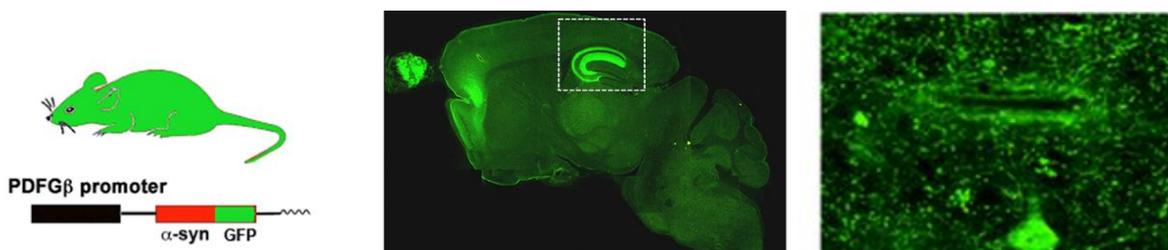
Figure 1. Construct for PDNG78 eGFP-ASYN transgenic mouse (*left*) and CNS expression levels of eGFP-ASYN (*middle and right panels*).

Study Objectives

The current studies were guided by three specific objectives including:

1. To evaluate the presence of retinal pathology in PDNG78 eGFP-ASYN transgenic mice & compare transgenic retinal GFP-ASYN expression with previous CNS observations.

1. To evaluate the presence of a progressive retinal transgenic phenotype for GFP-ASYN PDNG78 transgenic mice.

2. To determine the feasibility of and develop methods for longitudinal retinal evaluations for phenotypic progression studies and therapeutic evaluations.

Retinal Imaging Microscope and Procedure

The Phoenix Micron III Retinal Imaging Microscope (Phoenix Research Labs, Pleasanton, CA) was utilized for non-invasive brightfield and fluorescent retinal imaging studies in anesthetized PDNG78 transgenic and non-transgenic mice. The apparatus consists of a Xenon light source and CCD-camera coupled microscope for mouse retinal imaging resolution of 4 uM in a field of view of 1.8 mm. The procedure is non-invasive, however, it does require that the animal be anesthetized for optimal imaging conditions (to avoid movement-induced image artifacts). The entire procedure consists of sedation, dilation, imaging and recovery. The dilation and eye wetting reagents are available from Akorn Pharmaceuticals (Lake Forest, IL). Atropine sulfate 1% and phenylephrine HCl 2.5% solutions were applied to both eyes for dilation. Upon full dilation, animals were placed onto the positioning table (**Figure 2**), Gonak 2.5% solution was applied, and the objective was oriented for imaging. Mouse bright field image retinal maps (normal scan mode) were acquired followed by fluorescent images (progressive scans of 30) in the same orientation for each eye. The settings were kept consistent between subjects. The imaging session for each animal typically takes less than 5 minutes per animal to acquire bright field and fluorescent images of both eyes.


Figure 2. PDNG78 mouse positioned in Phoenix Micron III apparatus for retinal imaging.

Results

Objective 1: *Retinal imaging reveals clear retinal phenotype in eGFP-ASYN in PDNG78 transgenic mice*

Retinal imaging of non-transgenic and transgenic PDNG78 littermates revealed a clear difference in labeling **(Figure 3).** As previously reported in CNS evaluations, retinal imaging of eGFP-ASYN in PDNG78 transgenic mice demonstrated perivascular and nerve terminal-like GFP labeling.
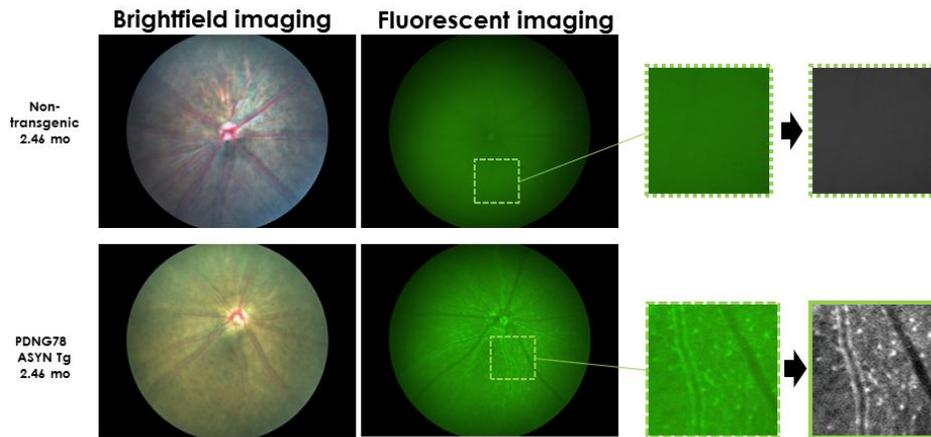
Figure 3. Fluorescent imaging of PDNG78 transgenic mice (*lower panels*) demonstrates increased eGFP-ASYN with accumulations similar to those observed in CNS studies.

Objective 2: *Retinal re-imaging of GFP-ASYN in PDNG78 transgenic mice demonstrates persistent eGFP-ASYN features that can be tracked over multiple imaging sessions*

Longitudinal imaging studies of mice are enabled with the Micron III and comparison of baseline scans with imaging 1 month later revealed persistent e-GFP-ASYN expression features in same mouse (**Figure 4**). These longitudinal evaluations illustrated the necessity of a consistent imaging angle for repeat imaging of the mouse retina.
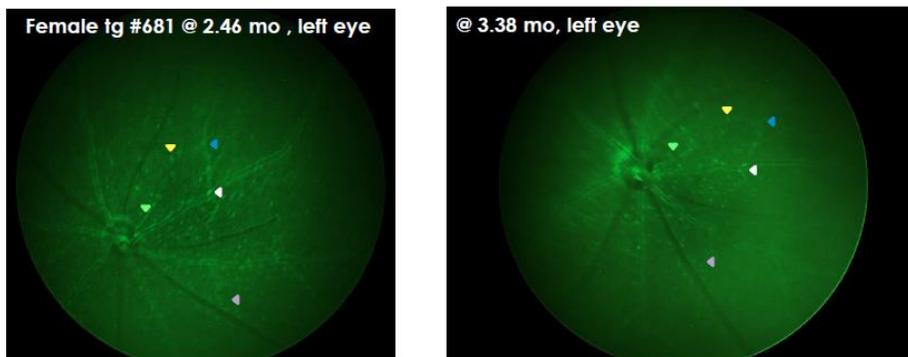
Figure 4. Identification of persistent eGFP-ASYN features in a PDNG78 transgenic mouse retina across multiple imaging sessions. Coinciding features are marked with matched arrowhead colors.

Objective 3: *Longitudinal evaluations of retinal GFP-ASYN expression in PDNG78 transgenic mice reveals persistent increased eGFP-ASYN signal compared to non-transgenic mice.*

Comparisons of non-transgenic and PDNG78 transgenic images over three imaging sessions revealed age-dependent increases in eGFP-ASYN occurring only in transgenic mice. The image analysis package ImageJ (NIH) was utilized to determine the percentage of area with eGFP-ASYN as well as total number of particles present. Representative images from one subject per group are shown in **Figure 5**, together with the group means for these measures. Data were analyzed via two-way repeated measure ANOVAs, with Sidak's post hoc comparisons (criteria for significance set at p<0.05). Group data are presented in **Figure 5** as the group means with statistically significant differences between groups at discrete time points noted with asterisks (**p<0.01 or ***p<0.001 versus non-transgenic mice*). There were statistically significant differences between non-transgenic and PDNG78 transgenic mice in the total number of particles at 2 months (**p<0.01*), and % of area at all measured time points (**p<0.01 and ***p<0.001*).
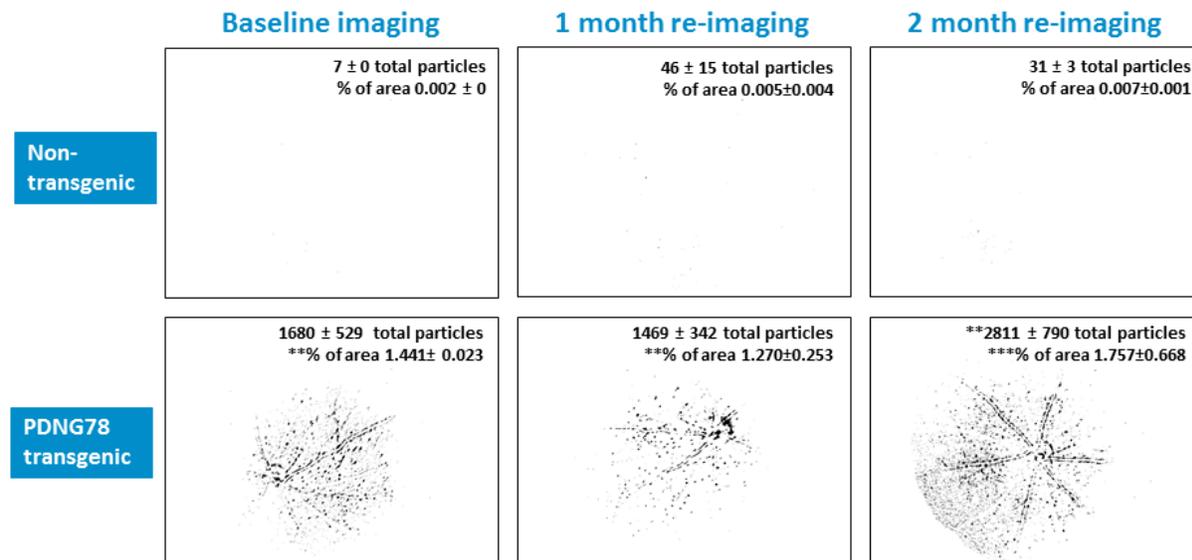
Figure 5. Multiple imaging of PDNG78 transgenic mice reveals statistically significant increases in eGFP-ASYN relative to non-transgenic control mice. Representative non-transgenic and PDNG78 transgenic mice demonstrate the expected differences in retinal eGFP-ASYN expression over three imaging sessions. Analysis of eGFP-ASYN as total particle counts and % of area revealed statistically significant differences between transgenic and non-transgenic mice at multiple time points (**p<0.01 or ***p<0.001 versus non-transgenic control mice*).

Conclusions

The present longitudinal studies support the use of the PDNG78 transgenic mouse represents a useful model system to evaluate ASYN-associated retinal pathology as an additional or surrogate marker for CNS neuropathology. In the absence of commercially available fluorescent imaging ligands targeting

ASYN, the PDNG78 transgenic mouse represents an important model system for therapeutic evaluations. Moreover, the Phoenix Micron III provides a means to non-invasive measures of progressive mouse retinal pathology with potential application to therapeutic evaluations. The utility of this imaging system and approach will be increased with the identification and use of fluorescent ligands targeting neurodegenerative disease relevant proteins of interest in other transgenic models without GFP-tagged proteins as well as in patients.

Acknowledgements

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