THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

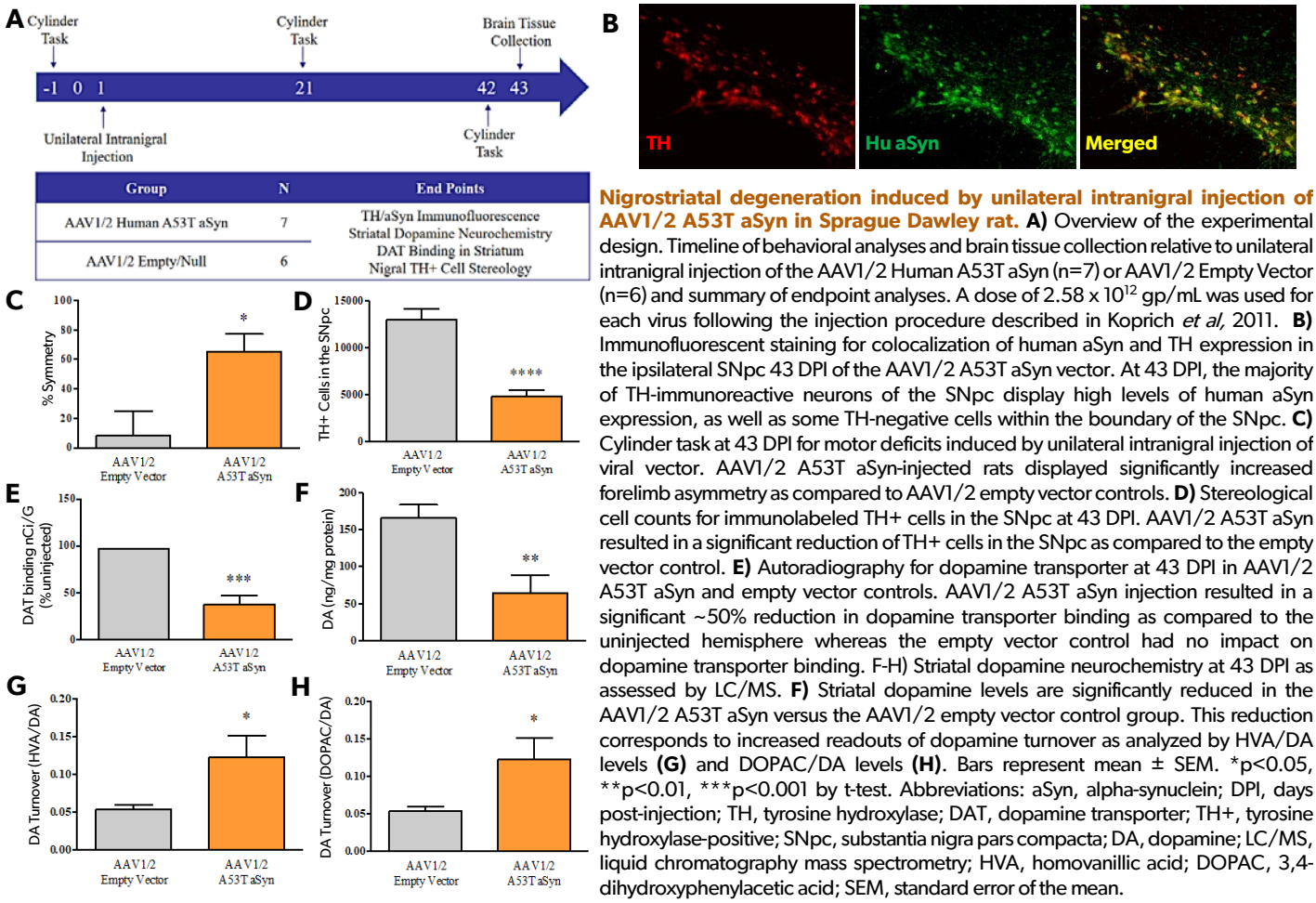
in partnership with

atuka | GD GeneDetect innovate | Vigene Biosciences A Charles River Company

HUMAN A53T ALPHA-SYNUCLEIN VIRAL VECTOR

MJFF has partnered with GeneDetect and Vigene Biosciences to make available the well-characterized adeno-associated viral vector expressing human A53T alpha-synuclein to model Parkinson's disease. This viral vector has been characterized in the mouse, rat, and non-human primate for ability to express alpha-synuclein, induce nigrostriatal degeneration, and model Parkinson's disease pathology (see Publications section). The current lot has been validated to ensure performance similar to the previously published batches. Viral vectors were designed and generated by GeneDetect, validated by Atuka, Inc., and are now available for purchase at Vigene Biosciences. Validation data is included below.

Transgene	Viral Vector Nomenclature	Catalog #
Human A53T aSyn	AAV1/2-CMV/CBA-**Human A53T aSyn**-WPRE-BGH-polyA	GD1001-RV
Empty Vector Control	AAV1/2-CMV/CBA-**Null/Empty**-WPRE-BGH-polyA	GD1004-RV



Nigrostriatal degeneration induced by unilateral intranigral injection of AAV1/2 A53T aSyn in Sprague Dawley rat. A) Overview of the experimental design. Timeline of behavioral analyses and brain tissue collection relative to unilateral intranigral injection of the AAV1/2 Human A53T aSyn (n=7) or AAV1/2 Empty Vector (n=6) and summary of endpoint analyses. A dose of 2.58 x 10^{12} gp/mL was used for each virus following the injection procedure described in Koprich *et al,* 2011. **B)** Immunofluorescent staining for colocalization of human aSyn and TH expression in the ipsilateral SNpc 43 DPI of the AAV1/2 A53T aSyn vector. At 43 DPI, the majority of TH-immunoreactive neurons of the SNpc display high levels of human aSyn expression, as well as some TH-negative cells within the boundary of the SNpc. **C)** Cylinder task at 43 DPI for motor deficits induced by unilateral intranigral injection of viral vector. AAV1/2 A53T aSyn-injected rats displayed significantly increased forelimb asymmetry as compared to AAV1/2 empty vector controls. **D)** Stereological cell counts for immunolabeled TH+ cells in the SNpc at 43 DPI. AAV1/2 A53T aSyn resulted in a significant reduction of TH+ cells in the SNpc as compared to the empty vector control. **E)** Autoradiography for dopamine transporter at 43 DPI in AAV1/2 A53T aSyn and empty vector controls. AAV1/2 A53T aSyn injection resulted in a significant ~50% reduction in dopamine transporter binding as compared to the uninjected hemisphere whereas the empty vector control had no impact on dopamine transporter binding. F-H) Striatal dopamine neurochemistry at 43 DPI as assessed by LC/MS. **F)** Striatal dopamine levels are significantly reduced in the AAV1/2 A53T aSyn versus the AAV1/2 empty vector control group. This reduction corresponds to increased readouts of dopamine turnover as analyzed by HVA/DA levels **(G)** and DOPAC/DA levels **(H)**. Bars represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 by t-test. Abbreviations: aSyn, alpha-synuclein; DPI, days post-injection; TH, tyrosine hydroxylase; DAT, dopamine transporter; TH+, tyrosine hydroxylase-positive; SNpc, substantia nigra pars compacta; DA, dopamine; LC/MS, liquid chromatography mass spectrometry; HVA, homovanillic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; SEM, standard error of the mean.

Publications:

- Koprich *et al.* (2010). Expression of human A53T alpha-synuclein in the rat substantia nigra using a novel AAV1/2 vector produces a rapidly evolving pathology with protein aggregation, dystrophic neurite architecture, and nigrostriatal degeneration with potential to model the pathology of Parkinson's disease. *Molecular Neurodegeneration,* 5:43.
- Koprich *et al.* (2011). Progressive neurodegeneration or endogenous compensation in an animal model of Parkinson's disease produced by decreasing doses of alpha-synuclein. *PLoS ONE,* 6(3): e17698.
- He *et al.* (2015). Treatment with trehalose prevents behavioral and neurochemical deficits produced in an AAV alpha-synuclein rat model of Parkinson's Disease. *Molecular Neurobiology,* 53(4): 2258-2268.
- Koprich *et al.* (2016). Towards a non-human primate model of alpha-synucleinopathy for development of therapeutics for Parkinson's disease: optimization of AAV1/2 delivery parameters to drive sustained expression of alpha-synuclein and dopaminergic degeneration in Macaque. *PLoS ONE,* 11(11): e0167235.
- Ip *et al.* (2017). AAV1/2-induced overexpression of A53T-alpha-synuclein in the substantia nigra results in degeneration of the nigrostriatal system with Lewy-like pathology and motor impairment: a new mouse model for Parkinson's disease. *Acta Neuropathologica Communications,* 5:11.
- Musacchio *et al.* (2017). Subthalamic nucleus deep brain stimulation is neuroprotective in the A53T α-synuclein Parkinson's disease rat model. *Annals of Neurology,* 81(6): 825-836.
- Gleave *et al.* (2017). Sirtuin 3 rescues neurons through the stabilisation of mitochondrial biogenetics in the virally-expressing mutant α-synuclein rat model of parkinsonism. *Neurobiology of Disease,* 106: 133-146.