Exploratory omics study reveals potential roles of sigma-2 receptor modulators in AAV1/2A53T-aSyn rat model of Parkinson's disease

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Introduction

Synucleinopathies, including Lewy body dementia (DLB) and Parkinson's disease (PD) comprise the second most common neurodegenerative disease worldwide. In synucleinopathies, alpha-synuclein (αSyn) oligomers cause neuronal dysfunction. Mutations in the αSyn gene, such as A53T, cause familial PD[1]. Ectopic expression of human A53T αSyn *in vivo* causes production of toxic αSyn oligomers, resulting in neuroinflammation and loss of dopaminergic neurons[2,3].

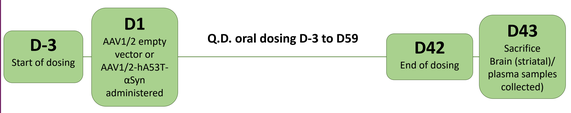
The sigma-2 receptor (S2R) is involved in pathways associated with age-related disease including autophagy, oxidative stress, and intracellular trafficking. We have shown that S2R modulation *in vitro* reverses αSyn oligomer-induced deficits in neuronal vesicle trafficking[4]. Given this data and S2R's functional overlap with pathways affected in PD, we hypothesized that S2R modulation would alter PD-related transcripts and pathways *in vivo*.

Methods

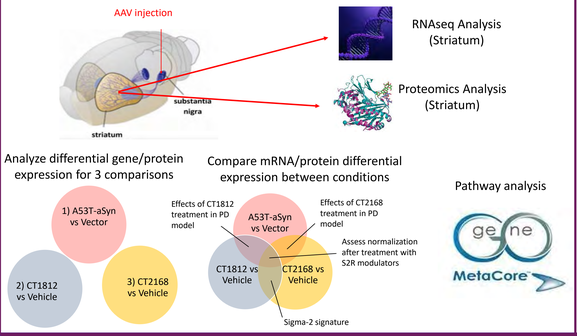
Goal) Determine effects of sigma-2 receptor modulators CT1812 and CT2168 in an *in vivo* PD model[3]

Model: AAV1/2-hA53T-αSyn vector administered unilaterally into the right substantia nigra of female Sprague Dawley rats (Charles River, Canada)

- Animals produce mutant αSyn in the substantia nigra
- Neuron loss and motor dysfunction occur by 6-weeks post-injection



Group	Test Item or Vehicle (PO, Q.D)	S2R Affinity (Ki)	Dose	AAV admin (D1)	Final N	% RO at S2 (Cerebellum)	%RO at S2 (Plasma)
1	Vehicle	---	0	EV	10	---	---
2	Vehicle	---	0	αSyn	10	---	---
3	CT1812	8.5nM	3mg/kg; q.d.	αSyn	10	69.8 ± 6.01	64.5 ± 10.53
4	CT2168	2nM	3mg/kg; q.d.	αSyn	10	88.6 ± 2.28	87.3 ± 2.20



Animals overexpressing mutant αSyn exhibit hallmark features of Parkinson's disease in striatal tissue, notably decreases in dopamine pathway-related proteins

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Human A53T-αSyn Protein Levels (Striatum) (ng/mL)				
	Vector	AAV +Veh	AAV +CT1812	AAV +CT2168
Mean	Not detected	188.34	180.14	182.39
SD	---	64.94	56.03	78.59

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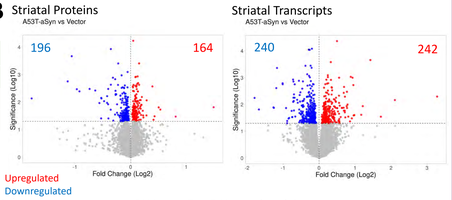

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Proteomics - Top 5 Upregulated Proteins

Gene	Protein	Protein Name	p-value	Log2FC
Prl	P01237	Prolactin	1.53E-02	1.50
Cpne1	Q8C166	Copine-1	3.35E-02	0.81
Cog7	Q3TR20	Conserved oligomeric Golgi complex subunit 7	1.86E-02	0.54
Rdh7	P55006	Retinol dehydrogenase 7	1.55E-02	0.51
Rsad1	A0A8I6AGR5	Radical S-adenosyl methionine domain containing 1	3.26E-02	0.43

RNAseq - Top 5 Upregulated Genes

Gene	Protein Name	p-value	Log2FC
Prl	Prolactin	5.09E-03	3.28
Calcr	Calcitonin receptor	6.86E-03	2.11
Gabre	Gamma-aminobutyric acid type A receptor subunit epsilon	1.15E-02	1.21
Kcnj6	Potassium inwardly rectifying channel subfamily J member 6	2.48E-02	0.98
Serinc2	Serine incorporator 2	2.55E-02	0.96

Proteomics – Top 5 Downregulated Proteins

Gene	Protein	Protein Name	p-value	Log2FC
Camkv	A0A0G2K1R5	CaM kinase-like vesicle-associated protein	7.31E-03	-1.78
Th	P04177	Tyrosine 3-monooxygenase	1.75E-03	-1.13
Aldh1a1	P24549	Aldehyde dehydrogenase 1A1	2.14E-04	-1.06
Slc6a3	P23977	Sodium-dependent dopamine transporter	3.37E-03	-0.97
Slc18a2	Q8BRU6	Dnal homolog subfamily C member 12	3.30E-03	-0.74

RNAseq – Top 5 Downregulated Genes

Gene	Protein Name	p-value	Log2FC
Car3	Carbonic anhydrase 3	5.72E-03	-1.79
Plac9	Placenta associated 9	1.27E-02	-1.27
Kcns1	Potassium Voltage-Gated Channel Modifier Subfamily S Member 1	2.45E-03	-1.19
Klk7	Kallikrein Related Peptidase 7	1.62E-03	-1.01
Hfe2	Transferrin receptor 2	1.76E-03	-1.01

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Proteomics – Metacore Pathway Analysis

Pathway Map (Proteomics) – A53T-αSyn vs Vector	p-value
Neurophysiological process: Constitutive and regulated NMDA receptor trafficking	2.50E-05
SCAP/SREBP transcriptional control of cholesterol and FA biosynthesis	3.24E-04
Neurophysiological process: Dopamine D2 receptor signaling in CNS	3.98E-04
Immune response: Lectin induced complement pathway	5.32E-04
Neurophysiological process: Synaptic vesicle fusion and recycling in nerve terminals	6.39E-04

RNAseq – Metacore Pathway Analysis

Pathway Map (RNAseq) – A53T-αSyn vs Vector	p-value
G-protein signaling: CDC42 inhibition and activation	4.57E-04 3.33E-03
Development: Role of CDKS in the nervous system	2.23E-03
Regulation of metabolism: GLP-1 signaling in beta cells	2.80E-03
Neurophysiological process: Regulation of intrinsic membrane properties and excitability of cortical pyramidal neurons	5.64E-03
Transport: Alpha-2 adrenergic receptor regulation of ion channels	5.82E-03

Figure 1. A) Total striatal human (transgene-derived) αSyn protein expression, quantified by ELISA. B) Significant differential protein and RNA expression in striatal tissue due to AAV1/2-A53T-αSyn treatment (p≤0.05) shown as a volcano plot. C) Top 5 positive and negative differentially expressed proteins (DEPs) and genes (DEGs) by fold change. D) Metacore Pathway Analysis (version 23.1.71200) using significant DEPs and DEGs (p≤0.05); non-relevant disease pathologies were excluded from top 5 list.

CT1812 treatment causes proteomic and transcriptomic changes in inflammation and stress response related genes

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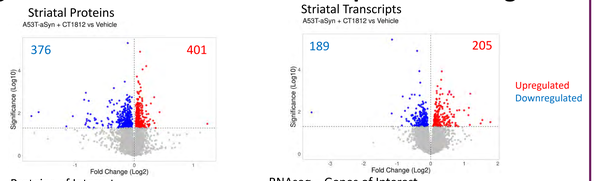

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Proteomics – Proteins of Interest

Gene	Protein Name	Relevance	p-value	Log2FC
Rdh7	Retinol dehydrogenase 7	Steroid metabolism	1.11E-03	-0.82
Ctnna2	Catenin alpha-2	Neuron migration/ development	1.24E-03	0.08
Ajm1	Apical junction component 1 homolog	Cell-cell junctions/ Plasma membrane	1.01E-03	0.05

RNAseq – Genes of Interest

Gene	Protein Name	Relevance	p-value	Log2FC
Apoa5	Apolipoprotein A5	HDL component/ Triglyceride regulator	1.21E-02	-1.16
Osgin1	Oxidative stress induced growth inhibitor 1	Oxidative stress/Autophagy	1.44E-03	0.35
Kcns1	Potassium voltage-gated channel modifier subfamily S member 1	Control of action potentials	6.52E-03	1.14

C

Proteomics – Metacore Pathway Analysis

Pathway Map (Proteomics) – A53T-αSyn + CT1812 vs A53T-αSyn	p-value
Neurophysiological process: Constitutive and activity-dependent synaptic AMPA receptor delivery	1.38E-05
Apoptosis and survival: Regulation of apoptosis by mitochondrial proteins	6.50E-05
Neurophysiological process: Constitutive and regulated NMDA receptor trafficking	1.98E-04
Neurophysiological process: Synaptic vesicle fusion and recycling in nerve terminals	2.84E-04
Transport: Clathrin-coated vesicle cycle	3.68E-04

RNAseq – Metacore Pathway Analysis

Pathway Map (RNAseq) – A53T-αSyn + CT1812 vs A53T-αSyn	p-value
Signal transduction: JNK pathway	3.67E-04
Signal transduction: IGF-1 receptor signaling pathway	9.78E-04
Development: Notch signaling inhibition	1.76E-03
Development: Thrombopoietin signaling via ERK1/2 and PI3K	1.87E-03
Development: EPO-induced MAPK pathway	2.85E-03

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Proteomics-RNAseq Overlap – Metacore Pathway Analysis

Pathway Map – A53T-αSyn + CT1812 vs A53T-αSyn	p-value
LRRK2 in neuronal apoptosis in Parkinson's disease	1.30E-02
Signal transduction: ERK1/2 signaling pathway	2.44E-02
Development: EGF-induced proliferation of type C cells in SVZ of adult brain	2.51E-02
Cell adhesion: Tight junctions	3.34E-02
Development: EPO-induced MAPK pathway	3.41E-02

Proteomics-RNAseq Overlap – Genes of Interest

Gene	Protein Name	Relevance	Protein p-value	Protein Log2FC	RNA p-value	RNA Log2FC
Prl	Prolactin	Growth/immune system growth	8.97E-03	-1.62	1.07E-02	-3.55
Sos2	SOS Ras/Rho guanine nucleotide exchange factor 2	Prolactin signaling/ Immune system	3.59E-02	-0.06	2.23E-02	-0.10
Prdx3	Peroxiredoxin 3	Antioxidant	2.28E-03	0.06	4.81E-02	0.13

Figure 2. A) Significant differential protein and RNA expression in striatal tissue of rats expressing mutant αSyn due to CT1812 treatment (p≤0.05); shown as a volcano plot. B) Differentially expressed proteins and RNA (p≤0.05) chosen based on relevance to disease pathology. C) Metacore Pathway Analysis (version 23.1.71200) using significant DEPs and DEGs (p≤0.05); non-relevant disease pathologies were excluded from top 5 list. D) Metacore Pathway Analysis using DEGs found to be significantly altered (p≤0.05) using proteomics and RNAseq (left); Differentially expressed genes identified by both proteomics and RNAseq (p≤0.05) chosen based on relevance to disease pathology.

CT2168 treatment causes proteomic and transcriptomic changes in the cholesterol transport pathway and upregulates dopamine pathway-related proteins

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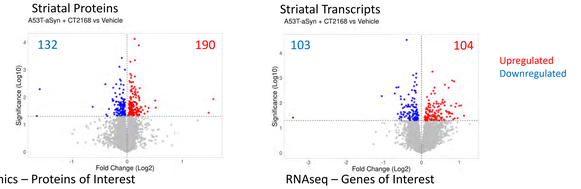

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Proteomics – Proteins of Interest

Gene	Protein Name	Relevance	p-value	Log2FC
Camkv	CaM kinase-like vesicle-associated protein	Glutamatergic synapse	1.18E-02	1.56
Slc17a6	Vesicular glutamate transporter 2	Glutamate transporter/ Neurotransmission	1.76E-03	0.14
Drd2	D(2) dopamine receptor	Dopamine	1.03E-02	0.22

RNAseq – Genes of Interest

Gene	Protein Name	Relevance	p-value	Log2FC
Nts	Neurotensin	Neuromodulator/ Neurotransmitter/Dopamine	2.07E-02	0.65
Hmgcr	3-hydroxy-3-methylglutaryl-CoA reductase	Cholesterol synthesis	9.67E-04	-0.14
Islr2	Immunoglobulin superfamily containing leucine rich repeat 2	Neural development	4.15E-03	0.35

C

Proteomics – Metacore Pathway Analysis

Pathway Map (Proteomics) – A53T-αSyn + CT2168 vs A53T-αSyn	p-value
SCAP/SREBP transcriptional control of cholesterol and FA biosynthesis	1.43E-04
Nicotine signaling in cholinergic neurons	2.86E-04
Immune response: Oncostatin M signaling via MAPK	7.61E-04
Development: EGFR signaling pathway	1.20E-03
Development: Estrogen-independent activation of ESR1 and ESR2	1.47E-03

RNAseq – Metacore Pathway Analysis

Pathway Map (RNAseq) – A53T-αSyn + CT2168 vs A53T-αSyn	p-value
Protein folding and maturation: Posttranslational processing of neuroendocrine peptides	1.61E-06
Cell cycle: Role of APC in cell cycles regulation	8.29E-05
Cell cycle: DNA replication – elongation and termination	1.02E-04
Immune response: ETV3 affect on CSF-1 promoted macrophage differentiation	3.38E-04
Cell cycle: Chromosome condensation in prometaphase	4.59E-04

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Proteomics-RNAseq Overlap – Metacore Pathway Analysis

Pathway Map – A53T-αSyn + CT2168 vs A53T-αSyn	p-value
Signal transduction: FGFR4 signaling	1.17E-04
Cholesterol and sphingolipid transport/Influx in the early endosome in lung (normal and CF)	6.69E-03
Transcription: Ligand-dependent activation of the ESR1/SP pathway	6.92E-03
Immune response: Oncostatin M signaling via MAPK	8.53E-03
Regulation of lipid metabolism: Regulation of lipid metabolism via LXR, NF-Y and SREBP	8.76E-03

Proteomics-RNAseq Overlap – Genes of Interest

Gene	Protein Name	Relevance	Protein p-value	Protein Log2FC	RNA p-value	RNA Log2FC
Prl	Prolactin	Growth/immune system growth	5.10E-03	-1.58	3.86E-02	-3.40
Ldlr	Low density lipoprotein receptor	LDL receptor/S2R-associated	7.06E-03	-0.21	4.19E-02	-0.22
Ltn1	Listerin E3 ubiquitin protein ligase 1	Parkinson's disease	9.95E-04	-0.04	2.95E-02	-0.12

Figure 3. A) Significant differential protein and RNA expression in striatal tissue of rats expressing mutant αSyn due to CT2168 treatment (p≤0.05); shown as a volcano plot. B) Differentially expressed proteins and RNA (p≤0.05) chosen based on relevance to disease pathology. C) Metacore Pathway Analysis (version 23.1.71200) using significant DEPs and DEGs (p≤0.05); non-relevant disease pathologies were excluded from top 5 list. D) Metacore Pathway Analysis using DEGs (p<0.05) identified using both proteomics and RNAseq (left); DEGs identified by both proteomics and RNAseq (p≤0.05) chosen based on relevance to disease pathology (right).

CT1812 and CT2168 treatment reverse proteomic and transcriptomic changes seen in animals overexpressing mutant αSyn

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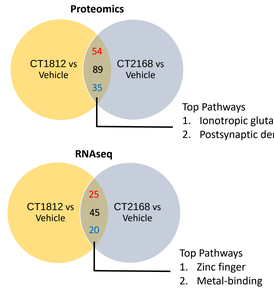

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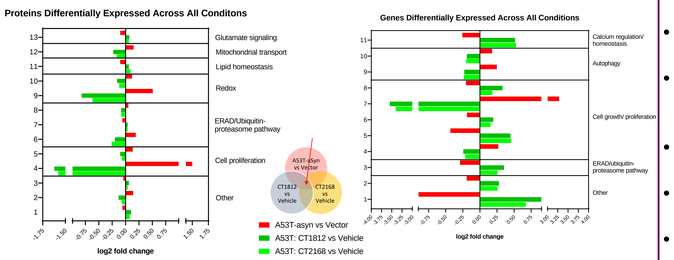

Figure 4. A) Number of significant differentially expressed proteins and genes between both S2R modulator treatments (S2R signature) (p≤0.05). Proteins/genes upregulated by both compounds shown in red, downregulated in blue. Lists next to the diagram show the top pathways (sorted by strength) identified by STRING (version 11.5) pathway analysis. B) Forest plots representing proteins (left) and genes (right) grouped by similar GO terms; Proteins and genes shown were altered by mutant αSyn expression (red) and normalized by treatment with both S2R modulators (green). Venn diagram in key indicates the comparison shown on the forest plots.

Conclusions

- This is the first study, to our knowledge, to elucidate the transcriptomic and proteomic profile of the A53T-αSyn PD model
- Mutant αSyn expression increased inflammatory pathway-related genes/proteins and decreased dopamine pathway-related proteins compared to control
- Sigma-2-receptor (S2R) modulator CT1812 impacted pathways involved in synaptic activity and function and transcripts/proteins known to protect against oxidative stress
  - CT1812 is currently in Phase 2 clinical trials for dementia with Lewy bodies (NCT05225415)
- S2R modulator CT2168 increased transcripts/proteins important to the dopamine pathway and decreased transcripts/proteins associated with PD and LDL receptor, which is relevant to S2R mechanism of action
- Both S2R modulators altered transcripts/proteins involved in signal transductions, the glutamatergic pathway, and reversed transcript/protein alterations seen in the mutant αSyn model
- These findings support the further development of sigma-2 receptor modulators for synucleinopathies

References:
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