A proteomic analysis to elucidate the role of sigma-2 receptor modulators CT1812 and CT2168 in a mouse model of Alzheimer's disease

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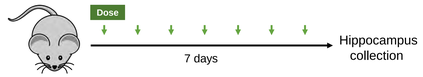
INTRODUCTION

Sigma-2 receptor (S2R) modulators can modulate amyloid-beta (Aβ) oligomer binding to neuronal synapses. The S2R modulator CT1812 is a first-in-class investigational therapeutic, currently in Phase 2 clinical trials[1] for Alzheimer's disease (AD). CT1812 selectively displaces Aβ oligomers from synapses[2] and clears them from the brain into the cerebrospinal fluid, restoring cognitive performance in a transgenic mouse model of AD[3]. To better understand and identify the biological processes that S2R modulators can impact, a proteomic analysis was performed in an *in vivo* mouse model of AD treated with CT1812[4] and a chemically distinct S2R modulator, CT2168.

METHODS

Five-month-old male non-transgenic mice (nTg) were dosed with vehicle and matched age male transgenic mice with the human APP with London (717) and Swedish (670/671) mutation hAPPsI (Tg) were dosed with vehicle or, either CT1812 (10 mg/kg) or CT2168 (5 mg/kg), given orally, once daily for 7 days. Animals were sacrificed 24 hr after last dose, and hippocampi collected. The study performed by QPS Custom-built research in Austria.

Study design:



Group	Genotype	Test Article	Dose (mg/kg)	Schedule	Route	Number animals
A	nTg	Vehicle	NA	Once daily for 7 days	Oral	10 (m)
B	hAPPsI (Tg)	Vehicle	NA	Once daily for 7 days	Oral	10 (m)
C	hAPPsI (Tg)	CT1812	10	Once daily for 7 days	Oral	10 (m)
D	hAPPsI (Tg)	CT2168	5	Once daily for 7 days	Oral	10 (m)

For the first time, unbiased proteomics, using tandem-mass tag mass spectrometry (TMT-MS), was conducted to characterize hippocampus proteome in hAPPsI Tg mice model and to examine differences in the hippocampus proteomes between Tg and nTg mice, and to assess effect of CT1812 and CT2168 compared to vehicle in Tg animals using differentially expression analysis. STRING and MetaCore pathway analyses were performed using protein lists of p ≤ 0.05.

RESULTS

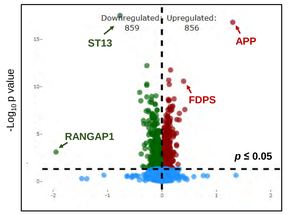
TOTAL DIFFERENTIALLY ABUNDANT PROTEINS IN PROTEOMIC ANALYSES

Condition	# DEGs (p ≤ 0.05)	# DEGs Upregulated	# DEGs downregulated
Tg vs. nTg	**1715**	856	859
Tg: CT1812 vs. Vehicle	**219**	114	105
Tg: CT2168 vs. Vehicle	**316**	181	135

Table 1. Summary of differentially abundant proteins in each treatment conditions in proteomic analyses.

hAPPsI MOUSE MODEL CHARACTERIZATION

A.



B.

Protein	UniProt ID	Log2FC	p-value
APP	A0A2I3BQZ9	1.30	1.52E-17
FDPS	A0A0G2JDJ5	0.42	2.59E-08
FDPS	Q3US29	0.40	2.62E-11
MSMO1	Q9CRA4	0.32	6.55E-08
DHCR24	Q8VCH6	0.32	3.23E-07
RANGAP1	Q91YS2	-1.95	7.93E-04
ST13	Q99L47	-0.77	2.97E-18
GPC4	P51655	-0.41	3.97E-10
TTC38	A3KMP2	-0.39	3.05E-09
SDHAF4	Q8BTE0	-0.38	1.29E-03

Figure 1. (A) Volcano plots to visualize the global proteomics change between Tg and nTg (hAPPsI) mouse model. Each data point in the scatter plot represents a protein. Green dots and red dots represent down and up regulated protein, respectively (p ≤ 0.05). (B) Top 5 up- and down-regulated protein show amyloid precursor protein (APP) as the most upregulated protein and RAN GTPase activating protein 1 (RANGAP1) as the most downregulated protein. Indicated proteins are the top 2 proteins on each fold change.

CT1812 AND CT2168 TREATMENT IMPACT THE PROTEOMIC PROFILE OF TREATED ANIMALS

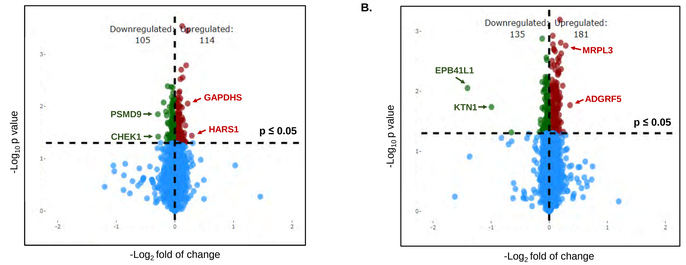


Figure 2. Volcano plots to visualize the global proteomic change after treatment with CT1812 (A) and CT2168 (B). Each data point in the scatter plot represents a gene. Green dots and red dots represent down- and up-regulated protein, respectively (p ≤ 0.05). Indicated proteins are the top 2 proteins on each fold change.

S2R MODULATORS ALTER PATHWAYS RELATED TO AD PATHOLOGY

A.

#	Top Proteomic Pathways (Tg CT1812 vs. Vehicle; p ≤ 0.05)	p-value
1	Development Negative regulation of WNT/Beta-catenin signaling in the cytoplasm	5.10E-03
2	DNA damage ATR activation by DNA damage	1.05E-02
3	Regulation of Adenylate cyclase and IMPA1 by lithium in major depressive disorder	1.50E-02
4	Transport Clathrin-coated vesicle cycle	2.29E-02
5	Cytoskeleton remodeling RalA regulation pathway	2.70E-02
6	Neurophysiological process Synaptic vesicle fusion and recycling in nerve terminals	7.30E-02

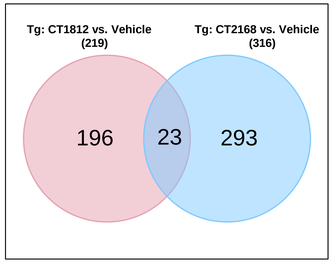
B.

#	Top Proteomic Pathways (Tg CT2168 vs. Vehicle; p ≤ 0.05)	p-value
1	Autophagy	1.01E-05
2	Cytoskeleton remodeling RalA regulation pathway	3.63E-04
3	Development Epigenetic and transcriptional regulation of oligodendrocyte precursor cell differentiation and myelination	5.93E-04
4	DNA damage ATM activation by DNA damage	1.32E-03
5	Signal transduction Muscarinic acetylcholine receptors signaling to second messengers	1.29E-02
6	Inhibition of remyelination in multiple sclerosis: role of cell-cell and ECM-cell interactions	1.67E-02

Table 2. MetaCore pathway analysis (v. 23.1.71200) of proteomics data using p-value criterion p ≤ 0.05. Pathways with CT1812 (A) and CT2168 (B) identified in non-relevant disease pathologies/organs were excluded from Top 6 pathways.

CT1812 AND CT2168 ALTER THE EXPRESSION OF 23 PROTEINS

A.



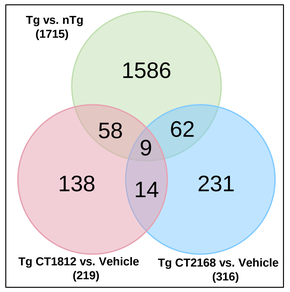
B.

Common Protein | Uniprot ID List	
AFF4 | Q9ESC8	NDOR1 | A2AI05
BAP1 | Q99PU7	OPA1 | P58281-2
CDA | P56389	PCNT | F8VPV0
COMMD6 | Q3V4B5	PRMT1 | Q9JIF0
CR1L | Q64735	RAP1B | Q99JI6
DNAH2 | D3Z667	RPL34 | Q9D1R9
DPF1 | D3YY44	RTL8B | Q9D1F0
FOXK2 | Q3UCQ1	SNX29 | Q9D3S3
GALT | A2AMS3	SULT4A1 | P63046
GDPD1 | Q9CRY7	THY1 | Q53YX2
INCENP | Q9WU62	TRIT1 | Q80UN9
NCAM1 | E9QB01	

Figure 3. (A) Venn diagram of common proteins of differential express (p ≤ 0.05) identified on proteomics of animals treated with CT1812 and CT2168. (B) Common proteins differentially expressed (p ≤ 0.05), in response to CT1812 and CT2168 treatment, at protein level include, for example **GALT[5]**, **NCAM1[6]**, and **OPA1[7]**, all of which are associated with AD phenotype.

COMMON PROTEINS BETWEEN nTg AND Tg CT1812 OR CT2168 TREATED ANIMALS

A.



B.

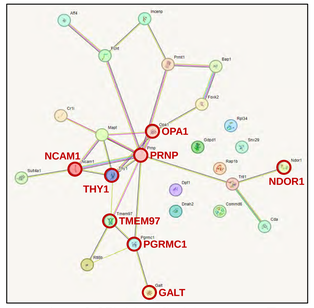


Figure 4. Venn diagram (A) of common differential abundant protein (p ≤ 0.05) identified between nTg and Tg treated animals with CT1812 and CT2168. Bitmap (B) with S2R complex components via STRING (version 12.0). TMEM97 (S2R), PRNP, and PGRMC1 were added to understand how they interacted with proteins identified in (A).

PROTEINS SHOWING NORMALIZATION TOWARDS HEALTHY nTg CONTROL

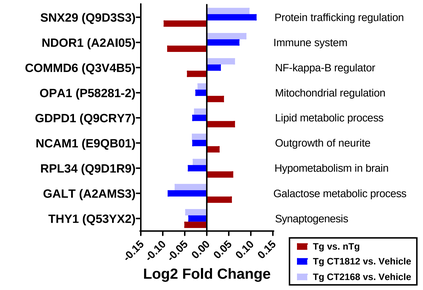


Figure 5. Forest Plot of common differential abundant protein (p ≤ 0.05) identified between nTg and Tg treated animals with CT1812 and CT2168. Proteins showing an aberrant expression in the Tg vs. nTg that are regulated in the opposing direction by CT1812 and CT2168 may underling a possible regulatory activity of the analyzed S2R modulators.

CONCLUSIONS

- **Elucidation of the proteomic profile for the hAPPsI transgenic mice were performed for the first time.**
- **Unbiased pathway analyses identify biological pathways including vesicle transport, autophagy, remyelination, synapse organization, and WNT/b-catenin signaling are impacted by treatment with two chemically distinct S2R modulators, CT1812 and CT2168.**
- **A S2R molecular signature was identified that was common to treatment effects with both chemically distinct S2R modulators, CT1812 and CT2168. Protein-protein interaction mapping illustrates a large degree of connectivity (p ≤ 0.05) including relevance with S2R components.**
- **A subset of proteins showing normalization towards healthy nTg control including proteins involved in trafficking, immune system, metabolic process, and synaptogenesis.**
- **These new findings can lead to further understanding of the molecular mechanism of action by which S2R modulators may be a promising therapeutic approach for AD patients.**

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REFERENCES:

1. Clinical trials NCT04735536, NCT03507790, NCT05531656.
2. Izzo NJ et al., Alzheimer's Dement. 2021 Aug 17(8):1365-1382.
3. Izzo NJ et al., PLoS One. 2014 Nov 12;9(11):e111898.
4. Di Caro V et al., AAIC 2023.
5. Coman DJ et al., Pediatric Research. 2010 Mar 67, 286-292.
6. Aisa B et al., J Alzheimers Dis. 2010 Jan 20(2):659-68.
7. Hoekstra JG et al., Alzheimers Res Ther. 2011 Jun 29;3(3):21

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