The Functional Role of *ENPP6* in the Pathogenesis of Psychosis in Alzheimer's Disease

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INTRODUCTION

Alzheimer's disease is a progressive, neurodegenerative disease that impacts thought, memory, and language. Recent literature published a systematic review that reported psychosis in 41% of patients diagnosed with Alzheimer's disease.

Figure 1A: Manhattan Plot

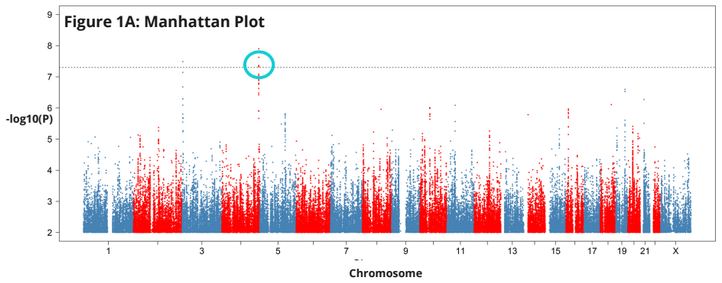
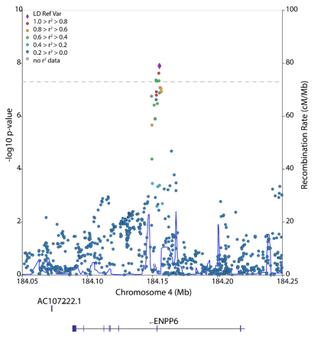


Figure 1B: Zoom Plot



Our lab conducted a genome-wide association meta-analysis for over 12,000 Alzheimer's disease subjects with psychosis (AD+P) and without psychosis (AD-P). One of the most significant genetic loci that was associated with psychosis was ectonucleotide phosphodiesterase 6 (*ENPP6*). (1)

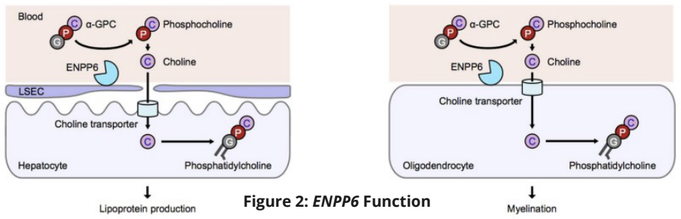


Figure 2: *ENPP6* Function

ENPP6 is a choline-specific phosphodiesterase involved in choline metabolic processes. The normal expression of *ENPP6* mRNA peaks as oligodendrocyte progenitor cells (OPCs) proliferate and declines during differentiation into mature oligodendrocytes in mouse models. There is uncertainty about its action in human models. (2)

METHODS

OPC/Oligodendrocytes are generated through induced pluripotent stem cells (iPSCs). The generation of OPC/Oligodendrocytes are examined by using four stage-specific markers: *PAX6*+, *OLIG2*, *O4*, and *MBP*. The markers are stained using immunofluorescence techniques. (3)

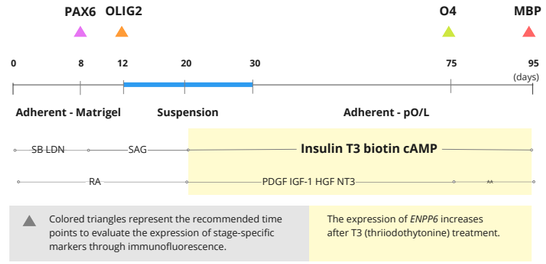
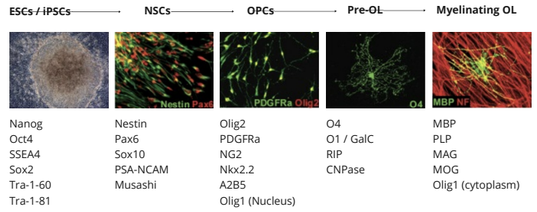


Figure 3: OPC/Oligodendrocytes protocol



Nanog	Nestin	Olig2	O4	MBP
Oct4	Pax6	PDGFRa	O1 / GalC	PLP
SSEA4	Sox10	NG2	RIP	MAG
Sox2	PSA-NCAM	Nkx2.2	CNPase	MOG
Tra-1-60	Musashi	A2B5		Olig1 (cytoplasm)
Tra-1-81		Olig1 (Nucleus)		

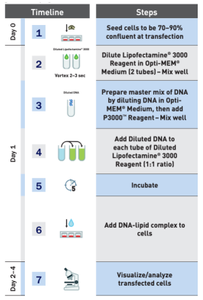


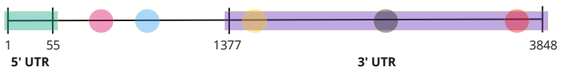
Figure 4: Lipofectamine 2000 Protocol

Target Sequence
CCACCTACTGCCTAGAATATA

Modified Sequence
+T*+A*+T*A*T*T*C*T*A*G*G*C*A*G*T*A*G*G*+T*+G*+G

*Phosphorothioate bonds
+Affinity Plus

Figure 5: ASO Location on *ENPP6* mRNA



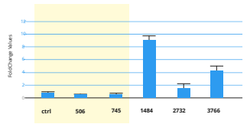
The lipofectamine 2000 protocol was used to knockdown *ENPP6* expression using ASOs. The reverse compliment of sequences were modified for ASO production. Phosphorothioate bonds were added to increase nuclease resistance and Affinity plus was added to increase affinity of the ASO to the target mRNA. RT-PCR and Western Blot protocols were used to assess RNA and protein level knockdown.

RESULTS

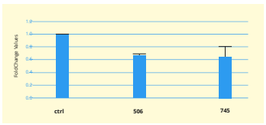
The impact of silencing the target gene was examined through quantitative reverse transcription polymerase chain reaction (RT-PCR) using primers from exon three and four. Five ASO's (506, 745, 1484, 2732, 3766) were screened to determine their *ENPP6* knockdown efficiency in the cell line SH-SY5Y.

Figure 5

***ENPP6* Expression After ASOs Incubation with SH-SY5Y Cells for 48 Hours**



***ENPP6* Expression After ASOs Incubation with SH-SY5Y Cells for 48 Hours**



The ASO's closer to the five-prime end of *ENPP6* had greater effects on knockdown compared to the three-prime end. The 506 and 745 were shown to individually knockdown *ENPP6* expression.

Figure 6

***ENPP6* Exon 3,4 with ASOs 506 & 745**

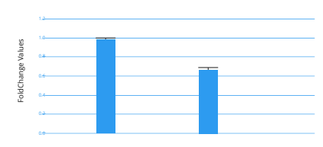
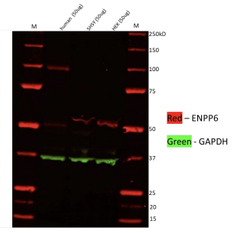


Figure 7

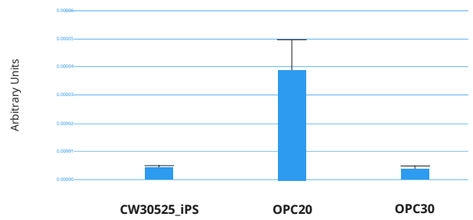


ENPP6 western blot

The combination of 506 and 745 did not display additive effects of knockdown. 1484 was shown to up-regulate *ENPP6* expression. At the protein level, the expected molecular weight for *ENPP6* is expected to be around 50 kDa. However, in the human postmortem sample, a band at 100 kDa appeared, which represents a dimer form of ENPP6.

Figure 8

***ENPP6* Exon 3, 4**



RT-PCR was conducted on iPS-derived OPCs prior and post a T3 treatment to asses *ENPP6* expression. Interestingly, the expression of ENPP6 was greater before T3 treatment, which differs from data presented in mouse models. iPS cells used to generate OPCs were used as a negative control.

DISCUSSION

OPC/ Oligodendrocytes were successfully generated from iPSCs. Two ASOs (506 and 745) were identified to successfully knockdown expression. We identified expression of *ENPP6* during OPC's maturation and differentiation, which can be utilized for downstream knockdown of *ENPP6*.

FUTURE DIRECTIONS

Our lab is currently exploring lentiviral-mediated knockdown of *ENPP6* in OPCs/Oligodendrocytes using the ASOs identified.

ACKNOWLEDGEMENTS

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