COVER SHEET

TITLE: A Quantitative Analysis of Proteomic Changes in Hippocampal Tissue of Alzheimer's Patients vs. Aged-Controls

AUTHOR'S NAME: Levi Martinka

MAJOR: Biology - Neuroscience option

DEPARTMENT: Department of Neurology

MENTOR: Corinna Burger

DEPARTMENT: Department of Neurology

MENTOR(2): N/a

DEPARTMENT(2): N/a

YEAR: Senior

(The following statement must be included if you want your paper included in the library's electronic repository.)

ABSTRACT

A Quantitative Analysis of Proteomic Changes in Hippocampal Tissue of Alzheimer's Patients vs. Aged-Controls

The purpose of this study was to identify changes in protein expression that occur in the hippocampus in Alzheimer's disease (AD). To achieve this, we performed a quantitative analysis comparing the protein levels from hippocampi of subjects with AD to aged-matched normal subjects. First, synaptoneurosome samples were prepared from hippocampal slices from deceased human patients. Then the samples were sent to the Mass Spectrometry Core, where they were analyzed to identify proteomic changes in expression of the sample tissue. The software Scaffold was used to validate MS/MS based peptide and protein identifications. Using the quantitative analysis of Scaffold, enrichment analysis was run using genes identified by Scaffold. From here identified ontologies and pathways were further explored for areas of interest related to AD. The results showed that 5 gene families– DPYSL, TUBB, ATP2B, PDE1 and DNM– might have a significant impact on the molecular mechanisms behind AD and need further investigation.

Levi Martinka / Biology - Neuroscience
Author Name/Major

Author Signature

5/1/2018
Date

NEUROLOGY
Mentor Name/Department

Mentor Signature

A Quantitative Analysis of Proteomic Changes in Hippocampal Tissue of Alzheimer's Patients vs. Aged-Controls

Abstract:

The purpose of this study was to identify changes in protein expression that occur in the hippocampus in Alzheimer's disease (AD). To achieve this, we performed a quantitative analysis comparing the protein levels in the synaptic region from hippocampi of subjects with AD to aged-matched normal subjects. First, synaptoneurosome samples were prepared from hippocampal slices from deceased human patients and enriched for perisynaptic proteins in order to increase the probability of detecting subtle changes in proteins at synaptic sites. Then the samples were sent to the Mass Spectrometry Core at the University of Wisconsin, where they were analyzed by tandem mass spectrometry (MS/MS) to identify proteomic changes in expression of the sample tissue. The software Scaffold was used to validate MS/MS based peptide and protein identifications received from the Mass Spectrometry Core. Using the quantitative analysis of Scaffold, enrichment analysis was run using genes identified by Scaffold as being significant. From here the identified ontologies and pathways were further explored for areas of interest related to AD. The results showed that 5 gene families– DPYSL, TUBB, ATP2B, PDE1 and DNM– might have a significant impact on the molecular mechanisms behind AD and need further investigation. This study will help to identify possible genes and biochemical pathways to focus on in further studies targeted to elucidate molecular mechanism of AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized, in part, by the accumulation of beta-amyloid (Aβ) and memory loss that mainly affects people over the age of 65 (Begcevic et al., 2013). Although the cause of AD is unknown, studies comparing the brain from individuals with AD to cognitively healthy individuals have shown that differences in mitochondrial function, calcium homeostasis and immune function may be critical to the development of AD (Begcevic et al., 2013)

As aging is the most significant risk factor of AD, there is a need to understand the mechanisms that occur during normal cognitive aging and how they may contribute to the pathologies observed in AD. Our lab previously completed genome-wide analyses examining transcriptional changes involved in learning and memory of aged rats in the CA1 and dentate regions of the hippocampus (Burger et al., 2007 and 2008). The hippocampus is a brain area that lies under the median temporal lobe and has been shown to play a critical role in learning and memory. These Burger et al. studies identified sets of 50 and 85 transcripts misregulated in CA1 and dentate respectively, in aged learning impaired rats.

The number of individuals with AD is expected to reach 14 million by 2050 (Neuner et al. 2017), so the identification of pathways and important genes is critical to understand the pathogenesis of the disease and for the development of effective therapies. Comparing the results of this study in humans to our previous findings in rats will help to identify genes of interest that overlap between the mechanisms of learning and memory in aging and with AD, and in doing so will help to target genes in human AD that we can study in rats.

Methods:

Hippocampal samples were obtained from 14 deceased male and female subjects: 7 with AD and 7 aged-controls, supplied by *The Wisconsin Alzheimer's Disease Research Center and the Wisconsin Brain Donor Program.* Synaptoneurosome samples were prepared from the slices (Cortese et al., 2018). Slices were homogenized in 200-300 mL of homogenization buffer (1 Tris, 1 sucrose, 0.5 EDTA,

and 0.25 EGTA [all in molar]) with protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO, USA) using a glass tissue grinder with a Teflon pestle. Nuclear material and unbroken cells were removed by centrifugation at 960 g for 15 minutes. The remaining supernatant was centrifuged at 15,000 g for 15 minutes yielding an S2 (cytosolic) fraction and a P2 (crude synaptic) fraction. The P2 synaptic pellet was then homogenized using a 0.5 mL plastic pestle in 100 mL homogenization buffer þ 0.5% SDS and sonicated. The P2 fraction is enriched for perisynaptic components including presynaptic and postsynaptic proteins, terminal mitochondria, and cytoplasm and synaptic vesicles in order to increase the probability of detecting subtle changes in proteins. Synaptic enrichment of the P2 fraction was confirmed by Western blot analysis using antibodies against synaptophysin (1:5000; 101-011; Synaptic Systems, Goet- tingen, Germany) and postsynaptic density 95 (PSD95; 1:1000; 3450; Cell Signaling, Danvers, MA, USA), common synaptic markers.

The samples were then sent to the Mass Spectrometry Core at the University of Wisconsin to be analyzed by tandem mass spectrometry (MS/MS) to identify changes in expression of the sample tissue. Tandem MS/MS uses collision-induced dissociation (CID) to fragment a precursor ion. A spectrum of ions is generated and the ion of interest is selectively transmitted into a collision cell. CID induces additional fragmentation in this cell and the resulting ions are mass analyzed as product ions.

The software Scaffold was used to validate MS/MS based peptide and protein identifications received from the Mass Spectrometry Core. Peptide identifications were accepted if they could be established at greater than 93.0% probability to achieve a false discovery rate (FDR; less than 1.0% by the Scaffold Local FDR algorithm). Protein identifications were accepted if they could be established at greater than 99.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides.

Results/Discussion:

1. Preliminary examination

Upon initial analysis, Scaffold identified 2351 individual proteins from the samples. After running a t-test comparing the AD samples to the aged-control samples, we were able to identify 55 statistically significant proteins at a p-value of .05. Of these 55 genes, 32 are up regulated in AD and 23 are down regulated in AD.

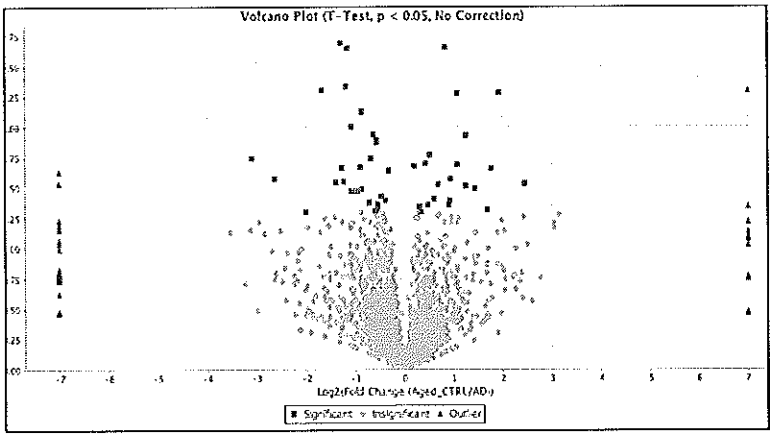


Fig. 1 For every gene, the fold change was plotted against the –logP value. Statistically significant differentially expressed genes, with a ≥1.25 or ≤-1.25, are depicted as green, insignificant as orange and outliers as red. Of the 2351 identified genes55 were differentially expressed (32 up- and 23 down-regulated).

Up regulated Gene name	Gene symbol	Accesion #	Parametric p value
Amyloid beta A4 protein	APP	P05067	0.002
Epimerase family protein SDR39U1	SDR39U1	Q9NRG7	0.0022

Calpain-1 catalytic subunit	CAPN1	P07384	0.0046
Long-chain-fatty-acid--CoA ligase 6	ACSL6	Q9UKU0	0.005
Membrane protein MLC1	MLC1	Q15049	0.0074
V-type proton ATPase subunit G 1	ATP6V1G1	O75348	0.0076
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	ATP2A2	P16615	0.01
Amine oxidase [flavin-containing] B	MAOB	P27338	0.012
Plasma membrane calcium-transporting ATPase 1	ATP2B1	P20020	0.013
Platelet-activating factor acetylhydrolase IB subunit gamma	PAFAH1B3	Q15102	0.013
Helicase SRCAP	SRCAP	Q6ZRS2	0.018
Plasma membrane calcium-transporting ATPase 4	ATP2B4	P23634	0.018
Histone H1.4	HIST1H1E	P10412	0.021
Thioredoxin-related transmembrane protein 4	TMX4	Q9H1E5	0.022
Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	PPP2R1A	P30153	0.023
Neurexin-3	NRXN3	Q9Y4C0	0.024
Synaptoporin	SYNPR	Q8TBG9	0.027
Palladin	PALLD	Q8WX93	0.028
UPF0317 protein C14orf159, mitochondrial	C14orf159	Q7Z3D6	0.029
Glycerol kinase	GK	P32189	0.029
Phosphatidate cytidylyltransferase 2	CDS2	O95674	0.033
NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial	NDUFS7	O75251	0.034
Ras-related protein Rab-6B	RAB6B	Q9NRW1	0.034
Plectin	PLEC	Q15149	0.037
Transgelin-2	TAGLN2	P37802	0.037
ADP/ATP translocase 2	SLC25A5	P05141	0.04
Erythrocyte band 7 integral membrane protein	STOM	P27105	0.042
LanC-like protein 1	LANCL1	O43813	0.043
Mitochondrial carrier homolog 2	MTCH2	Q9Y6C9	0.043
Amine oxidase [flavin-containing] A	MAOA	P21397	0.048
Cytochrome c oxidase subunit 7A-related protein, mitochondrial	COX7A2L	O14548	0.049
Solute carrier family 2, facilitated glucose transporter member 1	SLC2A1	P11166	0.05

Down regulated Gene name	Gene Symbol	Accesion #	Parametric p-value
Dihydropyrimidinase-related protein 4	DPYSL4	O14531	0.0022
Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1C	PDE1C	Q14123	0.0051
Kinesin light chain 2	KLC2	Q9H0B6	0.0052
Twinfilin-2	TWF2	Q6IBS0	0.0053
Ubiquilin-1	UBQLN1	Q9UMX0	0.012
T-complex protein 1 subunit alpha	TCP1	P17987	0.017
Disks large homolog 2	DLG2	E9PN83	0.02

STE20/SPS1-related proline-alanine-rich protein kinase	STK39	Q9UEW8	0.02
Tubulin beta-8 chain	TUBB8	Q3ZCM7	0.021
Neurosecretory protein VGF	VGF	O15240	0.022
S-adenosylmethionine synthase isoform type-2	MAT2A	P31153	0.027
La-related protein 1	LARP1	Q6PKG0	0.029
Galectin-1	LGALS1	P09382	0.03
Copine-1 (Fragment)	CPNE1	F2Z2V0	0.03
Epidermal growth factor receptor substrate 15	EPS15	P42566	0.032
Kinesin light chain 1	KLC1	E7EVH7	0.039
Bifunctional protein NCOAT	MGEA5	O60502	0.04
Protein NDRG1	NDRG1	Q92597	0.044
Rho guanine nucleotide exchange factor 2	ARHGEF2	V9GYM8	0.044
Glucose-induced degradation protein 8 homolog	GID8	Q9NWU2	0.045
Tubulin alpha-4A chain	TUBA4A	P68366	0.045
Breakpoint cluster region protein	BCR	P11274	0.048
Dynamin-3	DNM3	Q9UQ16	0.05

Tables 1 + 2 List of the 55 statistically significant genes. The up-regulated genes are in green (top) and the down-regulated genes are in red (bottom). The p-values were calculated from a t-test using the normative values for each of the AD and aged-control subjects. The genes listed in blue are the identified genes of interest.

When compared to the prior work (Burger et al., 2007 and 2008) we were able to identify 3 gene families that are statistically significant in the hippocampi of both rats and humans, DPYSL4, TUBB and ATP2B. We also established that the DNM and PDE1 families show promise for future AD studies.

2. DPYSL and TUBB:

In comparing to the proteomic analyses performed on the hippocampal dentate region, we found that proteins from the DPYSL and TUBB families were both down regulated in aged rats. More particularly, the study found both the collapsing-response mediated (DPYSL5) protein and the beta tubulin 3 (TUBB3) protein to be down regulated–both of which are known markers of immature neurons (Quach et al., 2013; Liang et al., 2008). Likewise, we found DPYSL4 and TUBB8 to be down

regulated in AD with p-values of 0.0022 and 0.021, respectively. Down regulation of these genes seems to indicate a decrease in neuronal development in this region associated with aging (Burger et al., 2008).

2.1 DPYSL family:

The DPYSL genes code for a family of proteins (CRMPs) that are expressed abundantly in the nervous system. These proteins are involved in the control of growth and collapse of neuritis, synaptic activity and nociceptor excitability (Quach et al., 2013). DPYSL5 is widespread throughout the CNS and a down regulation has been shown to lead to a decrease in filopodia and growth cone formation. In contrast, DPYSL4 is mostly localized in the hippocampus (Hotta et al., 2004). Previous studies have found that DPYSL4 regulates dendritic complexity in hippocampal neurons. DPYSL4 knockout animals have displayed impairment with abnormal long-term potentiation (LTP) and a deficit in prepulse inhibition (Quach et al 2008).

Because DPYSL4's expression is maintained throughout life in the hippocampus (Imperlini et al., 2014) it reasonable to hypothesize that it could be involved in aging and the pathophysiology of neurodegenerative diseases like AD. So, future studies need to look into the role of DPYSL4 as a neurite or dendrite outgrowth regulator. These studies should examine the mechanism of action on dendrites by looking into how the CRMP proteins react to calcium signaling. Also, the use of CRMP should also be looked into as a novel therapeutic agent to reduce or limit hippocampal dendritic

dystrophy.

2.2. TUBB family:

The TUBB family of proteins encode for beta tubulin proteins. These proteins form a dimer with alpha tubulin (TUBA) to act as a structural component of microtubules (Dubey et al., 2015). Our earlier genome wide study in rats is in accordance with other studies by identifying TUBB3 as a gene of interest in AD. Mutations in this gene have been shown to cause lower stability of microtubules and lead to cortical neuron migration and axonal guidance defects in mouse models (Dubey et al., 2015). Contrastingly, there have been few studies looking at the role of TUBB8 in the brain. TUBB8 is uniquely expressed in human oocytes and early embryos so much of the previous studies have looked into how mutations of TUBB8 can lead to oocyte meiotic arrest (Feng et al 2016).

However, TUBB8 has been shown to play a role in the CNS pathways such as the gap junction, cytoskeleton remodeling and receptor-mediated axon growth repulsion (Reuters, 13-15). Interestingly, the TUBB proteins interact with the DPYSL proteins in the axon growth repulsion mechanism. Future studies need to look at the levels of TUBB8 in neurodegenerative diseases to possibly identify as a gene of interest to focus on.

3. DNM3:

Many of the statistically significant genes have ontologies that deal with transport

and localization: APP, ATP2A2, ATP2B1, ATP2B4, ATP6V1G1, DNM3, RAB6B, SLC25A5, and SLC2A1. However, all of these genes are up regulated besides DNM3. This is interesting because of the shared functionality we would expect DNM3 to follow the trend of being upregulated. However, it is significantly downregulated with a p-value of 0.05. DNM3 encodes GTP-binding proteins that associate with microtubules and are involved in vesicular transport, particularly endocytosis. The Dynamin complex (DNM1-3) are important components in the cleavage of clathrin-coated vesicles and help in organelle division, viral resistance and mitochondrial fusion/fission (Singh et al., 2017)

Synaptic dysfunction is one of the most important events in the pathogenesis of AD. As previously stated, the accumulation of amyloid-β (Aβ) is one of the main causes of AD. Aβ has been shown to interact with DNM2, which is a homolog to DNM1 (Kamagata et al., 2009). In these studies DNM2 has been found to be down regulated in Late Onset AD (LOAD), suggesting that the cascade of neurodegeneration caused by Aβ involves the DNM proteins, although the mechanism behind this is unclear. Further studies need to pursue looking into the relationship between the DNM complex and Aβ. These studies need to particularly look at the individual roles of DNM1, DNM2 and DNM3, and how DNM3 plays a role in the production of Aβ plaques.

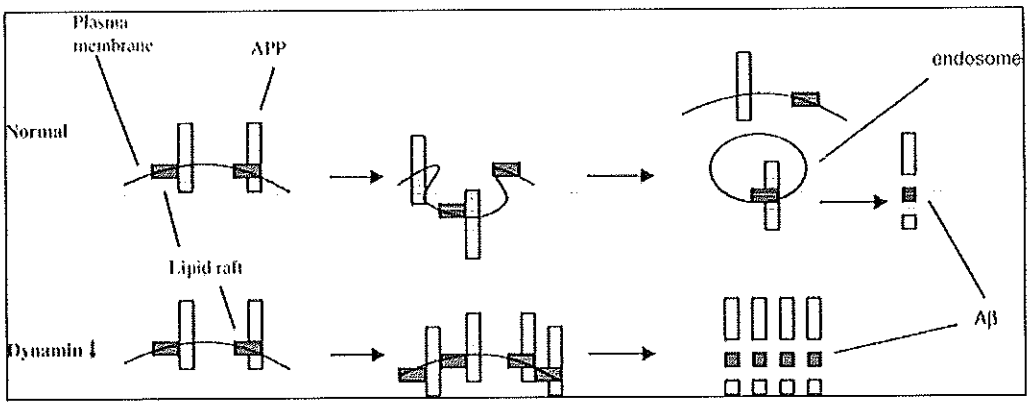
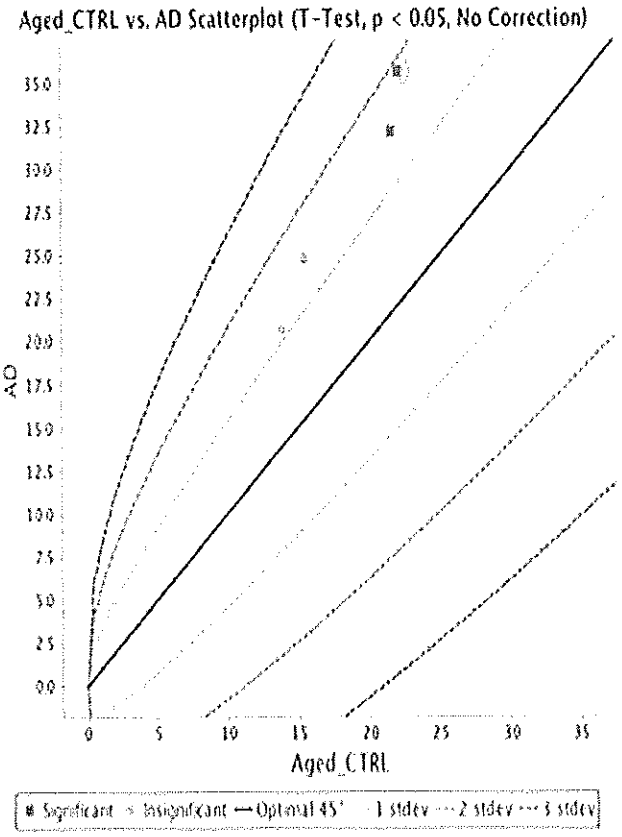


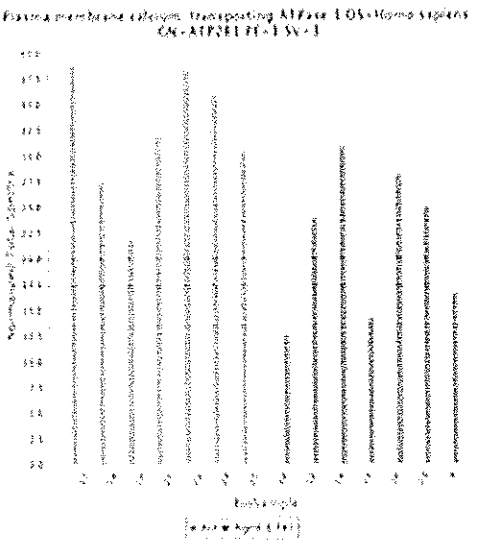
Fig. 2 Dysfunction of DNM2 increased Aβ generation as a result of the accumulation of APP in the plasma membrane. In normal conditions, APP is transported to plasma membrane through the endoplasmic reticulum and the Golgi apparatus and then is taken up in endosomes by endocytosis, where Aβ generation occurs. In contrast, neuronal cells that have lost DNM function may have an accumulation of APP in the plasma membrane, because the major role of DNM is in endocytosis. In DNM dominant negative neuronal cells increased Aβ production may occur due to the accumulation of APP and the presence of lipid raft or PS1 in the plasma membrane (Kamagata et al 2009).

4. ATP2B and PDE1:

4.1. ATP2B family:

In our previous paper, both Atp2b2 and Atp2b3 were found to be down regulated in the CA1 region of the hippocampus of rats that were superior learners. So, as expected, we found both ATP2B2 and ATP2B3 to be up regulated in the AD samples; However, these genes were not statistically significant, but we did find the related proteins, ATP2B1 and ATP2B4, to be significantly up regulated with p-values of 0.013 and 0.018, respectively.





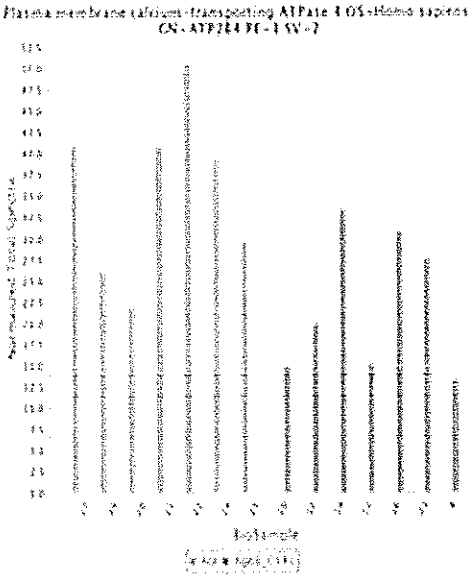


Fig. 3 The scatterplot on the left plots the mean normalized total spectra of each gene in the AD vs. the aged-control samples and identifies them as statistically significant or statistically insignificant. The order of genes (top to bottom) is ATP2B4, ATP2B4, ATP2B2, and ATP2B2 respectively.

Fig. 4 The 2 bar graphs show the normalized total spectra of ATP2B1 (top) and ATP2B4 (bottom) from each sample. The AD samples are in red and the aged-control samples are in blue

This family of genes code for plasma membrane Ca^{2+} pumps that play an important role in intracellular calcium homeostasis by removing bivalent calcium ions against very large concentration gradients (Shull, 2000). Failure of excitable cells to maintain the optimal Ca^{2+} cytosolic levels may be a common pathway leading to cell death. This is critical in many aging processes, including neuronal dysfunction underlying dementia. In AD, Ca^{2+} dysregulation seems to be highly influenced by the presence of neurotoxic Aβ in brains. This is important because the ATP2B proteins

are Ca^{2+}-regulatory proteins that may interact with Aβ (Mata et al., 2011). Suggesting that these proteins likely participate in many processes from development to aging.

Previous studies have shown that brain areas enriched with ATP2B4 show the highest inhibition by Aβ (Mata et al. 2011). Additionally ATP2B4 is uniquely found in lipid rafts. These membrane domains are characterized by abundance in cholesterol and glycosphingolipids that participate as platforms for cell signal transduction (Sepulveda et al., 2006). Thus, disruption of these rafts lead to a reduction of synapses and loss of dendritic spines leading to neurodegenerative processes. These observations identify the ATP2B4 proteins as a molecular mechanism involved in AD by its interaction with Aβ. Future investigations should look at other possible interactions with tau proteins, the other trademark of AD.

4.2 PDE1 family:

Because ATP2B1 and ATP2B4 show strong potential in affecting neurodegeration, we ran additional analysis to search for further interactions. GeneMANIA was used to identify genes that have shown interactions with these genes in previous studies. These interactions may include, predicted networks, physical interactions, shared protein domains, co-expression, co-localization and pathways. After running this analysis, 7 genes showed connections to both ATP2B1 and ATP2B4: ATP2A2, DLG2, DLG1, CALM1, HM13, SLC9A6, SLC9A7.

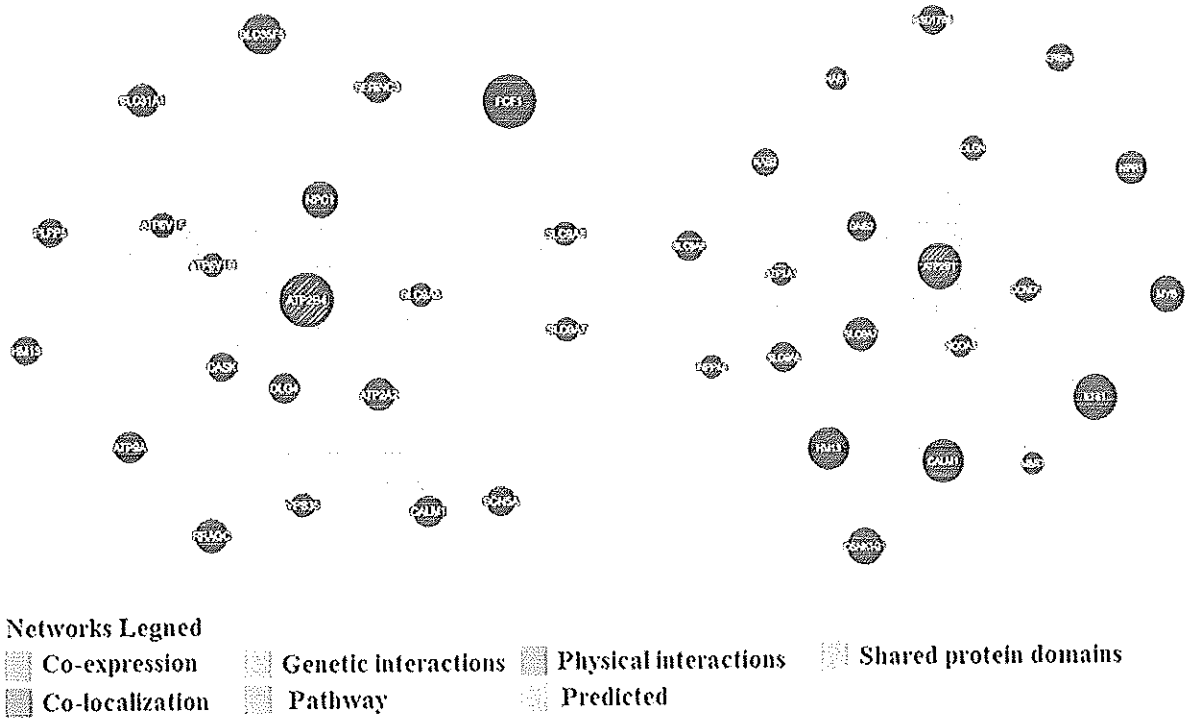


Fig. 5 Network of the ATP2B14 and ATP2B1 genes constructed using GeneMANIA. The ATP2B4 gene network (left) consists of 20 genes and the ATP2B1 gene network (right) also consists of 20 genes, 7 of which are shared.

These genes were then run through STRING, which is an analysis tool that summarizes the network of predicted associations for a group of genes. In doing this, we can see that CALM1 and ATP2B4 are nodes that show many associations.

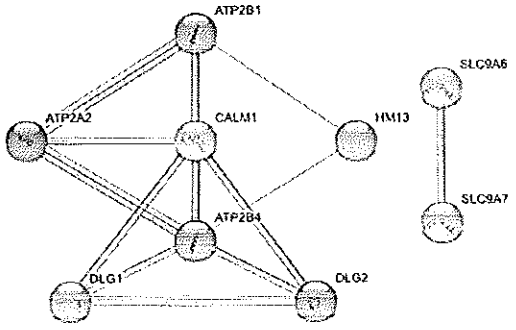


Fig. 6 Network of the predicted associations for the connected genes found using GeneMANIA. The nodes represent proteins, empty nodes have unknown 3D structure, filled nodes have known or predicted protein structure.

- Red line: indicates the presence of fusion evidence
- Green line: neighborhood evidence
- Blue line: co-occurrence evidence
- Purple line: experimental evidence
- Yellow line: textmining evidence
- Light blue line: database evidence

This set of genes was also run through the gene enrichment tool, GATHER, to identify pathways affected by these genes. We found that ATP2B1, ATP2B4, ATP2A2 and CALM1 all play a role in the Ca^{2+} signaling pathway. In this pathway CALM1

(Calmodulin 1) plays an important regulatory role that mediates the control of a large number of enzymes, ion channels, aquaporins and other proteins through calcium-binding (Berridge et al., 2003).

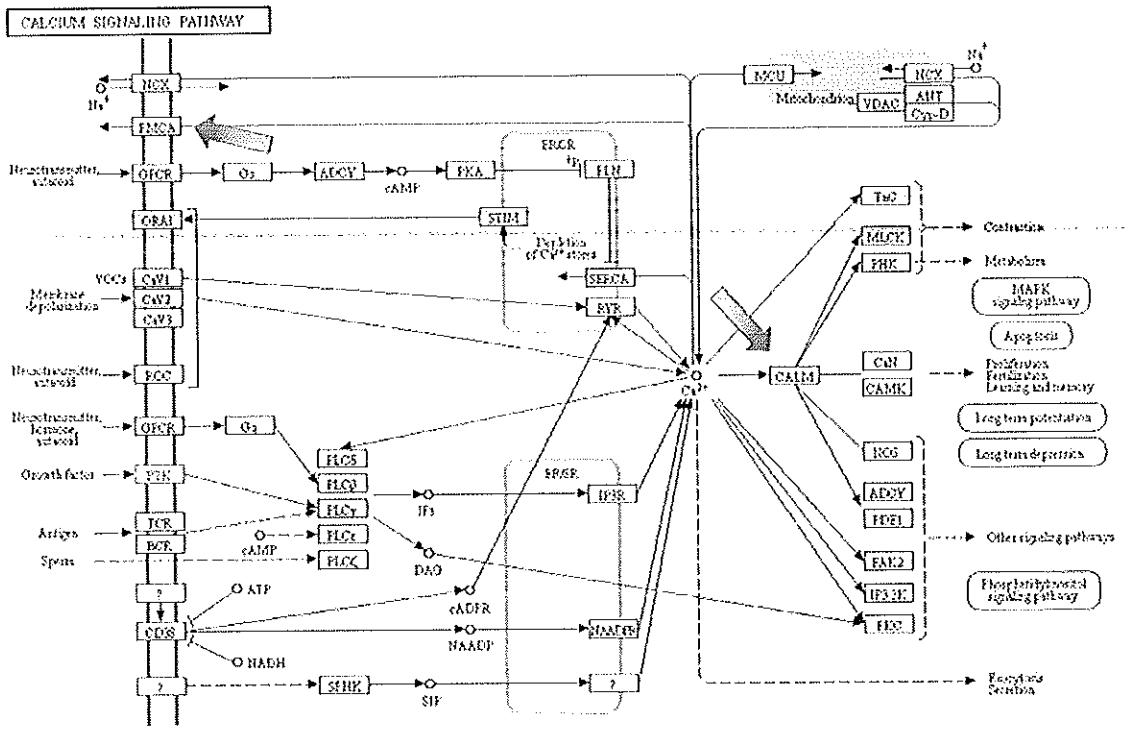


Fig. 7 Kegg pathway map visualization of the Calcium signaling pathway that depicts the molecular interaction and reaction network. The ATP2B proteins (PMCA) and CALM1 are pointed out using orange arrows.

Although we did not find CALM1 levels to be changed in our analysis, did find PDE1C to be significantly down regulated. The PDE1 family is a cyclic nucleotide phosphodiesterase that is activated by calcium in the presence of calmodulin (Wennogle et al., 2017). These genes code for proteins that are responsible for the breakdown of cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), that are important in neuroplasticity and protection. cAMP and cGMP serve to relay and amplify incoming signals at receptors on the cell surface (Bollen and Prickaerts, 2012).

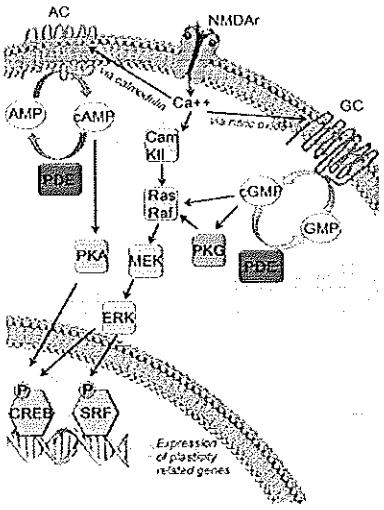


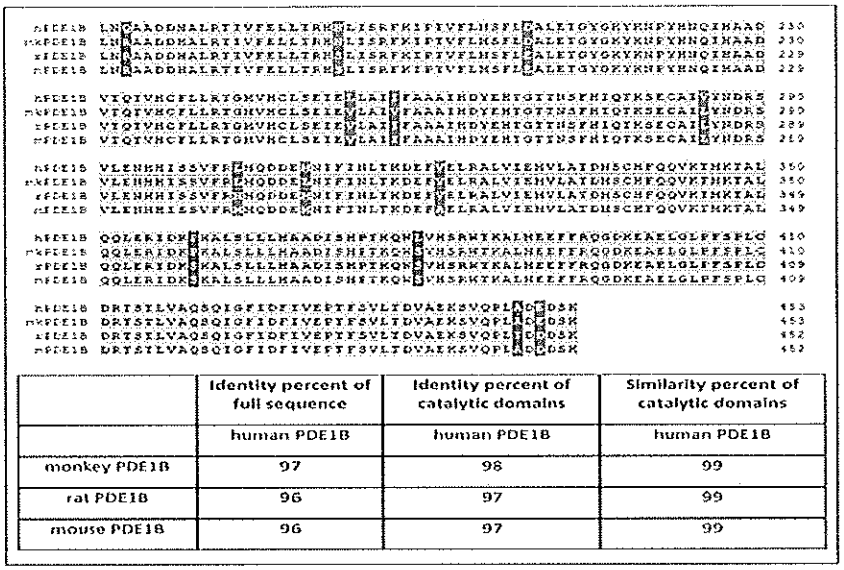
Fig 8 Inhibition of phosphodiesterase type 1 (PDE1) lead to the increase of cAMP and cGMP and ultimately to the expression of plasticity-related genes. AC, adenyl cyclase; GC, guanylate cyclase; CREB, cAMP responding element binding protein; SRF, serum response factor (Alexandre, 2011)

Of the 11 PDE families, only PED1 is activated in this manner (Alexandre, 2011), leading us to believe that PDE1 is involved when intra-cellular calcium levels are elevated and thus has an activity-dependent involvement in the control of cyclic nucleotides in excitatory neurons (Wennogle et al., 2017). Because the ATP2B family is up regulated, the intracellular calcium levels are lower, leading to expected lower levels of PED1 proteins. However, we found that PDE1A/B have no significant difference between the AD and controls. Due to these higher levels of PDE1A/B than expected, there are fewer cAMP and cGMP molecules than needed. Making PDE1A and PDE1B a potential target for drug therapy

Subcellular localization of PDE1 enzymes inside neurons is poorly researched, but the possibility exists of high enrichment is particular microenvironments. An example of this is that it is heavily localized with calcium-calmodulin activated kinase II (CamKII). CamKII plays an important role in synaptic plasticity and memory formation (Miller et al., 2011). Interestingly, CamKII is responsible for the phosphorylation of only PDE1B while protein kinase A (PKA) phosphorylates

PDE1A and PDE1C (Wennogle et al. 2017). This leads us to believe that CamKII and PKA concentrate the PDE1 family in these environments, giving us a way to target the specific areas of interest in the brain.

PDE1 isoforms are highly conserved across species, making them ideal proteins to study; however, they are also highly homologous. The ~85% homology between the isoforms makes it very difficult to discover an inhibitor of the drug specific to 1 or 2 isoforms (Wennogle et al. 2017).



	Identity percent of full sequence	Identity percent of catalytic domains	Similarity percent of catalytic domains
	human PDE1B	human PDE1B	human PDE1B
monkey PDE1B	97	98	99
rat PDE1B	96	97	99
mouse PDE1B	96	97	99

Fig. 9
Alignment of the amino acid sequence of the catalytic domain of PDE1B across species. Sequence alignment of the catalytic domains of PDE1Bs from different species.

h human, *mk* rhesus monkey, *r* rat, *m* mouse. *Blue* non-conserved substitution, *Dark Grey* conserved substitution, and *Light Grey* identical amino acids. Alignment of sequences was performed with the CLUSTAL W multiple sequence alignment program (Wennogle et al. 2017)

Though, there should be no downside to targeting all 3 isoforms –the rationale behind this is there is no known undesirable effect of the redundancy. But, if aiming only for certain tissues in the brain, PDE1B is a superior target since it is highly expressed in the striatum, hippocampus and prefrontal cortex (PFC) and can be localized by CamKII, while PDE1A and C are found in lesser levels and are more ubiquitous in the brain; PDE1A/C are also localized in cardiac and lung tissue.

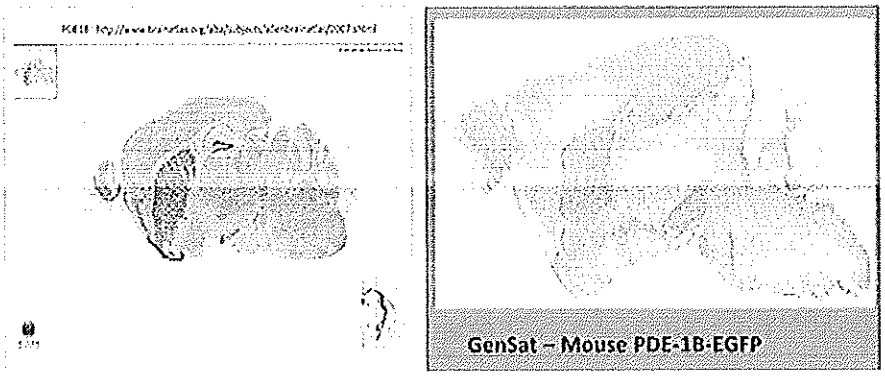


Fig. 10
Distribution of PDE1B in mouse brain as revealed by the Allen brain atlas (*left*) and GenSat (*right*) Technologies

When looking into potential drug therapies, we included all PDE1 isoforms in the enrichment but tried to focus on drugs that target PDE1B due to its abundance in the brain. In using the Comparative Toxicogenomics Database (CTD), we identified vinpocetine and copper as a potential therapeutic agents with specific affinity towards PDE1. Although, vinpocetine has been used as an AD drug therapy for the past 20 years, further studies should be performed to examine the efficacy of the drug towards PDE1B. In doing so the drug will be more successful in targeting specific tissues and will lead to better patient outcomes.

Acknowledgements

This research was supported by grant R01AG048172-03 to CB. I would like to thank *UW-Madison Biotechnology Center's Mass Spectrometry/Proteomics Facility, The Wisconsin Alzheimer's Disease Research Center and the Wisconsin Brain Donor Program.* I would also like to thank Professor Stephen Gammie, Andrew Olin, Kirstan Gimse and Corinna Burger, PhD, for their help, encouragement and support that made this possible.

Works Cited

1. Begcevic, Ilijana et al. "Semiquantitative Proteomic Analysis of Human Hippocampal Tissues from Alzheimer's Disease and Age-Matched Control Brains." *Clinical proteomics* 10.1 (2013): 5. *PMC.*
2. Neuner, Sarah et al. "Hippocampal proteomics defines pathways associated with memory decline and resilience in normal aging and Alzheimer's disease." *Behavioural Brain Research* 322.B (2017): 288-298. *ScienceDirect.*
3. Burger, Corinna et al. "Genome-wide analysis of aging and learning-related genes in the hippocampal dentate gyrus." *Neurobiology of Learning and Memory.* 89 (2008): 379-396. *ScienceDirect.*
4. Burger, Corinna et al. "Changes in transcription within the CA1 field of the hippocampus are associated with age-related spatial learning impairments." *Neurobiology of Learning and Memory.* 87.1 (2007): 21-41. *ScienceDirect.*
5. Cortese, Giuseppe et al. "Environmental enrichment improves hippocampal function in aged rats by enhancing learning and memory, LTP, and mGluR-Homer1c activity." *Neurobiology of Learning and Memory.* 63.1 (2018): 1-11. *ScienceDirect.*
6. Quach, Tam et al. "Mapping CRMP3 domains involved in dendrite morphogenesis and voltage-gated calcium channel regulation." *Journal of Cell Science.* 126 (2013): 4262-4273.
7. Liang, Winnie et al. "Altered neuronal gene expression in brain regions differentially affected by Alzheimer's disease: a reference data set." *Physiological Genomics.* 33.2 (2008): 240-256. *American Physiological Society.*
8. Hotta, Azusa et al. "Critical Role of Collapsin Respoinse Mediator Protein-associated Molecule CRAM for Filopodia and Growth Cone Development in Neurons." *Molecular Biology of the Cell.* 16.1 (2004): i-432. *Scientific Society Publisher Alliance.*
9. Quach, Tam et al. "CRMP3 is required for Hippocampal CA1 dendritic organization and plasticity." *The FASEB Journal.* 22.2 (2008).
10. Imperlini, Esther et al. "Altered Brain Protein Expression Profiles Are Associated with Molecular Neurological Dysfunction in the PKU Mouse Model." *Journal of Neurochemistry* 129.6 (2014): 1002–1012.
11. Dubey, J., Ratnakaran, N., Koushika, S. "Neurodegeneration and microtubule dynamics: death by a thousand cuts." *Frontiers in Cellular Neuroscience.* (2015).

12. Feng, Ruizhi et al. "Mutations in TUBB8 and Human Oocyte meiotic Arrest." *The New England Journal of Medicine.* 374 (2016): 223-232.
13. Reuters, Thomson. "Pathway Maps: Neurophysiological process_Receptor-Mediated Axon Growth Repulsion." *Life Sciences Research.* http://pathwaymaps.com/maps/527.
14. Reuters, Thomson. "Pathway Maps: Cell adhesion_Gap junctions." *Life Sciences Research.* http://pathwaymaps.com/maps/1485.
15. Reuters, Thomson. "Pathway Maps: Cytoskeleton remodeling_Neurofiliments." *Life Sciences Research.* http://pathwaymaps.com/maps/1491.
16. Shull, Gary "Gene knockout studies of Ca^{2+}-transporting ATPases." *European Journal of Biochemistry.* 267 (2000): 5284-5290.
17. Mata, Ana et al. "Impairment of the activity of the plasma membrane Ca^{2+}-ATPase in Alzheimer's disease." *Biochemistry Society Transactions.* 39.3 (2011): 819-822.
18. Sepulveda, Rosario et al. "The Plasma Membrane Ca^{2+}-ATPase Isofrorm 4 Is Localized in Lipid Rafts of Cerebellum Synaptic Plasma Membranes." *Journal od Biological Chemistry.* 281 (2006):447-453.
19. Berridge, Michael et al. "Calcium: Calcium signaling: dynamics, homeostasis and remodeling." *Nature Reviews Molecular Cell Biology.* 4 (2003): 517-529.
20. Wennogle, Lawrence et al. "Phosphodiesterase 1: A Unique Drug Target for Degenerative Diseases and Cognitive Dysfunction." *Phosphodiesterases: CNS Functions and Diseases.* Advances in Neurobiology. 15 (2017): 349-384.
21. Bollen, Eva and Prickaerts, Jos "Phosphodiesterases in neurodegenerative disorders." *IUBMB Life.* 64.12 (2012): 965-970.
22. Medina, Alexandre E. "Therapeutic Utility of Phosphodiesterase Type I Inhibitors in Neurological Conditions." *Frontiers in Neuroscience* 5 (2011): 21. *PMC.* 2018.
23. Miller, Stephan et al. "Disruption of Dendritic Translation of CaMKIIa Impairs Stabilization of Synaptic Plasticity and Memory Consolidation." *Neuron.* 36.3 (2002): 507-519. *ScienceDirect.*
24. Singh, Mahaveer et al., "Dynamin Functions and Ligand: Classical Mechanisms Behind." *Molecular Pharmacology.* 91.2 (2017): 123-134.
25. Kamagata, Eiichiro et al. "Decrease of dynamin 2 levels in late-onset Alzheimer's disease alters Aβ metabolism" *Biochemical and Biophysical Research Communications.* 379.3 (2009): 691-695