Title

Mutant Presenilin 1 Dysregulates Exosomal Proteome Cargo Produced by Human-Induced Pluripotent Stem Cell Neurons

Journal

ACS Omega, 6(20)

ISSN

2470-1343

Authors

Podvin, Sonia

Jones, Alexander

Liu, Qing

et al.

Publication Date

2021-05-25

DOI

10.1021/acsomega.1c00660

Peer reviewed

ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Mutant Presenilin 1 Dysregulates Exosomal Proteome Cargo Produced by Human-Induced Pluripotent Stem Cell Neurons

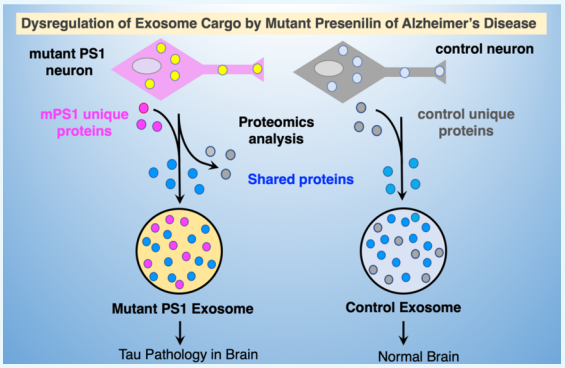
Sonia Podvin, Alexander Jones, Qing Liu, Brent Aulston, Charles Mosier, Janneca Ames, Charisse Winston, Christopher B. Lietz, Zhenze Jiang, Anthony J. O'Donoghue, Tsuneya Ikezu, Robert A. Rissman, Shauna H. Yuan, and Vivian Hook*

Cite This: *ACS Omega* 2021, 6, 13033−13056

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The accumulation and propagation of hyperphosphorylated tau (p-Tau) is a neuropathological hallmark occurring with neurodegeneration of Alzheimer's disease (AD). Extracellular vesicles, exosomes, have been shown to initiate tau propagation in the brain. Notably, exosomes from human-induced pluripotent stem cell (iPSC) neurons expressing the AD familial A246E mutant form of presenilin 1 (mPS1) are capable of inducing tau deposits in the mouse brain after *in vivo* injection. To gain insights into the exosome proteome cargo that participates in propagating tau pathology, this study conducted proteomic analysis of exosomes produced by human iPSC neurons expressing A246E mPS1. Significantly, mPS1 altered the profile of exosome cargo proteins to result in (1) proteins present only in mPS1 exosomes and not in controls, (2) the absence of proteins in the mPS1 exosomes which were present only in controls, and (3) shared proteins which were upregulated or downregulated in the mPS1 exosomes compared to controls. These results show that mPS1 dysregulates the proteome cargo of exosomes to result in the acquisition of proteins involved in the extracellular matrix and protease functions, deletion of proteins involved in RNA and protein translation systems along with proteasome and related functions, combined with the upregulation and downregulation of shared proteins, including the upregulation of amyloid precursor protein. Notably, mPS1 neuron-derived exosomes displayed altered profiles of protein phosphatases and kinases involved in regulating the status of p-tau. The dysregulation of exosome cargo proteins by mPS1 may be associated with the ability of mPS1 neuron-derived exosomes to propagate tau pathology.



INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder resulting in deficits in cognitive function. AD brain neuropathology displays progressive neuronal loss and severe neurodegeneration, with the accumulation of amyloid plaque deposits and neurofibrillary tangles (NFTs). NFTs result from the accumulation of hyperphosphorylated tau (p-Tau)[1−5] which lacks the ability to interact with microtubules, leading to detrimental effects on neuronal synaptic functions. Tau oligomers impair long-term potentiation and result in memory loss.[6] Tau undergoes cell−cell propagation in the brain cortex and hippocampus,[7−10] which leads to memory deficits and synaptic impairment.[11−15]

Recent evidence demonstrates that exosomes are involved in tau propagation.[16−19] Exosomes are released from neurons, glia, and many cell types and are characterized as extracellular vesicles of the endosomal origin[20−23] for the removal of cellular components. Exosomes participate in the transcellular shuttling of exosome cargo molecules consisting of proteins, RNAs, lipids, and metabolites.[24] Tau is present in exosomes from the cerebrospinal fluid of AD patients.[25] Studies of neuron-derived exosomes (NDEs) isolated from the plasma of AD patients demonstrate that p-Tau levels in NDE predict conversion from mild cognitive impairment to dementia of AD.[17] Significantly, the intracranial injection of AD patient-derived plasma NDEs into the mouse brain results in AD-like tau neuropathology. Furthermore, the inhibition of the exosome synthesis retards tau propagation in the mouse brain involving microglia.[16] These findings show that exosomes can mediate transcellular spreading of tau in the brain.

Familial AD (FAD) represents genetic forms of AD and sporadic AD represents AD patients with no known genetic mutation(s).[26−28] Gene mutations of FAD have provided much

Received: February 4, 2021
Accepted: April 16, 2021
Published: May 13, 2021

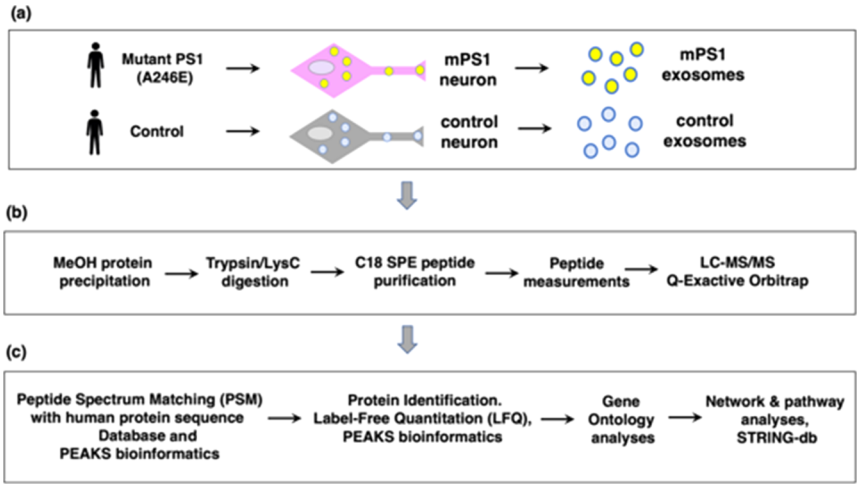


Figure 1. Proteomic study design of exosomes generated by mPS1 iPSC neurons and by control iPSC neurons. (a) mPS1 human neurons and control wild-type PS1 human neurons for exosome isolation. The iPSC neurons were derived (by a reprograming protocol) from a patient with A246E mPS1 and from a healthy control patient with wild-type PS1, as we have reported earlier.[19,34−37] It is known that reprograming of the fibroblast from biopsies into pluripotent stem cells and differentiation into neurons erases the aging phenotype and generates "age-equivalent" iPSC neurons.[38,39] The neuronal phenotype of the iPSC neurons has been confirmed by the presence of multiple neuronal markers representing synaptic neurotransmission. Exosomes generated by the neurons were released into media (three biological replicates) which was collected for exosome isolation. (b) Nano-LC−MS/MS tandem mass spectrometry of exosomes. Proteins of exosomes were collected by MeOH precipitation, digested with trypsin/LysC by peptide solid-phase extraction (SPE),[40] and subjected to nano-LC−MS/MS tandem mass spectrometry.[41] (c) Bioinformatics of proteomic data. MS/MS mass spectrometry data were analyzed for the peptide spectrum matching and protein identification, combined with quantification, by PEAKS (v. 8.5) software.[42,43] Proteomic data were analyzed for biological systems by GO[44,45] and for protein interaction networks by STRING-db.[46−48]

insight into molecular mechanisms of AD. These FAD gene mutations consist primarily of mutations of presenilin 1 (PS1), which represent most FAD, together with the mutations of presenilin 2 (PS2) and the amyloid precursor protein (APP).[29−31] Such FAD mutations result in progressive cognitive dysfunction and brain neuropathology consistent with AD.

To gain an understanding of the role of a FAD mutant PS1 (mPS1) in exosome-mediated tau neuropathology, we previously examined the role of the A246E mPS1[32,33] in regulating exosomal p-Tau produced by mPS1 patient-derived induced pluripotent stem cell (iPSC) neurons.[34] The mPS1 iPSC neurons displayed elevated p-Tau and secreted exosomes containing p-Tau. The intracranial injection of these exosomes into the mouse brain resulted in aggregated p-Tau in the hippocampus.[34] These results show that the A246E mPS1 participates in the exosome-mediated transmission of tau pathology.

These findings lead to the important question: What is the composition of the protein cargo of mPS1 NDEs? We hypothesized that the A246E mPS1 mutation may dysregulate the protein cargo of exosomes. To test this hypothesis, we performed a study to define the proteome of exosomes produced by patient-derived iPSC neurons with or without the mPS1 A246E mutation, which is achieved by nano-liquid chromatography with tandem mass spectrometry (LC−MS/MS)-based proteomics.

Significantly, our results demonstrate that mPS1 dysregulated the exosome cargo proteins. Global proteomic data were acquired by LC−MS/MS tandem mass spectrometry with label-free quantification and assessed for gene ontology (GO), STRING protein networks, brain functions, and AD mechanisms. Results showed that mPS1 alters the profile of exosome cargo proteins to result in (1) proteins present only in mPS1 exosomes, and not in controls, (2) the absence of proteins in the mPS1 exosomes, which were present only in controls, and (3) shared proteins which were upregulated or downregulated in the mPS1 exosomes compared to controls. These findings show that the A246E mutation of PS1 dysregulates the protein cargo of exosomes generated by human iPSC neurons.

RESULTS

Mutant PS1 Exosomes Induce Tau Deposits in the Mouse Brain. Deposits of accumulated tau in the brain is a characteristic of AD pathology and neurodegeneration. We found that when exosomes produced from iPSC neurons expressing the mutant A246E presenilin (mPS1) were injected into the mouse brain, tau pathology resulted which was observed as deposits of tau in the brain hippocampus (Figure S1). In contrast, control exosomes (from wild-type iPSC neurons) injected into the mouse brain had no effect.[34]

The functional difference of the mPS1 exosomes to induce tau deposits, compared to control exosomes, led to the purpose of this study to assess the proteome cargoes of mPS1 exosomes compared to the control exosomes.

Workflow Strategy to Assess the Protein Cargo of Exosomes Produced by mPS1 iPSC Neurons Compared to Controls. The proteomic strategy for analyses of exosomes generated by mPS1 and control iPSC neurons is conducted, as shown in Figure 1, using label-free quantitative mass spectrometry and bioinformatics tools for data analyses. This study was conducted by (a) isolation of exosomes from mPS1 iPSC neurons and from control iPSC neurons, with the confirmation of neuronal markers (Table S1), (b) nano-LC−MS/MS tandem mass spectrometry of tryptic digests of exosomes, and (c) bioinformatics to assess biological processes by GO and protein interaction networks by STRING-db.

Exosomes Secreted from mPS1 and Control Human iPSC Neurons. Exosomes isolated from mPS1 and control iPSC neurons were subjected to nanoparticle tracking analysis (NTA) to assess the distribution of exosome particle sizes

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(Figure S2). The mPS1 NDEs had a peak of particles with a diameter of ∼160 nm (∼100−300 nm). The control exosomes had a peak of particles with a diameter of ∼170 nm (∼100−300 nm). These vesicle diameters fell within the reported ranges of exosome diameters of approximately 50−150 nm.[49−51] These vesicles are enriched for exosome components including CD63 and CD81, as shown in the proteomic data for these exosomes (see Supporting Information Data S1). These markers indicate exosomes derived from endosomes.[20−23]

Protein Counts for Proteomic Data of mPS1 and Control Exosomes. Nano-LC−MS/MS-based proteomics identified 1117 total proteins from exosomes secreted by mPS1 and control iPSC neurons (Figure 2). For the mPS1 and control exosomes, 842 and 1008 proteins were identified, respectively. Proteins uniquely present in only the mPS1 exosomes numbered 109 and proteins present in only the control exosomes numbered 275. The mPS1 and control exosomes shared 733 proteins present in both groups.

a

Condition	# Proteins Identified
mPS1	842
mPS1 only	109
control	1008
control only	275
shared proteins	733
#total proteins, both groups	1117

b

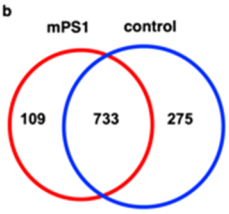


Figure 2. Protein identification counts of mPS1 and control exosome proteomic data. (a) Protein counts. The numbers of proteins identified in mPS1 and control exosomes are shown, including proteins present only in mPS1 or control exosomes, as well as shared proteins. (b) Venn diagram of unique and shared proteins of mPS1 and control exosomes. Proteins present only in mPS1 or control exosomes, or shared in both types of exosomes are shown.

Proteins Present Only in mPS1 Exosomes. The mPS1 exosomes contained 109 unique proteins (Figure 2, and listed in Supporting Information Data S1), which were absent in the control exosomes. GO analyses[45] revealed a significant enrichment of the mPS1 only proteins in biological pathways of the extracellular matrix (ECM) and structural organization, cell adhesion, development, and multicellular processes (Figure 3). The mPS1 only proteins were also enriched in the molecular binding of glycosaminoglycans, ECM, calcium, as well as endopeptidase activity. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses[52] indicated significant enrichment in protein homeostasis, ECM receptor interaction, glycosaminoglycan degradation, as well as protein degradation and processing in the lysosome and endoplasmic reticulum (ER), respectively. These findings suggest that proteins present in only mPS1 exosomes participate in ECM binding and endopeptidase functions, which involve protein processing by lysosomes and ER.

Protein network analyses of proteins present in only the mPS1 exosomes was evaluated by STRING.[46,47] The analysis showed that 38 out of the 109 mPS1 only proteins are in significant protein networks as nodes, as illustrated in Figure 4. The predicted protein interactions include functions of the extracellular structure and ECM and sulfur binding (Figure 4).

The top network hub proteins with the largest number of interactors displayed three to seven interactions for each hub (Table 1). The interactors for each hub protein are listed in Supporting Information Data S1. Among these top 11 hub proteins, a large portion represented collagen genes of COL8A1, COL5A3, COL6A2, COL6A3, and COL14A1, combined with proteases involved in procollagen processing [PCOLCE and tolloid-like 1 (TLL1)] and collagen fibril assembly [decorin (DCN)]. Collagen proteins are secreted from cells and deposited into the ECM as structural proteins and signaling molecules.[53] COL8A1 is a network-forming collagen involved in vessel wall integrity.[53] COL5A3 is a fibril-forming collagen.[53]

a

GO ID	GO Biological Process, Pathway	observed gene #	GO gene #	FDR
GO:0030198	Extracellular matrix organization	23	296	2.02E-16
GO:0043062	Extracellular structure organization	23	339	2.02E-16
GO:0007155	Cell adhesion	24	843	1.77E-08
GO:0032502	Developmental process	59	5401	3.31E-07
GO:0048856	Anatomical structure development	57	5085	3.31E-07
GO:0009888	Tissue development	27	1626	2.84E-05
GO:0032501	Multicellular organismal process	61	6507	3.88E-05

b

GO ID	GO Molecular Function, Pathway	observed gene #	GO gene #	FDR
GO:1901681	Sulfur compound binding	13	234	2.25E-07
GO:0005539	Glycosaminoglycan binding	12	219	6.17E-07
GO:0050840	Extracellular matrix binding	6	51	3.73E-05
GO:0005509	Calcium ion binding	15	700	0.00027
GO:0005488	Binding	85	11878	0.00029
GO:0004222	Metalloendopeptidase activity	6	110	0.0013
GO:0004175	Endopeptidase activity	10	399	0.0022

c

KEGG ID	KEGG Pathway	observed gene #	KEGG gene #	FDR
hsa04974	Protein digestion and absorption	8	90	5.06E-06
hsa04512	ECM receptor interaction	5	81	0.0046
hsa00531	Glyosaminoglycan degradation	3	19	0.0067
hsa04142	Lysosome	5	123	0.0147
hsa04141	Protein processing in ER	5	161	0.0375

Figure 3. GO analyses of proteins present only in mPS1 exosomes. GO analyses of proteins found only in mPS1 exosomes indicate involvement in (a) biological process pathways, (b) molecular function pathways, and (c) KEGG pathway. GO enrichment is significant with FDR <1%.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

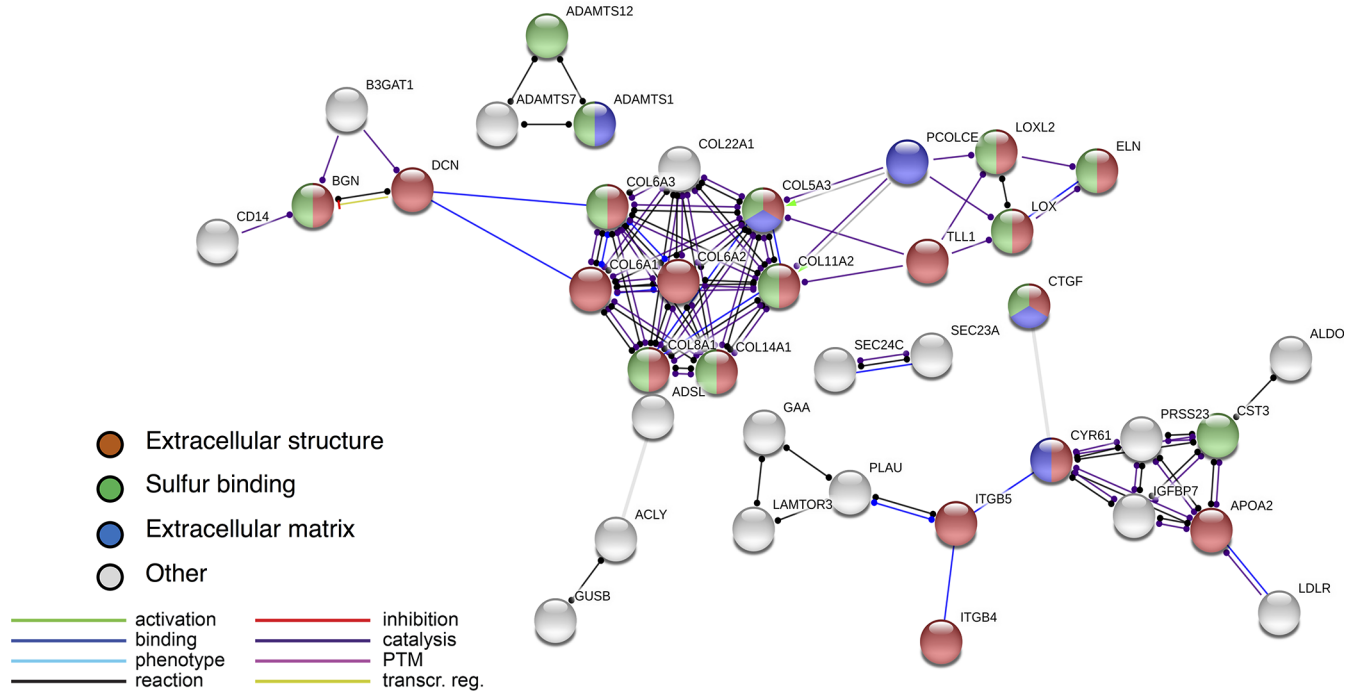


Figure 4. Protein network analyses of proteins present only in mPS1 exosomes. STRING-db protein interaction analyses indicate that 38 proteins (out of the 109 proteins identified only in mPS1 exosomes) are enriched for known protein−protein interactions. Interactions utilized scores set to high confidence (0.7) that predicted links existing among proteins.

Table 1. Hub Proteins of Networks Present Only in mPS1 Exosomes[a]

gene name	protein description	# nodes	functions in the brain, AD, related
COL5A3	collagen type V α-3 chain	6	fibril-forming collagen[53]
COL6A2	collagen type VI α-2 chain	3	beaded filament collagen[53]
			marker present in cerebral amyloid angiopathy[54]
COL6A3	collagen type VI α-3 chain	5	beaded filament collagen[53]
			related to dystonia, alcohol dependence, and congenital muscular dystrophy[55−57]
COL8A1	collagen type VIII α-1 chain	7	network-forming collagen[53]
			involved in vessel wall integrity[53]
COL14A1	collagen type XIV α-1 chain	4	fibril-associated collagen with interrupted triple helices[53]
DCN	decorin	4	role in collagen fibril assembly[60]
			stimulatory effect in autophagy and inflammation[61]
TLL1	tolloid-like 1, metalloendopeptidase	4	metalloprotease that cleaves fibrillar procollagens I, II, III, V, and VI[58]
			role in neurogenesis[58]
PCOLCE	procollagen endopeptidase enhancer 1	4	enhances procollagen C-proteinase activity that cleaves type I procollagen C-propeptide[59]
PRSS23	serine protease 23	4	vascular protease[62]
IGFBP7	insulin-like growth factor-binding protein 7	3	attenuates function of ILPs[63]
			upregulated in human AD brain[63]
			inhibition of IGFBP7 in AD mice restores memory impairment and associative learning behavior[63]
			biomarker for AD[63]
APOA2	apolipoprotein A2	3	role in lipid metabolism[64]
			associated with lifespan and cognitive function[64]

[a]Proteins identified only in the mutant PS1 exosomes are listed by gene name, number of interacting proteins terms "nodes", and description of the protein function. All proteins were identified with FDR less than 1% (see Experimental Procedures).

COL6A2 is a beaded filament[53] and has been found as a marker in cerebral amyloid angiopathy.[54] COL6A3 is also a beaded filament[53] and has roles in dystonia, alcohol dependence, and congenital muscular dystrophy[55−57] COL14A1 is a fibril-associated collagen.[53] The TLL1 metalloendopeptidase potentiates a procollagen processing protease known as bone morphogenetic protein.[53,58] The procollagen C-endopeptidase enhancer 1 protein (PCOLCE) promotes procollagen C-protease activity that cleaves type I procollagen C-propeptide.[59] DCN participates in the collagen fibril assembly and has a stimulatory effect in autophagy and inflammation.[60,61] Serine protease 23 (PRSS23) is a vascular protease.[62]

Several of the hub proteins have been shown to participate in AD-related functions of cognition and learning. IGFBP7, insulin-like growth factor-binding protein 7, a protein that attenuates the function of insulin-like peptides (ILPs), is

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

upregulated in the brains of AD patients and in a mouse model of AD (APP/S1-21 mice containing the Thy1 promoter-driven APP KM670/671NL and Thy1 promoter driven PS1 L166P transgenes);[63] the expression of IGFBP7 in mice results in impaired memory and significantly, the inhibition of IGFBP7 restores memory deficits. These findings support a role for IGFBP7 in AD-related memory loss, involving IGFBP7 attenuation of ILP function. With respect to APOA2, a protein involved in lipid metabolism, this protein is associated with cognitive function and lifespan.[64]

The mPS1 exosomes also contain ADAMTS1, ADAMTS7, and ADAMTS12 (Figure 4) which are not present in control exosomes. These three proteins are members of the ADAMTS protease family of zinc metallopeptidases, which are secreted and act on ECM components.[65,66] These components have been found to be associated with AD and related human diseases in genome-wide association studies (GWAS). Notably, ADAMTS1 has been found to be associated with the risk for late-onset AD in GWAS evaluations.[67] ADAMTS1 is also linked to degenerative intervertebral disc disease.[68] ADAMTS12 has been found to be associated with cerebral vascular aneurysm and pediatric stroke.[69] ADAMTS7 is linked to atherosclerosis and arterial disease.[67,70,71]

Among other proteins present only in mPS1 exosomes, several are involved in ECM functions. TGFBI (transforming growth factor-β-induced protein ig-h3) is an abundant protein based on its quantification (Figure 5, and Supporting Information Data S1). TGFBI is a secreted ECM protein that binds to type I, II, and IV collagens in cell−collagen interactions.[72−74] Type I and IV collagens are both present in mPS1 and control exosomes (see Supporting Information Data S1).

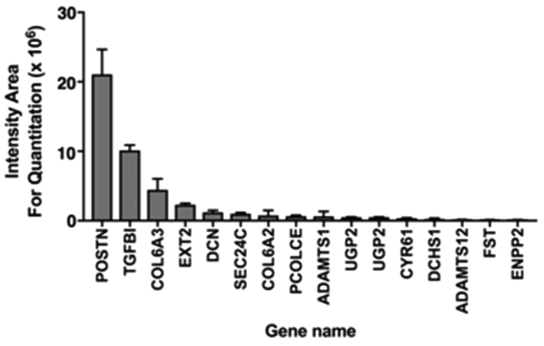


Figure 5. Abundance of quantifiable proteins present only in mPS1 exosomes. Quantifiable proteins are assessed for their abundance in the mPS1 exosomes by bar graphs and indicated by intensity areas from mass spectrometry data (conducted as described in the Experimental Procedures section).

The EXT2 protein, exostosin-2, is also present in only the mPS1 exosomes (Figure 5, and Supporting Information Data S1). EXT2 is a glycosyltransferase involved in the biosynthesis of heparin sulfate found at cell surfaces and in the ECM, participating in cell−matrix interactions.[75] Another protein of similar abundance is POSTN, which is a secreted ECM protein involved in cell adhesion and tissue regeneration;[76] POSTN participates in epithelial/mesenchymal interactions which are important in inflammation through the activation of NF-κB.

Overall, distinct proteins present in only the mPS1 exosomes (and not in control exosomes) possess ECM functions which include collagen isoforms and proteases for procollagen processing, ADAMTS zinc metallopeptidases which act on ECM substrates, and abundant TGFBI, EXT2, and POSTN which participate in ECM mechanisms. These findings suggest that the mPS1 exosomes uniquely contain protein cargo components involved in the ECM structure, function, and regulation.

Proteins Present Only in Control Exosomes and Absent in mPS1 Exosomes. Proteomic analyses of the control exosomes identified 275 unique proteins (Figure 2, and listed in Supporting Information Data S1), which were absent in the mPS1 exosomes. GO analyses indicated significant enrichment of the control only proteins in biological pathways of the cellular component organization and biogenesis in cellular processes, protein localization, and translational initiation (Figure 6). The control only proteins were also enriched in the molecular binding functions for protein, RNA, ribosomes, chromatin, and cell adhesion. Control only proteins were also enriched in proteasome activity. KEGG analyses indicated significant enrichment in ribosome, proteasome, and amino acid biosynthesis pathways. These results suggest that proteins present in only the control exosomes, and absent in the mPS1 exosomes, function in cellular protein localization and translational mechanisms, combined with binding functions of diverse molecules consisting of proteins, RNA, ribosomes, chromatin, as well as cell adhesion.

Protein network analyses of proteins present in only the control exosomes were assessed by STRING for predictions of protein−protein interaction networks. STRING analyses showed that 141 out of the 275 control only proteins are associated with significant protein networks, as illustrated in Figure 7. The top network hub proteins displayed 10 to 26 protein interactions for each hub (Table 2). The interacting proteins for the top hub proteins are listed in Supporting Information Data S1. The hub proteins with high numbers of protein interactions (Table 2) represent clustered networks of ribosomal proteins, protein phosphatases, translation initiation factors, RNA binding proteins for RNA processing and regulation, proteasome functions, and others (Figure 7). The presence of these hubs in only control exosome networks highlights the absence of these protein functions in the mPS1 exosomes.

Among proteins present in only the control exosomes, ribosome binding proteins comprise the largest group of hub proteins each having large numbers of interacting proteins (11−26 interactions for each hub). Such hub proteins consist of the 60S ribosomal proteins RPL4,[77] RPL7,[78,79] RPL12,[80] RPL13A[81] RPL18A[82] RPL19,[83] and RPL27A,[84] combined with the 40S ribosomal protein RPS2.[85] This ribosomal cluster of protein hubs (shown in Figure 7) includes the translation initiation factors EIF2S3, EIF3B, and EIF3J[86−88] as hub proteins (Table 2). This cluster of hubs for ribosomal and initiation factors participates in protein translation. The presence of these ribosomal proteins in only control exosomes suggests that the mPS1 exosomes may be compromised in protein translation functions.

Of notable interest is that only the control exosomes contain the hub protein PPP2R2A, serine/threonine protein phosphatase 2A regulatory subunit B α, a subunit of PP2A (Table 2), which is the major tau phosphatase.[89−91] PP2A is a heterotrimeric complex composed of catalytic, regulatory, and scaffolding subunits.[89,90] The PPP2R2 regulatory subunit of PP2A targets p-Tau as a substrate for PP2A.[92] Along with PPP2R2A, the control exosomes (not mPS1 exosomes) contain

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

a

GO ID	GO Biological Process, Pathway	observed gene #	GO gene #	FDR
GO:0009987	Cellular process	260	14652	7.22E-15
GO:0071840	Cellular component organization, biogenesis	140	5342	2.51E-13
GO:0016071	mRNA metabolic process	42	667	1.88E-12
GO:0034613	Cellular protein localization	58	1367	3.50E-11
GO:0044403	Sympiont process	39	650	3.52E-11
GO:0006413	Translational initiation	20	142	4.35E-11
GO:0016032	Viral process	35	571	2.90E-10

b

GO ID	GO Molecular Function, Pathway	observed gene #	GO gene #	FDR
GO:0005515	Protein binding	154	6605	1.79E-11
GO:0003723	RNA binding	43	850	1.96E-10
GO:0044877	Protein-containing complex binding	42	968	2.36E-08
GO:0003735	Structural constituent of ribosome	14	146	6.43E-06
GO:0003682	Chromatin binding	23	501	0.00011
GO:0036402	Proteasome-activating ATPase activity	4	6	0.0006
GO:0050839	Cell adhesion molecule binding	`2	200	0.00068

c

KEGG ID	KEGG Pathway	observed gene #	KEGG gene #	FDR
hsa03010	Ribosome	17	130	4.88E-09
hsa03050	Proteasome	8	43	5.00E-05
hsa01230	Biosynthesis amino acids	7	72	0.0081
hsa03040	Spliceosome	9	130	0.0081
hsa04144	Endocytosis	11	242	0.0262

Figure 6. GO analyses of proteins present only in control exosomes. GO analyses of proteins present only in control exosomes indicate involvement in (a) biological process pathways, (b) molecular function pathways, and (c) KEGG pathway. GO enrichment is significant with FDR at <1%.

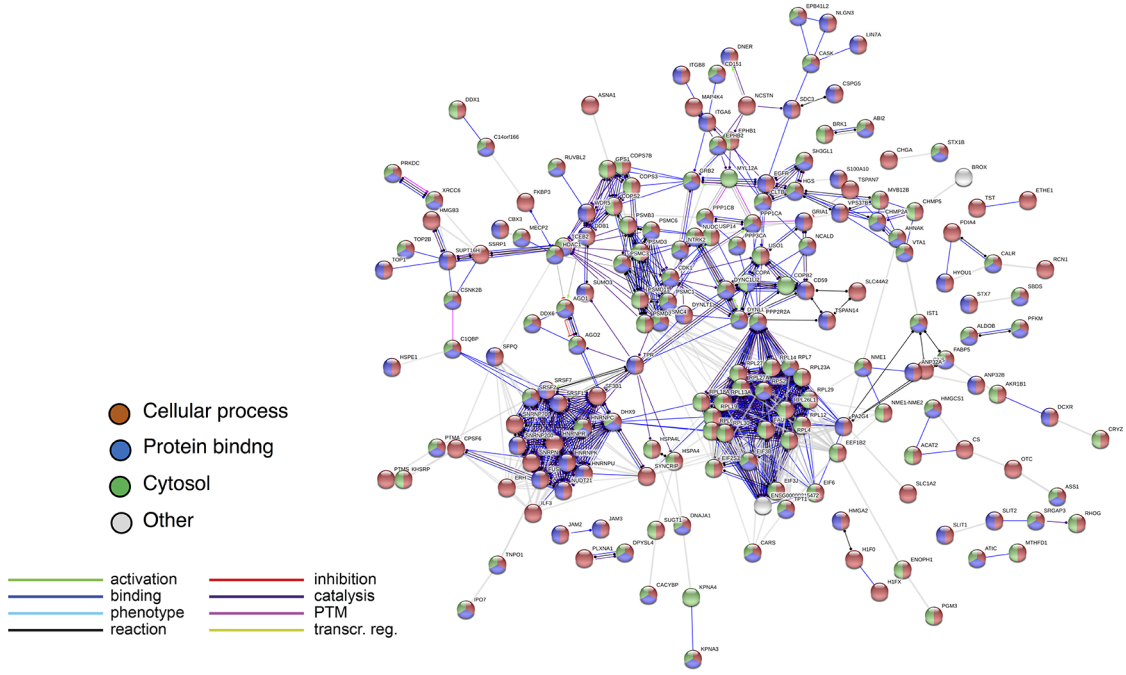


Figure 7. Protein network analyses of proteins present only in control exosomes. STRING-db network analyses indicate that 141 proteins (out of the 275 proteins present in only control exosomes) were enriched for known protein−protein interactions. Interactions utilized scores set to high confidence (0.7) to predict protein networks.

the three phosphatase catalytic subunits of PP1α, PP1β, and PP2B (calcineurin) (Supporting Information Data S1). The exclusion of these phosphatase subunits from the mPS1 exosomes, compared to their presence in the control exosomes, suggests that the absence of phosphatase components may promote p-Tau and its neuropathology, as previously observed with mPS1 exosome injections into the mouse brain.[36]

The control exosomes also uniquely contain hub proteins of networks for RNA binding and processing proteins (Table 2), which are absent in the mPS1 exosomes. These RNA-modulating hub proteins consist of SRSF1[93] and SRSF7[94] pre-messenger RNA (mRNA) splicing factors, the HNRNPL[95] component of ribonucleoprotein complexes of heterogeneous nuclear RNA in the nucleus, the RNA-binding proteins FUS[96−101] and PA2G (EBP1),[102] the ATP-dependent RNA helicase DHX9,[103] and the NUDT21[104] factor for 3′-RNA cleavage and polyadenylation.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

Table 2. Hub Proteins of Networks Present Only in Control Exosomes[a]

network cluster	gene name	protein description	# nodes	reported functions in AD or neurodegeneration
ribosomes	RPL4	60S ribosomal protein L4	22	None
	RPL7	60S ribosomal protein L7	17	forms aggregates with tau pathology[119]
	RPL12	60S ribosomal protein L12	15	associated with AD transcriptional changes[120]
	RPL13A	6S ribosomal protein L13a	11	None
	RPL18A	60S ribosomal protein L18a	26	None
	R	ribosomal protein L19	24	decreased synthesis in p-tau neurons[121]
	RPL27A	60S ribosomal protein L27a	16	None
	RPS2	40S ribosomal protein S2	16	None
translation initiation	ElF2S3	eukaryotic translation initiation factor 2 subunit 3	17	None
	ElF3B	eukaryotic translation initiation factor 3 subunit B	10	None
	EIF3J	eukaryotic translation initiation factor 3 subunit J	15	None
phosphatase	PPP2R2A	serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B α	17	dephosphorylation of p-tau[92]
RNA binding and processing	SRSF1	serine/arginine-rich-splicing factor 1	17	participates in binding to tau pre-mRNA[117]
	SRSF7	serine/arginine-rich-splicing factor 7	10	role in regulation of tau RNA alternative splicing[118]
	FUS	RNA-binding protein FUS	15	mutations in FUS are linked to familial ALS and FTLD[96−99,105,106]
	HNRNPU	heterogeneous nuclear ribonucleoprotein U	15	None
	PA2G4/EBP1	proliferation-assoc. protein 2G4	14	None
	DHX9	ATP-dependent RNA helicase	12	None
	NUDT21	cleavage and polyadenylation specificity factor subunit 5	12	None
proteasome	PSMC1	26S proteasome regulatory subunit 4	12	None
	PSMC4	26S proteasome regulatory subunit 68	14	dysregulated in AD and accumulates in Lewy bodies in PD[122]
	PSMD11	26S proteasome non-ATPase regulatory subunit 11	13	None

[a]Proteins identified only in the control PS1 exosomes are listed by gene name, number of interacting proteins terms "nodes", and description of protein function. All proteins were identified with FDR less than 1% (see Experimental Procedures).

a

GO ID	GO Biological Process, Pathway	observed gene #	GO gene #	FDR
GO:0016192	Vesicle-mediated transport	215	1699	1.85E-54
GO:0051179	Localization	385	5233	1.71E-49
GO:0006887	Exocytosis	133	774	4.54E-44
GO:0016043	Cellular component organization	368	5163	3.94E-43
GO:0032940	Secretion by cell	145	959	6.06E-43
GO:0006810	Transport	321	4130	8.58E-43
GO:0071840	Cellular component organization, biogenesis	374	5342	1.22E-42

b

GO ID	GO Molecular Function, Pathway	observed gene #	GO gene #	FDR
GO:0005515	Protein binding	433	6605	4.21E-46
GO:0043168	Anion binding	219	2696	2.77E-28
GO:0097367	Carbohydrate derivative binding	188	2163	7.15E-27
GO:0044877	Protein-containing complex binding	115	968	3.55E-25
GO:0036094	Small molecule binding	182	2460	1.43E-18
GO:0005102	Signaling receptor binding	132	1513	4.09E-18
GO:0005525	GTP binding	59	366	8.23E-18

c

KEGG ID	KEGG Pathway	observed gene #	KEGG gene #	FDR
hsa04144	Endocytosis	40	242	5.02E-12
hsa04512	ECM-receptor interaction	23	81	8.57E-11
hsa04810	Regulation of actin cytoskeleton	34	205	1.96E-10
hsa03050	Proteasome	16	43	4.07E-09
hsa04151	PI3K-Akt signaling pathway	41	348	8.63E-09

Figure 8. GO analyses of proteins shared by mPS1 and control exosomes. GO analyses of proteins identified in mPS1 and control exosomes indicate involvement in (a) biological process pathways, (b) molecular function pathways, and (c) KEGG pathway. GO enrichment is significant with FDR <1%.

Notably, the FUS RNA binding protein is significantly elevated in human brains of AD and frontotemporal lobular degeneration (FTLD) subjects.[96] Mutant forms of FUS are linked to amyotrophic lateral sclerosis (ALS) and FTLD.[97−99,105,106] The RNA-binding ability of mutant FUS is necessary for neurodegeneration.[97] Mutant FUS results in the defective RNA metabolism[99,100] and suppresses axonal protein synthesis.[99] FUS is normally present in nuclei[96] but mutant FUS accumulates in the cytoplasm of ALS and FTLD human brains[97,99−101] and results in an increase of toxicity involving

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

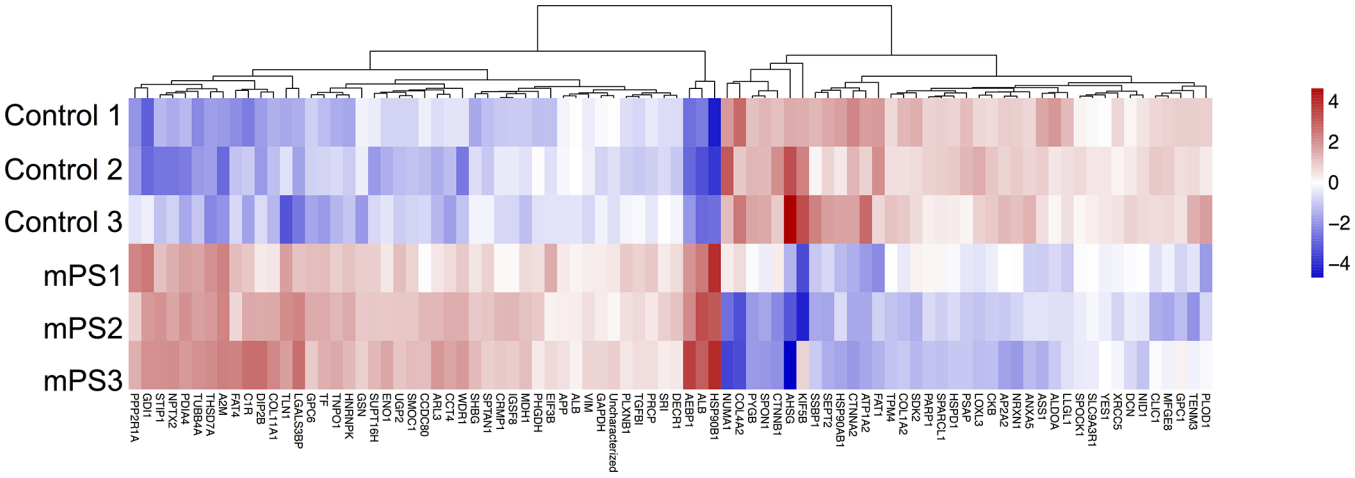


Figure 9. Upregulation and downregulation of shared proteins in mPS1 and control exosomes. Heat map illustration was conducted for the quantifiable proteins assessed by $\log_2$(mPS1/control) values (significance of $p < 0.05$). Quantified proteins shared between mPS1 and control exosomes were evaluated by comparing $\log_2$(mPS1/control), shown in heat maps.

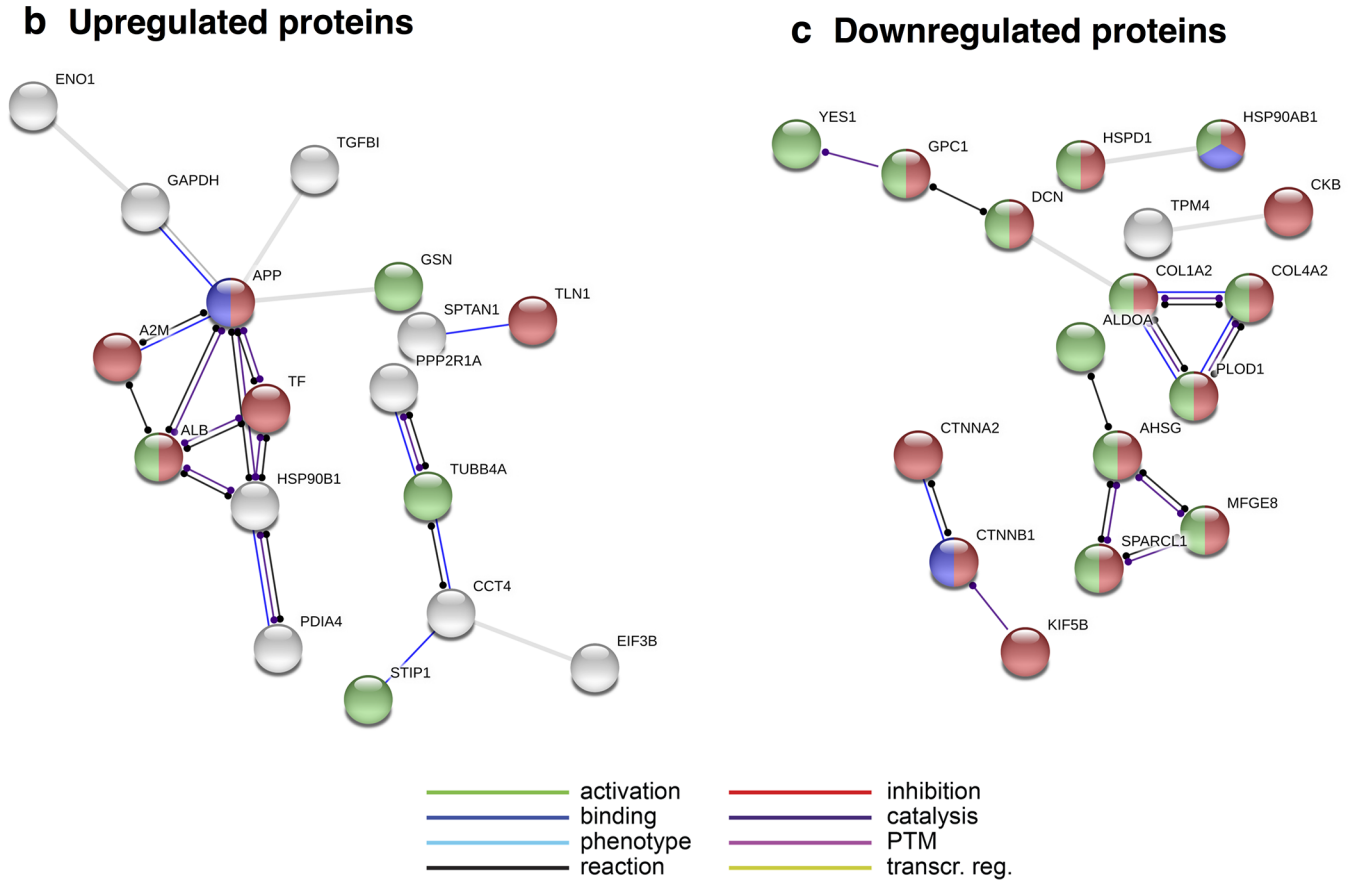


Figure 10. Network analyses of upregulated and downregulated proteins in mPS1 compared to control exosomes. (a) Upregulated proteins in mPS1. STRING-db analyses indicate that 17 proteins (out of the 46 upregulated shared proteins) were enriched with respect to known protein−protein interactions. (b) Downregulated proteins in mPS1. STRING-db analyses show that 17 proteins (out of the 39 downregulated shared proteins) were enriched with respect to known protein−protein interactions.

synaptic dysfunction and behavioral deficits in motor and cognitive functions.[99−101]

The control exosomes contain components of the proteasome complex consisting of a cluster of hub proteins consisting of PSMC1, PSMC4, and PMSD11 regulatory subunits of the 26S proteasome[107−109] (Table 2). This cluster interacts with COPS2, COPS3, and COPS7B[110−112] (Figure 7), components of the COP9 signalosome complex,[113,114] which is involved in the ubiquitin conjugation pathway through the regulation of E3 ligase complexes for the regulation of ubiquitin-targeted proteasome degradation of proteins.[113,115,116]

The absence of these protein network hubs in the PS1 exosomes, compared to their presence in only the control exosomes (Table 2), demonstrate that mPS1 exosomes display

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

Table 3. Tau Phosphatases and Kinases in mPS1 and Control Exosomes

gene name	description	mPS1 or control exosomes: only mPS1	only control	shared, $\log_2$ of mPS1/control	role in tau phosphorylation	references
			Phosphatases			
PPP1CA	serine/threonine protein phosphatase PP1-α catalytic subunit		+		PP1 α catalytic subunit contributes to de-phosphorylation of tau	89,342,343
PPP1CB	serine/threonine protein phosphatase PP1-β catalytic subunit		+		PP1 β catalytic subunit contributes to de-phosphorylation of tau	89,342,343
PPP3CA (calcineurin)	serine/threonine-protein phosphatase PP2B catalytic subunit		+		calcineurin de-phosphorylates pS262 and pS396 on tau, which are both found in parahelical filaments	89,342,343
PPP2R2A	serine/threonine protein phosphatase 2A 55 kDa regulatory subunit B α		+		B55α regulatory subunit of PP2A recognizes p-tau substrate for PP2A de-phosphorylation	89,92,342,345
PPP2R1A	serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A α			+, 1.670	this subunit of PP2A is required for tau (and other substrate) de-phosphorylation	89,92,342,345
			Kinases			
PRKDC	DNA-dependent protein kinase catalytic subunit		+		DNA-PK catalytic subunit phosphorylates tau *in vitro*	347
CSNK2B	casein kinase II subunit β		+		CK2 phosphorylates SET to induce PP2A inhibition resulting in hyper-phosphorylation of tau; CK2 is overactive in AD	349
CDK1	cyclin-dependent kinase 1		+		CDK has high affinity for phosphorylation of SP motifs of tau; CDK1 also inhibits PP2A *via* SET	346, 348
FYN	tyrosine protein kinase Fyn			+, 0.131	Fyn phosphorylates tau and also inhibits the main tau phosphatase PP2A by phosphorylation	341,350,351
MAPK3 (ERK1)	mitogen-activated protein kinase 3			+, −0.156	Erk1 is involved in abnormal tau phosphorylation at sites identified in AD brains	341,352
MAPK1 (ERK2)	mitogen-activated protein kinase 1			+, −0.768	Erk2 can phosphorylate tau at 15 sites *in vitro* and its increased activity can contribute to tauopathy	341,352,353
SRC	proto-oncogene tyrosine-protein kinase Src			+, na	Src phosphorylates tau present in NFTs	350

alterations in ribosomal proteins, serine/threonine-protein phosphatase, translation initiation factors, RNA binding proteins, and proteasome functions. Furthermore, several of the proteins present only in control exosomes are associated with tau with respect to binding to tau pre-mRNA by SRSF1,[117] the regulation of tau RNA alternative splicing by SRSF7,[118] RPL7 formation of aggregates with tau pathology,[119] association of RPL12 with AD transcriptional changes,[120] decreased synthesis of RPL19 in neurons containing p-Tau,[121] dysregulation of PSMC4 in AD,[122] and de-phosphorylation of tau by PPP2R2A.[92]

Proteins Shared by mPS1 and Control Exosomes: Upregulation and Downregulation by mPS1. Proteomic data showed that the shared proteins present in both the mPS1 and control exosomes consisted of 733 proteins (Figure 2, and listed in Supporting Information Data S1). GO analyses indicate that mPS1 and control exosomes share proteins functioning in biological processes of vesicle-mediated transport and localization, exocytosis and secretion, combined with biogenesis and organization of cellular components (Figure 8). Molecular functions of the shared proteins consist of binding proteins, anions, carbohydrates, protein complexes, signaling, GTP, and small molecules (Figure 8). KEGG pathway analyses indicate protein systems for endocytosis, ECM−receptor interaction, actin regulation, proteasome, and signaling systems.

The quantitated shared proteins were assessed for the upregulation or downregulation in mPS1 compared to control exosomes by heat maps (Figure 9). We also assessed proteins with $\log_2$(mPS1/control) ratios of ≥2, or ratios ≤−2, in another heat map illustration (Figure S3) that shows the high portion of proteins that were increased by 4- to 140-fold, or decreased by 75 to 99% in mPS1 exosomes compared to controls. Clearly, the substantial upregulation and downregulation of proteins occurred in mPS1 exosomes compared to control.

Upregulated Proteins. The most highly upregulated proteins in the mPS1 exosomes compared to controls consisted of HSP90B1, AEBP1, and ALB. HSP90B1 (GRP94), heat shock protein 90 β family member 1, is a molecular chaperone that functions in the processing and transport of secreted proteins;[123,124] brain levels of this chaperone are increased in a mouse model of AD and may participate in Aβ clearance.[125] AEBP1, AE binding protein, is a carboxypeptidase-like protein involved in collagen metabolism.[126] ALB, albumin, functions as a carrier protein for small molecules and for stabilizing extracellular fluid, such as blood.[127] STRING analyses of upregulated proteins in mPS1 compared to control exosomes, revealed APP which yields β-amyloid, as a hub protein interacting with HSP90B1, ALB, TF (transferrin), A2M (α2-macroglobulin protease inhibitor), TGFB1 (transforming growth factor β 1), as well as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Figure 10a).

Specific functions of the upregulated proteins and their relationships to tau and APP/β-amyloid of AD are provided in Table S2.[89,128−243] Biological functions of upregulated proteins consisting of trafficking and cell morphology (AEBP1, COL11A1, FAT4, GDI1, GPC6, GSN, SPTAN1, THSD7A, TLN1, TUBB4A, VIM, and WDR1), biochemical and binding (ALB, DECR1, ENO1, GAPDH, GPC6, MDH1, PHGDH, SHBG, SRI, TF, and UGP2), chaperone protein folding (CCT4, HSP90B1, PDIA4, and STIP1), proteases and protease inhibitors (A2M, C1R, PRCP, and THSD7A), synaptic function (CRMP1, NTPX2, and PLXNB1), development and growth (CCDC80, IGSF8, and TGFBI), transcriptional and nuclear regulation (DIP2B, SUPT16H, and TNPO1), cell signaling (ARL3), RNA features (HNRNPK), protein translation (EIF3B), and immune response (LGALS3BP) are listed in order of the largest to lowest number of proteins in these categories. Among these upregulated functions, several proteins are associated with p-Tau and APP/β-amyloid of AD. PPP2R1A

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

functions as a PP2A tau phosphatase[128,129] and, thus, the upregulation of PPP2R1A may increase p-Tau. The GAPDH protein has been shown to bind to tau.[130,131] Several proteins have been suggested as possible biomarkers of p-Tau or aggregated tau in NFTs which consist of A2M,[132,133] AEBP1,[134] MDH1,[135] and PLXNB.[136] Furthermore, APP and associated proteins with relationships with APP and β-amyloid were upregulated, consisting of A2M,[132,133] GAPDH,[130,131] GSN,[137,138] LGALS3BP,[139] PLXNB1,[136] and VIM.[140]

Downregulated Proteins. The most downregulated proteins in mPS1 compared to control exosomes consisted of NUMA1 (nuclear mitotic apparatus protein 1), COL4A2 (collagen type IV α 2 chain), PYGB (glycogen phosphorylase B), SPON1 (spondin 1), CTNNB1 (catenin β 1), AHSG (α 2-HS glycoprotein), and KIF5B (kinesin family member 5B) (see heat map in Figure 9). STRING network analyses of the downregulated proteins illustrated known interactions of COL1A2 and COL4A2 with PLOD1 (Figure 10b). Among the downregulated proteins, several participate in mechanisms of AD; SPON1 interacts with APOE and APP;[244,245] CTNNB1 interacts with presenilins[246] and participates in apoptosis;[247] and KIF5B kinesin participates in the axonal transport of APP.[248]

Specific functions of the downregulated proteins are provided in Table S3.[166,249−340] Biological functions of downregulated proteins consist of trafficking and cell morphology (AHSG, AP2A2, COL1A2, COL4A2, DCN, FAT1, KIF5B, LLGL1, NID1, NUMA1, SEPT2, and TPM4) biochemical and binding activities (ALDOA, ANXA5, ASS1, ATP1A2, CKB, CLIC1, LOXL3, PLOD1, PSAP, PYGB, SLC9A3R1, and SPOCK1), synaptic regulation (CTTNNA2 and SPON1), chaperone protein folding (HSP90B1 and HSPD1), development and growth (CTTNNA2, SPON1, and YES1), transcriptional nuclear regulation (PARP1, SSBP1, and XRCC5), and cell signaling (CTTNNB1, GPC1, and MFGE8).

Exosome Phosphatases and Kinases Associated with Tau Phosphorylation. Proteomic data from mPS1 and control exosomes were evaluated for phosphatase and kinase components known to participate in de-phosphorylation and phosphorylation of tau, respectively.[89,341,342] Several protein phosphatase components were absent in the mPS1 exosomes and present in only the control exosomes; these components consist of the phosphatase catalytic subunits PPP1CA, PPP1CB, and PPP3CA [serine/threonine protein phosphatase PP1-α catalytic subunit, serine/threonine protein phosphatase PP1-β catalytic subunit, and phosphatase PP2B catalytic subunit (calcineurin), respectively][89,341−343] (Table 3), combined with the regulatory phosphatase subunit of PPP2R2A (serine/threonine protein phosphatase 2A 55 kDa regulatory subunit B α isoform).[89,342−345] In addition, PPP2R1A (serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A α) is upregulated in mPS1 exosomes compared to the control (Table 3). Roles for these phosphatase components for de-phosphorylation of tau have been reported.[89,342−345] The absence of numerous protein phosphatase components may be consistent with facilitation of p-Tau formation by mPS1 exosomes.[36]

Several protein kinases with roles in the phosphorylation of tau were identified in only control exosomes, consisting of PRKDC, CSKN2, and CDK1 (DNA-dependent protein kinase catalytic subunit, casein kinase II subunit β, and cyclin-dependent kinase 1, respectively)[341,346−349] (Table 3). Among the proteins present in both mPS1 and control exosomes, FYN was moderately upregulated in mPS1 compared to control exosomes (Table 3). FYN has dual functions for the direct phosphorylation of tau and also phosphorylation of PP2A, which inhibits the PP2A-mediated de-phosphorylation of p-Tau.[341,350,351] The MAPK3 and MAPK1 (Erk1 and Erk2 protein kinases involved in tau phosphorylation)[341,352,353] are moderately downregulated in mPS1 compared to control exosomes (Table 3). The SRC kinase (proto-oncogene tyrosine-protein kinase Src) was present in both mPS1 and control exosomes; SRC participates in the phosphorylation of tau.[350] These findings suggest that the balance of several kinases is modified in the mPS1 exosomes compared to the control exosomes.

DISCUSSION

The exosome cargo produced by patient-derived iPSC neurons with the presenilin mutation A246E (mPS1) was investigated by proteomics and protein network analyses and compared to exosomes generated by normal human iPSC neurons derived from a nondemented control patient. These studies were designed to gain insights into the protein cargo of mPS1 exosomes which induce the propagation of tau pathology in the mouse brain.[34] Significantly, findings showed that mPS1 alters the profile of exosome cargo proteins to result in (1) proteins present only in mPS1 exosomes and not in controls, (2) the absence of proteins in the mPS1 exosomes which were present only in controls, and (3) shared proteins which were upregulated or downregulated in the mPS1 exosomes compared to controls (Figure 11). These data show that mPS1 dysregulates the proteome cargo of exosomes which result in the acquisition of proteins involved in ECM functions, deletion of proteins involved in RNA, and protein translation systems along with protease and related functions, combined with the

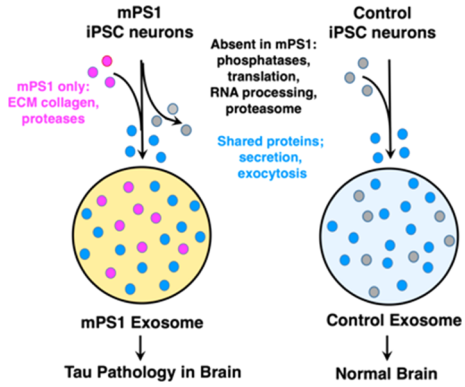


Figure 11. Mutant PS1 dysregulates exosome cargo through the acquisition and loss of proteins, combined with the upregulation and downregulation of proteins shared with control exosomes. The A246E mPS1 dysregulates the proteome cargo of exosomes generated by patient-derived iPSC neurons. Proteomic data demonstrated that mPS1 exosomes contain (1) proteins present only in mPS1 exosomes (pink circles) and not in controls, which included ECM collagen, proteases, and related components, (2) loss of proteins in the mPS1 exosomes (gray circles) which were present only in controls (gray circles), consisting of phosphatases, ribosomal, and protein translation proteins, RNA binding and processing proteins, proteasomes, and related, and (3) shared proteins (blue circles) for exocytotic, secretory, and related functions, many of which were upregulated or down-regulated in the mPS1 exosomes compared to controls. The mPS1 exosomes induce tau pathology in the mouse brain after the *in vivo* injection,[34] but the control exosomes have no effect.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

upregulation and downregulation of shared proteins (Figure 11). Notably, mPS1 exosomes display dysregulation of protein phosphatases and kinases known to be involved in regulating the phosphorylation status of tau. The altered profile of exosome cargo proteins by mPS1 may be associated with the ability of the mPS1 exosomes to propagate p-Tau neuropathology in the mouse brain.[34]

Tau undergoes phosphorylation and de-phosphorylation by protein kinases and phosphatases, respectively, to regulate the amount of p-Tau in AD and healthy brains. Hyperphosphorylation of tau occurs in AD and results in disrupted microtubule stability and deficits in axonal and synaptic functions.[354,355] Because the mPS1 exosomes result in the propagation of p-Tau in the mouse brain,[34] evaluation of mPS1 exosomes for regulators of phosphorylation was conducted. The most notable finding was that only the mPS1 exosomes lacked many phosphatase components known to participate in the de-phosphorylation of p-Tau that were present in control exosomes. The PPP2R2A regulatory subunit of protein phosphatase 2A was exclusively found in the control exosomes and not in mPS1 exosomes. PPP2R2A is a regulatory subunit that specifically targets p-Tau as the substrate for de-phosphorylation. The regulatory subunit with the catalytic and scaffolding subunits comprise the heterotrimeric phosphatase complex. There are numerous regulatory subunits for substrate recognition; therefore, it is significant that the tau-targeting subunit was present in only the control exosomes. In addition, the phosphatase catalytic subunits PPP1CA, PPP1CB, and PPP3CA were also exclusively found in the control exosomes and not in mPS1. The distinct loss of PPP2R2A by mPS1 exosomes for targeting tau as the substrate, combined with the loss of several catalytic subunits of protein phosphatases, may facilitate p-Tau involved in AD pathology.

With respect to protein kinases known to participate in phosphorylation of tau,[89] the PRKDC, CSKN2, and CDK1 kinases were identified in only the control exosomes and not the mPS1 exosomes. The tyrosine kinase FYN was moderately upregulated in mPS1 exosomes compared to controls; FYN has dual functions which include direct phosphorylation of tau and inhibiting PP2A the de-phosphorylation of p-Tau.[341,350,351] The MAPK3 and MAPK1 kinases were moderately downregulated in the mPS1 exosomes compared to controls. These findings suggest the dysregulation of the balance of several tau protein kinases in the mPS1 compared to control exosomes.

The mPS1 exosomes acquire distinct proteins which were absent in the control exosomes. Unique proteins present in only the mPS1 exosomes possess ECM functions consisting of collagen isoforms (collagen types V, VI, VIII, and XIV) and proteases (DCN protease and TLL1 metalloendopeptidase) for procollagen processing, ADAMTS zinc metallopeptidases which act on ECM substrates, and abundant TGFBI, EXT2, and POSTN which participate in ECM mechanisms. These findings suggest that the mPS1 exosomes uniquely contain protein cargo components involved in the ECM structure and function. Such ECM functions may participate in mPS1 exosome-mediated propagation of tau pathology in the brain.

Losses of proteins in mPS1 exosomes were observed because such proteins were present only in control exosomes. The control exosomes contain clusters of protein networks which function in ribosomal and initiation factors in protein translation systems, RNA binding and RNA processing systems, and proteasome components for protein degradation. The absence of these functional systems in the mPS1 exosomes suggests their lack of biosynthetic capacity for RNA processing and protein translation. Furthermore, the lack of proteasome components in the mPS1 exosomes could possibly contribute to the accumulation of misfolded proteins in tau pathology *via* the proteasome regulation of the misfolded protein response. Clearly, the mPS1 exosomes lack protein components integral to RNA-based protein translation combined with protein homeostasis. These findings demonstrate that mPS1 redirected the routing of approximately one-fourth of the normal exosomal proteins in a manner such that it prevents packaging into the mPS1 exosomes.

With respect to proteins that were absent in mPS1 exosomes compared to control exosomes, or absent in control exosomes compared to mPS1 exosomes, it is realized that such data are dependent on the mass spectrometry detection limits and dynamic range for data acquisition. Therefore, the "absence" of proteins is based on the mass spectrometry method. It is possible that proteins may be present at levels below the quantitative detection limit of the mass spectrometry method.

The proteomic data also show that a large portion of mPS1 and control exosome cargoes are common to both exosome types. These shared proteins represent fundamental exosome activities consisting of vesicle-mediated transport, exocytosis, and secretion processes, which involve molecular protein binding functions and enzymes. Among the shared proteins, mPS1 exosomes displayed a significant upregulation and downregulation of protein components (Figure 10). It is of interest that the APP was upregulated in mPS1 exosomes. APP serves as substrate of PS1, which is the aspartyl protease subunit of the γ-secretase complex which catalyzes processing of APP to generate Aβ peptides.[222] Thus, mPS1 elevates its APP substrate in exosomes. It will be of interest in future studies to examine whether mPS1 exosomes induce Aβ neuropathology combined with the propagation of tau pathology in the brain.

Along with APP, the mPS1 exosomes (compared to control exosomes) also display the strong upregulation of HSP90B1, AEBP1, and ALB proteins. Brain levels of the HSP90B1 chaperone are increased in a mouse model of AD and may participate in Aβ clearance.[243] The strong downregulation of proteins also occurs in the mPS1 exosomes (compared to controls) which consist of NUMA1 (nuclear mitotic apparatus protein 1), COL4A2 (collagen type IV α 2 chain), PYGB (glycogen phosphorylase B), SPON1 (spondin 1), CTNNB1 (catenin β 1), AHSG (α 2-HS glycoprotein), and KIF5B (kinesin family member 5B). Among these downregulated proteins, several participate in AD, consisting of SPON1 interaction with APOE and APP,[244,245] CTNNB1 interacts with presenilins[246] and participates in apoptosis,[247] and KIF5B participates in the axonal transport of APP.[264,265]

The A246E mPS1 is one of the numerous mutations reported for *PSEN1* in AD.[32,33,356,357] The results of this study demonstrating the mPS1 dysregulation of exosome proteins lead to the question of whether other *PSEN1* mutations of AD might dysregulate exosome cargo molecules. *PSEN1* mutations are the most common cause of early onset FAD. Mutant presenilins comprise the majority of the 150 presenilin mutations, which include about a dozen mutations of the homologous PS2 (*PSEN2*). It will be of interest to gain further insights into possible relationships of numerous presenilin mutations and exosome cargoes for the propagation of tau and related pathology in AD.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

EXPERIMENTAL PROCEDURES

Experimental Design. The workflow for proteomic analyses of exosomes isolated from human iPSC neurons expressing the A246E mPS1 or from control human iPSC neurons is shown in Figure 1. Human iPSC neurons were derived from the respective patient or healthy control tissue biopsies.[34,35] Each mPS1 and control group consisted of three biological replicates of neuronal cultures ($n = 3$) which allowed statistical evaluations (by Student's t-test, significance of $p < 0.05$). Exosomes were isolated from the media of neuronal cell cultures of the mPS1 and control groups using ExoQuick-TC (System Biosciences, Palo Alto, CA) (Figure 1a). Exosome proteins were digested with trypsin/LysC and subjected to nano-LC−MS/MS tandem mass spectrometry analysis on a Dionex UltiMate 3000 nano LC and an Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific, Carlsbad, CA) (Figure 1b). Samples were injected twice (in randomized order) into the nano-LC−MS/MS system for global proteomic analyses. Bioinformatic analyses of MS1 and MS2 data for peptide and protein identification and label-free quantitation (LFQ) used PEAKS Studio 8.5 (Bioinformatics Solutions Inc., Waterloo ON, Canada). PEAKS searched the human protein sequence database (UniprotKB/SwissProt 2018_2 with 71,783 entries) for peptide spectrum matches and protein identification with LFQ (Figure 1c).

The criteria for the inclusion of an identified or quantifiable protein in a biological replicate sample required that the protein was identified in at least one of the two technical replicates per biological sample. The criteria for the inclusion of an identified or quantifiable protein in either the mPS1 or control groups required that the protein was identified in at least two out of the three biological replicates per group. Quantifiable data of the mPS1 and control groups were compared by Student's t-test.

Human iPSC Neuronal Cultures: mPS1 and Control. Human iPSC neuronal cells were prepared from a control patient biopsy (non-demented) and from a patient possessing the A246E mutation in the *PSEN1* gene, using our published protocol.[19,34,35] The control iPSC neurons were derived from a biopsy of a non-demented male subject aged 86 years[36] and the mPS1 neurons were derived from a biopsy of a male subject, age d56 years, harboring the A246E mPS1 with AD onset at about 50 years;[37] all subject data have been deidentified. It is known that reprograming of the fibroblast from biopsies into pluripotent stem cells and differentiation into neurons erases the aging phenotype and generates "age-equivalent" iPSC neurons.[38,39] Therefore, the mPS1 and control iPSC neurons prepared for this study are "age-equivalent."

For the culture of iPSC neurons, neural stem cells plated at a density of 1.5×10^5 cells/cm^2 on Matrigel-coated (70 μg/mL, BD Bioscience) dishes and were grown to ∼80% confluence. Neuronal differentiation was initiated by the removal of basic fibroblast growth factor (bFGF, Biopioneer) from the media (DMEM−F-12, 1% N-2, 2% B-27, Pen−Strep, 20 ng/mL bFGF). Differentiation into neurons was indicated by neuronal markers of synaptic neurotransmission (shown in Table S1). Conditioned culture media was collected from cells at 3−4 day intervals. Cell viability in cultures was typically greater than 90−95% viable.

Exosome Isolation and NTA. The isolation of exosomes from cell culture medium used ExoQuick-TC (System Biosciences, Inc.) according to the provided protocol. The medium was incubated with ExoQuick-TC with rotation overnight at 4 °C, then centrifuged at 1500g for 30 min at 4 °C. The pellet was placed in phosphate-buffered saline with EDTA-free protease and a phosphatase inhibitor cocktail and stored at −70 °C. Protein levels of exosome preparations were measured using the bicinchoninic acid protein assay kit (Pierce Biotechnology). Exosomes (10 μg) were evaluated for the size distribution and concentration by NTA with a NanoSight LM10 instrument.

Trypsin/LysC Digestion and LC−MS/MS. Proteins of exosome samples (100 μg each) were precipitated in 90% ice-cold methanol on ice for 15 min, and then centrifuged for 30 min at 14,000g (4 °C). The resulting protein pellet was dried in a vacuum centrifuge, resuspended in urea buffer (8 M urea, 50 mM Tris−HCl, pH 8), and sonicated. For reduction, dithiothreitol (DTT, 100 mM stock) was added to obtain 5 mM DTT and samples were incubated at 55 °C for 45 min, and cooled at room temperature (RT) for 5 min. For cysteine alkylation, iodoacetamide (IAA, 200 mM stock in 50 mM Tris−HCl, pH 8) was added to obtain 15 mM IAA, incubated in the dark at RT for 30 min, and quenched by the addition of DTT to 5 μM. To lower the urea to less than 1 M, samples were diluted with 50 mM Tris−HCl, pH 8. Trypsin/LysC (Promega) was added to each sample at a ratio of 50:1 protein/trypsin (w/w) and incubated at RT for 18−24 h, and quenched by the addition of trifluoroacetic acid (TFA) to less than 0.5%. Samples were stored at −70 °C.

Tryptic peptides were purified and desalted on C18 stage tip SPE using Empore C18 wafers (3M), as reported in the field.[40] The stage tip was washed with acetonitrile (ACN) and equilibrated with 0.1% TFA. Samples were loaded, washed with 0.1% TFA, eluted with 50% ACN/0.1% TFA, dried in a vacuum centrifuge, resuspended in water and sonicated, and peptide levels were determined by the total peptide assay kit (Thermo Fisher). Samples were dried in a SpeedVac and stored at −70 °C.

LC−MS/MS Tandem Mass Spectrometry. LC−MS/MS was performed on a Dionex UltiMate 3000 nano-LC and an Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific). Peptide samples were re-suspended in 2% ACN, 0.1% TFA to 0.6 μg/μL peptide concentration. Each sample was injected twice (2.5 μg per injection) onto the nanoLC column (75 μm inner diameter, 360 μm outer diameter, and 25 cm length) packed with the BEH C18 (1.7 μm diameter) solid-phase material and heated to 65 °C with a column heater.[41] LC used a flow rate of 0.3 μL/min using a 120 min linear gradient of 5 to 25% ACN/0.1% formic acid, followed by a 5 min linear gradient of 25 to 95% ACN/0.1% formic acid. MS and MS/MS spectra were obtained in positive ion data-dependent mode. MS1 was acquired in the profile mode with a 3×10^6 automatic gain control target, 100 ms maximum injection time, a 310−1250 m/z window, and 70,000 resolution (at m/z 200). MS2 was acquired in the centroid mode with 1×10^5 AGC target, 50 ms maximum time for injection, 2×10^3 minimum precursor intensity, 35 s per 10 ppm dynamic exclusion, 17,500 resolution (at m/z 200), a first mass of m/z 150, and HCD collision energy of 28. A LC−MS/MS report is provided in Supporting Information S1. LC−MS/MS files are available at www.proteomexchange.org with the dataset identifier PXD019424, or at www.massive.ucsd.edu with dataset identifier MSV000085478 or ID = 229a900a721d40ada5e6fc806241-ba2b.

Protein Identification. MS and MS/MS data files were queried by PEAKS (v. 8.5) bioinformatic software[42] for peptide

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

identification and label-free quantitation (LFQ) analyses (next section). The data files were searched against the UniprotKB/SwissProt human protein sequence database (release 2018_02) having 71,783 entries. Peptide identifications included searching of a decoy-fusion spectrum library of human proteins, generated by PEAKS v. 8.5 from the human protein database of UniprotKB/SwissProt. PEAKS parameters for protein and peptide identification consisted of trypsin (cleavages at Arg and Lys, and two missed or nonspecific cleavages allowed), carbomidomethylation on Cys, oxidation of Met, pGlu, and N-terminal acetylation, and phosphorylation were included in the search parameters. Precursor mass error tolerance was 25 ppm, mass tolerance for the fragment ion was 0.01 Da, and threshold peptide scores of $-\log_{10} P \geq 32$. The threshold score was <1% FDR (false discovery rate), equivalent to $-\log_{10} P > 20$. The report of the PEAKS analyses is in Supporting Information S2. The threshold score for protein identification was $-\log_{10} P \geq 55$, equivalent to 1% FDR. The Supporting Information provides assigned peptide sequences and protein identifications (Supporting Information Data S1). Supporting Information Data S1 (master table of data) summarizes the identified and quantified proteins in mPS1 and control exosomes. Single peptides passing the criteria for protein identification are provided with MS/MS spectra in Supporting Information S3.

Protein Quantification. Label-free quantitation (LFQ) of proteins was assessed by PEAKS (v. 8.5) (Supporting Information Data S1). Extracted ion chromatographs of MS2 peaks were converted to the area under the curve, and the peak areas of MS2 of each peptide spectrum were summed to determine protein relative abundances. Spectra were filtered for quality parameters prior to LFQ, consisting of peptide quality of >0.3, abundance of 1×10^4, and present in 25% or more of technical replicates. Replicates were compared for retention time and isotope pattern for inclusion in quantitation analysis. The normalization of technical variations used the LOESS-G application.[43]

Analytical replicate reliability was restricted by $-\log_{10} P$ and quality assessed as $1/\log(\sigma)$, where σ is the variance between technical runs; $-\log_{10} P > 20$ represents 1% FDR. The imputation of the quantitative area with a value of 0 was achieved with a value representing the lower 5% of values within standard deviation (SD) of |1|. Multiple isoforms within a protein group were inspected to assure that the isoforms were assigned the same quantitation data. Biological replicate values for protein quantifications of mPS1 and control groups were averaged and SD computed, using Student's *t*-test to assess the significance ($p < 0.05$) of mPS1 compared to the control for significantly regulated proteins.

The PEAKS bioinformatic data analyses are provided in the master table of data (Supporting Information Data S1), which identifies the proteins present only in mPS1 exosomes, only in control exosomes, and proteins shared by the two groups. The Supporting Information Data S1 master table contains the lists of identified proteins, quantifiable proteins with their summed peak area values, and details of protein properties including % coverage and number of peptides per protein group.

GO and STRING-db Network Analyses. Identified and quantified proteins in experimental groups were evaluated for GO systems and protein−protein interactions using STRING-db (https://string-db.org/).

GO analyses indicated the significant enrichment of exosome protein groups to GO terms with FDR <1% using Benjamini−Hochberg.[44,45] FDR was assessed by hypergeometric testing, a probability distribution that assesses the significance of having hits within experimental gene sets compared to total genes in the GO pathway.

Protein networks were assessed by STRING (version 11.0)[46] (www.string-db.org). STRING utilizes a database of protein interactions data sets (DIP, BioGRID, HPRD, IntAct, MINT, and PDB). Significant protein−protein network enrichment was assessed by a probability *p*-value to indicate whether an experimental group of proteins have more interactions compared to interactions expected from a randomly selected protein group of the same size at a high confidence score of 0.7.[47]

Heat Maps of Significantly Upregulated and Downregulated Proteins in mPS1 and Control Exosomes. Quantifiable proteins shared by the mPS1 and control groups were analyzed by $\log_2$(mPS1/control) ratios and shown in heat maps for significant differences between mPS1 and control ($p < 0.05$). Power analysis of quantitative data were conducted using the pwr.2p.test function of the pwr package in R, with $n = 3$, significance set to 0.05 ($p < 0.05$), and power of 0.8 or greater. Heat maps were generated with the heat map function in R studio (https://www.rstudio.com/products/rstudio/).[48]

DATA AVAILABILITY

LC−MS/MS files are available at www.proteomexchange.org for identifier number PXD019424, or at www.massive.ucsd.edu under the identifier number MSV000085478 or ID = 229a900a721d40ada5e6fc806241ba2b.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00660.

Induction of tau deposits in the mouse brain by the injection of mPS1 exosomes obtained from iPSC neurons, NTA of mPS1 and control exosomes, substantial upregulation and downregulation of shared proteins in mPS1 and control exosomes, neuronal markers of mPS1 and control iPSC neurons representing synaptic neurotransmission, upregulated proteins in mPS1 exosomes compared to controls, downregulated proteins in mPS1 exosomes compared to controls, LC−MS/MS report, PEAKS report, and annotated MS/MS spectra for single-peptide identification of proteins (PDF)

Master table of data (XLSX)

AUTHOR INFORMATION

Corresponding Author

Vivian Hook − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States; Biomedical Sciences Graduate Program and Department of Neurosciences, School of Medicine, University of California, San Diego, San Diego 92093, California, United States;* orcid.org/0000-0001-6461-7024; Email: vhook@health.ucsd.edu

Authors

Sonia Podvin − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States*

Alexander Jones − *Biomedical Sciences Graduate Program, University of California, San Diego, San Diego 92093, California, United States*

Qing Liu − *Department of Neurosciences, School of Medicine, University of California, San Diego, San Diego 92093, California, United States*
Brent Aulston − *Department of Neurosciences, School of Medicine, University of California, San Diego, San Diego 92093, California, United States*
Charles Mosier − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States*
Janneca Ames − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States*
Charisse Winston − *Department of Neurosciences, School of Medicine, University of California, San Diego, San Diego 92093, California, United States*
Christopher B. Lietz − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States*
Zhenze Jiang − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States*
Anthony J. O'Donoghue − *Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego 92093, California, United States*
Tsuneya Ikezu − *Department of Pharmacology and Experimental Therapeutics, Department of Neurology, Alzheimer's Disease Research Center, Boston University, School of Medicine, Boston 02118, Massachusetts, United States;* orcid.org/0000-0002-3979-8596
Robert A. Rissman − *Department of Neurosciences, School of Medicine, University of California, San Diego, San Diego 92093, California, United States; Veterans Affairs San Diego Healthcare System, San Diego 92161, California, United States*
Shauna H. Yuan − *Department of Neurosciences, School of Medicine, University of California, San Diego, San Diego 92093, California, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c00660

Author Contributions

V.H., S.H.Y., and R.A.R. conceived the project idea, with scientific input from T.I. S.P., Q.L., B.A., and C.W., and C.B.L. performed the experiments. S.P., A.J., Z.J., and A.J.O. conducted analyses of proteomic data. S.P., A.J., and V.H. wrote the manuscript; S.H.Y., R.A.R., and T.I. edited the manuscript. S.P., A.J., C.M., J.A., Z.J., A.J.O., and V.H. analyzed the relevant literature on the proteomic data.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the NIH grant R56AG057469 (awarded to R.A.R., V.H., and T.I.), NIH R01NS094597 and R01NS109075 (awarded to V.H.), and ITN start-up funds to S.H.Y. A.J. was supported by NIH T32GM007752 (awarded to J. H. Brown), and C.B.L. was supported by NIH T32MH019934 (awarded to D. Jeste).

■ ABBREVIATIONS

AD, Alzheimer's disease
APP, amyloid precursor protein
FDR, false discovery rate
iPSC, induced pluripotent stem cell
KEGG, Kyoto Encyclopedia of Genes and Genomes
MAPT, tau
MS, mass spectrometry
NTA, nanoparticle tracking analysis
PS1, presenilin 1
SPE, solid-phase extraction

■ REFERENCES

(1) Wang, Y.; Mandelkow, E. Tau in physiology and pathology. *Nat. Rev. Neurosci.* **2016**, *17*, 22−35.
(2) Ballatore, C.; Lee, V. M.-Y.; Trojanowski, J. Q. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* **2007**, *8*, 663−672.
(3) Wang, J.-Z.; Xia, Y. Y.; Grundke-Iqbal, I.; Iqbal, K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. *J. Alzheimer's Dis.* **2013**, *33*, S123−S139.
(4) Kimura, T.; Sharma, G.; Ishiguro, K.; Hisanaga, S. I. Phospho-tau bar code: analysis of phosphoisotypes of tau and Its application to tauopathy. *Front. Neurosci.* **2018**, *12*, 44.
(5) Rajmohan, R.; Reddy, P. H. Amyloid-beta and phosphorylated tau accumulations cause abnormalities at synapses of Alzheimer's disease neurons. *J. Alzheimer's Dis.* **2017**, *57*, 975−999.
(6) Fá, M.; Puzzo, D.; Piacentini, R.; Staniszewski, A.; Zhang, H.; Baltrons, M. A.; Li Puma, D. D.; Chatterjee, I.; Li, J.; Saeed, F.; Berman, H. L.; Ripoli, C.; Gulisano, W.; Gonzalez, J.; Tian, H.; Costa, J. A.; Lopez, P.; Davidowitz, E.; Yu, W. H.; Haroutunian, V.; Brown, L. M.; Palmeri, A.; Sigurdsson, E. M.; Duff, K. E.; Teich, A. F.; Honig, L. S.; Sierks, M.; Moe, J. G.; D'Adamio, L.; Grassi, C.; Kanaan, N. M.; Fraser, P. E.; Arancio, O. Extracellular tau oligomers produce an immediate impairment of LTP and memory. *Sci. Rep.* **2016**, *6*, 19393.
(7) Clavaguera, F.; Bolmont, T.; Crowther, R. A.; Abramowski, D.; Frank, S.; Probst, A.; Fraser, G.; Stalder, A. K.; Beibel, M.; Staufenbiel, M.; Jucker, M.; Goedert, M.; Tolnay, M. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat. Cell Biol.* **2009**, *11*, 909−913.
(8) Ahmed, Z.; Cooper, J.; Murray, T. K.; Garn, K.; McNaughton, E.; Clarke, H.; Parhizkar, S.; Ward, M. A.; Cavallini, A.; Jackson, S.; Bose, S.; Clavaguera, F.; Tolnay, M.; Lavenir, I.; Goedert, M.; Hutton, M. L.; O'Neill, M. J. A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. *Acta Neuropathol.* **2014**, *127*, 667−683.
(9) Boluda, S.; Iba, M.; Zhang, B.; Raible, K. M.; Lee, V. M.-Y.; Trojanowski, J. Q. Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. *Acta Neuropathol.* **2015**, *129*, 221−237.
(10) Liu, L.; Drouet, V.; Wu, J. W.; Witter, M. P.; Small, S. A.; Clelland, C.; Duff, K. Trans-synaptic spread of tau pathology in vivo. *PLoS One* **2012**, *7*, No. e31302.
(11) Biundo, F.; Del Prete, D.; Zhang, H.; Arancio, O.; D'Adamio, L. A role for tau in learning, memory and synaptic plasticity. *Sci. Rep.* **2018**, *8*, 3184.
(12) Yin, Y.; Gao, D.; Wang, Y.; Wang, Z.-H.; Wang, X.; Ye, J.; Wu, D.; Fang, L.; Pi, G.; Yang, Y.; Wang, X.-C.; Lu, C.; Ye, K.; Wang, J.-Z. Tau accumulation induces synaptic impairment and memory deficit by calcineurin-mediated inactivation of nuclear CaMKIV/CREB signaling. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, E3773−E3781.
(13) Schindowski, K.; Bretteville, A.; Leroy, K.; Bégard, S.; Brion, J.-P.; Hamdane, M.; Buée, L. Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am. J. Pathol.* **2006**, *169*, 599−616.
(14) Congdon, E. E.; Sigurdsson, E. M. Tau-targeting therapies for Alzheimer disease. *Nat. Rev. Neurol.* **2018**, *14*, 399−415.

(15) Schroeder, S. K.; Joly-Amado, A.; Gordon, M. N.; Morgan, D. Tau-Directed Immunotherapy: A Promising Strategy for Treating Alzheimer's Disease and Other Tauopathies. *J. Neuroimmune Pharmacol.* **2016**, *11*, 9−25.
(16) Asai, H.; Ikezu, S.; Tsunoda, S.; Medalla, M.; Luebke, J.; Haydar, T.; Wolozin, B.; Butovsky, O.; Kügler, S.; Ikezu, T. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nat. Neurosci.* **2015**, *18*, 1584−1593.
(17) Winston, C. N.; Goetzl, E. J.; Akers, J. C.; Carter, B. S.; Rockenstein, E. M.; Galasko, D.; Masliah, E.; Rissman, R. A. Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimer's Dementia* **2016**, *3*, 63−72.
(18) Reilly, P.; Winston, C. N.; Baron, K. R.; Trejo, M.; Rockenstein, E. M.; Akers, J. C.; Kfoury, N.; Diamond, M.; Masliah, E.; Rissman, R. A.; Yuan, S. H. Novel human neuronal tau model exhibiting neurofibrillary tangles and transcellular propagation. *Neurobiol. Dis.* **2017**, *106*, 222−234.
(19) Winston, C. N.; Aulston, B.; Rockenstein, E. M.; Adame, A.; Prikhodko, O.; Dave, K. N.; Mishra, P.; Rissman, R. A.; Yuan, S. H. Neuronal exosome-derived human tau is toxic to recipient mouse neurons *in vivo*. *J. Alzheimer's Dis.* **2019**, *67*, 541−553.
(20) van Niel, G.; D'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 213−228.
(21) Budnik, V.; Ruiz-Cañada, C.; Wendler, F. Extracellular vesicles round off communication in the nervous system. *Nat. Rev. Neurosci.* **2016**, *17*, 160−172.
(22) Kowal, J.; Arras, G.; Colombo, M.; Jouve, M.; Morath, J. P.; Primdal-Bengtson, B.; Dingli, F.; Loew, D.; Tkach, M.; Théry, C. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, E968−E977.
(23) Levy, E. Exosomes in the Diseased Brain: First Insights from In vivo Studies. *Front. Neurosci.* **2017**, *11*, 142.
(24) You, Y.; Ikezu, T. Emerging roles of extracellular vesicles in neurodegenerative disorders. *Neurobiol. Dis.* **2019**, *130*, 104512.
(25) Saman, S.; Kim, W.; Raya, M.; Visnick, Y.; Miro, S.; Saman, S.; Jackson, B.; McKee, A. C.; Alvarez, V. E.; Lee, N. C. Y.; Hall, G. F. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J. Biol. Chem.* **2012**, *287*, 3842−3849.
(26) Masters, C. L.; Bateman, R.; Blennow, K.; Rowe, C. C.; Sperling, R. A.; Cummings, J. L. Alzheimer's disease. *Nat. Rev. Dis. Primers* **2015**, *1*, 15056.
(27) Piaceri, I.; Nacmias, B.; Sorbi, S. Genetics of familial and sporadic Alzheimer's disease. *Front. Biosci., Elite Ed.* **2013**, *5*, 167−177.
(28) Israel, M. A.; Yuan, S. H.; Bardy, C.; Reyna, S. M.; Mu, Y.; Herrera, C.; Hefferan, M. P.; Van Gorp, S.; Nazor, K. L.; Boscolo, F. S.; Carson, C. T.; Laurent, L. C.; Marsala, M.; Gage, F. H.; Remes, A. M.; Koo, E. H.; Goldstein, L. S. B. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* **2012**, *482*, 216−220.
(29) Lanoiselée, H.-M.; Nicolas, G.; Wallon, D.; Rovelet-Lecrux, A.; Lacour, M.; Rousseau, S.; Richard, A.-C.; Pasquier, F.; Rollin-Sillaire, A.; Martinaud, O.; Quillard-Muraine, M.; de la Sayette, V.; Boutoleau-Bretonniere, C.; Etcharry-Bouyx, F.; Chauviré, V.; Sarazin, M.; le Ber, I.; Epelbaum, S.; Jonveaux, T.; Rouaud, O.; Ceccaldi, M.; Félician, O.; Godefroy, O.; Formaglio, M.; Croisile, B.; Auriacombe, S.; Chamard, L.; Vincent, J.-L.; Sauvée, M.; Marelli-Tosi, C.; Gabelle, A.; Ozsancak, C.; Pariente, J.; Paquet, C.; Hannequin, D.; Campion, D. APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med.* **2017**, *14*, No. e1002270.
(30) Jiang, H.; Jayadev, S.; Lardelli, M.; Newman, M. A Review of the Familial Alzheimer's Disease Locus PRESENILIN 2 and Its Relationship to PRESENILIN 1. *J. Alzheimer's Dis.* **2018**, *66*, 1323−1339.
(31) Hunter, S.; Brayne, C. Understanding the roles of mutations in the amyloid precursor protein in Alzheimer disease. *Mol. Psychiatry* **2018**, *23*, 81−93.
(32) Sherrington, R.; Rogaev, E. I.; Liang, Y.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Chi, H.; Lin, C.; Li, G.; Holman, K.; Tsuda, T.; Mar, L.; Foncin, J.-F.; Bruni, A. C.; Montesi, M. P.; Sorbi, S.; Rainero, I.; Pinessi, L.; Nee, L.; Chumakov, I.; Pollen, D.; Brookes, A.; Sanseau, P.; Polinsky, R. J.; Wasco, W.; Da Silva, H. A. R.; Haines, J. L.; Pericak-Vance, M. A.; Tanzi, R. E.; Roses, A. D.; Fraser, P. E.; Rommens, J. M.; St George-Hyslop, P. H. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **1995**, *375*, 754−760.
(33) Lee, M. K.; Borchelt, D. R.; Kim, G.; Thinakaran, G.; Slunt, H. H.; Ratovitski, T.; Martin, L. J.; Kittur, A.; Gandy, S.; Levey, A. I.; Jenkins, N.; Copeland, N.; Price, D. L.; Sisodia, S. S. Hyperaccumulation of FAD-linked presenilin 1 variants in vivo. *Nat. Med.* **1997**, *3*, 756−760.
(34) Aulston, B.; Liu, Q.; Mante, M.; Florio, J.; Rissman, R. A.; Yuan, S. H. Extracellular vesicles isolated from familial Alzheimer's disease neuronal cultures induce aberrant tau phosphorylation in the wild-type mouse brain. *J. Alzheimer's Dis.* **2019**, *72*, 575−585.
(35) Yuan, S. H.; Martin, J.; Elia, J.; Flippin, J.; Paramban, R. I.; Hefferan, M. P.; Vidal, J. G.; Mu, Y.; Killian, R. L.; Israel, M. A.; Emre, N.; Marsala, S.; Marsala, M.; Gage, F. H.; Goldstein, L. S. B.; Carson, C. T. Cell-surface marker signatures for the isolation of neural stem cells, glia and neurons derived from human pluripotent stem cells. *PLoS One* **2011**, *6*, No. e17540.
(36) Israel, M. A.; Yuan, S. H.; Bardy, C.; Reyna, S. M.; Mu, Y.; Herrera, C.; Hefferan, M. P.; Van Gorp, S.; Nazor, K. L.; Boscolo, F. S.; Carson, C. T.; Laurent, L. C.; Marsala, M.; Gage, F. H.; Remes, A. M.; Koo, E. H.; Goldstein, L. S. B. Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* **2012**, *482*, 216−220.
(37) Liu, Q.; Waltz, S.; Woodruff, G.; Ouyang, J.; Israel, M. A.; Herrera, C.; Sarsoza, F.; Tanzi, R. E.; Koo, E. H.; Ringman, J. M.; Goldstein, L. S. B.; Wagner, S. L.; Yuan, S. H. Effect of potent γ-secretase modulator in human neurons derived from multiple presenilin 1-induced pluripotent stem cell mutant carriers. *JAMA Neurol.* **2014**, *71*, 1481−1489.
(38) Mertens, J.; Paquola, A. C. M.; Ku, M.; Hatch, E.; Böhnke, L.; Ladjevardi, S.; McGrath, S.; Campbell, B.; Lee, H.; Herdy, J. R.; Gonçalves, J. T.; Toda, T.; Kim, Y.; Winkler, J.; Yao, J.; Hetzer, M. W.; Gage, F. H. Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects. *Cell Stem Cell* **2015**, *17*, 705−718.
(39) Mertens, J.; Reid, D.; Lau, S.; Kim, Y.; Gage, F. H. Aging in a Dish: iPSC-Derived and Directly Induced Neurons for Studying Brain Aging and Age-Related Neurodegenerative Diseases. *Annu. Rev. Genet.* **2018**, *52*, 271−293.
(40) Rappsilber, J.; Mann, M.; Ishihama, Y. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. *Nat. Protoc.* **2007**, *2*, 1896−1906.
(41) Richards, A. L.; Hebert, A. S.; Ulbrich, A.; Bailey, D. J.; Coughlin, E. E.; Westphall, M. S.; Coon, J. J. One-hour proteome analysis in yeast. *Nat. Protoc.* **2015**, *10*, 701−714.
(42) Zhang, J.; Xin, L.; Shan, B.; Chen, W.; Xie, M.; Yuen, D.; Zhang, W.; Zhang, Z.; Lajoie, G. A.; Ma, B. PEAKS DB: De Novo Sequencing Assisted Database Search for Sensitive and Accurate Peptide Identification. *Mol. Cell. Proteomics* **2012**, *11*, M111.010587.
(43) Chawade, A.; Alexandersson, E.; Levander, F. Normalyzer: a tool for rapid evaluation of normalization methods for omics data sets. *J. Proteome Res.* **2014**, *13*, 3114−3120.
(44) Benjamini, Y.; Hochberg, Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.* **1995**, *57*, 289−300.
(45) Rivals, I.; Personnaz, L.; Taing, L.; Potier, M.-C. Enrichment or depletion of a GO category within a class of genes: which test? *Bioinformatics* **2007**, *23*, 401−407.
(46) Szklarczyk, D.; Gable, A. L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N. T.; Morris, J. H.; Bork,

P.; Jensen, L. J.; Mering, C. V. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **2019**, *47*, D607−D613.
(47) Franceschini, A.; Szklarczyk, D.; Frankild, S.; Kuhn, M.; Simonovic, M.; Roth, A.; Lin, J.; Minguez, P.; Bork, P.; von Mering, C.; Jensen, L. J. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res.* **2013**, *41*, D808−D815.
(48) Jayadeepa, R. M.; Ray, A.; Naik, D.; Sanyal, D. N.; Shah, D. Review and research analysis of computational target methods using BioRuby and in silico screening of herbal lead compounds against pancreatic cancer using R programming. *Curr. Drug Metab.* **2014**, *15*, 535−543.
(49) Paulaitis, M.; Agarwal, K.; Nana-Sinkam, P. Dynamic Scaling of Exosome Sizes. *Langmuir* **2018**, *34*, 9387−9393.
(50) Zabeo, D.; Cvjetkovic, A.; Lässer, C.; Schorb, M.; Lötvall, J.; Höög, J. L. Exosomes purified from a single cell type have diverse morphology. *J. Extracell. Vesicles* **2017**, *6*, 1329476.
(51) Agarwal, K.; Saji, M.; Lazaroff, S. M.; Palmer, A. F.; Ringel, M. D.; Paulaitis, M. E. Analysis of exosome release as a cellular response to MAPK pathway inhibition. *Langmuir* **2015**, *31*, 5440−5448.
(52) Kanehisa, M.; Furumichi, M.; Tanabe, M.; Sato, Y.; Morishima, K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* **2017**, *45*, D353−D361.
(53) Karsdal, M. A. *Biochemistry of Collagens, Laminins, and Elastin*, 1st ed.; Academic Press, 2016.
(54) Hondius, D. C.; Eigenhuis, K. N.; Morrema, T. H. J.; van der Schors, R. C.; van Nierop, P.; Bugiani, M.; Li, K. W.; Hoozemans, J. J. M.; Smit, A. B.; Rozemuller, A. J. M. Proteomics analysis identifies new markers associated with capillary cerebral amyloid angiopathy in Alzheimer's disease. *Acta Neuropathol. Commun.* **2018**, *6*, 46.
(55) Jochim, A.; Li, Y.; Zech, M.; Lam, D.; Gross, N.; Koch, K.; Zimmer, C.; Winkelmann, J.; Haslinger, B. Microstructural white matter abnormalities in patients with COL6A3 mutations (DYT27 dystonia). *Parkinsonism Relat. Disord.* **2018**, *46*, 74−78.
(56) Adkins, A. E.; Hack, L. M.; Bigdeli, T. B.; Williamson, V. S.; McMichael, G. O.; Mamdani, M.; Edwards, A. C.; Aliev, F.; Chan, R. F.; Bhandari, P.; Raabe, R. C.; Alaimo, J. T.; Blackwell, G. G.; Moscati, A.; Poland, R. S.; Rood, B.; Patterson, D. G.; Walsh, D.; Whitfield, J. B.; Zhu, G.; Montgomery, G. W.; Henders, A. K.; Martin, N. G.; Heath, A. C.; Madden, P. A. F.; Frank, J.; Ridinger, M.; Wodarz, N.; Soyka, M.; Zill, P.; Ising, M.; Nöthen, M. M.; Kiefer, F.; Rietschel, M.; Gelernter, J.; Sherva, R.; Koesterer, R.; Almasy, L.; Zhao, H.; Kranzler, H. R.; Farrer, L. A.; Maher, B. S.; Prescott, C. A.; Dick, D. M.; Bacanu, S. A.; Mathies, L. D.; Davies, A. G.; Vladimirov, V. I.; Grotewiel, M.; Bowers, M. S.; Bettinger, J. C.; Webb, B. T.; Miles, M. F.; Kendler, K. S.; Riley, B. P. Genomewide association study of alcohol dependence identifies risk loci altering ethanol-response behaviors in model organisms. *Alcohol.: Clin. Exp. Res.* **2017**, *41*, 911−928.
(57) Okada, M.; Kawahara, G.; Noguchi, S.; Sugie, K.; Murayama, K.; Nonaka, I.; Hayashi, Y. K.; Nishino, I. Primary collagen VI deficiency is the second most common congenital muscular dystrophy in Japan. *Neurology* **2007**, *69*, 1035−1042.
(58) Sieroń, A. L.; Stańczak, P. ASD–lessons on genetic background from transgenic mice with inactive gene encoding metalloprotease, Tolloid-like 1 (TLL1). *Med. Sci. Monit.* **2006**, *12*, RA17−RA22.
(59) Takahara, K.; Osborne, L.; Elliott, R. W.; Tsui, L.-C.; Scherer, S. W.; Greenspan, D. S. Fine mapping of the human and mouse genes for the type I procollagen COOH-terminal proteinase enhancer protein. *Genomics* **1996**, *31*, 253−256.
(60) Reed, C. C.; Iozzo, R. V. The role of decorin in collagen fibrillogenesis and skin homeostasis. *Glycoconjugate J.* **2002**, *19*, 249−255.
(61) Schaefer, L.; Tredup, C.; Gubbiotti, M. A.; Iozzo, R. V. Proteoglycan neofunctions: regulation of inflammation and autophagy in cancer biology. *FEBS J.* **2017**, *284*, 10−26.
(62) Chen, I.-H.; Wang, H.-H.; Hsieh, Y.-S.; Huang, W.-C.; Yeh, H.-I.; Chuang, Y.-J. PRSS23 is essential for the Snail-dependent endothelial-to-mesenchymal transition during valvulogenesis in zebrafish. *Cardiovasc. Res.* **2013**, *97*, 443−453.
(63) Agbemenyah, H. Y.; Agis-Balboa, R. C.; Burkhardt, S.; Delalle, I.; Fischer, A. Insulin growth factor binding protein 7 is a novel target to treat dementia. *Neurobiol. Dis.* **2014**, *62*, 135−143.
(64) Muenchhoff, J.; Song, F.; Poljak, A.; Crawford, J. D.; Mather, K. A.; Kochan, N. A.; Yang, Z.; Trollor, J. N.; Reppermund, S.; Maston, K.; Theobald, A.; Kirchner-Adelhardt, S.; Kwok, J. B.; Richmond, R. L.; McEvoy, M.; Attia, J.; Schofield, P. W.; Brodaty, H.; Sachdev, P. S. Plasma apolipoproteins and physical and cognitive health in very old individuals. *Neurobiol. Aging* **2017**, *55*, 49−60.
(65) Mead, T. J.; Apte, S. S. ADAMTS proteins in human disorders. *Matrix Biol.* **2018**, *71*−*72*, 225−239.
(66) Kelwick, R.; Desanlis, I.; Wheeler, G. N.; Edwards, D. R. The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. *Genome Biol.* **2015**, *16*, 113.
(67) Kunkle, B. W.; Grenier-Boley, B.; Sims, R.; Bis, J. C.; Damotte, V.; Naj, A. C.; Boland, A.; Vronskaya, M.; van der Lee, S. J.; Amlie-Wolf, A.; et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat. Genet.* **2019**, *51*, 414−430.
(68) Flister, M. J.; Tsaih, S.-W.; O'Meara, C. C.; Endres, B.; Hoffman, M. J.; Geurts, A. M.; Dwinell, M. R.; Lazar, J.; Jacob, H. J.; Moreno, C. Identifying multiple causative genes at a single GWAS locus. *Genome Res.* **2013**, *23*, 1996−2002.
(69) Bis, J. C.; Jian, X.; Kunkle, B. W.; Chen, Y.; Hamilton-Nelson, K. L.; Bush, W. S.; Salerno, W. J.; Lancour, D.; Ma, Y.; Renton, A. E.; et al. Whole exome sequencing study identifies novel rare and common Alzheimer's-Associated variants involved in immune response and transcriptional regulation. *Mol. Psychiatry* **2020**, *25*, 1859−1875.
(70) Vasquez, J. B.; Fardo, D. W.; Estus, S. ABCA7 expression is associated with Alzheimer's disease polymorphism and disease status. *Neurosci. Lett.* **2013**, *556*, 58−62.
(71) Speliotes, E. K.; Willer, C. J.; Berndt, S. I.; Monda, K. L.; Thorleifsson, G.; Jackson, A. U.; Lango Allen, H.; Lindgren, C. M.; Luan, J.; et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.* **2010**, *42*, 937−948.
(72) Han, K. E.; Choi, S.-i.; Kim, T.-i.; Maeng, Y.-S.; Stulting, R. D.; Ji, Y. W.; Kim, E. K. Pathogenesis and treatments of TGFBIH corneal dystrophies. *Prog. Retinal Eye Res.* **2016**, *50*, 67−88.
(73) Shelton, L.; Summers Rada, J. A. Inhibition of human scleral fibroblast cell attachment to collagen type I by TGFBIp. *Invest. Ophthalmol. Visual Sci.* **2009**, *50*, 3542−3552.
(74) Venkatraman, A.; Murugan, E.; Lin, S. J.; Peh, G. S. L.; Rajamani, L.; Mehta, J. S. Effect of osmolytes on in-vitro aggregation properties of peptides derived from TGFBIp. *Sci. Rep.* **2020**, *10*, 4011.
(75) Busse-Wicher, M.; Wicher, K. B.; Kusche-Gullberg, M. The extostosin family: proteins with many functions. *Matrix Biol.* **2014**, *35*, 25−33.
(76) Izuhara, K.; Nunomura, S.; Nanri, Y.; Ogawa, M.; Ono, J.; Mitamura, Y.; Yoshihara, T. Periostin in inflammation and allergy. *Cell. Mol. Life Sci.* **2017**, *74*, 4293−4303.
(77) Huber, F. M.; Hoelz, A. Molecular basis for protection of ribosomal protein L4 from cellular degradation. *Nat. Commun.* **2017**, *8*, 14354.
(78) Hemmerich, P.; Mikecz, A. v.; Neumann, F.; Sözeri, O.; Wolff-Vorbeck, G.; Zoebelein, R.; Krawinkel, U. Structural and functional properties of ribosomal protein L7 from humans and rodents. *Nucleic Acids Res.* **1993**, *21*, 223−231.
(79) Wu, W.-C.; Liu, H.-W.; Lin, A. Human ribosomal protein L7 displays an ER binding property and is involved in ribosome-ER association. *FEBS Lett.* **2007**, *581*, 651−657.
(80) Plafker, S. M.; Macara, I. G. Ribosomal protein L12 uses a distinct nuclear import pathway mediated by importin 11. *Mol. Cell. Biol.* **2002**, *22*, 1266−1275.
(81) Chaudhuri, S.; Vyas, K.; Kapasi, P.; Komar, A. A.; Dinman, J. D.; Barik, S.; Mazumder, B. Human ribosomal protein L13a is dispensable for canonical ribosome function but indispensable for efficient rRNA methylation. *RNA* **2007**, *13*, 2224−2237.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(82) Dhar, D.; Mapa, K.; Pudi, R.; Srinivasan, P.; Bodhinathan, K.; Das, S. Human ribosomal protein L18a interacts with hepatitis C virus internal ribosome entry site. *Arch. Virol.* **2006**, *151*, 509−524.
(83) Yang, E.-J.; Seo, J.-W.; Choi, I.-H. Ribosomal Protein L19 and L22 Modulate TLR3 Signaling. *Immune Netw.* **2011**, *11*, 155−162.
(84) Alkhatabi, H. A.; McLornan, D. P.; Kulasekararaj, A. G.; Malik, F.; Seidl, T.; Darling, D.; Gaken, J.; Mufti, G. J. RPL27A is a target of miR-595 and may contribute to the myelodysplastic phenotype through ribosomal dysgenesis. *Oncotarget* **2016**, *7*, 47875−47890.
(85) Kowalczyk, P.; Woszczyński, M.; Ostrowski, J. Increased expression of ribosomal protein S2 in liver tumors, posthepactomized livers, and proliferating hepatocytes in vitro. *Acta Biochim. Pol.* **2002**, *49*, 615−624.
(86) Lee, A. S. Y.; Kranzusch, P. J.; Cate, J. H. D. eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. *Nature* **2015**, *522*, 111−114.
(87) Young, D. J.; Guydosh, N. R. Hcr1/eIF3j Is a 60S Ribosomal Subunit Recycling Accessory Factor In Vivo. *Cell Rep.* **2019**, *28*, 39−50.e4.
(88) Choi, Y. J.; Lee, Y. S.; Lee, H. W.; Shim, D. M.; Seo, S. W. Silencing of translation initiation factor eIF3b promotes apoptosis in osteosarcoma cells. *Bone Joint Res.* **2017**, *6*, 186−193.
(89) Martin, L.; Latypova, X.; Wilson, C. M.; Magnaudeix, A.; Perrin, M.-L.; Terro, F. Tau protein phosphatases in Alzheimer's disease: the leading role of PP2A. *Ageing Res. Rev.* **2013**, *12*, 39−49.
(90) Sontag, J.-M.; Sontag, E. Protein phosphatase 2A dysfunction in Alzheimer's disease. *Front. Mol. Neurosci.* **2014**, *7*, 16.
(91) Taleski, G.; Sontag, E. Protein phosphatase 2A and tau: an orchestrated 'Pas de Deux'. *FEBS Lett.* **2018**, *592*, 1079−1095.
(92) Yu, U. Y.; Yoo, B. C.; Ahn, J.-H. Regulatory B subunits of protein phosphatase 2A are involved in site-specific regulation of tau protein phosphorylation. *Korean J. Physiol. Pharmacol.* **2014**, *18*, 155−161.
(93) Das, S.; Krainer, A. R. Emerging functions of SRSF1, splicing factor and oncoprotein, in RNA metabolism and cancer. *Mol. Cancer Res.* **2014**, *12*, 1195−1204.
(94) Saijo, S.; Kuwano, Y.; Masuda, K.; Nishikawa, T.; Rokutan, K.; Nishida, K. Serine/arginine-rich splicing factor 7 regulates p21-dependent growth arrest in colon cancer cells. *J. Med. Invest.* **2016**, *63*, 219−226.
(95) Yugami, M.; Kabe, Y.; Yamaguchi, Y.; Wada, T.; Handa, H. hnRNP-U enhances the expression of specific genes by stabilizing mRNA. *FEBS Lett.* **2007**, *581*, 1−7.
(96) Aoki, N.; Higashi, S.; Kawakami, I.; Kobayashi, Z.; Hosokawa, M.; Katsuse, O.; Togo, T.; Hirayasu, Y.; Akiyama, H. Localization of fused in sarcoma (FUS) protein to the post-synaptic density in the brain. *Acta Neuropathol.* **2012**, *124*, 383−394.
(97) Daigle, J. G.; Lanson, N. A., Jr.; Smith, R. B.; Casci, I.; Maltare, A.; Monaghan, J.; Nichols, C. D.; Kryndushkin, D.; Shewmaker, F.; Pandey, U. B. RNA-binding ability of FUS regulates neurodegeneration, cytoplasmic mislocalization and incorporation into stress granules associated with FUS carrying ALS-linked mutations. *Hum. Mol. Genet.* **2013**, *22*, 1193−1205.
(98) Ling, S.-C.; Dastidar, S. G.; Tokunaga, S.; Ho, W. Y.; Lim, K.; Ilieva, H.; Parone, P. A.; Tyan, S.-H.; Tse, T. M.; Chang, J.-C.; Platoshyn, O.; Bui, N. B.; Bui, A.; Vetto, A.; Sun, S.; McAlonis-Downes, M.; Han, J. S.; Swing, D.; Kapeli, K.; Yeo, G. W.; Tessarollo, L.; Marsala, M.; Shaw, C. E.; Tucker-Kellogg, G.; La Spada, A. R.; Lagier-Tourenne, C.; Da Cruz, S.; Cleveland, D. W. Overriding FUS autoregulation in mice triggers gain-of-toxic dysfunctions in RNA metabolism and autophagy-lysosome axis. *Elife* **2019**, *8*, No. e40811.
(99) López-Erauskin, J.; Tadokoro, T.; Baughn, M. W.; Myers, B.; McAlonis-Downes, M.; Chillon-Marinas, C.; Asiaban, J. N.; Artates, J.; Bui, A. T.; Vetto, A. P.; Lee, S. K.; Le, A. V.; Sun, Y.; Jambeau, M.; Boubaker, J.; Swing, D.; Qiu, J.; Hicks, G. G.; Ouyang, Z.; Fu, X.-D.; Tessarollo, L.; Ling, S.-C.; Parone, P. A.; Shaw, C. E.; Marsala, M.; Lagier-Tourenne, C.; Cleveland, D. W.; Da Cruz, S. ALS/FTD-Linked Mutation in FUS Suppresses Intra-axonal Protein Synthesis and Drives Disease Without Nuclear Loss-of-Function of FUS. *Neuron* **2018**, *100*, 816−830.e7.
(100) Ratti, A.; Buratti, E. Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J. Neurochem.* **2016**, *138*, 95−111.
(101) Scekic-Zahirovic, J.; Sendscheid, O.; El Oussini, H.; Jambeau, M.; Sun, Y.; Mersmann, S.; Wagner, M.; Dieterlé, S.; Sinniger, J.; Dirrig-Grosch, S.; Drenner, K.; Birling, M. C.; Qiu, J.; Zhou, Y.; Li, H.; Fu, X. D.; Rouaux, C.; Shelkovnikova, T.; Witting, A.; Ludolph, A. C.; Kiefer, F.; Storkebaum, E.; Lagier-Tourenne, C.; Dupuis, L. Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss. *EMBO J.* **2016**, *35*, 1077−1097.
(102) Squatrito, M.; Mancino, M.; Donzelli, M.; Areces, L. B.; Draetta, G. F. EBP1 is a nucleolar growth-regulating protein that is part of pre-ribosomal ribonucleoprotein complexes. *Oncogene* **2004**, *23*, 4454−4465.
(103) Hong, H.; An, O.; Chan, T. H. M.; Ng, V. H. E.; Kwok, H. S.; Lin, J. S.; Qi, L.; Han, J.; Tay, D. J. T.; Tang, S. J.; Yang, H.; Song, Y.; Bellido Molias, F.; Tenen, D. G.; Chen, L. Bidirectional regulation of adenosine-to-inosine (A-to-I) RNA editing by DEAH box helicase 9 (DHX9) in cancer. *Nucleic Acids Res.* **2018**, *46*, 7953−7969.
(104) Brumbaugh, J.; Di Stefano, B.; Wang, X.; Borkent, M.; Forouzmand, E.; Clowers, K. J.; Ji, F.; Schwarz, B. A.; Kalocsay, M.; Elledge, S. J.; Chen, Y.; Sadreyev, R. I.; Gygi, S. P.; Hu, G.; Shi, Y.; Hochedlinger, K. Nudt21 controls cell fate by connecting alternative polyadenylation to chromatin signaling. *Cell* **2018**, *172*, 106−120.e21.
(105) Vance, C.; Rogelj, B.; Hortobágyi, T.; De Vos, K. J.; Nishimura, A. L.; Sreedharan, J.; Hu, X.; Smith, B.; Ruddy, D.; Wright, P.; Ganesalingam, J.; Williams, K. L.; Tripathi, V.; Al-Saraj, S.; Al-Chalabi, A.; Leigh, P. N.; Blair, I. P.; Nicholson, G.; de Belleroche, J.; Gallo, J.-M.; Miller, C. C.; Shaw, C. E. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **2009**, *323*, 1208−1211.
(106) Kwiatkowski, T. J., Jr.; Bosco, D. A.; Leclerc, A. L.; Tamrazian, E.; Vanderburg, C. R.; Russ, C.; Davis, A.; Gilchrist, J.; Kasarskis, E. J.; Munsat, T.; Valdmanis, P.; Rouleau, G. A.; Hosler, B. A.; Cortelli, P.; de Jong, P. J.; Yoshinaga, Y.; Haines, J. L.; Pericak-Vance, M. A.; Yan, J.; Ticozzi, N.; Siddique, T.; McKenna-Yasek, D.; Sapp, P. C.; Horvitz, H. R.; Landers, J. E.; Brown, R. H., Jr. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **2009**, *323*, 1205−1208.
(107) Tanahashi, N.; Suzuki, M.; Fujiwara, T.; Takahashi, E.-i.; Shimbara, N.; Chung, C. H.; Tanaka, K. Chromosomal localization and immunological analysis of a family of human 26S proteasomal ATPases. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 229−232.
(108) Roelofs, J.; Park, S.; Haas, W.; Tian, G.; McAllister, F. E.; Huo, Y.; Lee, B.-H.; Zhang, F.; Shi, Y.; Gygi, S. P.; Finley, D. Chaperone-mediated pathway of proteasome regulatory particle assembly. *Nature* **2009**, *459*, 861−865.
(109) Vilchez, D.; Boyer, L.; Morantte, I.; Lutz, M.; Merkwirth, C.; Joyce, D.; Spencer, B.; Page, L.; Masliah, E.; Berggren, W. T.; Gage, F. H.; Dillin, A. Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. *Nature* **2012**, *489*, 304−308.
(110) Schaefer, L.; Beermann, M. L.; Miller, J. B. Coding sequence, genomic organization, chromosomal localization, and expression pattern of the signalosome component Cops2: the mouse homologue of Drosophila alien. *Genomics* **1999**, *56*, 310−316.
(111) Yan, T.; Wunder, J. S.; Gokgoz, N.; Gill, M.; Eskandarian, S.; Parkes, R. K.; Bull, S. B.; Bell, R. S.; Andrulis, I. L. COPS3 amplification and clinical outcome in osteosarcoma. *Cancer* **2007**, *109*, 1870−1876.
(112) Gaspar, L.; Howald, C.; Popadin, K.; Maier, B.; Mauvoisin, D.; Moriggi, E.; Gutierrez-Arcelus, M.; Falconnet, E.; Borel, C.; Kunz, D.; Kramer, A.; Gachon, F.; Dermitzakis, E. T.; Antonarakis, S. E.; Brown, S. A. The genomic landscape of human cellular circadian variation points to a novel role for the signalosome. *Elife* **2017**, *6*, No. e24994.
(113) Wei, N.; Deng, X. W. The COP9 signalosome. *Annu. Rev. Cell Dev. Biol.* **2003**, *19*, 261−286.
(114) Singh, A. K.; Chamovitz, D. A. Role of Cop9 Signalosome subunits in the environmental and hormonal balance of plant. *Biomolecules* **2019**, *9*, 224.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(115) Chung, D.; Dellaire, G. The Role of the COP9 Signalosome and Neddylation in DNA Damage Signaling and Repair. *Biomolecules* **2015**, *5*, 2388−2416.
(116) Bech-Otschir, D.; Seeger, M.; Dubiel, W. The COP9 signalosome: at the interface between signal transduction and ubiquitin-dependent proteolysis. *J. Cell Sci.* **2002**, *115*, 467−473.
(117) Shi, J.; Qian, W.; Yin, X.; Iqbal, K.; Grundke-Iqbal, I.; Gu, X.; Ding, F.; Gong, C.-X.; Liu, F. Cyclic AMP-dependent protein kinase regulates the alternative splicing of tau exon 10: a mechanism involved in tau pathology of Alzheimer disease. *J. Biol. Chem.* **2011**, *286*, 14639−14648.
(118) Welden, J. R.; van Doorn, J.; Nelson, P. T.; Stamm, S. The human MAPT locus generates circular RNAs. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2018**, *1864*, 2753−2760.
(119) Maziuk, B. F.; Apicco, D. J.; Cruz, A. L.; Jiang, L.; Ash, P. E. A.; da Rocha, E. L.; Zhang, C.; Yu, W. H.; Leszyk, J.; Abisambra, J. F.; Li, H.; Wolozin, B. RNA binding proteins co-localize with small tau inclusions in tauopathy. *Acta Neuropathol. Commun.* **2018**, *6*, 71.
(120) Rahman, M. R.; Islam, T.; Turanli, B.; Zaman, T.; Faruquee, H. M.; Rahman, M. M.; Mollah, M. N. H.; Nanda, R. K.; Arga, K. Y.; Gov, E.; Moni, M. A. Network-based approach to identify molecular signatures and therapeutic agents in Alzheimer's disease. *Comput. Biol. Chem.* **2019**, *78*, 431−439.
(121) Evans, H. T.; Benetatos, J.; van Roijen, M.; Bodea, L. G.; Götz, J. Decreased synthesis of ribosomal proteins in tauopathy revealed by non-canonical amino acid labelling. *EMBO J.* **2019**, *38*, No. e101174.
(122) Grünblatt, E.; Ruder, J.; Monoranu, C. M.; Riederer, P.; Youdim, M. B.; Mandel, S. A. Differential alterations in metabolism and proteolysis-related rroteins in human parkinson's disease substantia nigra. *Neurotoxic. Res.* **2018**, *33*, 560−568.
(123) Randow, F.; Seed, B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. *Nat. Cell Biol.* **2001**, *3*, 891−896.
(124) Yang, Y.; Liu, B.; Dai, J.; Srivastava, P. K.; Zammit, D. J.; Lefrançois, L.; Li, Z. Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. *Immunity* **2007**, *26*, 215−226.
(125) Zhang, M.; Qian, C.; Zheng, Z.-G.; Qian, F.; Wang, Y.; Thu, P. M.; Zhang, X.; Zhou, Y.; Tu, L.; Liu, Q.; Li, H.-J.; Yang, H.; Li, P.; Xu, X. Jujuboside A promotes Aβ clearance and ameliorates cognitive deficiency in Alzheimer's disease through activating Axl/HSP90/PPARγ pathway. *Theranostics* **2018**, *8*, 4262−4278.
(126) Blackburn, P. R.; Xu, Z.; Tumelty, K. E.; Zhao, R. W.; Monis, W. J.; Harris, K. G.; Gass, J. M.; Cousin, M. A.; Boczek, N. J.; Mitkov, M. V.; Cappel, M. A.; Francomano, C. A.; Parisi, J. E.; Klee, E. W.; Faqeih, E.; Alkuraya, F. S.; Layne, M. D.; McDonnell, N. B.; Atwal, P. S. Bi-allelic Alterations in AEBP1 Lead to Defective Collagen Assembly and Connective Tissue Structure Resulting in a Variant of Ehlers-Danlos Syndrome. *Am. J. Hum. Genet.* **2018**, *102*, 696−705.
(127) Minghetti, P. P.; Ruffner, D. E.; Kuang, W. J.; Dennison, O. E.; Hawkins, J. W.; Beattie, W. G.; Dugaiczyk, A. Molecular structure of the human albumin gene is revealed by nucleotide sequence within q11-22 of chromosome 4. *J. Biol. Chem.* **1986**, *261*, 6747−6757.
(128) Calin, G. A.; di Iasio, M. G.; Caprini, E.; Vorechovsky, I.; Natali, P. G.; Sozzi, G.; Croce, C. M.; Barbanti-Brodano, G.; Russo, G.; Negrini, M. Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene* **2000**, *19*, 1191−1195.
(129) Javadpour, P.; Dargahi, L.; Ahmadiani, A.; Ghasemi, R. To be or not to be: PP2A as a dual player in CNS functions, its role in neurodegeneration, and its interaction with brain insulin signaling. *Cell. Mol. Life Sci.* **2019**, *76*, 2277−2297.
(130) El Kadmiri, N.; Slassi, I.; El Moutawakil, B.; Nadifi, S.; Tadevosyan, A.; Hachem, A.; Soukri, A. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Alzheimer's disease. *Pathol. Biol.* **2014**, *62*, 333−336.
(131) Gerszon, J.; Rodacka, A. Oxidatively modified glyceraldehyde-3-phosphate dehydrogenase in neurodegenerative processes and the role of low molecular weight compounds in counteracting its aggregation and nuclear translocation. *Ageing Res. Rev.* **2018**, *48*, 21−31.
(132) Varma, V. R.; Varma, S.; An, Y.; Hohman, T. J.; Seddighi, S.; Casanova, R.; Beri, A.; Dammer, E. B.; Seyfried, N. T.; Pletnikova, O.; Moghekar, A.; Wilson, M. R.; Lah, J. J.; O'Brien, R. J.; Levey, A. I.; Troncoso, J. C.; Albert, M. S.; Thambisetty, M. Alpha-2 macroglobulin in Alzheimer's disease: a marker of neuronal injury through the RCAN1 pathway. *Mol. Psychiatry* **2017**, *22*, 13−23.
(133) Tanskanen, M.; Peuralinna, T.; Polvikoski, T.; Notkola, I. L.; Sulkava, R.; Hardy, J.; Singleton, A.; Kiuru-Enari, S.; Paetau, A.; Tienari, P. J.; Myllykangas, L. Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. *Ann. Med.* **2008**, *40*, 232−239.
(134) Shijo, M.; Honda, H.; Suzuki, S. O.; Hamasaki, H.; Hokama, M.; Abolhassani, N.; Nakabeppu, Y.; Ninomiya, T.; Kitazono, T.; Iwaki, T. Association of adipocyte enhancer-binding protein 1 with Alzheimer's disease pathology in human hippocampi. *Brain Pathol.* **2018**, *28*, 58−71.
(135) Recabarren, D.; Alarcón, M. Gene networks in neurodegenerative disorders. *Life Sci.* **2017**, *183*, 83−97.
(136) Yu, L.; Petyuk, V. A.; Gaiteri, C.; Mostafavi, S.; Young-Pearse, T.; Shah, R. C.; Buchman, A. S.; Schneider, J. A.; Piehowski, P. D.; Sontag, R. L.; Fillmore, T. L.; Shi, T.; Smith, R. D.; De Jager, P. L.; Bennett, D. A. Targeted brain proteomics uncover multiple pathways to Alzheimer's dementia. *Ann. Neurol.* **2018**, *84*, 78−88.
(137) Carro, E. Gelsolin as therapeutic target in Alzheimer's disease. *Expert Opin. Ther. Targets* **2010**, *14*, 585−592.
(138) Chauhan, V.; Ji, L.; Chauhan, A. Anti-amyloidogenic, anti-oxidant and anti-apoptotic role of gelsolin in Alzheimer's disease. *Biogerontology* **2008**, *9*, 381−389.
(139) Seki, T.; Kanagawa, M.; Kobayashi, K.; Kowa, H.; Yahata, N.; Maruyama, K.; Iwata, N.; Inoue, H.; Toda, T. Galectin 3-binding protein suppresses amyloid-β production by modulating β-cleavage of amyloid precursor protein. *J. Biol. Chem.* **2020**, *295*, 3678−3691.
(140) Kamphuis, W.; Kooijman, L.; Orre, M.; Stassen, O.; Pekny, M.; Hol, E. M. GFAP and vimentin deficiency alters gene expression in astrocytes and microglia in wild-type mice and changes the transcriptional response of reactive glia in mouse model for Alzheimer's disease. *Glia* **2015**, *63*, 1036−1056.
(141) Eitzen, G.; Will, E.; Gallwitz, D.; Haas, A.; Wickner, W. Sequential action of two GTPases to promote vacuole docking and fusion. *EMBO J.* **2000**, *19*, 6713−6720.
(142) Garrett, M. D.; Zahner, J. E.; Cheney, C. M.; Novick, P. J. GDI1 encodes a GDP dissociation inhibitor that plays an essential role in the yeast secretory pathway. *EMBO J.* **1994**, *13*, 1718−1728.
(143) Owen, J. B.; Di Domenico, F.; Sultana, R.; Perluigi, M.; Cini, C.; Pierce, W. M.; Butterfield, D. A. Proteomics-determined differences in the concanavalin-A-fractionated proteome of hippocampus and inferior parietal lobule in subjects with Alzheimer's disease and mild cognitive impairment: implications for progression of AD. *J. Proteome Res.* **2009**, *8*, 471−482.
(144) Scheper, W.; Zwart, R.; van der Sluijs, P.; Annaert, W.; Gool, W. A.; Baas, F. Alzheimer's presenilin 1 is a putative membrane receptor for rab GDP dissociation inhibitor. *Hum. Mol. Genet.* **2000**, *9*, 303−310.
(145) Lackie, R. E.; Maciejewski, A.; Ostapchenko, V. G.; Marques-Lopes, J.; Choy, W. Y.; Duennwald, M. L.; Prado, V. F.; Prado, M. A. M. The Hsp70/Hsp90 Chaperone Machinery in Neurodegenerative Diseases. *Front. Neurosci.* **2017**, *11*, 254.
(146) Schmid, A. B.; Lagleder, S.; Gräwert, M. A.; Röhl, A.; Hagn, F.; Wandinger, S. K.; Cox, M. B.; Demmer, O.; Richter, K.; Groll, M.; Kessler, H.; Buchner, J. The architecture of functional modules in the Hsp90 co-chaperone Sti1/Hop. *EMBO J.* **2012**, *31*, 1506−1517.
(147) Maciejewski, A.; Ostapchenko, V. G.; Beraldo, F. H.; Prado, V. F.; Prado, M. A. M.; Choy, W.-Y. Domains of STIP1 responsible for regulating PrPC-dependent amyloid-β oligomer toxicity. *Biochem. J.* **2016**, *473*, 2119−2130.
(148) Chapman, G.; Shanmugalingam, U.; Smith, P. D. The Role of Neuronal Pentraxin 2 (NP2) in Regulating Glutamatergic Signaling and Neuropathology. *Front. Cell. Neurosci.* **2020**, *13*, 575.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(149) Lee, S.-J.; Wei, M.; Zhang, C.; Maxeiner, S.; Pak, C.; Calado Botelho, S.; Trotter, J.; Sterky, F. H.; Südhof, T. C. Presynaptic Neuronal Pentraxin Receptor Organizes Excitatory and Inhibitory Synapses. *J. Neurosci.* **2017**, *37*, 1062−1080.
(150) Bjartmar, L.; Huberman, A. D.; Ullian, E. M.; Rentería, R. C.; Liu, X.; Xu, W.; Prezioso, J.; Susman, M. W.; Stellwagen, D.; Stokes, C. C.; Cho, R.; Worley, P.; Malenka, R. C.; Ball, S.; Peachey, N. S.; Copenhagen, D.; Chapman, B.; Nakamoto, M.; Barres, B. A.; Perin, M. S. Neuronal pentraxins mediate synaptic refinement in the developing visual system. *J. Neurosci.* **2006**, *26*, 6269−6281.
(151) Swanson, A.; Wolf, T.; Sitzmann, A.; Willette, A. A. Neuroinflammation in Alzheimer's disease: Pleiotropic roles for cytokines and neuronal pentraxins. *Behav. Brain Res.* **2018**, *347*, 49−56.
(152) Xiao, M. F.; Xu, D.; Craig, M. T.; Pelkey, K. A.; Chien, C. C.; Shi, Y.; Zhang, J.; Resnick, S.; Pletnikova, O.; Salmon, D.; Brewer, J.; Edland, S.; Wegiel, J.; Tycko, B.; Savonenko, A.; Reeves, R. H.; Troncoso, J. C.; McBain, C. J.; Galasko, D.; Worley, P. F. NPTX2 and cognitive dysfunction in Alzheimer's Disease. *Elife* **2017**, *6*, No. e23798.
(153) Wang, Z.; Zhang, H.; Cheng, Q. PDIA4: The basic characteristics, functions and its potential connection with cancer. *Biomed. Pharmacother.* **2020**, *122*, 109688.
(154) Schwaller, M.; Wilkinson, B.; Gilbert, H. F. Reduction-reoxidation cycles contribute to catalysis of disulfide isomeriza,tion by protein-disulfide isomerase. *J. Biol. Chem.* **2003**, *278*, 7154−7159.
(155) Fujiwara, K.; Tilney, L. G. Substructural analysis of the microtubule and its polymorphic forms. *Ann. N.Y. Acad. Sci.* **1975**, *253*, 27−50.
(156) Wawro, M. E.; Sobierajska, K.; Ciszewski, W. M.; Wagner, W.; Frontczak, M.; Wieczorek, K.; Niewiarowska, J. Tubulin beta 3 and 4 are involved in the generation of early fibrotic stages. *Cell. Signalling* **2017**, *38*, 26−38.
(157) Wang, C. H.; Su, P. T.; Du, X. Y.; Kuo, M. W.; Lin, C. Y.; Yang, C. C.; Chan, H. S.; Chang, S. J.; Kuo, C.; Seo, K.; Leung, L. L.; Chuang, Y. J. Thrombospondin type I domain containing 7A (THSD7A) mediates endothelial cell migration and tube formation. *J. Cell. Physiol.* **2010**, *222*, 685−694.
(158) Adams, J. C. Thrombospondin-1. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 861−865.
(159) Rao, K. V. R.; Curtis, K. M.; Johnstone, J. T.; Norenberg, M. D. Amyloid-β inhibits thrombospondin 1 release from cultured astrocytes: effects on synaptic protein expression. *J. Neuropathol. Exp. Neurol.* **2013**, *72*, 735−744.
(160) Kim, D. H.; Lim, H.; Lee, D.; Choi, S. J.; Oh, W.; Yang, Y. S.; Oh, J. S.; Hwang, H. H.; Jeon, H. B. Thrombospondin-1 secreted by human umbilical cord blood-derived mesenchymal stem cells rescues neurons from synaptic dysfunction in Alzheimer's disease model. *Sci. Rep.* **2018**, *8*, 354.
(161) Borth, W. Alpha 2-macroglobulin, a multifunctional binding protein with targeting characteristics. *FASEB J.* **1992**, *6*, 3345−3353.
(162) Borth, W. Alpha 2-macroglobulin. A multifunctional binding and targeting protein with possible roles in immunity and auto-immunity. *Ann. N.Y. Acad. Sci.* **1994**, *737*, 267−272.
(163) Zhang, X.; Liu, J.; Liang, X.; Chen, J.; Hong, J.; Li, L.; He, Q.; Cai, X. History and progression of Fat cadherins in health and disease. *OncoTargets Ther.* **2016**, *9*, 7337−7343.
(164) Katoh, M. Function and cancer genomics of FAT family genes (review). *Int. J. Oncol.* **2012**, *41*, 1913−1918.
(165) Arlaud, G. J.; Gaboriaud, C.; Thielens, N. M.; Rossi, V.; Bersch, B.; Hernandez, J.-F.; Fontecilla-Camps, J. C. Structural biology of C1: dissection of a complex molecular machinery. *Immunol. Rev.* **2001**, *180*, 136−145.
(166) Arlaud, G.; Gaboriaud, C.; Garnier, G.; Circolo, A.; Thielens, N. M.; Budayova-Spano, M.; Fontecilla-Camps, J. C.; Volanakis, J. E. Structure, function and molecular genetics of human and murine C1r. *Immunobiology* **2002**, *205*, 365−382.
(167) Yasojima, K.; Schwab, C.; McGeer, E. G.; McGeer, P. L. Up-regulated production and activation of the complement system in Alzheimer's disease brain. *Am. J. Pathol.* **1999**, *154*, 927−936.
(168) Vázquez-Villa, F.; García-Ocaña, M.; Galván, J. A.; García-Martínez, J.; García-Pravia, C.; Menéndez-Rodríguez, P.; González-del Rey, C.; Barneo-Serra, L.; de Los Toyos, J. R. COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression. *Tumor Biol.* **2015**, *36*, 2213−2222.
(169) Yoshioka, H.; Greenwel, P.; Inoguchi, K.; Truter, S.; Inagaki, Y.; Ninomiya, Y.; Ramirez, F. Structural and functional analysis of the promoter of the human alpha 1(XI) collagen gene. *J. Biol. Chem.* **1995**, *270*, 418−424.
(170) Gao, H.; Tao, Y.; He, Q.; Song, F.; Saffen, D. Functional enrichment analysis of three Alzheimer's disease genome-wide association studies identities DAB1 as a novel candidate liability/protective gene. *Biochem. Biophys. Res. Commun.* **2015**, *463*, 490−495.
(171) Gough, R. E.; Goult, B. T. The tale of two talins - two isoforms to fine-tune integrin signalling. *FEBS Lett.* **2018**, *592*, 2108−2125.
(172) Ullrich, A.; Sures, I.; D'Egidio, M.; Jallal, B.; Powell, T. J.; Herbst, R.; Dreps, A.; Azam, M.; Rubinstein, M.; Natoli, C.; et al. The secreted tumor-associated antigen 90K is a potent immune stimulator. *J. Biol. Chem.* **1994**, *269*, 18401−18407.
(173) Tinari, N.; Kuwabara, I.; Huflejt, M. E.; Shen, P. F.; Iacobelli, S.; Liu, F.-T. Glycoprotein 90K/MAC-2BP interacts with galectin-1 and mediates galectin-1-induced cell aggregation. *Int. J. Cancer* **2001**, *91*, 167−172.
(174) De Cat, B.; David, G. Developmental roles of the glypicans. *Semin. Cell Dev. Biol.* **2001**, *12*, 117−125.
(175) Filmus, J.; Capurro, M.; Rast, J. Glypicans. *Genome Biol.* **2008**, *9*, 224.
(176) Wang, T.; Xu, S.-F.; Fan, Y.-G.; Li, L.-B.; Guo, C. Iron Pathophysiology in Alzheimer's Diseases. *Adv. Exp. Med. Biol.* **2019**, *1173*, 67−104.
(177) Crichton, R. R.; Charloteaux-Wauters, M. Iron transport and storage. *Eur. J. Biochem.* **1987**, *164*, 485−506.
(178) Wang, T.; Xu, S.-F.; Fan, Y.-G.; Li, L.-B.; Guo, C. Iron Pathophysiology in Alzheimer's Diseases. *Adv. Exp. Med. Biol.* **2019**, *1173*, 67−104.
(179) Cahill, C. M.; Lahiri, D. K.; Huang, X.; Rogers, J. T. Amyloid precursor protein and alpha synuclein translation, implications for iron and inflammation in neurodegenerative diseases. *Biochim. Biophys. Acta* **2009**, *1790*, 615−628.
(180) Twyffels, L.; Gueydan, C.; Kruys, V. Transportin-1 and Transportin-2: protein nuclear import and beyond. *FEBS Lett.* **2014**, *588*, 1857−1868.
(181) Wilson, K. L. Nuclear import pathway key to rescuing dominant progerin phenotypes. *Sci. Signaling* **2018**, *11*, No. eaat9448.
(182) Wang, Z.; Qiu, H.; He, J.; Liu, L.; Xue, W.; Fox, A.; Tickner, J.; Xu, J. The emerging roles of hnRNPK. *J. Cell. Physiol.* **2020**, *235*, 1995−2008.
(183) Xu, Y.; Wu, W.; Han, Q.; Wang, Y.; Li, C.; Zhang, P.; Xu, H. New Insights into the Interplay between non-coding RNAs and RNA-binding protein HnRNPK in regulating cellular functions. *Cells* **2019**, *8*, 62.
(184) Kwiatkowski, D. J. Functions of gelsolin: motility, signaling, apoptosis, cancer. *Curr. Opin. Cell Biol.* **1999**, *11*, 103−108.
(185) Yin, H. L. Gelsolin: calcium- and polyphosphoinositide-regulated actin-modulating protein. *Bioessays* **1987**, *7*, 176−179.
(186) Orphanides, G.; Wu, W.-H.; Lane, W. S.; Hampsey, M.; Reinberg, D. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature* **1999**, *400*, 284−288.
(187) Keller, D. M.; Zeng, X.; Wang, Y.; Zhang, Q. H.; Kapoor, M.; Shu, H.; Goodman, R.; Lozano, G.; Zhao, Y.; Lu, H. A DNA damage-induced p53 serine 392 kinase complex contains CK2, hSpt16, and SSRP1. *Mol. Cell* **2001**, *7*, 283−292.
(188) Lopez-Alemany, R.; Suelves, M.; Diaz-Ramos, A.; Vidal, B.; Munoz-Canoves, P. Alpha-enolase plasminogen receptor in myogenesis. *Front. Biosci.* **2005**, *10*, 30−36.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(189) Ji, H.; Wang, J.; Guo, J.; Li, Y.; Lian, S.; Guo, W.; Yang, H.; Kong, F.; Zhen, L.; Guo, L.; Liu, Y. Progress in the biological function of alpha-enolase. *Anim. Nutr.* **2016**, *2*, 12−17.
(190) Butterfield, D. A.; Poon, H. F.; St. Clair, D.; Keller, J. N.; Pierce, W. M.; Klein, J. B.; Markesbery, W. R. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol. Dis.* **2006**, *22*, 223−232.
(191) Zeng, C.; Xing, W.; Liu, Y. Identification of UGP2 as a progression marker that promotes cell growth and motility in human glioma. *J. Cell. Biochem.* **2019**, *120*, 12489−12499.
(192) Perenthaler, E.; Nikoncuk, A.; Yousefi, S.; Berdowski, W. M.; Alsagob, M.; Capo, I.; van der Linde, H. C.; van den Berg, P.; Jacobs, E. H.; Putar, D.; et al. Loss of UGP2 in brain leads to a severe epileptic encephalopathy, emphasizing that bi-allelic isoform-specific start-loss mutations of essential genes can cause genetic diseases. *Acta Neuropathol.* **2020**, *139*, 415−442.
(193) Slavotinek, A. M. Eye development genes and known syndromes. *Mol. Genet. Metab.* **2011**, *104*, 448−456.
(194) Makridakis, M.; Roubelakis, M. G.; Vlahou, A. Stem cells: insights into the secretome. *Biochim. Biophys. Acta* **2013**, *1834*, 2380−2384.
(195) Della Noce, I.; Carra, S.; Brusegan, C.; Critelli, R.; Frassine, A.; De Lorenzo, C.; Giordano, A.; Bellipanni, G.; Villa, E.; Cotelli, F.; Pistocchi, A.; Schepis, F. The Coiled-Coil Domain Containing 80 (ccdc80) gene regulates gadd45β2 expression in the developing somites of zebrafish as a new player of the hedgehog pathway. *J. Cell. Physiol.* **2015**, *230*, 821−830.
(196) Fansa, E. K.; Wittinghofer, A. Sorting of lipidated cargo by the Arl2/Arl3 system. *Small GTPases* **2016**, *7*, 222−230.
(197) Graham, T. R. Membrane targeting: getting Arl to the Golgi. *Curr. Biol.* **2004**, *14*, R483−R485.
(198) Llorca, O.; McCormack, E. A.; Hynes, G.; Grantham, J.; Cordell, J.; Carrascosa, J. L.; Willison, K. R.; Fernandez, J. J.; Valpuesta, J. M. Eukaryotic type II chaperonin CCT interacts with actin through specific subunits. *Nature* **1999**, *402*, 693−696.
(199) Pavel, M.; Imarisio, S.; Menzies, F. M.; Jimenez-Sanchez, M.; Siddiqi, F. H.; Wu, X.; Renna, M.; O'Kane, C. J.; Crowther, D. C.; Rubinsztein, D. C. CCT complex restricts neuropathogenic protein aggregation via autophagy. *Nat. Commun.* **2016**, *7*, 13821.
(200) Fujibuchi, T.; Abe, Y.; Takeuchi, T.; Imai, Y.; Kamei, Y.; Murase, R.; Ueda, N.; Shigemoto, K.; Yamamoto, H.; Kito, K. AIP1/WDR1 supports mitotic cell rounding. *Biochem. Biophys. Res. Commun.* **2005**, *327*, 268−275.
(201) Kato, A.; Kurita, S.; Hayashi, A.; Kaji, N.; Ohashi, K.; Mizuno, K. Critical roles of actin-interacting protein 1 in cytokinesis and chemotactic migration of mammalian cells. *Biochem. J.* **2008**, *414*, 261−270.
(202) Hammond, G. L.; Bocchinfuso, W. P. Sex hormone-binding globulin: gene organization and structure/function analyses. *Horm. Res.* **1996**, *45*, 197−201.
(203) Caldwell, J.; Jirikowski, G. Sex hormone binding globulin and aging. *Horm. Metab. Res.* **2009**, *41*, 173−182.
(204) Holland, J.; Bandelow, S.; Hogervorst, E. Testosterone levels and cognition in elderly men: a review. *Maturitas* **2011**, *69*, 322−337.
(205) Huang, C. Y.-M.; Zhang, C.; Ho, T. S.-Y.; Oses-Prieto, J.; Burlingame, A. L.; Lalonde, J.; Noebels, J. L.; Leterrier, C.; Rasband, M. N. αII Spectrin Forms a Periodic Cytoskeleton at the Axon Initial Segment and Is Required for Nervous System Function. *J. Neurosci.* **2017**, *37*, 11311−11322.
(206) Ackermann, A.; Brieger, A. The Role of Nonerythroid Spectrin αII in Cancer. *J. Oncol.* **2019**, *2019*, 7079604.
(207) Nakamura, F.; Kumeta, K.; Hida, T.; Isono, T.; Nakayama, Y.; Kuramata-Matsuoka, E.; Yamashita, N.; Uchida, Y.; Ogura, K.; Gengyo-Ando, K.; Mitani, S.; Ogino, T.; Goshima, Y. Amino- and carboxyl-terminal domains of Filamin-A interact with CRMP1 to mediate Sema3A signalling. *Nat. Commun.* **2014**, *5*, 5325.
(208) Yamashita, N.; Goshima, Y. Collapsin response mediator proteins regulate neuronal development and plasticity by switching their phosphorylation status. *Mol. Neurobiol.* **2012**, *45*, 234−246.
(209) Hooli, B. V.; Kovacs-Vajna, Z. M.; Mullin, K.; Blumenthal, M. A.; Mattheisen, M.; Zhang, C.; Lange, C.; Mohapatra, G.; Bertram, L.; Tanzi, R. E. Rare autosomal copy number variations in early-onset familial Alzheimer's disease. *Mol. Psychiatry* **2014**, *19*, 676−681.
(210) Bradshaw, N. J.; Korth, C. Protein misassembly and aggregation as potential convergence points for non-genetic causes of chronic mental illness. *Mol. Psychiatry* **2019**, *24*, 936−951.
(211) Ray, A.; Treloar, H. B. IgSF8: a developmentally and functionally regulated cell adhesion molecule in olfactory sensory neuron axons and synapses. *Mol. Cell. Neurosci.* **2012**, *50*, 238−249.
(212) Murdoch, J. N.; Doudney, K.; Gerrelli, D.; Wortham, N.; Paternotte, C.; Stanier, P.; Copp, A. J. Genomic organization and embryonic expression of Igsf8, an immunoglobulin superfamily member implicated in development of the nervous system and organ epithelia. *Mol. Cell. Neurosci.* **2003**, *22*, 62−74.
(213) Lo, A. S.-Y.; Liew, C.-T.; Ngai, S.-M.; Tsui, S. K.-W.; Fung, K.-P.; Lee, C.-Y.; Waye, M. M.-Y. Developmental regulation and cellular distribution of human cytosolic malate dehydrogenase (MDH1). *J. Cell. Biochem.* **2005**, *94*, 763−773.
(214) Lee, S.-M.; Dho, S. H.; Ju, S.-K.; Maeng, J.-S.; Kim, J.-Y.; Kwon, K.-S. Cytosolic malate dehydrogenase regulates senescence in human fibroblasts. *Biogerontology* **2012**, *13*, 525−536.
(215) Mullarky, E.; Mattaini, K. R.; Vander Heiden, M. G.; Cantley, L. C.; Locasale, J. W. PHGDH amplification and altered glucose metabolism in human melanoma. *Pigm. Cell Melanoma Res.* **2011**, *24*, 1112−1115.
(216) Zhao, X.; Fu, J.; Du, J.; Xu, W. The Role of D-3-Phosphoglycerate Dehydrogenase in Cancer. *Int. J. Biol. Sci.* **2020**, *16*, 1495−1506.
(217) Yan, Z.; Zhou, Z.; Wu, Q.; Chen, Z. B.; Koo, E. H.; Zhong, S. Presymptomatic Increase of an Extracellular RNA in Blood Plasma Associates with the Development of Alzheimer's Disease. *Curr. Biol.* **2020**, *30*, 1771−1782.e3.
(218) Chaudhuri, J.; Chakrabarti, A.; Maitra, U. Biochemical characterization of mammalian translation initiation factor 3 (eIF3). Molecular cloning reveals that p110 subunit is the mammalian homologue of Saccharomyces cerevisiae protein Prt1. *J. Biol. Chem.* **1997**, *272*, 30975−30983.
(219) Feng, X.; Li, J.; Liu, P. The Biological Roles of Translation Initiation Factor 3b. *Int. J. Biol. Sci.* **2018**, *14*, 1630−1635.
(220) Baumkötter, F.; Schmidt, N.; Vargas, C.; Schilling, S.; Weber, R.; Wagner, K.; Fiedler, S.; Klug, W.; Radzimanowski, J.; Nickolaus, S.; Keller, S.; Eggert, S.; Wild, K.; Kins, S. Amyloid precursor protein dimerization and synaptogenic function depend on copper binding to the growth factor-like domain. *J. Neurosci.* **2014**, *34*, 11159−11172.
(221) Nguyen, K. V. β-Amyloid precursor protein (APP) and the human diseases. *AIMS Neurosci.* **2019**, *6*, 273−281.
(222) Haass, C.; Kaether, C.; Thinakaran, G.; Sisodia, S. Trafficking and proteolytic processing of APP. *Cold Spring Harbor Perspect. Med.* **2012**, *2*, a006270.
(223) Lott, I.; Head, E.; Doran, E.; Busciglio, J. Beta-amyloid, oxidative stress and down syndrome. *Curr. Alzheimer Res.* **2006**, *3*, 521−528.
(224) Lu, J.; Stewart, A. J.; Sadler, P. J.; Pinheiro, T. J.; Blindauer, C. A. Albumin as a zinc carrier: properties of its high-affinity zinc-binding site. *Biochem. Soc. Trans.* **2008**, *36*, 1317−1321.
(225) Erickson, M. A.; Banks, W. A. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **2013**, *33*, 1500−1513.
(226) Lazarides, E. Intermediate filaments: a chemically heterogeneous, developmentally regulated class of proteins. *Annu. Rev. Biochem.* **1982**, *51*, 219−250.
(227) Steinert, P. M.; Jones, J. C.; Goldman, R. D. Intermediate filaments. *J. Cell Biol.* **1984**, *99*, 22s−27s.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(228) Nicholls, C.; Li, H.; Liu, J. P. GAPDH: a common enzyme with uncommon functions. *Clin. Exp. Pharmacol. Physiol.* **2012**, *39*, 674−679.
(229) Seidler, N. W. Basic biology of GAPDH. *Adv. Exp. Med. Biol.* **2013**, *985*, 1−36.
(230) Tong, Y.; Hota, P. K.; Penachioni, J. Y.; Hamaneh, M. B.; Kim, S.; Alviani, R. S.; Shen, L.; He, H.; Tempel, W.; Tamagnone, L.; Park, H.-W.; Buck, M. Structure and function of the intracellular region of the plexin-b1 transmembrane receptor. *J. Biol. Chem.* **2009**, *284*, 35962−35972.
(231) Janssen, B. J. C.; Robinson, R. A.; Pérez-Brangulí, F.; Bell, C. H.; Mitchell, K. J.; Siebold, C.; Jones, E. Y. Structural basis of semaphorin-plexin signalling. *Nature* **2010**, *467*, 1118−1122.
(232) Kannabiran, C.; Klintworth, G. K. TGFBI gene mutations in corneal dystrophies. *Hum. Mutat.* **2006**, *27*, 615−625.
(233) Karring, H.; Runager, K.; Valnickova, Z.; Thøgersen, I. B.; Møller-Pedersen, T.; Klintworth, G. K.; Enghild, J. J. Differential expression and processing of transforming growth factor beta induced protein (TGFBIp) in the normal human cornea during postnatal development and aging. *Exp. Eye Res.* **2010**, *90*, 57−62.
(234) Skidgel, R. A.; Erdös, E. G. Cellular carboxypeptidases. *Immunol. Rev.* **1998**, *161*, 129−141.
(235) Diano, S. New aspects of melanocortin signaling: a role for PRCP in α-MSH degradation. *Front. Neuroendocrinol.* **2011**, *32*, 70−83.
(236) Maki, M.; Kitaura, Y.; Satoh, H.; Ohkouchi, S.; Shibata, H. Structures, functions and molecular evolution of the penta-EF-hand Ca2+-binding proteins. *Biochim. Biophys. Acta* **2002**, *1600*, 51−60.
(237) Ursini-Siegel, J.; Rajput, A. B.; Lu, H.; Sanguin-Gendreau, V.; Zuo, D.; Papavasiliou, V.; Lavoie, C.; Turpin, J.; Cianflone, K.; Huntsman, D. G.; Muller, W. J. Elevated expression of DecR1 impairs ErbB2/Neu-induced mammary tumor development. *Mol. Cell. Biol.* **2007**, *27*, 6361−6371.
(238) Young, C. D.; Anderson, S. M. Sugar and fat—that's where it's at: metabolic changes in tumors. *Breast Cancer Res.* **2008**, *10*, 202.
(239) Layne, M. D.; Endege, W. O.; Jain, M. K.; Yet, S.-F.; Hsieh, C.-M.; Chin, M. T.; Perrella, M. A.; Blanar, M. A.; Haber, E.; Lee, M.-E. Aortic carboxypeptidase-like protein, a novel protein with discoidin and carboxypeptidase-like domains, is up-regulated during vascular smooth muscle cell differentiation. *J. Biol. Chem.* **1998**, *273*, 15654−15660.
(240) Sorisky, A.; Gagnon, A.; Abaiian, K. Aortic carboxypeptidase-like protein (ACLP): what is a protein with a name like that doing in adipose tissue? *Horm. Metab. Res.* **2002**, *34*, 764−766.
(241) Majdalawieh, A.; Ro, H. S. Regulation of IkappaBalpha function and NF-kappaB signaling: AEBP1 is a novel proinflammatory mediator in macrophages. *Mediators Inflammation* **2010**, *2010*, 823821.
(242) Li, P.; Zhang, M.; Zou, Y.; Sun, Z.; Sun, C.; Geng, Z.; Xu, W.; Wang, D. Interaction of heat shock protein 90 B1 (Hsp90B1) with liposome reveals its potential role in protection the integrity of lipid membranes. *Int. J. Biol. Macromol.* **2018**, *106*, 1250−1257.
(243) Zhang, M.; Qian, C.; Zheng, Z.-G.; Qian, F.; Wang, Y.; Thu, P. M.; Zhang, X.; Zhou, Y.; Tu, L.; Liu, Q.; Li, H.-J.; Yang, H.; Li, P.; Xu, X. Jujuboside A promotes Aβ clearance and ameliorates cognitive deficiency in Alzheimer's disease through activating Axl/HSP90/PPARγ pathway. *Theranostics* **2018**, *8*, 4262−4278.
(244) Liu, Z.; Dai, X.; Tao, W.; Liu, H.; Li, H.; Yang, C.; Zhang, J.; Li, X.; Chen, Y.; Ma, C.; Pei, J.; Mao, H.; Chen, K.; Zhang, Z. APOE influences working memory in non-demented elderly through an interaction with SPON1 rs2618516. *Hum. Brain Mapp.* **2018**, *39*, 2859−2867.
(245) Ho, A.; Südhof, T. C. Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2548−2553.
(246) Fraser, P. E.; Yang, D.-S.; Yu, G.; Lévesque, L.; Nishimura, M.; Arawaka, S.; Serpell, L. C.; Rogaeva, E.; St George-Hyslop, P. Presenilin structure, function and role in Alzheimer disease. *Biochim. Biophys. Acta* **2000**, *1502*, 1−15.
(247) Chen, Y. Z. APP induces neuronal apoptosis through APP-BP1-mediated downregulation of beta-catenin. *Apoptosis* **2004**, *9*, 415−422.
(248) Chiba, K.; Araseki, M.; Nozawa, K.; Furukori, K.; Araki, Y.; Matsushima, T.; Nakaya, T.; Hata, S.; Saito, Y.; Uchida, S.; Okada, Y.; Nairn, A. C.; Davis, R. J.; Yamamoto, T.; Kinjo, M.; Taru, H.; Suzuki, T. Quantitative analysis of APP axonal transport in neurons: role of JIP1 in enhanced APP anterograde transport. *Mol. Biol. Cell* **2014**, *25*, 3569−3580.
(249) Sun, Q.-Y.; Schatten, H. Role of NuMA in vertebrate cells: review of an intriguing multi-functional protein. *Front. Biosci.* **2006**, *11*, 1137−1146.
(250) Meuwissen, M. E. C.; Halley, D. J. J.; Smit, L. S.; Lequin, M. H.; Cobben, J. M.; de Coo, R.; van Harssel, J.; Sallevelt, S.; Woldringh, G.; van der Knaap, M. S.; de Vries, L. S.; Mancini, G. M. S. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. *Genet. Med.* **2015**, *17*, 843−853.
(251) Mathieu, C.; de la Sierra-Gallay, I. L.; Duval, R.; Xu, X.; Cocaign, A.; Léger, T.; Woffendin, G.; Camadro, J.-M.; Etchebest, C.; Haouz, A.; Dupret, J.-M.; Rodrigues-Lima, F. Insights into brain glycogen metabolism: the structure of human brain glycogen phosphorylasse. *J. Biol. Chem.* **2016**, *291*, 18072−18083.
(252) Newgard, C. B.; Littman, D. R.; van Genderen, C.; Smith, M.; Fletterick, R. J. Human brain glycogen phosphorylase. Cloning, sequence analysis, chromosomal mapping, tissue expression, and comparison with the human liver and muscle isozymes. *J. Biol. Chem.* **1988**, *263*, 3850−3857.
(253) San Segundo-Acosta, P.; Montero-Calle, A.; Fuentes, M.; Rábano, A.; Villalba, M.; Barderas, R. Identification of Alzheimer's Disease Autoantibodies and Their Target Biomarkers by Phage Microarrays. *J. Proteome Res.* **2019**, *18*, 2940−2953.
(254) Feinstein, Y.; Klar, A. The neuronal class 2 TSR proteins F-spondin and Mindin: a small family with divergent biological activities. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 975−980.
(255) Adams, J. C.; Tucker, R. P. The thrombospondin type 1 repeat (TSR) superfamily: diverse proteins with related roles in neuronal development. *Dev. Dyn.* **2000**, *218*, 280−299.
(256) Ho, A.; Südhof, T. C. Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2548−2553.
(257) Hoe, H.-S.; Rebeck, G. W. Regulated proteolysis of APP and ApoE receptors. *Mol. Neurobiol.* **2008**, *37*, 64−72.
(258) Bahmanyar, S.; Kaplan, D. D.; Deluca, J. G.; Giddings, T. H., Jr.; O'Toole, E. T.; Winey, M.; Salmon, E. D.; Casey, P. J.; Nelson, W. J.; Barth, A. I. M. β-Catenin is a Nek2 substrate involved in centrosome separation. *Genes Dev.* **2008**, *22*, 91−105.
(259) Weiske, J.; Albring, K. F.; Huber, O. The tumor suppressor Fhit acts as a repressor of beta-catenin transcriptional activity. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 20344−20349.
(260) Fuentealba, R. A.; Farias, G.; Scheu, J.; Bronfman, M.; Marzolo, M. P.; Inestrosa, N. C. Signal transduction during amyloid-beta-peptide neurotoxicity: role in Alzheimer disease. *Brain Res. Rev.* **2004**, *47*, 275−289.
(261) Chen, Y. Z. APP induces neuronal apoptosis through APP-BP1-mediated downregulation of beta-catenin. *Apoptosis* **2004**, *9*, 415−422.
(262) Geroldi, D.; Minoretti, P.; Bianchi, M.; Di Vito, C.; Reino, M.; Bertona, M.; Emanuele, E. Genetic association of alpha2-Heremans-Schmid glycoprotein polymorphism with late-onset Alzheimer's disease in Italians. *Neurosci. Lett.* **2005**, *386*, 176−178.
(263) Splinter, D.; Tanenbaum, M. E.; Lindqvist, A.; Jaarsma, D.; Flotho, A.; Yu, K. L.; Grigoriev, I.; Engelsma, D.; Haasdijk, E. D.; Keijzer, N.; Demmers, J.; Fornerod, M.; Melchior, F.; Hoogenraad, C. C.; Medema, R. H.; Akhmanova, A. Bicaudal D2, dynein, and kinesin-1 associate with nuclear pore complexes and regulate centrosome and nuclear positioning during mitotic entry. *PLoS Biol.* **2010**, *8*, No. e1000350.
(264) Kamal, A.; Almenar-Queralt, A.; LeBlanc, J. F.; Roberts, E. A.; Goldstein, L. S. B. Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. *Nature* **2001**, *414*, 643−648.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(265) Zheng, Y.; Tian, S.; Peng, X.; Yang, J.; Fu, Y.; Jiao, Y.; Zhao, J.; He, J.; Hong, T. Kinesin-1 inhibits the aggregation of amyloid-β peptide as detected by fluorescence cross-correlation spectroscopy. *FEBS Lett.* **2016**, *590*, 1028−1037.
(266) Huang, J.; Gong, Z.; Ghosal, G.; Chen, J. SOSS complexes participate in the maintenance of genomic stability. *Mol. Cell* **2009**, *35*, 384−393.
(267) Kuo, Y.-C.; Shen, Y.-R.; Chen, H.-I.; Lin, Y.-H.; Wang, Y.-Y.; Chen, Y.-R.; Wang, C.-Y.; Kuo, P.-L. SEPT12 orchestrates the formation of mammalian sperm annulus by organizing core octameric complexes with other SEPT proteins. *J. Cell Sci.* **2015**, *128*, 923−934.
(268) Retzlaff, M.; Stahl, M.; Eberl, H. C.; Lagleder, S.; Beck, J.; Kessler, H.; Buchner, J. Hsp90 is regulated by a switch point in the C-terminal domain. *EMBO Rep.* **2009**, *10*, 1147−1153.
(269) Verma, S.; Goyal, S.; Jamal, S.; Singh, A.; Grover, A. Hsp90: Friends, clients and natural foes. *Biochimie* **2016**, *127*, 227−240.
(270) Schaffer, A. E.; Breuss, M. W.; Caglayan, A. O.; Al-Sanaa, N.; Al-Abdulwahed, H. Y.; Kaymakçalan, H.; Yılmaz, C.; Zaki, M. S.; Rosti, R. O.; Copeland, B.; et al. Biallelic loss of human CTNNA2, encoding αN-catenin, leads to ARP2/3 complex overactivity and disordered cortical neuronal migration. *Nat. Genet.* **2018**, *50*, 1093−1101.
(271) Cummings, A. C.; Jiang, L.; Velez Edwards, D. R.; McCauley, J. L.; Laux, R.; McFarland, L. L.; Fuzzell, D.; Knebusch, C.; Caywood, L.; Reinhart-Mercer, L.; Nations, L.; Gilbert, J. R.; Konidari, I.; Tramontana, M.; Cuccaro, M. L.; Scott, W. K.; Pericak-Vance, M. A.; Haines, J. L. Genome-wide association and linkage study in the Amish detects a novel candidate late-onset Alzheimer disease gene. *Ann. Hum. Genet.* **2012**, *76*, 342−351.
(272) Moseley, A. E.; Lieske, S. P.; Wetzel, R. K.; James, P. F.; He, S.; Shelly, D. A.; Paul, R. J.; Boivin, G. P.; Witte, D. P.; Ramirez, J. M.; Sweadner, K. J.; Lingrel, J. B. The Na,K-ATPase alpha 2 isoform is expressed in neurons, and its absence disrupts neuronal activity in newborn mice. *J. Biol. Chem.* **2003**, *278*, 5317−5324.
(273) Sopko, R.; McNeill, H. The skinny on Fat: an enormous cadherin that regulates cell adhesion, tissue growth, and planar cell polarity. *Curr. Opin. Cell Biol.* **2009**, *21*, 717−723.
(274) Avilés, E. C.; Goodrich, L. V. Configuring a robust nervous system with Fat cadherins. *Semin. Cell Dev. Biol.* **2017**, *69*, 91−101.
(275) Crabos, M.; Yamakado, T.; Heizmann, C. W.; Cerletti, N.; Bühler, F. R.; Erne, P. The calcium binding protein tropomyosin in human platelets and cardiac tissue: elevation in hypertensive cardiac hypertrophy. *Eur. J. Clin. Invest.* **1991**, *21*, 472−478.
(276) Karsenty, G.; Park, R.-W. Regulation of type I collagen genes expression. *Int. Rev. Immunol.* **1995**, *12*, 177−185.
(277) Kehrel, B. Platelet-collagen interactions. *Semin. Thromb. Hemostasis* **1995**, *21*, 123−129.
(278) Coulson, E. J.; Barrett, G. L.; Storey, E.; Bartlett, P. F.; Beyreuther, K.; Masters, C. L. Down-regulation of the amyloid protein precursor of Alzheimer's disease by antisense oligonucleotides reduces neuronal adhesion to specific substrata. *Brain Res.* **1997**, *770*, 72−80.
(279) Yamagata, M.; Weiner, J. A.; Sanes, J. R. Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. *Cell* **2002**, *110*, 649−660.
(280) Tang, H.; Chang, H.; Dong, Y.; Guo, L.; Shi, X.; Wu, Y.; Huang, Y.; He, Y. Architecture of cell-cell adhesion mediated by sidekicks. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, 9246−9251.
(281) Ahel, I.; Ahel, D.; Matsusaka, T.; Clark, A. J.; Pines, J.; Boulton, S. J.; West, S. C. Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. *Nature* **2008**, *451*, 81−85.
(282) Reinemund, J.; Seidel, K.; Steckelings, U. M.; Zaade, D.; Klare, S.; Rompe, F.; Katerbaum, M.; Schacherl, J.; Li, Y.; Menk, M.; Schefe, J. H.; Goldin-Lang, P.; Szabo, C.; Olah, G.; Unger, T.; Funke-Kaiser, H. Poly(ADP-ribose) polymerase-1 (PARP-1) transcriptionally regulates angiotensin AT2 receptor (AT2R) and AT2R binding protein (ATBP) genes. *Biochem. Pharmacol.* **2009**, *77*, 1795−1805.
(283) Martire, S.; Mosca, L.; d'Erme, M. PARP-1 involvement in neurodegeneration: A focus on Alzheimer's and Parkinson's diseases. *Mech. Ageing Dev.* **2015**, *146−148*, 53−64.
(284) Zeng, J.; Libien, J.; Shaik, F.; Wolk, J.; Hernández, A. I. Nucleolar PARP-1 Expression Is Decreased in Alzheimer's Disease: Consequences for Epigenetic Regulation of rDNA and Cognition. *Neural Plast.* **2016**, *2016*, 8987928.
(285) Vafadar-Isfahani, B.; Ball, G.; Coveney, C.; Lemetre, C.; Boocock, D.; Minthon, L.; Hansson, O.; Miles, A. K.; Janciauskiene, S. M.; Warden, D.; Smith, A. D.; Wilcock, G.; Kalsheker, N.; Rees, R.; Matharoo-Ball, B.; Morgan, K. Identification of SPARC-like 1 protein as part of a biomarker panel for Alzheimer's disease in cerebrospinal fluid. *J. Alzheimer's Dis.* **2012**, *28*, 625−636.
(286) Levy-Rimler, G.; Viitanen, P.; Weiss, C.; Sharkia, R.; Greenberg, A.; Niv, A.; Lustig, A.; Delarea, Y.; Azem, A. The effect of nucleotides and mitochondrial chaperonin 10 on the structure and chaperone activity of mitochondrial chaperonin 60. *Eur. J. Biochem.* **2001**, *268*, 3465−3472.
(287) Walls, K. C.; Coskun, P.; Gallegos-Perez, J. L.; Zadourian, N.; Freude, K.; Rasool, S.; Blurton-Jones, M.; Green, K. N.; LaFerla, F. M. Swedish Alzheimer mutation induces mitochondrial dysfunction mediated by HSP60 mislocalization of amyloid precursor protein (APP) and beta-amyloid. *J. Biol. Chem.* **2012**, *287*, 30317−30327.
(288) Campanella, C.; Pace, A.; Caruso Bavisotto, C.; Marzullo, P.; Marino Gammazza, A.; Buscemi, S.; Palumbo Piccionello, A. Heat Shock Proteins in Alzheimer's Disease: Role and Targeting. *Int. J. Mol. Sci.* **2018**, *19*, 2603.
(289) Kishimoto, Y.; Hiraiwa, M.; O'Brien, J. Saposins: structure, function, distribution, and molecular genetics. *J. Lipid Res.* **1992**, *33*, 1255−1267.
(290) Paushter, D. H.; Du, H.; Feng, T.; Hu, F. The lysosomal function of progranulin, a guardian against neurodegeneration. *Acta Neuropathol.* **2018**, *136*, 1−17.
(291) Nicholson, A. M.; Finch, N. A.; Almeida, M.; Perkerson, R. B.; van Blitterswijk, M.; Wojtas, A.; Cenik, B.; Rotondo, S.; Inskeep, V.; Almasy, L.; Dyer, T.; Peralta, J.; Jun, G.; Wood, A. R.; Frayling, T. M.; Fuchsberger, C.; Fowler, S.; Teslovich, T. M.; Manning, A. K.; Kumar, S.; Rademakers, R. Prosaposin is a regulator of progranulin levels and oligomerization. *Nat. Commun.* **2016**, *7*, 11992.
(292) Andersson, A.; Remnestål, J.; Nellgård, B.; Vunk, H.; Kotol, D.; Edfors, F.; Uhlén, M.; Schwenk, J. M.; Ilag, L. L.; Zetterberg, H.; Blennow, K.; Månberg, A.; Nilsson, P.; Fredolini, C. Development of parallel reaction monitoring assays for cerebrospinal fluid proteins associated with Alzheimer's disease. *Clin. Chim. Acta* **2019**, *494*, 79−93.
(293) Csiszar, K. Lysyl oxidases: a novel multifunctional amine oxidase family. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *70*, 1−32.
(294) Lee, J.-E.; Kim, Y. A tissue-specific variant of the human lysyl oxidase-like protein 3 (LOXL3) functions as an amine oxidase with substrate specificity. *J. Biol. Chem.* **2006**, *281*, 37282−37290.
(295) Simionescu-Bankston, A.; Pichavant, C.; Canner, J. P.; Apponi, L. H.; Wang, Y.; Steeds, C.; Olthoff, J. T.; Belanto, J. J.; Ervasti, J. M.; Pavlath, G. K. Creatine kinase B is necessary to limit myoblast fusion during myogenesis. *Am. J. Physiol.: Cell Physiol.* **2015**, *308*, C919−C931.
(296) Hernández, F.; Cuadros, R.; Ollá, I.; García, C.; Ferrer, I.; Perry, G.; Avila, J. Differences in structure and function between human and murine tau. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2019**, *1865*, 2024−2030.
(297) Ju, T.-C.; Lin, Y.-S.; Chern, Y. Energy dysfunction in Huntington's disease: insights from PGC-1α, AMPK, and CKB. *Cell. Mol. Life Sci.* **2012**, *69*, 4107−4120.
(298) Montgomery, M. K.; Bayliss, J.; Keenan, S.; Rhost, S.; Ting, S. B.; Watt, M. J. The role ofAp2a2 in PPARα-mediated regulation of lipolysis in adipose tissue. *FASEB J.* **2019**, *33*, 13267−13279.
(299) Ting, S. B.; Deneault, E.; Hope, K.; Cellot, S.; Chagraoui, J.; Mayotte, N.; Dorn, J. F.; Laverdure, J.-P.; Harvey, M.; Hawkins, E. D.; Russell, S. M.; Maddox, P. S.; Iscove, N. N.; Sauvageau, G. Asymmetric segregation and self-renewal of hematopoietic stem and progenitor cells with endocytic Ap2a2. *Blood* **2012**, *119*, 2510−2522.
(300) Espinosa, A.; Hernández-Olasagarre, B.; Moreno-Grau, S.; Kleineidam, L.; Heilmann-Heimbach, S.; Hernández, I.; Wolfsgruber, S.; Wagner, H.; Rosende-Roca, M.; Mauleón, A.; et al. Exploring genetic

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

associations of Alzheimer's disease Loci With Mild cognitive impairment neurocognitive endophenotypes. *Front. Aging Neurosci.* **2018**, *10*, 340.
(301) Katsumata, Y.; Fardo, D. W.; Bachstetter, A. D.; Artiushin, S. C.; Wang, W. X.; Wei, A.; Brzezinski, L. J.; Nelson, B. G.; Huang, Q.; Abner, E. L.; Anderson, S.; Patel, I.; Shaw, B. C.; Price, D. A.; Niedowicz, D. M.; Wilcock, D. W.; Jicha, G. A.; Neltner, J. H.; Van Eldik, L. J.; Estus, S.; Nelson, P. T. Alzheimer disease pathology-associated polymorphism in a complex variable Number of tandem repeat region within the MUC6 gene, near the AP2A2 gene. *J. Neuropathol. Exp. Neurol.* **2020**, *79*, 3−21.
(302) Reissner, C.; Runkel, F.; Missler, M. Neurexins. *Genome Biol.* **2013**, *14*, 213.
(303) Sindi, I. A.; Tannenberg, R. K.; Dodd, P. R. A Role for the neurexin-neuroligin complex in Alzheimer's disease. *Neurobiol. Aging* **2014**, *35*, 746−756.
(304) Martinez-Mir, A.; González-Pérez, A.; Gayán, J.; Antúnez, C.; Marín, J.; Boada, M.; Lopez-Arrieta, J. M.; Fernández, E.; Ramírez-Lorca, R.; Sáez, M. E.; Ruiz, A.; Scholl, F. G.; Real, L. M. Genetic study of neurexin and neuroligin genes in Alzheimer's disease. *J. Alzheimer's Dis.* **2013**, *35*, 403−412.
(305) Bouter, A.; Carmeille, R.; Gounou, C.; Bouvet, F.; Degrelle, S. A.; Evain-Brion, D.; Brisson, A. R. Review: Annexin-A5 and cell membrane repair. *Placenta* **2015**, *36*, S43−S49.
(306) Boersma, H. H.; Kietselaer, B. L.; Stolk, L. M.; Bennaghmouch, A.; Hofstra, L.; Narula, J.; Heidendal, G. A.; Reutelingsperger, C. P. Past, present, and future of annexin A5: from protein discovery to clinical applications. *J. Nucl. Med.* **2005**, *46*, 2035−2050.
(307) Correani, V.; Di Francesco, L.; Mignogna, G.; Fabrizi, C.; Leone, S.; Giorgi, A.; Passeri, A.; Casata, R.; Fumagalli, L.; Maras, B.; Schininà, M. E. Plasma membrane protein profiling in beta-amyloid-treated microglia cell line. *Proteomics* **2017**, *17*, 1600439.
(308) Muraoka, S.; DeLeo, A. M.; Sethi, M. K.; Yukawa-Takamatsu, K.; Yang, Z.; Ko, J.; Hogan, J. D.; Ruan, Z.; You, Y.; Wang, Y.; Medalla, M.; Ikezu, S.; Chen, M.; Xia, W.; Gorantla, S.; Gendelman, H. E.; Issadore, D.; Zaia, J.; Ikezu, T. Proteomic and biological profiling of extracellular vesicles from Alzheimer's disease human brain tissues. *Alzheimer's Dementia* **2020**, *16*, 896−907.
(309) Diez-Fernandez, C.; Rüfenacht, V.; Häberle, J. Mutations in the human argininosuccinate synthetase (ASS1) gene, impact on patients, common changes, and structural considerations. *Hum. Mutat.* **2017**, *38*, 471−484.
(310) Engel, K.; Höhne, W.; Häberle, J. Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. *Hum. Mutat.* **2009**, *30*, 300−307.
(311) Haas, J.; Storch-Hagenlocher, B.; Biessmann, A.; Wildemann, B. Inducible nitric oxide synthase and argininosuccinate synthetase: co-induction in brain tissue of patients with Alzheimer's dementia and following stimulation with beta-amyloid 1-42 in vitro. *Neurosci. Lett.* **2002**, *322*, 121−125.
(312) Ye, F.; Chen, Y.; Xia, L.; Lian, J.; Yang, S. Aldolase A overexpression is associated with poor prognosis and promotes tumor progression by the epithelial-mesenchymal transition in colon cancer. *Biochem. Biophys. Res. Commun.* **2018**, *497*, 639−645.
(313) Zafar, S.; Shafiq, M.; Younas, N.; Schmitz, M.; Ferrer, I.; Zerr, I. Prion protein interactome: identifying novel targets in slowly and rapidly progressive forms of Alzheimer's disease. *J. Alzheimer's Dis.* **2017**, *59*, 265−275.
(314) Jossin, Y.; Lee, M.; Klezovitch, O.; Kon, E.; Cossard, A.; Lien, W.-H.; Fernandez, T. E.; Cooper, J. A.; Vasioukhin, V. Llgl1 connects cell polarity with cell-cell adhesion in embryonic neural stem cells. *Dev. Cell* **2017**, *41*, 481−495.e5.
(315) Russ, A.; Louderbough, J. M. V.; Zarnescu, D.; Schroeder, J. A. Hugl1 and Hugl2 in mammary epithelial cells: polarity, proliferation, and differentiation. *PLoS One* **2012**, *7*, No. e47734.
(316) Edgell, C.-J. S.; BaSalamah, M. A.; Marr, H. S. Testican-1: a differentially expressed proteoglycan with protease inhibiting activities. *Int. Rev. Cytol.* **2004**, *236*, 101−122.
(317) Röll, S.; Seul, J.; Paulsson, M.; Hartmann, U. Testican-1 is dispensable for mouse development. *Matrix Biol.* **2006**, *25*, 373−381.
(318) Barrera-Ocampo, A.; Arlt, S.; Matschke, J.; Hartmann, U.; Puig, B.; Ferrer, I.; Zürbig, P.; Glatzel, M.; Sepulveda-Falla, D.; Jahn, H. Amyloid-β precursor protein modulates the sorting of testican-1 and contributes to Its accumulation in brain tissue and cerebrospinal fluid from patients with Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **2016**, *75*, 903−916.
(319) Shenolikar, S.; Weinman, E. J. NHERF: targeting and trafficking membrane proteins. *Am. J. Physiol.: Renal Physiol.* **2001**, *280*, F389−F395.
(320) Weinman, E. J.; Hall, R. A.; Friedman, P. A.; Liu-Chen, L.-Y.; Shenolikar, S. The association of NHERF adaptor proteins with g protein-coupled receptors and receptor tyrosine kinases. *Annu. Rev. Physiol.* **2006**, *68*, 491−505.
(321) Kriz, V.; Korinek, V. Wnt, RSPO and hippo signalling in the intestine and intestinal stem cells. *Genes* **2018**, *9*, 20.
(322) Courtneidge, S. A.; Dhand, R.; Pilat, D.; Twamley, G. M.; Waterfield, M. D.; Roussel, M. F. Activation of Src family kinases by colony stimulating factor-1, and their association with its receptor. *EMBO J.* **1993**, *12*, 943−950.
(323) Xu, M.; Liu, Y.; Huang, Y.; Wang, J.; Yan, J.; Zhang, L.; Zhang, C. Re-exploring the core genes and modules in the human frontal cortex during chronological aging: insights from network-based analysis of transcriptomic studies. *Aging* **2018**, *10*, 2816−2831.
(324) Roberts, S. A.; Strande, N.; Burkhalter, M. D.; Strom, C.; Havener, J. M.; Hasty, P.; Ramsden, D. A. Ku is a 5′-dRP/AP lyase that excises nucleotide damage near broken ends. *Nature* **2010**, *464*, 1214−1217.
(325) Tuteja, N.; Tuteja, R.; Ochem, A.; Taneja, P.; Huang, N. W.; Simoncsits, A.; Susic, S.; Rahman, K.; Marusic, L.; Chen, J.; et al. Human DNA helicase II: a novel DNA unwinding enzyme identified as the Ku autoantigen. *EMBO J.* **1994**, *13*, 4991−5001.
(326) Stanescu, V. The small proteoglycans of cartilage matrix. *Semin. Arthritis Rheum.* **1990**, *20*, 51−64.
(327) Snow, A. D.; Mar, H.; Nochlin, D.; Kresse, H.; Wight, T. N. Peripheral distribution of dermatan sulfate proteoglycans (decorin) in amyloid-containing plaques and their presence in neurofibrillary tangles of Alzheimer's disease. *J. Histochem. Cytochem.* **1992**, *40*, 105−113.
(328) Lam, V.; Takechi, R.; Pallebage-Gamarallage, M. M. S.; Galloway, S.; Mamo, J. C. L. Colocalisation of plasma derived apo B lipoproteins with cerebral proteoglycans in a transgenic-amyloid model of Alzheimer's disease. *Neurosci. Lett.* **2011**, *492*, 160−164.
(329) Timpl, R. Structure and biological activity of basement membrane proteins. *Eur. J. Biochem.* **1989**, *180*, 487−502.
(330) Narindrasorasak, S.; Altman, R. A.; Gonzalez-DeWhitt, P.; Greenberg, B. D.; Kisilevsky, R. An interaction between basement membrane and Alzheimer amyloid precursor proteins suggests a role in the pathogenesis of Alzheimer's disease. *Lab. Invest.* **1995**, *72*, 272−282.
(331) Averaimo, S.; Milton, R. H.; Duchen, M. R.; Mazzanti, M. Chloride intracellular channel 1 (CLIC1): Sensor and effector during oxidative stress. *FEBS Lett.* **2010**, *584*, 2076−2084.
(332) Novarino, G.; Fabrizi, C.; Tonini, R.; Denti, M. A.; Malchiodi-Albedi, F.; Lauro, G. M.; Sacchetti, B.; Paradisi, S.; Ferroni, A.; Curmi, P. M.; Breit, S. N.; Mazzanti, M. Involvement of the intracellular ion channel CLIC1 in microglia mediated beta-amyloid-induced neurotoxicity. *J. Neurosci.* **2004**, *24*, 5322−5330.
(333) Sabha, B. H.; Alzahrani, F.; Almehdar, H. A.; Uversky, V. N.; Redwan, E. M. Disorder in milk proteins: lactadherin multifunctionality and structure. *Curr. Protein Pept. Sci.* **2018**, *19*, 983−997.
(334) Neniskyte, U.; Brown, G. C. Lactadherin/MFG-E8 is essential for microglia-mediated neuronal loss and phagoptosis induced by amyloid β. *J. Neurochem.* **2013**, *126*, 312−317.
(335) Filmus, J.; Capurro, M.; Rast, J. Glypicans. *Genome Biol.* **2008**, *9*, 224.
(336) Lorente-Gea, L.; García, B.; Martín, C.; Ordiales, H.; García-Suárez, O.; Piña-Batista, K. M.; Merayo-Lloves, J.; Quirós, L. M.; Fernández-Vega, I. Heparan sulfate proteoglycans undergo differential expression alterations in Alzheimer disease brains. *J. Neuropathol. Exp. Neurol.* **2020**, *79*, 474−483.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056

(337) Berns, D. S.; DeNardo, L. A.; Pederick, D. T.; Luo, L. Teneurin-3 controls topographic circuit assembly in the hippocampus. *Nature* **2018**, *554*, 328−333.
(338) Antinucci, P.; Nikolaou, N.; Meyer, M. P.; Hindges, R. Teneurin-3 specifies morphological and functional connectivity of retinal ganglion cells in the vertebrate visual system. *Cell Rep.* **2013**, *5*, 582−592.
(339) Walker, L. C.; Overstreet, M. A.; Siddiqui, A.; De Paepe, A.; Ceylaner, G.; Malfait, F.; Symoens, S.; Atsawasuwan, P.; Yamauchi, M.; Ceylaner, S.; Bank, R. A.; Yeowell, H. N. A novel mutation in the lysyl hydroxylase 1 gene causes decreased lysyl hydroxylase activity in an Ehlers-Danlos VIA patient. *J. Invest. Dermatol.* **2005**, *124*, 914−918.
(340) Chong, M. S.; Goh, L. K.; Lim, W. S.; Chan, M.; Tay, L.; Chen, G.; Feng, L.; Ng, T. P.; Tan, C. H.; Lee, T. S. Gene expression profiling of peripheral blood leukocytes shows consistent longitudinal down-regulation of TOMM40 and upregulation of KIR2DL5A, PLOD1, and SLC2A8 among fast progressors in early Alzheimer's disease. *J. Alzheimer's Dis.* **2013**, *34*, 399−405.
(341) Martin, L.; Latypova, X.; Wilson, C. M.; Magnaudeix, A.; Perrin, M.-L.; Yardin, C.; Terro, F. Tau protein kinases: involvement in Alzheimer's disease. *Ageing Res. Rev.* **2013**, *12*, 289−309.
(342) Braithwaite, S. P.; Stock, J. B.; Lombroso, P. J.; Nairn, A. C. Protein phosphatases and Alzheimer's disease. *Prog. Mol. Biol. Transl. Sci.* **2012**, *106*, 343−379.
(343) Taymans, J.-M.; Baekelandt, V. Phosphatases of alpha-synuclein, LRRK2, and tau: important players in the phosphorylation-dependent pathology of Parkinsonism. *Front. Genet.* **2014**, *5*, 382.
(344) Xu, Y.; Chen, Y.; Zhang, P.; Jeffrey, P. D.; Shi, Y. Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation. *Mol. Cell* **2008**, *31*, 873−885.
(345) Sontag, E.; Luangpirom, A.; Hladik, C.; Mudrak, I.; Ogris, E.; Speciale, S.; White, C. L., 3rd Altered expression levels of the protein phosphatase 2A ABalphaC enzyme are associated with Alzheimer disease pathology. *J. Neuropathol. Exp. Neurol.* **2004**, *63*, 287−301.
(346) Steinhilb, M. L.; Dias-Santagata, D.; Mulkearns, E. E.; Shulman, J. M.; Biernat, J.; Mandelkow, E.-M.; Feany, M. B. S/P and T/P phosphorylation is critical for tau neurotoxicity in Drosophila. *J. Neurosci. Res.* **2007**, *85*, 1271−1278.
(347) Wu, J. M.; Chen, Y. P.; An, S. J.; Perruccio, L.; Abdelghany, M.; Carter, T. H. Phosphorylation of protein tau by double-stranded DNA-dependent protein kinase. *Biochem. Biophys. Res. Commun.* **1993**, *193*, 13−18.
(348) Yin, L.; Zeng, Y.; Xiao, Y.; Chen, Y.; Shen, H.; Dong, J. Cyclin-dependent kinase 1-mediated phosphorylation of SET at serine 7 is essential for its oncogenic activity. *Cell Death Dis.* **2019**, *10*, 385.
(349) Zhang, Q.; Xia, Y.; Wang, Y.; Shentu, Y.; Zeng, K.; Mahaman, Y. A. R.; Huang, F.; Wu, M.; Ke, D.; Wang, Q.; Zhang, B.; Liu, R.; Wang, J. Z.; Ye, K.; Wang, X. CK2 phosphorylating I2(PP2A)/SET mediates tau pathology and cognitive impairment. *Front. Mol. Neurosci.* **2018**, *11*, 146.
(350) Lee, G. Tau and src family tyrosine kinases. *Biochim. Biophys. Acta* **2005**, *1739*, 323−330.
(351) Lee, G.; Thangavel, R.; Sharma, V. M.; Litersky, J. M.; Bhaskar, K.; Fang, S. M.; Andreadis, A.; Van Hoesen, G.; Ksiezak-Reding, H. Phosphorylation of tau by fyn: implications for Alzheimer's disease. *J. Neurosci.* **2004**, *24*, 2304−2312.
(352) Ferrer, I.; Blanco, R.; Carmona, M.; Ribera, R.; Goutan, E.; Puig, B.; Rey, M. J.; Cardozo, A.; Viñals, F.; Ribalta, T. Phosphorylated map kinase (ERK1, ERK2) expression is associated with early tau deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Brain Pathol.* **2001**, *11*, 144−158.
(353) Qi, H.; Prabakaran, S.; Cantrelle, F.-X.; Chambraud, B.; Gunawardena, J.; Lippens, G.; Landrieu, I. Characterization of Neuronal Tau Protein as a Target of Extracellular Signal-regulated Kinase. *J. Biol. Chem.* **2016**, *291*, 7742−7753.
(354) Wang, J.-Z.; Xia, Y. Y.; Grundke-Iqbal, I.; Iqbal, K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. *J. Alzheimer's Dis.* **2013**, *33*, S123−S139.
(355) Ballatore, C.; Lee, V. M.-Y.; Trojanowski, J. Q. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* **2007**, *8*, 663−672.
(356) De Strooper, B.; Iwatsubo, T.; Wolfe, M. S. Presenilins and γ-secretase: structure, function, and role in Alzheimer Disease. *Cold Spring Harbor Perspect. Med.* **2012**, *2*, a006304.
(357) Wolfe, M. S. Processive proteolysis by γ-secretase and the mechanism of Alzheimer's disease. *Biol. Chem.* **2012**, *393*, 899−905.

https://doi.org/10.1021/acsomega.1c00660
ACS Omega 2021, 6, 13033−13056