Resource

Cell Reports

Amyloid Accumulation Drives Proteome-wide Alterations in Mouse Models of Alzheimer’s Disease-like Pathology

Graphical Abstract

Highlights

- Proteomics of mouse brains with AD-like pathology reveals stark proteome remodeling
- Dysregulation of ApoE levels associated with Aβ clearance rather than production
- Co-expression analysis found distinctly impaired synapse and mitochondria modules
- AD AMPARs have reduced levels of TARPγ-2, which may compromise synapses in AD

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In Brief

Savas et al. achieve proteome-wide measurements of protein abundance in brain extracts from three mouse models of AD-like pathology. These analyses revealed Aβ, brain region, and age-dependent alteration of protein levels of several functional categories. This resource provides a global protein expression atlas of AD mouse model brain proteomes.

Data and Software Availability

PXD005595

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Amyloid Accumulation Drives Proteome-wide Alterations in Mouse Models of Alzheimer’s Disease-like Pathology

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SUMMARY

Amyloid beta (Aβ) peptides impair multiple cellular pathways and play a causative role in Alzheimer’s disease (AD) pathology, but how the brain proteome is remodeled by this process is unknown. To identify protein networks associated with AD-like pathology, we performed global quantitative proteomic analysis in three mouse models at young and old ages. Our analysis revealed a robust increase in Apolipoprotein E (ApoE) levels in nearly all brain regions with increased Aβ levels. Taken together with prior findings on ApoE driving Aβ accumulation, this analysis points to a pathological dysregulation of the ApoE-Aβ axis. We also found dysregulation of protein networks involved in excitatory synaptic transmission. Analysis of the AMPA receptor (AMPAR) complex revealed specific loss of TARPγ-2, a key AMPAR-trafficking protein. Expression of TARPγ-2 in hAPP transgenic mice restored AMPA currents. This proteomic database represents a resource for the identification of protein alterations responsible for AD.

INTRODUCTION

Alzheimer’s disease (AD) is a progressive brain disorder that is the leading cause of dementia in adults, resulting in impaired memory and cognition. Pathological hallmarks include amyloid plaques, neurofibrillary tangles, astrogliosis, and changes in brain vasculature, which culminate in neurodegeneration. Abundant evidence shows AD brain pathology is complex and represents feedback and feedforward responses in multiple cell types (De Strooper and Karran, 2016). Many mechanisms have been proposed to explain AD, but a unifying and universally accepted description has yet to be achieved. There is near unanimous agreement that β-amyloid (Aβ) peptides generated by proteolytic processing of amyloid precursor protein (APP) represent a key toxic species. The amyloid cascade hypothesis has provided a useful theoretical framework for the basis of AD (Selkoe and Hardy, 2016), yet current evidence suggests a more complicated pathological etiology (Herrup, 2015). One major challenge impeding our understanding of AD pathology is that the molecular perturbations vary based on the stage of disease and brain region. Furthermore, even individuals with mutations in APP or Presenilin-1 (PS1) take decades to develop AD, suggesting that long incubation periods or aging is required. Several molecular pathways, including impaired synaptic transmission, hampered protein degradation dynamics, defective mitochondrial function, and inflammatory responses, all play important roles in AD pathogenesis (Akiyama et al., 2000; Lin and Beal, 2006; Mori et al., 1987; Sheng et al., 2012).

Dynamic changes in protein abundances represent key cellular responses to cope with cellular insults. However, broad toxicity, such as the accumulation of Aβ peptides, likely affects many proteins and pathways. We hypothesized this must ultimately result in proteome remodeling (Powers et al., 2009). For example, proteins physically associated with Aβ plaques accumulate while proteins localized to synapses have reduced levels in AD (Lassmann et al., 1992). To understand the effects of Aβ peptides on the brain proteome, we performed quantitative proteomic analysis of extracts from the frontal cortex (FC),
hippocampus (HIP), and cerebellum (CB) of transgenic mice expressing mutated hAPP or hAPP/PS1 (Borchelt et al., 1996; Mucke et al., 2000). While these models have provided insight into AD pathology, they are limited by the overexpression of APP fragments outside Aβ in non-relevant cells (Saito et al., 2014). We also performed proteomic analysis of BRI-Aβ42 mouse brain extracts overexpressing Aβ42 without additional APP fragments (McGowan et al., 2005). To temporally resolve changes in protein abundance, we performed our analysis at ages before and after plaques form to find early and potentially reversible alterations.

We report an AD proteomic resource of 18,882 quantified proteins (10,288 genes) from mouse model brain region extracts. To delineate proximal effects of Aβ on the brain proteome, we determined the level of Aβ and amyloid plaques, and we performed our analyses at asymptomatic and symptomatric time points, based on previous reports (Dodart et al., 1999; Lalonde et al., 2005). We observed an age-dependent increase in proteome remodeling, region-specific patterns, and we found many proteins genetically linked to late-onset AD (LOAD), such as ApoE, as significantly altered. ApoE plays a key role in regulating Aβ metabolism and is co-deposited in plaques (Liu et al., 2013; Namba et al., 1991). ApoE also seems to play a role in Aβ aggregation (Bales et al., 1997). However, how ApoE levels are altered in AD remains unclear (Gupta et al., 2011; Hesse et al., 2000; Kuo et al., 2000; Liu et al., 2013). We found ApoE significantly increased in all three models and only in those datasets with increased Aβ levels. Overall, we identified a small panel of proteins significantly altered in multiple models. Co-expression analysis revealed two distinct modules (MEs) involved with synaptic transmission in the HIP and two MEs related to mitochondria in the FC. Biochemical purification of AMPARs, which represented core ME proteins, showed a specific loss of TARPγ-2 (known as stargazin). To assess if our proteomics can lead to important conclusions, we tested and found that correcting TARPγ-2 levels can restore glutamatergic synaptic transmission in vivo.

**RESULTS**

**Remodeled Proteomes in hAPP and hAPP/PS1 Brains**

To determine how Aβ peptide toxicity affects the brain proteome, we developed a quantitative proteomic strategy using nitrogen-15 (15N)-labeled mice (McClatchy et al., 2007; Wu et al., 2004). The 15N heavy brains served as internal standards for proteome-wide quantitation of transgenic AD mouse models (Tg-AD) relative to non-transgenic (Non-Tg) littersmates that were unlabeled and light. The mixed light and heavy proteins were digested to peptides, separated using multi-dimensional chromatography, and analyzed by tandem mass spectrometry (MS/MS) (Figure 1A). We compared the FC, HIP, and CB between age-matched Tg-AD or Non-Tg littersmates using the same whole-brain heavy reference proteome, and we performed our analysis at presumptively asymptomatic (3-month) and symptomatic (12-month) time points to identify age-dependent changes in protein abundance. In total, our MS analysis provided relative quantification for >3,800 different proteins in each dataset of three biological replicates per group (Figure 1B).

By analyzing regional brain proteomes of two Tg-AD models with different degrees of pathology, we identified unknown AD pathological mechanisms and confirmed several previously reported. To identify proximal Aβ targets, we first analyzed mice overexpressing APPswell (hAPP) at moderate levels. The hAPP mice have hampered synaptic transmission at 2–4 months, but they do not develop plaques before 10 months (Hsia et al., 1999; Mucke et al., 2000). We also analyzed a double Tg-AD mouse model overexpressing APPswel/PSEN1(A246E) (hAPP/PS1) (Borchelt et al., 1996). hAPP/PS1 mice have plaques, dystrophic neurites, and behavioral deficits by 9 months (Wang et al., 2003). To document the pathological state of our hAPP mice at 3 months, we performed Aβ1–42 ELISA, and we found a significant, ~50%, increase in the level of Aβ1–42 in the FC and HIP, but not the CB (Figure S1A). At 12 months, Aβ1–42 levels in the FC and HIP were more significantly increased compared to the Non-Tg littersmates, while the CB did not possess significantly increased Aβ1–42 levels. The absence of a significant increase in Aβ1–42 levels in the CB of the hAPP provided a negative control for proteins sensitive to APP fragments outside Aβ. Aβ1–42 ELISA showed a large and significant increase in the Aβ1–42 level in all three brain regions at 3 and 12 months in hAPP/PS1 (Figure S1B). We analyzed the amyloid plaque load in the HIP by Congo red and Thioflavin S staining in both models, and we confirmed 3-month hAPP mice lacked plaques, the number of plaques increased with age in both models, and the plaque load even at 12 months was moderate for both models (Figures S1C–S1P). Based on these results and previous behavioral analyses, we consider the HIP and FC datasets from 3-month hAPP mice to best represent early stages of pathology.

Our analysis revealed a global imbalance in protein levels: about 5% of the quantified proteins had a change of >50% at 3 months and an even larger percentage altered >50% at 12 months in 11 of 12 of our brain region–matched comparisons (Figures 1C and 1D). These measurements were made from, on average, ≥30 different peptides per protein, and >28% of proteins were quantified in 50% of our samples (Figures S1Q and S1R). The total number of significantly altered proteins increased from 3 to 12 months in all brain regions and models. Overall, the HIP possessed the greatest number of significantly (false discovery rate [FDR]-adjusted [adj] p value < 0.05) altered proteins (Figure 1E). We compared the levels of all the significantly altered proteins in the same Tg-AD model and brain region between the 3- and 12-month datasets. For FC of hAPP, but not hAPP/PS1, mice, we found that the difference between the average protein fold change at 3–12 months was significantly increased (−0.0621 ± 0.389 versus 0.214 ± 0.320, mean ± SD; p value = 1.34E−8), consistent with an accumulation of protein in the context of more severe pathology (Figures S1S and S1T). For the HIP, we found a significant increase between 3 and 12 months in hAPP/PS1 (−0.266 ± 0.619 versus −0.155 ± 0.604, mean ± SD; p value = 0.0240). For the CB, we found a significant increase in the hAPP/PS1 model (−0.00866 ± 0.554 versus 0.209 + 0.489, mean ± SD; p value = 3.34E−4).

We determined the number of significantly altered proteins in each brain region, and we identified those altered in multiple
Figure 1. Quantitative MS Analysis of Tg-AD Brains

(A) Experimental workflow. $^{14}$N ratio $^{15}$N is calculated and normalized to controls.

(B) Quantified protein counts for 12 datasets.

(C) Brain region proteome remodeling of hAPP at 3 and 12 months.

(D) Brain region proteome remodeling of hAPP/PS1 at 3 and 12 months.

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brain regions. Each brain region in both models had hundreds of significantly altered proteins (Figures 1F and 1G; Tables S1 and S2). Bioinformatics to determine the cellular compartments to which the altered proteins localized showed they reside in a wide range of compartments (Figure S1U). We found 42 proteins significantly altered in the FC of both models (Figure 1H; Table S3), and similarly for the HIP we found 184 (Figure 1I; Table S3). To determine how many of our altered proteins have yet to be reported, we performed a literature search, and we found 17 FC and 92 HIP unreported in AD or AD models (Table S3). We next used hierarchical clustering to compare protein profiles, and we found region-specific patterns (Figures S2A–S2C). Principal component analysis showed the 3- and 12-month FC proteomes of both models are most similar to each other, CB both time points for hAPP or hAPP/PS1 were most similar, and HIP datasets were least similar between the models and time points (Figures S2D–S2F).

Connecting Proteins to Genes that Predispose for AD

We tested the connection between genes linked to LOAD and those proteins we found with altered abundance (Bertram et al., 2007). Our screen identified 23 significantly altered proteins genetically linked to LOAD (Figure 2A). APOE and GOT1 were altered in six datasets, GSTM1 in five, and TF in four. We extracted the model, time point, and brain region where these alterations occurred. Interestingly, we found that APOE was significantly altered only in those datasets with significantly increased Aβ levels. Other proteins, such as BIN1 and CLU, were only altered in hAPP/PS1 mice (Figure 2B). These findings show that a large number of genes genetically linked to AD also have altered protein products in mouse model brains of AD-like pathology.

We generated rank-ordered summary confidence plots for the significant proteins (FDR-adj p value < 0.05). In hAPP mice, α-spectrin 2, which is known to be altered in AD, was the only protein identified as significantly altered in all four datasets with elevated Aβ (Figure 3A). We found 27 significantly altered proteins in three of four hAPP datasets, 125 in two, and 691 in one. Similarly, only microtubule-associated protein 2 (Map2) was significantly altered in all six hAPP/PS1 datasets, three proteins in five, 20 in four, 36 in three, 214 in two, and 469 in one (Figure S2G). Next, we compared the top 30 proteins (~4%) between the models, and we found nine proteins met this criterion in both models. These mostly confidently altered proteins included ApoE, Glial fibrillary acidic protein (GFAP), Solute Carrier Family 12 Member 5 (Slc12a5), neurofilament medium peptide (Nefm), clathrin heavy chain 1, Ankyrin 2 (Ank2), spectrin I-III (Spnb3), aconitase 2 (Ac02), and dynnein cytoplasmic 1 heavy chain 1 (Dynch1). These results are consistent with previous findings that the structural proteins Nefm and Spnb3 are altered in AD brains (Ciavardelli et al., 2010; Masliah et al., 1990). Slc12a5, a major chloride extruder, shifts the effect of GABA, is linked to epilepsy, and may be relevant in AD (Lagostena et al., 2010; Palop and Mucke, 2009). Ank2, GFAP, Aco2, Cnp, and Dynch11 have also been implicated in AD (Burbaeva et al., 2005; Kamphuis et al., 2014; Lazarov et al., 2002; Mangialasche et al., 2015; Silva et al., 2013; Soler-Lopez et al., 2011; Wang et al., 2003).

Our finding that ApoE was among the most confidently altered proteins moved us to examine its level relative to APP and Aβ. In 3-month hAPP mice, APP was increased ~2-fold in all brain regions and more so at 12 months (Figure 3B). For hAPP/PS1, APP levels were slightly higher at both time points and in all brain regions. When we examined ApoE, which in mice is required for Aβ pathologies (Bales et al., 1997), we found its levels significantly increased in the FC and HIP at 12 months in hAPP. In hAPP/PS1, ApoE had significantly increased levels at both time points in the FC and at 12-month HIP and CB (Figure 3C). Interestingly, ApoE levels were higher exclusively in those brain regions with elevated Aβ levels, since it was unchanged at both time points in the CB of hAPP (Figure S1A; Figure 3B). Altogether, ApoE was increased in five of the five possible 12-month datasets with the highest Aβ levels.

To specifically explore Aβ42-dependent proteome remodeling, we performed label-free quantitative proteomic analysis of the FC, HIP, and CB extracts of BRI-Aβ42 mice at 3 and 12 months (Figure S3A) (McGowan et al., 2005; Park et al., 2008). BRI-Aβ42 was only detected in mice carrying the transgene and overall levels increased over time (Figure S3B). Our BRI-Aβ42 proteomics quantified >4,000 proteins in each dataset and identified hundreds of proteins with significantly altered levels (Figure S3C; Table S4). As expected, we found no significant changes in APP levels, but we did detect a significant increase in ApoE. We confirmed increased ApoE levels in BRI-Aβ42 FC and HIP by western blot (WB) (Figure 3D). Altogether, these results strongly suggest that Aβ42 accumulation leads to a concomitant increase in ApoE in vivo, which likely has unexpected and important pathological consequences.

To test the confidence of our MS-based protein measurements, we performed semiquantitative WB analyses of six proteins. Indeed, these results unanimously confirmed proteins with both increased and decreased levels (Figures S3D–S3H). Moreover, BRI-Aβ42 proteomic analysis also provided additional independent confirmation of many proteins found altered in hAPP and hAPP/PS1. Altogether, these results show that our proteomic analysis possesses sufficient analytical power to reveal confident changes in protein levels relevant to AD pathology.

**Protein Co-expression Network Analysis of Tg-AD Brains**

We hypothesized that consensus weighted gene/protein co-expression analysis (WGCNA), which identifies correlated patterns of protein levels in individual brain regions across the two time points and models, would allow us to delineate groups of co-regulated proteins (Langfelder and Horvath, 2007). We

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(E) Bar graphs of age-dependent increase of the number of significantly altered proteins (FDR-adj p value < 0.05).
(F and G) Venn diagrams of significantly altered proteins in hAPP (F) black outline, CB lacking increased Aβ levels, and (G) hAPP/PS1.
(H) FC summary for both models.
(I) HIP summary for both models. (B and C) Solid bars are proteins with a >50% change in ratio 2. See also Figures S1 and S6 and Tables S1, S2, S3, and S6.
were able to generate consensus networks across genotypes and time points for both the FC and HIP datasets, but not the CB one. We identified 25 FC and 10 HIP MEs across 30 FC and 34 HIP datasets (Figure 4A; Figure S4A; Table S5). WGCNA reduced thousands of protein measurements into 35 MEs that represent core proteome-remodeling programs responding to Aβ (Zhang et al., 2013). Direct comparison of the two topological overlap matrices from the Tg-AD or Non-Tg datasets showed that Aβ significantly (Z statistics p value < 1E–04) remodels several distinct molecular interaction networks (Figure 4B; Figure S4B). To test if the MEs were enriched for distinct functions (Zhang et al., 2013), we subjected each ME to gene ontology cell component (GO: CC) enrichment analysis. Indeed, we identified significant GO assignments for seven of 10 HIP

**Figure 2. Altered Protein Abundance of Genes Linked to LOAD**

(A) Significant (FDR-adj p value < 0.05) protein changes mapped to 153 genes previously linked to LOAD (dash, not quantified).

(B) Brain region(s) and age(s) with significant changes. Red, FC; orange, HIP; teal, CB. #hAPP CB that lacks Aβ accumulation;,IDE1 is shown twice because of sequence variations; +A2M+ was not identified, related alpha-2-macroglobulin protein Pzp is reported; #CYP2D6 is not in the mouse genome, we measured related Cyp2d22.
Figure 3. Most Significantly Altered Proteins from Brain Regions with Elevated Aβ

(A) Summary confidence index (Experimental Procedures) plot for all significantly (FDR-adj p value < 0.05) altered proteins in the four datasets with significantly increased Aβ levels in hAPP. Proteins with small p values >0 but <3.42E−13 are graphed as 1E−13 (see Table S1); proteins in the top 30 of both models are in bold.

(B) APP levels in Tg-AD datasets revealed a significant increase compared to Non-Tg controls (n = 8–32 peptides).

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MEs. These included myelin sheath, regulation of actin polymerization, mitochondrion, synapse, adherens junction, and basal laminae (adj p values $6.41\times 10^{-4}$–$1.96\times 10^{-17}$) (Table S5), consistent with previous findings (Bartzokis et al., 2007; Terry et al., 1991; Yamaguchi et al., 1992).

By decomposing our MEs into individual time points, we identified shared and unique protein expression patterns between the two models (Zhang and Horvath, 2005), and by using a meta-analysis of correlations of ME eigengenproteins (summary expression profile) (Langfelder et al., 2016), we measured the relationship between MEs and each model at two time points. We found that six HIP and two FC MEs had significant (Z statistic and corresponding meta-analysis p value < 0.05) differences in abundance for each time point and model (Figure 4C; Figures S4C and S4D). We found both synapse MEs (ME1 and ME3) in the HIP to have reduced protein levels. However, the myelin sheath (ME2) had only slightly altered expression patterns, except at 12-month hAPP that showed a significant increase (Figure 4C). Bioinformatics of the FC MEs resulted in significant but less dramatic eigengenproteins (Figure S4D). Interestingly, only myelin sheath and mitochondrion MEs were found in both the FC and HIP (Table S5). The strongest associations were in the HIP. Noticeably, in hAPP mice, the synapse MEs (1 and 3) were significantly decreased at 3 months (Figure 4C), which is before Aβ plaques form (Figures S1D and S1K), consistent with previous reports that synaptic deficits are caused by soluble Aβ peptides (Hsia et al., 1999). In contrast, the hAPP/PS1 synapse MEs were only slightly reduced at 3 months (Figure 4C), but Aβ plaques were already formed (Figures S1E and S1L). The HIP myelin sheath (ME2) also provided a contrasting view of the two models (Figure 4C, Figure S4D). In hAPP there were only slight differences in eigenprotein levels at both ages, while in hAPP/PS1 we observed a substantial decrease at 3 months and a more dramatic reduction at 12 months. Meta-analysis statistics allowed us to rank each ME in two Tg-AD MEs, and they highlighted related but distinct changes in the proteomes of the HIP and FC.

**Refining MEs to Untangle Pathology**

The interpretation of affected protein pathway perturbations in Tg-AD HIP and FC depends on confident ME characterizations. To identify the most confident groups of proteins in our datasets, we first considered the most significant HIP MEs. ME2 and ME4 were the among the most significant and highly populated MEs, which included myelin sheath, regulation of actin polymerization, mitochondrion, synapse, adherens junction, and basal laminae. These included myelin sheath proteins (adj p value = $2.22\times 10^{-15}$; n = 213) and mitochondria, respectively (adj p value = $1.57\times 10^{-12}$; n = 117). Since these proteins are known to be impaired in AD, it confirms our analysis strategy (Bartzokis, 2004; Hirai et al., 2001).

We focused on synapse-related HIP ME1 and ME3, and we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to test if these MEs are significantly enriched in proteins with shared functional assignments (Kanehisa and Goto, 2000). Interestingly, for ME1, we found long-term potentiation (LTP) to be the predominant assignment (Figure 5A). This result is consistent with previous reports showing that HIP LTP impairments may represent the basis of hampered memory formation in AD patients (Chapman et al., 1999). ME3 was enriched for synaptic vesicle cycle. Consistently, when we used the GO biological processes (GO: BP) database (von Mering et al., 2005) to examine ME1 and ME3, we found these MEs were significantly enriched for synapse plasticity/actin organization and modulation of synaptic transmission/vesicle transport processes (Figure 5B). These results suggest that synaptic transmission may be altered in HIP by multiple mechanisms in AD-like pathology. To confirm this strategy, we next explored if our MEs relate to previously characterized AD pathology in the FC. We first used KEGG to test if our MEs were enriched for proteins assigned to the AD pathway. We found that ME1, ME2, and ME4 were significantly enriched in proteins previously associated with AD. To extend these findings, we subjected these three MEs to GO: BP analysis. This analysis showed that these BP terms, namely, ME1, excitatory postsynaptic potential; ME2, oxidative-reduction process; and ME4, hydrogen ion transporter activity, were significantly enriched (Figure S4E), and all of these pathways are linked to AD pathology.

We examined the expression pattern of the individual proteins of the most significant MEs, which provided us with insight into the core protein network perturbations. First, we inspected the hippocampal LTP module (ME1), which contained proteins such as GluA2 and GluN2AB (Figure 5C). Overall, these proteins showed progressive and significant reduction in levels in hAPP, but they were mostly upregulated at 3 and downregulated at 12 months in hAPP/PS1. For the FC and CB in both models, proteins in this ME generally had increased levels. We obtained similar results for the ME1 proteins associated with actin organization, a key process for synapse remodeling (Figure 5D). In general, the proteins comprising the second HIP synapse-related module (ME3) showed a more robust reduction in levels in hAPP compared to hAPP/PS1 (Figure 5E). ME2 and ME4, from the FC datasets, were involved with mitochondria, and they had mostly reduced expression in the HIP but increased expression in the FC and CB (Figures S4F and S4G). Finally, we performed network analysis based on the continuous measure of membership and connectivity based on WGCNA to determine the top 50 hub proteins in HIP ME1 and ME3 (Langfelder et al., 2016). These hub protein alterations were similar to the eigengenproteins of these MEs, and they largely reflected function of corresponding MEs. Several core hub proteins identified in ME1, including Wasf1 and Nckap1 (Figures 5F and 5G), play critical roles regulating spine structure and have been implicated in AD (Ceglia et al., 2015; Govek et al., 2005; Kim et al., 2006; Yamamoto et al., 2001).

**AMPARs as Core Synaptic Complexes Altered in AD**

WGCNA identified the core AMPAR subunit GluA2 as a top hub gene of ME1 in the HIP. Additional GluA2 regulatory proteins, such as Nsf and Camk2b (Braithwaite et al., 2002; Kristensen et al., 2004) were significantly increased at 12 months in the FC and HIP, but not the CB. In hAPP/PS1, ApoE levels are significantly increased at 3 months in the FC, and at 12 mo in all three brain regions (n = 17–39 peptides).
et al., 2011; Shanks et al., 2012), were also identified as hub genes (Figure 5F). This finding suggests that GluA2-containing protein networks may represent key potential targets for AD therapeutic intervention. However, GluA2 plays essential synaptic functions in learning and memory, and, thus, the possibility of directly manipulating it is problematic. Thus, we focused on finding key components of AMPAR complexes contributing in AD. To identify the earliest alterations, we examined the levels of AMPAR subunits in the HIP and FC datasets from hAPP at 3 and 12 months. At 3 months, the only significantly altered

**Figure 4. Consensus Protein Co-expression Network Analysis of Tg-AD Brains**  
(A) HIP protein clustering trees from both Tg-AD models and time points (top). Each ME is in non-gray (middle). The two rows of heatmaps below show the association of individual proteins with Q for Non-Tg or Tg-AD groups (bottom). Blue and red shading indicates proteins with reduced or increased expression, respectively, with increasing Q. Black bars show altered protein groups.  
(B) Individual topological overlap matrices of significantly (Z statistics p value < 1E−04) differentially connected MEs in the HIP between Tg-AD and Non-Tg datasets in both models. ME assignment based on most significant GO assignment (number for ME1, see Table S5). Shown are all MEs with significant p values (<1E−4).  
(C) Summary expression value (eigenprotein) from the indicated HIP datasets. All Non-Tg/Tg-AD comparisons have a p value < 0.05 calculated from Z statistics. Bar graphs show mean ± SEM. See also Figure S4 and Table S5.
AMPAR proteins were TARP\(\gamma\)-2 in the HIP (1.253 ± 0.162 versus 0.847 ± 0.231, mean ± SD; \(p\) value = 4.29E-04) and TARP\(\gamma\)-3 in the FC (1.165 ± 0.0354 versus 2.380 ± 0.139, mean ± SD; \(p\) value = 7.21E-04) (Figure S5A). This result contrasted results at 12 months that showed both GluA1 and 2 significantly down-regulated in the HIP (Figure S5B). Based on the key effects of TARP\(\gamma\)-2/3 levels are altered in the context of intact AMPARs in AD model brains. Indeed, AMPARs immunopurified from hAPP brains with anti-GluA2 antibodies analyzed by WB and MS showed decreased levels of TARP\(\gamma\)-2/3, strongly suggesting AMPARs in AD model brains have altered interactions with

Figure 5. Synaptic Proteins in ME1 and ME3 Have Specific Changes in Protein Levels
(A) HIP ME1 and ME3 are enriched for proteins with distinct synaptic functions based on KEGG. Scatterplot shows enrichment FDR (y axis) versus the number of proteins per KEGG pathway (x axis).
(B) HIP ME1 and ME3 are enriched in proteins with distinct synaptic functions based on GO: BP.
(C–E) Protein expression matrix (ratio 2) for the indicated proteins in ME1 KEGG (C), ME1 GO: BP (D), ME3 GO: BP (E) for both Tg-AD models and time points. Black bars show datasets with contrasting protein expression patterns. Below bar graph based on hypergeometric enrichment \(p\) values, dotted line indicates an adj \(p\) value < 0.05.
(F and G) Network of top 50 hub proteins in ME1 (F) and ME3 (G) from the HIP (8 most connected, large; next 16, medium; remaining 26, small). (A and B) Most significant KEGG and GO: BP terms for ME1 and ME3 are underlined. See also Table S5.
TARP proteins (Figure 6A; Figure S5C). To test if AMPAR complexes are remodeled in AD human cortex, we immunopurified AMPARs, analyzed the precipitates with MS, and confirmed reduced TARPγ-2 levels in the context of human AD pathology (Figure 6B).

Figure 6. AMPAR Complexes Are Hub Proteins Altered in AD

(A) WBs of AMPAR complexes from hAPP or Non-Tg brains. Less TARPγ-2 was recovered in the hAPP brain compared to Non-Tg after normalizing to the recovery of GluA2 (1.0 versus 0.63).

(B) Semiquantitative MS analysis of AMPAR complexes from AD or healthy control brains show AMPARs in AD cortex have reduced levels of TARPγ-2 associated normalized to GluA2 (healthy cortex = 0.082 ± 0.011 versus AD cortex = 0.035 ± 0.0058). Bar shows mean ± SD for AD brains (n = 4 patients) and controls (n = 2). White bars, controls; solid bars, AD.

(C) Strategy to test if injected LVs expressing FLAG-TARPγ-2-IRES-GFP can rescue AMPA defect in hAPP mice.

(D) Image of simultaneously recorded uninfected and infected CA1 cells in a hAPP HIP brain slice.

(E) AMPA currents are not significantly different in Non-Tg mice (TARPγ-2, 70.248 ± 11.479; uninfected, 59.896 ± 11.020; n = 7; p value = 0.379).

(F) AMPA currents are significantly increased in TARPγ-2-expressing cells in hAPP mice (TARPγ-2, 134.67 ± 27.812 pA; uninfected, 48.331 pA; n = 7; p value = 0.00385).

(G) NMDA currents are not significantly different in Non-Tg mice (TARPγ-2, 114.23 ± 22.5515; uninfected, 121.669 ± 29.973; n = 9; p value = 0.7089).

(H) NMDA currents are not significantly different in hAPP mice (TARPγ-2, 112.148 ± 16.537; uninfected, 88.666 ± 0.301; n = 5; p value = 0.3016). (E–H) Mean ± SEM. **p value < 0.01 and ***p < 0.001 by Student’s t test. See also Figure S5.

Impaired excitatory synaptic transmission and AMPAR dysfunction have been implicated in AD HIP, however, the details are unclear (Hsia et al., 1999; Hsieh et al., 2006). Consistently, we observed a significant reduction in the strength of evoked field potentials in hAPP HIP slices relative to control littermates (Figures S5D–S5F). We hypothesized that the reduction of TARPγ-2 in AD could lead to the destabilization of synaptic AMPARs, since TARPγ-2 anchors AMPAR subunits to PSD-95 and this interaction can help deliver AMPARs to synapses (Schnell et al., 2002). We wondered if TARPγ-2 might affect basal synaptic transmission in hAPP mice. To test this, we injected lentiviruses (LVs) that overexpressed TARPγ-2 into CA1 in Tg-AD and Non-Tg littermates (Figure 6C and 6D; Figure S5G). Consistent with previous findings (Schnell et al., 2002), we found overexpression of TARPγ-2 had no effect on basal AMPAR excitatory postsynaptic currents (EPSCs) in Non-Tg animals, but we found that TARPγ-2 overexpression resulted in a significant increase in AMPAR EPSCs in hAPP mice.
(Figures 6E and 6F). Additionally, we observed no effects on NMDA receptor (NMDAR) EPSCs (Figures 6G and 6H). These results support TARPγ-2 as a potential target for treatment in AD.

**DISCUSSION**

Proteomic characterization of AD model brains shows elevated Aβ levels cause proteome remodeling in multiple cell types and pathways. Most ApoE is produced by glia and is likely involved in multiple mechanisms important to AD (Holtzman et al., 2012). Our proteomics showed that ApoE levels are increased in brain regions with high levels of Aβ, but not in the CB of hAPP, suggesting that it is primarily involved in Aβ clearance rather than production. This result may contrast previous results showing that the APP intracellular domain can drive ApoE gene transcription, and it may highlight differences between AD models and the complexity of ApoE biology (Liu et al., 2007). Recently, an allele-specific role of ApoE in regulating complement C1q protein had an important role in regulating the rate of synaptic pruning (Chung et al., 2016). It is possible that the synaptic protein defects we identified could be due to altered ApoE levels at tripartite synapses.

Many previous reports have described impaired synaptic transmission and an overall reduction in the number of synapses in the HIP of AD models and patients before the appearance of plaques (Shankar et al., 2008; Terry et al., 1991). We confirmed distinct components of excitatory synapses are altered prior to plaque formation. WGCNA revealed two synaptic MEs with reduced protein levels at early and late time points. ME1 is enriched in proteins involved with LTP and actin cyto-skeleton, both of which have been implicated as playing key roles in AD (Sheng et al., 2012). ME3 is enriched in proteins involved with pre-synaptic functions. Among the top 50 hub proteins in ME1 were AMPAR and NMDAR subunits. Examination of AMPAR components showed a pioneering reduction in protein abundance in the auxiliary subunits TARPγ-2/3. It was not until the later time point that GluA1 and 2 had significantly reduced levels in the HIP of hAPP mice. AMPAR density was shown reduced at perforated synapses and synapses onto parvalbumin (PV)-positive neurons in the CA1 region of TARPγ-2 knockout (KO) mice; interestingly, we found significantly reduced levels of PV in the HIP of both models (Yamasaki et al., 2016). Furthermore, pore-forming GluA3 and -4 subunits were not significantly altered. In other AD models, they have been shown to be important Aβ substrates (Reinders et al., 2016). AMPAR downscaling and removal have been reported in multiple AD models, but the precise mechanism(s) have remained vague (Chang et al., 2006; Hsia et al., 1999; Hsieh et al., 2006). These findings motivated us to test whether restoring TARPγ-2 levels could rescue AMPA defects in hAPP mice. Indeed, overexpression of TARPγ-2 caused a large increase in AMPAR-, but not NMDAR-, mediated currents. Our results suggest that GluA1/2-containing AMPARs that are known to be enriched with TARPγ-2/3 may represent key Aβ targets due to their restricted expression patterns at the affected synapses (Schwenk et al., 2012). Finally, while both NMDARs and AMPARs play key roles in the establishment and maintenance of LTP, our results highlight a potential mechanism by which reduced expression of TARPγ-2 could result in impaired delivery of AMPARs to spines and, thus, compromise LTP in AD patients. TARP subtypes have been shown to differentially influence AMPAR gating (Milestone et al., 2007), and TARPγ-2 may specifically represent a therapeutic target to restore cognitive function in AD patients.

We determined protein substrates and mechanisms of Aβ toxicity to dissect the amyloid cascade hypothesis by using three complementary mouse models of AD-like pathology (Table S6). To maximize the accessibility of our results, we have generated an online interactive AD model protein expression database (Proteomics INTEGRator) as a resource for the entire AD research community (http://sealion.scripps.edu/pint/?project=3dc7c1ac0789307a79ba07c6a397b2d21ef). This resource allows users to query proteins of interest, visualize quantified peptides, and perform enrichment analyses (Figure S6). The importance of determining protein abundances is particularly relevant for the investigation of AD, because altered proteostasis and reduced protein degradation capacities are hallmarks (Powers et al., 2009; Rubinstein, 2006). However, our study is not without limitations, since we failed to accurately quantify many proteins expressed at low levels, and our results are limited by protein abundance averaging among the multiple cell types present in the brain.

Our results show that Aβ causes a broad and progressive alteration in the level of many functionally and spatially linked components of the brain proteome. These findings, and those recently published by others (Seyfried et al., 2017), raise the possibility that studying groups of co-expressed proteins might be advantageous over the study of individual proteins, due to the highly complex response of various cell types to toxic Aβ.

**EXPERIMENTAL PROCEDURES**

**Mice**

Ten C57BL/6 female mice were metabolically labeled with 15N-rich, Spirulina-based diet (Cambridge Isotopes) for 12 weeks starting at post-natal day (P)21 (Wu et al., 2004). The 15N protein enrichment was calculated based on the shape of the peptide isotope envelope, and in the brain it was determined to be 90%–95% (MacCoss et al., 2003). For the hAPP model, we used the less aggressive J9 line, except for the AMPAR immunoprecipitation-MS/WBs and whole-cell electrophysiology, in which, out of necessity, we used J20 mice (RRID: IMSR_JAX:004662) (Mucke et al., 2003). All hAPP mice were on a C57/B6 background. The hAPP/PS1 mice used were (B6C3-Tg[APPswe, PSEN1dE9]85Dbo/Mmjax–129x1/SvJ) (RRID: MMRRC_037564-JAX) (Borchelt et al., 1996; Cohen et al., 2009). The BRI-Aj42 mice were C57BL/6J (RRID: IMSR_JAX:007182) (McGowan et al., 2005). Institutional Animal Care and Use Committees of UCSD, Salk Institute, and the Scripps Research Institute approved all animal procedures and studies. Most of the Tg-AD mice used in this study were female (3 months, 3.0–3.6 months; and 12 months, 12.2–15.0 months). Brains were split into two hemispheres, one for proteomic analysis and ELISA and the other for histology. Mice were from the Masliah, Koo, and Dillin lab colonies or Jackson Laboratory. See the Supplemental Experimental Procedures for details.

**MS Analysis**

Brain region homogenous corresponding to 100 µg, based on BCA assay, was digested to peptides with trypsin overnight and was processed for multidimensional protein identification technology (MudPIT), as previously described (Savas et al., 2015). The peptide-loaded column was placed in line with an Agilent 1200 HPLC and analyzed with an 11-step method. MS analyses were completed with Thermo Orbitrap Velos or Orbitrap Fusion Tribrid mass spectrometers (see Table S6). Protein identification and quantification and
analysis were done with ProLuCID, DTASelect2, Census, and QuantCompare within the Integrated Proteomics Pipeline (IP2) environment. Tandem mass spectra were matched to sequences using the ProLuCID algorithm with 50-ppm peptide mass tolerance for precursor ions and 400 ppm for fragment ions. Each dataset had an ~1% FDR rate at the protein level based on the target-decoy strategy. Since we analyzed brain tissue that has many cell types and mRNA alternative splicing, we chose large databases to maximize our analysis potential. All the peptide identifications have been mapped to genes, and the details can be found in our online database where all proteins were also mapped to UniprotKB accessions.

For MS1-based quantification, Census first calculates the elemental compositions and corresponding isotopic distributions for both the unlabeled and labeled peptides. All isotopes with greater than 5% of the calculated isotope cluster base peak abundance were used. MS1 files were used to generate chromatograms from the m/z range surrounding both the unlabeled and labeled precursor peptides. The core of Census is a linear least-squares correlation that is used to calculate the ratio (i.e., slope of the line) and closeness of fit (i.e., correlation coefficient \( r^2 \)) between the data points of the unlabeled and labeled ion chromatograms. In this study, only peptide ratios with the coefficient correlation values \( r^2 \) greater than 0.5 were used for further analysis. The Grubbs test \( p \) value < 0.01 was used to remove outlier peptides. Final protein ratios were generated with QuantCompare, which uses Log 2-fold change on the biological replicates. The statistical significance labeled of the differential expression of all proteins was assessed using a two-tailed one-sample t test on their corresponding peptide quantification ratios between both conditions. The obtained \( p \) values were FDR-adjusted for multiple hypothesis testing using the Benjamin-Hochberg correction (Benjamini and Hochberg, 1995). Proteins with FDR-adjusted \( p \) values < 0.05 and for which quantification measurements were obtained in at least two biological replicates in both conditions were considered for further analyses. We also performed label-free qualitative analysis using Census within IP2. See the Supplemental Experimental Procedures for details.

**Statistical and Bioinformatic Analyses**
We compared our proteomic data to the previously described potential AD susceptibility genes (Bertram et al., 2007). For all heatmaps, we used 3 biological replicates for each experimental group, and we required each protein to be quantified in a minimum of 57 (or a total of 72) datasets and to be quantified in each brain region. We used the Genes to Cognition protein database to identify synaptic proteins, Mitocarta2.0 for mitochondrial proteins, and IntrraDE to identify the immunological proteins. Boxplots (Figure 3) define the 25th and 75th percentiles, and statistical analysis was performed with Student’s t test with tails and equal variance. For the quantification of APP levels, only peptide sequences with 100% homology between human and mouse were used. Bar graphs (Figure S1) show mean ± SD with Student’s t test with 1 tail and unequal variance (Figures S1A and S1B) and with 2 tails and equal variance (Figures S11 and S1P). Bar graphs for human AMPAR purifications with MS analysis and statistics were performed with Student’s t test with 1 tail and two-sample equal variance (Figure 6B). WB quantification bar graphs (Figure S3) show mean ± SEM and Student’s t test with 1 tail and two-sample equal variance. We used the program Venn Diagram Plotter to construct two- and three-circle Venn diagrams (aka Euler diagrams). Detailed descriptions of each analysis, the tools used, and the specific criteria are in the Supplemental Experimental Procedures.

**WGCNA**
The consensus weighted correlation networks were constructed across both Non-Tg and Tg-AD groups for each brain region with R (Langfelder and Horvath, 2008; Zhang and Horvath, 2005). Prior to analysis, protein IDs with 14 or more missing values were removed, resulting in three pairs of datasets: 1,912 protein IDs for the HIP, 2,146 protein IDs for the FC, and 2,169 protein IDs for the CB. The specific datasets for each analysis are as follows (dataset numbers correspond to the numbering indicated in Table S6): FC = 137–139, 124–127, 89–92, 77–79, 112–116, 101–103, 65–67, and 53–57; and HIP = 140–144, 128–131, 93–95, 80–84, 117–119, 104–108, 68–72, and 58–61. The soft power threshold was set to 12 for the HIP groups and 6 for the FC groups to arrive at the network adjacency. See the Supplemental Experimental Procedures for details.

**Affinity Purification of AMPA Receptor Complexes**
We used our previously reported method to purify native AMPARs (Nakagawa et al., 2005). Human brain (cortex) was obtained through the National Disease Research Interchange (NDRI) (Researcher: Yates [code YAJJ2], TSRI: IRB-11-5719). The antigen of the antibody against GluA2 is conserved in rat and human. See the Supplemental Experimental Procedures for details.

**Electrophysiology**
Brains were cut into 300-μm sagittal sections on a vibratome and placed in ice-cold carbogenated artificial cerebrospinal fluid. Whole-cell recordings were made using 3- to 5-M \( \mu \) pipettes filled with an internal solution that contained 123 mM Cs-gluconate, 8 mM NaCl, 1 mM CaCl2, 10 mM EGTA, 10 mM HEPE, and 10 mM glucose (pH 7.3) with CsOH. 280–290 mM Osm. Responses were evoked by stimulating axons in the stratum radiatum with a platinum 2-contact cluster electrode 100–200 μm lateral to the recording site. See the Supplemental Experimental Procedures for details.

**DATA AND SOFTWARE AVAILABILITY**
The accession numbers for the raw MS data reported in this paper are ProteomeXchange: PXD005595 and MassIVE: MSV000080431.

**SUPPLEMENTAL INFORMATION**
Supplemental Information includes Supplemental Experimental Procedures, six figures, and six tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2017.11.009.

**AUTHOR CONTRIBUTIONS**

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