Amyloid Precursor Protein (APP) May Act as a Substrate and a Recognition Unit for CRL4<sup>CRBN</sup> and Stub1 E3 Ligases Facilitating Ubiquitination of Proteins Involved in Presynaptic Functions and Neurodegeneration*

The amyloid precursor protein (APP), whose mutations cause Alzheimer disease, plays an important in vivo role and facilitates transmitter release. Because the APP cytosolic region (ACR) is essential for these functions, we have characterized its brain interactome. We found that the ACR interacts with proteins that regulate the ubiquitin-proteasome system, predominantly with the E3 ubiquitin-protein ligases Stub1, which binds the NH<sub>2</sub> terminus of the ACR, and CRL4<sup>CRBN</sup>, which is formed by Cul4a/b, Ddb1, and Crbn, and interacts with the COOH terminus of the ACR via Crbn. APP shares essential functions with APP-like protein-2 (APLP2) but not APP-like protein-1 (APLP1). Noteworthy, APLP2, but not APLP1, interacts with Stub1 and CRL4<sup>CRBN</sup>, pointing to a functional pathway shared only by APP and APLP2. In vitro ubiquitination/ubiquitome analysis indicates that these E3 ligases are enzymatically active and ubiquitinate the ACR residues Lys<sup>649</sup>/650/651/676/688. Deletion of Crbn reduces ubiquitination of Lys<sup>676</sup> suggesting that Lys<sup>676</sup> is physiologically ubiquitinated by CRL4<sup>CRBN</sup>. The ACR facilitated in vitro ubiquitination of presynaptic proteins that regulate exocytosis, suggesting a mechanism by which APP tunes transmitter release. Other dementia-related proteins, namely Tau and apoE, interact with and are ubiquitinated via the ACR in vivo. This, and the evidence that Crbn and Cul4B are linked to intellectual disability, prompts us to hypothesize a pathogenic mechanism, in which APP acts as a modulator of E3 ubiquitin-protein ligase(s), shared by distinct neuronal disorders. The well described accumulation of ubiquitinated protein inclusions in neurodegenerative diseases and the link between the ubiquitin-proteasome system and neurodegeneration make this concept plausible.

Processing of APP<sup>2</sup> plays an important role in the central nervous system. A polymorphism in APP that reduces APP processing protects from sporadic Alzheimer disease (AD) (1). In contrast, mutations in APP and in genes that regulate APP processing, such as PSENs and BRI2/ITM2B, cause familial demen-
tias (2–12). APP is cleaved by β-secretase/BACE1 into a soluble ectodomain (soluble APPβ) and the COOH-terminal fragment β-CTF. Alternatively, α-secretase cleaves APP into soluble APPα and a shorter COOH-terminal fragment, α-CTF. β-CTF and α-CTF can be cleaved by γ-secretase to produce Aβ and the APP intracellular domain (AID) or P3 and AID, respectively (13–17). AID contains the ACR plus a few amino acids derived from the trans-membrane region of APP. AID is released in the cytosol upon production. Another processing pathway involves cleavage of APP in the ACR by caspase-6, -3, and -8 (18–24). Sequential γ-secretase/caspase processing can potentially generate the NH<sub>2</sub>- and COOH-terminal cytosolic peptides JCas and Ccas (23, 24).

In vivo studies have identified an essential role for the ACR in the patterning of neuromuscular junction and survival and in synaptic transmission (25–29). Other studies have suggested that release of AID modulates apoptosis, gene transcription, and Ca<sup>2+</sup> homeostasis (23, 30–40). The caspase-derived APP fragments Ccas and JCas also possess toxic activities (22–24). Overall, these data indicate that the ACR is functionally important in vivo.

APP belongs to a protein family that includes APLP1 and APLP2. APLP1 and APLP2 are processed like APP (41–45) and release intracellular peptides, called ALID1 and ALID2, respectively, that, like AID, can potentially regulate transcription (42, 46). The evidence that Aplp2-KO and App-KO mice are viable and show normal synaptic vesicle release, whereas the combined App/Aplp2-dKO mice develop neuromuscular junction deficits, die shortly after birth, and have altered synaptic vesicle exocytosis (28, 47), illustrates the functional redundancy of APP and APLP2.

The ACR is short and does not possess enzymatic activity, suggesting that it may function by modulating the activity of interacting proteins. As discussed above, several APP metabo-

---

* This work was supported by National Institutes of Health Grants R01AG033007, R01AG041531, R21AG048971, and R01AG052286 from NIA (to L. D.), The Hartwell Foundation (to A. M. R.), and The BrightFocus (to D. P.). The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

1 To whom correspondence should be addressed. E-mail: luciano.dadamio@einstein.yu.edu.

2 The abbreviations used are: APP, amyloid precursor protein; AD, Alzheimer disease; ACR, APP cytosolic region; UPS, ubiquitin-proteasome system; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; WB, Western blotting; CRL, Cullin-RING ubiquitin ligase; K- UB, lysine residues ubiquitinated; dKO, double knock-out; DUB, deubiquitinating enzyme; NSAF, normalized spectral abundance factor; CTF, COOH-terminal fragment; AID, APP intracellular domain.
Modulation of E3 Ligases by APP

lites contain the ACR. These include the membrane-bound full-length APP, β-CTF, and α-CTF and the soluble AID peptide. Thus, complexes formed by ACR-interacting proteins may have distinct functional properties depending on the ACR-containing APP metabolite with which they interact.

Using an unbiased proteomic approach, we have characterized the ACR brain interactome (28, 48). Here, we show that the ACR interacts with several proteins that regulate the UPS. The E3 ubiquitin-protein ligase Stub1 and the E3 ubiquitin-protein ligase complex CRL4CRBN, which is formed by Cul4a/b, Ddb1, and Crbn (49), are the most abundant UPS-related proteins interacting with the ACR. By performing ubiquitome analysis on the ACR brain interactome and total mouse brain, we generated evidence implicating APP in the ubiquitination of ACR-interacting proteins, and E3 ubiquitin-protein ligases, including CRL4CRBN, in the ubiquitination of APP. Moreover, we found that APLP2, but not APLP1, can potentially exert a similar role. Finally, we observed that several of the UPS-related ACR interactors and proteins ubiquitinated in vitro in an ACR-dependent manner are genetically linked to neurodegeneration.

Ubiquitination can either modulate protein function or promote protein degradation by the proteasomal and the autophagic/lysosomal pathway. Integrity of these two pathways is important for normal aging and to ensure efficient turnover of both functional and defective proteins. The finding that APP may play a role in the ubiquitination of proteins linked to neurodegenerative diseases suggests that dysregulation of a functional network in which APP functions as a modulator of E3 ubiquitin-protein ligase(s) could be a pathogenic mechanism shared by numerous neuronal disorders.

Results

APP Interacts, via Its ACR, with Proteins That Regulate the UPS—In vivo observations underscore a key physiological and pathological role of the ACR (25, 27–29, 48, 50, 51, 53, 54). This short sequence lacks enzymatic activity and may function as a docking domain for cytosolic as well as membrane-bound proteins (46, 55–72). To explore the potential role of the ACR, we used a proteomic approach (28, 48). Five synthetic peptides, i.e., control St peptide, St-ACR, St-ACR with on Tyr682 (St-ACR Tyr(P)), St-ACR with phosphorylation on Thr668 (St-ACR Thr(P)), and St-ACR with phosphorylation on both Thr668 and Tyr682 (St-ACR Thr(P) Tyr(P)), were immobilized on Streptactin resin. The numbering of phosphorylated residues is based on the APP isoform of 695 amino acids. Mouse brain fractions were first passed twice through Streptactin resin columns to remove proteins that bind to the Streptactin resin. Then, they were applied in parallel on separate columns packed with either Streptactin-St-, Streptactin-St-ACR-, Streptactin-St-ACRThr(P)-, Streptactin-St-ACR Tyr(P)-, or Streptactin-St-ACR Thr(P) Tyr(P)-coated resin. After extensive washing, the St, St-ACR, St-ACRThr(P)-, St-ACR Tyr(P)-, and St-ACR Thr(P) Tyr(P) peptides were eluted, together with proteins specifically bound to them, with desthiobiotin. Eluted proteins were digested with trypsin and identified by nano-LC/MS/MS. We used St-ACR, St-ACR Thr(P), St-ACR Tyr(P), and St-ACR Thr(P) Tyr(P) peptides for two reasons. First is to identify interactions that are regulated by phosphorylation on these two residues. This goal is justified by previous reports showing that phosphorylation of either Thr668 or Tyr682 can modulate interaction of APP with some binding partners (73). Second is that the use of four independent ACR baits can help to pinpoint interactions that are possibly biologically relevant.

Analysis of proteins that interact with at least one St-ACR bait but not with the St control showed that the ACR can potentially interact with several proteins involved in the UPS. These include the following: 1) ubiquitin-like modifier-activating enzyme E1 and ubiquitin-conjugating enzyme E2 (Table 1); 2) E3 ubiquitin-protein ligase or components of E3 protein ligase complexes (Table 1); 3) proteins that regulate the E3 ligase activity (Table 2); 4) proteasome subunits (Table 2); 5) deubiquitinase; and 6) ubiquilins. Ubiquitin-like modifier-activating enzyme E1 (Uba1) and ubiquitin-conjugating enzyme E2-O (Ube2o) and E2-R2 (Ube2r2) were found in St-ACR pulldowns.

Nineteen E3 ubiquitin-protein ligases were found in St-ACR pulldowns as follows: Arih1, Hecw1, Huw1, Kcmf1, Nedd4, Park7, Rnf14, Trim32, Ube3a, Ubr3, Ubr4, Ubr5, and STIP1 homology and U box-containing protein 1 (Stub1). In addition, other proteins pulled down by the ACR are components of E3 protein ligase complexes as follows: Cullin-4a (Cul4a); Cullin-4b (Cul4b); DNA damage-binding protein 1 (Ddb1); DDB1 and CUL4-associated factors 5 (Dcaf5) and 8 (Dcaf8); F-box only proteins 3 (Fbox03) and 21 (Fbox21); F-box/LRR-repeat protein 16 (Fbx16); and Cereblon (Crbn). Cullins provide a scaffold for E3 ubiquitin ligases and combine with RING proteins to form Cullin-RING ubiquitin ligases (CRLs). Ddb1 primarily functions as a core component of the Cul4a- and Cul4b-based E3 ubiquitin ligase complexes (CRL4, E3 cullin 4-RING ligase) and serves as an adaptor protein that interacts with Dcaf class of proteins (74, 75). Dcaf5 are substrate specificity receptors that form the substrate-presenting side of the CRL4 complex (74, 75). Three of the proteins isolated are Dcaf: Dcaf5, Dcaf8, and Crbn. F-box proteins, such as Fbox21, Fbox03, and Fbx16, are substrate recognition components of SKP1-CUL1-F-box protein) E3 ubiquitin ligase complex (76, 77).

The E3 ubiquitin-protein ligase activity of CRL complexes is dependent on the neddylation of the cullin subunit and is inhibited by the association of the deneddylated cullin subunit with the Cullin-associated NEDD8-disassociated protein 1 (TIP120A/CAND1) (78). CRL deneddylation is performed by the COP9 signalosome complex, a protein complex with isopeptidase activity that catalyzes the hydrolysis of NEDD8 protein from the cullins (79). Cand1 and all eight subunits composing the COP9 signalosomes (Cops1/2/3/4/5/6/7/8/9) were isolated in our ST-ACR pulldown experiments.

Proteasomes are ATP-dependent proteolytic complexes that degrade poly-ubiquitinated cytosolic and nuclear proteins. The proteasome most exclusively used in mammals is the cytosolic 26S proteasome (80). We found the following components of the proteasome among the potential ACR interactors: 26S protease regulatory subunit 4 (Psmc1); 6B (Psmc4); 8 (Psmc5); 10B (Psmc6); 26S proteasome non-ATPase regulatory subunits-1 (Psmd1); -2 (Psmd2); -4 (Psmd4); -7 (Psmd7); -8 (Psmd8); -11 (Psmd11); -12 (Psmd12); -13 (Psmd13); and -14 (Psmd14).
### TABLE 1

**E1, E2, and E3 interacting with the ACR, Ddb1, Crbn, Stub1, Cul4a, and Cul4b, are the most abundant UPS-linked proteins that bind to the ACR**

Table contains the list of proteins identified (1st column); the database accession numbers (2nd column); the molecular mass in kDa (3rd column); and the NSAF (4th to 8th columns. Phosphorylation of Thr668 and/or Tyr682 does not significantly alter binding of these five proteins.

| Proteins | UniProtKB/Swiss-Prot | kDa  | St | ACR | ACR\(^{Thr(P)}\) | ACR\(^{Thr(P)}\) |
|----------|-----------------------|------|----|-----|-----------------|-----------------|
| E1/E2    |                       |      |    |     |                 |                 |
| Uba1     | Q02053                | 118  | 0  | 0   | 0               | 0               |
| Ubc2o    | Q6ZP73                | 141  | 0  | 0   | 0.0001          | 0.0001          |
| Ubc2r2   | Q6ZWZ2                | 27   | 0  | 0.0005 | 0             | 0               |
| E3       |                       |      |    |     |                 |                 |
| Arih1    | Q9Z21K5               | 64   | 0  | 0.0005 | 0             | 0.0002          |
| Hecw1    | Q8K4P8                | 179  | 0  | 0   | 0               | 0               |
| Huw1     | Q7TMY8                | 483  | 0  | 0   | 0               | 0               |
| Kcmf1    | Q80UY2                | 42   | 0  | 0.0002 | 0             | 0               |
| Nedd4    | P49635                | 103  | 0  | 0.0003 | 0             | 0               |
| Park7    | Q99LXO                | 20   | 0  | 0.0004 | 0             | 0               |
| Rnf14    | Q9J190                | 55   | 0  | 0.0002 | 0             | 0               |
| Trim32   | Q8CH72                | 72   | 0  | 0.0008 | 0.0002 | 0.0011          |
| Ube3a    | O087S9                | 101  | 0  | 0   | 0               | 0               |
| Ubr3     | Q5U430                | 213  | 0  | 0.0001 | 0             | 0               |
| Ubr4     | A2AN08                | 572  | 0  | 0.0002 | 0.0010 | 0.0005          |
| Ubr5     | Q80TP3                | 308  | 0  | 0   | 0.0001          | 0               |
| Stub1    | Q9WUD1                | 35   | 0  | 0.0093 | 0             | 0.0001          |
| Cul4a    | Q3TCH7                | 88   | 0  | 0.0015 | 0.00030 | 0.0009         |
| Cul4b    | A2A432                | 111  | 0  | 0.0003 | 0.0009 | 0.0004          |
| Ddb1     | Q3U1J4                | 127  | 0.0019 | 0.0141 | 0.0228 | 0.0114          |
| Dcaf5    | Q80T85                | 104  | 0  | 0.0004 | 0             | 0.0001          |
| Dcaf8    | Q8N7N5                | 66   | 0  | 0.0002 | 0             | 0.0003          |
| Fbxo3    | Q9D6C3                | 55   | 0  | 0.0002 | 0             | 0               |
| Fbxo21   | Q8VDH1                | 72   | 0  | 0.0001 | 0.0003 | 0.0001          |
| Fbx116   | A2RT62                | 52   | 0  | 0.0006 | 0             | 0               |
| Crbn     | Q8C7D2                | 51   | 0  | 0.0061 | 0.0129 | 0.0053          |

### TABLE 2

**Other UPS-related proteins interacting with the ACR**

Table is organized same as Table 1. Binding of Pin1 and Grb2 (bottom two entries) is dependent on phosphorylation of Thr668 and Tyr682, respectively.

| E3 regulators | UniProtKB/Swiss-Prot | kDa  | St | ACR | ACR\(^{Thr(P)}\) | ACR\(^{Thr(P)}\) |
|---------------|----------------------|------|----|-----|-----------------|-----------------|
| E3 regulators | Cand1                | Q6ZQ38 | 136 | 0  | 0               | 0              |
|              | Cops1                | Q9FLQ4 | 53  | 0.0007 | 0             | 0              |
|              | Cops2                | P61202 | 52  | 0.0011 | 0             | 0              |
|              | Cops3                | Q88543 | 48  | 0.0008 | 0             | 0              |
|              | Cops4                | Q88544 | 46  | 0.0018 | 0             | 0              |
|              | Cops5                | O35864 | 38  | 0.0013 | 0             | 0              |
|              | Cops6                | O88545 | 36  | 0.0011 | 0             | 0              |
|              | Cops7a               | Q9CZ04 | 30  | 0.0007 | 0             | 0              |
|              | Cops7b               | Q8BV13 | 30  | 0.0003 | 0             | 0              |
|              | Cops8                | Q8BV7  | 23  | 0.0004 | 0             | 0              |
| Proteasome   | Psmc1                | P62192 | 49  | 0  | 0               | 0              |
|              | Psmc4                | P54775 | 47  | 0  | 0               | 0              |
|              | Psmc5                | P62166 | 46  | 0  | 0.0003 | 0             |
|              | Psmc6                | P62334 | 44  | 0  | 0               | 0              |
|              | Psmc1                | Q3TCH7 | 106 | 0  | 0               | 0              |
|              | Psmc2                | Q8MDM4 | 100 | 0  | 0.0001 | 0             |
|              | Psmc4                | O35226 | 41  | 0  | 0.0002 | 0.0003 | 0               |
|              | Psmc7                | P26516 | 37  | 0  | 0               | 0.0002 | 0               |
|              | Psmc8                | Q8XZ6  | 40  | 0  | 0.0002 | 0.0003 | 0               |
|              | Psmc11               | Q8RZ32 | 47  | 0  | 0               | 0               |
|              | Psmc12               | Q9D8W5 | 53  | 0  | 0.0002 | 0.0003 | 0               |
|              | Psmc13               | Q9WV7  | 43  | 0  | 0               | 0.0002 | 0               |
|              | Psmc14               | O35593 | 35  | 0  | 0               | 0.0002 | 0.0002 | 0               |
| DUBs         | Usp9x                | P70398 | 291 | 0  | 0.0005 | 0.0001 | 0.0003 | 0               |
|              | Usp5                 | P56399 | 96  | 0  | 0.0013 | 0.0003 | 0.0002 | 0               |
|              | Usp7                 | Q6A4J8 | 128 | 0  | 0.0002 | 0             |
|              | Usp10                | P52479 | 87  | 0  | 0               |
|              | Otubah               | Q7TQ3  | 31  | 0  | 0.0008 | 0             |
| Ubiquilins   | Ubgl1                | QQR317 | 62  | 0  | 0               | 0.0004 | 0               |
|              | Ubgl2                | UBQL2  | 67  | 0  | 0.0003 | 0.0006 | 0.0007 | 0.0003          |
|              | Ubgl4                | Q9NB8  | 64  | 0  | 0               | 0.0003 | 0               |
| Phosphorylation-dependent | Grb2     | Q60631 | 25  | 0.0007 | 0.0008 | 0.0753 | 0.0350 | 0.0005 |
|              | Pin1                 | Q9QUR7 | 18  | 0  | 0               | 0.0057 | 0            | 0.0030 |
Modulation of E3 Ligases by APP

Deubiquitinating enzymes (DUBs) are a large group of proteases that cleave ubiquitin from proteins and other molecules. Ubiquitination of a protein can have several biological consequences as follows: target the protein for degradation via the proteasome and autophagosome/lysosome; coordinate the cellular localization of proteins; activate and inactivate proteins; and modulate protein-protein interactions. DUBs can reverse these effects by cleaving off ubiquitin from substrate protein. In humans there are nearly 100 DUB genes, which can be classified into two main classes, cysteine proteases and metalloproteases (81). The cysteine proteases include many types of proteases, including ubiquitin-specific proteases and ovarian tumor proteases. Among the ACR-interacting proteins, we found five ubiquitin-specific proteases and one out: ubiquitin carboxyl-terminal hydrolase-9x (Usp9x); -5 (Usp5); -7 (Usp7); -10 (Usp10); and ubiquitin thioesterase Otub1.

Ubiquilins contain an NH2-terminal ubiquitin-like domain and a COOH-terminal ubiquitin-associated domain. They physically associate with both proteasomes and ubiquitin ligases and thus are thought to functionally link the ubiquitination machinery to the proteasome (82). Three ubiquilins were isolated in our screening as follows: ubiquilin-1 (Ubqln1), -2 (Ubqln2), and -4 (Ubqln4).

CRL4CRBN E3 Ligase Complex and the E3 Ligase Stub1 Bind the COOH- and NH2-terminal Regions of the ACR, Respectively—The UPS/ACR-interacting network is presumably formed via the direct interaction of the ACR with one or more UPS-linked proteins, and the other proteins are most likely indirectly associated with the ACR, for example via secondary interactions (i.e. mediated by binding to a direct ACR interactor) or tertiary interactions (i.e. mediated by binding to a protein that binds a direct ACR interactor) and so forth. It is reasonable to suppose that proteins binding to the ACR directly will be enriched more efficiently than proteins that bind indirectly. Normalized spectral abundance factor (NSAF) analysis indicates that Stub1, Ddb1, Crbn, Cul4a, and Cul4b are the five most abundant UPS-related proteins present in the St-ACR, St-ACRThr(P), St-ACRTyr(P), and St-ACRThrP(TyrP) pulldowns (Table 1; NSAF values for St-negative control, St-ACR, St-ACRThr(P), St-ACRTyr(P), and St-ACRThrP(TyrP) pulldowns were as follows: for Ddb1, 0.0019, 0.0141, 0.0228, 0.0114, and 0.0149; for Cul4a, 0, 0.0015, 0.003, 0.0009, and 0.0019; for Cul4b, 0, 0.0003, 0.0009, 0.0004, and 0.0007; for Crbn, 0, 0.0061, 0.0129, 0.0053, and 0.0061; and for Stub1, 0, 0.0091, 0.0072, 0.0055, and 0.0046). This quantitative analysis suggests that Stub1, Ddb1, Crbn, Cul4a, and Cul4b are the most likely UPS-related proteins to directly interact with APP and that phosphorylation of the ACR on either Thr668 or Tyr682 does not appreciably alter their binding to the ACR.

As a further step toward discriminating biologically relevant interactions from background noise, we used the above proteomic approach to identify the brain proteins interacting with the NH2 terminus (JCasp) and COOH terminus (Ccas) subdomains of the ACR (Fig. 1A, schematic). In this experiment as well we used five baits as follows: the negative control St peptide, St-Ccas, St-Ccas with phosphorylation on Tyr682 (St-Ccas Tyr(P)), St-Ccas with phosphorylation on Thr668 (St-Ccas Thr(P)), and St-JCasp.

To confirm the accuracy of the proteins detected in the proteomic analysis, we performed Western blotting analysis with pulldown lysates. As shown in Fig. 1B, Crbn, Cul4a, and Ddb1 were readily detected in St-CcasThr(P), St-CcasTyr(P), and St-Ccas but not in St and St-JCasp pulldowns. On the contrary, Stub1 was found exclusively in St-JCasp pulldowns. Grb2, whose interaction with APP requires phosphorylation of Tyr682
of all the UPS-linked proteins pulled down with St-ACR, the CRL4CRBN E3 ligase complex components and E3 ligase Stub1 are the most likely to interact directly with APP in vivo. Incidentally, an interaction between Stub1 and APP in vivo was previously described (85).

Crbn Mediates Binding of the CRL4CRBN E3 Ligase Complex to the ACR—Next, we investigated whether Ddb1, Cul4a, and Cul4b interact with the ACR as a complex. Ddb1 and Cul4a bind several substrate-recognition subunits, called Dcafs. Indeed, Crbn is essentially a Dcaf. If the ACR interacted with the CRL4CRBN E3 ligase complex either via Cul4a, Cul4b, or Ddb1, several Dcafs should have been isolated by St-ACR. However, Crbn was the only Dcaf abundantly present in the pulldown with the four St-ACR baits (St-ACR, St-ACRThr(P), St-ACRTyr(P), and St-ACRThr(P)Tyr(P)) and the three St-Ccas baits (St-Ccas, St-CcasThr(P), and St-CcasTyr(P)); Dcaf5 and Dcaf8 were detected but in very low amounts and only in St-ACR and St-ACRThr(P) pulldowns (NSAF for Dcaf5-derived peptides was 0.0004 and 0.0001 in St-ACR and St-ACRThr(P) pulldowns, respectively; NSAF for Dcaf8-derived peptides was 0.0002 and 0.0003 in St-ACR and St-ACRThr(P) pulldowns, respectively) and not in St-ACRThr(P), St-ACR Thr(P)Tyr(P), St-Ccas, Ccas Thr(P), and St-Ccas Tyr(P) pulldowns (NSAF were 0 for both proteins in all five samples). Altogether, these data suggest that Dcaf5 and Dcaf8 may either have been non-specifically isolated in the St-ACR and St-ACRThr(P) pulldown or that Dcaf5 and Dcaf8 can weakly and indirectly interact with the ACR.

To directly test whether Crbn mediates the interaction of CRL4CRBN with APP, we performed St-ACRThr(P)Tyr(P) pulldowns using brain lysates isolated either from wild type (WT) or Crbn-KO mice (86). Both proteomic (Table 4) and Western blotting analysis (Fig. 2) of these pulldowns show St-ACR binds Ddb1 and Cul4 when challenged with brain lysates isolated from WT mice but not when the brain lysates were derived from Crbn-KO (86) animals, albeit Ddb1 and Cul4 were equally

|                  | UniProtKB/Swiss-Prot | Molecular mass | St   | CcasThr(P) | CcasTyr(P) | Ccas | JCas |
|------------------|----------------------|---------------|------|------------|------------|------|------|
|                  |                      | kDa           |      |            |            |      |      |
| E1 and E2        |                      |               |      |            |            |      |      |
| Uba1             | Q02053               | 118           | 0    | 0          | 0          | 0    | 0.0001 |
| Ubc2c            | Q6ZP13               | 141           | 0    | 0          | 0          | 0    | 0.0001 |
| Ubc2r3           | Q6ZW72               | 27            | 0    | 0          | 0          | 0    | 0.0016 |
| E3               |                      |               |      |            |            |      |      |
| Ahrl1            | Q9Z1K5               | 64            | 0    | 0          | 0          | 0    | 0.001 |
| Ned4             | P46935               | 103           | 0    | 0          | 0          | 0    | 0.0003 |
| Park7            | Q99LX0               | 20            | 0.0012 | 0.0017 | 0.0004 | 0.0022 | 0.002 |
| Ubr4             | A2A0N8               | 572           | 0    | 0          | 0          | 0    | 0.0002 |
| Stub1            | Q9WUD1               | 35            | 0    | 0          | 0          | 0    | 0.0004 |
| Cu4a             | Q3TCH7               | 88            | 0    | 0.0047     | 0.003     | 0.0026 | 0     |
| Cu4b             | A2A432               | 111           | 0    | 0.0011     | 0.0006    | 0.0008 | 0     |
| Ddb1             | Q3U1J4               | 127           | 0.0005 | 0.0478 | 0.0273 | 0.0375 | 0.0012 |
| Crbn             | Q8C7D2               | 51            | 0    | 0.0252     | 0.0167    | 0.0213 | 0     |
| E3 regulators    |                      |               |      |            |            |      |      |
| Cand1            | Q6ZQ38               | 136           | 0.0002 | 0       | 0          | 0    | 0.0001 |
| Cops1            | Q99LD4               | 53            | 0    | 0          | 0          | 0    | 0.0018 |
| Cops2            | P61202               | 52            | 0    | 0          | 0          | 0    | 0.0022 |
| Cops3            | Q88543               | 48            | 0    | 0          | 0          | 0    | 0.0004 |
| Cops4            | Q88544               | 46            | 0    | 0          | 0          | 0    | 0.0003 |
| Cops5            | Q5S664               | 38            | 0    | 0          | 0          | 0    | 0.0011 |
| Cops6            | Q88545               | 36            | 0    | 0          | 0          | 0    | 0.0015 |
| Cops7a           | Q9C7D4               | 30            | 0    | 0          | 0          | 0    | 0.0022 |
| Cops7b           | Q8BV13               | 30            | 0    | 0          | 0          | 0    | 0.0004 |
| Cops8            | Q8BV7               | 23            | 0    | 0          | 0          | 0    | 0.0005 |
| DUB              |                      |               |      |            |            |      |      |
| Usp5             | P56399               | 96            | 0    | 0.0001     | 0          | 0    | 0.0019 |
| Otub1            | Q7TQL3               | 31            | 0    | 0.0004     | 0          | 0    | 0.0007 |
| Proteasome       |                      |               |      |            |            |      |      |
| Psmd11           | Q8BG32               | 47            | 0.0005 | 0       | 0          | 0    | 0.0003 |
| Psmd12           | Q9D8W5               | 53            | 0    | 0.0004     | 0          | 0    | 0.0002 |
| Psmd13           | Q9WV2                | 43            | 0    | 0          | 0          | 0    | 0.0003 |
| Ubiquitin        |                      |               |      |            |            |      |      |
| Ubqln2           | Q9QZM0               | 67            | 0    | 0.0002     | 0.0001    | 0.0005 | 0.0002 |
| Phosphorylation-dependent |               |               |      |            |            |      |      |
| Grb2             | Q60631               | 25            | 0.0062 | 0.0008 | 0.178     | 0.0005 | 0     |
| Pin1             | Q9QUR7               | 18            | 0    | 0.0126     | 0          | 0    | 0     |
**Modulation of E3 Ligases by APP**

**TABLE 4**

| Proteins | UniProtKB/Swiss-Prot | Molecular mass (kDa) | WT NSAF | Crbn-KO NSAF |
|----------|----------------------|----------------------|---------|-------------|
| Stub1    | Q9WUD1               | 35                   | 0.001   | 0.002       |
| Ddb1     | Q3U114               | 127                  | 0.006   | 0           |
| Crbn     | Q8CD72               | 51                   | 0.004   | 0           |
| Cul4a    | Q3TCH7               | 88                   | 0.001   | 0           |
| Grb2     | Q69621               | 25                   | 0.013   | 0.018       |
| Pin1     | Q9QUR7               | 18                   | 0.003   | 0.004       |

**FIGURE 2.** **Crbn mediates the interaction of CRL4^{CRBN} with APP.** Western blotting analysis shows that with St and St-ACR^{Thr(P)Tyr(P)} binds Ddb1 only when Crbn is present in brain lysates, indicating that Crbn mediates the binding of CRL4^{CRBN} to the ACR. Binding of Grb2 and Pin 1 to St-ACR^{Thr(P)Tyr(P)} is not dependent on Crbn. The WB shown is representative of 4 independent experiments.

expressed in both WT and Crbn-KO lysates (Fig. 2). These data strongly suggest that Cul4a, Ddb1, and Crbn bind to the ACR as a complex and that the APP cytoplasmic tail binds CRL4^{CRBN} via Crbn. On the contrary and as predictable, Stub1 (Table 4), Grb2, and Pin1 (Table 4 and Fig. 2) bind to St-ACR^{Thr(P)Tyr(P)} independently of Crbn.

**COOH Terminus of APP Binds the Substrate Recognition Pocket of Crbn—** Although Thr^{668} and Tyr^{682} are in the Ccas ACR domain, phosphorylation of these residues does not modulate binding of CRL4^{CRBN} suggesting that they are not involved in the CRL4^{CRBN} APP interaction. To further confirm this, we performed pulldowns with NH3-terminal Ccas deletions and found that the COOH-terminal 12 amino acids of APP (St-C-12, which do non include Thr^{680} and Tyr^{682}) retain full binding capabilities for CRL4^{CRBN}, whereas the COOH-terminal eight amino acids (St-C-8) do not (Fig. 3A). Next, we performed pulldowns using a series of COOH-terminal deletions of St-C-12. Removal of the last one and two amino acids of APP reduces the binding of Crbn and Ddb1 by ~50% and ~100%, respectively. Essentially, when the NH2-terminal amino acids of the 12-mer are deleted, binding was greatly reduced, as in the case with deleting the COOH-terminal two amino acids. It is therefore possible that reducing the ACR peptide below the length of 12 amino acids disrupts the peptide’s secondary structure, thereby reducing Crbn and Ddb1 binding. Overall, the data indicate that the COOH-terminal 12 amino acids of APP contain the docking site for CRL4^{CRBN}.

**Crbn is the substrate recognition subunit of CRL4^{CRBN} (49).** To test whether APP binds the substrate-recognition pocket of Crbn, we pre-incubated brain lysates with either 10 or 100 μM concentrations of either thalidomide (Thal) or lenalidomide (Len), two compounds that bind the substrate-recognition pocket of Crbn (87–89). After 1 h, lysates were used for pulldown experiments with St-CcasTyr^{P} (P). Both thalidomide and lenalidomide reduced binding of Crbn and Ddb1 to St-CcasTyr^{P} in a dose-dependent manner (Fig. 3C). Lenalidomide, which binds Crbn more efficiently than thalidomide, is more effective. As expected, neither thalidomide nor lenalidomide interfered with binding of Grb2 to St-CcasTyr^{P} (Fig. 3C). In summary, the extreme COOH terminus of the ACR interacts with brain-derived CRL4^{CRBN} via the substrate-binding pocket of Crbn (model shown in Fig. 3D), suggesting that APP may be a substrate of the CRL4^{CRBN} E3 ligase.

**APLP2, but not APLP1, Bind Stub1 and CRL4^{CRBN}—** The APP protein family includes two other members, APLP1 and APLP2. These three proteins are functionally redundant, particularly APP and APLP2 (25, 45, 47, 90). APLP1 and APLP2 cytoplasmic regions (named AL1CR and AL2CR, respectively) are similar to that of APP. The ACR and the AL2CR share 66% identity and 16% similarity; the ACR and AL1CR share 53% identity and 21% similarity; and the AL2CR and the AL1CR share 62% identity and 18% similarity (Fig. 4A). As a first step toward determining whether APLP1 and/or APLP2 may functionally interface with UPS-related proteins, we characterized the brain interactome of the AL1CR and the AL2CR. We synthesized the control St-only peptides, St-AL1CR and St-AL2CR. As shown in Table 5, St-AL2CR interacts with UPS-related proteins found in the ACR pulldown were Nedd4, Arih1, and Ubr4. As was the case for St-ACR pulldowns, Ddb1, Crbn, Stub1 and Cul4a were the four most abundant UPS-related proteins found in the St-AL2CR pulldown. In contrast, UPS-related proteins were scarcely represented in AL1CR pulldowns.

The COOH terminus of the ACR is essential for binding CRL4^{CRBN}; the AL2CR binds CRL4^{CRBN}, but the AL1CR does not; the COOH-terminal four amino acids of the ACR share 75% identity with the corresponding region of the AL2CR and AL1CR, respectively (Fig. 4A). These observations suggest that, like for the ACR, the COOH-terminal region of the AL2CR binds CRL4^{CRBN} via the substrate-binding pocket of Crbn. To test this, we determined whether Crbn is required for binding of Ddb1 to the AL2CR. As shown in Fig. 4C, St-AL2CR binds Ddb1 when challenged with brain lysates isolated from WT animals but not when the input material is isolated from Crbn-KO mice. Next, we tested whether thalidomide and lenalidomide interfere with the AL2CR-Crbn interaction; indeed, both thalidomide and lenalidomide reduced binding of Crbn to St-AL2CR (Fig. 4D). Thus, like for the ACR, the AL2CR binds CRL4^{CRBN} via the substrate-binding pocket of Crbn. Overall, these data suggest that APP and APLP2, but not APLP1, may functionally interface with CRL4^{CRBN} and Stub1.
FIGURE 3. APP-CRL4<sup>Crbn</sup> complex is mediated by the interaction of the COOH terminus of APP with the substrate recognition pocket of Crbn. A, sequences of the APP intracellular regions used as baits (St-Ccas, St-C28, St-C20, St-C16, St-C12, St-C8, and St-C4) in the proteomic experiments are shown. Western blotting analysis of pulldowns with brains isolated from WT mice show that the COOH-terminal 12 amino acids of APP retain full binding activity to Crbn and Ddb1. The WB shown is representative of two independent experiments. B, sequences of the APP intracellular regions used as baits (St-C12, St-C12C1, St-C12C2, St-C12C3, St-C12C5, and St-C12C7) in the proteomic experiments are shown. The WB shown is representative of two independent experiments. C, Western blotting analysis shows that thalidomide (Thal.) and lenalidomide (Len.), two compounds that bind to the substrate recognition pocket of Crbn, reduce binding of Crbn and Ddb1 from brain lysates to St-Ccas<sup>Tyr(P)</sup> in a dose-dependent manner. Thalidomide and lenalidomide do not interfere with binding of Grb2 to St-Ccas<sup>Tyr(P)</sup>. Because Pin1 binding is dependent on phosphorylation of Thr<sup>668</sup> of APP, Pin1 does not bind the St-Ccas<sup>Tyr(P)</sup> bait and is used as a negative control to show binding specificity. The WB shown is representative of three independent experiments. D, schematic model representing how Crbn mediated binding of CRL4<sup>Crbn</sup> to the COOH terminus of APP, via its substrate recognition pocket.

FIGURE 4. AL2CR, but not the AL1CR, binds CRL4<sup>Crbn</sup> via the substrate recognition pocket of Crbn. A, alignments of the ACR, AL1CR, and AL2CR show that the ACR and the AL2CR are the most conserved intracellular regions of the APP protein family. B, Western blotting analysis of pulldowns with brains isolated from WT mice shows that the AL2CR, but not the AL1CR, interacts with Cul4a, Ddb1, and Crbn. The WB shown is representative of two independent experiments. C, St-AL2CR binds Ddb1 in lysate from WT but not from Crbn-KO mice. The WB shown is representative of three independent experiments. D, Western blotting analysis of St-AL2CR pulldowns from brains isolated from WT mice shows that incubation of the lysates with either thalidomide and lenalidomide prior to pulldowns interferes with the AL2CR-Crbn interaction. The WB shown is representative of two independent experiments.
Modulation of E3 Ligases by APP

ACR and the AL2CR Pull Down Active E3 Ubiquitin-Protein Ligase(s)—Ubiquitination of proteins is a multistep reaction. First, an E1 ubiquitin-activating enzyme activates ubiquitin. Next, ubiquitin is transferred on one of many ubiquitin-conjugating enzyme (E2s). The E2 is loaded on an E3 ubiquitin-protein ligase, which directs the mono- and poly-ubiquitination of substrate proteins. The presence of many of these components in St-ACR and St-AL2CR pulldowns prompted us to test whether ubiquitinating activity could be detected. To this end, we performed an in vitro ubiquitination assay using FLAG-ubiquitin as an external source of ubiquitin. The reactions were analyzed by Western blotting using an α-FLAG antibody to determine whether the FLAG-ubiquitin (~9 kDa) was incorporated into larger complexes. A smear of FLAG-ubiquitinated proteins was clearly detected in the St-ACR and St-AL2CR pulldowns after supplemental addition of recombinant E2 Ubch5a/Ube2D1 (Fig. 5A). Overimposed on the smear was a ladder of bands starting at ~25 kDa, which are ~9 kDa apart (Fig. 5A). The ~25-kDa band is compatible with mono-ubiquitinated recombinant E2; the higher bands likely represent recombinant E2 linked to a progressively increasing number of ubiquitin molecules. Addition of recombinant E1 Ube1, albeit not necessary, further potentiated the ubiquitination reactions (Fig. 5A). The observation that only recombinant E2 is required to initiate ubiquitination activity (Fig. 5A) indicates that St-ACR and St-AL2CR bring down from the mouse brain functional E1 and E3 ubiquitin-protein ligase(s). Of note, neither the smear nor the ladder was visible in the in vitro ubiquitination assay of St and St-AL1CR eluates (Fig. 5A), further stressing that APP and APLP2, but not APLP1, may functionally interface with the ubiquitination system.

Because active E3 ubiquitin-protein ligases undergo auto-ubiquitination, we tested whether Crbn was ubiquitinated in vitro. The St-ACR in vitro ubiquitination assay was analyzed by Western blotting with an α-Crbn antibody; in addition to full-length Crbn, a ladder of signals compatible with Crbn molecules linked to an increasing number of ubiquitins was detected (Fig. 5B). This evidence suggests that St-ACR interacts with an active brain-derived CRL4CRBN complex.

ACR Facilitates Ubiquitination of ACR-Interacting Proteins in Vitro—The smear detected by the α-CRBN antibody hints at ubiquitination of numerous proteins. To test this hypothesis, we performed another in vitro ubiquitination assay on St-ACRThr(P)Tyr(P) pulldown because it includes proteins that interact with APP in a Thr(P)668- and Tyr(P)682-dependent manner. The reaction was immunoprecipitated with the α-CRBN M2-agarose beads, and proteins were eluted from the M2-agarose beads using a competing 3×FLAG peptide. To identify the lysine residues ubiquitinated (K-ub) in vitro, the eluted material was subjected to the ubiquitin remnant motif (K-e-GG) assay (UbiScan). In parallel, we performed UbiScan experiments on total mouse brain lysates to determine whether K-ubs found in the in vitro assay are also ubiquitinated in vivo.

As reported in Table 6, several brain-derived proteins associated with the ACR bait were ubiquitinated. These proteins can be separated into four groups as follows: (a) group 1, proteins of the ubiquitin-conjugating system; (b) group 2, presynaptic proteins; (c) group 3, other proteins implicated in AD; and (d) group 4, phosphorylation-dependent ACR interactors. Group 1: consistent with the Western blot showing ubiquitination of Crbn (Fig. 5B), we found six K-ub from Crbn, four of which have been found in vivo. Two other components of

### TABLE 5

| UPS-related proteins, including Ddb1, Crbn, Stub1, Cul4a, and Cul4b, bind to the AL2CR but not to the AL1CR |
|---|---|---|---|---|---|---|
| Proteins | UniProtKB/Swiss-Prot | Molecular mass | St | AL1CR | AL2CR |
|---|---|---|---|---|---|
| Ddb1 | Q3UIH4 | 127 | 0.0021 | 0.0014 | 0.0356 |
| Crbn | Q8C7D2 | 51 | 0 | 0 | 0.01810 |
| Stub1 | Q9WUD1 | 35 | 0 | 0 | 0.01427 |
| Cul4a | Q3U1J4 | 127 | 0.0021 | 0.0014 | 0.0356 |
| E3 Nedd4 | P4695 | 103 | 0 | 0 | 0.00264 |
| Otub1 | Q7TQ83 | 31 | 0 | 0 | 0.00244 |
| Usp5 | P56399 | 96 | 0 | 0.0007 | 0.01819 |
| Uba1 | Q02053 | 118 | 0 | 0 | 0.00103 |
| Cops4 | O68544 | 46 | 0 | 0 | 0.00099 |
| Fbxl16 | A2RT62 | 52 | 0 | 0 | 0.00058 |
| Fbxo3 | Q9D6C3 | 55 | 0 | 0 | 0.00055 |
| E3 Arh1 | Q921K5 | 64 | 0 | 0 | 0.00047 |
| Dcl4b | Q8N7N5 | 66 | 0 | 0 | 0.00046 |
| Ubcn2 | Q9Q2M0 | 67 | 0 | 0 | 0.00045 |
| E3 Ubr4 | A2A06 | 572 | 0 | 0 | 0.00024 |
| Usp9x | P70398 | 291 | 0 | 0 | 0.00021 |

(For A and B, the table contains the lists of proteins identified (1st column); the database accession numbers (2nd column); the molecular mass in kDa (3rd column); NSAF of pulldown of WT mouse brains with St alone (4th column); St-AL1CR (5th column); and NSAF of pulldown of WT mouse brains with St-AL2CR (6th column).)
CRL4<sup>CRBN</sup> were ubiquitinated (23 K-ub for Cul4a and 10 K-ub for Cul4b, six of which have been found <em>in vitro</em>). Other E3 ubiquitin-protein ligases ubiquitinated are as follows: Stub1 (Lys345, new), Arih1 (Lys457, new), Mib2 (Lys426, Lys435, new, and Lys<sup>639</sup> known), Nedd4 (Lys445, found in our mouse brain UbiScan; Lys453, previously found <em>in vivo</em>), Cbl-b (Lys477 and Lys<sup>415</sup> new; Lys491, found in our mouse brain UbiScan; Lys516, previously found), Syn1 (m*445, Lys426, Lys435, new), and Lys615, new; Lys491, found in our mouse brain UbiScan; Lys453, previously found <em>in vitro</em>). Table 7 reports database accession numbers.

Table 6

| Ubiquitin conjugating system | Cul4a | Cul4b | Crbn | Chip | Arom1 | Mib2 | Nedd4 | Cbl-b | Ube1 | Ube2n | Ube2o |
|-----------------------------|-------|-------|------|------|-------|------|-------|-------|------|-------|-------|
|                             | 8, 23, h33, 42, 46, 65, 71, 97, m/h104, 133, 134, 191, 330, 404, 411, 416, m645, 473, 708, 724, 734, 749 | m112, 297, m315, 398, 460, 495, m676, 684, 931, 939 | h/e46, h119, m/n303, 395, 410, c416 | h/126 | 457 | 344, 369, 426, 435 | m*445, m/h453 | 37, m*491, h516, 615 | 28 | m/h802 | m/h/r82, m/h/r92, m/h/r94 |
|                             |       |       |      |      |       |      |       |       |      |       |       |
|                             |       |       |      |      |       |      |       |       |      |       |       |

Predictive targets

| Group 1: in vivo ubiquitination (lysines 32, 473, 708, 724, 728, 734, 749) | Cul4a | Cul4b | Crbn | Chip | Arom1 | Mib2 | Nedd4 | Cbl-b | Ube1 | Ube2n | Ube2o |
|-----------------------------------------------------------------------|-------|-------|------|------|-------|------|-------|-------|------|-------|-------|
| in vivo ubiquitinated lysines                                           |       |       |      |      |       |      |       |       |      |       |       |
| Group 2: in vitro ubiquitination                                       | Cul4a | Cul4b | Crbn | Chip | Arom1 | Mib2 | Nedd4 | Cbl-b | Ube1 | Ube2n | Ube2o |
| in vitro ubiquitinated lysines                                          |       |       |      |      |       |      |       |       |      |       |       |

Table 6 continues...

Ubiquitination of these presynaptic proteins is not entirely surprising. Indeed, the interaction of the ACR with Syt1, Syt2, Sv2a, Sv2b, Snap-25, and Vamp2 was reported previously. Moreover, two other studies have shown that APP interacts <em>in vivo</em> with Syn1, Syt1, Vamp2, Syn2, Sv2a, Snap-25, and Rab3A (91, 92). In Table 7, we show proteomic data substantiating the association of the ACR with Scamp1, Atp6v0a1, Vglu1, Rab14, Rab3a, Syn2, and Syn1.

Group 3: this group includes the tubulin-binding protein Tau (93) and the cholesterol transporter apoE (94). Both proteins were detected in St-ACR pulldowns (Table 7). The two Tau K-ubs found <em>in vitro</em> (Lys<sup>455</sup> and Lys<sup>613</sup>) were also identified in our mouse brain UbiScan. It is worth noting that Stub1 can mediate Tau ubiquitination (95, 96) suggesting that the ACR may facilitate Tau ubiquitination via interaction with Stub1 on one side and Tau on the other side. Alternatively, the ACR may interact with Tau indirectly, via Stub1. It is worth noting that a previous study has described a physical interaction between APP and Tau <em>in vivo</em> (97). ApoE was ubiquitinated on Lys<sup>105</sup>. ApoE is a secreted protein and its ubiquitination may be an <em>in vitro</em> artifact. However, ubiquitination of Lys<sup>105</sup> of apoE has been reported <em>in vivo</em>, suggesting that apoE is, at least in a small fraction or in certain conditions, resident at the synol. Indeed, previous work has shown that apoE is cleaved by a neuro-specific chymotrypsin-like serine protease that generates bioactive...
**Modulation of E3 Ligases by APP**

In this study, we provide in vitro evidence suggesting that APP may function as a substrate recognition unit for one or more E3 ubiquitin-protein ligases. This hypothesis is supported by the following findings. 1) The cluster of brain proteins that may interact with APP via the ACR is rich in proteins that bind APP and are ubiquitinated in an ACR-dependent manner. 2) APP may interact with E3 ubiquitin-protein ligases that are enzymatically active and ubiquitinate the five lysine residues present in segments that are exposed to the cytosol.

**FIGURE 6.** Only lysine residues present in cytosolic domains of transmembrane proteins are ubiquitinated in vitro. Schematic representation of single pass and multipass transmembrane presynaptic vesicle proteins that interact with the ACR and are ubiquitinated in vitro in an ACR-dependent manner. Notably, all the lysine residues ubiquitinated in vitro are in segments that are exposed to the cytosol.

**TABLE 7**

| Proteins | kDa | St | ACRTyr(P) | ACRTyr(P) | ACRThr(P) | ACRThr(P) |
|----------|-----|----|-----------|-----------|-----------|-----------|
| Vglu1    | 62  | 0  | 0.0013    | 0.0015    | 0.0004    | 0.0031    |
| Atp6v0a1 | 96  | 0  | 0.0012    | 0.0014    | 0.0003    | 0.0024    |
| Scamp1   | 38  | 0  | 0.0002    | 0         | 0         | 0.0003    |
| Rab14    | 24  | 0  | 0.0025    | 0.0030    | 0.0036    | 0.0035    |
| Rab3a    | 25  | 0  | 0.0019    | 0.0024    | 0.0028    | 0.0025    |
| Syn2     | 63  | 0  | 0.0016    | 0.0017    | 0.0011    | 0.0008    |
| Syn1     | 74  | 0  | 0.0023    | 0.0042    | 0.0023    | 0.0022    |
| ApoE     | 36  | 0  | 0.0020    | 0.0039    | 0.0030    | 0.0033    |
| Tau      | 76  | 0  | 0.0010    | 0.0006    | 0.0004    | 0.0012    |

**Discussion**

In this study, we provide in vitro evidence suggesting that APP may function as a substrate recognition unit for one or more E3 ubiquitin-protein ligases. This hypothesis is supported by the following findings. 1) The cluster of brain proteins that may interact with APP via the ACR is rich in proteins that regulate the UPS (Table 1). 2) The E3 ubiquitin-protein ligases CRL4CRBN and Stub1 are the most abundant UPS-related proteins interacting with the ACR (Tables 1 and 3 and Fig. 1B). 3) The E3 ubiquitin-protein ligase interacting with the ACR is enzymatically active and ubiquitinates the five lysine residues present in segments that are exposed to the cytosol.
The APP cytosolic domain facilitates ubiquitination of interact-
ing proteins in vitro. The five cytoplasmic APP residues (Lys649, Lys650, Lys651, Lys688) are targets of other UPS-linked proteins isolated by St-ACR and five ubiquitinated in vitro are also ubiquitinated in vitro in ACR pulldowns (Table 9) suggesting that the E3 ubiquitin-protein ligase(s) present in the pulldown interaction may be responsible for ubiquitination of APP in vivo. The evidence that ubiquitination of Lys676, but not that of the other four ACR lysine residues, is significantly reduced in the absence of CRL4CRBN (Table 10) suggests that the E3 ubiquitin-protein ligase(s) present in the pulldown interaction may be responsible for ubiquitination of APP in vivo. The five cytoplasmic APP residues (Lys649, Lys650, Lys651, Lys676, and Lys688), which are ubiquitinated in vitro, are also ubiquitinated in vitro in ACR pulldowns (Table 9) suggesting that the E3 ubiquitin-protein ligase(s) present in the pulldown interaction may be responsible for ubiquitination of APP in vivo. The evidence that ubiquitination of Lys676, but not that of the other four ACR lysine residues, is significantly reduced in the absence of CRL4CRBN (Table 10) suggests the following: 1) Lys676 of APP may be physiologically ubiquitinated mainly, but not exclusively, by the CRL4CRBN E3 ubiquitin-protein ligase; 2) Lys649, Lys650, Lys651, and Lys688 are probably targets of other E3 ubiquitin-protein ligases, although a role for CRL4CRBN cannot be formally excluded, like Stub1 that is very abundant in the ACR pulldown. Because the subunits of CRL4CRBN undergo auto-ubiquitination (Table 6 and Fig. 5B), the possibility that APP may at the same time act as a substrate recognition unit and a substrate of a CRL4CRBN/APP E3 ubiquitin-protein ligase is not far-fetched.

APP belongs to a protein family that includes APLP1 and APLP2. Analysis of single and double knock-out (KO and dKO) mice has shown that App-KO, Aplp1-KO, Aplp2-KO, and App/ Aplp1-dKO have minor deficits. In contrast, App/Aplp2-dKO mice have severe neuromuscular junctions deficits, are significantly smaller than App-KO and Aplp2-KO mice, and die within the first 28 days of life (25, 43, 102, 103). These data indicate that APP and APLP2 share some essential function that cannot be compensated for by APLP1. However, the molecular mechanisms mediating this essential function (or functions) of APP and APLP2 are unclear. Here, we show that the brain interactomes of the ACR and the AL2CR, but not of the AL1CR, share many UPS-related proteins, including Stub1 and CRL4CRBN (Table 5 and Fig. 4B). Moreover, the AL2CR interacts, like the ACR, with the substrate recognition pocket of Crbn (Fig. 4, C and D). Finally, the ACR and the AL2CR brain interactomes possess E3 ubiquitin-protein ligase activity, whereas the brain interactome of the AL1CR does not (Fig. 5A). Altogether, these data suggest that both APP and APLP2 may possess E3 ubiquitin-protein ligase substrate recognition activity, which is not compensated for by APLP1. Thus, the loss of this activity may mechanistically cause the severe phenotype of App/Aplp2-dKO mice.

APP facilitates glutamatergic transmitter release, likely through the interaction with the neurotransmitter release machinery (28). In addition, the APP intracellular domain has been linked to many other pathological and functional pathways, including caspase activation, transcription, Ca2+ flux, and neurodegeneration (28, 31, 32, 34, 40, 46, 64, 104, 106–108). It is tempting to speculate that APP may affect all these seemingly unrelated pathways via a single mechanism of action. Indeed, by acting as a substrate recognition unit for E3 ubiquitin-protein ligase(s), APP could modulate ubiquitination of proteins involved in these processes (model in Fig. 7). Thus, using a single modus operandi, APP may regulate disparate and seemingly unrelated signaling pathways. As we show here, many of the proteins that control synaptic vesicle functions and interact with APP are ubiquitinated in vitro in an ACR-dependent manner (Table 6). Consequently, APP may aid synaptic vesicle activity by modulating ubiquitination of these proteins, thereby altering their stability and/or their function.

Eleven UPS-linked proteins isolated by St-ACR and five brain-derived proteins that are ubiquitinated in vitro in an ACR-dependent manner are associated with genetic diseases of the central nervous system and neuromuscular system. These include HUWE1, CRBN, CUL4B, USP9X, PARK7, STUB1,

---

**TABLE 8**

In vitro ubiquitination of ACR-interacting proteins occurs on a subset of the lysine residues ubiquitinated in vivo

The 1st column lists some of the proteins ubiquitinated in vivo in an ACR-dependent manner. The 2nd column reports all the lysine residues found ubiquitinated in the mouse brain lysate UbiScan that we performed (in vivo ubiquitinations). The 3rd column reports the lysine residues found ubiquitinated in our in vitro ubiquitination/UbiScan experiment.

| Protein | K-ub found in our UbiScans from mouse brains | K-ub found in the in vitro ubiquitination/UbiScan assay of ACRWT-pulldown |
|---------|---------------------------------------------|----------------------------------------------------------------------------|
| Scamp1  | 52, 65, 78, 89, 298, 311, 334               | 334                                                                        |
| SV2a    | 32, 143, 375, 396                           | 32                                                                         |
| Syt1    | 98, 104, 107, 189, 190, 191, 200, 213, 267, 272, 288, 297, 321, 332, 366, 369, 420, 421 | 98, 104, 190, 200, 369                                                     |
| SNAP-25 | 40, 96, 102, 103, 184, 189, 201             | 103                                                                        |
| ApoE    | 85, 86, 105, 173, 252                       | 105                                                                        |
| Tau     | 455, 546, 551, 566, 603, 609, 613, 623, 632, 635, 645, 661, 662, 667, 677, 730 | 455, 613                                                                  |

**TABLE 9**

E3 ligases interacting with the ACR ubiqutinate the five lysine residues of the ACR

The 1st column shows the site of modification for the peptide assignment. The 2nd column reports the amino acid sequence for the peptide assignment with the ubiquitinated lysine (*). The 3rd column reports the relative fold-change between the integrated peak area of the experimental (numerator, Crbn-KO) and control (denominator, WT) conditions. A negative value indicates the peptide is more abundant in the control condition.

| Site | Peptide | Normalized fold change Crbn-KO/WT |
|------|---------|----------------------------------|
| 649  | K*KQYTSIHHGVVEVDAAVTPEER          | 1.4                              |
| 650  | KK*KQYTSIHHGVVEVDAAVTPEER         | 1.3                              |
| 651  | KK*QYTSIHHGVVEVDAAVTPEER          | 1.4                              |
| 676  | HLSK*MQGGYNENPTYK                 | −3.9                             |
| 688  | MQGGYNENPTYK*FFCQCN               | −1.2                             |

**TABLE 10**

Ubiquitination of Lys676 is significantly reduced in the absence of CRL4CRBN

Table shows the parent ion intensity observed during the MS at its chromatographic apex for the two WT samples (1st and 2nd columns) and the two Crbn-KO samples (3rd and 4th columns).

| Normalized intensity | WT (1) | WT (2) | Crbn-KO (1) | Crbn-KO (2) |
|----------------------|--------|--------|-------------|-------------|
| 5,690,496            | 6,303,560 | 9,363,865 | 9,691,813 |
| 429,867              | 688,670  | 835,455  | 739,774    |
| 1,480,860            | 1,749,112 | 2,392,320 | 2,704,107 |
| 6,532,470            | 6,297,230 | 1,840,820 | 1,776,503 |
| 37,117,469           | 32,761,543 | 38,646,971 | 26,595,257 |
Modulation of E3 Ligases by APP

Ubiquitinations of APP and APP-interacting proteins may lead to:
1) Degradation by proteasome/lysosome/autophagosome
2) Modification of function
DCAF8, TRIM32, UBE3A, UBQL2, UBA1, SYT2, SNAP-25, SYN1, APOE, and TAU (109–139).

These observations suggest a molecular and functional connection between APP and other proteins genetically involved in Alzheimer and other neurodegenerative diseases. Tau is the main component of neuronal tangles that characterize AD, and TAU mutations are linked to genetic forms of frontotemporal dementia. Previous studies have shown that APP and Tau interact in vivo (97). Interestingly, Stub1 mediates ubiquitination of Tau, and Tau is polyubiquitinated at several sites in AD patients (140–142). Thus, a role of APP in Tau ubiquitination would be both biologically and pathologically significant. APOE is the main genetic risk factor for sporadic AD, with the APOE4 allele increasing the risk of developing late onset AD (137). Our in vitro evidence suggesting that APP may both interact with and regulate ubiquitination of apoE is inciting and needs to be developed further. Four of the proteins discussed in this study (HUWE1, CRBN, CUL4B, and USP9X) are genetically linked to dementia. Previous studies have shown that APP and Tau interaction between APP and other proteins genetically involved in neurodegenerative diseases and the link between mutations in proteins involved in the UPS and neurodegenerative disorders are both established truths (52, 105, 143).

Several APP-derived metabolites contain the ACR and can interact with Stub1 and CRL4CRBN (Fig. 7E). These include the following: 1) full-length APP; 2) β-CTF and α-CTF, which are the products of β- and α-secretase cleavage of full-length APP; and 3) AID/AICD peptide, which is produced by γ-secretase cleavage of β-CTF and α-CTF. Like full-length APP, β-CTF and α-CTF are membrane-bound; in principle, full-length APP-Stub1/CRL4CRBN, β-CTF-Stub1/CRL4CRBN, and α-CTF-Stub1/CRL4CRBN complexes could have distinct functions, although this concept may not be immediately obvious. In contrast, processing of β-CTF and α-CTF by γ-secretase would release AID-Stub1/CRL4CRBN complexes from membranes. This may have several predictable biological consequences, including a down-modulation of the APP-dependent ubiquitination of trans-membrane proteins.

FIGURE 7. APP could be both a substrate and a substrate recognition subunit of Stub1 and CRL4CRBN E3 protein ligases. A. CRL4CRBN mediates ubiquitination of lysine residue(s) present in the cytoplasmic tail of APP (with Lys735 being the most likely candidate). B, Stub1 could also be involved in the ubiquitination of cytoplasmic APP lysine residue(s). C, APP could bridge cytosolic and membrane-bound proteins to CRL4CRBN de facto functioning as a substrate recognition unit of a CRL4CRBN-APP E3 ubiquitin-protein ligase. D, in this final model APP is postulated to act as a substrate recognition unit for a Stub1-APP E3 ubiquitin-protein ligase, mediating ubiquitination of cytosolic and/or membrane-bound proteins that interact with the ACR ubiquitination of APP, and APP-binding proteins could lead to functional modification of or to degradation of the ubiquitinated proteins by the proteasome, autophagosomes, and/or lysosomes. E, some of the APP-derived metabolites that contain the ACR and can potentially interact with Stub1 and CRL4CRBN Processing of full-length APP by β-, α-, and γ-secretase can have several functional consequences. For example, it is possible that full-length APP-Stub1/CRL4CRBN, β-CTF-Stub1/CRL4CRBN, and α-CTF-Stub1/CRL4CRBN complexes have distinct functions, i.e., that the ectodomain of APP may influence the function of the ACR. That processing of β-CTF and α-CTF by γ-secretase could have functional consequences is obvious. Indeed, AID-Stub1/CRL4CRBN complexes are released from membranes. This may, among other things, result into down-modulation of the APP-dependent ubiquitination of trans-membrane proteins. F, cleavage of APP-Stub1/CRL4CRBN, β-CTF-Stub1/CRL4CRBN, α-CTF-Stub1/CRL4CRBN, and AID-Stub1/CRL4CRBN by caspases could functionally separate the activities linked to the various ACR-Stub1 and ACR-CRL4CRBN complexes.
Modulation of E3 Ligases by APP

EERLHSKMQNGYENPTYKFFEQMQ; St-JCasp, WSHQQKEV-KMLKKKQYTSIHGVVEVD; St-C4, WSHQQKEFK-MQN; St-C8, WSHQQKEFKKEFQMQN; St-C12, WSHQQKEKNTYPYKFFE-EQMQN; St-C20, WSHQQKEKMMQNGYENPTYKFFE-QMQN; St-C24, WSHQQKEKRLHSMQNGYENPTYKFF-EQMQN; St-C28, WSHQQKEKTPERHLHSMQNGYENPTYKFFE-QMQN; St-C282, WSHQQKEKTPERHLHSMQNGYENPTYKFFE-QMQN; St-C2863, WSHQQKEKTPERHLHSMQNGYENPTYKFFE-QMQN; St-C2865, WSHQQKEKTPERHLHSMQNGYENPTYKFFE-QMQN; St-C2867, WSHQQKEKTPERHLHSMQNGYENPTYKFFE-QMQN; St-AL1CR, WSHQQKEKRRKPYGAI-SHGVVEVDPMLTLEEQQQLRELQRHGYENPTYRFLEERP; and St-AL2CR, WSHQQKEKLRKKQYGTTISHGEVDPMLTPEE-RHLNKQMNHGYNPTYKYLQMQI.

Pulldown Assays with St-peptides—The St-peptides were immobilized on StrepTactin column (catalog no. 2-1209-550, IBA-GmbH, Goettingen, Germany). S2 plus LS1 brain fractions were pre-cleared on StrepTactin columns containing no St-peptides. Pre-cleared S2 plus LS1 brain fractions were next passed through the StrepTactin column loaded with St-peptides. The columns were then washed, and St-peptides, together with brain proteins specifically bound to the St-peptide, were eluted with desthiobiotin following the manufacturer’s recommendations. In some pulldowns, brain lysates were incubated for 1 h at 4 °C with the indicated concentrations of either lonalidomide (catalog no. T2800, lot 2570277, LKT Laboratories, Inc., St. Paul, MN) or thalidomide (catalog no. 0652, batch 11A/141284, Tocris Bioscience, Bristol, UK), prior to pulldown with St-peptides.

In Vitro Ubiquitination Assay—Pulldown samples were incubated in 50 mM Tris, pH 7.6, 5 mM MgCl2, 2 mM ATP, 0.6 mM DTT, with/without 40 ng of the E1 UBE1 (catalog no. E-305, lot 16114714, BostonBiochem), 16114714, BostonBiochem), 0.3 μg of the E2 UbcH5a/UBE2D1 (catalog no. E2-616, Lot 04201314C, BostonBiochem). Reactions were incubated in 50 mM Tris, pH 7.6, 5 mM MgCl2, 2 mM ATP, 0.6 mM DTT, with/without 40 ng of the E1 UBE1 (catalog no. E-305, lot 16114714, BostonBiochem), 0.3 μg of the E2 UbcH5a/UBE2D1 (catalog no. E2-616, Lot 04201314C, BostonBiochem), 1 μg of ubiquitin-FLAG (catalog no. U-211, Lot DBGI0215011, BostonBiochem), 1 μg recombinant human HA ubiquitin aldehyde, C terminus (catalog no. U-556, lot 0AB03101C, BostonBiochem). Reactions were incubated overnight at 30 °C. The final volume of the reaction was 30 μl/sample.

Immunoprecipitation of the in Vitro Ubiquitination Assays—To isolate proteins ubiquitinated in vitro, the in vitro ubiquitination assay performed on St-ACR pulldown was incubated with FLAG-M2 affinity gel (catalog no. A2220, lot SLBF8148, Sigma), under constant rotation for 3 h at 4 °C. The agarose beads were collected by centrifugation and washed five times in phosphate-buffered saline plus 0.05% Tween. After washing, the proteins bound to FLAG-M2 affinity gel were eluted by incubation with 100 μM concentration of the 3×FLAG competing peptide (catalog no. F4799, lot SLBG0131V, Sigma).

Mouse Brain Preparation for UbiScan—To process the brain tissue for UbiScan analysis, brains from at least eight animals of the same genotype for each experiment were cut into small pieces and lysed in freshly prepared urea lysis buffer (20 mM Hepes, 9 M urea, 1 mM sodium orthovanadate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerol phosphate), using 4 ml of buffer for 100 mg of tissue. Samples were homogenized twice using a Polytron set to maximum speed, using 20-ml-long pulses. Between pulses, samples were chilled on ice for 1 min. Successively, samples were sonicated using a micropipet set to 15-watt output using three bursts of 30 s each. Between bursts, samples were chilled on ice for 1 min. Finally, the lysates were cleared from debris by centrifugation at 20,000 × g for 15 min at 4 °C.

UbiScan Analysis—This analysis was performed by the PTM-Scan Facility at Cell Signaling Technology. Briefly, samples were digested with trypsin; after digestion, peptides were loaded directly onto a 10 cm × 75 μm PicoFrit capillary column packed with Magic C18 AQ reversed-phase resin. The column was developed with a 90-min linear gradient of acetonitrile in 0.125% formic acid delivered at 280 nl/min. Ubiquitinated peptides were enriched by immunoprecipitation with the ubiquitin branch motif antibody (K-e-GG) (catalog no. 3925). Samples were subjected to LC-MS/MS analysis using LTQ-Orbitrap-Velos, ESI-CID. MS parameter settings are as follows: MS run time of 96 min; MS1 scan range (300.0–1500.0); top 20 MS/MS (minimum signal 500; isolation width 2.0; normalized collision energy 35.0; activation-Q 0.250; activation time 20.0; lock mass 371.101237; charge state rejection enabled, charge state 1+ rejected; dynamic exclusion enabled, repeat count 1; repeat duration 35.0; exclusion list size 500; exclusion duration 40.0; exclusion mass width relative to mass; exclusion mass width 10 ppm). MS/MS spectra were evaluated using SEQUEST 3G and the CORE platform from Harvard University. Searches were performed against the most recent update of the NCBI Mus musculus database with mass accuracy of ±50 ppm for precursor ions and 1 Da for product ions. Results were filtered with mass accuracy of ±5 ppm on precursor ions and presence of the intended motif (K-e-GG).

Western Blotting (WB) Analysis and Antibodies Specificity—Samples were separated on 4–20% SDS-PAGE (catalog no. 345-0125, Bio-Rad) and transferred onto nitrocellulose membranes (catalog no. 10600012, GE Healthcare). The following data presented here support the specificity of the antibodies as follows. (a) A band of the predicted size for Ddb1, Pin1, Stub-1, Grb2, Cul4a, and Crbn are detected both in total lysates and in pulldown samples. (b) These bands are visible only in pulldown samples in which Ddb1, Pin1, Stub-1, Grb2, Cul4a, and Crbn are detected by nano-LC/MS/MS. (c) Crbn is not detectable in samples derived from Crbn-KO brains. The following antibodies were used in WB: anti-Ddb1 (catalog no. 6998s, lot 1, rabbit monoclonal antibody, Cell Signaling Technology, dilution 1:300); anti-Pin-1 (catalog no. 3972s, lot 2, rabbit polyclonal antibody, Cell Signaling Technology, dilution 1:300); anti-Grb2 (catalog no. 3972s, lot 2, rabbit polyclonal antibody, Cell Signaling Technology, dilution 1:1000); anti-Stub1 (catalog no. 2080s, lot 2, rabbit polyclonal antibody, Cell Signaling Technology, dilution 1:300); anti-Cul4a (catalog no. 14851-1-AP, lot 1, rabbit polyclonal antibody, Proteintech, dilution 1:300); anti-Crbn (catalog no. HPA045910, lot Q103829, rabbit polyclonal antibody, Sigma, dilution 1:500); and anti-FLAG M2 (catalog no. A2220, lot SLBF8148, mouse monoclonal antibody, Sigma, dilution 1:1000). This antibody detects a signals-only in samples in which ubiquitin-FLAG and the ubiquitination machinery are present (Fig. 5A). Incubations with primary antibodies were performed in phosphate-buffered saline plus 0.05%
TWEEN 20 (catalog no. H5151, Promega) and 5% bovine serum albumin fraction V (BSA, catalog no. BP1605-100, lot 146598, Fisher) overnight in a cold room. We used the following HRP-conjugated secondary antibodies. For WB with mouse monoclonal primary antibodies, we used the goat anti-mouse HRP-conjugated antibody (catalog no. 1031-05, lot I0912-ML33B, Southern Biotech, dilution 1:1000). For WB with rabbit primary antibodies, we used two goat anti-mouse HRP-conjugated secondary antibodies (catalog no. 4050-05, lot E0513-ZF44, Southern Biotech, dilution 1:1000; catalog no. 7074, lot 25, Cell Signaling Technology, dilution 1:1000). Membranes were incubated with secondary antibodies in phosphate-buffered saline plus 0.05% Tween 20 and 5% Blotto-Grade blocker nonfat dry milk (catalog no. 170-6404, Bio-Rad), at room temperature for 1 h. After washing in phosphate-buffered saline plus 0.05% Tween, WB was developed using either SuperSignal West Dura stable peroxidase (catalog no. 34076, lot QL226061, Pierce) or SuperSignal West Pico stable peroxidase (catalog no. 1859674, lot OB182893, Pierce). Signals were acquired using ImageQuant LAS 4000 mini and ImageQuant LAS 4000 software (GE Healthcare).

Mass Spectrometry—MS Bioworks, LLC, Ann Arbor, MI, performed this analysis. The volume of each pulldown sample was reduced to 50 μl by vacuum centrifugation; 20 μl of each concentrated sample was processed by SDS-PAGE using a 10% BisTris NuPAGE gel (Invitrogen); with the MES buffer system was operated in data-dependent mode, with MS/MS performed in the Orbitrap at 70,000 and 17,500 full spectrometer was operated in data-dependent mode, with MS low by alkylation with 50 mM iodoacetamide at room temperature; digested with trypsin (Promega) at 37 °C for 4 h; quenched with formic acid; and the supernatant was analyzed directly without further processing. Each digest was analyzed by nano-LC/MS/MS with a Waters NanoAcuity HPLC system interfaced to a ThermoFisher Q Exactive mass spectrometer. Peptides were loaded on a trapping column and eluted over a 75-μm analytical column at 350 nl/min; both columns were packed with Jupiter Proteo resin (Phenomenex). The mass spectrometer was operated in data-dependent mode, with MS and MS/MS performed in the Orbitrap at 70,000 and 17,500 full width at half-maximum resolution, respectively. The 15 most abundant ions were selected for MS/MS.

Author Contributions—L. D. conceived and coordinated the study and wrote the paper. D. D. P. and L. D. designed, performed, and analyzed all the experiments. D. D. P. contributed to writing the paper. A. M. R. provided reagents/knock-out mice and contributed to writing the paper. R. C. R. generated knock-out mice. L. D. and R. C. R. conceived and coordinated the study. L. D. prepared the tables. All authors contributed to writing the paper. A. M. R. provided reagents/knock-out mice and contributed to writing the paper. D. D. P. and L. D. designed, performed, and wrote the paper. R. C. R. generated knock-out mice. L. D. and R. C. R. conceived and coordinated the study. L. D. and R. C. R. conceived and coordinated the study.

References
1. Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., et al. (2012) A mutation in APP protects against Alzheimer’s disease and age-related cognitive decline. Nature 488, 96–99
2. Matsuda, S., Giliberto, L., Matsuda, Y., Davies, P., McGowan, E., Pickford, F., Ghiso, J., Frangione, B., and D’Adamo, L. (2005) The familial dementia BRI2 gene binds the Alzheimer gene amyloid-β precursor protein and inhibits amyloid-β production. J. Biol. Chem. 280, 28912–28916
3. Egashira, N., Iwasaki, K., Takashima, A., Watanabe, T., Kawabe, H., Matsuda, T., Mishima, K., Chiiori, S., Nishimura, R., and Fujiwara, M. (2005) Altered depression-related behavior and neurochemical changes in se-rotonergic neurons in mutant R406W human τ transgenic mice. Brain Res. 1059, 7–12
4. Matsuda, S., Giliberto, L., Matsuda, Y., McGowan, E. M., and D’Adamo, L. (2008) BRI2 inhibits amyloid-β-peptide precursor protein processing by interfering with the docking of secretases to the substrate. J. Neurosci. 28, 8668–8676
5. Matsuda, S., Matsuda, Y., and D’Adamo, L. (2009) BRI3 inhibits amyloid precursor protein processing in a mechanistically distinct manner from its homologue dementia gene BRI2. J. Biol. Chem. 284, 15815–15825
6. Tanzi, R. E. (2012) The genetics of Alzheimer disease. Cold Spring Harb. Perspect. Med. 2, a006629
7. Vidal, R., Frangione, B., Rostagno, A., Mead, S., Rêvész, T., Plant, G., and Ghiso, J. (1999) A stop-codon mutation in the BRI gene associated with familial British dementia. Nature 399, 776–781
8. Garringer, H. J., Murrell, J., D’Adamo, L., Ghetti, B., and Vidal, R. (2010) Modeling familial British and Danish dementia. Brain Struct. Function 214, 235–244
9. Vidal, R., Revesz, T., Rostagno, A., Kim, E., Holton, J. L., Bek, T., Bojsen-Møller, M., Braendgaard, H., Plant, G., Ghiso, J., and Frangione, B. (2000) A decoder duplication in the 3’ region of the BRI gene originates an amyloid peptide that is associated with dementia in a Danish kindred. Proc. Natl. Acad. Sci. U.S.A. 97, 4920–4925
10. De Strooper, B. (2007) Loss-of-function presenilin mutations in Alzheimer disease. talking point on the role of presenilin mutations in Alzheimer disease. EMBO Rep. 8, 141–146
11. De Strooper, B., Vassar, R., and Golde, T. (2010) The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat. Rev. Neurosci. 11, 99–107
12. De Strooper, B., and Voet, T. (2012) Alzheimer’s disease: a protective mutation. Nature 488, 38–39
13. Baulac, S., LaVoie, M. J., Kimberly, W. T., Strahle, J., Wolfe, M. S., Selkoe, D. J., and Xia, W. (2003) Functional γ-secretase complex assembly in Golgi/trans-Golgi network: interactions among presenilin, nicastrin, Aph1, Pen-2, and γ-secretase substrates. Neuron Biol. Dis. 14, 194–204
14. Borchelt, D. R., Lee, M. K., Gonzales, V., Slunt, H. L., Ratovitski, T., Jenkins, N. A., Copeland, N. G., Price, D. L., and Sisodia, S. S. (2002) Accumulation of proteolytic fragments of mutant presenilin 1 and accelerated amyloid deposition are co-regulated in transgenic mice. Neurobiol. Aging 23, 171–177
15. Bennett, B. D., Babu-Khan, S., Loeloff, R., Louis, J. C., Curran, E., Citron, M., and Vassar, R. (2000) Expression analysis of BACE2 in brain and peripheral tissues. J. Biol. Chem. 275, 20647–20651
16. Annaert, W., Cuppers, P., Safiig, P., and De Strooper, B. (2000) Presenilin function in APP processing. Ann. N.Y. Acad. Sci. 920, 158–164
17. De Strooper, B. (2003) Aph-1, Pen-2, and nicastrin with presenilin generate an active γ-Secretase complex. Neuron 38, 9–12
18. Pellegrini, L., Passer, B. J., Tabaton, M., Ganjei, J. K., and D’Adamo, L. (1999) Alternative, non-secretase processing of Alzheimer’s β-amyloid precursor protein during apoptosis by caspase-6 and -8. J. Biol. Chem. 274, 21011–21016
19. Gervaist, F. G., Xu, D., Robertson, G. S., Vaillancourt, J. P., Zhi, Y., Huang, J., LeBlanc, A., Smith, D., Rigby, M., Shearman, M. S., Clarke, E. E., Zheng, H., Van Der Ploeg, L. H., Rufolo, S. C., Thornberry, N. A., et al. (1999) Involvement of caspases in proteolytic cleavage of Alzheimer’s amyloid-β precursor protein and amyloidogenic Aβ peptide formation. Cell 97, 395–406
20. Weidemann, A., Paliga, K., Dürrwang, U., Reinhard, F. B., Schuckert, O., Evin, G., and Masters, C. L. (1999) Proteolytic processing of the Alzheimer’s disease amyloid precursor protein within its cytoplasmic domain.
Modulation of E3 Ligases by APP

by caspase-like proteases. J. Biol. Chem. 274, 5823–5829

21. Passer, B. J., Pellegrini, L., Vito, P., Ganjei, J. K., and D’Adamio, L. (1999) Interaction of Alzheimer’s presenilin-1 and presenilin-2 with Bcl-X(L). A potential role in modulating the threshold of cell death. J. Biol. Chem. 274, 24007–24013

22. Lu, D. C., Rabizadeh, S., Chandra, S., Shaya, R. F., Ellery, L. M., Ye, X., Salvesen, G. S., Koo, E. H., and Bredesen, D. E. (2000) A second cytotoxic proteolytic peptide derived from amyloid β-protein precursor. Nat. Med. 6, 397–404

23. Madeira, A., Pommet, J. M., Prochiantz, A., and Allinquant, B. (2005) SET protein (TAF1β, I2PP2A) is involved in neuronal apoptosis induced by an amyloid precursor protein cytoplasmic subdomain. FASEB J. 19, 1905–1907

24. Bertrand, E., Brouillet, E., Caillet, I., Boulliott, C., Cole, G. M., Prochiantz, A., and Allinquant, B. (2001) A short cytoplasmic domain of the amyloid precursor protein induces apoptosis in vitro and in vivo. Mol. Cell. Neurosci. 18, 503–511

25. Barbagallo, A. P., Wang, Z., Zheng, H., and D’Adamio, L. (2011) A single tyrosine residue in the amyloid precursor protein intracellular domain is essential for developmental function. J. Biol. Chem. 286, 8717–8721

26. Li, H., Wang, Z., Wang, B., Guo, Q., Dolios, G., Tabuchi, K., Hammer, R. E., Sůdhof, T. C., Wang, R., and Zheng, H. (2010) Genetic dissection of the amyloid precursor protein in developmental function and amyloid pathogenesis. J. Biol. Chem. 285, 30598–30605

27. Barbagallo, A. P., Wang, Z., Zheng, H., and D’Adamio, L. (2011) The intracellular threonine of amyloid precursor protein that is essential for docking of Pin1 is dispensable for developmental function. PLoS One 6, e18006

28. Fanutza, T., Del Prete, D., Ford, M. J., Castillo, P. E., and D’Adamio, L. (2015) APP and APLP2 interact with the synaptic release machinery and facilitate transmitter release at hippocampal synapses. Elife 4, e09743

29. Klevanski, M., Herrmann, U., Weyer, S. W., Fol, R., Cartier, N., Wolfner, D. P., Caldwell, J. H., Korte, M., and Müller, U. C. (2015) The APP intracellular domain is required for normal synaptic morphology, synaptic plasticity, and hippocampus-dependent behavior. J. Neurosci. 35, 16018–16033

30. Hamid, R., Kliger, E., Willem, M., Vassallo, N., Kostka, M., Bornhövd, C., Pardossi-Piquard, R., Petit, A., Kawarai, T., Sunyach, C., Alves da Costa, C., Vincent, B., Ring, S., D’Adamio, L., Shen, J., Müller, U., St George-Hyslop, P., and da Costa, C. A. (2007) The γ-secretase-derived APP intracellular domain fragments regulate p53. Curr. Alzheimer Res. 4, 423–426

31. Leissring, M. A., Murphy, M. P., Mead, T. R., Akbari, Y., Sugarman, M. C., Jannatipour, M., Anliker, B., Müller, U., Saftig, P., De Strooper, B., Wolfe, M. S., Golde, T. E., and LaFerla, F. M. (2002) A physiologic signaling role for the γ-secretase-derived intracellular fragment of APP. Proc. Natl. Acad. Sci. U.S.A. 99, 4697–4702

32. Müller, U. C., and Zheng, H. (2012) Physiological functions of APP family proteins. Cold Spring Harb. Perspect. Med 2, a006288

33. Scheinfeld, M. H., Ghezri, E., Laky, K., Fowlkes, B. J., and D’Adamio, L. (2002) Processing of β-amyloid precursor-like protein-1 and -2 by γ-secretase regulates transcription. J. Biol. Chem. 277, 44195–44201

34. Heber, S., Herm, J., Gajic, V., Hainfellner, J., Aguzzi, A., Rülicke, T., von Kretzschmar, H., von Koch, C., Sisodia, S., Tremml, P., Lipp, H. P., Wolfner, D., and Müller, U. (2000) Mice with combined gene knockouts reveal essential and partially redundant functions of amyloid precursor protein family members. J. Neurosci. 20, 7951–7963

35. Herms, J., Anliker, B., Heber, S., Ring, S., Fuhrmann, M., Kretzschmar, H., Sisodia, S., and Müller, U. (2004) Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. EMBO J. 23, 4106–4115

36. von Koch, C. S., Zheng, H., Chen, H., Trumbauer, M., Thinakaran, G., van der Ploeg, L. H., Price, D. L., and Sisodia, S. S. (1997) Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. Neurobiol. Aging 18, 661–669

37. Scheinfeld, M. H., Matsuoka, S., and D’Adamio, L. (2003) JNK-interacting protein-1 promotes transcription of Aβ protein precursor but not Aβ precursor-like proteins, mechanistically different than Fe65. Proc. Natl. Acad. Sci. U.S.A. 100, 1729–1734

38. Wang, P., Yang, G., Mosier, D. R., Chang, P., Zaidi, T., Gong, Y. D., Zhao, N. M., Dominguez, B. Lee, K. F., Gan, W. B., and Zheng, H. (2005) Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-Like protein 2. J. Neurosci. 25, 1219–1225

39. Del Prete, D., Lombino, F., Liu, X., and D’Adamio, L. (2014) APP is cleaved by Bace1 in pre-synaptic vesicles and establishes a pre-synaptic interaction, via its intracellular domain, with molecular complexes that regulate pre-synaptic vesicles functions. PLoS One 9, e108576

40. Ito, T., Ando, H., Suzuki, T., Ogura, T., Hotta, K., Imamura, Y., Yamaguchi, Y., and Handa, H. (2010) Identification of a primary target of thalidomide in human brain. Science 327, 1345–1350

41. Lombino, F., Biundo, F., Tamayev, R., Arancio, O., and D’Adamio, L. (2013) An intracellular threonine of amyloid-β precursor protein mediates synaptic plasticity deficits and memory loss. PLoS One 8, e57120

42. Tamayev, R., and D’Adamio, L. (2012) Inhibition of γ-secretase worsens memory deficits in a genetically congruous mouse model of Danish dementia.. Mol. Neurodegener. 7, 19

43. Riederer, B. M., Leuba, G., Vernay, A., and Riederer, I. M. (2011) The role of the ubiquitin proteasome system in Alzheimer’s disease. Exp. Biol. Med. 236, 268–276

44. Matrone, C., Lusvietto, S., La Rosa, L. R., Tamayev, R., Pigaturo, A., Camu, N., Yang, L., Barbagallo, A. P., Biundo, F., Lombino, F., Zheng, H., Ammassari-Teule, M., and D’Adamio, L. (2012) Tyr682 in the α-secretase precursor intracellular domain regulates synaptic connectivity, cholinergic function, and cognitive performance. Aging Cell 11, 1084–1093

45. Barbagallo, A. P., Weldon, R., Tamayev, R., Zhou, D., Giliberto, L., Foreman, O., and D’Adamio, L. (2010) Tyr682 in the α-secretase precursor intracellular domain regulates synaptic connectivity, cholinergic function, and cognitive performance. Aging Cell 11, 1084–1093

46. Zhou, D., Zambrano, N., Russo, T., and D’Adamio, L. (2009) Phosphorylation of their intracellular domains. Mol. Neurodegener. 4, 28
ylation of a tyrosine in the amyloid-β protein precursor intracellular domain inhibits F65 binding and signaling. J. Alzheimers Dis. 16, 301–307.

57. Ghersi, E., Vito, P., Lopez, P., Abdallah, M., and D’Adamio, L. (2004) The intracellular localization of amyloid β protein precursor (AβPP) intracellular domain associated protein-1 (AIDA-1) is regulated by AβPP and alternative splicing. J. Alzheimers Dis. 6, 67–78.

58. Ghersi, E., Noviello, C., and D’Adamio, L. (2004) Amyloid-β protein precursor (AβPP) intracellular domain-associated protein-1 proteins bind to AβPP and modulate its processing in an isoform-specific manner. J. Biol. Chem. 279, 49105–49112.

59. Zhou, D., Noviello, C., D’Ambrosio, C., Scalon, A., and D’Adamio, L. (2004) Growth factor receptor-bound protein 2 interaction with the tyrosine-phosphorylated tail of amyloid β protein precursor is mediated by its Src homology 2 domain. J. Biol. Chem. 279, 25374–25380.

60. Scheinfeld, M. H., Ghersi, E., Davies, P., and D’Adamio, L. (2003) Amyloid β protein precursor is phosphorylated by JNK-1 independent of, yet facilitated by, INK-interacting protein (IIP)-1. J. Biol. Chem. 278, 42058–42063.

61. Matsuda, S., Matsuda, Y., and D’Adamio, L. (2003) Amyloid β protein precursor (AβPP), but not AβPP-like protein 2, is bridged to the kinesis light chain by the scaffold protein JNK-interacting protein 1. J. Biol. Chem. 278, 38601–38606.

62. Noviello, C., Vito, P., Lopez, P., Abdallah, M., and D’Adamio, L. (2003) Autosomal recessive hypercholesterolemia protein interacts with and regulates the cell surface level of Alzheimer’s amyloid β peptide precursors. J. Biol. Chem. 278, 31834–31847.

63. Tarr, P. E., Roncarati, R., Pellici, G., Pellici, P. G., and D’Adamio, L. (2002) Tyrosine phosphorylation of the β-amyloid protein precursor cytoplasmic tail promotes interaction with Shc. J. Biol. Chem. 277, 16798–16804.

64. Roncarati, R., Rastan, S., Scheinfeld, M. H., Berechid, B. E., Lopez, P. A., Meucci, O., McGlade, J. C., Rakic, P., and D’Adamio, L. (2002) The γ-secretase-generated intracellular domain of β-amyloid protein precursor binds Numb and inhibits Notch signaling. Proc. Natl. Acad. Sci. U.S.A. 99, 7102–7107.

65. Scheinfeld, M. H., Roncarati, R., Vito, P., Lopez, P. A., Abdallah, M., and D’Adamio, L. (2002) The γ-secretase-generated intracellular domain of β-amyloid precursor protein binds Numb and inhibits Notch signaling. Proc. Natl. Acad. Sci. U.S.A. 99, 7102–7107.

66. Bauza, I., Faraonio, R., Minopoli, G., Zambrano, N., and Russo, T. (1998) The region of the Fe65 protein homologous to the phosphotyrosine interaction with Shc/Grb2 adaptor proteins in reactive astrocytes of Alzheimer’s disease brain. J. Biol. Chem. 273, 1695, 874–903.

67. Kumar, P., Ambasta, R. K., Veereshswaraya, V., Rosen, K. M., Kosik, K. S., Band, H., Mestril, R., Patterson, C., and Querfurth, H. W. (2007) CHIP and HSIPs interact with β-APP in a proteasome-dependent manner and influence Aβ metabolism. Hum. Mol. Genet. 16, 848–864.

68. Rajadhyaksha, A. M., Ra, S., Kishinevsky, S., Lee, A. S., Romanienko, P., DuBoff, M., Yang, C., Zapan, B., Byrne, M., Daruwalla, Z. R., Mark, W., Kosofsky, B. E., Toth, M., and Higgins, J. I. (2012) Behavioral characterization of cereblon forebrain-specific conditional null mice: a model for human non-syndromic intellectual disability. Behav. Brain Res. 226, 428–434.

69. Hartmann, M. D., Boichenko, I., Coles, M., Zanini, F., Lupas, A. N., and Hernandez Alvarez, B. (2014) Thalidomide mimics uridine binding to an aromatic cage in cereblon. J. Struct. Biol. 188, 225–232.

70. Chamberlain, P. P., Lopez-Girona, A., Miller, K., Carmel, G., Pagirigan, B., Chie-Leon, B., Rychar, E., Corral, L. G., Ren, Y. J., Wang, M., Riley, M., Delker, S. L., Ito, T., Ando, H., Mori, T., et al. (2014) Structure of the human Cereblon-DDB1-LENALIDOMIDE complex reveals basis for responsiveness to thalidomide analogs. Nat. Struct. Mol. Biol. 21, 803–809.

71. Fischer, E. S., Böhm, K., Lyeadre, J. R., Yang, H., Stadler, M. B., Cavagnini, F., Nagel, J., Seleru, F., Acker, V., Lingaraju, G. M., Tichkule, R. B., Schebesta, M., Forrester, W. C., Schirle, M., Hassiepen, U., et al. (2014) Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide. Nature 512, 49–53.

72. Dawson, G. R., Seabrook, G. R., Zheng, H., Smith, D. W., Graham, S., O'Dowd, G., Bowery, B. J., Boyce, S., Trumbauer, M. E., Chen, H. Y., Van der Ploeg, L. H., and Sirinathsinghji, D. J. (2004) Age-related cognitive marker density in mice lacking the amyloid β precursor protein intracellular domain. FEBS J. 270, 30853–30865.

73. Borg, J. P., Ooi, J., Levy, E., and Logulis, B. (1996) The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPty motif of amyloid precursor protein. Mol. Cell. Biol. 16, 6229–6241.

74. McLoughlin, D. M., and Miller, C. C. (1996) The intracellular cytoplasmic domain of the Alzheimer’s disease amyloid precursor protein interacts with phosphotyrosine-binding domain proteins in the yeast two-hybrid system. FEBS Lett. 397, 197–200.

75. Lu, P. J., Wuif, G., Zhou, X. Z., Davies, P., and Lu, K. P. (1999) The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. Nature 399, 784–788.

76. Tamayev, R., Zhou, D., and D’Adamio, L. (2009) The interactor of the amyloid β precursor protein family members is shaped by phosphorylation of their intracellular domains. Mol. Neurodegener. 4, 28.

77. Sang, Y., Yan, F., and Ren, X. (2015) The role and mechanism of CRL4 E3 ubiquitin ligase in cancer and its potential therapy implications. Oncotarget 6, 42590–42602.

78. Jackson, S., and Xiong, Y. (2009) CUL4s: the CUL4-RING E3 ubiquitin ligases. Trends Biochem. Sci. 34, 562–570.

79. Skaar, J. R., Pagan, J. K., and Pagano, M. (2014) SCF ubiquitin ligase-targeted therapies. Nat. Rev. Drug Discov. 13, 889–903.

80. Dahlmann, B. (2016) Mammalian proteasome subtypes: their diversity in structure and function. Arch. Biochem. Biophys. 591, 132–140.

81. Coyne, E. S., and Wing, S. S. (2016) The business of deubiquitination—location, location, location. F1000Res 5, f1000.

82. Lee, D. Y., and Brown, E. I. (2012) Ubiquilins in the crosstalk among proteolytic pathways. Biol. Chem. 393, 441–447.

83. Russo, C., Venezia, V., Repetto, E., Nizzari, M., Violani, E., Carlo, P., and Schettini, G. (2005) The amyloid precursor protein and its network of interacting proteins: physiological and pathological implications. Brain Res. Brain Res. Rev. 48, 257–264.

84. Pastorino, L., Sun, A., Lu, P. J., Zhou, X. Z., Balastik, M., Finn, G., Wulf, G., Lim, J., Li, S. H., Li, X., Xia, W., Nicholson, L. K., and Lu, K. P. (2006) The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-β production. Nature 440, 528–534.

85. Kumar, P., Ambasta, R. K., Veereshwaraya, V., Rosen, K. M., Kosik, K. S., Band, H., Mestril, R., Patterson, C., and Querfurth, H. W. (2007) CHIP and HSIPs interact with β-APP in a proteasome-dependent manner and influence Aβ metabolism. Hum. Mol. Genet. 16, 848–864.

86. Rajadhyaksha, A. M., Ra, S., Kishinevsky, S., Lee, A. S., Romanienko, P., DuBoff, M., Yang, C., Zapan, B., Byrne, M., Daruwalla, Z. R., Mark, W., Kosofsky, B. E., Toth, M., and Higgins, J. I. (2012) Behavioral characterization of cereblon forebrain-specific conditional null mice: a model for human non-syndromic intellectual disability. Behav. Brain Res. 226, 428–434.
Nitsch, R. M., and Konietzko, U. (2012) Interactome of the amyloid precursor protein APP in brain reveals a protein network involved in synaptic vesicle turnover and a close association with Synaptotagmin-1. J. Proteome Res. 11, 4075–4090

Sabbage, J. J., and Dickey, C. A. (2016) The metathoracic nature of the tau protein: dynamic flexiblility comes at a cost. Front. Neurosci. 10, 3

Holtzman, D. M., Herz, J., and Bu, G. (2012) Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb. Perspect. Med. 2, a006312

Petruelli, L., Dickson, D., Kehoe, K., Taylor, J., Snyder, H., Grover, A., De Lucia, M., McGowan, E., Lewis, J., Prihar, G., Kim, J., Dillmann, W. H., Browne, S. E., Hall, A., Voellmy, R., et al. (2004) CHIP and Hsp70 regulate \( \tau \) ubiquitination, degradation and aggregation. Hum. Mol. Genet. 13, 703–714

Shimura, H., Schwartz, D., Gygi, S. P., and Kosik, K. S. (2004) CHIP-Hsc70 complex ubiquitimates phosphorylated \( \tau \) and enhances cell survival. J. Biol. Chem. 279, 4869–4876

Islam, K., and Levy, E. (1997) Carboxy-terminal fragments of \( \beta \)-amyloid precursor protein bind to microtubules and the associated protein \( \tau \). J. Am. Pathol. 151, 265–271

Harris, F. M., Brecht, W. J., Xu, Q., Tesser, I., Kekonius, L., Wyss-Coray, T., Fish, J. D., Maslah, E., Hopkins, P. C., Searce-Levie, K., Weisgraber, K. H., Muckley, L., Mahley, R. W., and Huang, Y. (2003) Carboxy-terminal-truncated apolipoprotein E4 causes Alzheimer’s disease-like neurodegeneration and behavioral deficits in transgenic mice. Proc. Natl. Acad. Sci. U.S.A. 100, 10966–10971

Brecht, W. J., Harris, F. M., Chang, S., Tesser, I., Yu, G. Q., Xu, Q., Dee Fish, J., Wyss-Coray, T., Buttini, M., Muckley, L., Mahley, R. W., and Huang, Y. (2004) Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. J. Neurosci. 24, 2527–2534

Wenner, C., Lorkowski, S., Engel, T., and Cullen, P. (2001) Apolipoprotein E in macrophages and hepatocytes is degraded via the proteasomal pathway. Biochem. Biophys. Res. Commun. 282, 608–614

Morl, E., Chamoun, Z., Lasiecka, Z. M., Chan, R. B., Williamson, R. L., Veranoveta, C., Dall’Armi, C., Simoes, S., Point Du Jour, K. S., McCabe, B. D., Small, S. A., and Di Paolo, G. (2013) Phosphatidylinositol-3-phosphate regulates sorting and processing of amyloid precursor protein through the endosomal system. Nat. Commun. 4, 2250

Wang, Z., Wang, B., Yang, L., Guo, Q., Aithmitti, N., Songyang, Z., and Zheng, H. (2009) Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis. J. Neurosci. 29, 10788–10801

Yang, G., Gong, Y. D., Gong, K., Jiang, W. L., Kwon, E., Wang, P., Zheng, H., Zhang, X. F., Gan, W. B., and Zhao, N. M. (2005) Reduced synaptic vesicle density and active zone size in mice lacking amyloid precursor protein (APP) and APP-like protein 2. Neurosci. Lett. 384, 66–71

Li, M., Pehar, M., Liu, Y., Bhattacharrya, A., Zhang, S. C., O’Riordan, K. J., Burger, C., D’Adamo, L., and Puglielli, L. (2015) The amyloid precursor protein (APP) intracellular domain regulates translation of p44, a short isoform of p53, through an IRES-dependent mechanism. Neurobiol. Aging 36, 2725–2733

Keller, J. N., Hanny, K. B., and Markesbery, W. R. (2000) Impaired proteasome function in Alzheimer’s disease. J. Neurochem. 75, 436–439

Tamayev, R., Matsuda, S., Arancio, O., and D’Adamio, L. (2012) \( \beta \)- but not \( \gamma \)-secretase proteolysis of APP causes synaptic and memory deficits in mice. EMBO Mol. Med. 4, 171–179

Liu, W., Meng, M., Zhang, B., Du, L., Pan, Y., Yang, P., Gu, Z., Zhou, Q., and Cao, Z. (2015) Dehydroepiandrosterone effectively inhibits human gastric cancer cell-mediated vasculogenic mimicry with low toxicity. Toxicol. Appl. Pharmacol. 287, 98–110

Cao, X., and Südfloh, T. C. (2004) Dissection of amyloid-\( \beta \) precursor protein-dependent transcriptional transactivation. J. Biol. Chem. 279, 24601–24611

Froyen, G., Corbett, M., Vandewalle, J., Jarvela, I., Lawrence, O., Meldrum, C., Bauters, M., Govaerts, K., Vandeule, L., Van Esch, H., Chelly, J., Sanlaville, D., van Bokhoven, H., Ropers, H. H., Laumonnier, F., et al. (2008) Submicroscopic duplications of the hydroxysteroid dehydroge-
124. Cordoba, M., Rodriguez-Quiroga, S., Gatto, E. M., Alurralde, A., and Kauffman, M. A. (2014) Ataxia plus myoclonus in a 23-year-old patient due to STUB1 mutations. *Neurology* **83**, 287–288

125. Klein, C. J., Wu, Y., Vogel, P., Goebel, H. H., Bönnemann, C., Zülsky, K., Botuyan, M. V., Duan, X., Middha, S., Atkinson, E. J., Mer, G., and Dyck, P. J. (2014) Ubiquitin ligase defect by DCAF8 mutation causes HMSN2 with giant axons. *Neurology* **82**, 873–878

126. Frosk, P., Weiler, T., Nylen, E., Sudha, T., Greenberg, C. R., Morgan, K., Fujiwara, T. M., and Wrognemann, K. (2002) Limb-girdle muscular dystrophy type 2H associated with mutation in TRIM32, a putative E3-ubiquitin-ligase gene. *Am. J. Hum. Genet.* **70**, 663–672

127. Schosser, B. G., Frosk, P., Engel, A. G., Klutzy, U., Lochmüller, H., and Wrognemann, K. (2005) Commonality of TRIM32 mutation in causing sarcotubular myopathy and LGMD2H. *Ann. Neurol.* **57**, 591–595

128. Saccone, V., Palmieri, M., Passamano, L., Piluso, G., Meroni, G., Polito, L., and Nigro, V. (2008) Mutations that impair interaction properties of TRIM32 associated with limb-girdle muscular dystrophy 2H. *Hum. Mutat.* **29**, 240–247

129. Sell, G. L., and Margolis, S. S. (2015) From UBE3A to Angelman syndrome: a substrate perspective. *Front. Neurosci.* **9**, 322

130. Finsterer, J., and Burgunder, J. M. (2014) Recent progress in the genetics of motor neuron disease. *Eur. J. Med. Genet.* **57**, 103–112

131. Dlamini, N., Josifova, D. J., Paine, S. M., Wraige, E., Pitt, M., Murphy, A. J., King, A., Buk, S., Smith, F., Abbas, S., Sewry, C., Jacques, T. S., and Jungbluth, H. (2013) Clinical and neuropathological features of X-linked spastic ataxia type 2 (SMAX2) associated with a novel mutation in the UBA1 gene. *Neuromuscul. Disord.* **23**, 391–398

132. Iqbal, Z., Willemsen, M. H., Papon, M. A., Musante, L., Benevento, M., Hu, H., Venselaar, H., Wissink-Lindhout, W. M., Vulto-van Silfhout, A. T., Vissers, L. E., de Brouwer, A. P., Marouillat, S., Wienker, T. F., Ropers, H. H., Kahrizi, K., et al. (2015) Homozygous SLC6A17 mutations cause autosomal-recessive intellectual disability with progressive tremor, speech impairment, and behavioral problems. *Am. J. Hum. Genet.* **96**, 386–396

133. Bourassa, C. V., Meijer, J. A., Merner, N. D., Mered, K. K., Stefanelli, M. G., Hodgkinson, K., Ives, E. J., Pryse-Phillips, W., Jog, M., Boycott, K., Grimes, D. A., Goobie, S., Leckey, R., Dion, P. A., and Rouleau, G. A. (2012) VAMP1 mutation causes dominant hereditary spastic ataxia in Newfoundland families. *Am. J. Hum. Genet.* **91**, 548–552

134. Herrmann, D. N., Horvath, R., Rowden, J. E., Gonzalez, M., Gonzales, M., Sanchez-Mejias, A., Guan, Z., Whittaker, R. G., Almodovar, J. L., Lane, M., Bansagi, B., Pyle, A., Boczonadi, V., Lochmüller, H., Griffin, H., et al. (2014) Synaptotagmin 2 mutations cause an autosomal-dominant form of Lambert-Eaton myasthenic syndrome and nonprogressive motor neuropathy. *Am. J. Hum. Genet.* **95**, 332–339

135. Shen, X. M., Selcen, D., Brengman, J., and Engel, A. G. (2014) Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability. *Neurology* **83**, 2247–2255

136. Garcia, C. C., Blair, H. J., Seager, M., Coulthard, A., Tennant, S., Buddles, M., Curtis, A., and Goodship, J. A. (2004) Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. *J. Med. Genet.* **41**, 183–186

137. Sanders, A. M., Strittmatter, W. J., Schmechel, D., George-Hyslop, P. H., Pericak-Vance, M. A., Joo, S. H., Rosi, B. L., Gusella, J. F., Crapper-Malcolm, D. R., and Alberts, M. J. (1993) Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer’s disease. *Neurology* **43**, 1467–1472

138. Baizabal-Carvallo, J. F., and Jankovic, J. (2016) Parkinsonism, movement disorders and genetics in frontotemporal dementia. *Nat. Rev. Neurol.* **12**, 175–185

139. Iqbal, K., Liu, F., and Gong, C. X. (2016) Tau and neurodegenerative disease: the story so far. *Nat. Rev. Neurol.* **12**, 15–27

140. Thomas, S. N., Cripps, D., and Yang, A. J. (2009) Proteomic analysis of protein phosphorylation and ubiquitination in Alzheimer’s disease. *Methods Mol. Biol.* **566**, 109–121

141. Cripps, D., Thomas, S. N., Jeng, Y., Yang, F., Davies, P., and Yang, A. J. (2006) Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J. Biol. Chem.* **281**, 10825–10838

142. Morris, M., Knudsen, G. M., Maeda, S., Trinidad, J. C., Ioanoviciu, A., Burlingame, A. L., and Mucke, L. (2015) Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. *Nat. Neurosci.* **18**, 1183–1189

143. Lim, K. L. (2007) Ubiquitin-proteasome system dysfunction in Parkinson’s disease: current evidence and controversies. *Expert Rev. Proteomics* **4**, 769–781